APRIL, NO. 4

MAY, NO. 5

JOURNAL OF DAIRY SCIENCE

VOLUME XXXI MAY, 1948 NUMBER 5

THE EFFECT OF CLIPPING THE UDDERS OF COWS ON THE QUALITY OF MILK

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The effect of clipping of udders of cows upon the quality of milk has been the subject of original study of but a few investigators (2, 3). The information available, though brief, indicates benefit from the practice. Among milk producers and quality supervisors there seems to be varied opinion as to the benefit of the clipping of udders. In view of the increasing emphasis on the methods for obtaining milk of good quality, a study was made of the effect of clipping of udders and adjacent areas on certain quality properties of milk.

EXPERIMENTAL PROCEDURE

Handling of Cows. The experiment was divided into four periods as follows: (a) Control period, milking by machine. November 20 to December 2, 1946. (b) Second period, milking by machine. December 3, 1946, to January 30, 1947. (c) Third period, milking by hand. February 6 to March 26, 1947. (d) Fourth period, milking by machine. March 26 to April 4, 1947. In the control period, prior to clipping any of the animals, the numbers of bacteria in the milk of the individual cows milked by machine were determined. For the second period, alternate cows, as they stood in line, were clipped. The animals were reclipped during the third period. The clipped area was posterior to a line from the pinbones to the navel, including thighs, flanks, and udder, and the tail except for the switch. The area clipped is illustrated in figure 1. As they freshened and were introduced in the milking line, alternate cows were clipped.

The conditions in the barn were comparable to those usually found in a city fluid milk area. Wood shavings were used liberally for bedding, but not in excess. The cows were groomed once daily, usually during the morning but not immediately before the milkings. The night's accumulation of manure was in the gutters at the time of the morning milking. The cows seldom were soiled at milking time to a degree greater than that

Received for publication August 15, 1947.

[1] Project supported by grant from Sunbeam Corporation, Chicago, Ill.

of having loose bedding and dirt clinging to the body. The herd was kept indoors without access to an exercise yard.

Treatment of milking utensils. All utensils used in this experiment were new. Prior to each period of use, four disassembled milking machine units, excluding the pails, were sterilized in a steam autoclave. The stainless steel machine pails and milk cans were jet steamed for a period of 3 minutes, then filled with a solution of 200 p.p.m. available chlorine for 1 hour, drained, and covered with parchment paper. The units then were assembled and immersed in a solution containing 200 p.p.m. of chlorine. Two gallons of sterile distilled water added in three portions was used to rinse the chlorine from one of the machine pails chosen at random. The

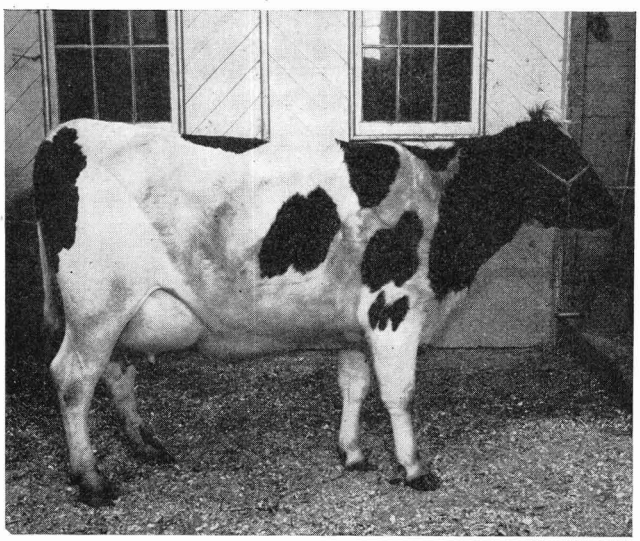

FIG. 1. One of the cows used in the experiment, showing the area clipped.

machine head then was adjusted and another 2 gallons of sterile distilled water drawn through the teat cup assembly and into the pail. The average bacteria count per ml. of the rinse water was ascertained to be 2 in the evening and 1 in the morning tests.

Just before milking, the udder of each cow was wiped with an individual cloth previously immersed in a warm solution of 200 p.p.m. available chlorine. The cows were fore-milked into a strip cup, and this milk was discarded. The sterilized machine unit was attached with special care to avoid contamination. The cows were machine stripped. The intact milking unit was taken immediately into the milk house for sampling and weighing the milk. The milk then was carefully transferred to identified sterile covered 10-gallon cans. The milk was not strained. The machine

was partially dismantled and all parts which came in contact with milk were rinsed twice in warm tap water, followed by an immersion rinse in a solution containing 200 p.p.m. available chlorine. The reassembled unit then was returned for immediate use on other animals. Terminating each milking period, the machines were water rinsed, completely disassembled, washed and scrubbed, rinsed in hot water, and stored on racks for later milking periods.

For hand milking, open-top oval dairy pails were sterilized, as were the milking machine pails. The cows' udders were wiped in the same manner as when machine milked. The six milkers immersed their hands in a solution containing 200 p.p.m. chlorine prior to each milking, after which they touched nothing except the cows' teats until each milking assignment was completed. The milking stools were handled for the milkers by assistants. To obtain balanced results, the milkers alternated at random between clipped and non-clipped cows. The milk obtained by hand milking of each cow was immediately transferred to the milk house for sampling.

Milk sampling procedures. The following procedures were used for bacteriological analysis of the milk: (a) A sample from each pail of milk from each cow was obtained immediately after it was conveyed to the milk house. (b) Separate composite samples of the milk from the clipped and non-clipped cows, respectively, were taken immediately from each 10-gallon can filled during the milkings. (c) Separate composite samples and off-the-bottom sediment tests of the milk from the clipped and non-clipped cows were taken from each can at the time the milk was delivered to the dairy plant.

The samples of milk, transferred by means of a sterile glass tube thief to sterile screw cap bottles, immediately were placed in ice water and so kept until the bacteriological tests were undertaken, regularly within 60 minutes. The filled milk cans were stored in a water immersion refrigerator at 33 and 34° F. The milk in cans was held until approximately 7:00 a.m., when it was trucked to the dairy plant, a distance of approximately 0.25 mile (milkings were begun at 3:30 a.m. and p.m.).

Methods of testing. Standard bacteria plate count estimates of the milk were made as described in Standard Methods for the Examination of Dairy Products, 8th Edition (1). The raw milk samples were plated in duplicate at dilutions of one in ten. Samples pasteurized in the laboratory were plated without dilution. The milk was pasteurized at 143° F. in a thermo controlled bath for 30 minutes, using 5-ml. portions and a blank open tube for thermometer observation. All counts are reported as bacteria per ml.

The presence of extraneous material in the milk was determined by use of a Langsenkamp-Wheeler off-the-bottom sediment tester which withdrew a 1-pint sample from each 10-gallon can of composited milk.

RESULTS

In table 1 are presented the bacteria counts of the milk (weighted arithmetic averages) from those cows which were milked throughout the first and the second periods. In the first or control period, all udders were unclipped; in the second period, udders of alternate cows were clipped. The average count per ml. of the milk from those cows not clipped was determined as 1,308 in the control period and 1,869 per ml. in the second

TABLE 1

Weighted arithmetic average count per ml. of samples of milk taken from individual milkings obtained by machine during preliminary period and after clipping part of cows

Treatment after preliminary period	No. of cows	Prior to clipping any cows		After clipping part of the cows	
		No. of samples	Av. count per ml.	No. of samples	Av. count per ml.
Unclipped	10	19	1308	84	1869
Clipped	13	22	1629	92	1397

period. The count per ml. of the milk from cows subsequently clipped decreased from the control period average of 1,629 to 1,397. Since, during the second period, the average count of milk from the cows remaining unclipped increased, while that of the milk from the cows clipped decreased, the apparent over-all difference in numbers of organisms in the milk appears to indicate beneficial effects of clipping.

In table 2 are presented the weighted arithmetic averages of the counts per ml. of milk from cows during the second period, when milked by machine, and during the third period, when milked by hand. The results are presented on the basis of both morning and evening milkings. The number of cows involved in this analysis varied due to drying-off and

TABLE 2

Weighted arithmetic average count per ml. of samples of milk taken from individual milkings

Time of milking	Clipped			Unclipped		
	No. of cows	No. of samples	Weighted arithmetic av. count per ml.	No. of cows	No. of samples	Weighted arithmetic av. count per ml.
Machine milked						
Evening	13	92	1548	23	115	1805
Morning	13	93	1375	16	101	1317
Hand milked						
Evening	14	83	877	16	83	1484
Morning	14	102	830	16	104	1143

TABLE 3

Weighted arithmetic count per ml. of composite samples of milk from clipped and unclipped groups of cows

Time of milking	Clipped		Unclipped	
	No. of samples	Av. count per ml.	No. of samples	Av. count per ml.
		Machine milked		
Evening	8	1590	8	2381
Morning	8	1254	8	1245
		Hand milked		
Evening	6	566	7	1250
Morning	8	771	8	1000

freshening. In addition to these natural causes, some samples were omitted because of explainable contamination, such as dropping of teat cups into the bedding, cows kicking into milk pails during hand milking, and sudden evidence of mastitis. The results show that the average count per ml. of the milk obtained by machine milking from clipped cows differed but slightly (4.4 per cent more in morning and 2.4 per cent less in evening) from that similarly obtained from unclipped cows. On the other hand, the results show that the average counts of the milk obtained by hand milking from clipped cows were less (40.9 per cent for morning and 28.0 per cent for evening) than those similarly obtained from unclipped cows.

In table 3 are presented the average counts per ml. of the composite samples of milk obtained from filled 10-gallon cans. The average count per ml. of this milk from the clipped cows was definitely less than that from the non-clipped cows. The over-all difference approximated 30 per cent. A similar relationship in bacteria numbers of the evening's milk refrigerated for approximately 14 hours before being sampled also was

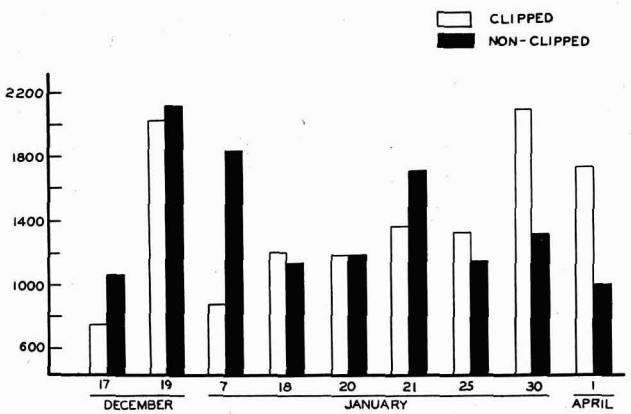

FIG. 2. Average bacteria count of individual milkings of cows milked by machine in morning.

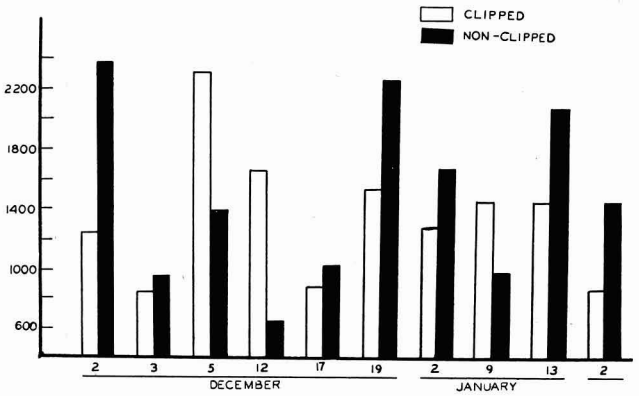

FIG. 3. Average bacteria count of individual milkings of cows milked by machine in evening.

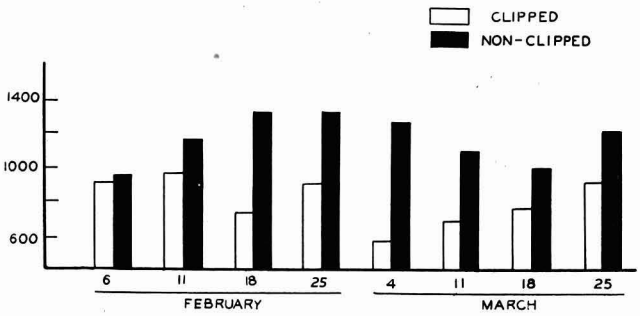

FIG. 4. Average bacteria count of individual milkings of cows milked by hand in morning.

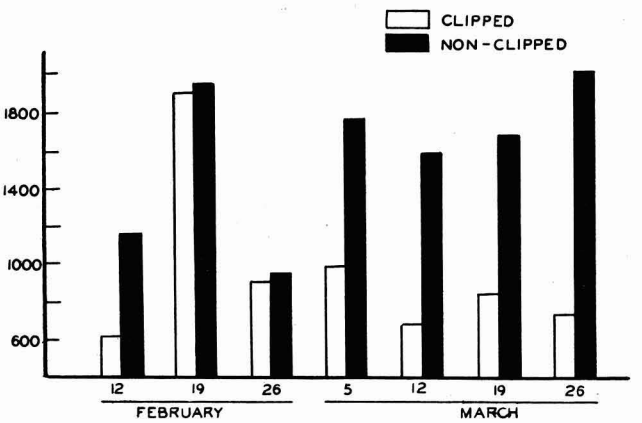

FIG. 5. Average bacteria count of individual milkings of cows milked by hand in evening.

observed. The counts of the composite samples subsequently pasteurized ranged from 3 to 13 per ml. and were so low that no significance could be attached to the figures.

The average counts per ml. of the milkings from the clipped and non-clipped cows, during the morning and evening milking, for each milking period included in the study, are presented in figures 2, 3, 4 and 5. An analysis of variance of the data presented in the graphs shows there is a significant difference in the count per ml. of the milks obtained by hand milking between the clipped and non-clipped cows. The difference in the counts per ml. of the milks obtained by machine milking from clipped and non-clipped cows was not statistically significant.

The tests for extraneous material present in the milks were conducted at the time the milks were delivered to the platform of the plant. No

TABLE 4

Frequency of the grades (Wisconsin Standards) of the tests for extraneous material in milk

	Grades			
	1	2	3	4
Machine milking				
Clipped cows	1	16	7	1
Unclipped cows	.....	14	10	0
Hand milking				
Clipped cows	.....	.....	8	20
Unclipped cows	.....	.....	5	18

difference in milks from clipped or unclipped cows could be determined. However, the amount of extraneous material in the milk obtained by hand milking was much greater than in that obtained by machine. The summary of the tests is tabulated in table 4.

The udders usually were clean prior to washing except for loose dirt or shavings clinging to the hair. The time spent in washing the udders prior to milking did not differ appreciably between the clipped and unclipped cows. This was used as the routine stimulus for let-down of milk, and the time spent was more than adequate to cleanse the teats and udder of all visible dirt.

DISCUSSION AND SUMMARY

The effect of the clipping of cows upon the quality of milk was determined by colony plate count and tests for presence of extraneous material. The clipping tended to lower the average bacteria counts per ml. of the milk, whether the milking was done by machine or by hand. The average counts per ml. of the milk obtained by machine from clipped and unclipped cows were 3,042 and 3,458, and by hand 1,643 and 2,996, respectively. The advantages of clipping were statistically significant for the milks obtained by hand milking.

The average counts per ml. of the milk obtained by machine were greater than those of the milk obtained by hand. This might be due to the ends of the teats being bathed to some extent by milk during machine milking, resulting in rinsing of organisms into the milk. When the milking is performed by hand, the bathing action does not occur. Although the average bacteria count per ml. of the milk obtained by machine was greater than when obtained by hand, the amount of extraneous material present was observed to be greater in the milk obtained by hand. The clipping of cows caused no measurable difference in the amount of extraneous material in milk when obtained by either machine or hand milking.

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STORAGE AND TREATMENT OF MILKING MACHINE INFLATIONS UNDER FARM CONDITIONS[1,2]

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Difficulty in producing high quality milk frequently has been attributed to using milking machines that were not in the proper state of sanitation. Many procedures have been advocated as a means of sanitizing the rubber inflations and tubing. Most of these procedures were discussed in a previous article (3) in which the authors presented results of a laboratory study of rubber inflations for milking machines. The study reported: (a) the extent of fat absorption, (b) the extent of storage solution absorption, (c) the deteriorating effect of inorganic chlorine, and (d) the advantage of boiling rubber parts in lye solution at intervals as a means of saponifying entrained fat, thus improving their sanitary condition.

Mallman *et al.* (4) recently reported a study in which producers used a variety of sanitizers. This study indicated that cationic germicides were more effective as sanitizing agents than lye and chlorine, as measured by lower total and thermoduric counts of milk.

Dahlberg (2) also recently reported results of milking machine sanitizing studies and found high bacterial counts with dry-stored inflations and low counts and clean tubes with lye solution on rack storage. He reported that dry storage following washing and rinsing with cationic germicides was not satisfactory.

Investigation seemed desirable to determine certain storage and treatment practices under practical farm conditions. To this end farm studies were made over an extended period on the treatment and storage of rubber inflations and tubing of milking machines, studying the physical and bacteriological cleanliness of the rubber and the bacterial population of milk produced through their use.

EXPERIMENTAL PROCEDURE

Preliminary observation of producer methods. Producers of milk from one dairy plant were used for this study over an 18-month period. The early portion of the study consisted of making inspection of milking machines for cleanliness and method employed for storage between use, while weekly bacterial counts were made on milk from each producer.

Received for publication December 10, 1947.

1 Journal Article no. 919 (n.s.), Michigan Agricultural Experiment Station.

2 This study was made possible through a grant from Swift and Company, Chicago, Illinois, for research in quality milk and cream.

During this period certain storage practices were found to give satisfactory results and others were observed to have some objectionable features that deemed them undesirable for rubber storage. The producers having generally satisfactory results were those using lye and cationic germicide solutions, and dry storage following scalding with very hot water. Acid solution had no appreciable germicidal property and inorganic chlorine caused gross deterioration of rubber. This portion of the study was continued by making bacterial counts of sterile water rinses of the inspected rubber parts and bacterial counts of the milk produced through their use.

Based on these observations, further study was made comparing dry, lye and cationic solution storage under conditions wherein the washing procedure employed before their use would be the same. Producers operating two milking machines each were chosen for this study. Four different storage treatments were compared: (a) dry storage following the regular washing procedure, (b) dry storage following washing and rinsing, after which a subsequent rinse with 1 gallon of 200 p.p.m. cationic germicide solution was used, (c) washing followed by solution storage, 200 p.p.m. cationic germicide, and (d) solution storage with 0.5 per cent lye solution. All milking machines were washed using a washing powder consisting of anionic synthetic detergent and near-neutral polyphosphate. Long tube milkers generally were washed by a flush washing procedure, while short tube milkers generally were washed disassembled.

Each producer having two milker units was asked to follow different designated storage procedures on each unit as a means of comparing the sanitary condition of the inflations and tubing affected thereby, thus eliminating the factor of washing effectiveness, which was assumed to be the same for both units. Examinations and bacterial counts were made over a period of 5 to 10 weeks, the greatest number involving ten weekly examinations.

Bacterial counts of inflations were made from water rinses, using 500 ml. of sterilized water, chilled by icing to ice water temperature. This water was emptied into the cups and tubing of long tube milkers while supporting these parts in an upright position. After filling, the water was returned to the water jar by discharging through the rubber tubing. Short tube milker inflations were filled after pinching shut the tubing near the outlet. Four inflations from each short tub milker unit were rinsed similarly. The samples again were placed in iced water and within 4 hours were plated for total count, using standard methods (1). Thermoduric counts of rinse water were made by plating the water after laboratory pasteurization at 143° F. for 30 minutes.

Weigh-can samples of each producer's milk supply were taken the day after examining inflations. These were plated for total and thermoduric count. Examination for physical cleanliness of inflations and tubing was

made by scraping the inner lining of inflations with a spatula and rodding the tubing. Evidence of soiling was noted.

RESULTS

Use of dry storage with and without cationic germicide rinse. Bacterial counts resulting from dry storage as obtained from three producers are shown in table 1. A survey of bacterial counts of inflations shows no important improvement in sanitation when 200 p.p.m. cationic germicide solutions were used for rinsing following washing. This was true when high bacterial counts were obtained with producers 3*L* and 17*L*, as well as when low counts were secured by producer 36*L*, whose low counts were attributed to rinsing the washed utensils with a liberal quantity of very

TABLE 1

Influence of cationic rinse on bacterial population of rubber inflations

Producer	Method of storage[a]	No. of trials	Bacterial count/ml.			
			Inflation and tubing		Milk	
			Total	Thermoduric	Total	Thermoduric
36*L*[b]	A	10	5,000	1,300	25,000	600
	B	10	3,000	1,500	100,000	600
	C	10	4,000	1,200		
3*L*	A	11	550,000	4,600	40,000	1,300
	B	9	220,000	2,100	46,000	500
	C	9	650,000	1,600		
17*L*	B	5	2,800,000	5,600		
	C	5	1,400,000	5,000	160,000	12,000

[a] A = dry, preliminary trials; B = dry; C = dry, cationic germicide rinse.
[b] L = long tube milker.

hot water. Total and thermoduric counts of inflation rinses from producers 3*L* and 17*L* were excessively high, and the cationic rinse gave insufficient germicidal property by this method of application.

Total and thermoduric counts of milk produced with the inflations also are shown in the table. No attempt was made to relate these counts to the particular sanitizing treatment applied to inflation assemblies, since it was not practical to segregate milk produced by each milker unit. Normally low thermoduric counts were obtained from producers 36*L* and 3*L*, while those of 17*L* were high, as were also the total counts of inflations from this producer. Averages obscure the conditions that were involved in this producer's counts. These conditions are presented in detail in table 5.

Dry storage versus storage in cationic solution. Bacterial counts of rubber inflations and tubing secured when comparing dry storage following washing with solution storage using 200 p.p.m. cationic germicide solution are shown in table 2.

Three producers' machines were observed. The most pronounced difference in results was secured with producer 1*S*, for whom cationic solution storage results were very satisfactory, with an average count of 11,000 for the nine trials. The dry storage inflations, while similarly washed, were excessively high in count, averaging 3,800,000 for the nine trials. The extreme difference secured with this producer is accounted for by the observation that washing and rinsing were done with medium warm water and no attempt was made to sanitize the dry storage inflations.

Producers 2*S* and 26*L* had low bacterial counts on both dry and cationic solution trials, counts that were markedly lower than in the preceding preliminary trials. Both producers were habitually careful about cleaning their milkers and were discovered to be somewhat reluctant to

TABLE 2

Influence of cationic solution storage on bacterial population of rubber inflations

Producer	Method of storage[a]	No. of trials	Bacterial count/ml.			
			Inflation and tubing		Milk	
			Total	Thermoduric	Total	Thermoduric
1*S*[b]	A	9	170,000	3,100	39,000	700
	B	9	3,800,000	4,700	250,000	5,200
	C	9	11,000	1,700		
2*S*	A	7	30,000	3,000	35,000	800
	B	10	2,500	1,000	11,000	500
	C	10	3,500	2,300		
26*L*	A	7	58,000	1,900	230,000	1,300
	B	6	1,300	700	830,000	900
	C	6	500	500		

[a] A = dry, preliminary trials; B = dry; C = cationic solution.
[b] S = short tube milker; L = long tube milker.

use sanitizing solution storage. They therefore were concerned about having the dry storage inflations as bacteria-free as those stored with cationic solution and used very hot water for sanitizing following washing.

All of the machines in table 2 were washed disassembled. Machines and inflations of producers 2*S* and 26*L* always were found in an excellent state of cleanliness, while those of 1*S* at times were criticized for being slightly slimy in the upper portion of the inflations.

The conditions of the milking machine tubing and inflations were not necessarily reflected in the total bacterial counts of the milk. High bacterial counts were obtained in milk samples from producer 26*L*, in spite of his use of clean and well sanitized inflations and tubing. The high counts were attributed to delayed and inadequate cooling caused by the milk house being located near the farm residence across a highway from the barn. Also, this producer had more milk than could be accommodated in his cooling tank. However, there was considerable increase in thermoduric

count on the milk in samples from producer 1*S* when the milker inflation total counts were high.

Trials comparing solution storage using 0.5 per cent lye and 200 p.p.m. cationic germicide are shown by bacterial counts listed in table 3. Most apparent is a reduction of bacterial count when solution storage was used, in contrast to the situation when dry storage was employed by the various producers during the preliminary trials.

When long tube milkers requiring solution racks were used for storage of inflations and tubing, as shown by producers 5*L*, 9*L*, 15*L*, 23*L*, and 59*L*

TABLE 3

Influence of cationic solution and lye solution storage on bacterial population of rubber inflations

Producer	Method of storage[a]	No. of trials	Bacterial count/ml.			
			Inflation and tubing		Milk	
			Total	Thermoduric	Total	Thermoduric
5*L*	A	11	7,800	1,400	47,000	1,200
	B	9	2,000	800		
	C	9	2,000	1,000	48,000	6,200
9*L*	B	6	5,900	800		
	C	6	6,600	600	31,000	600
15*L*	A	11	390,000	6,000	9,600	1,000
	B	9	1,400	1,500		
	C	9	1,400	600	10,000	600
23*L*	A	11	140,000	3,500	100,000	
	B	8	1,100	800		
	C	8	1,300	500	83,000	900
59*L*	A	8	170,000	13,000		
	B	9	7,600	2,800		
	C	9	1,100	600	28,000	400
61*S*	D[b]	11	13,000	4,000	44,000	4,700
	B	9	24,000	1,900		
	C	9	1,300	800	10,000	600

[a] A = dry, preliminary trials; B = lye solution; C = cationic solution.
[b] D = lye solution, preliminary trials.

lye and cationic germicide solutions were equally effective as sanitizing agents, as indicated by total bacterial counts of rinses. A consistent, though small, decrease in thermoduric bacterial counts of rinse samples was secured in favor of cationic germicide solution storage over lye solution storage. Likewise, there was a general reduction in milk thermoduric counts when solution storage was used compared with dry storage. One exception to this is observed in producer 5*L*. Although the counts on the inflations and tubing were low, this producer was not successful in maintaining general physical cleanliness of the rubber parts of his machines. Usually spatula scrapings from the inflations gave heavy milk sludge deposits. This condition was found to be caused by dipping milk-coated

teat cups in warm chlorine solution of 200 p.p.m. strength before milking the next cow, without previously rinsing off the milk in cold water. Such a treatment caused a slimy film that was not removed readily during washing.

When inflations were stored in solution jars, as was required of the short tube inflations of producer 61*S*, cationic solution storage was more effective than lye as a sanitizing agent. The inflations stored in the cationic solution yielded an average rinse count of 1,300 in contrast to 24,000 for lye storage.

Higher rinse counts were secured using lye in jar storage that were obtained with lye used in solution racks. This was considered to be due to repeated use of the same lye solution for a 7–day period in jar storage, whereas a fresh lye solution was applied between each milking when rack storage was used.

Physical cleanliness and bacterial cleanliness. A tabulation of the

TABLE 4

The relationship between bacterial count and physical cleanliness of milker inflations

Bacterial count of milker inflations	Appearance of milker inflations			
	Clean		Not clean	
	No.	%	No.	%
<10,000	242	51.0	34	24
10,000—100,000	97	20.4	25	18
100,000—1,000,000	88	18.3	33	23
>1,000,000	49	10.3	50	35

milking machines examined over the entire course of the study was made to determine to what extent cleanliness by physical examination was verified by the bacterial counts obtained. This included 617 examinations. Of this number, 475 were noted as being clean and 142 as not clean. The bacterial counts of both groups are shown in table 4. In accordance with the data presented, it would appear that milking machines can be judged for bacteriological cleanliness by physical examination with only a fair degree of success, for in 51 per cent of the milkers rated clean, the bacterial counts were less than 10,000, which probably could be considered a "fair" count for inflations stored dry. However, 29 per cent of the milkers that appeared clean were highly contaminated.

There was less relationship between appearance of milker inflations in the "not clean" group. Here 24 per cent had counts of less than 10,000. These figures likely were not representative of average conditions, since the number of samples was relatively small and among them were inflations that frequently were found to retain an oily wax-like deposit as the result of storage in cationic germicide and the inflations of producer 5*L*

that were doused with warm chlorine solution without first rinsing off adhering milk with clear water.

Producer reaction. Some objections to cationic solutions were expressed by producers. One expressed dislike for cationic detergents because they made the rubber feel "dead" and because they caused the rubber to become coated with a slippery film. Occasionally this would cause the milk pail gaskets to be sucked into the milker pails. It was discovered that this condition occurred mainly when the milk films were not completely washed off the rubber before placing the parts in the solution jar. Lye solution was preferred by this operator because it served better as a detergent.

TABLE 5

The relationship between sanitization treatment and bacterial condition of a single set of milking machine inflations (producer 17L)

Date	Bacterial count/ml.			
	Inflations		Milk	
	Total	Thermoduric	Total	Thermoduric
2–5[a]	3,000	1,000	10,000	2,400
2–19[a]	10,000	500	16,000	500
3–27[b]	310,000	5,000	30,000	400
	510,000[c]	6,000		
4–3[b]	52,000	5,000	7,000	300
	200,000[c]	3,000		
4–10[b]	1,000,000	2,000	88,000	4,000
	1,300,000[c]	2,500		
4–16[b]	10,000,000	2,500	930,000	600,000
	75,000,000[c]	2,500		
4–24[b]	30,000,000	25,000	6,000,000	900,000
	21,000,000[c]	50,000		
5–1[a]	3,500[d]	4,500	22,000	400
	10,000	4,000		

[a] Hot water used.
[b] Cold water used.
[c] Rinsed with 200 p.p.m. cationic solution after washing.
[d] Stored on solution rack with 200 p.p.m. cationic solution.

Some objection also was expressed with respect to cationic solution forming an oily and somewhat wax-like film when used for sanitizing milker pails. This condition also was found inside inflations. At no time during the course of this study was milkstone a problem. Only during the initial portion of the study was it noticed. The anionic detergent combined with near-neutral polyphosphate was effective in its removal as well as in its prevention. A soft gel-like slime occasionally was found when examining inflations for cleanliness by spatula scraping. This usually was found when chlorine solutions were used for sanitizing without properly removing all milk film by rinsing in water.

Most difficulty with bacterial contamination occurred where washing with detergent and cold or only moderately warm water was used, after which the inflations were stored dry. An illustration of the result of such washing was well demonstrated by producer 17*L*, who had been producing milk for several months with a good record of low bacterial counts. His hot water heater was sent away for repair for several weeks during the course of the study. Results as shown in table 5 reveal a progressive degree of contamination that first was made evident by increase in the rinse count of inflations. Milk samples remained normal during the first 2 weeks of cold water washing and then greatly increased in total and thermoduric counts. Immediate reduction in counts followed the return to hot water rinsing after washing. Similar patterns were discernible with other producers that washed their machines but did not follow up with effective germicidal treatment. This would support a concept that inflations are not a formidable source of bacteria until contamination has penetrated the rubber pores.

DISCUSSION

The results of storing rubber milker inflations under practical farm conditions indicate that lye and cationic germicide solution storage provide an assurance of better sanitation than does dry storage. Germicidal properties of 0.5 per cent lye and 200 p.p.m. cationic germicidal solutions appear to be of practically equal value where fresh solutions of lye are applied, such as is made possible with solution rack storage. When jar or bath storage was used, cationic solutions provided better sanitizing than did lye. This would indicate that the cationic solutions are more durable as germicides than lye and that when lye jar storage is used for rubber parts, fresh solutions should be prepared more frequently than once each week, as was used in the study.

The cationic solution applied as a rinse following washing was not satisfactory as a means of sanitizing the rubber parts. This was indicated by high bacterial counts in the inflations as well as in milk produced with the inflations. Apparently more intimate exposure to the cationic germicide was required than was possible by drawing 1 gallon of solution through the cups and tubing.

The authors previously reported (3) that rubber inflations were capable of absorbing storage solutions to an appreciable degree. Butterfat and unquestionably bacterial contamination likewise have been found to be absorbed by rubber. With these absorbing qualities definitely shown, it would seem logical to conclude that the problem of sanitizing rubber is not just a matter of treating the surface but of penetrating the pores either by prolonged contact with penetrable germicidal solution or with heat. Hence, the lack of effective germicidal treatment by rinsing and

retaining only a surface film could be expected. It likewise would follow that a combined washing-sanitizing compound, such as frequently has been sought, would not prove effective unless followed by solution storage.

The observations indicate that rinse counts do not represent the entire bacterial contamination but only a portion of that present on the surface of the rubber. Low rinse counts therefore should not be accepted with complete assurance that inflations are sanitized satisfactorily unless the milk produced with them yields low total and thermoduric counts.

In this study, milker pails were not examined for bacterial contamination but only for appearance of cleanliness. General observation led to the belief that the greatest contamination came from milker inflations and tubing.

CONCLUSIONS

Farm application of milker inflation storage employing 0.5 per cent lye solution, 200 p.p.m. cationic germicide and dry storage was observed through means of farm inspection, sterile rinse counts of inflations and bacterial counts of milk. Comparison of storage treatment was made using two different procedures on each of two designated milkers on each farm.

The lye and cationic solutions appeared to have equal germicidal value as measured by total rinse counts when solution rack storage was used. The cationic germicide solution caused greater reduction in thermoduric count of rinse water samples than did lye. Lye solution had less germicidal effectiveness than the cationic germicide when immersion storage was used.

Dry storage was least satisfactory in maintaining uniformly low counts of inflations, and high thermoduric counts were associated with cold water washing followed by dry storage. Dry storage after washing and "sanitizing" with 1 gallon of 200 p.p.m. cationic solution was not satisfactory. Some objection to physical properties of the cationic germicide was registered.

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SWEET POTATO MEAL VERSUS GROUND CORN IN THE RATION OF DAIRY COWS[1]

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Raw sweet potatoes have been used as a feed for domestic animals for some time. However, it has been only during the past decade that sweet potato meal has gained recognition as a possible substitute for corn in the ration of livestock. Several southern investigators (6, 7, 8, 9, 10, 11, 12, 13) have found dried sweet potatoes to be worth approximately 90 to 95 per cent as much as corn for fattening beef cattle. Hogs and mules preferred rations in which the sweet potato meal did not replace more than 50 per cent of the corn in the ration. Massey (14) at the Georgia Experiment Station conducted a series of experiments in which he studied the comparative value of sweet potato meal and ground corn as a feed for ewes. The 300 ewes that were fed these rations during a period of 3 years favored the sweet potato meal from a palatability standpoint and gained as much during the gestation period when fed the sweet potato meal ration as when fed the corn meal ration. The ewes that received the sweet potato meal ration produced more milk after the lambs were born and thereby gave the lambs a faster growing start.

Louisiana workers (15) report that dehydrated sweet potatoes have approximately 88 per cent of the value of yellow corn meal and are approximately 17 per cent more valuable than ground snapped corn, including cob and shuck, for milk production. Good quality dehydrated sweet potatoes contained from 76 to 81 per cent T.D.N. and a poor quality product contained 71 per cent T.D.N. on the dry basis. Vitamin A analyses of the butterfat from milk produced by cows fed sweet potatoes were 19 per cent higher than those of butterfat produced by cows fed ground corn. The basal feeds were common lespedeza hay, alyce clover hay, or kobe lespedeza hay and cottonseed meal. Briggs *et al.* (2) of the Oklahoma Station recently reported that on the dry matter basis the average T.D.N. value of dried sweet potatoes for steers was 86.05 as compared to 85.80 for the corn. The basal feeds were alfalfa hay or prairie hay and cottonseed meal. Copeland (5) of Texas reported that one could expect 3.08 per cent more milk as a result of feeding corn than when feeding sweet potato meal. It also was noted that butter produced from cows on the sweet potato meal contained 37.98 I.U. of vitamin A per g. as compared with 31.11 I.U. from butter produced by cows fed yellow corn.

Received for publication December 13, 1947.

[1] This paper is part of a thesis presented by John H. Thomason to the Graduate School of the University of Georgia in partial fulfillment of the requirements for the degree of Master of Science in Agriculture.

Massey (14), in a study with 48 cows, reports that the cows which received the sweet potato meal ration produced 9.3 per cent more milk than did those fed the corn ration.

EXPERIMENTAL PROCEDURE

In the spring of 1946, eight Jersey cows of the University of Georgia dairy herd were paired into two groups (Groups *A* and *B*) and fed comparative rations of sweet potato meal and ground snapped white corn. The constituents of the concentrate mixtures used in the experiment are shown in table 1. The mixtures were the same except that an equal amount of sweet potato meal was substituted for the corn in mixture 2 and equal parts of corn and sweet potato meal were used in mixture 3. The cows also were fed mixed lespedeza and grass hay *ad libitum*. The animals were quartered in a dry lot except when being milked by machine twice daily.

TABLE 1

Constituents of grain mixtures used in experiment

Constituents	Mixture 1	Mixture 2	Mixture 3
	(*lb.*)	(*lb.*)	(*lb.*)
Ground snapped white corn	200		100
Sweet potato meal	.....	200	100
Oats	100	100	100
Wheat bran	100	100	100
Cottonseed meal	135	135	135
Bonemeal	9	9	9
Salt	4.5	4.5	4.5

The double reversal method of experimentation (1, 4, 18) was employed in this work. Both Groups *A* and *B* were fed mixture 3, which contained equal parts of corn and sweet potato meal, during a preliminary period of 14 days. Then Groups A and B were fed mixtures 1 and 2, respectively, during the first experimental period. The groups were changed alternately from one ration to the other during the second, third, and fourth experimental periods, each of which extended for 28 days.

When the experiment was about one-third over, cows no. 7 and 8 had to be withdrawn from the experiment due to the incidence of mastitis. In view of the fact that these two cows were paired together before the experiment started, their removal affected the experiment only insofar as the number of cows in each group was reduced by one.

Analysis of variance (4) was used in the statistical treatment of the liveweight and milk and butterfat production data according to two different methods. Method 1 tripled the differences between the response of the cows on the two feeds during the second and third experimental periods $(-a + 3b - 3c + d)$ and gave equal weight to the differences during the first and

fourth periods. Method 2 (3) consisted of giving equal weight ($a–b + c–d$) to the differences incurred during each experimental period.

RESULTS

Chemical analysis of feeds. Sufficient quantities of both rations were mixed bimonthly so as to keep a fresh supply of feed on hand at all times. A representative sample from each batch of feed was analyzed for dry matter, ether extract, crude protein, nitrogen-free extract, crude fiber, ash and moisture. The data shown in table 2 reveal that the corn ration contained 0.70 per cent more moisture, 1.6 per cent more fiber, 0.50 per cent more protein, 0.30 per cent more fat, 1.0 per cent less ash and 2.20 per cent less nitrogen-free extract than did the sweet potato meal ration. Very little difference in the chemical composition of the two mixtures was apparent.

The work of Briggs *et al.* (2) and Rusoff *et al.* (15) indicates that the

TABLE 2

Average chemical analyses of experimental rations[a]

Mixture	Constituents					
	Moisture	Fiber	Protein	Ash	Fat	Nitrogen-free extract
1	9.8	8.8	16.9	5.2	4.3	55.0
2	9.1	7.2	16.4	6.2	4.0	57.2

[a] Chemical analyses were made by personnel of the State Chemists Department.

apparent digestion of coefficients of the protein, fat and fiber of dehydrated sweet potato meal may be rather low. The Louisiana workers (15) point out that since the N.F.E. made up over 84 per cent of the dry matter in the dehydrated sweet potato, the apparent lack of digestibility of the other constituents had but little effect on the T.D.N. content and that the low content of protein precludes this product from being an important source of this nutrient. It would appear from these reports (2, 15) that the sweet potato meal ration (mixture 2) fed in this experiment may have been a little lower in digestible protein than the corn ration (mixture 1).

Palatability. All of the cows relished the sweet potato meal (Porto Rico variety) ration from the start of the experiment; when the cows were changed from one ration to the other, they did not eat the corn ration as readily as they did the sweet potato meal ration. This is in agreement with the results obtained by Massey (14) of the Georgia Experiment Station and Seath (16) and Seath *et al.* (17). The Porto Rico variety of sweet potato was fed in these studies. The Louisiana workers (17) observed in another study that, when the high-starch sweet potato variety, L–45, was compared with ground yellow corn meal in the ration,

from 1 to 4 days were required for all of the cows to become accustomed to the change in the rations. Then they ate the ration containing sweet potato meal as readily as they did the one containing corn meal. In regard to the varying degrees of palatability of sweet potato meal as reported, one should remember that this product is a relatively new livestock feed, the quality of which has not yet been standardized. The proportion of sweet potato meal to other constituents and the number of various ingredients used in the ration probably would have a definite relationship to the palatability of the ration containing the sweet potato meal. From the standpoint of color and palatability, the product used in this experiment was excellent.

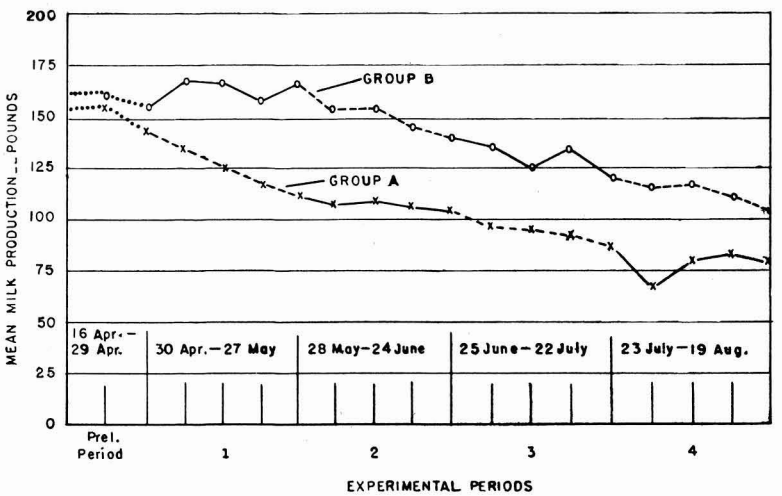

FIG. 1. Curves showing mean milk yield of cows on experiment. (Group $A = X$; Group $B = O$. Mixture 1 = - - - - - -; Mixture 2 = ————; Mixture 3 = ...)

The cows fed the sweet potato meal appeared to have a sleeker hair coat than did those fed the corn ration. There was no indication of any unusual looseness in the feces of the animals as the result of feeding the sweet potato meal; the feces of these cows were not as loose as those from the regular herd cows which were on green pasture. Massey (14) likewise found no digestive disturbances among the cows fed sweet potato meal, although it seemed to have a more laxative effect than did the corn meal.

Liveweight. The liveweight average per cow when fed the corn ration was 798 lb. as compared to 801 lb. when fed the sweet potato meal ration. Statistical treatment of the data according to the methods described previously gave *F*-tests which indicate that there was no significant difference between the effect of the two feeds on the liveweights of the cows.

Milk and butterfat yields. A comparative study of the effect of corn and sweet potato meal on milk and butterfat production was made during each of the experimental periods. The milk production data (Fig. 1) reveal that although the level of production of Group B was higher than that of Group *A*, the trend of the lactation curves of the two groups of cows was very much the same throughout the experiment. The level of production of the two groups was about the same at the start of the study. The sharp drop in the curve of Group *A* during the period July 23 through July 29, inclusive, was attributed to a case of foot rot, which one of the cows had during that period. Group *A* was being fed sweet potato meal ration during this period.

From the standpoint of total production, when the cows were fed the corn ration (mixture 1) they produced 5,752.1 lb. of milk as compared to 5,741.2 lb. when fed the sweet potato meal ration (mixture 2). The butterfat yields of the cows when fed each of the experimental rations were calculated from a bimonthly butterfat test and the actual milk production. When fed the corn ration, the cows produced a total of 267.58 lb. of butterfat as compared to 268.72 lb. when fed the sweet potato meal ration. The *F*-tests indicate that there was no significant difference between the effects of the two feeds on the amount of milk and butterfat produced.

SUMMARY

A comparative study of the effect of ground snapped white corn and sweet potato meal on the liveweight and milk and butterfat production of dairy cows was conducted during the spring and summer of 1946. Analysis of variance revealed no significant differences in the milk and butterfat production or in the liveweights of cows when the sweet potato meal or corn constituted 36 per cent of the concentrate mixture. The sweet potato meal was as palatable as the ground corn when each of these concentrates constituted 36 per cent of the concentrate mixture. No excessive or objectionable laxative effect upon the digestive system of the cows was noted when they were fed the sweet potato meal mixture. The cows fed the sweet potato meal had a sleeker, brighter hair coat than did those fed the corn ration.

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CORRELATION BETWEEN THE LACTOSE CONTENT OF MILK AND THE CEREBROSIDE AND CHOLINE CONTENT OF BRAIN

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Although the composition of cerebrosides is well known, their function is not understood. They are found in almost all tissues but are present in largest amounts in the brain. As the cerebrosides are relatively stable under various conditions (14, 15), they are believed to function as basic structural elements. Although the cerebroside composition of different brains has been studied (9,12,14,15), a systematic study of their relation to other lipids has not yet been made.

The cerebrosides have a galactose residue; sphingomyelins have a choline phosphoric acid residue. This led to the belief (1) that the two radicals might be interchangeable, that galactose might act as a choline sparer in the organism. If true, this would throw some light on the functions of lactose in milk, and also on the functions of cerebrosides and sphingomyelins. If there is such sparing of choline by galactose, it may be of significance in lipotropy and related phenomena. This problem was studied by feeding different levels of lactose. However, large amounts of lactose exert an unfavorable effect, apparently by competition with the glucose at the tissue centers where glucose is metabolized. A report (13) appeared on the effect of lactose feeding on the cerebroside content of the rat brain, but not on the change in choline distribution. Therefore, the author has attempted to find out if there is a correlation between the lactose percentage in milk and the cerebroside content of the brain in several species of mammals.

METHODS

The brains of the various freshly killed animals were weighed, dried in a vacuum oven for 48 hours, pulverized, weighed, and extracted with absolute methanol in a Soxhlet apparatus for 36 hours. The choline in the extract was determined by the method of Glick (6) and the cerebrosides by the method of Brückner (2, 3). Figures 1 and 2 and table 1 show the correlation between choline and galactose in the brain and the lactose in the milk. The effects of age, gestation, and cortical differentiation on the cerebrosides and choline contents of the several brains are presented in tables 2 to 4.

RESULTS AND DISCUSSION

No definite major function has yet been ascribed to galactose, although many minor functions have been attributed to it (1). Biochemically,

Received for publication December 15, 1947.

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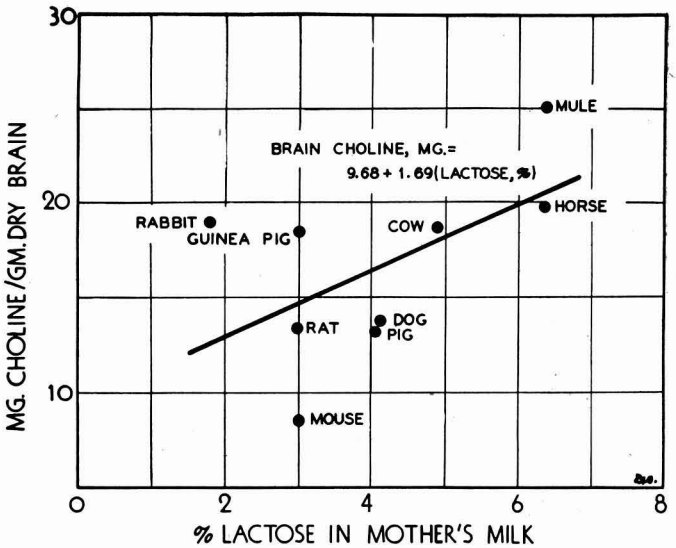

FIG. 1. Correlation of brain galactose with lactose percentage in milk of different species. (Each data point represents one animal.)

galactose is more resistant to oxidation in the body than is glucose. This greater stability of lactose led to the belief that galactoses form a hydrophilic group attached to the sphingosine base. As glucose may form a

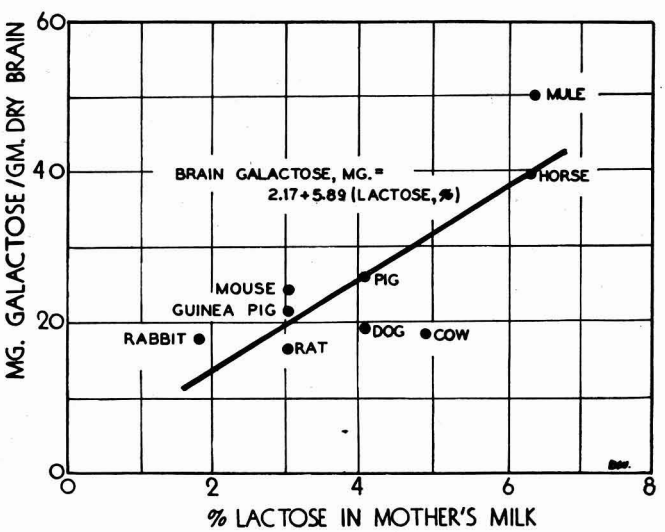

FIG. 2. Correlation of brain choline with lactose percentage in milk of different species.

similar hydrophilic group, gluco-cerebrosides would be expected to exist in the body, and, indeed, gluco-cerebrosides have been isolated from the spleens of patients suffering from Gaucher's disease (5, 7, 11) though the normal galacto-cerebrosides also were present (10). Since then gluco-cerebrosides also have been found in the normal cattle spleen (18) but never in the brain. It is likely that gluco-cerebrosides normally are present side by side with galacto-cerebrosides but, since glucose is the only oxidative substrate for the brain, it is quickly oxidized, and, therefore, not detectable in the brain. Galactose, on the other hand, being resistant to oxidation, forms stable galactosides of brain cerebrosides. This assumption is substantiated by the presence of both gluco- and galacto-cerebrosides in the spleen (8) but only galacto-cerebroside in the brain.

TABLE 1

Choline and galactose content of cerebrosides

Species	No. estimations	Choline (% of dry matter)	Galactose (% of dry matter)
Rat	24	1.346	1.667
Mouse	12	0.846	2.456
Rabbit	18	1.878	1.797
Pig	10	1.306	2.621
Guinea pig	10	1.831	2.133
Dog	6	1.368	1.915
Cow	12	1.826	2.239
Horse	6	1.979	3.995
Mule	10	2.507	5.053

It is true that galactose is synthesized by all animals, but in the mammalian series preformed galactose, supplied by lactose, is likely to determine the galactoside content in the phylogenetic scale of evolution. Feeding of lactose in nontoxic amounts increases galactoside formation in the rat brain (13).

If the galactose can replace the choline phosphoric acid of sphingomyelins, an inverse relationship may be expected between galactose and sphingomyelin contents of brain. Data on rat brain lipids (14, 15) show that as cerebrosides increase with age, sphingomyelins decrease. However, this is true only when these analyses are expressed as percentages of total brain lipids; both cerebrosides and sphingomyelins show an increase in absolute amounts with increasing age. Figure 2 shows that when the choline contents of the brains of different mammals are compared with the lactose contents of milk, a positive correlation is obtained, although a negative correlation was expected. Even if sphingomyelins decrease with increase of galactosides, there may be a simultaneous increase in other choline-containing lipids. As sphingomyelins were not estimated, this question cannot be settled. However, it should be noted, that the correlation between lactose and choline is not very high, and, if a concomitant increase

in lecithins or choline fractions other than sphingomyelins occurs, a negative correlation may be found between lactose and sphingomyelins. It would be interesting to estimate the galactose and sphingomyelin content of the brains of experimental animals fed graded amounts of galactose and choline.

TABLE 2

Influence of age on choline and galactose content of brains

Species	Age	Choline (% of dry matter)	Galactose (% of dry matter)
Rat	8 days	2.244	1.385
	14 days	1.718	1.304
	Adult	1.346	1.667
Mouse	20 days	1.630	1.180
	Adult	0.846	2.456
Rabbit	22 days *foeti*	1.477	1.599
	Adult	1.878	1.797

TABLE 3

Influence of gestation on choline and galactose content of brain

Species	Choline (% of dry matter)		Galactose (% of dry matter)	
	Nonpregnant	Pregnant	Nonpregnant	Pregnant
Rat	1.346	1.493	1.667	2.568
Rabbit	1.878	1.274	1.797	2.684

TABLE 4

Choline and galactose content of cortex and medulla as compared to that of the composite sample of the brain

Species	Choline (% of dry matter)			Galactose (% of dry matter)		
	Cortex	Medulla	Composite	Cortex	Medulla	Composite
Cow	1.755	1.833	1.826	2.185	2.243	2.239
Dog	1.337	1.378	1.368	2.053	1.902	1.915

Table 2 shows that there is an increase in the cerebroside content of the brain with age, confirming earlier results (14, 15). Table 3 shows an increase in brain cerebroside with increasing period of gestation, as might be expected, since lactose formation begins in the mother during the gestation period. Table 4 shows no difference in cerebroside content of different species associated with difference in the degrees of cortical differentiation or with different proportions of cortical and medullary tissues.

SUMMARY

1. Nine species of mammals have been examined for the cerebroside and choline contents of their brains. Brain cerebrosides show a high positive correlation with the lactose percentage of respective milks. There

is a slight positive correlation between the choline content of brains and the lactose content of the mothers' milk.

It is suggested that sphingosine base can reversibly take up galactose or choline phosphoric acid to form cerebrosides or sphingomyelins and, thus, cerebrosides may function as choline sparers.

2. There is an increase of cerebrosides with age and gestation. There is no significant difference in the cerebroside distribution between cortex and medulla of the brains of the species studied.

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ESTIMATING THE AMOUNT OF FEED DERIVED FROM PASTURE BY COWS IN THE CONNECTICUT DAIRY HERD IMPROVEMENT ASSOCIATION

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In many problems of farm management, estimates of the contribution of pasture to total annual feed consumption of dairy cows are desirable. The most common methods for determining pasture yields may be classified broadly under three headings: (a) Yields per acre of pasture based on agronomic data, a procedure which involves clipping, drying, and chemical analysis of the pasture grasses. (b) By quantitative grazing, which measures the weight changes of livestock and involves estimating the yield of pasture from the recorded weight changes. (c) On the basis of milk production records, when total feed requirements are estimated, barn feeds are known, and pasture appears as a residual. The purpose of this paper is to enlarge on the latter method.

EXPERIMENTAL PROCEDURE

A measure of energetic efficiency of milk production which describes the input-output relationship of milk production was selected. The measure was applied to production and feed consumption records by month of lactation for cows freshening in the winter months when barn feed equaled total feed input. The computed efficiencies of milk production for the various months of lactation were applied to the milk production records for cows freshening in the various months and estimates were made of the total nutrients required by month of lactation. After the total nutrients required were computed by month of freshening and month of lactation, the contribution of pasture was determined by deducting the known barn feeds from the estimated total requirements.

A measure of energetic efficiency of milk production. To obtain an estimate of a cow's consumption of total digestible nutrients (T.D.N.), certain measures which describe the cow's ability to convert feed into milk are used. These measures of energetic efficiency of milk production are based on records of milk output, feed intake, weight of cows, and so on.

Various types of dairy-merit indices have been developed. Davis *et al.* (3) proposed a dairy-merit index relating fat-corrected milk (F.C.M.) and weight (W). Brody and Nisbet (2) and Kleiber (7) relate

Received for publication December 31, 1947.

[1] The authors are indebted to Dr. S. Johnson and Dr. R. G. Bressler, Jr., for their helpful suggestions.

milk production to the three-fourths power of body weight. Other indices relating the amount of feed consumption and the amount of milk production, however, are much more applicable to this analysis. Brody (1) has developed a relationship which defines dairy merit as the ratio of milk-energy production to T.D.N. energy consumption. Based on the ratios of 1 lb. of milk to equal 340 calories and 1 lb. of T.D.N. to equal 1,814 calories, the relationship is:

$$\text{Dairy-merit ratio} = \frac{340 \times \text{lb. 4\% F.C.M. produced}}{1{,}814 \times \text{lb. of T.D.N. consumed}}$$

Brody used this for comparing milk producing capabilities of various cows within breeds and between breeds and also to compare different mammals. He found that dairy merit varied between different mammals and that the larger breeds of dairy cows had a lower dairy merit than the smaller breeds.

Dairy merit by month of lactation. Brody's analysis and use of the concept of dairy merit was entirely on an annual basis. In this study his measure is adapted to monthly input-output data in order to develop dairy-merit indices which lead to a determination of the total T.D.N. consumption and the feed derived from pasture. With the established measures of dairy merit by month of lactation, records of milk production may be used to derive monthly feed requirements. Deducting recorded amounts of T.D.N. in barn feed from these required nutrients should give the contribution of pasture.

The records from ten D.H.I.A. herds over a period of 10 years from 1935 through 1944 served as the source for input-output records. A total of 1,200 lactation records from Holstein cows was obtained, with 100 records in each month of freshening. While these records are subject to the usual limitations in applying a single day's performance to estimate an entire month, the records of concentrate feeding were taken on an individual basis and appeared to be reasonably accurate. The records of barn-fed roughages, however, were recorded as the average of several cows or the entire herd. This method of computing roughage consumption would tend, among other things, to obscure any possible differences that may have been associated with the stage of lactation.

The input-output relationships developed from D.H.I.A. data were based only on those months when pasture was unimportant. This was done on an average basis by combining figures for the barn-feeding months from December through March for all months of freshening, and so obtaining average inputs and outputs for each month in the lactation cycle.

RESULTS

The dairy-merit ratios for the different months of lactation, calculated according to Brody's formula, are given in table 1. Since feed inputs

do not decline as rapidly as milk production during the lactation period, dairy merit decreases throughout the period. Disregarding the results for the first partial month, dairy merit averaged about 34 per cent during the second month and 20 per cent during the ninth month, an average decline of 2 per cent per month. The tenth through twelfth months of lactation are subject to more rapidly declining milk production and dairy merit, presumably as a result of advancing gestation.

TABLE 1

Estimates of dairy merit by months during the lactation period

Mo. of lactation	Av. lb. of milk per month[a]	Av. lb. of T.D.N. per month[a]	% dairy merit
1	448	195	43.3[b]
2	1160	635	34.2
3	1048	626	31.3
4	958	614	29.2
5	870	594	27.4
6	799	576	26.0
7	736	558	24.7
8	650	538	22.6
9	557	520	20.1
10	393	490	15.0
11	267	475	10.5
12	162	456	6.7

[a] Milk production has been converted to 4% F.C.M., while feed inputs have been converted to pounds of T.D.N. on the basis of 0.75 per lb. of grain, 0.50 per lb. of hay, and 0.18 per lb. of ensilage.

[b] Based on production and feed for an average of 10.8 days during the month. Since feed inputs were allocated in the ratio of 10.8 to 30, the amount fed after freshening probably is underestimated and the calculated lactational efficiency correspondingly high.

Estimating required nutrients, and the contribution of pasture. The dairy-merit ratio defined by Brody may be converted to give directly the amount of feed obtained from pasture. The formula is as follows.

$$\text{Pounds of T.D.N. from pasture} = \frac{340 \times \text{F.C.M.}}{1{,}814 \times \text{D.M.}} - \text{Pounds of T.D.N. from barn feed}$$

where D.M. represents the previously calculated dairy-merit ratios.

A first attempt at an estimate of pasture will consider the feed equivalent obtained from pasture for cows freshening in the month of January. Table 2 summarizes the computations. These estimates indicate that such cows obtained about 6 per cent of their April feed requirements from pasture, 50 per cent of their May requirements, 74 per cent of their June requirements, and so on. On an annual basis, pasture accounted for 37 per cent of the total feed requirements.

Monthly feed requirements from cows calving in each month of freshening were computed in a manner similar to that for cows freshening in January. Having computed the feed requirements and the breakdown of source of feed, whether from pasture or from barn feeds, it is possible

to combine feed records by month of lactation and by month of freshening. The monthly composite feed records for a herd organization with equal numbers of cows freshening in each of the calendar months may be obtained in this manner. Table 3 summarizes the amounts of recorded nutrients from grain, hay and ensilage, and the total required T.D.N. based on milk production. The amount of pasture was found by deducting the total recorded T.D.N. per day from the total required T.D.N. per day. When the cows are not getting pasture, this figure would be expected to

TABLE 2

Estimates of monthly feed requirements and nutrients obtained from pasture for cows freshening in January

Month	Fat-corrected milk per month	Barn-fed T.D.N. per month	Estimated T.D.N[a]		
			From all sources	From pasture	
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(%)
Jan.[b]	457	556	543	− 13[c]	− 2.4[d]
Feb.	1058	597	580	− 17	− 2.9
March	1079	633	646	+ 13	+ 2.0
April	951	571	610	+ 39	+ 6.4
May	980	332	670	+338	+50.4
June	906	172	653	+481	+73.7
July	825	179	626	+447	+71.4
Aug.	756	181	627	+446	+71.1
Sept.	654	207	610	+403	+66.1
Oct.	536	276	670	+394	+58.8
Nov.	306	435	546	+111	+20.3
Dec.	186	478	520	+ 42	+ 8.1
Total	8694	4617	7301	2684	36.8

[a] Using estimated dairy merit from table 1.
[b] Since cows in the sample freshened throughout the entire month, the data applied to an average period of only 10.8 milking days.
[c] Difference between T.D.N. from all sources and barn-fed T.D.N.
[d] Per cent pasture is of total feed requirements.

be zero and any plus or minus amounts would be due to inaccuracy of the method. The fact that these differences are quite small, however, suggests that the errors in the use of this method are not large.

On an annual basis (table 3), considering a herd with equal numbers of cows freshening in each of the calendar months, pasture accounts for 36 per cent of the total T.D.N., grain contributes 27 per cent, hay 21 per cent and ensilage 16 per cent of the total nutrients. The contribution of pasture to total feed requirements of cows freshening in the various months varies from a high of 38 per cent for cows freshening in March to a low of 33 per cent for cows freshening in August. For brevity, tables similar to table 2 are not presented for cows freshening in the months from February through December.

TABLE 3

Estimates of nutrients obtained from pasture in different months on 10 D.H.I.A. farms in Connecticut, based on 10-year records of production of milk and consumption of grain, hay, and ensilage

Item	Calendar months												Total for year	% of total T.D.N.
	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.		
	(*av. lb. per day*)												(*lb.*)	
Milk produced														
4% F.C.M.	22.1	22.0	22.5	23.0	25.6	25.9	24.3	24.2	24.1	23.5	21.7	22.0	.....	.....
Total required T.D.N.														
Based on milk production	18.2	17.9	18.4	18.9	20.9	21.8	20.1	19.8	20.1	19.7	18.0	18.4	6966.0	.....
T.D.N. furnished & recorded														
Grain	5.5	5.9	5.8	5.9	5.1	4.6	4.7	4.7	4.9	5.1	5.4	5.4	1890.0	27.1
Hay	7.4	7.6	7.6	7.0	2.6	0.2	0.2	0.2	0.5	1.6	6.4	7.5	1464.0	21.0
Ensilage	5.2	5.1	4.7	4.2	1.6	0.3	0.6	0.9	2.1	3.4	4.7	5.3	1143.0	16.4
Total	18.1	18.6	18.1	17.1	9.3	5.1	5.5	5.8	7.5	10.1	16.5	18.2	.....	.....
T.D.N. furnished by pasture														
Difference between required and recorded T.D.N.	0.1	−0.7	0.3	1.8	11.6	16.7	14.6	14.0	12.6	9.6	1.5	0.2	2469.0	35.5
% of required T.D.N. furnished by pasture	(0.5)	(−3.9)	(1.6)	9.5	55.5	76.6	72.6	70.7	62.7	48.7	8.3	(1.1)	.....	.....

DISCUSSION

There are several limitations to the measure used in this analysis. As stated by Brody, dairy merit varies from cow to cow and is affected by such factors as changes in body weight and size. Since no weight data were available for the D.H.I.A. records used in this analysis, changes in body weight due to overfeeding or loss of flesh as well as to natural growth may have influenced the dairy-merit indices. Dairy merit is affected by age, which includes a joint relationship of increased body size and advanced maturity as related to increased milk production. This relationship is particularly important when working with lactation curves, since the slope of the production curve varies for cows of different ages; therefore, results are affected by the age of the cows in the sample. Dairy merit is a function of feeding levels and not a constant; the curvilinear total input-output curve based on smoothed data as given by Jensen *et al.* (6) would correspond to dairy-merit ratios increasing from 23.0 per cent at 6,000 lb. of 4 per cent F.C.M. output to 25.4 per cent at 8,000 lb. of 4 per cent F.C.M., and then decreasing to 23.6 per cent at 10,000 lb. of 4 per cent F.C.M. Dairy merit varies with environment, especially temperature. Regan and Richardson (9) found that higher temperatures had an adverse effect upon milk production. Some degree of error in the estimation of pasture could be explained on the basis of temperature, since dairy-merit indices were based on the winter months when temperature, as well as the other environmental conditions, was fairly constant.

While the measure under discussion has these limitations, it may prove particularly useful in two cases. First, as shown above, an estimate of the pasture consumption of a herd can be computed. Second, this measure could be used as a means of estimating pasture yields on a seasonal per acre basis. This would provide another measure for estimating pasture consumption on individual farms as well as an additional check on pasture studies involving lactating dairy cows, in which clipping and grazing methods are used. No significant changes would need to be made in the managerial techniques now used in the grazing methods as employed by Hodgson and Shepherd (5) or in the procedures recommended by the Joint Committee of the American Society of Agronomy, American Dairy Science Association and American Society of Animal Production (8). The results, however, would provide another comparison between the clipping (actual growth) and the grazing (actual consumption) methods. Hodgson *et al.* (4) found some divergence in the two methods which might be verified and explained by the use of a third comparison.

SUMMARY

The residual method was used in order to determine the contribution of pasture to total feed supply for a sample of 1,200 Holstein cows from

10 D.H.I.A. farms in Connecticut. One of Brody's measures of dairy merit was combined with the input-output records of the 1,200 cows. Dairy-merit indices were computed for each month of lactation on the basis of winter feed and production records. Pasture contributions then were determined by month of lactation for cows freshening in the various months. Pasture accounted for 38 per cent of the total feed for a cow freshening in March and 33 per cent of the feed for a cow freshening in August, on an annual basis. These were the highest and the lowest percentages, respectively.

On a yearly herd basis, using an equal number of cows freshening in each month, pasture accounted for 36 per cent of the total T.D.N. intake, grain for 27 per cent, hay for 21 per cent, and ensilage for 16 per cent of the total nutrients.

While there are limitations, this method may be used in computing pasture yield on a seasonal per acre basis. This would provide an additional check on pasture studies in which clipping and grazing methods are used.

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EFFECT OF WATER SPRINKLING WITH AND WITHOUT AIR MOVEMENT ON COOLING DAIRY COWS

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The knowledge that cattle produce some insensible perspiration yet lack true sweat glands (1) has caused a great deal of speculation among livestock men as to just how much effect artificial sprinkling or wetting by rain may have on cooling cows during warm weather. Likewise, there is much difference of opinion as to how much effect air movement, such as wind, may have on the cooling of cows. This applies to cows prior to sprinkling as well as after sprinkling.

In a previous experiment at this Station (4) it was found that milk cows when removed from the sunshine to shade cooled much faster when sprinkled with water than when not sprinkled. Body temperatures for those sprinkled reduced to normal levels in 1 hour, while those not sprinkled averaged approximately 0.5° F. higher. Also, the reduction in respiration rate was almost twice as great for those sprinkled as for those not sprinkled. Unpublished results of other Louisiana Station experiments (3) have shown that natural rain tends to cool milk cows rapidly. Experimental work in India (2) has shown this same tendency, with the body temperatures of water buffaloes, hill cattle, and sheep dropping considerably and those of zebu cattle showing somewhat less reduction due to heavy natural showers. It was found that wetting animals by hosing for 3 minutes was as effective in cooling water buffaloes as was allowing them to wallow for 20 minutes or for 1 hour.

In a later Indian experiment (5), 15 water buffaloes produced significantly more milk when wetting of their bodies was carried on by splashing. The authors concluded that body wetting was essential for water buffaloes during the hot months.

As reported by Kendall (1), the water lost by insensible perspiration by cattle on a maintenance ration was two to three times greater than that passed in the urine. This loss, he reports, may vary 12 or more lb. per day, with decreases accompanying a drop in air temperature. This large moisture loss, while not true sweat, appears to be relatively important, and the variations normally occurring may be associated with air movement as well as with changes in air temperatures. This experiment was designed to determine the importance of these factors plus others associated with air movement and body sprinkling on the cooling of dairy cows.

Received for publication January 12, 1948.

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EXPERIMENTAL PROCEDURE

Six Jersey cows in milk were utilized in a 2×2 factorial design (6), being divided as equally as possible into two groups of three cows each. Twelve warm clear days between July 11 and August 5, 1947, were selected for conducting this experiment. On each day selected all six cows were tied outside in the sun between 12:00 noon and 2:00 p.m., after which records were made of respiration rates taken from flank movements and of rectal body temperatures. Following this, three of the cows were completely wet by use of a hand sprinkler containing water averaging approximately 85° F. in temperature. Then the cows were put into a special section of the experimental barn. This section was enclosed and rectangular in shape with a 40-inch exhaust fan at one end. Panel-like doors could be opened at the far end when the fan was running to permit circulation of air. The area was divided into three stalls but the sides of the stalls were not solid, thus allowing air circulation. Two cows were tied in each stall, one at each end, and changes from stall to stall were made systematically from day to day. On half of the days the fan was started as soon as the cows entered the stall and continued running for 1 hour. During the other days the cows remained in the stalls without the fan being in operation. Tests made for wind velocity by use of a Taylor Biram-type anemometer were rather unsatisfactory but showed practically no detectable movement near the floor and from 40 to 240 feet per minute at levels between the cows' bellies and their backs. The division of the cows and treatments is given in table 1.

TABLE 1

Factorial design for determining effectiveness of water sprinkling on cooling dairy cows (6 Jersey cows)

Test days	Fan operation	Sprinkling procedure[a]	
		Group A	Group B
1, 5, 9	Without fan	Dry	Wet
2, 6, 10	Without fan	Wet	Dry
4, 7, 11	With fan	Wet	Dry
3, 8, 12	With fan	Dry	Wet

[a] 3 cows in each group.

As shown, each treatment was performed on three different test days. This particular design, as can be seen, has the effects of fan versus no fan confounded with differences caused by variations between test days (6). The effects of treatments as described were determined by noting changes in body temperature and respiration rate after cows had remained 0.5 hour and 1 hour in the special section of the experimental barn.

RESULTS

Records on cows while in sun. After the cows had remained in the

TABLE 2

Average body temperatures and respiration rates of individual cows after standing in sun for 2 hours

Cow no.	Body temperature (°F.)		Respiration rate (times per min.)	
	Range	Av.	Range	Av.
X1	102.1–104.7	103.47	80–130	106.3
4	101.5–103.4	102.46	66–115	89.3
X5	101.3–105.1	103.36	92–138	112.5
12	103.8–107.4	105.91	136–175	153.9
X4	101.2–104.0	102.84	75–132	104.9
X7	101.5–104.6	102.92	68–106	86.8
Av.	.....	103.49	.....	109.0

sunshine for 2 hours, the rectal body temperatures (table 2) ranged from 101.2 to 107.4° F. and averaged 103.49° F. The average temperature prior to the cooling treatments thus was approximately 2.0° F. higher than that which usually is considered normal. Respiration rates varied from 66 to 175 per minute, with an average of 109. This average was more than twice as high as were the respiration rates during cool weather.

The variations shown in these preliminary records prior to cooling appear extremely large. Much of this variation was due to differences

TABLE 3

Comparative average reductions in body temperatures of cows due to wetting and air movement

Test days	Atmospheric records at 2 p.m.		Decrease in body temperature at end of 1 hr.	
	Temperatures	Humidity	Dry group	Sprinkled group
	(° F.)	(%)	(° F.)	(° F.)
	Without fan			
1	90	36	0.63	0.83
2	92	45	0.77	1.77
5	94	46	0.53	1.07
6	87	29	0.47	1.07
9	93	50	0.70	1.07
10	92	48	1.10	1.77
Av.	91.3	42.3	0.70	1.26
	With fan			
4	92	42	1.47	1.77
3	90	61	1.00	1.43
7	97	47	1.30	1.77
8	94	49	1.33	2.03
11	94	43	1.70	1.90
12	96	44	1.53	2.17
Av.	93.8	47.7	1.39	1.84

between individual cows as well as to the day to day variability found in air temperatures (table 3). Air temperatures ranged from 87 to 97° F. and averaged 91.3° F. on days when the fan was not used and 93.8° F. on the days when the fan was used.

Influence of cooling on body temperatures. Statistically significant differences (6) were found in favor of sprinkling as contrasted to not sprinkling and in favor of air movement caused by fan as compared to no fan (tables 3, 4 and 5). Cows left dry without fan showed an average decrease in body temperature after 0.5 hour of 0.28° F. as contrasted to 0.62° F. with fan alone, 0.83° F. for sprinkling alone, and a decrease of 1.42° F. for cows receiving both sprinkling and fan. At the end of 1 hour for the non-sprinkled and non-fan treatment, the decrease in body temperatures averaged only 0.70° F., and for sprinkling plus fan, 1.84° F.

TABLE 4

Effect of sprinkling and air movement on changes in body temperature and respiration rate at end of 0.5 hour

Cow no.	Av. reduction in body temperature after 0.5 hr.				Av. reduction in respiration rate after 0.5 hr.			
	Without fan		With fan		Without fan		With fan	
	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled
	(° *F.*)	(° *F.*)	(° *F.*)	(° *F.*)	(*times per minute*)			
X1	0.93	0.53	0.57	1.30	24.0	35.7	35.3	41.3
4	0.33	0.57	0.73	0.83	25.0	32.0	22.0	52.0
X5	0.33	0.97	0.90	1.23	25.7	30.0	37.7	40.7
12	−0.10	1.70	0.53	2.47	− 3.0	64.3	18.0	43.0
X4	0.10	0.57	0.37	1.50	16.7	31.0	26.7	48.0
X7	0.40	0.63	0.63	1.20	10.0	30.7	23.0	29.0
Av.	0.28	0.83	0.62	1.42	16.4	37.3	27.1	42.3

The trend appears to be consistent whether considered for individual test days (table 3) or for individual cows (tables 4 and 5), except in the case of the cows sprinkled without fan; those cows had cooled more at the end of 0.5 hour than had the dry cows with the benefit of fan. This was reversed, however, at the end of 1 hour, when the fan-treated cows were the cooler, thus showing the slower cooling effect produced by the fan alone.

Influence of cooling on respiration rates. For cows receiving the sprinkling treatment in this experiment, respiration rates per minute decreased more on an average at the end of 0.5 hour of cooling (table 4) than at the end of 1 hour (table 5). The cows left dry either with or without fan failed to follow this trend, *i.e.*, those without fan decreased an average of 16.4 respirations per minute at the end of 0.5 hour and 20.8 at the end of 1 hour. With fan, the decrease was 27.1 at the end of 0.5 hour and 37.1 at the end of 1 hour. In contrast, those sprinkled decreased

37.3 at the end of 0.5 hour and 31.8 at the end of 1 hour; those with sprinkling and fan reduced 42.3 times per minute in 0.5 hour and 35.3 at the end of 1 hour.

Cow no. 12 (table 4), on an average, failed to decrease either in respiration rate or body temperature at the end of 0.5 hour when left dry and without fan. At the end of 1 hour a slight average reduction in respiration of 3 times per minute was shown, while body temperature also showed an average decrease. This particular cow suffered much from exposure to sunshine and had high body temperatures and respiration rates prior to

TABLE 5

Effect of sprinkling and air movement on changes in body temperature and respiration rate at end of 1 hour

Cow no.	Av. reduction in body temperature after 1 hr.				Av. reduction in respiration rate after 1 hr.			
	Without fan		With fan		Without fan		With fan	
	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled	Dry	Sprinkled
	(° *F.*)	(° *F.*)	(° *F.*)	(° *F.*)	(*times per minute*)			
X1	1.13	1.20	1.53	1.97	34.3	35.0	44.0	40.3
4	0.43	0.83	1.00	1.27	21.7	31.3	38.7	41.3
X5	0.77	0.93	1.80	1.87	28.0	25.7	47.3	34.0
12	0.77	2.90	1.27	2.70	3.0	64.0	36.7	34.3
X4	0.40	0.80	1.30	1.70	23.3	15.3	29.0	39.7
X7	0.70	0.90	1.43	1.57	14.7	19.7	26.7	22.3
Av.	0.70	1.26	1.39	1.84	20.8	31.8	37.1	35.3

cooling. The fact that she had calved only shortly prior to the start of the experiment partially explains her unusual reactions.

DISCUSSION

Results from this experiment give information in addition to that already published (4) concerning the cooling of cows in Louisiana. Of particular interest is the added information that the circulation of air by a fan greatly increases the cooling of both the non-sprinkled and sprinkled cows. For the non-sprinkled cows fanning increased the cooling rate by convection because the ambient air temperature was below that of the body. Perhaps evaporation of insensible perspiration also is a factor, but this is a problem which remains to be investigated. There is need for partitioning the heat loss between convection, vaporization, and radiation, all of which are affected by the vapor pressure of the air and skin and by the air movement.

These trials showed that the least cooling of cows took place without any sprinkling or air circulation and the best results were secured when cows were first sprinkled and then subjected to air circulation. The use of sprinkling alone or fan alone produced essentially equal results, with

sprinkling producing the greater drop in both body temperature and respiration rate at the end of 0.5 hour (table 4) and the fan being the more effective by the end of 1 hour (table 5). In these comparisons it is of interest that sprinkling alone produced a lower respiration rate at the end of 0.5 hour than was present after 1 hour. This was not true for body temperature, which is much slower to respond to cooling treatments. The fact that the cows were sprinkled just once and with water at approximately 85° F. probably helps explain why the maximum reduction in respiration was secured at the end of the 0.5-hour period.

SUMMARY AND CONCLUSIONS

When cows were removed from sunshine, sprinkled with water and then subjected to a gentle breeze produced by a fan, they showed rapid changes toward normal body temperature and respiration rate. Shade alone showed a small change in that direction, while the fan alone and sprinkling without a fan were intermediate in their effects.

The results of this experiment give valuable information on how rain and wind as produced by nature tend to cool milking cows during summer months. The results also suggest the need for further experimental work on how mechanical sprayers and fans may be utilized economically during summer when nature is not producing wind or rain.

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MEASUREMENT OF FLUORESCENT MATERIALS IN MILK AND MILK PRODUCTS[1]

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A number of workers in recent years have shown that various dehydrated food products and extracts thereof emit blue fluorescence when subjected to ultraviolet radiation. The intensity of fluorescence increases during storage of the products, and, in some cases, is correlated with loss of palatability, as Fryd and Hanson (8) found with dried eggs. Others, notably at the National Research Council of Canada (28, 36, 40, 41, 42, 43), have demonstrated that heat treatment and storage of dried egg increase the blue fluorescence of a 10 per cent potassium chloride extract (26, 27) of the defatted powder. While Pearce (21) at first postulated that the active fluorescing materials in such extracts are hydrolytic products of the proteins, it appears much more likely that they arise by interaction of the proteins with reducing sugars (1, 13, 20, 25, 33). Furthermore, although Pearce and Thistle (26) and Thistle *et al.* (35) concluded that loss of palatability of dried egg is correlated significantly with increase in fluorescence of the salt-soluble constituents, others (2, 7) have presented evidence indicating a much closer relation between flavor and fluorescence arising in the lipide fraction by reaction of lipide amines with aldehydes (5, 6). Fluorescence has been suggested as an index of storage deterioration of dehydrated pork, dried banana, dried parsnip, ration biscuits, and butter (22) and also for following development of rancidity in lard (10).

Only very meager data are available on the fluorescence of milk, dairy products or milk constituents. It is well known that normal fresh milk emits greenish-yellow fluorescence when irradiated with ultraviolet light (3, 30) and that this fluorescence is due principally to riboflavin (34, 44). Gerngross and Schultz (9) observed that irradiation of milk causes an alteration in fluorescence from yellow to blue, perhaps by conversion of riboflavin to lumichrome (17), although Raoul's (31) data do not substantiate this explanation. Radley (29), finding that severe heat treatment and roller drying of milk shifted its fluorescence to the blue, attributed this change also to a breakdown of riboflavin.

To date the only published quantitative data on the fluorescence of milk other than that due to riboflavin are those of Pearce (22, 23), who studied the salt-soluble fluorescent compounds of defatted[2] dry whole milk.

Received for publication January 20, 1948.

[1] Paper no. 2370, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

[2] It is not clear in the original just what solvent was used to extract the fat or how complete an extraction was attained.

He found: (a) greater initial blue fluorescence in extracts from spray dried than in those from roller dried milks and (b) decided increases upon storage. Since the increase in fluorescence was only slightly correlated with loss of palatability, Pearce (22, 23, 24) has concluded that fluorescence is not a satisfactory index of palatability in milk powder.

The fluorescence of the lipides of milk has not been studied extensively. Morgan and MacLennan (19)[3] found that the fluorescence of butter and butterfat is yellow in contrast to that of margarine, which is blue, and Pearce (22) has reported a decidedly greater blue fluorescence in a butter serum (in 10 per cent potassium chloride solution) from rancid than from fresh butter.[4] This latter observation has been made the basis of a method for assessing deterioration in butter (11).

The fact that several proteins, including casein, exhibit fluorescence when illuminated with ultraviolet has been demonstrated by Reeder and Nelson (32) and also by Vlés (39).

The work reported in this paper represents an attempt to develop a method capable of distinguishing among blue fluorescing materials produced in milk by such reactions as (a) breakdown of riboflavin or other reactions producing soluble blue-fluorescing material (b) lipide-amine-aldehyde interaction, and (c) interaction of protein and sugar. It was hoped that such a method would prove of value in following the course of storage deteriorations of dry milk products and possibly might serve as an objective criterion of palatability changes.

METHOD

In attempting to segregate fluorescing compounds from the several possible sources, a standard empirical method was devised involving fractionation of the constituents of milk into four categories: (I) those soluble in 67 per cent acetone; (II) those insoluble in 67 per cent acetone but soluble in acetone-ether (20:80); (III) those insoluble in (I) or (II) but soluble in 10 per cent potassium chloride; and (IV) those insoluble in (I), (II), or (III). This method is applicable to fluid milk or to dry milk reconstituted to a fresh basis. For dry whole milk, a 4-g. sample may be reconstituted by shaking with 31 ml. of distilled water.

The fractionating procedure is as follows: Add two volumes of acetone to one volume of milk or reconstituted milk, mix thoroughly, and filter through 15 cm. paper (S. and S. no. 597) which previously has been extracted exhaustively with 67 per cent acetone (2 acetone + 1 water). Wash the precipitate on the filter with two successive 10-ml. portions of 67 per cent acetone and make the combined filtrate and washings up to 100 ml. with 67 per cent acetone. This constitutes extract I.

[3] See also review by Déribéré (4).

[4] It is not stated whether the sample exhibited hydrolytic or oxidative rancidity.

Grind the precipitated residue from the first extraction with 20 ml. of C.P. acetone, transfer to a 250-ml. Erlenmeyer flask stoppered with a foil-covered cork, shake mechanically for 10 minutes and filter through 15 cm. paper (S. and S. no. 597). Further extract by shaking mechanically for 10 minutes with each of two successive 40-ml. portions of anhydrous ether. The combined acetone-ether filtrates made up to 100 ml. with ether constitute extract II. Although the solvent extracts small amounts of fluorescing material from the filter paper, no appreciable error is introduced if the blank is filtered in the same manner as the sample.

Dry the remaining protein residue by exposure to air at room temperature for 1 hour. Shake a 1-g. sample of it with 25 ml. of 10 per cent potassium chloride solution for 10 minutes and filter, again using S. and S. no. 597, 15 cm. paper. Wash the residue with two successive 25-ml. portions of the 10 per cent potassium chloride solution and make up to 100 ml. with 10 per cent potassium chloride. This is extract III. The 10 per cent potassium chloride does not extract fluorescing materials from the paper.

Extracts prepared in this manner were crystal clear. It will be noted that extract I is essentially identical to the filtrate used by Hand (12) for determination of riboflavin. To determine riboflavin, prepare tubes as follows and measure their fluorescence with the Coleman photofluorometer, using filters *B*–2 and *PC*–2 (see Hoffer *et al.* (15)).

Reading *A*—1 ml. filtrate + 9 ml. 67 per cent acetone + 1 ml. water
" *B*—1 ml. filtrate + 9 ml. 67 per cent acetone + 1 ml. riboflavin solution containing 1γ/ml.
" *C*—Same as *B* but with fluorescence quenched with approximately 20 mg. of sodium hydrosulfite.

Then:

$$\left(\frac{A-C}{B-A}\right) = \gamma \text{ riboflavin per ml. filtrate}$$

$$\left(\frac{A-C}{B-A}\right) \times \frac{100}{1000} \times \frac{100}{4} = \text{mg. riboflavin per 100 g. powder.}$$

Determine blue fluorescence in each extract with the Coleman photofluorometer using filters *B*–1 and *PC*–1. Adjust the galvanometer to read 70 with a quinine sulfate solution containing 0.2γ/ml. Make blank determinations and report results in terms of net galvanometer readings multiplied by any necessary dilution factor.

For fresh whole milk and fresh dry whole milk it usually was necessary to dilute an aliquot of the acetone extract with an equal volume of the 67 per cent acetone solvent in order to obtain a reading on the scale. The ether and potassium chloride extracts from these products gave readings on the scale without dilution. In many aged samples, considerable dilu-

tion of the acetone and potassium chloride extracts was necessary, but rarely was it found necessary to dilute the ether extracts. It appeared desirable to express all results on a common basis and consequently net fluorescence readings were multiplied by dilution factors to convert them to the basis of fluorescence intensity of the 100-ml. extracts. For example,

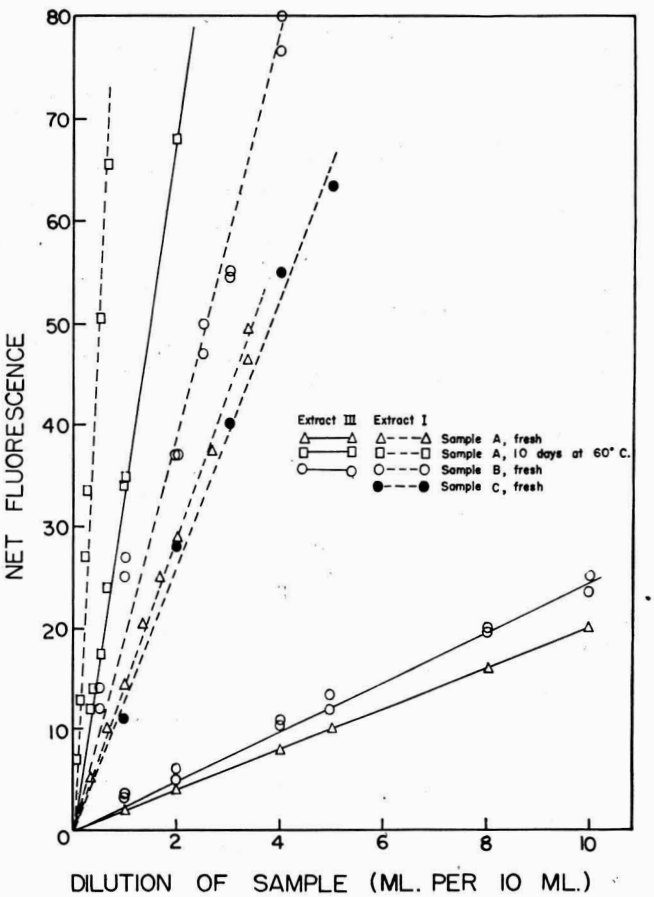

FIG. 1. Relation of fluorescence to dilution of extracts I and III. Indicated aliquots of the extracts were diluted to 10 ml. for fluorescence determination.

a sample diluted by ten times and reading 82.5 with a blank of 31.5 would have a net fluorescence of $(82.5 - 31.5) \times 10$ or 510. This procedure appears justified by the fact that over the range covered by the instrument the relation between net fluorescence and concentration of the acetone or potassium chloride extracts is linear and passes through the origin, as is illustrated in figure 1.

MATERIALS

1. *Dried milk* was prepared from University herd milk with the experimental drying equipment of the University of Minnesota.

2. *Casein* was prepared from fresh skim milk by the method of Van Slyke and Baker (38) as modified by Van Slyke (37), with the exception that the extraction with fat solvents was omitted. Calcium phosphocaseinate sols, used as a base for several series of simplified systems, were prepared by dispersing 100 g. of dry casein in 3 l. of saturated limewater, chilling in an ice bath and adding slowly with vigorous agitation 350 ml. of a solution containing 2.98 g. magnesium oxide, 34.47 g. potassium dihydrogen phosphate and 21.95 g. citric acid monohydrate per liter. During this back titration the following materials also were added: 1.014 g. potassium sulfate, 1.260 g. potassium carbonate, 5.600 g. sodium chloride, 1.264 g. potassium chloride, and 3.556 g. calcium hydroxide. The final pH of the sol was 6.7; it was very milky and reasonably stable.

3. *Milk serum protein* was prepared by removal of casein from skim milk with acetate buffer at pH 4.7, followed by exhaustive dialysis of the serum, concentration by pervaporation, freezing, and drying from the frozen state.

4. The *lactose* employed was of USP grade.

5. *Milk fat* usually was obtained by rendering butter and decanting and filtering the fat layer. For one experiment a sample of fat was extracted from whole milk by a macro-adaptation of the Roese-Gottlieb method. Milk fat was incorporated into the simplified systems by emulsification with a hand homogenizer.

6. The *phospholipide-protein-complex* constituting the so-called "fat globule membrane" was obtained by concentrating and drying from the frozen state the buttermilk and butter serum obtained by churning cream washed by the method of Jenness and Palmer (16).

7. *Riboflavin* was obtained from the Eastman Kodak Company.

8. *Ascorbic acid* was obtained from Hoffmann-LaRoche, Inc.

EXPERIMENTAL

Partition of milk constituents among the various fractions. Some analyses of the several extracts were undertaken to ascertain how the constituents of milk are partitioned by the method used. All of these analyses were made on extracts from whole milk samples, six being dry and one liquid. The data are presented in table 1.

Extract I contains 36–43 per cent of the total dry matter of the 4-g. sample. The largest component of this dry matter is lactose; in fact, nearly all of the lactose of the sample appears in this extract. Undoubtedly the failure to recover the lactose quantitatively in extract I is attributable to incomplete washing of the sugar from the precipitate. The lactose de-

terminations were made by evaporating an aliquot of the extract nearly to dryness, making up a definite volume with zinc sulfate and sodium hydroxide solutions according to McDowell (18), filtering and determining lactose in the filtrate by the chloramine-T method of Hinton and Macara (14).

Some nitrogenous material is present in extract I. The amounts of 15.5 to 18.6 mg. per 100 ml. of extract correspond to 46.5–55.8 mg. per 100 g. of milk (12 per cent solids) and hence are somewhat greater than the usual non-protein nitrogen content of milk. Of course extract I also

TABLE 1

Analyses of extracts

Sample	Extract I			Extract II		Extract III
	Dry matter	Nitrogen	Lactose	Dry matter	Lipide P	Protein[a]
	(*% of sample*)	(*mg./100 ml.*)	(*% of sample*)	(*% of sample*)	(*mg./100 g. fat*)	(*mg./100 ml.*)
Dry whole milk						
1	.....	.....	.....	27.0	4.12	.....
2	.....	.....	.....	28.1	4.95	.....
3	40.2	18.2	.....	25.6	.....	74.0
4	42.7	18.6	.....	25.8	.....	72.3
5*a*	36.4	15.5	29.8[b]	29.8[c]	3.10[c]	62.0
b	.....	.....	.....	28.0	.....	85.7
c	.....	.....	.....	30.6	.....	54.4
6	39.4	.....	31.2[b]	26.1	.....	.....
Liquid whole milk						
7[d]	.....	.....	.....	25.5	2.55	83.0

[a] Calculated as total nitrogen × 6.38.
[b] Lactose content of dry milk determined directly was 32.5 and 35.6% for samples 5*a* and 6, respectively.
[c] Mojonnier extract of sample 5*a* yielded 31.2% fat and 27.7 mg. lipide P/100 g. fat.
[d] Calculations made on basis of solids in sample.

contains riboflavin and undoubtedly a number of other minor soluble constituents. It contains very little, if any, fat.

Extract II contains the bulk of the fat of the sample. The amount of fat extracted in this way falls somewhat short of that extracted by the Mojonnier method. The amount of lipide phosphorus in this extract is only a small fraction of that present in the milk and extractable by the Mojonnier method.

The amount of dry matter extracted from 1 g. of defatted protein by the 10 per cent potassium chloride treatment amounts to 175 to 200 mg. The amount of nitrogen extracted by this treatment is equivalent to 54 to 86 mg. of protein. Actually only a portion of this nitrogen represents protein, since it was found that for sample 5*c*, at least, only approximately

80 per cent of the nitrogen was nondialyzable. Since this extract contains no fat, the non-nitrogenous portion must be composed of minerals and possibly lactose.

The residue that is not dispersed by 10 per cent potassium chloride undoubtedly is largely protein.

Fluorescence of milk constituents. The fluorescent characteristics of several milk constituents were studied using the *B*–1 and *PC*–1 filters in the Coleman instrument. The plot of fluorescence of riboflavin solutions as a function of concentration in figure 2 shows a linear relationship. It is

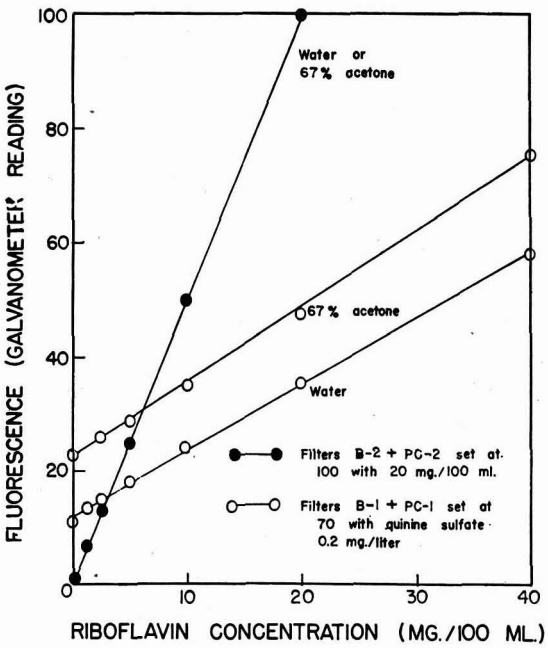

FIG. 2. Fluorescence of riboflavin as a function of concentration.

evident that considerable of the fluorescence of riboflavin is transmitted by filter *PC*–1 and consequently that riboflavin accounts for a large portion of the fluorescence of extract I.

In figure 3 the fluorescence of several milk fat solutions is plotted as a function of concentration. The relation is approximately linear. Fat obtained by Roese-Gottlieb extraction exhibits a much higher fluorescence than that prepared by churning and rendering butter. This comparison between churned and Roese-Gottlieb extracted fat had to be made with ether as solvent, because the Roese-Gottlieb extracted fat was not completely soluble in the 20:80 acetone-ether mixture. Undoubtedly this phenomenon, as well as the higher fluorescence of the Roese-Gottlieb extracted fat, is due to the presence of phospholipides.

The fat obtained in extract II from whole milk exhibits a *net* fluorescence intermediate between those of the Roese-Gottlieb extracted fats and the churned fats, which is in accord with the previously established fact that extract II contains a portion but not all of the phospholipide of the milk.

The data in table 2 show that both casein and milk serum protein sols in phosphate buffer exhibit blue fluorescence but that the latter fluoresces much more intensely. The fluorescence of milk serum protein preparations may vary considerably, but the fluorescence exhibited by a given

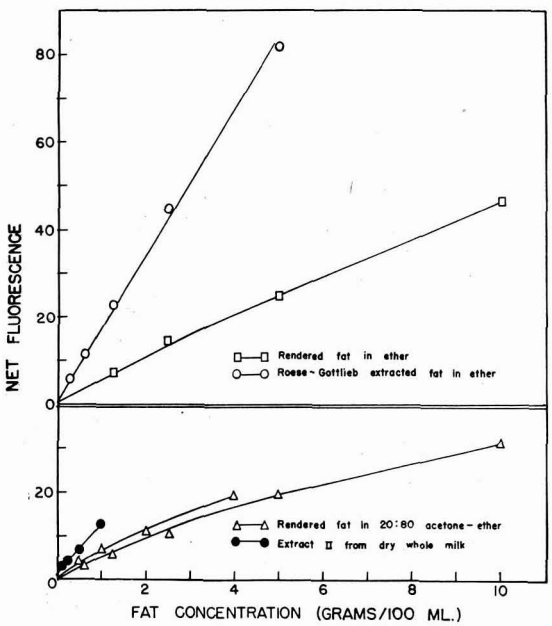

FIG. 3. Comparison of fluorescence of rendered butterfat, extracted fat, and extract II as a function of concentration. Fluorescence of the ether blank was 5.0; that of the 20: 80 acetone-ether was 12.0.

preparation in phosphate buffer is approximately identical to that in 10 per cent potassium chloride solution at the same concentration. Casein alone is only slightly dispersible in 10 per cent potassium chloride, but the portion that does dissolve exhibits a considerable fluorescence.

Extraction of the defatted protein fraction of milk by the regular treatment with 10 per cent potassium chloride results in dispersion of only 54–86 mg. of protein from a 1-g. sample, as may be seen in table 1. Furthermore, it was found that grinding of this material with the 10 per cent potassium chloride solvent is without effect on amount of fluorescent material extracted. Variations in the amount of protein extracted do not

TABLE 2

Fluorescence of milk protein sols

Casein sols			Milk serum protein sols		
Sample	Conc.	Net fluorescence	Sample	Conc.	Net fluorescence
In phosphate at pH 6.6	(*g./100 ml.*)		In phosphate at pH 6.9	(*g./100 ml.*)	
1	0.94	19.0	1	0.54	23.0
2	0.47	10.0	2*a*	0.78	88.0
3	0.23	5.5	2*b*	0.39	48.5
			2*c*	0.20	25.0
Fraction soluble in 10% KCl			In 10% KCl		
Regular[a]	0.0063	5.0	2*a*	0.77	Too high
1 grinding[b]	0.0207	17.5	2*b*	0.38	49.5
3 grindings[b]	0.0344	21.0	2*c*	0.19	24.0

[a] 1-g. samples of casein treated by the method described herein for preparing extract III.

[b] Grinding of casein samples with 10% KCl was used in addition to the shaking employed in the regular method.

appear to be closely related either to the treatment of the product or to the fluorescence of the extract.

Solutions of lactose in water or in phosphate buffer at pH 6.6 and $\mu = 0.1$ were found to fluoresce only negligibly more than the blank.

Fluorescence of systems of milk constituents. In order to obtain further information on the contributions of the various milk constituents to

TABLE 3

Fluorescence of simplified systems of milk constituents

System	Constituents[a]	Net fluorescence						
		Extract I		Extract II		Extract III		
		B[b]	C	B	C	A	B	C
		(*per 4 g. complete system*)				(*per g. protein*)		
1	Phosphocaseinate	.....	10.1	.....	.....	.....	.....	8
2	1 + lactose	10.1	16.0	3.8	1.7	3	3	7
3	2 + serum prot.	15.3	25.8	3.5	3.7	4	4	16
4	2 + fat	9.4	17.7	8.5	9.3	3	3	5
5	4 + F.G.M.[c]	17.0	20.7	10.4	9.4	4	5	5
6	5 + serum prot.	24.0	32.0	11.0	10.0	8	6	17
7	6 + riboflavin	51.0	70.0	10.0	10.0	6	6	15
8	7 + ascorbic	51.0	.....	10.0	.....	8	6	.....
Usual value for fresh dry whole milk		100.0		11.0		12.0		

[a] Ratio of constituents was as follows: 1.00 casein : 2.15 lactose : 0.30 serum protein: 1.52 fat : 0.04 F.G.M. : 0.000075 riboflavin : 0.0010 ascorbic acid.

[b] Letters designate replicate series.

[c] Fat globule "membrane".

the fluorescence of the several extracts, the standard method described in this paper was applied to a series of simplified systems of milk constituents, each of which was dried from the frozen state.

Table 3 gives the data for fluorescence of these systems, while table 4 gives the contribution of each constituent to the fluorescence. For extracts I and II, fluorescence has been calculated on the basis of 4 g. of the complete system (*i.e.*, the system containing all of the constituents), while for extract III the data are expressed on the basis of 1 g. of protein taken for extraction. The fluorescence of extract I from the system containing all of the constituents mentioned approaches but does not attain that obtained from whole milk. Riboflavin evidently is the major contributor to the fluorescence of extract I, although smaller increments of fluorescent

TABLE 4

Contributions of constituents to fluorescence of simplified systems of milk constituents

Constituent	Contribution to fluorescence						
	Extract I		Extract II		Extract III		
	B	C	B	C	A	B	C
	(*per 4 g. complete system*)				(*per g. protein*)		
Caseinate	.....	10.1	.....	.....	.....	.....	8
Caseinate + lactose	10.1	16.0	3.8	1.7	3	3	7
Serum protein[a]	4.2	9.8	−0.3	2.0	1	1	9
Serum protein	7.0	11.3	0.6	0.6	4	1	12
Milk fat	−0.7	1.7	4.7	7.6	0	0	−2
F.G.M.	7.6	3.0	1.9	0.1	1	2	0
Riboflavin	27.0	38.0	−1.0	0.0	−2	0	−2
Ascorbic	0.0	.....	0.0	.....	2	0	.....

[a] First value given for serum protein is computed as difference between systems 2 and 3, the second as difference between systems 5 and 6.

materials are extracted from the proteins. The serum protein preparation used in series *C* evidently carries more fluorescing materials than that used in series *B*.

Tables 3 and 4 show that the fluorescence of extract II is due principally to the lipides and that the characteristic fluorescence of whole milk extracts is satisfactorily reproduced in systems containing milk fat and fat globule "membrane". The proteins or materials associated with them in the defatted residue are responsible for the fluorescence extractable by 10 per cent potassium chloride from that residue. As expected, fat and riboflavin contribute nothing to the fluorescence of this extract.

Fluorescence of whole milk and the influence of processing. The method described herein was applied to 32-ml. samples of liquid whole milk, skim milk and butter milk, all from the same lot, and also to 4-g. samples of dry whole milk, dry skim milk, and dry buttermilk from a second lot. Table 5 indicates that the fluorescence of extract I from these products is approximately equal when expressed on the basis of fat-free solids in the

TABLE 5

Comparison of fluorescence of extracts from whole milk, skim milk, and buttermilk

Sample	Riboflavin	Net fluorescence			
		Extract I		Extract II	Extract III
	(*mg./100 g. solids*)	(*per 4 g. solids*)	(*per 4 g. fat-free solids*)	(*per 4 g. solids*)	(*per g. defatted protein*)
Series I—Liquid					
Whole milk	1.33	108	150	10	11.0
Skim milk	2.00	157	157	14	9.0
Buttermilk	1.67	147	147	25	17.0
Series II—Dry					
Whole milk	1.14	100	139	13	14.5
Skim milk	1.47	130	130	7	18.0
Buttermilk	1.59	146	146	20	22.2

sample. Buttermilk yielded the most fluorescing materials in extract II, probably because of its higher phospholipide content, but the reason for the high fluorescence of extract III from buttermilk is not apparent. Furthermore, the authors are unable to account for the anomalous relations exhibited by extracts II and III of the skim milk samples in that the liquid and dry samples differ considerably.

The effects of variations in pasteurization temperature, of condensing, and of spray drying were studied on a lot of mixed whole milk. The sample size was adjusted to furnish 4 g. of solids in each case. The results, shown in table 6, indicate that heat treatment in the range of 145 to 195° F.

TABLE 6

Effect of heat treatment, condensing and drying on fluorescence of extracts

Sample	Pasteurization temp. for 30 min.	Riboflavin	Net fluorescence		
			Extract I	Extract II	Extract III
	(° *F.*)	(*mg./100 g. solids*)	(*per 4 g. solids*)		(*per g. defatted protein*)
Whole milk	Raw	0.99	108	12	10.5
" "	145	1.05	116	12	9.5
" "	155	1.05	116	11	11.5
" "	165	1.09	114	14	12.5
" "	175	1.10	124	14	13.0
" "	185	1.10	125	13	13.0
" "	195	1.15	132	13	14.5
Condensed	165	1.22	138	12	9.5
Spray dried	165	1.10	120	12	15.0
Spray dried	150	.....	87	12	7.4
Normal	185	.....	95	12	7.4
Spray dried	150	.....	94	12	10.1
High temp.	185	.....	93	13	7.4

for 30 minutes has some effect in increasing fluorescence in extract I, possibly some in extract III, but none in extract II. The effects of condensing and spray drying, if any, do not appear to be very marked.

In another experiment, also recorded in table 6, in which pasteurizing treatments of 150 and 185° F. were combined with normal and high temperature drying conditions, no significant effects of either treatment on fluorescence were noted.

DISCUSSION

While the procedure adopted for fractionating the constituents of milk is rather empirical, it is felt that a reasonable approach has been made to determining which of those constituents are capable of emitting blue fluorescence when illuminated with ultraviolet. Furthermore, it is considered that the scheme which has been evolved may prove of use in following changes occurring during storage of dry whole milk.

Riboflavin appears to be the principal fluorescent material of extract I but very evidently certain other materials also are involved. In the simplified systems some of the "non-riboflavin fluorescence" was contributed by the protein preparations used, but any speculation on the nature of the specific protein fraction that may be responsible for this effect is fruitless at present. Whole milk evidently contains fluorescent materials soluble in 67 per cent acetone other than those included in the simplified systems, because in no case did the most complete simplified system yield fluorescence in extract I equaling that of whole milk.

The fluorescence of extract II appears to depend, in part, on its phospholipide content. Evidently the major portion of these lipides is not extracted at all but remains in the final residue. Consequently, variations in the fluorescence of this extract might be expected to depend on the extent of extraction of phospholipides. The most satisfactory scheme would be one which extracted all of the phospholipides, but under the empirical conditions used, the proportion of phospholipide extracted probably is sufficiently constant so that no great variations in fluorescence are introduced from that cause.

Ten per cent potassium chloride disperses a fraction of milk protein which is associated with a certain amount of fluorescing ability. No exhaustive attempt was made to determine just which protein fraction is involved. However, the data secured on fluorescence of milk proteins in 10 per cent potassium chloride indicate that both casein and at least some of the serum protein fractions are involved. Here again it might be argued that the ideal situation would be to disperse all of the protein in extract III, leaving no undispersed residue, but to date it has not proved possible to prepare such a dispersion that is satisfactory for fluorescence measurements.

The data fail to indicate any pronounced change in fluorescence proper-

ties during the processing involved in manufacture of dry whole milk. The procedure employed in this study is being applied to following the changes occurring during heat treatment of milk and storage of dry whole milk.

SUMMARY AND CONCLUSIONS

A method is presented for evaluating the fluorescence characteristics of milk by fractionating the constituents of milk into (I) those materials soluble in 67 per cent acetone, (II) those insoluble in (I) but soluble in 20:80 acetone-ether, (III) those insoluble in (I) or (II) but soluble in 10 per cent potassium chloride, and (IV) those insoluble in (I), (II) or (III), and determining the blue fluorescence of the solutions of (I), (II) and (III).

As might be expected, riboflavin is the largest contributor to the fluorescence of extract I, although proteins and probably other constituents also contribute. The lipides, particularly the phospholipides, are the fluorescent compounds of extract II, while the proteins contribute the fluorescent materials dispersible in 10 per cent potassium chloride.

The normal processing of dry whole milk appears to be without effect on the fluorescence of these extracts.

ACKNOWLEDGMENTS

The research which this paper reports was undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

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THE RELATIONSHIP OF THE CHANGE IN pH EFFECTED BY INCUBATION TO OTHER SEMEN CHARACTERISTICS[1]

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A number of simple, rapid tests have been applied to the evaluation of semen, although no single measure is presently recognized as an adequate index of fertilizing capacity. The reports of several workers (1, 2, 4, 5, 8, 15, 17) would indicate that the initial pH of semen may be related to semen quality and/or used as a criterion of relative fertility. Significant correlations have been found between the initial pH and spermatozoa concentration (1, 2, 3, 9, 14, 15, 17), ejaculate volume (1, 2, 9, 17), spermatozoa motility (1, 2, 6, 9, 11, 17), sugar content (5) and buffer capacity (4) of semen, and the glucose loss, lactic acid gain and viability following incubation at 46.5° C. for 1 hour (17). The initial pH was inversely related to these semen characteristics. However, Swanson and Herman (16) found no appreciable relationship between the pH of fresh semen and conception rate.

Although the initial pH was found to be helpful in the evaluation of semen in a number of studies, several reports would indicate that the final pH and/or the change in pH effected by incubation and/or storage of semen provides a more satisfactory index (6, 11, 13, 19, 20). The final pH of semen following incubation at 37° C. and storage at 40° F. was correlated with the concentration, motility, oxygen consumption and fertility of spermatozoa (13) and the conception rate (11), respectively. Although Anderson (4) found no significant correlation between the change in pH upon incubating semen at 37° C. for 1 hour and the buffer capacity or specific gravity of semen, a high correlation was found between the change in pH and the spermatozoa concentration (6, 13), initial motility (6, 13), oxygen consumption (13) and fertility (13, 19, 20, 21) of spermatozoa. Measuring the drop in pH of semen stored at 27 to 29° C. and at 10° C., Dougherty and Ewalt (10) found the decrease in pH to be related to the motility and viability of spermatozoa. These workers showed that refrigeration and chemical inactivation, both of which inhibit motility, also prevent rapid decreases in pH. Several workers (6, 13) believe that the change in pH of semen is a reflection of the general metabolism of spermatozoa involving the number and activity of spermatozoa, respiration and glycolysis. For development of the maximum increase in acidity during

Received for publication January 21, 1948.

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incubation, Anderson (6) suggested the necessity of a high concentration of actively motile spermatozoa, an adequate quantity of glucose or other reducible sugar, and conditions favorable for the retention in the semen of acid products, *i.e.*, anaerobic conditions.

The present study was initiated when it was observed that, of a large number of semen quality tests employed in this laboratory, the change in pH during incubation seemed to be giving the best over-all estimate of semen quality. Since most of the data recorded in the literature represent a relatively limited number of ejaculates and since the relationships of the pH change to only a few semen characteristics are reported, the purpose of this investigation was to contribute additional data to the subject and to determine the value of the change in pH effected by incubation as an index of semen quality.

EXPERIMENTAL PROCEDURE

The relationship of the change in pH upon incubation of semen at 37° C. for 1 hour to concentration, initial motility, and viability of spermatozoa and percentage of abnormal spermatozoa, was studied in 203 semen specimens from 11 bulls. The average sample consisted of 2.33 ejaculates (range, 1–5 ejaculates).

Soon after the semen was ejaculated, the initial motility was estimated by using a constant temperature stage incubator adjusted to 100° F., the initial pH was measured with a Beckman glass electrode potentiometer, and an aliquot of each sample was incubated at 37° C. for 1 hour, after which time the pH was measured again. The spermatozoa concentration was determined with a cytometer, and the percentage of morphologically abnormal spermatozoa was estimated in fixed and stained semen smears. The initial motility ratings used in this study represent gradations of 1 to 20 units and are approximately equivalent to per cent motility divided by five. Viability was measured as the percentage of the initial motility persisting at 100 hours subsequent to ejaculation.

RESULTS AND DISCUSSION

The pertinent data obtained in this study are shown in table 1. Highly significant positive correlations were found between the pH change of incubated semen and each of the characteristics, of concentration, initial motility, and viability of spermatozoa, whereas a significant negative correlation existed between the pH drop and the percentage of morphologically abnormal spermatozoa. Of these semen characteristics, the concentration and initial motility of spermatozoa showed the highest degree of relationship to the decrease in pH. These correlation coefficients (0.460 and 0.436, respectively) are remarkably similar to those reported by Anderson (6) (0.476 and 0.450, respectively). From a casual observation, these coeffi-

TABLE 1

Correlation coefficients showing interrelationship of various semen characteristics

Semen characteristics	1 pH change	2 Viability	3 Spermatozoa concentration	4 Initial motility	5 Abnormal spermatozoa
1 pH change	.....	0.2062 ± 0.0675**	0.4600 ± 0.0556**	0.4364 ± 0.0571**	− 0.3237 ± 0.0631**
2 Viability	.....	.....	0.2639 ± 0.0656**	0.2277 ± 0.0669**	− 0.0276 ± 0.0705
3 Spermatozoa concentration	.....	.....	.....	0.3464 ± 0.0621**	− 0.2489 ± 0.0662**
4 Initial motility	.....	.....	.....	.....	− 0.3557 ± 0.0616**
5 Abnormal spermatozoa	.....	.....	.....	.....	.....
Means ± standard error	0.336 ± 0.016[a]	35.29 ± 2.32[b]	0.993 ± 0.023[c]	13.1 ± 0.345[d]	13.7 ± 0.630[e]
			$R_{1.2345} = 0.5658 \pm 0.0484$		
Range	0.0–1.64	0.0–128[f]	0.21–2.04	0.1–19.8	2.0–43.0

[a] Units of pH.
[b] Percentage of the initial motility persisting at 100 hours subsequent to ejaculation.
[c] Millions per mm.^{3}
[d] Motility value × 5 is approximately equivalent to per cent motility.
[e] Per cent morphologically abnormal.
[f] Three values found above 100%; 12 values above 88%.
** Highly significant.

cients may appear to be low. However, they were calculated from the ungrouped data of individual semen samples.

The multiple correlation coefficient (0.5658 ± 0.0484) between the pH change and the four semen characteristics studied was highly significant. The pH change of incubated semen would seem to have great potential worth in the evaluation of semen, since it is related to a number of different characteristics.

It long has been known that lactic acid formation occurs during the storage of semen and is accompanied by a decrease in the quantity of glucose or other reducing sugars, a reduction in motility and an increase in acidity (18). The incubation of semen increases the rate of these reactions, allowing measurable differences in the end-products within short periods of time. Since the rate and the extent of the decrease in pH were found to be proportional to both motility and concentration of spermatozoa in this study as well as in others (6, 13), the pH drop effected by incubation appeared to be a quantitative reaction dependent upon the metabolic activity of the individual spermatozoan and the total number of spermatozoa present. This provided an indirect measure of the over-all metabolism of semen. That the effects of the percentage of morphologically abnormal spermatozoa reflect upon the change in pH of a semen specimen is indicated by the inverse relationship between these two characteristics (correlation coefficient $= -0.324$). This phenomenon, in which a large number of morphologically abnormal spermatozoa minimized the extent to which pH was changed, would suggest that these spermatozoa are participating in katabolism either very little or not at all. Since deformed spermatozoa tend to impede the motility of the more normal cells, they perhaps further inhibit the rate of metabolism of the total specimen, producing less lactic acid and other acid products, thereby resulting in a lesser degree of pH drop. Table 1 shows a highly significant inverse relationship between the percentage of morphologically abnormal spermatozoa and the initial motility. The abnormal spermatozoa commonly appear to survive the normal spermatozoa, as determined by the maintenance of motility. This observation would indicate the possibility of energy conservation in abnormal spermatozoa and would be in accord with the observations previously discussed. Some studies suggest that results of measures of spermatozoa metabolism, such as the rate of respiration (18) and the rate of glycolysis (7), provide the best single indications of fertility. However, the measures of oxygen consumption and products of glycolysis of spermatozoa are tedious, necessitate special apparatus and conditions restricted to well-equipped laboratories, and require personnel of specialized training, thereby limiting their field usage. A high degree of relationship exists between the pH change and oxygen consumption (13), a number of other semen characteristics and fertility (13, 19, 20, 21). It would seem that complex,

time consuming tests and the use of a large number of simple semen quality tests would be largely obviated by the use of the pH change upon incubation as a single measure of semen quality. Although a pH-meter was employed in this study, a series of indicators could be readily adapted to field use as suggested by Laing (13). Incubation of semen for 1 hour produces greater changes in semen pH than does incubation for 0.5 hour; however, some artificial breeding units may find the shorter incubation period more compatible with their semen collection schedule. Information provided by this test would be available for the inseminator when needed, *i.e.*, before dilution of the semen and before insemination of the cow.

SUMMARY

1. A study was made of the relationship of the decrease in pH during incubation of 203 semen specimens composed of 473 ejaculates from 11 bulls to other semen characteristics.

2. Highly significant coefficients of correlation found between the change in pH and other characteristics of semen were: concentration of spermatozoa, 0.46; initial motility of spermatozoa, 0.44; viability of spermatozoa, 0.21; and percentage of morphologically abnormal spermatozoa, −0.32. The multiple coefficient of correlation (0.57) between these four semen characteristics and the drop in pH was highly significant. These data suggest a significant relationship of the pH change in incubated semen to a number of different semen characteristics and indicate its value as an indirect measure of over-all semen metabolism.

3. The results of this study, in combination with those of other investigations involving fertility data, would seem to indicate that the change in pH effected by incubation is probably the best simple, quick test of semen quality available at the present time.

The authors are indebted to Prof. C. E. Shuart and Messrs. Martin Struble and D. Eby for the feeding and management of the bulls and many other services.

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ASSOCIATION ANNOUNCEMENTS

PROGRAM

FORTY-THIRD ANNUAL MEETING

OF THE

AMERICAN DAIRY SCIENCE ASSOCIATION

UNIVERSITY OF GEORGIA
ATHENS, GEORGIA
JUNE 14-16, 1948

PROGRAM COMMITTEE

GENERAL:
FORDYCE ELY, *Chairman*
Ohio State University
JENNINGS B. FRYE
University of Georgia

EXTENSION:
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Bureau of Dairy Industry
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University of Kentucky

REGISTRATION

SOULE HALL
UNIVERSITY OF GEORGIA

Meetings will be held in the buildings on the campus of the College of Agriculture of the University of Georgia. Headquarters will be in Soule Hall.

The opening session will be in Hardman Hall. All sectional meetings will be in Dawson Hall. Rooms there will be assigned based on the needs of the sections. The general business session on Wednesday afternoon will be in the Auditorium, Dawson Hall.

PROJECTION EQUIPMENT

Lanterns will be available in all lecture rooms for projection of standard and $2'' \times 2''$ slides. Projectors for 16 mm. movies will be available by ar-

rangement. Please advise respective section chairmen by mail of the type of projection equipment, if any, which will be needed for the presentation of your paper.

COMMITTEE MEETINGS

Those wishing rooms for Extension, Production, and Manufacturing Section Committee meetings should write or contact Jennings B. Frye, Jr., Dairy Department, University of Georgia.

SPECIAL MEETINGS

Groups wishing rooms and equipment for special meetings before, during, or after the regular sessions will please contact Jennings B. Frye, Jr., of the Dairy Department, University of Georgia. Provision can be made for a limited number of breakfasts, luncheons, or dinners for special groups.

EXTENSION EXHIBITS

Extension Dairymen desiring space for exhibition of material will please contact Frank W. Fitch, Extension Dairyman, University of Georgia. The exhibits will be in the Auditorium, Dawson Hall.

PROGRAM OF ENTERTAINMENT

(Principally for the Ladies)

Monday, June 14

10:00 A.M. TOUR—Antebellum Homes of Athens

3:00 P.M. TEA—Founders Memorial Gardens
Compliments of Landscape Architecture Department and Georgia Feed Manufacturers Association

8:00 P.M.* ENTERTAINMENT—Physical Education Building
Early American Dances—The Atlanta Promenade Club
Negro Spirituals
Group Singing

Tuesday, June 15

MORNING FREE. Convenient busses to shopping district

12:30 P.M. LUNCHEON—Athens Country Club
Compliments of Kraft Foods Company

3:30 P.M. ART EXHIBIT AND DEMONSTRATION—Fine Arts Department

8:00 P.M.* RECEPTION AND DANCE—Memorial Hall
(Formal for Ladies)

Wednesday, June 16

3:00 P.M. BRIDGE—Physical Education Building

7:00 P.M.* BARBECUE—Amphitheatre

8:30 P.M.* INSTALLATION OF OFFICERS AND PRESENTATION OF AWARDS—Amphitheatre

* These features open to all in attendance. Other events open to ladies only. Admission to all events by badge only.

GENERAL PROGRAM

Monday, June 14

Eastern Standard Time

9:30–12:00 OPENING SESSIONS, *Hardman Hall*

H. B. HENDERSON, *Dairy Department, University of Georgia, presiding*

Invocation

DR. PAUL C. HOWLE, *Pastor, First Christian Church, Athens*

Introduction of Officers and Guests

Address of Welcome

H. W. CALDWELL, *President, University of Georgia*

Presidential Address

PAUL H. TRACY, *President, American Dairy Science Association*

Intermission

Music

Guest Speaker

PAUL W. CHAPMAN, *Dean, College of Agriculture, University of Georgia*

Announcements

1:30– 4:30 SECTIONAL MEETINGS

Production Section A

Genetics and Endocrine Investigations
Dawson Hall

Production Section B

Type, Vitamins, Metabolism, Techniques
Dawson Hall

Manufacturing Section

Dry Milk, Condensed Milk, Ice Cream, Cream
Dawson Hall

Extension Section

Records and Interpretations
Dawson Hall

4:30– 5:30 COMMITTEE MEETINGS

8:00 ENTERTAINMENT, *Physical Education Building*
Early American Dances—Atlanta Promenade Club
Negro Spirituals
Group Singing

Tuesday, June 15

9:00–12:00 SECTIONAL MEETINGS

Production Section A
Calf Problems
Dawson Hall

Production Section B
Artificial Breeding
Dawson Hall

Manufacturing Section
Pasteurization, Microbiology, Cheese
Dawson Hall

Extension Section
Teaching Methods and Exhibits
Dawson Hall

1:30– 4:00 SECTIONAL MEETINGS

Joint Meeting of Production and Extension Sections
Dawson Hall

Manufacturing Section
Cheese
Dawson Hall

4:00– 5:30 PRODUCTION AND EXTENSION SECTIONS

Committee Reports
MANUFACTURING SECTION

Business Meeting

8:00 RECEPTION AND DANCE, *Memorial Hall*
(Formal for Ladies)

Wednesday, June 16

9:00–11:00 SECTIONAL MEETINGS

Production Section A
Parturient Changes in Blood and in Mammary Secretions
Dawson Hall

Production Section B
Forages, Hay
Dawson Hall

Manufacturing Section A
Chemistry
Dawson Hall

Manufacturing Section B
Homogenized Milk, Sanitation, Microbiology
Dawson Hall

Extension Section
4-H Clubs and Testing Rules
Dawson Hall

11:00–12:00 BUSINESS MEETINGS
Production Section, *Dawson Hall*
Manufacturing Section, *Dawson Hall*
Extension Section, *Dawson Hall*

1:00 GROUP PICTURE, *Amphitheatre*

1:30– 3:00 SECTIONAL MEETINGS

Production Section A
Feeding and Management
Dawson Hall

Production Section B
Forages, Pastures
Dawson Hall

Manufacturing Section
Dairy Sanitation Symposium
Dawson Hall

3:00– 5:00 GENERAL BUSINESS SESSION, *Auditorium, Dawson Hall*

7:00 BARBECUE, *Amphitheatre*
INSTALLATION OF OFFICERS AND PRESENTATION OF AWARDS

PROGRAM OF MANUFACTURING SECTION

Monday, June 14

Afternoon Session

1:30– 4:30 **DRY MILK, CONDENSED MILK, ICE CREAM, CREAM.** D. V. JOSEPHSON, *Chairman.*

M1 The Effect of the Addition of Ascorbic Acid to Milk on the Keeping Quality of Its Dried Product. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*

M2 The Formation and Preservation of Antioxidants by Special Methods of Processing in the Preparation of Dried Milk. GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*

M3 The Effect of Heat Treatment on the Reducing Systems of Milk. S. T. COULTER, HERBERT HARLAND, AND ROBERT JENNESS, *University of Minnesota.*

M3-a The Heat Treatment of Milk Necessary to Prevent Lipolytic Activity in Its Dried Product (A Preliminary Report). GEORGE R. GREENBANK AND PHILIP A. WRIGHT, *Bureau of Dairy Industry, U.S.D.A.*

M4 The Isolation of Compounds Responsible for the Stale Flavor Developed in Dried Whole Milk. I. The Distribution of Stale Flavor between the Fractions of Reconstituted Stale Whole Milk Powder. R. M. WHITNEY AND P. H. TRACY, *University of Illinois.*

M5 A Solubility Method for the Determination of Alpha and Beta Lactose in Dry Products of Milk. R. P. CHOI, C. W. TATTER, AND B. W. FAIRBANKS, *American Dry Milk Institute, Inc., Chicago, Illinois.*

M6 The Viscosity and Heat Stability of Milks Subjected to High Temperature Processing. B. H. WEBB AND C. F. HUFNAGEL, *Bureau of Dairy Industry, U.S.D.A.*

FIFTEEN MINUTE RECESS.

M7 The Microbiological Keeping Quality of Bulk Condensed Milk. A. M. PEARSON AND F. E. NELSON, *Iowa State College.*

M8 The Use of Sweetened Condensed Whole Milk in the Manufacture of Caramels. J. J. SHEURING AND P. H. TRACY, *University of Illinois.*

M9 Influence of the Mineral Content of Water on the Properties of Ice Cream Mixes. ROBERT A. HIBBS AND W. A. KRIENKE, *University of Florida.*

M10 Observations on the Effects of Various Stabilizing and Emulsifying Materials on the Properties of Ice Cream. W. S. ARBUCKLE, R. B. REDFERN, AND L. F. BLANTON. *North Carolina State College.*

M11 The Effect of a Mannitol of Beef Fat on the Whipping Qualiites, Body and Texture of Ice Cream. RALPH NADEN, J. J. SHEURING AND P. H. TRACY, *University of Illinois.*

M12 A Study of the Fat Emulsion in Natural and Artificial Creams. W. E. SNYDER, *University of Georgia,* AND H. H. SOMMER, *University of Wisconsin.*

4:30– 5:30 **COMMITTEE MEETINGS.**

Tuesday, June 15

Morning Session

9:00–12:00 **PASTEURIZATION, MICROBIOLOGY, CHEESE.** E. M. BARKER, *Chairman.*

M13 Preservation of Milk for the Phosphatase Test. GEORGE P. SANDERS AND OSCAR S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*

M14 Differentiation of Microbial Phosphatases from Milk Phosphatase. RALPH P. TITTSTLER, OSCAR S. SAGER, AND GEORGE P. SANDERS, *Bureau of Dairy Industry, U.S.D.A.*

M15 A Solution for Time and Temperature Relationships for Inactivating the Phosphatase Enzyme in Milk. JOHN HETRICK, *Dean Milk Co., Rockford, Illinois,* AND P. H. TRACY, *University of Illinois.*

M16 Isolation of Heat-induced Flavor Compounds from Milk. STUART PATTON AND DONALD V. JOSEPHSON, *Ohio State University.*

M17 Some Observations on the Efficiency of High-temperature Short-time Pasteurization of Chocolate Milk. MARVIN L. SPECK AND CHARLES D. COLVARD, *North Carolina State College.*

M18 Use of the Direct Microscopic Method for Pasteurized Dairy Products. M. J. PRUCHA AND VIRGINIA FRAZEE, *University of Illinois.*

M19 Bacteriophage Production by Cultures of *Streptococcus lactis.* F. J. BABEL, *Purdue University.*

M20 Electron Microscope Studies of Bacteriophages Active against *Streptococcus lactis.* C. E. PARMELEE, P. H. CARR, AND F. E. NELSON, *Iowa State College.*

M21 Some Factors Affecting the Rate of Acid Production by Cheese Cultures. H. C. OLSON AND FRANCIS D. COHENOUR, *Oklahoma A. and M. College.*

M22 Methods of Controlling the pH of Fermenting Dairy Products and the Effects of pH Control. WAYNE I. TRETSVEN, *Chicago, Illinois.*

M23 Chemical Changes Occurring in Limburger Cheese during Accelerated Ripening. W. K. STONE AND S. L. TUCKEY, *University of Illinois.*

M24 A Preliminary Note on the Pasteurization of American Cheddar Cheese by Radio-frequency Heat. F. V. KOSIKOWSKY, B. L. HERRINGTON, AND A. C. DAHLBERG, *Cornell University.*

M25 Increasing Efficiency and Reducing Costs in the Manufacture of Cheddar Cheese. D. M. IRVINE AND WALTER V. PRICE, *University of Wisconsin.*

Tuesday, June 15

Afternoon Session

1:30– 4:00 **CHEESE.** P. R. ELLIKER, *Chairman.*

M26 The Use of Nonfat Dry Milk Solids in the Manufacture of Cheddar Cheese from High Fat Content Milk. G. H. WILSTER AND C. E. JOHNSON, *Oregon State College.*

M27 The Problem of Sampling Cheddar Cheese for Analysis. WILLIAM C. WINDER AND WALTER V. PRICE, *University of Wisconsin.*

M28 The Influence of *Oospora lactis* in Promoting Changes in the Constants of Cheese Fat during Ripening of Cheddar Cheese. S. L. TUCKEY, W. O. NELSON, AND R. V. HUSSONG, *University of Illinois.*

M29 Studies of Sources of the Typical Flavor in Cheddar Cheese. HAROLD E. CALBERT AND WALTER V. PRICE, *University of Wisconsin.*

M30 The Influence of Temperature of Ripening on the Concentration of Tyramine in American Cheddar Cheese. A. C. DAHLBERG AND F. V. KOSIKOWSKY, *Cornell University.*

M31 Methods for Studying the Ripening of Cheese. H. H. SOMMER AND W. J. HARPER, *University of Wisconsin.*

M32 The Effect of Added Amino Acids on Flavor Development in Cheddar Cheese. R. J. BAKER AND F. E. NELSON, *Iowa State College.*

M33 Studies of Amino Acids in Cheddar Cheese during Ripening. W. J. HARPER AND A. M. SWANSON, *University of Wisconsin.*

M34 The Influence of Various Lactobacilli and Certain Streptococci on the Chemical Changes, Flavor Develop-

ment, and Quality of Cheddar Cheese. R. P. TITTSLER, GEORGE P. SANDERS, H. R. LOCHRY, AND O. S. SAGER, *Bureau of Dairy Industry, U.S.D.A.*

4:00– 5:00 **BUSINESS MEETING.**

Wednesday, June 16

Morning Session

9:00–11:00 SECTION A, **CHEMISTRY.** D. V. JOSEPHSON, *Chairman.*

M35 Some Physiological Effects of Dietary Lactose. JESSIE ELIZABETH FISCHER AND T. S. SUTTON, *Ohio State University.*

M36 The Limitations of the Refractometer Readings of Milk Serums in Detecting Watered Milk. L. R. ARRINGTON AND W. L. FOUTS, *University of Florida.*

M37 The Application of Flame Photometry to Determinations of Calcium, Potassium and Sodium in Milk. W. A. KRIENKE AND NATHAN GAMMON, *University of Florida.*

M38 A Rapid Method for the Determination of Nitrogen in Milk Products by Direct Nesslerization of the Digested Sample. J. H. HETRICK, *Dean Milk Company, Rockford, Illinois,* AND R. M. WHITNEY, *University of Illinois.*

M39 Studies on Separation and Fractionation of Casein. E. C. HAGBERG AND A. M. SWANSON, *University of Wisconsin.*

M40 The Fractionation of Milk Fat by Molecular Distillation. E. L. JACK AND MRS. L. B. OLSEN, *University of California.*

M41 The Measurement of Free Fatty Acids in Dairy Products. H. A. HOLLENDER AND H. H. SOMMER, *University of Wisconsin.*

M42 The Determination of Linoleic Acid in Milk Fat. P. S. SCHAFFER AND GEORGE E. HOLM, *Bureau of Dairy Industry, U.S.D.A.*

M43 Retention of Ascorbic Acid, Changes in Oxidation-reduction Potential, and the Prevention of an Oxidized Flavor during Freezing Preservation of Milk. R. W. BELL, *Bureau of Dairy Industry, U.S.D.A.*

M44 The Effects of the Treatment of Milk and the Subsequent Storage of Cream and Butter below Freezing

Temperatures upon the Sensitivity of Fat to Oxidative Deterioration as Determined by the Re-emulsification Test. VLADIMIR N. KRUKOVSKY, E. S. GUTHRIE AND FRANK WHITING, *Cornell University.*

M45 Ascorbic Acid Oxidation in Milk by Preformed H_2O_2. VLADIMIR N. KRUKOVSKY, *Cornell University.*

Wednesday, June 16

Morning Session

9:00–11:00 SECTION B, **HOMOGENIZED MILK, SANITATION, MICROBIOLOGY.** E. M. BARKER, *Chairman.*

M46 Stimulation of the Oxidized Flavors in Homogenized Milk by Light as Governed by the Vitamin C Content of the Milk. E. S. GUTHRIE AND VLADIMIR N. KRUKOVSKY, *Cornell University.*

M47 Studies on Seepage from Bottles of Homogenized Milk. E. O. HERREID, *University of Illinois.*

M48 The Leucocyte Count of the Complete Milking of Normal Animals for Complete Lactation Periods. E. O. ANDERSON, *University of Connecticut.*

M49 Effect of Some Water Constituents on Quarternaries. W. S. MUELLER AND D. B. SEELEY, *University of Massachusetts.*

M50 Germicidal Effectiveness of Certain Hypochlorides and Quaternary Ammonium Compounds under Simulated Plant Conditions. P. R. ELLIKER, *Oregon State College,* AND K. R. SPURGEON, *Purdue University.*

M51 Sanitizing Milk Cans in Mechanical Can Washers. G. W. REINBOLD, S. L. TUCKEY, R. V. HUSSONG, AND P. H. TRACY, *University of Illinois.*

M52 Some Factors Involved in Developing a Sediment Test for One-pint Samples of Cream Taken Off the Bottom of the Container. RAYMOND W. MYKLEBY AND BEN M. ZAKARIASEN, *Land-O-Lakes Creameries, Inc., Minneapolis, Minnesota.*

M53 A Skunk-like Odor of Bacterial Origin in Farm-separated Cream. T. J. CLAYDON, *Kansas State College.*

M54 Coliform Bacteria in Butter. R. N. SINGH AND F. E. NELSON, *Iowa State College.*

M55 The Effect of *Streptococcus lactis* and Coliform Organisms on Soluble Nitrogen in Milk. E. B. COLLINS AND F. E. NELSON, *Iowa State College.*

11:00–12:00 **BUSINESS MEETING.**

Wednesday, June 16

Afternoon Session

1:30– 3:00 **SYMPOSIUM ON ASPECTS OF SANITATION IN THE DAIRY INDUSTRY.** P. R. ELLIKER, *Chairman.* K. G. WECKEL, *Leader.*

Chemical and Physical Aspects of Cleaning Dairy Equipment. H. G. HARDING AND H. A. TREBLER, *National Dairy Research Laboratories, Inc., Baltimore, Maryland.*

Aspects of Quarternary Compounds. LUTHER BLACK, *U. S. Public Health Service, Cincinnati, Ohio.*

Bacteriophage and Its Relation to Sanitary Practices. F. J. BABEL, *Purdue University.*

3:00– 5:00 **GENERAL BUSINESS SESSION,** *Auditorium, Dawson Hall.*

7:00 **BARBECUE,** *Amphitheatre.*

PROGRAM OF PRODUCTION SECTION

Monday, June 14

Afternoon Session

1:30– 4:30 SECTION A, **GENETICS AND ENDOCRINE INVESTIGATIONS.** G. H. WISE, *Chairman.*

P1 The Relative Merits of a Cow's Own Record and Her Progeny Test for Predicting the Butterfat Production of Her Future Daughters. W. J. TYLER AND GEORGE HYATT, JR., *West Virginia University.*

P2 Preliminary Results from the Crossing of Two Inbred Lines of Holsteins on Growth and Milk Production. N. P. RALSTON, S. W. MEAD, AND W. M. REGAN, *University of California.*

P3 Genetic Variation in the Levels of Blood Plasma Carotene and Vitamin A in Dairy Cattle. R. E. MATHER, *New Jersey Agricultural Experiment Station.*

P4 Measurement of the Rate of Endocrine Gland Secretion as a Tool in the Genetic Selection of Dairy Cattle. C. W. TURNER, *University of Missouri.*

P5 Thyroid Secretion Rate and Its Relation to Various Physiological Processes. VICTOR HURST, *University of Missouri.*

P6 The Effect of Low Levels of Thyroprotein Feeding upon Milk and Milk Fat Production, Body Weight, Body Temperature, Heart Rate and Respiration Rate of Dairy Cows. R. G. SWANSON AND C. B. KNODT, *Pennsylvania State College.*

P7 Effects of Feeding Thyroprotein to Milking Cows in Summer. K. E. GARDNER AND T. W. MILLEN, *University of Illinois.*

P8 Effects of Feeding Thyroprotein during Successive Lactations. J. W. THOMAS AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

P9 Factors Controlling the Extent of Duct Growth in Mammary Glands. I. The Influence of an Estrogen in a Hereford Heifer. RALPH P. REECE, *New Jersey Agricultural Experiment Station.*

P10 The Value of Oxytocin for Reducing Fluctuations in Milk and Fat Yield during Experimental Periods. H. P. ADAMS AND N. N. ALLEN, *University of Wisconsin.*

P11 The Role of Certain Hormones in Spermatogenesis. J. D. SAMPATH KUMARAN, *University of Missouri.*

Monday, June 14
Afternoon Session

1:30– 4:30 SECTION B, **TYPE, VITAMINS, METABOLISM, TECHNIQUES.** L. A. MOORE, *Chairman.*

P12 The Relationship between Type Rating of Ayrshire Females as Young Heifers and as Cows. GEORGE HYATT, JR., AND W. J. TYLER, *West Virginia University.*

P13 The Effect of Certain Vitamins and Minerals on Blood Carotene Values of Dairy Animals. DWIGHT ESPE, *North Dakota Agricultural College.*

P14 Effect of Certain Soybean Products on the Concentrations of Carotene and Vitamin A in the Milk and in the Blood Plasma of Dairy Cows. R. L. SQUIBB, C. Y. CANNON, AND R. S. ALLEN, *Iowa State College.*

P15 Further Studies on the Relationship between the Feeding of Soybeans and the Vitamin A Requirements of Dairy Cattle. M. F. ELLMORE, J. C. SHAW, AND B. C.

HATZIOLOS, *University of Maryland,* AND L. A. MOORE AND J. F. SYKES, *Bureau of Dairy Industry, U.S.D.A.*

P16 The Influence of Tocopherols on the Fat Content of Milk. F. WHITING AND J. K. LOOSLI, *Cornell University.*

P17 Covitamin Studies of Milk Fats from Four Breeds of Dairy Cattle. V. N. KRUKOVSKY AND F. WHITING, *Cornell University.*

P18 Heat Production and Cardiorespiratory Activities during Gestation and Lactation in Jersey Cattle. S. BRODY, D. M. WORSTELL, H. H. KIBLER, AND A. C. RAGSDALE, *University of Missouri.*

P19 A Biochemical and Histo-pathological Study of Ketosis in Dairy Cattle. J. C. SHAW, B. C. HATZIOLOS, AND V. P. SAARINEN, *University of Maryland.*

P20 A Study of Sampling at Various Stages of Milking in Determining the Bacterial Flora of the Udders of Dairy Cows. E. M. KESLER, C. B. KNODT, AND J. T. REID, *Pennsylvania State College.*

P21 A Permanent and Convenient Rumen Fistula for Dairy Cows. G. E. STODDARD AND N. N. ALLEN, *University of Wisconsin.*

P21-a Studies Bearing on the Bloat Problem. H. H. COLE AND MAX KLEISER, *University of California.*

4:30– 5:30 **COMMITTEE MEETINGS.**

Tuesday, June 15

Morning Session

9:00–12:00 SECTION A, **CALF PROBLEMS.** G. H. WISE, *Chairman.*

P22 Calf Losses in a Self-contained Herd over a Period of 17 Years. R. E. JOHNSON, E. L. JUNGHERR, AND W. N. PLASTRIDGE, *University of Connecticut.*

P23 The Effect of Prepartum Vitamin A Supplementation on the Newborn Calf. A. A. SPIELMAN, H. D. EATON, J. K. LOOSLI, AND K. L. TURK, *Cornell University.*

P24 The Utilization of Fetal Liver Stores of Vitamin A by the Newborn Calf. A. A. SPIELMAN, H. D. EATON, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, *University of Connecticut*

P25 Effect of the Method of Administration of Carotene and of Vitamin A upon the Rate at Which They Are Absorbed from the Alimentary Tract of Dairy Calves. N. L. JACOBSON, G. H. WISE, AND R. S. ALLEN, *Iowa State College.*

P26 Some Irregular Fluctuations in the Vitamin A Level of Blood Plasma Produced in Calves by Ration Changes. W. C. JACOBSON AND J. W. THOMAS, *Bureau of Dairy Industry, U.S.D.A.*

P27 Influence of the Ration on Some Blood Vitamin Constituents of the Young Dairy Calf. JOHN W. HIBBS AND W. D. POUNDEN, *Ohio Agricultural Experiment Station.*

P28 Influence of the Ration on the Digestive Tract Microorganisms of the Young Dairy Calf. W. D. POUNDEN AND JOHN W. HIBBS, *Ohio Agricultural Experiment Station.*

P29 Relation of Aerobic Bacterial Flora to Consistency of Feces. M. D. VAN PELT, R. E. JOHNSON, AND W. N. PLASTRIDGE, *University of Connecticut.*

P30 Raising Dairy Calves without Colostrum. J. T. MILES, S. A. HINTON, AND HOMER PATRICK, *University of Tennessee.*

P31 A Comparison of Corn Starch, Dextrin and Corn Sugar as the Principal Carbohydrate Source in Synthetic Rations for Calves. R. J. FLIPSE, C. F. HUFFMAN, C. W. DUNCAN, AND F. THORP, *Michigan State College.*

P32 Effect of Tryptophan in the Diet on the Excretion of Niacin and Its Metabolic Products in Dairy Calves. G. C. ESH AND T. S. SUTTON, *Ohio State University.*

P33 Performance of Calves on a Photolysed Milk Diet. R. G. WARNER AND T. S. SUTTON, *Ohio State University.*

P34 Anemia in Young Calves and Its Alleviation by Iron. W. C. JACOBSON AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

PAUL H. PHILLIPS, *Discussion Leader.*

9:00–12:00 SECTION B, **ARTIFICIAL BREEDING.** L. A. MOORE, *Chairman.*

P35 A Method of Evaluating Bull Semen. TOM LUDWICK, D. OLDS, AND M. CARPENTER, *University of Kentucky.*

P36 Vital Staining of Bovine Spermatozoa with an Eosin-aniline Blue Staining Mixture. H. E. SHAFFER AND J. O. ALMQUIST, *Pennsylvania State College.*

P37 Turbidometric Assay of Hyaluronidase in Bull Semen. JOHN P. MIXNER AND JAMES E. JOHNSTON, *New Jersey Agricultural Experiment Station.*

P38 Hyaluronidase and Bull Semen. J. E. JOHNSON, E. J. STONE, AND J. P. MIXNER, *New Jersey Agricultural Experiment Station.*

P39 Effect of Testis Biopsy on Semen Characteristics. J. F. SYKES, W. J. SWEETMAN, AND P. C. UNDERWOOD, *Bureau of Dairy Industry, U.S.D.A.*

P40 Spermatozoa Behavior in Bovine Cervical Mucus at Varying Stages of Estrus. H. A. HERMAN AND OTIS H. HORTON, *University of Missouri.*

P41 Varying the Proportion of Egg Yolk in Diluters for Bull Semen. ERIC W. SWANSON, *University of Tennessee.*

P42 A Study of the Types of Bacteria in Bovine Semen and Their Effect upon Motility. J. E. EDMONDSON, K. L. TALLMAN, AND H. A. HERMAN, *University of Missouri.*

P43 Effect of Penicillin upon the Fertility of Semen from Relatively Infertile Bulls. J. O. ALMQUIST, *Pennsylvania State College.*

P44 Breeding Results with Bovine Semen Treated with Varying Amounts of Thyroxine. A. B. SCHULTZE AND H. P. DAVIS, *University of Nebraska.*

P45 Measuring Breeding Efficiency by Pregnancy Examinations and by Non-returns. G. R. BARRETT, L. E. CASIDA, AND C. A. LLOYD, *University of Wisconsin.*

P46 Order Number of Insemination and Conception Rate. G. R. BARRETT, C. A. LLOYD, AND R. A. CARPENTER, *University of Wisconsin.*

G. W. SALISBURY, *Discussion Leader.*

Tuesday, June 15

Afternoon Session

1:30– 4:30 **JOINT MEETING WITH EXTENSION SECTION.** E. H. LOVELAND AND G. H. WISE, *Co-Chairmen.*

Symposium—Reproductive Problems in Dairy Cattle. L. A. MOORE, *Leader.*

1. Infectious Disease as a Cause of Infertility. D. E. BARTLETT, *Bureau of Animal Industry, U.S.D.A.*
2. Functional Causes of Infertility and Methods of Treatment.
   a. Hormone Disturbances } S. A. ASDELL, *Cor-*
   b. Nutrition Disturbances } *nell University.*
   c. Inheritance. L. O. GILMORE, *University of Minnesota.*
3. Possible Modes of Approach to a Study of Infertility. J. F. SYKES, *Bureau of Dairy Industry, U.S.D.A.*
4. Activities of the Reproduction Committee of the Dairy Cattle Breeding Research Council of the Purebred Dairy Cattle Association. P. H. PHILLIPS, *University of Wisconsin.*

4:30– 5:30 **COMMITTEE REPORTS.**

Dairy Cattle Health Committee. L. A. MOORE, *Chairman.*

Dairy Cattle Breeding Committee. E. J. PERRY, *Chairman.*

Breeds Relations Committee. H. A. HERMAN, *Chairman.*

1. Program of Purebred Dairy Cattle Association. G. A. BOWLING, *Sec.-Treas.*

Wednesday, June 16

Morning Session

9:00–11:00 SECTION A, **PARTURIENT CHANGES IN BLOOD AND IN MAMMARY SECRETIONS.** G. H. WISE, *Chairman.*

P47 The Effect of Udder Inflation of Cows with Parturient Paresis on Blood Calcium, Magnesium and Inorganic Phosphorus. VEARL R. SMITH AND R. P. NEIDERMEIER, *University of Wisconsin.*

P48 A Study of Citric Acid Levels in the Blood and Urine of Cows at Time of Parturition. T. H. BLOSSER, VEARL R. SMITH, AND H. A. LARDY, *University of Wisconsin.*

P49 The Effect of Prepartum Milking on Some Blood Constituents of the Cow. R. E. JOHNSON, H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, AND R. J. SLATE, *University of Connecticut.*

P50 A Study of Some Blood Constituents of Cows not Milked Following Parturition. R. P. NEIDERMEIER AND VEARL R. SMITH, *University of Wisconsin.*

P51 The Effect of Preparturient Milking on the Composition of Colostrum. A. H. VAN LANDINGHAM, C. E. WEAKLEY, R. A. ACKERMAN, AND GEORGE HYATT, JR., *West Virginia University.*

P52 The Effect of Prepartum Milking on the Carotene and Vitamin A and Proximate Composition of Colostrum. H. D. EATON, A. A. SPIELMAN, R. E. JOHNSON, L. D. MATTERSON, AND R. J. SLATE, *Unversity of Connecticut.*

P53 The Carotene and Vitamin A and Proximate Composition of Portions of the First Milking Postpartum. H. D. EATON, A. A. SPIELMAN, L. D. MATTERSON, R. E. JOHNSON, AND R. J. SLATE, *University of Connecticut.*

P54 The Effect of the Form of Vitamin A and of Tocopherol Supplements of the Ration on the Concentration of Vitamin A and Carotenoids of Colostrum and Early Milk. D. B. PARRISH, GEORGE H. WISE, AND J. S. HUGHES, *Kansas State College.*

T. S. SUTTON, *Discussion Leader.*

9:00–11:00 SECTION B, **FORAGES, HAY.** L. A. MOORE, *Chairman.*

P55 Comparison of Barn-cured and Field-cured Alfalfa Hay. GILBERT H. ROLLINS AND PAUL M. REAVES, *Virginia Polytechnic Institute.*

P56 Studies on Mow Curing of Baled Hay. W. A. KING, J. W. WILBUR, S. M. HAUGE, AND A. W. COOPEL, *Purdue University.*

P57 Stack Finishing of Baled Hay with and without Heat. K. A. KENDALL, W. B. NEVENS, AND J. H. RAMSER, *University of Illinois.*

P58 Conservation of Nutrients and Feeding Value of Wilted Silage, Barn-cured Hay and a Poor Quality Field-cured Hay. J. B. SHEPHERD, L. G. SCHOENLEBER, H. G. WISEMAN, C. G. MELIN, W. J. SWEETMAN, W. H. HOSTERMAN, AND H. M. TYSDAL, *Bureau of Dairy Industry; Bureau of Plant Industry, Soils and Agricultural Engineering; and Production and Marketing Administration.*

P59 Vitamin D Content of Forages as Affected by Various Curing Procedures. J. W. THOMAS AND L. A. MOORE, *Bureau of Dairy Industry, U.S.D.A.*

P60 Comparison of Early-cut and Late-cut Lespedeza Hay for Milk Production. C. E. WYLIE, J. A. EWING,

ERIC W. SWANSON, AND J. N. MADDUX, *University of Tennessee.*

P61 The Influence of Various Hays on the Production, Vitamin Content, and Flavor of Milk. J. K. LOOSLI, V. N. KRUKOVSKY, AND G. P. LOFGREEN, *Cornell University.*

P62 Comparison of Digestion Coefficients of Sun-cured and Mow-cured Hays from the Same Field. O. M. CAMBURN, *University of Vermont.*

11:00–12:00 **BUSINESS MEETING.**

Wednesday, June 16

Afternoon Session

1:30– 3:00 SECTION A, **FEEDING AND MANAGEMENT.** G. H. WISE, *Chairman.*

P63 Lactating Factors for Dairy Cows in Dried Grapefruit Peel. R. N. DAVIS AND A. R. KEMMERER, *University of Arizona.*

P65 The Growth of Dairy Heifers Reared on Maximum Roughage with Varying Amounts of Grain. O. T. STALLCUP, H. A. HERMAN, AND A. C. RAGSDALE, *University of Missouri.*

P66 Wintering Dairy Heifers on Legume Hay. S. A. HINTON, J. T. MILES, AND C. E. WYLIE, *University of Tennessee.*

P67 Observations on Calves Dehorned with Antimony Trichloride-salicylic Acid-collodion Preparation. G. E. STODDARD, *University of Wisconsin.*

P68 Comparison between Various Methods of Cooling Dairy Cows in Summer. D. M. SEATH AND G. D. MILLER, *Louisiana Agricultural Experiment Station.*

P69 Relationship of Management to the Let-down of Milk. C. E. KNOOP, *Ohio Agricultural Experiment Station.*

P70 The Effect of Time of Milking after Milk Excretion on Total Milk Production. G. M. WARD AND VEARL R. SMITH, *University of Wisconsin.*

1:30– 3:00 SECTION B, **FORAGES, PASTURES.** L. A. MOORE, *Chairman.*

P71 Silage or Winter Pasture for Dairy Cattle. C. E. WYLIE, S. A. HINTON, AND L. R. NEEL, *University of Tennessee.*

P72 Sweet Sudan as a Forage Crop for Dairy Cattle. K. A. KENDALL AND W. B. NEVENS, *University of Illinois.*

P73 Pastures in Relation to Dairy Development in the South. R. H. LUSH, *University of Tennessee.*

P74 Irrigated Pastures for Dairy Cows. JOHN EWING, NELSON MADDUX, C. E. WYLIE, AND R. H. LUSH, *University of Tennessee.*

P75 Increasing the Production of Permanent Pastures through Renovation. J. B. SHEPHERD, R. E. WAGNER, R. E. HODGSON, W. J. SWEETMAN, AND C. G. MELIN, *Bureau of Dairy Industry* and *Bureau of Plant Industry, Soils, and Agricultural Engineering, U.S.D.A.*

P76 Effect of Intermittent and Limited Winter Grazing of Rye Pasture on the Carotene and Vitamin A Content of Cows' Milk. R. G. WASHBURN AND C. F. MONROE, *Ohio Agricultural Experiment Station.*

3:00– 5:00 **GENERAL BUSINESS SESSION.** *Auditorium, Dawson Hall.*

7:00 **BARBECUE,** *Amphitheatre.*

PROGRAM OF EXTENSION SECTION

Monday, June 14

Afternoon Session

1:30– 4:30 **RECORDS AND INTERPRETATION.** E. H. LOVELAND, *Chairman.*

Opening Business Session.

E1 Report of Dairy Records Committee. CHARLES GEARHART, *Pennsylvania State College.*

E2 Seven Years of Central Laboratory Testing. J. E. STOLLARD, *University of Wisconsin.*

Discussion.

Tuesday, June 15

Morning Session

9:00–12:00 **TEACHING METHODS AND EXHIBITS.**—G. HEEBINK, *Chairman,*

E3 Report of Committee on Teaching Methods. I. L. PARKIN, *Pennsylvania State College.*

E4 Interdepartmental Cooperation on Dairy Extension. EVERT WALLENFELDT, GEORGE WERNER, AND CARL NEITZKE, *University of Wisconsin.*

Explanation and Discussion of Exhibits, *Auditorium, Dawson Hall*

Afternoon Session

1:30– 4:00 **JOINT MEETING OF EXTENSION AND PRODUCTION SECTIONS.** E. H. LOVELAND AND G. H. WISE, *Co-Chairmen.*

(See Production Section Program)

Wednesday, June 16

Morning Session

9:00–11:00 **4-H CLUB AND TESTING RULES.** E. H. LOVELAND, *Chairman.*

E5 Systems Used in Obtaining 4-H Club Calves. RALPH PORTERFIELD, *University of Maryland.*

E6 National and Regional 4-H Dairy Contests. M. J. REGAN, *University of Missouri.*

E7 Adoption of Practices as the Result of 4-H Dairy Work. J. C. NAGEOTTE, *Pennsylvania State College.*

E8 Suggested Revision of D.H.I.A. Rules and Regulations. CHARLES GEARHART, *Pennsylvania State College.*

Discussion.

Afternoon Session

3:00– 5:00 **GENERAL BUSINESS SESSION,** *Auditorium, Dawson Hall.*

7:00 **BARBECUE,** *Amphitheatre.*