LIPASE ACTIVITY IN BOVINE MILK AND BLOOD

By

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Submitted to the faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
July 28, 1967

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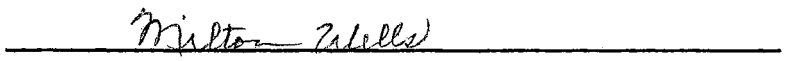
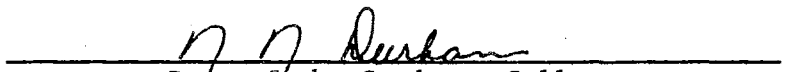
Thesis Approved:

Thesis Adviser





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ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. James B. Mickle, his major adviser, for his supervision and constructive criticism during the experimental work and the writing of this thesis. Appreciation also is expressed to Dr. S. D. Musgrave, Head of the Dairy Science Department, who was helpful in every way. The author wants to thank Mrs. Olive Pryor, Mr. Mack Tietz, and Mr. Don Haggerty, who helped with the experimental work.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Rancidity is a disagreeable flavor defect which sometimes occurs in raw milk. This actually is a combination of several flavors rather than a single one. It is caused by the enzyme lipase which hydrolyzes milk fat producing free fatty acids that are responsible for the flavor defects. When it occurs, this flavor results in lost sales; thus it is of importance to the dairy industry in Oklahoma and throughout the nation. A great number of papers have been published about this flavor defect, most of which have no bearing on the present study; however, they have been reviewed by Johnson (6) and by Kolar (8).

Several workers have concluded that the tendency for raw milk to develop rancidity is a function of the individual cow and that this tendency varies from day-to-day in the same cow (2, 5). Although much has been accomplished recently towards understanding the actions of lipase in other systems, little progress has been made toward understanding the causes for the differences in milk lipase activity among cows.

It is known that the lipase activity of the liver is higher in a laying hen than it is in an unmature bird (4). In addition, lipase activity in the mammary gland of guinea pigs has been found to increase markedly at parturition (9, 10), although Baldwin has reported no such increase in the mammary tissue of cows (1). Although these literature reports provide no clear-cut answers, it appears that there might be

some relation between the reproductive functions of the animal body and the lipase activity of certain of its components. In view of this, a study was made at OSU of the relationship between estrous cycles of dairy cows and blood serum lipase activity (3). In connection with this study, the relation between blood and milk lipase activity was measured. These variables and their relation to rancid flavors are the subject of the present report.

CHAPTER II

METHODS

The four cows used in this work were fed the same ration and were housed under similar conditions throughout the experiment (3). Information concerning ages, calving, estrus, and health for these animals is summarized in Table I. Sampling was started the first time a cow was observed in heat after she went on experiment. Blood and milk samples were obtained once a week during the middle of the estrus cycle, and daily samples were taken starting two or three days before the day of observed heat and ending two or three days after the day of heat. Weekly samples of blood and milk were taken after the cows were bred, at the end of the third or fourth estrous cycle.

When taking samples, 200 ml aliquots of the afternoon milking were cooled to 0-5C in less than 5 minutes and held at that temperature until analyzed two days later. After milking, 50 ml blood samples were taken from the jugular vein of each cow and put into centrifuge tubes containing 5 ml of a 3.2% sodium citrate solution. These mixtures were held at 0-5C until analyzed two days later. Blood lipase activity was determined by the procedure of Kern et al. (7), as modified by Haggerty (3). The procedure for determining milk lipase activity is summarized below.

1. Twenty-five ml of skim milk (obtained by centrifuging the samples) were added to 25 ml of 0.2M "Tham" buffer at pH 9.6[1] and 2 ml of tributyrin substrate.[2]

2. Duplicate determinations were prepared for each milk sample. The lipase in one duplicate, marked "blank," was inactivated immediately by adding H Cl to reduce the pH to 2.0 or less. The other duplicate, marked "test," was incubated one hour at 37C in a shaking water bath, after which it was also inactivated with H Cl.

3. The fatty acids in both determinations (blank and test) were extracted with ether, then titrated with KOH to a thymolphtholein end-point. The results were corrected for the blank, then expressed as grams of butyric acid released per ml of skim milk.

An effort was made to relate these milk lipase activity values to the acid degree value (ADV) milk and to its rancid taste. ADV values were determined by the procedure of Thomas et al. (11) and tasted by two or three "trained" judges.

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[1]A buffer pH of 8.5 was used in preliminary work which caused higher results than were obtained with the pH 9.6 buffer. However, the differences obtained with the two buffers were consistent; thus, either buffer could be used to demonstrate differences among milk samples.

[2]This substrate contained 15% tributyrin and 30% bovine albumin (Fraction V from Sigma Chemical Co.). The mixture was warmed then homogenized until a stable emulsion was formed.

TABLE I

AGE, CALVING, ESTROUS, AND HEALTH[a] INFORMATION FOR FOUR COWS

Identification Number	Breed	Calving Date	Age[b] Years-Months	Date of 1st Heat
477	Jersey	9/21/66	5-2	10/26/66
488	Ayrshire	9/6/66	5-11	10/21/66
561	Ayrshire	9/19/66	4-1	10/11/66
564	Ayrshire	9/22/66	4-0	10/20/66

[a]Health Records:

488 - Antibiotics 10-12 and 13
Glucose and B-12 11-7, treated for bloat 12-13.

561 - Antibiotics mastitis 9-20 and 2-2.

564 - Antibiotics mastitis 1-1 and 1-18.

[b]Age on October 1, 1966.

CHAPTER III

RESULTS AND DISCUSSION

The graphs (Figures I-IV) of the lipase data (Table II) show maximum blood lipase values within 24 hours of the day when the cow was observed to be in "heat." As Haggerty has observed (3), the relationship between observed heat and high blood lipase activity would probably have been more exact had samples been taken more often than once a day. These high lipase values associated with heat were preceded by low lipase values--the minimum values occurring one or two days before estrus. In addition, the maximum value of heat was followed by low lipase values with the minimum occurring one or two days after estrus. Blood lipase activity values during the middle of the estrus cycle were erractic; apparently the hormone actively associated with estrus was not the dominant factor during this part of the cycle.

Nearly all of the changes in blood lipase activity were reflected by similar changes in milk lipase activity. The relative lipase activity of the milk was much greater than that of the blood, however; so the changes were magnified in the milk. The relationship between milk and blood lipase was not exact in terms of time, and changes often occurred in the blood the day before they occurred in the milk.

The milk lipase data of the first two heat periods were analyzed statistically with an analysis of variance technique. For this, the maximum lipase values obtained within 24 hours of observed heat were

chosen together with the minimum values occurring one or two days before and after the day of heat. One milk lipase value representing an approximate average of the middle of the cycle also was chosen. These calculations (Table IV) indicated a significant difference between periods of the cycle ($P \leq 0.01$). Most of this difference could be accounted for by the variance between the low lipase activities before and after heat, as compared to the high values obtained during heat and during the middle of the cycle. There was a difference among cows ($P < 0.05$), and the values obtained during the second estrus cycle were higher than those obtained during the first cycle ($P < 0.05$). The remainder of the data indicated that this gradual increase in lipase activity continued even after the cows had been bred.

When considering all the data, Cow 477 appears to have a higher average milk lipase activity than the other three cows. This animal also had the best health record (Table I). She was the only Jersey on the experiment, the other three animals being Aryshires. As such, her average milk production was somewhat lower than the other three animals (Table III) and the average fat content of her milk was higher. These data cannot demonstrate whether these factors caused the observed differences in lipase activity. However, their relationships might be interesting subjects for future study.

An effort was made to relate the lipase values of this study (with tributyrin substrates) to the taste of the milk and to its "acid Degree Value" (ADV). This latter value is a measurement of lipase activity used in some of the earlier research on rancid flavors (6, 11). Although 31 different milk samples were tested, none of these tasted rancid; so no positive correlation could be demonstrated. However, all the samples

had ADV values of less than 3.0 and butyric acid values of less than 30. Thus, values higher than these appear to be necessary before the milk will taste rancid.

TABLE II

LIPASE ACTIVITY IN MILK AND BLOOD OF INDIVIDUAL COWS
(BUTYRIC ACID PER ML OF BLOOD OR MILK)

	COW NUMBER							
	477		488		561		564	
Date	Blood	Milk	Blood	Milk	Blood	Milk	Blood	Milk
	(g x 10,000)							
Oct. 11					5.1	18.8		
17					5.1	20.3		
20					5.1	21.5	6.8	19.0
21			6.4	19.4				
22							8.6	27.3
23			5.5	25.7				
24			5.5	24.7	5.9	21.5	6.7	25.7
26	9.3	28.1			8.2	25.7		
27	5.4	26.7	5.7	23.1				22.5
28	7.5	28.3			6.7	19.7		
29					8.0*	26.7		
30					6.7	16.9		
31							9.3	18.7
Nov. 1					6.7	21.7	10.0	20.1
3	10.3	30.6	5.1	23.2	5.9	27.4	8.7	26.0
5			4.6	25.2			5.7	23.4
6			5.9	27.3			8.0	22.7
7	7.7	28.0	4.9	27.4	5.9	15.9	5.9	21.7
8			5.7				6.2	20.4
9			5.9*	21.5			8.5*	19.0
10	8.0	31.3	7.2	21.5	8.5	18.7	8.0	18.7
11	8.0	26.2	6.4	22.5			8.2	23.9
12	9.3	25.0						
13	8.2	28.3			7.5	18.3		
14	8.0	28.3	7.2		6.2	20.8	6.4	23.1
15	8.5	28.0			7.7*	24.8		
16	8.7	24.6			6.2	22.7		
17	8.5		7.2		7.7		8.0	
18	8.7*	27.9						
19	6.4	27.4	8.0	25.5	7.2	24.3	10.0	24.1
20	8.2	27.0						
21	8.0	24.1	6.4				8.0	21.1
23	9.0	29.0	5.9				10.3	24.3
26			5.4	25.5			12.3	22.4
27			6.7	21.9			10.7	26.7
28	9.2	27.4	8.5	26.2	8.0	24.8	11.5	24.8
29			9.0	25.7			12.0	23.5
30			7.7	26.7				25.1

TABLE II (Continued)

Date	COW NUMBER 477 Blood	477 Milk	488 Blood	488 Milk	561 Blood	561 Milk	564 Blood	564 Milk
				(g x 10,000)				
Dec. 1	8.2	24.6	7.5	24.6			10.5	19.2
2			6.5	26.5			8.7	19.0
3							11.2	27.9
5			7.2	28.4	8.5	26.9	10.0	26.3
6	9.0	28.0						
7	10.2	29.4						
8	9.3*	32.6	7.2		8.4*	22.9	12.6	26.5
9	7.8	27.5			5.1	22.9		
10	7.5	32.2			8.4	29.4		
12	10.5	32.4	9.0	30.4	9.0	23.0	9.6	25.5
15			8.1	24.3				
16			5.0	25.7			8.6	19.5
17			5.3	25.5			7.4	20.3
18			5.6	23.7				
19			8.0	21.7			5.0	23.7
20	7.7	26.7	5.0	23.5	6.8	20.3	8.0	25.9
21			5.7	26.0			8.0	22.1
22				24.9			9.5	17.1
28	8.6	29.0			7.4	23.3		
Jan. 3	7.4	33.2	5.6	30.8	8.9	25.7	9.5	25.7
11	8.3	29.3	6.2	19.5	8.0	23.5	7.1	21.5
17	8.0	24.6	8.0	28.1	8.3	20.5	7.7	21.4
24	8.2	31.4	7.2	32.0	6.9	24.0	6.5	19.3
31	7.9	34.7	6.9	22.6	7.9	23.8	9.2	24.0
Feb. 8	13.7	29.0	8.4	26.9	8.1	20.5	10.6	21.0
14	6.2	26.0	5.9	28.6	6.2	27.7	11.5	27.1
21	9.3	27.1	7.2	27.7	8.4	23.7	10.3	22.2
28	7.8	29.0	5.9	25.6	10.6	22.7	10.6	25.0
Mar. 7	8.4	32.0	10.5	22.9	8.4	26.5	9.0	26.7
14	7.5	30.5	11.2	26.0	10.3	23.1	6.8	18.0
21	8.0	34.3	8.2	29.4	11.2	32.3	8.0	19.3
28	8.0	25.3	8.5	19.3	9.8	20.7	6.9	19.3
Apr. 4	9.8	32.3	8.0	21.8	9.8	24.8	9.2	23.1

TABLE III

MILK PRODUCTION OF COWS ON AFTERNOONS OF SAMPLING

Date	COW NUMBER 477	488	561	564
		lb		
Oct. 11			23.0	
13				
17			27.0	
20			29.0	23.0
21		27.0		
22			26.0	
23		25.0		
24		36.0	30.0	24.0
26	14.0		28.0	
27	22.0	29.0		29.0
28	22.0		31.0	
29			28.0	
30			29.5	
31	21.0		28.0	24.0
Nov. 1			28.0	23.5
2			28.0	28.5
3	19.0	27.0	27.5	24.0
5		32.0		22.0
6		21.0		18.5
7	21.0	12.0	27.0	29.0
8		24.0		32.5
9		19.0		25.0
10	20.0	23.0	26.5	25.0
11	20.5	26.5		31.0
12	22.0			
13	18.0		21.5	
14	19.5	36.0	29.0	28.0
15	21.5		26.0	
16	21.0		23.5	
17				
18	19.0			
19	17.5	29.0	24.0	20.5
20	20.0			
21				24.5
23	21.5	12.0		24.0
26		28.5		25.0
27		18.0		18.0
28	18.0	25.0	22.5	24.0
29		28.5		29.0
30		30.0		25.5

TABLE III (Continued)

Date	COW NUMBER 477	488	561	564
		lb		
Dec. 1	20.0	23.0		24.0
2		24.0		28.0
3				25.0
5		27.0	19.0	23.0
6	19.0			
7	20.5			
8	17.0		16.0	23.0
9	17.0		19.0	
10	16.0		21.5	
12	19.0	26.0	21.0	19.0
15		6.0		
16		16.0		20.0
17		18.5		20.0
18		20.0		19.0
19		28.0		20.0
20	15.5	28.0	20.0	19.0
21		20.0		19.0
22		24.0		19.0
28	13.0		17.0	
Jan. 3	12.5	21.0	18.0	18.0
11	11.5	19.0	12.0	16.0
17	14.1	19.0	16.5	18.0
24	14.0	20.5	15.0	17.5
31	14.0	16.0	14.0	15.0
Feb. 8	13.0	21.5	16.5	14.0
14	16.0	22.0	15.0	15.5
21	15.0	21.0	14.5	14.0
28	13.5	21.0	13.5	16.0
Mar. 7	12.0	18.0	13.0	12.0
14	12.0	19.5	13.0	12.5
21	12.0	19.0	13.0	6.5
28	13.0	14.0	11.5	11.0

TABLE IV

ANALYSIS OF VARIANCE FOR MILK LIPASE ACTIVITY DATA

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F
Total	311.50	31	-	-
Estrus Cycles	23.80	1	23.80	7.30[a]
Cows	125.03	3	41.68	12.79[b]
Periods of Cycle	76.20	3	25.40	7.79[b]
(Before and After heat vs. other two periods)	(75.65)	(1)	75.65	23.21[b]
Interactions				
Estrus x Cows	2.81	3	0.94	0.29
Estrus x Periods	2.06	3	0.69	0.21
Cows x Periods	52.25	9	5.81	1.78
Estrus x Cows x Periods (error)	29.35	9	3.26	-

[a] $P < 0.05$

[b] $P < 0.01$

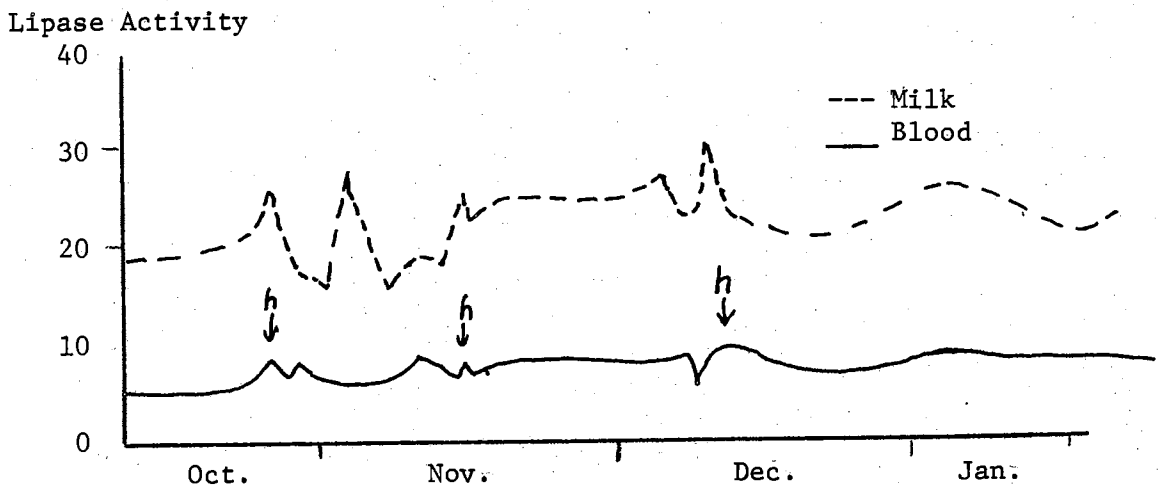

Figure 1. Lipase Activity of Milk and Blood. Cow 561.

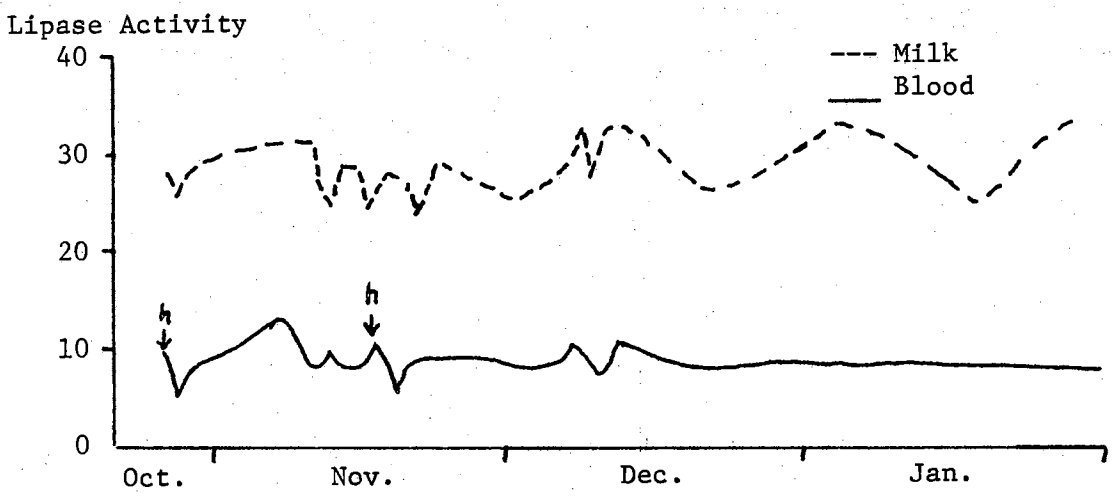

Figure 2. Lipase Activity of Milk and Blood. Cow 477.

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h. Day of observed heat.

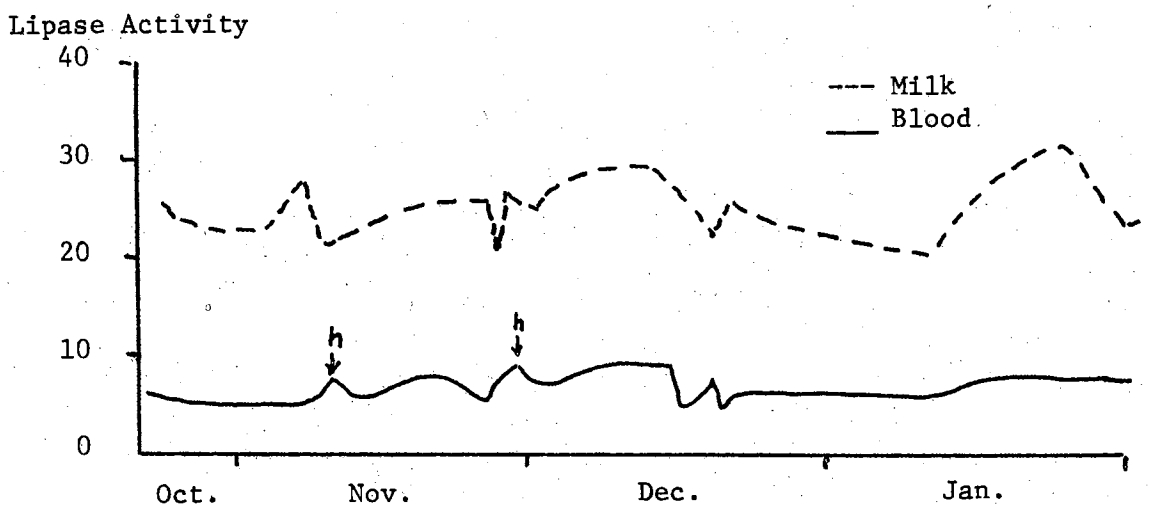

Figure 3. Lipase Activity of Milk and Blood. Cow 488.

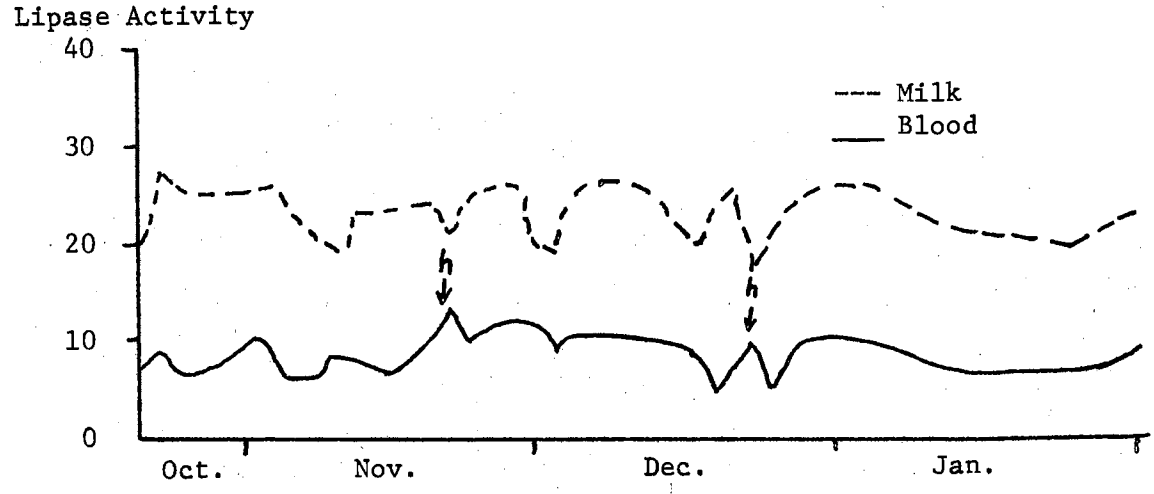

Figure 4. Lipase Activity of Milk and Blood. Cow 564.

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h. Day of observed heat.

CHAPTER IV

SUMMARY AND CONCLUSIONS

The purpose of this experiment was to measure the relationship between milk and blood lipase in individual cows. The relationships of these two variables to the estrus cycles of the cows and the rancid flavors in their milk also were observed. Milk and blood samples were taken from four cows throughout their lactation. Daily samples were obtained from each cow starting two or three days before each day of "heat" and continuing until two or three days after the day of heat. Weekly samples were taken during the middle of each estrous cycle, and this weekly sampling continued after the cows were bred at the end of the third or fourth estrous cycle.

High blood lipase values occurred within 24 hours of observed "heat." These maximum values were bracketed by lower values with minimums occurring one or two days before and one or two days after the day of "heat." Changes in blood lipase activity were reflected, and magnified, in the milk, although these changes often occurred a day after they were observed in the blood. Most of the milk samples tested in this study were not rancid; however, milk lipase activities higher than 30 (units of butyric acid) might indicate potential rancidity.

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