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A CRITICAL STUDY OF THE UNITED STATES PUBLIC HEALTH SERVICE DEFINITION FOR HOMOGENIZED MILK WITH SOME RECOMMENDATIONS[1]

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Homogenized milk was first defined by the United States Public Health Service in the 1939 edition of the recommended Milk Ordinance and Code.

In the intervening years this definition has been adopted by some health agencies, made the basis of a modified definition by others and has been used by many milk dealers as a standard for efficient processing with which their product could be compared. There has been, however, no commonly accepted procedure for carrying out the determinations involved in the definition.

Several investigators (2, 3, 9, 11, 13) have found that the definition is not easy to comply with and have criticized it for failing to specify a definite method of obtaining the top 100 cc. of a quart bottle of milk for analysis, for not stating the temperature at which the milk should be stored and for failing to specify a method of analysis. They have shown that different methods for removing the upper layer of milk from a quart bottle, after storage, result in varying fat contents but unfortunately different procedures for accomplishing this have been proposed. A further suggestion on this matter was recently made by Levine and Feingold (7) who recommend the mixing of a sample, its transference to a "Cream-top" type of milk bottle for storage and the use of a specially constructed plunger which allows the top 100 cc. of the milk to be poured off without disturbing the remainder. No evidence is presented to show that remixing of the homogenized milk and a second gravitation do not affect the results, or that the peculiar shape of this type of bottle has no influence on gravitation.

A microscopic technique for ascertaining the thoroughness of homogenization of milk has been described by Farrall, Walts and Hanson (5). The

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[2] Fellow under Dairy Industries Supply Association Fellowship which made possible the larger portion of these studies.

Farrall index was found by Doan, Josephson and Adams (3) to correlate quite closely with the index (per cent difference) involved in the U. S. Public Health Service definition.

The study reported in this paper was undertaken for the purpose of investigating further some of the unspecified variables in the U. S. Public Health Service definition for homogenized milk with the object of making recommendations in the interest of uniformity and accuracy. A second object was to evaluate the possibilities of a modified Farrall index as an alternative or additional method of judging the sufficiency of processing of homogenized milk.

Since this study was completed, Snyder and Sommer (8) have proposed a centrifugal method for rating the efficiency of homogenization based on the use of Babcock equipment.

In this paper the U. S. Public Health Service "percentage difference" is referred to as the U.S.P.H.S. index of homogenization.

METHODS

Mixed herd milk containing between 4.0 and 4.2 per cent of fat was clarified cold, heated to 135° F., homogenized, pasteurized at 143° F. for 30 minutes and cooled over a small surface cooler, in all cases unless otherwise specified. Duplicate quart bottles were analyzed for each of the samples taken and the samples from each bottle were analyzed in duplicate by a modification of the Babcock test (3). A limited number of Mojonnier tests were made as a verification of the Babcock results.

Six different methods of removing the top 100 cc. of gravitated bottles of homogenized milk were subjected to study. These methods were as follows:

Decanting. The cap was removed from the bottle and wiped across the lip to remove any creamy material adhering. The top 100 cc. of milk were poured slowly and carefully into a graduated beaker.

Pipetting. The bottle cap was treated as above. The bottle was placed on a low table or stool to permit using a 100 cc. pipette in a vertical position. The material around the cap seat was first drawn into the pipette. Then the tip was brought flush with the surface of the milk and, using continuous slow suction, the milk was drawn into the pipette. This was accomplished in such a way that only the immediate surface was obtained, air bubbles accompanying the milk throughout the entire procedure.

Siphon-opening-up. This siphon is illustrated in figure 1. The stopper is of such size that it fits exactly into the bottle seat, a size 10 being required for a bottle with a No. 2 finish. The siphon was placed carefully into the bottle after removing the cap and after previously adjusting the delivery tube to deliver 95 cc. of milk before air bubbles appeared. The upper layer of milk was blown into a 100 cc. graduated cylinder. Air came over with

the milk when the cylinder registered 95 cc. By blowing more forcibly at this point the level was raised to 100 cc. by milk drawn from the immediate surface in the bottle.

Siphon-opening-down. This siphon is identical with that shown in figure 1 except that the intake of the delivery tube (B) is not bent upward but is straight. The manner of use was identical with the above.

Inverting bottle. The bottle was stoppered with a two-hole rubber stopper, which fitted the bottle seat exactly and through which a glass tube extended to the very bottom of the bottle. To the upper end of the tube was attached a short rubber tube. A glass delivery tube, cut flush with the bottom of the stopper, was inserted into the other hole. The stopper and tubes might be inserted in the bottle either before or after storage of the milk. In use, the bottle was quickly inverted, bringing the delivery tube over a graduated cylinder and by immediately blowing gently into the rubber tube the flow of milk was started. The rate and amount was controlled by pinching the rubber tube.

Drawing off under layer. A hole about 5 mm. in diameter was bored through the side of a quart bottle flush with the inside bottom and closed with a small rubber stopper. The bottle was filled with milk, stored as usual and then the stopper was loosened and 846 cc. of milk drawn off. It was again stoppered and the 100 cc. remaining in the bottle was well mixed by inversion and rotation.

Three different makes of piston homogenizers were employed in the study. Two had the usual plug type homogenizer valves and one was equipped with a valve consisting of a cone of fine compressed stainless steel wire which was held by tension in a suitable body or seat.

Curd tension determinations were carried out in accordance with the procedure approved by the American Dairy Science Association (6).

Farrall indices were determined using the special eyepiece micrometer described by Farrall, Walts and Hanson (5) and now procurable from the Creamery Package Manufacturing Company. This consists of a large square 100 microns on a side subdivided into 16 smaller squares, or fields, 25 microns on a side. Through the middle of the large square is a scale 100 microns long graduated into two micron subdivisions. The eyepiece micrometer is standardized against a stage micrometer using the oil immersion objective and a 10× eyepiece by raising or lowering the draw tube.

The milk sample is adjusted to room temperature and 0.4 cc. are added to 10 cc. of 40 per cent glycerine solution, using accurate pipettes, and thoroughly mixed. A special slide is prepared by placing strips of narrow cellophane tape (Scotch Tape) across the narrow dimension of an ordinary well-cleaned glass slide, about one-half inch apart. The tape should measure about 70 microns in thickness and should be unwrinkled and free from air bubbles when in place. A thread of white vaseline is then drawn down the

inside edge of each strip of tape and across the open ends of the enclosed cell. A square or rectangular cover glass having one dimension the same as the width of the slide is cleaned and held ready. A drop or two of the milk glycerine mixture is placed in the cell area with a glass rod and thoroughly spread around and mixed. The cover glass is then immediately placed over the sample and pressure applied with a pencil or similar object along the edges of the tape. If these operations are properly carried out the sample fills, or nearly fills, the cell and exhibits a homogeneous appearance when viewed toward the light. The depth of the cell corresponds

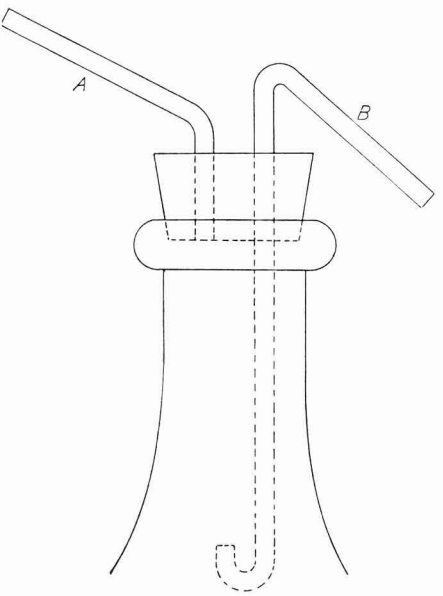

FIG. 1. Siphon-opening-up.

approximately to the thickness of the cellophane and should be uniform. The slide is allowed to stand for 15 minutes or longer to allow the fat globules to come to the top of the cell. It is then ready for observation.

It is well to check the uniformity of the slide by observation under the 16 mm. and 4 mm. objectives, then shift to the oil immersion objective and record the size-class and number of all fat globules over 2 microns in diameter in each of 15 adjacent small fields. The slide is moved and observations are made on another 15 consecutive small fields. The size-classes are as follows: 2.0 to 2.5 μ; 2.5 to 3.0 μ; 3.0 to 4.0 μ; 4.0 to 5.0 μ; 5.0 to 6.0 μ; and 6.0 to 7.0 μ. The Farrall index may be defined as the number of fat globules 2 microns in diameter which could be obtained from all the fat appearing in globules larger than 2 microns in diameter in five small fields

when the observation is carried out according to the above directions. To calculate the index it is necessary to multiply the fat globules in each size-class by a factor which will convert them to an equivalent number of globules of 2 microns in diameter. The factors for the size ranges as given above are: 1.4, 2.6, 5.4, 11.4, 21.0 and 34.0 respectively. Since 15 fields are measured the final tabulation, after conversion, is divided by three or if both observations of the slide (30 small fields) are tabulated together, then the index is obtained by dividing by six. With this modified procedure considerably lower indices were obtained than those reported by the originators of the method or by Doan, Josephson and Adams (3).

EXPERIMENTAL

Method of removing top of bottle. The six different methods of removing the top 100 cc. of milk from quart bottles of gravitated homogenized milk were compared in a series of trials using a piston, plug type valve, homogenizer. Processing was accomplished in different trials at low pressure (300–1000 lbs.) medium pressure (1500–2500 lbs.) and at high pressure (3300–3800 lbs.) and duplicate bottles of milk were analyzed in every case for each of the six methods. Fat analyses by the modified Babcock test were made in duplicate and the average results used in calculating the U. S. Public Health Service homogenization index (per cent difference between the fat test of the upper 100 cc. and the fat test of the remainder). The results of this study are shown in table 1.

The highest index values were obtained in all pressure ranges when separation was accomplished by "decanting" or by using the 100 cc. pipette. Undoubtedly these two methods demonstrate more accurately the true condition in gravitated milk than do any of the others. However, these methods are less easy to standardize, the human factor is a more important variable and even in the hands of a careful individual operator the variations in results between duplicate bottles are greater and less consistent. The most erratic results were obtained where the "inversion of the bottle" method of separation was utilized. With poorly homogenized milk this method was particularly unsatisfactory. The method of drawing off the under layer resulted in higher indices than were obtained with the siphons but variability was greater. Of the two siphons employed, the "siphon-opening-up" gave higher results and results with smaller variabilities. In fact considering all pressure ranges of homogenization the "siphon-opening-up" produced more closely agreeing results than did any of the other methods of separation employed. Its particular advantage is that once the siphon is properly set, it is much less affected by the idiosyncrasies of different operators. This is illustrated by the results shown in table 2 which presents the data obtained by different operators using the "pipette" and the "siphon-opening-up" methods of separation with milk homogenized at

TABLE 1

The effect of method of removing the top 100 cc. of milk on the U. S. Public Health Service index and on duplicability of results

Method	Milk homogenized at low pressure				Milk homogenized at medium pressure				Milk homogenized at high pressure			
	Trial	Indices	Average	Variation	Trial	Indices	Average	Variation	Trial	Indices	Average	Variation
Decantation	1	24.4 27.4	25.90	3.0	5	14.1 13.1	13.60	1.0	9	4.8 4.8	4.80	0
	2	34.4 37.7	36.05	3.3	6	11.1 9.4	10.25	1.7	10	4.2 4.8	4.50	0.6
	3	48.5 49.6	49.05	1.1	7	11.4 12.4	11.90	1.0	11	2.4 4.2	3.30	1.8
	4	50.4 47.2	48.80	3.2	8	20.1 18.6	19.35	1.5	12	6.4 6.4	6.40	0
	Average	.....	39.95	2.65	...	.....	13.77	1.30	.....	.....	4.75	0.60
Pipetting (100 cc.)	1	27.4 28.5	28.05	1.1	5	13.1 11.0	12.05	2.1	9	3.7 4.2	3.95	0.5
	2	37.4 37.0	37.20	0.4	6	10.9 11.5	11.20	0.6	10	4.8 5.4	5.10	0.6
	3	46.4 46.9	46.65	0.5	7	11.9 12.5	12.20	0.6	11	4.2 3.0	3.60	1.2
	4	43.5 47.4	45.45	3.9	8	18.3 19.6	19.45	1.3	12	5.7 5.8	5.75	0.1
	Average	.....	39.34	1.47	...	.....	13.72	1.15	.....	.....	4.60	0.60
Siphon-opening-up	1	16.7 16.0	16.35	0.7	5	8.9 7.7	8.30	1.2	9	2.4 3.0	2.70	0.6
	2	24.6 23.7	24.05	0.9	6	6.9 6.8	6.85	0.1	10	3.1 2.5	2.80	0.6
	3	34.0 33.0	33.50	1.0	7	8.7 9.3	9.00	0.6	11	2.9 3.7	3.30	0.8
	4	33.6 31.8	32.70	1.8	8	13.8 12.5	13.00	1.3	12	2.9 3.6	3.25	0.7
	Average	.....	26.65	1.10	...	.....	9.29	0.80	.....	.....	3.01	0.60

TABLE 1—(*Continued*)

Method	Milk homogenized at low pressure				Milk homogenized at medium pressure				Milk homogenized at high pressure			
	Trial	Indices	Average	Variation	Trial	Indices	Average	Variation	Trial	Indices	Average	Variation
Siphon-opening-down	1	15.3 14.9	15.10	0.4	5	8.5 6.8	7.65	1.7	9	3.1 1.9	2.50	1.2
	2	21.6 21.6	21.60	0	6	6.8 6.4	6.60	0.4	10	1.8 3.1	2.45	1.3
	3	30.8 29.4	30.10	1.4	7	5.6 6.2	5.90	0.6	11	2.4 1.8	2.10	0.6
	4	32.4 30.0	31.20	2.4	8	12.3 10.1	11.20	2.2	12	3.0 2.4	3.30	0.6
	Average	.....	24.50	1.95	...	.....	7.84	1.22	.....	.....	2.59	0.92
Bottle inversion	1	11.6 14.0	12.80	2.4	5	6.8 8.0	7.40	1.2	9	3.1 1.9	2.50	1.2
	2	7.1 −3.9	2.10	11.0	6	6.3 3.5	4.90	2.8	10	3.6 2.5	3.05	1.1
	3	9.8 −7.0	1.40	16.8	7	4.4 1.9	3.15	2.5	11	2.4 2.4	2.40	0
	4	−7.1 −3.3	−5.20	2.8	8	8.9 7.6	8.25	1.3	12	4.1 3.5	3.80	0.6
	Average	.....	2.77	8.25	...	.....	5.92	1.95	.....	.....	2.94	0.72
Drawing off under layer	1	24.2 21.7	22.90	2.5	5	11.7 9.5	10.60	2.2	9	3.7 2.5	3.10	1.2
	2	30.9 28.0	29.45	2.9	6	7.3 7.8	7.55	0.5	10	4.8 3.1	3.95	1.7
	3	46.2 46.2	46.20	0	7	8.2 9.5	8.85	1.3	11	3.1 0.6	1.85	2.5
	4	48.5 49.3	48.90	0.8	8	9.7 11.8	10.75	2.1	12	3.5 4.7	4.10	1.2
	Average	.....	36.86	1.55	...	.....	9.44	1.52	.....	.....	3.25	1.65

three different levels of efficiency. It should be noted that the operators were carefully instructed in the method of using the pipette. Had they not been, the results obtained would have shown considerably greater variability.

TABLE 2

Duplicability of results among different operators using the "pipette" and "siphon-opening-up" methods of separating the upper layer of a milk bottle for determining the U. S. Public Health Service homogenization index

Milk sample	Operator	Pipette				Siphon-opening-up			
		Bottle No.	Index	Individual variation	Individual deviation from mean of all	Bottle No.	Index	Individual variation	Individual deviation from mean of all
1 1500 lbs.	A	1	17.88	1.69	1.01	1	11.95	1.85	0.48
		2	19.57		0.68	2	10.10		1.37
	B	1	19.77	1.22	0.88	1	10.98	0.13	0.49
		2	18.55		0.34	2	11.11		0.37
	C	1	20.39	2.69	1.50	1	12.08	1.10	0.61
		2	17.70		1.19	2	10.98		0.49
	D	1	18.75	0.20	0.14	1	11.82	0.94	0.35
		2	18.55		0.34	2	12.76		1.29
		Mean	18.89	1.45	0.76	.....	11.47	1.00	0.67
2 2500 lbs.	A	1	10.41	0.19	0.85	1	8.24	1.10	0.91
		2	10.22		1.04	2	7.14		0.19
	B	1	12.48	1.01	1.22	1	6.41	0.73	0.92
		2	11.47		0.21	2	7.14		0.19
	C	1	10.50	1.40	0.76	1	7.59	0.36	0.26
		2	11.90		0.64	2	7.23		0.10
	D	1	11.17	0.76	0.09	1	7.74	0.60	0.41
		2	11.93		0.67	2	7.14		0.19
		Mean	11.26	0.84	0.68	.....	7.33	0.69	0.40
3 3500 lbs.	A	1	7.50	0.28	0.07	1	4.88	1.13	0.89
		2	7.78		0.21	2	3.75		0.24
	B	1	8.54	0.76	0.97	1	3.70	0.57	0.29
		2	7.78		0.21	2	4.27		0.28
	C	1	6.71	0.52	0.86	1	3.66	0.61	0.33
		2	7.23		0.34	2	4.27		0.28
	D	1	6.63	1.75	0.94	1	3.66	0.09	0.33
		2	8.38		0.81	2	3.75		0.24
		Mean	7.57	0.82	0.55	.....	3.99	0.60	0.36

Storage temperature. The U. S. Public Health Service definition for homogenized milk does not mention the temperature of storage which is a consideration of importance. U. S. Public Health Service indices were determined in a series of trials where all factors were kept constant except temperature of storage. Duplicate fat tests on duplicate bottles of homogenized milk were obtained at each of the three temperatures used, namely 32° F., 40° F. and 50° F. Separation of the upper 100 cc. of milk was accomplished using the "pipette" and the "siphon-opening-up" methods after 48 hours of storage. The results are presented in table 3.

Storage temperatures of 32° F. produce indices averaging one-fourth lower and temperatures of 50° F. produce indices averaging one-third higher than are obtained at 40° F.

Length of storage or gravitation period. One of the obvious disadvantages of the U. S. Public Health Service index of homogenization is the 48-hour period of storage which delays the obtaining of results. It was considered possible that a 24-hour storage period might be utilized in an effort to partially overcome this disadvantage. Accordingly several trials were made comparing U. S. Public Health Service indices obtained at 24 hours with those at 48 hours. The other conditions of the experiments were identical with the foregoing study except that the storage temperature was 40° F. in all cases. The results obtained are shown in table 4.

TABLE 3

The effect of temperature of storage on the U. S. Public Health Service homogenization index
(Time of storage 48 hours)

Trial	"Pipette" method of separation			"Siphon-opening-up" method of separation		
	Temperature of storage			Temperature of storage		
	32° F.	40° F.	50° F.	32° F.	40° F.	50° F.
	index	*index*	*index*	*index*	*index*	*index*
1	21.4	27.4	32.2	11.5	15.3	19.9
2	5.9	8.4	10.8	3.6	6.0	7.8
3	4.0	4.9	9.0	3.0	4.2	7.3
4	16.4	21.2	26.6	9.8	13.2	16.5
5	3.5	4.5	8.2	2.7	3.7	5.6
6	8.1	9.4	12.4	5.6	7.6	9.0
Average	9.88	12.63	16.53	6.03	8.33	11.01
% variation from 40° F.	−21.8	0	+30.9	−27.6	0	+32.2

It appears that the U. S. Public Health Service index, when measured after 24 hours of storage is approximately 50 per cent of the value found after 48 hours of storage.

Delayed storage. Samples of homogenized milk are frequently picked up on the streets by health department inspectors and conveyed to laboratories for examination. Some dealers send samples to commercial laboratories for analysis. Under such conditions the milk is usually remixed and then stored for a 48-hour gravitation period and it becomes important to know whether such gravitation, remixing and a second gravitation have any influence on the homogenization index.

Comparative trials were made with different samples of milk homogenized at various efficiencies. One set of bottles of each sample was allowed to gravitate for 12 hours at refrigerator temperature after which the bottles

were thoroughly remixed and then stored for 24 and 48 hours. The other set of bottles was stored for 24 and 48 hours in the usual manner. The two previously used methods of removing the upper layers for analysis were employed. Table 5 shows the data obtained in this study.

TABLE 4

The effect of period of storage on the U. S. Public Health Service homogenization index
(Temperature of storage 40° F.)

Trial	"Pipette" method of separation			"Siphon-opening-up" method of separation		
	Storage period		% 24-hr. reading is of 48-hr.	Storage period		% 24-hr. reading is of 48-hr.
	24 hrs.	48 hrs.		24 hrs.	48 hrs.	
	index	*index*		*index*	*index*	
1	15.2	27.4	56.3	8.7	15.3	56.9
2	12.6	23.2	54.3	6.6	14.1	46.8
3	13.6	24.8	54.9	11.7	20.9	56.0
4	6.6	13.0	50.8	4.0	8.2	48.8
5	9.6	15.3	62.7	6.0	9.7	61.8
6	7.5	14.0	53.6	4.4	8.0	55.0
7	8.0	16.1	49.7	5.4	10.0	54.0
8	7.5	13.5	55.3	3.3	8.1	40.7
9	4.3	6.7	63.2	2.7	5.0	54.0
10	3.8	8.4	45.3	2.2	6.0	36.7
11	2.7	5.4	50.0	1.2	3.7	32.5
12	2.7	4.9	55.1	2.2	4.2	52.4
Average	7.84	14.4	54.4	4.87	9.43	51.6

There is a slight tendency for delayed storage to reduce the indices but the differences on the whole are well within experimental error.

TABLE 5

The effect of delayed storage on the U. S. Public Health Service homogenization index
(Temperature of storage 40–45° F.)

Trial	Storage period	Immediate storage	Storage delayed 12 hours, sample remixed	Variation between immediate and delayed storage
	hours	*index*	*index*	
1	24	9.65	7.95	−1.70
	48	15.30	16.10	+0.80
2	24	5.95	5.40	−0.55
	48	9.70	10.00	+0.30
3	24	7.45	7.50	+0.05
	48	14.00	13.50	−0.50
4	24	4.45	3.30	−1.15
	48	8.05	8.00	−0.05
5	24	2.65	2.15	−0.50
	48	5.40	5.75	+0.35
6	24	2.10	2.00	−0.10
	48	4.35	4.15	−0.20
Average	.....	7.42	7.14	−0.28

Temperature of pasteurization. Homogenized milk in some markets is pasteurized by the long hold method at higher than normal temperatures. Such high temperature pasteurization might be expected to interfere with the rise of fat globules in homogenized milk just as it does in unhomogenized milk and might therefore contribute to erroneous conclusions in cases where the U. S. Public Health Service index is utilized as an empirical measure of the efficiency of homogenizer operation. To test this expectation a series of samples was pasteurized at 143° F. and 160° F. for 30 minutes respectively after homogenizing at various pressures with different machines. The U. S. Public Health Service homogenization indices were determined in duplicate and in identical fashion.

The results of this comparison, shown in table 6, indicate that higher

TABLE 6

The effect of higher than normal pasteurizing temperatures on the U. S. Public Health Service homogenization index

(Separation accomplished by using the "pipette")

Sample	Pasteurizing treatment		Difference	Sample	Pasteurizing treatment		Difference
	143° F.—30′	160° F.—30′			143° F.—30′	160° F.—30′	
	index	*index*			*index*	*index*	
1	25.8	21.4	−4.4	9	7.9	6.7	−1.2
2	22.7	18.1	−4.6	10	7.1	6.4	−0.7
3	18.5	13.6	−4.9	11	6.7	5.2	−1.5
4	17.9	16.5	−1.4	12	5.3	4.2	−1.1
5	11.9	9.0	−2.9	13	5.3	5.7	+0.4
6	11.7	10.4	−1.3	14	5.2	5.4	+0.2
7	11.4	10.8	−0.6	15	5.2	4.0	−0.8
8	9.3	8.5	−0.8	16	3.8	4.1	+0.3

temperatures of pasteurization do influence the ability of the fat globules of homogenized milk to rise. However, the effect is most noticeable in the case of ineffectively homogenized milk and practically disappears when milk is very efficiently homogenized. Nevertheless the pasteurizing temperature must be considered as a factor exercising an influence on the U. S. Public Health Service homogenization index. It might be mentioned, in passing that samples 1, 2, 5, 6, 9, 10, 13 and 14, in the table, were homogenized at pressures of from 500 to 2500 pounds using a piston homogenizer equipped with a cone of compressed stainless steel wire as the homogenizing valve. while the others were homogenized at pressures of from 1000 to 4000 pounds utilizing a piston homogenizer with the conventional plug type valve. Pressure for pressure the former type of valve produced a much more efficiently homogenized milk than the latter type throughout these studies.

Homogenization index and curd tension. One of the objects of the homogenization of milk is to reduce curd tension and produce a more di-

gestible product. It has been frequently stated that homogenizing pressures in excess of approximately 2500 pounds (piston machines with plug type valve) have relatively little effect in further reducing the tension (1, 10, 12). The effects of increasing pressures of homogenization on curd tension and on the U. S. Public Health Service index are illustrated in figure 2. These data represent averages obtained with the same lots of milk used for table 6 and include the same two pasteurization treatments. The average curd tension of the raw milk utilized was 41 grams.

Figure 2 shows very definitely that curd tension drops sharply on homogenization, even at low pressure but that increasing pressures cause only a small additional reduction which becomes negligible when pressures be-

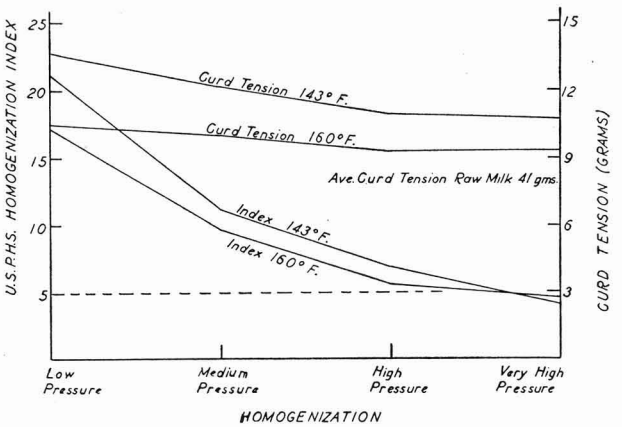

FIG. 2. Effect of increasing pressures of homogenization on curd tension and on the U.S.P.H.S. homogenization index.

tween "medium" and "high" are used (approximately 2500 pounds with plug type valves). The U. S. Public Health Service indices, on the other hand, continue to show decreases up to the "very high" pressure levels (3500–4000 pounds with plug type valves). The curd tension is no longer significantly affected by increasing pressures at a point which fails to produce homogenized milk conforming to the U. S. Public Health Service definition. In fact the indices range from 6.0 to 10.0 at this point, averaging about 9.0 with the "pipette" method of separating and about 7.0 with the "siphon-opening-up" method. It is felt that this constitutes good evidence for the contention that the U. S. Public Health Service definition of homogenized milk is unnecessarily stringent.

The Farrall index of homogenization. A number of samples of milk homogenized at various levels of efficiency, for which U.S.P.H.S. indices (using the siphon-opening-up method) had been obtained, were examined

under the microscope and Farrall indices determined using the modified procedure previously described. A rather good correlation between the two indices was obtained as shown by figure 3. The Farrall values, however, were considerably lower than have previously been reported (3, 5) due probably to the modifications employed.

Milk, sufficiently homogenized to reduce its curd tension to the practical minimum, was found to have a Farrall index of 12.0 or less, equivalent to a U.S.P.H.S. index of approximately 8.0 or under, when determined using the "siphon-opening-up" method of separation.

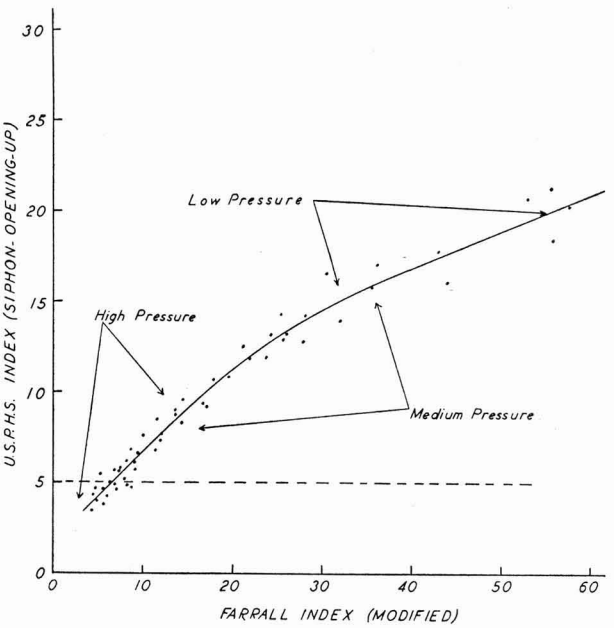

FIG. 3. Relationship between Farrall index of homogenization and the U.S.P.H.S. index.

The Farrall index is no less subject to the idiosyncrasies of the technician than the U.S.P.H.S. index. The procedure is highly conventional and somewhat tedious but the important advantages are that the results are immediately available, they are apparently not affected by the heat treatment used and a much smaller sample is required.

SUMMARY AND CONCLUSIONS

The U. S. Public Health Service definition for homogenized milk is practically meaningless because it does not specify in detail the procedure to be followed in separating the upper 100 cc. from the remainder of the quart

bottle and because it does not state the temperature at which the bottles must be held during the 48-hour gravitation period.

Differences of as much as 100 per cent in the U.S.P.H.S. index are found when different, commonly used, methods of separating the upper layers from gravitated quart bottles of homogenized milk are employed. Variations of about 25 per cent and 30 per cent, respectively, are noted when the storage temperature is 32° F. and 50° F. as compared with 40° F.

The most consistent and satisfactory method of accomplishing the separation of the upper layer of homogenized milk in gravitated bottles and the method which best lends itself to exact description is that termed the "siphon-opening-up" method in this study. A sketch of this siphon appears as figure 1.

A storage period of 24 hours for gravitation in place of 48 hours gives U.S.P.H.S. indices which are roughly one-half the 48-hour values. It appears that a 24-hour storage period might be employed with an appropriate adjustment of the expected index, as a means of reducing the time required to obtain results.

Samples of homogenized milk allowed to gravitate partially may be remixed and stored for 48 hours without appreciably affecting the U.S.P.H.S. index.

High pasteurizing temperatures lower the U.S.P.H.S. indices of milk which has not been well homogenized. The effect on well-homogenized milk however is negligible.

Milk homogenized so effectively that the U.S.P.H.S. index is less than 10.0 in general will not show a further reduction in curd tension by elevating the homogenizing pressure, although the index will become progressively lower as the pressure increases up to 4000 pounds with the usual piston type homogenizers. Thus an insistence on a U.S.P.H.S. index of 5.0 does not result in a product with significantly lower curd tension than could be had at an index of 10.0, but it does require the use of excessive pressures of homogenization which depreciate equipment more rapidly.

If a definition of homogenization based on gravitation is to be used, then it seems desirable to raise the required index to 7.0 or 8.0 and to specify the "siphon-opening-up" procedure for removing the upper layer of milk as well as to specify in detail its manner of use.[3]

The Modified Farrall index of homogenization, as described, correlates quite well with the U.S.P.H.S. index. It has the advantage of giving immediate results and is probably a more accurate measure of the degree of

[3] Since this paper was written, the U. S. Public Health Service has announced a change in the definition of homogenized milk as contained in the "Milk Ordinance and Code." The allowable percentage difference between the fat content of the top 100 cc. of milk and the remainder in a quart container after 48 hours standing has been raised from 5 to 10 per cent. No specifications regarding the temperature of gravitation or the method of removing the upper layer, however, has been incorporated.

homogenization inasmuch as the size of the fat globules is the criterion rather than their ability to rise, which ability is affected by some factors other than size.

A definition of homogenized milk might be stated in terms of the Modified Farrall index in which case a value of 12.0 would appear to be a satisfactory limit. In such an event the gravitation method could be employed as an approximate method by those not equipped for determining the Farrall index.

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FACTORS AFFECTING THE DEVELOPMENT OF ACIDITY IN PASTEURIZED SKIM MILK INOCULATED WITH COMMERCIAL LACTIC STARTERS*

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INTRODUCTION

An active starter is generally considered one of the prerequisites for making a high quality cheese. Slow starters have caused difficulties in commercial plants and several theories as to what may be the cause of the "slowing up" of the activity of starters have been formulated. The inability of certain strains of streptococci to grow at cheese scalding temperature appears to be a cause of slowness in cheese making (7, 8, 17). The presence of a bacteriophage also has been given as a cause for slow starters (1, 9, 19). Multiple strains of streptococci are less susceptible to attack by phage than when only one or two strains are present in the culture (1, 9, 19). Slow starters develop sooner with light inoculations, than when heavy inoculation is practiced (1, 6, 20). The phage is introduced by air contamination (21) and has been found associated with equipment, walls and floor which have come in contact with whey (9). Aeration of the milk is a possible source of slow acid development (6, 17, 20).

Highest quality milk of high total solids is desirable for starters (2, 4, 10). The presence of certain "non-acid" streptococci is given as a cause of slow acid production (3, 16). Mastitis milk will slow up the development of acid by the starter organisms (5, 14). Proper pasteurization of the milk will partially overcome this retarding effect in some samples (12). The exact procedure to which milk is subjected during pasteurization and cooling prior to incubation is of prime importance (6, 15). Milk of late lactation is suggested as one of the causes of slow starters (6). Constant overripening of the starter is suggested as a cause of slow acid development (4). However, Dolby (7) has shown that, given the right treatment, there should be no difficulty in producing cheese of normal acidity using starters of widely varying activity.

Whitehead and Cox (18) state that the organisms selected for active starters must have two properties; "(a) They must be active acid formers at temperatures of 20° C. to 30° C. (b) They must be relatively unaffected in their own growth and morphology at 37° C." Whitehead and Cox (13) describe a method for determining the relative activity of starters. The principle of the test is to simulate the cheese making process and compare

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the relative (not the actual) amounts of acidity developed in milk from the same source by the several starters being compared. Acidity titrations are made at several definitely spaced time intervals, up to the time of salting the curd. By this method, the relative activity of the starters undergoing observation can be determined accurately. Whitehead (16) suggests that the milk used for starter propagation should also be checked frequently by using a starter having vigorous activity.

The object of this study was to determine the effect of several different factors on the development of acid in pasteurized skim milk inoculated with commercial lactic starters.

MATERIALS AND METHODS

Milk. Grade A pasteurized skim milk was used for all the experiments and for the preparation of the starter. The milk ranged in acidity from 0.15 per cent to 0.175 per cent, and had a pH range of 6.55 to 6.70. The quality of this milk was such that when held at 86° F. for 8 hours, it did not show a measurable change in acidity.

Origin of starters. The starters used were originally prepared from commercial starters as follows:

A. A commercial lactic ferment culture in powder form.
B. A commercial lactic ferment culture in liquid form.
C. A commercial lactic butter culture in liquid form.

Daily preparation of starters. The starters were transferred either daily or on alternate days and incubated at 70° to 72° F. Soon after the starters had clotted, they were cooled in a refrigerator at 31° F. The starters were carried in skim milk with frequent transfers for a period of at least one month before the experiments were commenced. The following procedure was used in preparing the starters for an experiment: 80 ml. of pasteurized skim milk were measured into a 125 ml. Erlenmyer flask, covered with butter paper and the whole steamed one hour in an Arnold steam sterilizer. The flasks were then cooled in running cold water and placed in the 31°F. refrigerator until required for inoculation that evening. As required for use, each flask of milk was raised to 70° F., inoculated with 1 per cent of the required starter and incubated at from 70° to 72° F. By varying the time of incubation, fresh starters were obtained at different acidities. The refrigerated starters, tables 1 and 2, were held 24 hours at 31° F. after clotting.

Titratable acidity. The titratable acidity was determined by the method given in the Laboratory Manual (11) using a 9 gram sample. The results were expressed as percentage lactic acid.

pH tests. A Beckman pH meter (Industrial Model M) with glass electrode was used in making the readings. All pH determinations were made at 25° C. and expressed at that temperature.

Purity of starters. At intervals during the experiments the starters were examined for purity by the catalase test, microscopically using the Gram stain, and for coli organisms by the presumptive test using Bacto Brilliant Green Bile 2.0 per cent.

To determine the effect of various factors on the rate of acid development by starter in skim milk the following procedure was used: pasteurized skim milk was obtained the preceding day in a sterile can and stored overnight in a 31° F. refrigerator. Next morning, determinations were made of the pH and percentage acidity on the milk and starter. One thousand grams of the refrigerated skim milk were poured into a sterile two-quart jar and inoculated with 7.5 grams of starter. Uniform mixing was obtained by rotating the jar for one minute. For the determination of the temperature acidity curves, in which case more milk was required, a sterile jug holding 3.5 quarts was used and mixing was done for one minute with a large sterile spoon. In all cases the inoculation of starter was at the rate of 0.75 per cent by weight.

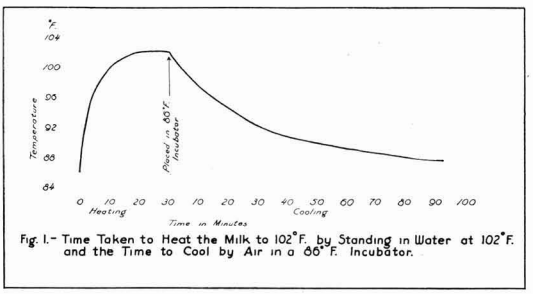

Fig. 1.- Time Taken to Heat the Milk to 102°F. by Standing in Water at 102°F. and the Time to Cool by Air in a 86° F. Incubator.

EXPERIMENTAL

Approximately 200 ml. of the cold mixture was poured into the required number of sterile 250-ml. beakers. The acidity and pH of the inoculated cold milk in the jar was then determined. Checked dairy thermometers were placed in each beaker of milk which was raised to the required temperature by standing in a water bath. The beakers were then covered with a lid and placed in the incubator at the temperature to which the milk had been tempered. At the end of the specified times, the pH and acidity were again determined.

Where cheese making temperatures were simulated, the two beakers of the same inoculated milk were held for two hours at 86° F. One beaker was then placed in the water bath at 102° F. and raised to that temperature so as to be similar to the scalding temperature during cheese making (figure 1). The other beaker was held at 86° F. during the entire experiment. After the allotted period for the scald the beaker of milk was placed back in the 86° F. incubator for the remainder of the test period so that the milk gradually dropped in temperature (figure 1).

The effect of temperature of incubation on the development of acidity. Using the methods as stated, the development of acidity in the skim milk by the three different starters at 8 different temperatures was measured after four, six, and eight hours. Typical cases of the development of acidity plotted against temperature are given in figures two, three and four, for each starter. Figures two, three and four show:

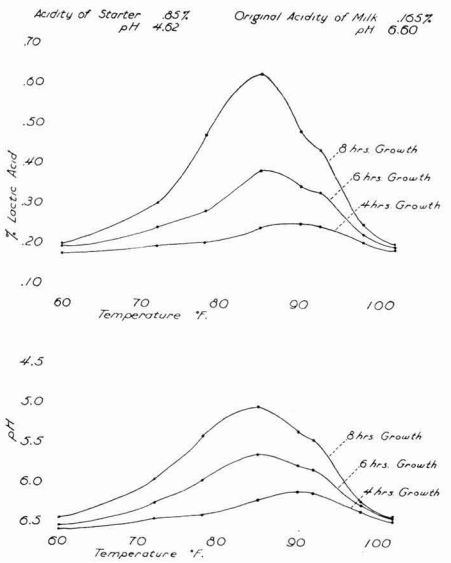

Fig. 2 - Effect of Temperature on the Production of Lactic Acid by a .75 per cent Inoculation of Commercial Starter A into Pasteurized Skim Milk.

1. The maximum acid development in eight hours took place between 85° and 90° F. for all three starters. However, the C starter had the slightly highest optimum.

2. The development of acidity at 77° F. and 95° F. was approximately one half that of the optimum.

3. The development of acidity at 60° F. and 100° F. was insignificant during the eight hours of the test.

4. There was considerable difference in the rate of the development of acidity by the different starters. The difference between starters remained nearly constant during the whole period of the study.

Starter B was the most active in developing acid.

Starter A was next most active in developing acid.

Starter C was the slowest in developing acid.

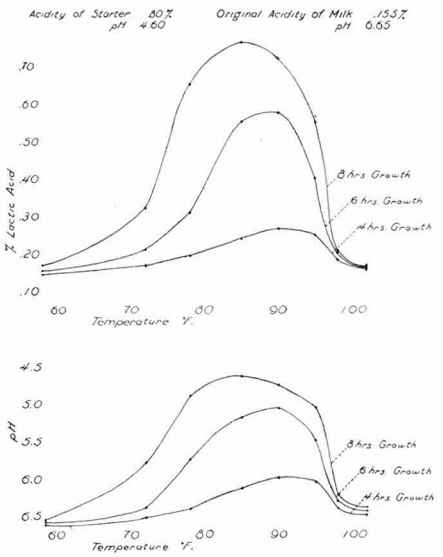

Fig. 3 - Effect of Temperature on the Production of Lactic Acid by a .75 per cent Inoculation of Commercial Starter B into Pasteurized Skim Milk.

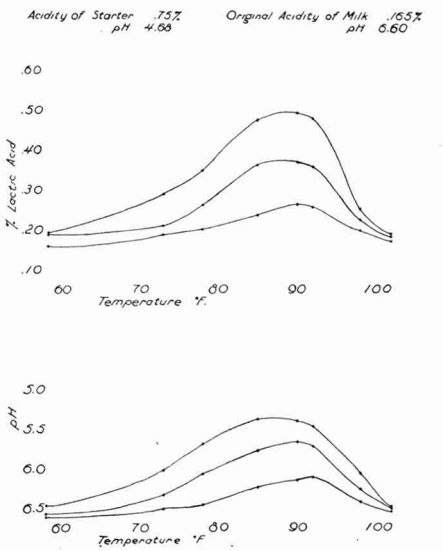

Fig. 4 - Effect of Temperature on the Production of Lactic Acid by a .75 per cent Inoculation of Commercial Starter C into Pasteurized Skim Milk.

The effect of acidity of the starter and refrigeration on the development of acidity at 86° F. The three starters were developed to four different acidities by varying the incubation time. Their acidities, as shown in table 1, cover the usual range found in starters used for cheese making. The development of acidity inoculation with a starter and a subsequent eight hour growth period at 86° F. of these starters in skim milk, as given in table 1, show:

1. The three different starters demonstrated approximately the same respective activity as they did in the experiments shown in figures 2, 3, and 4.

2. It is very doubtful if the difference in the development of acidity and pH of the milk produced by the same starters of varying acidities can be considered significant when grown at approximately optimum temperatures. There is no trend for the high acid starter to be less active.

3. Refrigeration of the starters for 24 hours by the method used had not changed the activity of the starters when grown at approximately optimum temperatures for the development of acid, over that of the same fresh starter.

The effect of acidity of the starter and refrigeration on the development of acidity at cheddar cheese making temperature. The same starters as used in the previous experiment were used to determine the effect of cheddar cheese making temperatures, figure 1, on the rate of development of acidity. The only factor that was different in the data in table 1 from those in table 2 was the temperature at which the milk was held. The development of acidity after an inoculation with 0.75 per cent starter and an 8-hour growth period at cheese making temperatures in shown in table 2.

1. The three different starters showed that under these temperatures, the B starter was most active, whereas the A and C were about the same.

2. The scalding temperature of 102° F. greatly retarded the development of acidity in all three cases.

3. Under cheddar cheese making temperatures refrigeration of the starter caused a slight retarding of acid development with the A starter and a quite definite retarding with the B starter.

The data in this study on refrigeration of starters are not sufficient. The problem of the activity of refrigerated starters for cheese making requires further investigation due to the number of varying factors involved. Such factors are temperature and time of holding the starter, and the use to which the starter is put.

The effect on the development of acidity by differences in the time of holding at the scalding temperature. Since in the previous experiment it was shown that a normal scalding time reduced the rate of development of acidity, it was considered desirable to determine what would be the effect if the scalding time were reduced or increased.

TABLE 1

Effect of the acidity of lactic starters used for inoculation on the subsequent development of acidity during 8 hours growth at 86° F. in pasteurized skim milk, 0.75% inoculation used

Experiment	Starter A				Starter B				Starter C			
	Starter acidity		Milk acidity		Starter acidity		Milk acidity		Starter acidity		Milk acidity	
	%	*pH*	%	*pH*	%	*pH*	%	*pH*	%	*pH*	%	*pH*
a. Fresh starter	0.68	4.70	0.52	5.12	0.60	5.09	0.84	4.61	0.68	4.84	0.46	5.29
	0.75	4.72	0.46	5.25	0.80	4.87	0.84	4.61	0.77	4.70	0.46	5.29
	0.85	4.50	0.52	5.12	0.95	4.54	0.83	4.67	0.82	4.54	0.50	5.11
	0.89	4.42	0.50	5.20	1.14	4.42	0.82	4.67	0.98	4.49	0.50	5.15
b. Fresh starter	0.62	4.84	0.50	5.26	0.70	4.90	0.80	4.54	0.53	4.95	0.49	5.25
	0.62	4.84	0.55	5.30	0.83	4.67	0.80	4.45	0.80	4.50	0.53	5.14
	0.81	4.58	0.51	5.18	0.93	4.50	0.78	4.50	0.69	4.65	0.54	5.17
	0.96	4.43	0.61	4.95	1.00	4.41	0.81	4.53	0.91	4.40	0.53	5.23
c. Refrigerated starter*	0.68	4.75	0.47	5.31	0.57	5.30	0.77	4.60	.....	.....	.....	.....
	0.70	4.71	0.49	5.22	0.70	4.85	0.77	4.62	.....	.....	.....	.....
	0.77	4.50	0.53	5.15	0.85	4.55	0.80	4.61	.....	.....	.....	.....
	0.89	4.42	0.50	5.25	0.94	4.48	0.80	4.65	.....	.....	.....	.....
d. Refrigerated starter*	0.59	4.99	0.54	5.28	0.64	5.05	0.84	4.62	.....	.....	.....	.....
	0.57	5.08	0.49	5.43	0.80	4.70	0.83	4.60	.....	.....	.....	.....
	0.71	4.65	0.50	5.25	0.84	4.53	0.84	4.60	.....	.....	.....	.....
	0.88	4.50	0.53	5.20	0.95	4.37	0.85	4.68	.....	.....	.....	.....

* In Experiment c the same starters as for Experiment a were held 24 hrs. refrigerated.
In Experiment d the same starters as for Experiment b were held 24 hrs. refrigerated.

TABLE 2

Effect of cheese-making temperatures (figure 1) on the development of acidity by lactic starters during 8 hours growth in pasteurized skim milk 0.75% inoculation used

Experiment	Starter A				Starter B				Starter C			
	Starter acidity		Milk acidity		Starter acidity		Milk acidity		Starter acidity		Milk acidity	
	%	*pH*	%	*pH*	%	*pH*	%	*pH*	%	*pH*	%	*pH*
a. Fresh starter	0.68	4.70	0.32	5.70	0.60	5.09	0.46	5.39	0.68	4.84	0.27	5.92
	0.75	4.72	0.31	5.78	0.80	4.87	0.48	5.33	0.77	4.70	0.28	5.95
	0.85	4.50	0.31	5.78	0.95	4.54	0.44	5.50	0.82	4.54	0.27	5.94
	0.89	4.42	0.31	5.77	1.14	4.42	0.43	5.50	0.98	4.49	0.28	5.89
b. Fresh starter	0.62	4.84	0.31	5.81	0.70	4.90	0.49	5.30	0.53	4.95	0.31	5.88
	0.62	4.84	0.29	5.82	0.83	4.67	0.51	5.72	0.80	4.50	0.33	5.83
	0.81	4.58	0.31	5.77	0.93	4.50	0.47	5.30	0.69	4.65	0.33	5.84
	0.96	4.43	0.32	5.68	1.00	4.41	0.38	5.55	0.91	4.40	0.32	5.90
c. Refrigerated starter*	0.68	4.75	0.31	5.87	0.57	5.30	0.39	5.51	.....	.....	.....	.....
	0.70	4.71	0.34	5.80	0.70	4.85	0.31	5.84	.....	.....	.....	.....
	0.77	4.50	0.31	5.90	0.85	4.55	0.39	5.61	.....	.....	.....	.....
	0.89	4.42	0.31	5.90	0.94	4.48	0.32	5.82	.....	.....	.....	.....
d. Refrigerated starter*	0.59	4.99	0.29	5.95	0.64	5.05	0.31	5.88	.....	.....	.....	.....
	0.57	5.08	0.25	6.21	0.80	4.70	0.37	5.63	.....	.....	.....	.....
	0.71	4.65	0.28	5.92	.....	.....	.....	.....	.....	.....	.....	.....
	0.88	4.50	0.28	5.97	.....	.....	.....	.....	.....	.....	.....	.....

* In Experiment c the same starters as for Experiment a were held 24 hrs. refrigerated.
In Experiment d the same starters as for Experiment b were held 24 hrs. refrigerated.

The results given in table 3 show that the scald greatly reduces the rate of acid production as in the previous experiment, table 2. Also, the longer the scald, the less acid will be developed in 12 hours.

TABLE 3

Effect on the development of acidity in pasteurized skim milk of prolonging the time of holding at a scalding temperature of 102° F. 0.75% inoculation used

Starter	Experiment	Starter acidity		Held at 86° F. for 12 hrs. acidity		Held at 86° F. for 12 hrs. with the exception of the scalding period of					
						*1½ hrs. scald acidity		*2½ hrs. scald acidity		*3½ hrs. scald acidity	
		%	*pH*	%	*pH*	%	*pH*	%	*pH*	%	*pH*
A	1	0.87	4.48	0.75	4.80	0.51	5.38	0.38	5.58	0.35	5.72
	2	0.85	4.52	0.73	4.80	0.48	5.35	0.37	5.65	0.33	5.81
B	1	1.00	4.45	0.90	4.41	0.56	5.11	0.44	5.48	0.34	5.80
	2	0.87	4.47	0.79	4.37	0.73	4.56	0.56	5.17	0.44	5.48
C	1	1.01	4.48	0.71	4.81	0.50	5.41	0.36	5.79	0.31	5.98
	2	0.82	4.55	0.71	4.59	0.59	4.92	0.46	5.50	0.35	5.80

* Time held in water bath at 102° F.

DISCUSSION OF RESULTS

From the data presented, it is shown that the rate of acid development by a lactic starter during American cheddar cheese making is likely to be affected by: 1. The origin of the starter. 2. The retarding effect of the scalding temperature or cook. 3. The time the curd is held at scalding temperature in the whey after cooking the curd.

The acidity of fresh starters had little effect on the rate of acid development during American cheddar cheese making. However, the data do not consider repeated high acid development of starters. With the greater importance of the starter when pasteurized milk is made into American cheddar cheese, it would be desirable that commercial starters have standard activity ratings, and a heat tolerance figure, both made under specified conditions. Some cheese makers prefer to use very active starters, others, medium active, and some slow acid producing starters.

SUMMARY AND CONCLUSIONS

1. Data are presented to show:
   a. The effect of the temperature of incubation on the development of acidity in skim milk by commercial starters.
   b. The effect of the acidity of the starter on the development of acidity during definite periods at 86° F. and also at temperatures and times similar to that of American cheddar cheese making, when using pasteurized milk.

c. The effect of the time the starter organisms are held at scalding temperature (102° F.) in skim milk on their subsequent acid development in skim milk.

2. The optimum development of acidity for the three starters used took place at about 86° F. The development of acidity at 60° F. and 100° F. was insignificant during the eight hours of the test.

3. The acidity of fresh starters in the range usually found in the cheese factory had little effect on the rate of acid development in skim milk.

4. A cooking temperature of 102° F. greatly retarded the development of acidity and the longer the starter organisms were held at scalding temperature, the slower was their subsequent development of acidity when returned to 86° F.

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A COMPARISON OF ALFALFA–BROME GRASS SILAGE AND CORN SILAGE FOR DAIRY COWS*

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Dairy cows require rations of high nutritive value for efficient production. A major portion of the cow's ration is either pasture grasses or dry roughages. The pasture grasses are usually of high nutritive value, being especially rich in vitamins. The dry roughages are usually of lower nutritive value because of the loss of nutrients during harvesting due to unfavorable climatic conditions.

Reed and Fitch (20) were the first investigators in this country to study the preservation of alfalfa for silage and found that molasses definitely aided in preservation of this silage as well as increasing its palatability. Since the report of these workers there has been considerable research concerning the nutritive value of these silages.

Many workers have reported that grass or legume silages and corn silage were about equal in feeding value for milk production. Bender and co-workers (4) found that molasses grass silage will replace corn silage or hay without any marked change in milk production, and when molasses silage replaced both corn silage and hay, production was maintained, but there was a slight loss in body weight. Atkeson and Anderson (1) found that sweet clover silage and corn silage were practically equal for milk production.

Hegsted *et al.* (8) reported that when the protein intake was equalized, rations containing corn silage, A.I.V. silage, or molasses alfalfa silage were equally satisfactory for milk production. Similar reports have been made by other workers (4, 16). Hegsted *et al.* (8) reported better preservation of both protein and carotene in the A.I.V. silage than in molasses silage. However Bechdel and associates (3) have recently reported that, after seven months of storage, molasses silage contained three to five times as much carotene as silages preserved with phosphoric acid. Johnson and co-workers (12) also reported that the vitamin A potency of butter was lower when cows were fed silage preserved with low amounts of phosphoric acid than when preserved by molasses or the A.I.V. method.

Most investigators agree that the carotene content of legume and grass crops can be better preserved by ensiling than by drying and that legume and grass silages usually contain considerable more carotene than corn silage (7, 9, 11, 14, 15, 19, 21, 23). The work on preservation and nutritive value

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of grass silage has been thoroughly reviewed by Bender (5) and Huffman (10).

Although considerable work has been done to determine the relative feeding values of many grass silages there are no available data on alfalfa-brome grass silage. A mixture of alfalfa and brome grass is becoming important as a rotation pasture crop for dairy farms. If this mixture can profitably be made into a palatable silage of good feeding quality, its value will be even greater. Therefore the purpose of this experiment was to compare the value of alfalfa-brome grass silage with corn silage for milk production, body maintenance, and as a source of carotene when used as a succulent roughage for dairy cows. Two feeding trials comparing these silages have been completed.

EXPERIMENTAL

Part I. Feeding trials. The alfalfa-brome grass used in these trials was produced by seeding nine pounds of alfalfa and nine pounds of brome grass to the acre. The silage was made from the first cutting the second and third years after seeding. Blackstrap molasses was used as a preservative. Approximately 80 pounds were added per ton of green material as it went into the silo. The corn silage was made from hybrid corn cut at the usual stage of harvesting corn for silage. The alfalfa-brome grass silage was stored in an 8×24-foot silo and the corn silage in a 12×35-foot silo. Both silages were considered to be of good quality. The cows used in each of the two feeding trials were divided into two equal groups with respect to breed, stage of lactation, milk production, and body weight. Two groups of five cows each were used in the first trial and two groups of six cows each were used in the second trial.

Group I received corn silage and Group II alfalfa-brome grass silage. The silages were fed at the rate of approximately three pounds of silage for each 100 pounds of body weight. In addition the cows received a limited amount of alfalfa hay and a balanced grain mixture consisting of 400 pounds of ground yellow corn, 100 pounds of ground oats, 100 pounds of wheat bran, 50 pounds of linseed oil meal, 50 pounds of cotton seed meal, ten pounds of salt, seven pounds of ground limestone, and seven pounds of steamed bone meal. The grain was fed according to production. The length of the feeding trials was ten weeks following a ten-day preliminary period during which feeding schedules were adjusted and the cows became accustomed to the silages. The average composition of the feeds used in these feeding trials is given in table 1.

The results of the two trials are summarized in table 2. In the first trial the milk production of the cows on alfalfa-brome grass silage was slightly higher than the cows fed corn silage. This was reversed in the second trial, the corn silage group producing a little more milk than the group on the alfalfa-brome grass silage. However, the average daily milk production of

TABLE 1

The average composition of feeds used in the feeding trials

Components	Alfalfa-brome grass silage	Corn silage	Alfalfa hay	Grain mixture
Moisture	66.4	68.4	13.2	11.6
Crude protein	4.9	2.3	13.8	13.2
Ether extract	2.3	1.4	3.5	4.7
Crude fiber	10.4	7.2	30.6	5.7
Nitrogen free extract	16.0	20.7	38.9	64.8

the two groups in each trial were closely parallel and the differences may be considered insignificant. Cows fed the corn silage maintained slightly better body condition than did the cows on alfalfa-brome grass silage.

Part II. Vitamin A and carotene studies. Since silage is one of the most important sources of vitamin A in winter rations of dairy cows, studies were made during the second trial to determine the effects of the amount of

TABLE 2

A summary of feeding trials (Length of trials—10 weeks)

	Trial I		Trial II		Average of two trials	
	Corn silage	Alfalfa brome silage	Corn silage	Alfalfa brome silage	Corn silage	Alfalfa brome silage
Number of cows	5	5	6	6	11	11
Total pounds of milk	9980.6	10855.2	10385.8	10131.3	20366.4	20986.5
Av. daily milk	28.5	31.0	24.7	24.1	26.4	27.2
Total pounds of fat	413.5	413.3	445.6	458.6	859.1	871.9
Av. daily fat	1.18	1.18	1.06	1.09	1.12	1.13
Total pounds of 4% F. C. M.	10217.6	10570.8	10828.1	10933.5	21045.7	21504.3
Av. daily 4% F. C. M.	29.2	30.2	25.8	26.0	27.3	27.9
Total pounds of grain consumed	3950.0	3965.0	3510.7	3668.0	7460.7	7633.0
Av. daily grain consumed	11.3	11.3	8.4	8.7	9.7	9.9
Total pounds of hay consumed	3323.8	3284.0	4176.0	4086.7	7499.8	7370.7
Av. daily hay consumed	9.5	9.4	9.9	9.7	9.7	9.6
Total pounds of silage consumed	10102.0	9584.0	12797.0	12405.0	22899.0	21989.0
Av. daily silage consumed	28.9	27.0	30.5	29.5	29.7	28.6
Av. initial body weight (pounds)	1100	1088	1037	1007	1068	1047
Av. final body weight (pounds)	1111	1083	1098	1042	1104	1062
Av. gain in body weight (pounds)	+11	−5	+61	+35	+36	+15

carotene in the two silages upon the carotene content of the blood plasma and carotene and vitamin A content of the milk fat secreted by the cows. The silages, blood plasmas, and butterfats were analyzed for carotene. The vitamin A potencies of the butterfats were determined by bioassay.

Silage carotene. The method used for the determination of carotene in these silages was essentially that of Beadle and Zscheile (2). The carotene was extracted from the samples with diacetone in a Waring Blendor after which it was transferred from diacetone to heptane by successive extractions in a separatory funnel. Carotenols and chlorophyll were removed by three extractions with diacetone solution (six per cent water). After saponification with 25 ml. of 25 per cent potassium hydroxide in methyl alcohol the heptane solution was washed with water until alkali free. The carotene solution was then dried with anhydrous sodium sulfate and made to volume. The carotene values were determined by absorption at a wave length of 455 millimicron using a Bausch and Lomb Visual Spectrophotometer. The values obtained by this method were checked and agreed closely with results obtained by using Moore's method (18). The results of these carotene determinations are shown in figure 1.

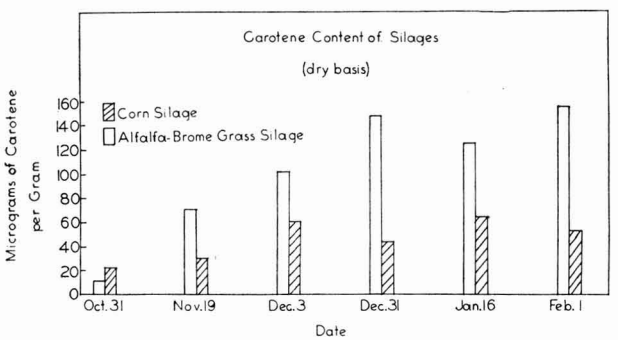

FIG. 1. Showing the carotene content of corn silage and alfalfa-brome grass silage at successive intervals during the feeding experiment.

Analyses of the alfalfa-brome grass silage showed, in general, an increase in carotene on successive dates as samples were taken from successive depths in the silo. The increase of carotene in the corn silage, up to December third, was definite but not as great as that of the alfalfa-brome grass silage. The low carotene values of the silages near the top of the silo indicated that the deterioration was greatest in the upper part and that preservation was much better in the lower part.

Blood plasma carotene. Blood plasma carotenes were determined biweekly from five cows from each group using Moore's method (17). The average blood plasma carotene values for each group are given in figure 2.

The initial blood plasma carotenes were fairly high because the cows had been on fall pasture prior to the experiment. The blood plasma carotenes of both groups declined until about December eighth, after which the corn silage group continued to decline, while the alfalfa-brome grass silage group began a steady rise. At the termination of the study the blood plasma values of the alfalfa-brome grass cows were more than twice the values of the corn silage cows.

Butterfat carotene. The milk from three cows of each group was collected separately for 24-hour periods bi-weekly, separated and churned. The butter was rendered at 55° C. and filtered to free it from curd and water. A weighed amount of butterfat was dissolved in heptane, made to volume, and the carotene concentration determined by reading at 455 millimicrons. It is recognized that carotene values obtained by this procedure may be somewhat high due to the presence of some xanthophyll and acid formed pigments (13). Blood plasma carotenes by Moore's method (17) are probably also correspondingly high. The relationship between average butterfat carotene and the blood plasma carotene of the three cows of each group is presented in figure 2. The carotene values of the butterfat closely parallel the carotene values of the blood plasma.

Vitamin A potency of butterfat. For the determination of the vitamin A potency of the butterfat, samples were collected at three periods; at the beginning, near the middle, and at the end of the experiment. Equal amounts of butterfat from two cows of each group were made into composite samples. These samples were bioassayed for vitamin A by the usual rat-growth methods, using United States Pharmacopoeia Reference Oil diluted with Wesson oil as a standard.

As shown in figure 2 the vitamin A potencies of the butterfats were closely related to the carotene content of the butter and blood plasma. This graph also shows that under the conditions of this experiment alfalfa-brome grass silage was superior to corn silage as a source of carotene. This difference became more apparent toward the latter part of the experiment.

DISCUSSION

The results of these feeding trials indicate that the silage made from this alfalfa-brome grass mixture was equal to the corn silage in feeding value for milk production.

At the beginning of the feeding period, the alfalfa-brome silage did not appear to be as palatable as the corn silage, but after the cows became accustomed to the ration, there seemed to be little difference in palatability between the two silages.

Although there was little difference between these two silages for milk and butterfat production, the alfalfa-brome silage had a distinct advantage as a source of carotene. This became increasingly apparent in the better

preserved silages in the lower part of the silos. This difference in carotene content was reflected in increased carotene contents of the blood plasmas and butterfats and also in higher vitamin A values of the butterfats.

It may seem peculiar that the differences in carotene contents of the two silages were not reflected in the carotene contents of blood plasmas and butterfats during the early part of the experiment. Apparently, the carotene

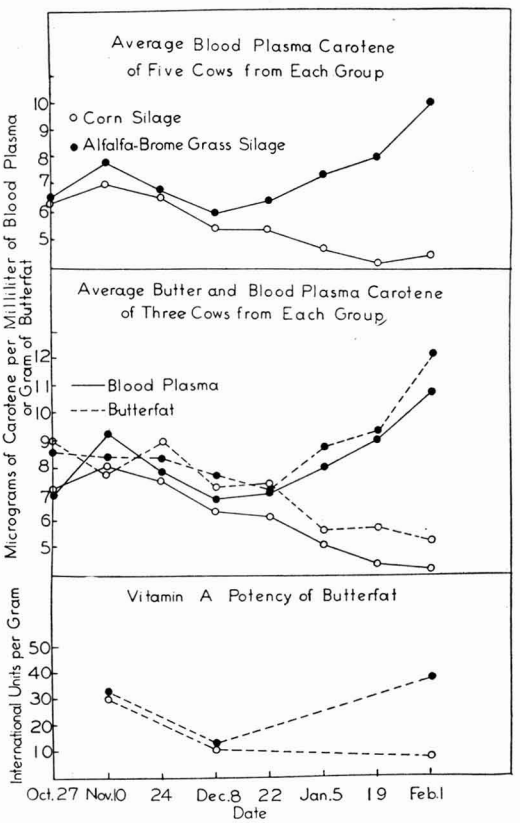

FIG. 2. Showing the effect of the silages upon the carotene contents of the blood plasmas, and the carotene and vitamin A potencies of the butterfats.

intakes of the cows were too low to have a marked effect on the butterfat. This may be explained on the basis of the work of Wilbur, Hilton and Hauge (22). They found that when cows were fed increasing amounts of carotene there was no increase in vitamin A potency of the butterfat until the cows received at least 300,000 Sherman units of vitamin A daily in the form of carotene. Although 550,000 units daily is required for complete saturation,

saturation appears to begin at an intake of approximately 300,000 units. An intake below this level allows desaturation. Calculations of the approximate carotene intakes show that the cows on alfalfa-brome grass silage had an intake of 300,000 units or less during November and the first part of December. In December the carotene intake increased to amounts well above this value, and was reflected in increased carotene and vitamin A content of the butterfats. The carotene intakes of the cows on corn silage were below this level throughout the trial and resulted in low carotene and vitamin A values of the butterfat. It is recognized however that corn silage as well as grass silage varies in carotene content, depending upon stage of maturity, methods of harvesting, weather conditions prevailing during ensiling and possibly differences in varieties of the plants. From these and other studies, it may be concluded that alfalfa-brome silage or other grass silages may be important sources of carotene to insure a satisfactory intake of carotene for the production of milk of high vitamin A potency during the winter feeding periods.

SUMMARY

1. Alfalfa-brome grass silage and corn silage were found to be very similar in feeding value for milk production.

2. The alfalfa-brome grass silage was higher in carotene than the corn silage. This higher carotene content was reflected in higher carotene values of the blood plasma and butterfat, and in higher total vitamin A values of the milk fat secreted by the cows.

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BLOAT IN CATTLE AND COMPOSITION OF RUMEN GASES

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Problem. Ruminants fed exclusively on succulent soft feed often develop a dangerous inflation of the rumen, resulting from accumulation of gas, a condition known as bloat. According to Cole, Mead, and Kleiber (1), who experimented on cows with rumen fistulae and made observations in the field, "Bloat is caused not by excessive gas formation but by interference with belching. It results most frequently from absence of the stimuli necessary for belching." An important condition for normal belching seems to be the presence of prickly material in the rumen. Dougherty (2) noticed that hydrogen sulfide or carbon monoxide injected into the rumen "caused a distinct paralysis of the organ when sufficient concentrations were reached." He found comparatively large amounts of hydrogen sulfide in gas and ingesta taken from a heifer that had died of bloat. Olson (3) reported later, "The amount of hydrogen sulfide in bloated animals may be ten to twenty times that of animals on dry feed" and "Alfalfa under certain conditions is very high in hydrogen sulfide." According to him, these facts suggest that "this gas plays a significant role in the cause and death from bloat in the bovine."

The experiments reported in this paper were carried out in order to investigate this possible relation between bloat and the chemical composition of rumen gases, especially the hydrogen sulfide content.

EXPERIMENTAL

Method. The storing of moist gas samples for determining small concentrations of hydrogen sulfide is complicated by the solubility of this gas in water or aqueous solutions and by its reaction with mercury. To avoid these difficulties, we measured the amount of gas (as taken directly from the rumen) that contains a given amount of hydrogen sulfide instead of using the ordinary method of measuring the amount of hydrogen sulfide in a given amount of gas. We thus measured the amount of gas which we had to pump from the rumen through 200 cc. of 0.001 N iodine solution for the reduction of the iodine, as indicated by the disappearance of the blue color that had been produced in the iodine solution by added starch. With relatively high concentrations of sulfide and iodine, errors occur because part of the sulfide is oxidized to sulfate instead of sulfur. Since, however, the hydroxide sulfide concentration in our absorbing liquid is very low and since also the iodine solution used was only 0.001 normal, this direct iodometric titration of hydrogen sulfied should produce correct results (4).

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The 0.1 N stock solution of iodine, prepared according to Treadwell (5), contained 12.7 g. iodine and 24.0 g. potassium iodide per liter. It was kept in the dark in a brown bottle. Just before the measurements in the field, 2 ml. of the stock solution was diluted to 200 ml. and 2 ml. of a solution containing 5 g. of starch per liter was added. The iodine solution was titrated with 0.1 N sodium thiosulfate solution.

Figure 1 shows the apparatus for pumping rumen gas through the iodine solution. Trocar *T*, with a side outlet, is inserted into the rumen of the cow.[1] Stopcock S_1 is turned first so that the rumen gas bypasses the

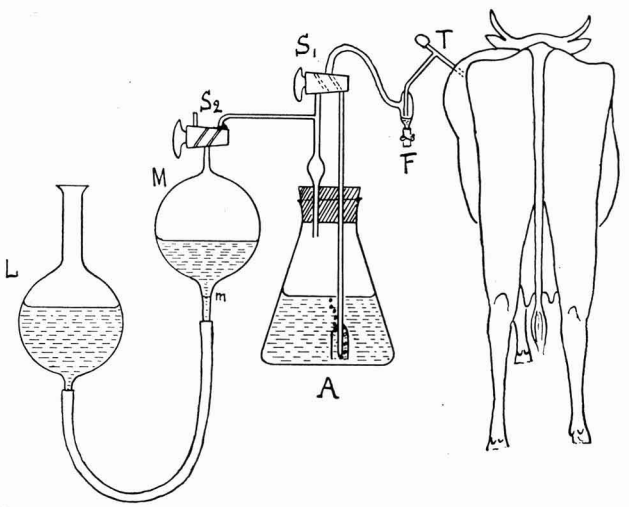

FIG. 1. Measuring H_2S in rumen gas.

A = Absorber containing 200 cc. $\frac{n}{1000}$ I solution with starch.
F = Trap for liquid.
M = Measuring bulb of gas pump 150 cc. capacity.
S = Three-way stopcock.
L = Leveling bulb of gas pump.
m = Calibration mark on measuring bulb.
T = Trocar.

absorber, entering directly the 150-ml. measuring bulb *M* while water flows from this bulb to the leveling bulb *L*. When the gas reaches the mark *m* of the measuring bulb, stopcock S_2 is turned so that raising the leveling bulb expels the gas to outside. After 3 bulbs full of gas have thus been driven out, stopcock S_1 is turned so that the rumen gas, aspired by lowering the

[1] We obtained a sample of rumen gas from a bloated cow by a stomach tube inserted through the nose and then connected to our apparatus. For this operation we are indebted to Dr. John Britton, Division of Veterinary Science. The hydrogen sulfide content in this gas sample was 0.04 per cent. This value was not used for the calculation of our means.

leveling bulb *L*, bubbles through the iodine solution in absorber *A*, passing a double-action bubbler as shown in figure 1. The apparatus, mounted on an ordinary laboratory stand with elongated rod, was easily carried to the pasture where the rumen puncture was performed.

The number of bulbs full of rumen gas necessary to bleach the iodine solution, multiplied by the volume of bulb *M* and by the temperature-pressure factor, indicates the amount of rumen gas that reduces the 0.2 milliequivalent of iodine in the absorbing liquid. We assume that the reducing agent is hydrogen sulfide alone, and thus the reduction of 0.2 milliequivalent of iodine indicates 0.1 millimol or 2.2 ml. hydrogen sulfide.

The following example illustrates the procedure of calculation:

Cow 624. Time: October 16, 1942; 2:30 P.M. Condition: Not bloated. Test No. 3.

Number of strokes of pump for bleaching iodine solution: 13.

Volume of measuring bulb, 144 ml.; reduced to standard condition, 130 ml.

Volume of rumen gas bubbled through absorber: $0.130 \times 13 = 1.7$ liters.

According to calibration with $Na_2S_2O_3$, the iodine solution contained $0.85 \times 0.2 = 0.17$ equivalents of I.

Reduction of this amount by H_2S indicates $0.85 \times 2.2 = 1.9$ ml. H_2S.

Since 1.7 liters rumen gas contain 1.9 ml. H_2S, 0.1 liter = 100 ml. rumen gas contains $0.1 \frac{1.9}{1.7} = 0.11$ ml. H_2S.

At the end of 3 such measurements of the hydrogen sulfide content, a sample of the sulfide-free gas was kept in bulb *M* and later analyzed for carbon dioxide, methane and oxygen in a standard Burrell gas-analysis apparatus.

In two such analyses rather low methane and correspondingly high nitrogen values were obtained. We suspect that in these two cases the platinum wire for combustion of methane was kept at too low a temperature and that this condition resulted in incomplete combustion. This explanation is strengthened by an abnormal combustion quotient: $\frac{CO_2 \text{ formed}}{O_2 \text{ used up}}$ of 0.13. For methane combustion this quotient should be 0.50. The average quotient of our normal analyses was actually 0.515 ± 0.017 ml. carbon dioxide per ml. oxygen.

The cows from which the gas samples were taken were pastured daily on alfalfa together with a group of 5 to 17 other cows. All were in lactation. They received concentrates at night. On 5 nights concentrates were the only supplement to the alfalfa pasture. Under those conditions at least 1 in 16 cows bloated on the alfalfa pasture next morning. On two days following the nights without roughage, gas samples were taken from bloated cows; on 3 days from cows that were not bloated. One night the cows were given fine alfalfa hay. The next day 1 of 15 cows bloated on the alfalfa pasture. That day's gas sample was, however, taken from a cow that was not bloated.

On two nights the cows had access to Sudan hay in addition to their normal ration of concentrates. There was no bloat on the alfalfa pasture on either of the following days, on each of which a sample of rumen gas was taken.

Results. Table 1 shows the results from these trials. The data are arranged approximately according to the intensity of the bloat, as observed when the gas samples were taken and as measured by the ratio of bloated cows to total cows on the pasture. The concentration of hydrogen sulfide in the rumen gas ranges from 0.08 to 0.16 per cent. It shows no correlation to the intensity of bloat. The mean of the 8 trials is 0.11 ± 0.01 per cent hydrogen sulfide.

This concentration is considerably above those observed by Dougherty (2), who reports "as much as 0.03 per cent hydrogen sulfide in rumen gas." Dougherty found an exceptionally high value of 0.15 per cent in the rumen gas taken from a heifer that had died of bloat. This concentration is within the range of our results obtained on nonbloated cows.

TABLE 1

Composition of rumen gas from cows on alfalfa pasture

Date 1942 Oct.	Roughage fed night before gas sample was taken	Condition of cows			Composition of rumen gas volume per cent				
		Total number of cows on pasture	Number of cows bloated	Cows from which gas sample was taken	H_2S	CO_2	CH_4	O_2	N_2
					%	%	%	%	%
27	No	6	3	Bloated	0.08	67.8	28.8	0.2	3.1
26	"	6	2	"	0.11	.....	.....	.....	.....
15	"	16	6	Not bloated	0.15	66.9	22.1	0.4	10.4
23	"	13	2	" "	0.09	.....	.....	.....	.....
10	"	16	1	" "	0.12	.....	.....	.....	.....
14	Fine alfalfa hay	15	1	" "	0.16	69.0	.....	0.5	.....
8	Sudan hay	18	0	" "	0.08	.....	.....	.....	.....
16	" "	16	0	" "	0.11	65.7	26.8	1.0	6.4

All our cows were on alfalfa pasture. Possibly cows on other pasture or on dry feed have a smaller percentage of hydrogen sulfide in their rumen gas. Our results demonstrate, however, that the relatively high hydrogen sulfide concentration in rumen gas of cows on alfalfa pasture does not necessarily lead to bloat. Most likely, therefore, hydrogen sulfide concentration plays no very significant role as a condition producing bloat. Conceivably, on the other hand, this poisonous gas is involved in the fatal consequences of bloat. Any poison that destroys blood and tissue pigments involved in respiration would be particularly dangerous in a condition such as bloat, which interferes mechanically with normal respiration. Even the accumulation of carbon dioxide in the rumen is much more disastrous than the accumulation of an equal pressure of methane or oxygen (1).

Only three samples of rumen gas were completely analyzed. The concentrations of carbon dioxide and methane are within the range of results obtained earlier (1). The concentration of oxygen in the rumen gas was very low, a fact that also confirms previous observations.

SUMMARY

The hydrogen sulfide content of the rumen gas of bloated and nonbloated cows on alfalfa pasture was measured in 8 trials by an iodometric method especially adapted for this purpose.

The rumen gas contained, on the average of 26 titrations, 0.11 ± 0.01 per cent hydrogen sulfide by volume. There was no relation between hydrogen sulfide concentration and bloat.

The rumen gas contained on the average 67 per cent carbon dioxide, 26 per cent methane, and less than 1 per cent oxygen. The concentration of these gases was not related to bloat.

This result supports the theory that bloat is caused, not by abnormal gas formation, but by a lack of belching.

ACKNOWLEDGMENT

We are grateful to Mr. Th. Chernikoff for his valuable help in taking the gas samples and in their analysis.

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PHOSPHOLIPIDS IN DAIRY PRODUCTS. II. DETERMINATION OF PHOSPHOLIPIDS AND LECITHIN IN LIPIDS EXTRACTED FROM DAIRY PRODUCTS[1]

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INTRODUCTION

In a previous paper (2) the application of the Roman method to the analysis of extracted milk fat for choline was discussed. In this article the continuation of that investigation with regard to the analysis of milk lipids for phospholipids and choline is described.

REVIEW OF LITERATURE

There are a number of reviews of the literature pertaining to phospholipids in milk and its products in bulletin form and in other articles (7, 8, 10, 14, 15).

The extraction and isolation of these materials has been reported by a number of workers (3, 11, 12, 16, 18, 20, 23). Studies of the phosphorus-containing lipids have also been made by Gould and co-workers (5), Heinemann (6) and Perlman (17). Lobstein and Flatter (13), using several procedures of extraction showed that the amount of these substances in milk was about 0.03 per cent. They pointed out that the phospholipids formed a colloidal complex with the milk proteins which was insoluble in ether but capable of dissociation in alcohol and acetone. Holm, Wright and Deysher (8) concluded that phospholipid material was not completely extracted from milk unless hot alcohol was first used. They recommended extraction with hot alcohol then ether, followed by drying of the water-alcohol-ether extract. This residue was combined with the material precipitated from the milk by the alcohol, and the whole dried with anhydrous sodium sulfate. The dried mass was then extracted with chloroform to obtain the lipids. Holwerda (9) reported that there is a phosphoric ester present in milk which makes results based on phosphorus too high. Toyeau (24), and Toyeau and Marchebouef (25) stated that phospholipids were not complex with boiling alcohol. The use of soaps, such as sodium oleate, freed part of the lipid from the protein-phospholipid complex. The Mojonnier modification of the Roese-Gottlieb method has also been used to obtain the fat for analysis (5, 6, 10, 17), and this method was used in this investigation.

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Kurtz, Jamieson and Holm (12) found that the lecithin-cephalin preparation, which they obtained from dried buttermilk powder, contained 56 per cent lecithin. The ratio of lecithin to cephalin to sphingomyelin as reported by Kurtz (21) was 8.4 to 4.5 to 1.0. Reward (18), in the fractionation of lecithin from cephalin by solution in absolute ethanol, obtained 58 per cent lecithin in the material isolated from dried whole milk.

EXPERIMENTAL

Whole milk samples were first analyzed. The fat was extracted by the Mojonnier modification of the Roese-Gottlieb method using 25 ml. of each ether in the last extraction and shaking the flasks for at least one minute before centrifuging. The solvent was evaporated from the fat on the hot plate until from 96 to 99 per cent of the extract was fat, in some cases a vacuum oven was used. The extract was collected from a number of flasks and analyzed for phosphorus and choline. In the data given here, choline is calculated to lecithin, using a factor of 806/121. A blank of 0.15 ml. of 0.01 normal sodium thiosulfate was taken in calculating the choline in each instance.

A composite sample of milk, containing 5.35 per cent fat, was analyzed, using varying conditions of extraction. The results are shown in table 1.

TABLE 1

Analysis of a composite sample of whole milk

Sample	Fat hydrolyzed	Aliquot	Thiosulfate (0.0102 N)	Choline	Choline per gram of fat	Lecithin in the fat
	gm.		*ml.*	*mg.*	*mg.*	%
1	15.10	1/5	7.32	5.29	0.350	0.23
2	7.52	1/5	3.60	2.60	0.345	0.23
3	12.17	1/5	5.18	3.74	0.307	0.21

Sample 1 was extracted in the usual manner, as was Sample 2, but in the latter case choline was added to the milk before it was extracted in the form of a solution of choline chloride. In the case of Sample 3, the milk was treated with ammonia and alcohol, and then refluxed on the steam bath before extraction with ether. The amount of total phospholipids in the fat was found to be 0.55 per cent. Hydrolyzates were made up to 25 ml. The average agreement between choline titrations was 0.05 ml. of thiosulfate. Each milliliter of the 0.0102 normal thiosulfate was taken as equivalent to 0.1443 milligrams of choline. The percentage of fat in the extracts were 96, 96, and 98.5; weights of fat used for hydrolysis were corrected to 100.

Twenty samples of milk from cows of different breeds were analyzed for total phospholipids and choline. The extracted material contained from 96 to 99 per cent of fat. The amount of sample used for hydrolysis was from 3 to 11 grams, the average being about 6 grams. Hydrolyzates were

made up to 25 ml. Five of the samples were hydrolyzed in duplicate, in the other cases one hydrolysis for each sample was made. The results are shown in table 2.

TABLE 2

Average values for phospholipids in extracted milk fat

Breed	Number of samples	Average fat in milk	Total phospholipids in fat			Choline per gram of fat		Average lecithin in the fat	Average lecithin in total phospholipids
			Low	High	Ave.	Low	High		
		%	%	%	%	*mg.*	*mg.*	%	%
Ayrshire	5	4.30	0.53	0.84	0.71	0.41	0.55	0.31	44
Guernsey	5	5.18	0.47	0.76	0.67	0.33	0.51	0.29	43
Holstein	7	3.92	0.54	0.99	0.77	0.39	0.61	0.30	39
Jersey	3	4.68	0.58	0.76	0.67	0.21	0.42	0.24	36

The average agreement between duplicates for total phospholipids was 0.056 per cent; between lecithin values the average difference was 0.015 per cent, and the average difference between titration values for choline was 0.10 ml.; the average titration was 3.71 ml. of the thiosulfate.

The results for cream are shown in table 3.

TABLE 3

Average values for phospholipids in fat extracted from cream

Samples	Fat in cream	Total phospholipids in fat	Choline per gram of fat	Lecithin in fat	Lecithin in phospholipids
	%	%	*mg.*	%	%
Sweet cream	Approx. 35	0.24	0.16	0.11	46
Sweet cream	Approx. 35	0.28	0.12	0.03	29
Sour cream	32.75	0.49	0.31	0.21	43
Sour cream	32.52	0.39	0.25	0.17	44
Sour cream	32.18	0.51	0.21	0.14	27
Sour cream	30.12	0.42	0.24	0.16	38

In the case of the first sample, unpasteurized sweet cream of about 35 per cent butterfat was extracted on the large scale. After removal of the solvent to the extent of 99.5 per cent fat, 30-gram samples were hydrolyzed with 5 normal sulfuric acid, and the acid removed as barium sulfate after the hydrolysis. The amount of choline obtained in the case of four such samples was from 4.5 to 5.1 milligrams, or a variation of 0.15 to 0.17 milligrams per gram of fat. This represented a variation in lecithin of from 0.10 to 0.11 per cent. The other cream samples represented in the table were extracted in the usual way, but using 3 to 5 ml. of cream in the extraction flask. Solvents were removed to the extent of 99.5 per cent or negligible amounts in the first three samples, and to the extent of 95.2 per cent, 88.7 per cent, and 82.8 per cent fat in the last three samples. The corrected

amount of fat hydrolyzed in each case is shown in the table. In the case of fat extracted from cream and butter, it was necessary to hydrolyze the amounts of fat shown in order to have a high enough titration value. In the case of the cream samples, a blank of 0.15 ml. of N/100 thiosulfate was taken, the average titration difference was 0.14 ml., and there was an overall average titration of 3.72 ml. of thiosulfate for the data shown.

In the case of butter samples, one to two grams of the butter were extracted in each flask. It was found in the case of experimental butter, freshly churned, that from 8- to 10-gram samples of the fat were sufficient to obtain a titration value. In the case of some commercial butter samples, it was necessary to boil 20 to 40 grams of the fat with the acid. The results for some of the butter samples are reported in table 4.

TABLE 4

Average amounts of phospholipids in fat extracted from butter

Sample	Fat	Total phospholipids	Choline per gram of fat	Lecithin in fat	Lecithin in phospholipids
	%	%	*mg.*	%	%
1	78.2	0.15	0.148	0.099	60
1	78.2	0.15	0.132	0.089	
2	75.0	0.15	0.123	0.082	57
2	75.0	0.15	0.136	0.091	
3	78.2	0.11	0.071	0.047	43
4	80.2	0.18	0.111	0.074	41
5	81.6	0.17	0.119	0.079	46
6	80.8	0.20	0.121	0.081	40
7	78.6	0.17	0.131	0.087	51
8	.....	0.14	0.049	0.033	.....
9	.....	0.13	0.035	0.023	.....

Samples 8 and 9 were taken from Samples 1 and 2 which had been stored for about three months in the cold and then held at room temperature for two weeks. Forty grams of fat were used for Samples 8 and 9 to obtain titration values of about 2 ml. of 0.0102 normal thiosulfate. In the case of the other samples 8 to 20 grams were used. A blank of 0.15 ml. of thiosulfate was used. The average titration value was 3.74 ml. for the data shown, and the average difference 0.08 ml. between duplicates on hydrolyzates. There was no difference between the average values for the total phospholipid determinations.

A number of samples of buttermilk were analyzed for total phospholipids and choline. Representative data are given in table 5.

In the case of buttermilk fat, as well as that of skimmilk and separator slime, irregular results were obtained in some instances. Thus, in the data shown, the average variations between duplicates for total phospholipids amounted to 0.38 per cent, in other instances agreement to 0.01 per cent was obtained. The average difference between choline determinations

on hydrolyzates was 0.10 ml., and the average titration value was 3.25 ml.; a blank of 0.15 ml. was used. Samples hydrolyzed in duplicate also showed more differences, but in the case of other products these differences were small. Thus, in one case above, a difference of 0.43 milligrams choline per gram of fat was obtained, and in another case, there was a difference of 0.21 milligrams. Since 0.4 to 1.5 grams of extracted lipids were hydrolyzed, these differences represented variations of from 0.14 to 0.24 per cent of lecithin in the fat.

TABLE 5

Average values for phospholipids and lecithin in fat extracted from buttermilk

Sample	Fat in buttermilk	Phospholipids in fat	Choline per gram fat	Lecithin in fat	Lecithin in total phospholipids
	%	%	*mg.*	%	%
1	0.99	5.79	4.10	2.72	47
2	0.41	13.38	8.79	5.85	44
3	0.61	9.04	5.58	3.72	41
4	0.52	6.50*	3.81	2.54	39
4	"	"	4.24	2.82	41
5	0.71	19.90	3.98	2.65	13
6	0.54	9.10	3.76	2.50	27
7	0.59	8.63	3.10	1.90	22

* One sample.

DISCUSSION

The results of table 1 show that there is no inclusion of free choline salts in milk in the extracted lipids. Thus any considerable splitting of choline from the phospholipids may be followed.

In the case of milk fat from cows of various breeds, the average of total phospholipids and lecithin in the fat is about the same. The range of values obtained for each set of cows tested showed no variations of interest. In tests on a few cows having mastitis, there was no noticeable increase in phospholipid material in the fat over the amount in normal milk. If the amount of phospholipids in milk is about 0.03 per cent, and 56 per cent of this is lecithin, then the amount of choline per gram of fat would be approximately 0.44 milligrams for 4 per cent milk. This figure is in the range of values shown.

In the data for cream samples, the amount of total phospholipids and the amount of choline associated with the fat is proportionately less than for whole milk fat. The ratio of total phospholipids to lecithin is not significantly different from that for whole milk fat.

In the butter samples one-third to one-fourth of the amount of phospholipids and choline was associated with the fat as in the case of whole milk. The results indicated that about 43 milligrams of choline were present per pound of the experimental butter. A number of commercial butter samples showed a variation of from 0.01 to 0.10 milligrams of choline

per gram of fat. The amount of total phospholipids averaged about 0.15 per cent of the fat. At these low amounts of choline however, the results may be low due to losses during the analysis.

Samples of evaporated milk, which were extracted, contained from 0.26 to 0.43 milligrams of choline per gram of fat, and from 0.42 to 0.50 per cent phospholipids in the fat.

The results for a few samples of skimmed milk fat indicated that from 4.0 to 16.8 milligrams of choline per gram of extracted fat were present. The results of table 5 show the relatively large amounts of these substances present in buttermilk fat. The irregularities in results may have been due to non-homogeneous samples, and the fact that it was difficult to obtain clear hydrolyzates from these lipids. Further work is needed on these substances.

A sample of colostrum contained 6.99 per cent fat, of which 0.83 per cent was phospholipids. The choline present amounted to 0.76 milligrams per gram of fat.

The ratio of lecithin to total phospholipids averaged 46 per cent for whole milk fat, 54 per cent for fat from cream, 48 per cent for butter lipids, and 34 per cent for buttermilk lipids.

Any evaluation of the above results for choline should take into consideration the limitations of the method used. These are, the method of extraction of the lipids, the problem of loss of choline during the procedure, especially in the concentration of hydrolyzates, and the difficulties in the periodide precipitation and titration. According to Roman (22) in the range of choline concentration concerned in this analysis choline losses up to 10 per cent may be found. A number of recovery experiments in this investigation showed that from 93 to 102 per cent of the calculated amount of choline was found, with a general average of 97 per cent. This problem needs further investigation, since during recovery experiments in which choline was determined in a solution of choline chloride, by the chloroplatinate method, the choline solution was evaporated almost to dryness in a stream of nitrogen at 40° to 50° C. under vacuum and the residue taken up in absolute alcohol and similarly evaporated twice more. In these instances there was no appreciable loss, for the chloroplatinate determination checked with the amount present by direct weight.

The difficulties involved in carrying out the analyses described, indicate that further work on the phospholipids of milk fat would require a study of extraction methods, the use of Reinecke's salt for choline precipitation and the avoidance of concentrations of large amounts of hydrolyzate.

SUMMARY

Data concerning the amounts of phospholipids and choline present in milk fat for various products are given, and the application of the periodide method for the analysis for choline is discussed.

The average total phospholipids and lecithin in the milk fat from cows of various breeds was about the same.

There was no noticeable increase in the phospholipid material of the milk fat from the few cows having mastitis than that in normal milk.

The amount of total phospholipids and choline associated with the fat in cream was proportionately less than for whole milk fat. However, the ratio of total phospholipids to lecithin was not significantly different from that for whole milk fat.

One-third to one-fourth of the amount of phospholipids and choline was associated with the fat of butter as compared to that in whole milk. The results indicated that about 43 milligrams of choline were present per pound of experimental butter.

Evaporated milk contained from 0.26 to 0.43 milligrams of choline per gram of fat, and from 0.42 to 0.50 per cent phospholipids in the fat.

The ratio of lecithin to total phospholipids averaged 46 per cent for whole milk fat, 54 per cent for fat from cream, 48 per cent for butter lipids, and 34 per cent for buttermilk lipids.

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EFFECT OF GROWTH OF *PSEUDOMONAS PUTREFACIENS* ON DIACETYL AND FLAVOR OF BUTTER[1]

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The cheesy and putrid defects[2] of butter are among the most serious manufacturing problems confronting the butter industry of Indiana. Numerous reports emphasize the significance of this problem elsewhere. Studies in this laboratory indicate that in the surrounding region the putrid defect and perhaps in most cases the pronounced cheesy defect are due to species of *Pseudomonas putrefaciens*. Certain of the less pronounced cheesy defects, however, appear to be due to other related water bacteria capable of some lipolytic and proteolytic action on milk and butter constituents.

During preliminary investigations on the nature and cause of these defects it was repeatedly noted in butter containing neither salt nor starter that the first apparent change produced by *Ps. putrefaciens* was loss of the typical full, clean odor. The butter at this stage appeared to have completely lost its normal odor and instead was characterized by a distinctly flat and in some cases by a slightly "oxidized-like" odor.

When butter was made with added diacetyl, the occurrence of cheesy or putrid defects was invariably preceded by almost complete destruction of all diacetyl aroma. The obvious conclusion was that *Ps. putrefaciens* and possibly related organisms destroyed the compounds essential to high aroma of butter. The ability of *Ps. putrefaciens* to destroy added diacetyl in milk and butter was therefore studied.

HISTORICAL

The subject of cheesy, also sometimes referred to as putrid, rabbito, surface taint, fetid, or limburger defect in butter has been reviewed (2, 3, 5, 6, 7, 14, 23). These papers, with many others, indicate the widespread occurrence of this general type of defect.

Considering the tremendous volume of research on production of aroma compounds in dairy products and other foods, comparatively few reports are available on the effect of microorganisms on diacetyl. Neuberg and Nord (13) and Nagelschmidt (12) demonstrated the ability of yeasts to reduce diacetyl to 2, 3-butylene glycol. Michaelian and co-workers (10) noted a

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[2] The defects referred to in this paper have, in every case, been produced by bacteria growing in the butter after its manufacture and should not be confused with the cheesy defect of cream which may be carried into butter.

rapid decrease of diacetyl in a well-ripened butter culture when neutralized to a low acidity and held at a temperature favorable for development of citric acid fermenting streptococci. Subsequent studies (4) showed that *Streptococcus citrovorus* and *Streptococcus paracitrovorus* reduce diacetyl to acetylmethylcarbinol and 2, 3-butylene glycol. Wiley *et al.* (21) reported that betacocci produced diacetyl rapidly and then actively destroyed it. Virtanen and Kontio (20) investigated the action on diacetyl of various microorganisms isolated from butter. Diacetyl and acetylmethylcarbinol were added to sterile milk and respective lots inoculated and incubated at 19–21° C. for periods up to 160 hours. *B. punctatum* destroyed about 90 per cent of both compounds. *B. vulgatus* destroyed 30 to 50 per cent of the diacetyl but only 9 per cent of the carbinol. A mixture of yeasts destroyed 30 to 40 per cent of the diacetyl and as much as 30 per cent of the acetylmethylcarbinol. Certain strains of the colon-aerogenes group, green fluorescent bacteria and a number of species of aerobic spore formers are able to destroy acetylmethylcarbinol (22).

Slatter and Hammer (18) and Prill and Hammer (16) observed both increases and decreases in the diacetyl and acetylmethylcarbinol content of unsalted butters made from sweet cream with a butter culture. Salt appeared to prevent significant increase or decrease of diacetyl and acetylmethylcarbinol. However, in one lot of butter containing only 0.75 per cent salt there occurred a relatively rapid disappearance of these compounds. The increases and decreases were attributed to aroma bacteria from the starter. Toth (19) reported that in butter prepared from sour cream, diacetyl increased for some days during storage and later decreased. In butter from sweet cream a constant decrease took place during storage. According to Mohr *et al.* (11) the diacetyl content of butter during storage may remain constant or may decrease. In one trial they noted a pronounced decrease of diacetyl in unsalted but not in salted butter stored at 10° C. for 10 days. The diacetyl content of salted and unsalted butters decreased at about the same rate when the butters were stored at −20° C. Bunger (1) reported increases followed by decreases of diacetyl in butter.

Some investigators (8, 9, 17, 23) have indicated that diacetyl has an inhibitory effect on bacteria. The inhibiting effect of diacetyl on growth of various organisms in sterile skim milk has been observed by us. However, the inhibiting concentrations were higher than would occur in average butter.

EXPERIMENTAL

The apparent ability of putrid butter organisms to destroy diacetyl was noted both in commercial salted butter and in unsalted laboratory samples. Since in laboratory churnings the usual percentage of salt appeared to inhibit development of *Ps. putrefaciens,* no salt was added to the experimental butter. Under commercial conditions the salt sometimes provides

less assurance of complete inhibition of this organism than under laboratory conditions.

Sterilized cream in 2000-ml. quantities was churned at about 7.2° C. (45° F.) in sterile "Dazey" churns. The butter was inoculated with *Ps. putrefaciens* by adding a sufficient quantity of a 24-hour beef infusion broth culture to provide about 1,000,000 cells per ml. of wash water. After one washing with either sterile or contaminated water, the butter was worked in the churns with large, sterile, metal spoons. A volume of water equal to the original volume of cream was used. This appeared to adequately wash the butter granules and the large number of organisms in the water provided sufficient inoculation of the butter.

Diacetyl in the form of starter distillate was added to the butter after washing and partial working. Five per cent of starter was added a short time before churning to some lots of cream. The starters used did not appear to have a high aroma, but the butters prepared from them always had a desirable aroma. After thorough working, the butter was placed in

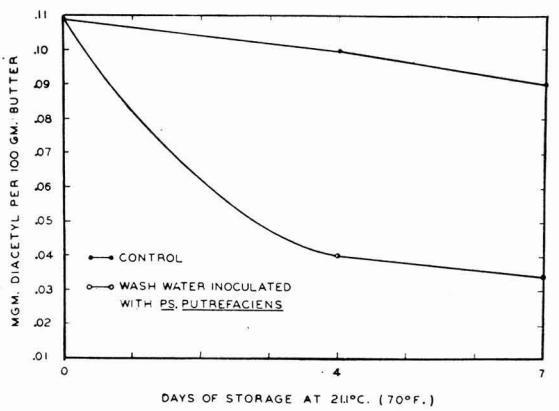

FIG. 1. Effect of growth of *Pseudomonas putrefaciens* on diacetyl added in the form of starter distillate to butter.

approximately 100-gram amounts in sterile 4-ounce sample jars and stored at 21.1° C. (70° F.). Samples were examined for odor and flavor, daily, and for diacetyl content on the day of preparation and again after 4 and 7 days of storage. The method for determining diacetyl was essentially that of Prill and Hammer (15).

Figures 1 and 2 present a summary of the results of several experiments. The analyses substantiated earlier observations on loss of aroma in butter due to growth of *Ps. putrefaciens*. Within 7 days at 21.1° C. the diacetyl content was reduced from 0.109 mgm. to 0.034 mgm. per 100 gm. In the sterile control only a slight drop occurred during the 7-day period.

The diacetyl contents of the starter butters were comparatively low. One

evident reason for this was the fact that the starter culture employed had a low diacetyl content. Also the fact that the starter was added only shortly before churning undoubtedly resulted in the butter carrying over less diacetyl from the starter than would have been true if starter and cream had been in contact for a period of several hours.

The starter butter containing no *Ps. putrefaciens* showed a slight increase in diacetyl. Such increases were noted in other experiments. In some samples containing starter plus added starter distillate, marked decreases occurred. This might be expected in the light of other reported investigations. When *Ps. putrefaciens* was present, the diacetyl in starter butter decreased slightly. The main purpose of adding starter in this study was to determine the effect of *Ps. putrefaciens* on diacetyl in butter when its activity was inhibited by starter bacteria. Cultures of *Streptococcus lactis* added in the same manner as starter cultures also effectively inhibited development of *Ps. putrefaciens.*

The aroma and clean, full flavor of both starter and uninoculated starter distillate samples were maintained throughout the storage period. Samples of inoculated butter containing starter distillate became flat and lost all aroma in 24 to 48 hours and within several hours thereafter became definitely putrid.

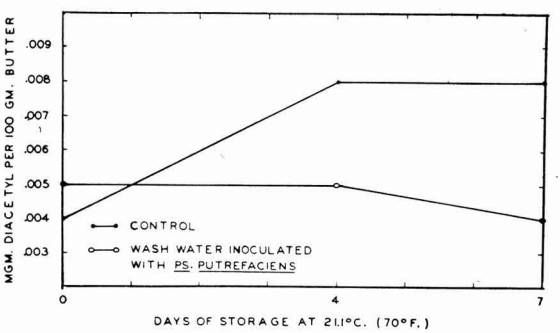

FIG. 2. Effect of *Pseudomonas putrefaciens* on diacetyl in butter made with starter.

Results of other experiments showed comparatively rapid destruction of diacetyl at refrigeration temperatures when active development of *Ps. putrefaciens* occurred in the butter. In one such trial the diacetyl content fell from 0.080 mgm. per 100 grams of butter to 0.040 mgm. in 4 days at 7.2° C. and in the corresponding sample at 21.1° C. it fell to 0.025 mgm. in 2 days.

DISCUSSION

In general the results indicated relatively rapid destruction of diacetyl in butter under conditions favorable to the growth of *Ps. putrefaciens.* The rapid disappearance at 7.2° C. (45° F.) coincided with observations on commercial samples that developed a cheesy or putrid defect under normal

storage and retail conditions. It is probable that *Ps. putrefaciens* rapidly destroyed the diacetyl in the aqueous phase. Diacetyl in the fat phase may have been sufficiently protected so that little was affected by the organisms. This explanation might account for the complete lack of aroma following growth while one-third to one-fourth of the original diacetyl still remained intact in the butter.

The diacetyl contents of starter butters, both control and inoculated, were low. Nevertheless the butter had a definite, pleasant aroma. It is possible that the aroma in the starter butter was enhanced or maintained by continual slow production of diacetyl during storage of the butter not only in the control sample but also in that where *Ps. putrefaciens* was slightly active. In the non-starter butter containing *Ps. putrefaciens* the opposite condition may have been true, namely, the organisms destroyed all diacetyl that might have been able to contribute to the aroma of the butter.

It has been suggested that diacetyl may be the component that enables butter starters to inhibit development of *Ps. putrefaciens* in butter (23). The low diacetyl contents in the starter butter, however, suggest that it is the activity of the lactic acid bacteria (probably lactic acid production) rather than presence of diacetyl that inhibits action of *Ps. putrefaciens* in butter made with starter. The ability of *Streptococcus lactis* alone to inhibit *Ps. putrefaciens* in butter further substantiates this conclusion.

One fact that should be borne in mind is that commercial butters present a somewhat different problem than laboratory churned samples. For example neither salt nor starter definitely insures the inhibition of *Ps. putrefaciens* in commercial butter. Various factors such as methods of working, printing, storage and distribution influence the activity of the microorganisms involved. However, the close correlation between changes occurring in commercial and laboratory samples definitely indicates a similarity in the sequence of changes taking place under the two different conditions.

One of the most troublesome angles in this problem, particularly to the creameryman who has been without the technical advice of a control laboratory, has been the failure to recognize the nature of the defect caused by *Ps. putrefaciens* and related bacteria. The cheesy and putrid defects sometimes have not been recognized by the butter manufacturer or handler except where they have progressed to an advanced stage. Possibly the term surface taint would be more suggestive for this defect; however, the so-called cheesy or putrid defect is usually not confined to the surface. Many commercial samples received in this laboratory or picked up in retail outlets appear to run through the whole series of changes to the final cheesy or putrid state only after periods considerably longer than they ordinarily would be stored in the household refrigerator. This means that such samples, when in the hands of the creameryman, wholesaler and retailer, and often the final purchaser, show only a flat, disagreeable odor and flavor difficult to identify. Many creamerymen are aware that their butter is off

in flavor but recognize it only as flat. Part of a recent large shipment was returned to one manufacturer with the complaint that it was "flat and metallic." This butter had a pleasing, full flavor with high aroma at the time of shipment from the creamery. The cause of this "flat" flavor was *Ps. putrefaciens*. This fact was indicated by bacteriological examination and also because the "flat" flavor was followed by the development of a typical putrid flavor. This type of flavor defect seems more common in commercial butter than is generally realized. Results to be published later indicate that species other than *Ps. putrefaciens* and the aroma bacteria may destroy diacetyl in butter.

The "metallic" flavor mentioned above may have been confused with the tallowy flavor reported by Toth (19) and others to accompany reduction of diacetyl in butter. In such occurrences the fat apparently is oxidized. When the masking effect of a strong aroma has been removed by the action of microorganisms or other factors, a tallowy or oxidized flavor might become more apparent. On the other hand our studies and those of others (23) indicate that an oxidation of certain compounds in the butter may possibly be due to *Ps. putrefaciens*. The flavor developed would undoubtedly be masked by the pronounced cheesy or putrid flavor following active growth of the organism.

SUMMARY

Observations on both commercial and laboratory samples of butter in which *Ps. putrefaciens* developed showed that the first apparent stage of decomposition by this organism was a complete loss of typical butter aroma. The butter became flat in odor and flavor. This stage was then followed by development of typical cheesy or putrid flavors.

Chemical analyses indicated that during a 7-day storage period at 21.1° C. the diacetyl content of the sterile control butter samples remained fairly constant. More than half the diacetyl was destroyed under the same conditions in similar lots of butter contaminated with *Ps. putrefaciens*.

The diacetyl content of butter prepared with starter and contaminated with *Ps. putrefaciens* remained relatively stable over a 7-day storage period at 21.1° C., although some destruction of diacetyl occurred.

Results and observations indicate that the ability of *Ps. putrefaciens* and other organisms to produce a flat-flavored butter is perhaps more prevalent than is commonly realized.

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THE EFFECT OF COCOA UPON THE UTILIZATION OF THE CALCIUM AND PHOSPHORUS OF MILK*

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To what extent the chocolate flavoring material affects the nutritive value of milk is a question which has been the subject of investigation (9, 11, 12, 13) but has not yet been completely answered. Numerous investigations (3, 5, 6, 16, 17, 19) have shown that the calcium of spinach, beet greens and other oxalic acid-rich vegetables is poorly utilized. Therefore, the presence in cocoa of considerable quantities of oxalic acid (1, 7, and this study) suggested the possibility of its interference with the utilization of the calcium of the milk or of the diet.

Since calcium is one of the minerals most often lacking or at a borderline level in the modern diet, and since cocoa and chocolate flavored foods are used so widely, it seemed advisable to determine whether or not cocoa interfered with the absorption and utilization of calcium. It also seemed advisable to determine whether or not cocoa interfered with the absorption of phosphorus, since milk contains a liberal amount of this important mineral, which is nutritionally closely connected with calcium.

EXPERIMENTAL PROCEDURE

Nine litters containing 63 albino rats 25 to 27 days of age were selected for the experiment. The animals of each litter were grouped according to weight and sex into groups of three. One rat of each group was sacrificed in order that the calcium and phosphorus content at that age might be determined. One of the remaining animals was fed whole milk powder and sugar, or Diet I, and the other was fed whole milk powder, sugar, and cocoa, or Diet II. The composition of both diets is given in table 1, and the analyses of the cocoa powder and whole milk powder used for this study are given in table 2. A slightly modified method of paired feeding was used throughout the experiment. All animals within a pair were fed the same amount of basal ration (milk powder, sugar and mineral mixture) so that the only variable in the ration was the cocoa fed as a supplement. Slight differences in calcium and phosphorus content of the two diets were due to the fact that it is very difficult to equalize calcium and phosphorus content and caloric value, and maintain the proportions of a representative chocolate milk.

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The required amount of cocoa for Diet II was mixed with the basal ration, and 0.24 grams of basal ration (Diet I) was fed for each gram of the cocoa-supplemented ration (Diet II). This feeding procedure has the advantage in that it eliminates weighing and mixing daily portions of the cocoa supplement with the basal ration. The basal ration intake of a pair was restricted

TABLE 1

*Composition of diets**

	Amount in 84 grams of Diet I—control diet (Whole milk powder + sugar)	Amount in 100 grams of Diet II—cocoa diet (Whole milk powder + sugar + cocoa)
	gms.	*gms.*
Whole milk powder	52.90	52.90
Cane sugar	31.10	31.10
Cocoa powder	0.00	16.00
Calcium	0.4655	0.4911
Phosphorus	0.3968	0.5152
$\frac{Ca}{P}$ ratio	1.17	0.95
Caloric value	392 Cal.	435 Cal.

* The composition of the diets is given as amount of constituents in 84 grams of Diet I and in 100 grams of Diet II, because a modified method of paired feeding was used in which 0.84 gram of Diet I was fed for every 1 gram of Diet II. A more detailed explanation for this procedure is given in the text.

Note:—Fe, Cu, and Mn were fed to both groups as explained in the text.

to the amount consumed by the member eating the less, which in most instances was the animal receiving the cocoa diet. However, an attempt was made not to feed more than the amount which would be eaten within 24 hours. The whole milk powder was used because it has many obvious

TABLE 2

Analyses of cocoa and whole milk powder

Constituents	Cocoa	Whole milk powder
	Per cent	*Per cent*
Moisture	5.40	1.75
Fat	11.23	27.50
Protein	22.62*	27.50
Ash	5.98	6.00
Lactose	.....	37.25
Tannic substances	12.15	.....
Theobromine	1.65	.....
Caffeine	0.10	.....
Oxalic acid	0.54	.....
Fiber	5.25	.....
Calcium	0.16	0.88
Phosphorus	0.74	0.75
Iron	0.0134	.....
Sodium	0.048	.....
Potassium	1.69	.....

* Nitrogen (minus the theobromine and caffeine nitrogen) $\times 6.25$.

advantages in experimental feeding over fluid milk. All rats were fed daily a mineral mixture consisting of iron, copper, and manganese salts, as recommended by Elvehjem *et al.* (4). The salt solutions were mixed with a 10 per cent sugar solution and fed in supplement dishes. The rats consumed it greedily as soon as it was placed in the cages.

The animals were placed in individual galvanized iron cages which rested on raised screening of three mesh to the inch. Food was fed in porcelain cups of approximately 50-ml. capacity, placed within metal cups to catch spillage. Distilled water was supplied ad libitum. The rats were weighed at weekly intervals, and the weighings were made 6 to 8 hours after the animals had received their daily feeding.

After five weeks on experiment, the animals were killed with chloroform, the fur brushed carefully to remove any food particles, the gastrointestinal tract dissected out, and the contents removed. The tract was weighed before and after the contents were removed and then discarded to avoid the possibility of the inclusion of traces of food. This is justifiable since the calcium content is known to be negligible. The net body weight was then calculated as the final weight minus the weight of the gastrointestinal contents. The rats were frozen and placed in cold storage (0 to – 10° F.) until ashing and chemical analyses could be performed.

At a suitable time, rats were taken from the cold room, thawed and ashed in weighed vitreosil dishes in a muffle furnace at a temperature below red heat (approximately 500° C.), until the ash reached a constant weight, usually about 3 days. The ash was then dissolved in 100 ml. of 1:4 HCl and made up to a volume of 500 ml. Calcium was determined by the Sherman and MacLeod (15) method. The calcium oxalate was titrated with potassium permanganate instead of being determined gravimetrically. Phosphorus was determined by the Truog and Meyer method (20), using an Evelyn photoelectric colorimeter.

RESULTS AND DISCUSSION

The effect of cocoa upon growth. Although observations of the effect of cocoa upon growth were only incidental in this study, they are, nevertheless, significant. From figure 1 and table 3, it may be seen that the rate of growth during the five-week experimental period was higher for both male and female rats on the control diet than for the cocoa-fed rats. The decrease in rate of growth of cocoa-fed animals was 10.59 and 12.31 per cent for males and females, respectively. This difference in rate of growth was statistically highly significant when analyzed by Student's (18) method. The finding of a decreased growth rate is in accordance with the results of previous investigations (9, 12, 13). The decrease in rate of growth is even more striking in view of the fact that cocoa-fed rats actually received more calories, more protein, more calcium, and more phosphorus than the control rats, as cocoa was fed as a supplement to the basal ration.

There are several possible explanations as to the manner in which cocoa brings about a retarded growth rate. The work of Lipman and Mueller (11) indicates that the decrease in digestibility of milk proteins when cococa is added to a milk diet may be responsible for part of the observed slower growth of cocoa-fed rats. Results by Mueller (12) indicate that the tannic substances of cocoa are responsible in part for the decreased growth rate, while the theobromine has no significant effect. Kohman (10) and Speirs

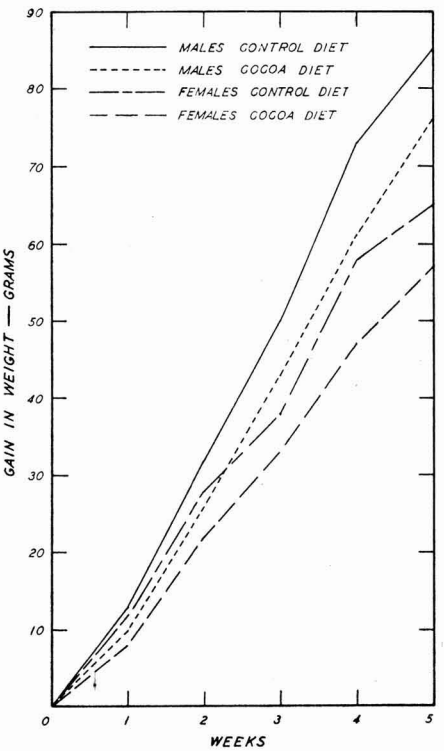

FIG. 1. Comparative growth of rats fed control and cocoa diets.

(17) as well as other investigators have found that rats receiving vegetables high in soluble oxalate do not grow as well as those fed food low in oxalate content. Therefore, the presence of soluble oxalate or oxalic acid in cocoa may be a factor in explaining the inferior growth of rats receiving the cocoa supplement.

The effect of cocoa upon calcium utilization. The effect of cocoa upon the utilization of the calcium of milk may be seen from the data presented in table 3 and figure 2. The percentage of calcium in the body after the five-week experimental period was higher for control rats than for cocoa-fed rats.

The bodies of control males and females contained 0.942 and 0.983 per cent, respectively, as compared with 0.847 and 0.913 per cent for cocoa-fed rats. This finding is even more striking since the rats on the cocoa ration received slightly greater amounts of both calcium and phosphorus in the diet.

TABLE 3

*Table of average values for rats fed control and cocoa diets**

	Male		Female	
	Control diet	Cocoa diet	Control diet	Cocoa diet
Growth				
Number of animals	12	12	9	9
Weight gain—grams	85	76 (± 1.74)†	65	57 (± 2.01)
Total basal ration intake—grams	237.7	237.7	218.4	218.4
Total cocoa intake—grams	0.0	45.3	0.0	41.6
Total food intake—grams	237.7	283.0	218.4	260.0
Calcium				
Net body weight—grams	123.89	113.67	103.87	95.61
Ash weight—grams	4.161	3.600	3.590	3.187
Calcium in body at 60 days				
Grams	1.167	0.963	1.021	0.873
Per cent net weight	0.942	0.847	0.983	0.913
Calcium in body at 25 days—grams	0.343	0.343	0.349	0.349
Calcium retained—grams	0.824	0.620	0.672	0.524
Calcium intake—grams	1.317	1.390	1.210	1.277
Per cent calcium retained	62.6	44.6	55.5	41.0
Calcium retained per 100 gm. net body weight—grams	0.665	0.545 (± 0.013)	0.647	0.548 (± 0.014)
Phosphorus				
Phosphorus in body at 60 days				
Grams	0.741	0.631	0.628	0.559
Per cent net weight	0.598	0.555	0.605	0.585
Phosphorus in body at 25 days—grams	0.243	0.243	0.240	0.240
Phosphorus retained—grams	0.498	0.388	0.388	0.319
Phosphorus intake—grams	1.123	1.458	1.032	1.340
Per cent phosphorus retained	44.3	26.6	37.6	23.8
Phosphorus retained per 100 gm. net body weight—grams	0.402	0.341 (± 0.007)	0.374	0.334 (± 0.011)

* Experimental period was 5 weeks.

† Figures in parentheses express standard error of mean difference between control and cocoa-fed rats.

It was also noted that the percentage of calcium in the body is higher for female rats than for male rats. This is true both of the 25-day-old and the 60-day-old animals. The percentage of ash was also higher in females. These results are in accordance with those of Cox and Imboden (2), Sherman *et al.* (14, 15), and other workers.

Calcium retention, calculated by subtracting the amount of calcium present in littermate controls sacrificed at 25 to 27 days of age from the amount

present in the rats after five weeks on experiment, is greater for the animals on Diet I. The cocoa-fed rats showed a decrease in the actual grams of calcium retained of 24.8 per cent for males and 22.0 per cent for females. Because it was felt that the difference in weights of the control and cocoa-fed rats would give an unfair representation of retention, these values were again calculated on a 100-gram basis. According to these calculations, the cocoa-fed rats showed a decreased calcium retention of 18.0 per cent for males and 15.3 per cent for females. These figures are statistically highly significant.

The observed reduction in calcium retention in cocoa-fed rats cannot be attributed entirely to insoluble calcium oxalate. Calculations have shown that the amount of oxalic acid in the cocoa used can tie up empirically only

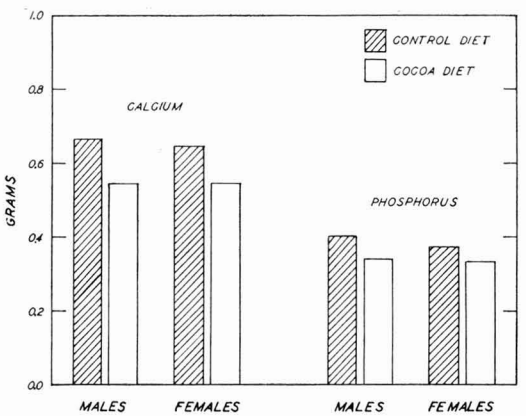

FIG. 2. The effect of cocoa on the amount of calcium and phosphorus retained per 100 grams net body weight.

approximately 7.8 per cent of the calcium of Diet II. Nothing seems to be known about the availability of the calcium of cocoa. Even if it be assumed that all of the cocoa calcium is unavailable, and if this amount is added to the calculated unavailable calcium, the combined total will not account for all of the observed reduction. To what the additional decrease in calcium retention may be attributed is not clear. Numerous investigators have studied the relation of the pH of intestinal contents and feces to calcification, and the general theory is that an increase in acidity aids the absorption of calcium and phosphorus. However, a more recent study by Jones (8) throws some doubt on this theory. Mueller and Ritchie (13) reported that the pH of feces of rats receiving a diet containing 5 per cent cocoa was slightly higher than that of control rats receiving a cocoa-free diet. Whether the 16 per cent cocoa diet used in this study may have increased the pH of the intestinal contents with consequent decreased absorption is not known. These investigators also reported that when the rats were fed fluid milk containing 7 and 10 per cent cocoa, the feces in the intestinal

tract were very hard and there was a greater accumulation of food material in the ceca than was the case in the control group. In this study no indications of obstruction were apparent upon removal of the gastrointestinal tract, but it was noted that the feces were harder and drier than those from the cocoa-free diet. Whether this condition had any adverse effect on the absorption of the calcium is problematical.

The effect of cocoa upon phosphorus utilization. The effect of cocoa upon phosphorus utilization is no less important than its effect upon calcium utilization. Average figures and statistical analyses are presented in table 3 and figure 2. The bodies of control rats after five weeks on experiment contained a higher percentage of phosphorus than those of cocoa-fed rats. These results were noted in both sexes. Control males contained 0.598 per cent phosphorus as compared with 0.555 per cent for cocoa-fed males, and control females contained 0.605 per cent as compared with 0.585 per cent for females receiving cocoa. As with calcium, females, both at 25 days of age and at 60, contained a higher percentage of phosphorus than males. Phosphorus retention was lower in cocoa-fed rats of both sexes than in controls. Males showed a decrease of 22.1 per cent and females a decrease of 17.8 per cent in the actual grams of phosphorus retained. When the phosphorus retention values were calculated on an equal weight basis the cocoa-fed rats showed a decreased phosphorus retention of 15.2 per cent for males and 10.7 per cent for females. These values are highly significant statistically.

To what the reduction in phosphorus retention may be attributed is not clear. There seems to be no known substance in cocoa which would bind phosphorus in a manner similar to the calcium. Since the complete composition of cocoa powder is probably not yet known, there may be substances present which act in this manner. Other possible explanations discussed for calcium may also play a role in the decreased phosphorus utilization.

SUMMARY AND CONCLUSIONS

1. A group of 63 young rats, 36 males and 27 females, was used. One-third of this number of animals was sacrificed at the beginning of the experiment to determine calcium and phosphorus content. Half the remaining rats received a control diet of whole milk powder and sugar, and half received a diet of whole milk powder, sugar, and cocoa. Iron, copper and manganese salts were fed to both groups. The experimental period lasted five weeks.

2. Cocoa-fed rats showed a significantly lower rate of growth than control rats.

3. Cocoa-fed rats showed a significantly lower calcium and phosphorus content of the body and calcium and phosphorus retention than control rats.

4. The decrease in calcium retention was greater than could be accounted for by the oxalic acid present in the cocoa. The cause for the decreased phosphorus retention is not clear.

5. While results obtained with small animals cannot always be applied directly to human beings, yet the fundamental facts obtained in this study would seem to indicate that the indiscriminate and excessive use of chocolate-flavored foods, especially in a diet already low in calcium, should not be recommended.

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INFLUENCE OF VARIOUS TREATMENTS ON THE BACTERIA CONTENT OF FROZEN CREAM[1]

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The storage of cream in a frozen state has certain economic advantages provided it can be done successfully. It permits the utilization without churning of surplus cream produced at the peak of production over a longer period of time. This provides a more economical fat supply, since losses due to churning are eliminated and a greater spread of a lower priced fat is possible.

The freezing of cream for storage presents two major problems. One is the preservation of the normal flavor. The other is the de-emulsification or "oiling-off" of the fat as a result of freezing. A series of experiments was planned to determine the best method of treating cream prior to freezing and during storage.

PLAN OF EXPERIMENTS

Thirty gallons of cream ranging in fat from 45 to 60 per cent, with an average of 51.75 per cent, were processed and frozen monthly throughout the year. Portions of this cream were pasteurized at 150° F. for 30 minutes, 165° F. for 15 minutes, and 185° F. for 5 minutes. Portions of each of these lots were then homogenized at 0, 1500, and 3000 pounds pressure. These were further subdivided into 4 samples; one being retained as a control with nothing added, 1 ppm. copper was added to a second, 10 per cent sucrose was added to the third and 1 ppm. copper and 10 per cent sucrose were added to the fourth. Samples which had received these various treatments were then placed in glass, paper, and tin containers, were frozen at – 5° F. to – 10° F. and stored at this temperature for six months and one year.

At the end of these periods they were examined for flavor, carotene, pH, oxidation-reduction potential, acidity, the de-emulsification of fat and the bacteria content.

BACTERIOLOGICAL EXAMINATION

This paper is concerned primarily with the bacteriological findings. The results of the rest of the work are published elsewhere (3, 5, 6, 7). Total plate counts were made according to Standard Methods (4). All plates were made in duplicate from suitable dilutions and averaged.

It was thought advisable to determine the number of lipolytic as well as the total number of bacteria present in the cream. The method used to determine the lipolytic bacteria was one devised and tested in this labora-

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TABLE 1

Influence of homogenizing on the bacteria count of cream pasteurized at different temperatures and stored in glass, paper, and tin (averages of 12 monthly samples)

Heat treatment	Stored in	Period of storage in months	Bacterial plate count of cream homogenized at					
			0 lbs.		1500 lbs.		3000 lbs.	
			Total	Lipolytic	Total	Lipolytic	Total	Lipolytic
Unpast.	Glass	Fresh*	2,498,000	3,300,000				
		6	1,638,000	1,202,000				
		12	680,000	84,000				
Past. at 150° F. for thirty minutes	Glass	Fresh	6,795	5,732	8,680	8,285	12,350	3,450
		6	6,681	779	7,287	1,018	12,429	2,917
		12	5,458	436	5,690	1,122	7,750	2,591
	Paper	Fresh	6,795	5,732	8,680	8,285	12,350	3,450
		6	5,011	598	7,880	705	13,793	1,057
		12	3,300	500	7,580	1,290	9,990	1,295
	Tin	Fresh	6,795	5,732	8,680	8,285	12,350	3,450
		6	6,632	542	7,854	809	9,154	1,432
		12	4,345	1,095	5,680	1,530	5,947	1,435
Past. at 165° F. for fifteen minutes	Glass	Fresh	1,518	810	2,084	1,041	6,718	3,143
		6	1,904	373	1,848	166	3,247	164
		12	1,277	575	1,694	329	1,792	645
	Paper	Fresh	1,518	810	2,084	1,041	6,718	3,143
		6	1,364	311	1,518	223	2,317	313
		12	930	505	769	332	911	486
	Tin	Fresh	1,518	810	2,084	1,041	6,718	3,143
		6	1,809	839	2,518	394	3,641	455
		12	1,154	586	1,769	323	2,830	186
Past. at 185° F. for five minutes	Glass	Fresh	659	409	615	513	397	226
		6	770	360	940	175	840	750
		12	712	108	1,032	217	570	138
	Paper	Fresh	659	409	615	513	397	226
		6	900	285	943	414	970	350
		12	520	95	1,114	173	530	110
	Tin	Fresh	659	409	615	513	397	226
		6	770	491	880	563	1,111	355
		12	816	73	600	232	935	127

* Fresh = sample immediately after pasteurizing.

tory (2).[2] Briefly the method is as follows: One ml. of 20 per cent sterile cream and 0.5 ml. of 0.1 per cent Nile blue sulfate solution were added to 100 ml. of melted and cooled (55° C.) tryptose agar. The flask was rotated to effect an even distribution of the milk fat and dye throughout the agar, and the plates poured before the agar solidified. Tests showed that there was no inhibition of the lipolytic bacteria by this concentration of the dye. With this concentration of dye, the agar loses most of its blue color during the incubation period. The colonies producing lipase hydrolyzed the fat molecules to glycerol and fatty acids. In the presence of fatty acids, the Nile blue sulfate turns deep blue, so the lipolytic colonies were various shades of blue depending upon their ability to produce lipase. Due to the use of tryptose the lipolytic counts, especially on the fresh cream, are sometimes greater than the total counts on Standard agar.

All data recorded in tables 1 and 2 represent arithmetical averages of duplicate plates from monthly samples taken throughout the year. The arithmetical average was used since the counts were grouped sufficiently closely to make this method applicable. Each plate count, therefore, represents the average of 24 plate counts. While there are some objections to this method of presenting the data, it was considered advisable to condense them in this way because they were so voluminous, and, also, to facilitate obtaining an over-all rather than a detailed monthly picture.

INFLUENCE OF HOMOGENIZING AND PASTEURIZING

The first two factors considered were the influence of homogenizing and pasteurizing on the bacteria content of the cream as well as the influence of the type of container used to store the cream during the freezing period.

A good grade of sweet cream was used. The bacteria count of this cream ranged from a few thousand to a few millions. The only real high count cream was encountered in July. As the fresh cream was held in storage at $-5°$ to $-10°$ F. the counts gradually decreased. The lipolytic plate counts showed the same trend.

Taking the bacteria plate counts of the cream which was not homogenized as the base, the data in table 1 show that as the homogenizing pressure was increased there was a corresponding increase in the bacteria count. This increase was very consistent in the cream pasteurized at 150° F. for 30 minutes for both the total count and the lipolytic count. As the pasteurizing temperature was increased, to 165° F. for 15 minutes, the total plate count still showed a consistent increase in the number of bacteria but the lipolytic count was more variable. When the pasteurizing temperature reached 185° F. for 5 minutes, both the total and lipolytic counts showed considerable variation as the homogenizing pressure was increased. This was doubtless due to the small number of bacteria present.

[2] Data used by permission of Graduate Council.

TABLE 2

Influence of copper, sucrose and combinations of the two on the bacterial count of non-homogenized cream held in the frozen state in glass, paper, and tin containers (averages of 12 monthly samples)

Heat treatment of sample	Type of container for storage	Period of storage in months	Bacterial plate count of non-homogenized cream to which was added							
			Control		One ppm. of copper		10 per cent sucrose		One ppm. copper and 10 per cent sucrose	
			Total	Lipolytic	Total	Lipolytic	Total	Lipolytic	Total	Lipolytic
Past. at 150° F. for thirty minutes	Glass	Fresh*	6,795	5,732	10,770	5,160	9,030	4,135	8,722	5,544
		6	6,681	779	7,656	972	5,212	536	4,906	736
		12	5,458	436	4,445	432	5,472	664	3,500	918
	Paper	Fresh	6,795	5,732	10,770	5,160	9,030	4,135	8,722	5,544
		6	5,011	598	5,539	489	7,695	464	5,614	594
		12	3,300	500	4,100	510	6,200	590	7,040	1,015
	Tin	Fresh	6,795	5,732	10,770	5,160	9,030	4,135	8,722	5,544
		6	6,632	542	5,014	571	5,839	506	5,087	638
		12	4,345	1,095	5,265	680	4,693	835	5,580	995
Past. at 165° F. for fifteen minutes	Glass	Fresh	1,575	813	1,542	3,664	2,582	1,926	2,487	1,595
		6	1,904	373	1,998	477	1,720	286	1,504	430
		12	1,277	575	1,748	835	1,600	958	1,769	579
	Paper	Fresh	1.575	813	1,542	3,664	2,582	1,926	2,487	1,595
		6	1,364	311	1,370	409	1,000	289	1,093	380
		12	930	505	1,230	486	1,330	664	1,334	427
	Tin	Fresh	1,575	813	1,542	3,664	2,582	1,926	2,487	1,595
		6	1,809	839	1,577	673	1,606	451	1,495	461
		12	1,154	586	1,848	614	1,659	550	1,568	450
Past. at 185° F. for five minutes	Glass	Fresh	659	409	680	645	592	244	675	203
		6	770	360	747	284	786	133	708	120
		12	712	108	630	192	577	125	812	142
	Paper	Fresh	659	409	680	645	592	244	675	203
		6	900	285	891	193	670	107	677	108
		12	520	95	864	214	705	86	628	73
	Tin	Fresh	659	409	650	645	592	244	675	203
		6	770	491	785	530	852	191	782	218
		12	816	73	727	123	500	132	586	95

* Fresh = sample immediately after pasteurization.

The increase in the bacteria plate count with increasing homogenizing pressures was no doubt apparent rather than real since it has been shown by Fabian (1) that this was the case in ice cream mix. He showed that the increase in the bacteria content of the mix was due to the breaking-up of bacteria clumps and to contamination from the homogenizer. The type of homogenizer used in these experiments permitted the disassembling and thorough sterilizing of all parts so that contamination of the homogenizer may be ruled out.

The data presented in table 1 leave little doubt as to the best time and temperature to use in pasteurizing cream. There is a definite and steady decrease in the total and lipolytic plate counts as the temperature is increased even though the time was decreased.

INFLUENCE OF COPPER AND SUCROSE

1. *Non-homogenized cream.* In table 2 are presented the bacteriological results of the addition of one part per million of copper, 10 per cent sucrose and a combination of the two. An analysis of the data shows that none of them had any pronounced effect on the bacteria. In most cases the bacteria count was higher in the samples of cream to which copper and sugar were added than the control. However, the samples of cream to which both copper and sugar were added showed slightly fewer bacteria on the whole than did those samples to which copper or sugar alone were added, but more bacteria than the control. From a bacteriological standpoint there appears to be no advantage in adding sucrose to cream for storage. On the contrary, samples of cream to which sucrose was added had in general a slightly higher bacteria count.

2. *Homogenized cream.* Total and lipolytic bacteria counts were made also on samples of cream which had been homogenized at 1500 and 3000 lbs. and to which one part per million of copper, 10 per cent sucrose and a combination of the two had been added. Counts were made on samples of this cream shortly after it had been so treated and at the end of six and twelve months. These data would correspond to table 1 except one table would be the bacteriological data for cream homogenized at 1500 lbs. and the other for cream homogenized at 3000 lbs. These tables are not included since they show no additional facts not already brought out in tables 1 and 2.

The addition of one ppm. of copper and 10 per cent sugar and combinations of the two to cream homogenized at 1500 and 3000 lbs. do not materially affect the bacteria count. There is a similar increase in bacteria count of the homogenized cream as shown in table 1 and a similar difference in bacteria count in the treated samples over the controls as shown in table 2. These data simply confirmed and amplified the data already given in tables 1 and 2.

INFLUENCE OF KIND OF CONTAINER USED

The cream was stored in three different materials, glass, paper, and tin. The data in table 2 indicate that the kind of material in which cream was held during storage had no influence on the bacteria count of the cream.

INFLUENCE OF STORAGE

Bacterial analysis of samples of cream taken immediately after pasteurization and at the end of six and 12 months' storage at temperatures ranging between $-5°$ F. and $-10°$ F. showed that the bacteria gradually die (tables 1 and 2).

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SUMMARY

Bacterial plate counts of monthly samples of cream over a period of a year showed that:

1. Of the three methods used to pasteurize cream, 150° F. for 30 min., 165° F. for 15 min. and 185° F. for 5 min., the greatest reduction of bacteria occurred at the highest temperature and the shortest time.

2. The bacterial plate count of cream increased with increased homogenizing pressures.

3. The addition of one ppm. of copper, 10 per cent sucrose or a combination of the two had little or no influence on the bacteria count of the cream. There was a slight increase in the bacteria plate count in some instances over that of the control.

4. There was no significant difference in the bacteria plate count of cream stored in glass, paper, or tin, during refrigerator storage.

5. The bacteria plate count of the cream decreased during storage at $-5°$ F. to $-10°$ F. over a period of one year.

6. The addition of one ppm. of copper, 10 per cent sucrose or a combination of the two had no significant effect on the bacteria plate count of cream homogenized at 1500 and 3000 lbs. pressure.

CONCLUSION

From a bacteriological standpoint there is no reason why clean, wholesome, fresh cream cannot be pasteurized and stored for a period of one year in glass, paper, or tin containers at a temperature ranging from $-5°$ F. to $-10°$ F.

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THE USE OF FIRST RECORDS VERSUS THE AVERAGE OF ALL RECORDS IN DAM–DAUGHTER COMPARISONS WHEN PROVING SIRES*

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INTRODUCTION

Since the acceptance of the proved sire theory as a positive instrument in dairy herd improvement several methods of measuring the approximate transmitting ability of sires have been advocated. Most of these methods are based on dam-daughter comparisons, but there has been a constant shifting of ideas as to the type of record, or records, to be used in making these dam-daughter comparisons.

Since the Bureau of Dairy Industry and the breed registry organizations have changed from the use of calendar-year records to the use of lactation records, all dam-daughter comparisons are based on lactation records of not over 365 days in length. Records, in order that they may be compared readily, are, in most cases, placed on an equivalent basis and there has been a rather general acceptance of the mature equivalent as the proper basis for comparisons. With the choice of using the first records, the best records or the average of all available records there has been a tendency to shift from the comparison of single equivalent records for dams and daughters to a comparison of the average of all mature equivalent records of each dam and daughter (1, 2, 4, 6, 10). The use of averages has been injected into the mechanics of determining dam-daughter comparisons because of the fact that the average of all records gives a better picture of the life-time production of the animals involved.

When the Ayrshire Breeders' Association adopted its "Approved Sire Plan," a condition of the plan was that all dam-daughter comparisons should be reported on the basis of first dam and daughter records calculated to a mature equivalent, two-time milking, 305-day record, and also on the averages of all records of both dams and daughters after they had first been converted to mature equivalents. These two calculations of dam-daughter comparisons for each sire presented an opportunity to compare the two methods and to determine the amount of variation between them.

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[2] Ayrshire Breeders' Association, Brandon, Vermont.

PRESENTATION OF DATA

At the time this study was undertaken 169 Ayrshire sires had been analyzed under the rules of the Ayrshire Approved Sire Plan (1). Each sire had a minimum of 10 dam-daughter pairs and a total of 3388 dam-daughter comparisons were involved. The percentage variation between the averages obtained by using first records only and by using the average of all records, and the percentage variation between the equal parent indices obtained from the two different averages were calculated. These results are

TABLE 1

Frequency distribution of percentage variations between the averages of first records and the averages of all records for 169 sires

Class mark	Daughters' average		Dams' average		Sire indices	
Per cent variation*	Milk	Fat	Milk	Fat	Milk	Fat
−28						
−26						
−24	Class interval = 2% (1.00 to 2.99)					
−22					1	
−20					0	
−18					1	1
−16					4	1
−14					1	4
−12				1	3	4
−10		1	4	1	4	7
− 8	3	1	5	3	9	8
− 6	4	3	4	6	7	8
− 4	7	5	17	17	18	13
− 2	12	16	21	16	15	15
0	63	53	29	19	15	21
2	42	41	28	27	32	28
4	20	28	22	31	12	17
6	12	10	10	17	9	13
8	3	6	13	10	18	10
10	2	4	6	5	6	9
12	1	0	6	10	7	3
14		1	2	3	2	2
16			1	0	2	1
18			1	1	0	1
20				1	0	1
22				1	1	1
24					0	1
26					0	
28					2	
Total number	169	169	169	169	169	169

* The minus variations indicate that the averages of first records are lower than the averages of all records.

shown in a frequency distribution in table 1. The percentage variation was determined by dividing the first records by the average of all records in every case.

Table 2 shows correlation coefficients for daughters' records, dams' records, and sires' indices when calculated on first records and on the average of all records.

Table 3 contains coefficients of variation of averages of first records and the averages of all records.

TABLE 2

Correlation coefficients for daughters' records, dams' records, and sire indices when calculated on first and on the average of all records

First versus average of all records for	Correlation coefficient
Daughters'—Milk production	0.9506
—Fat production	0.9532
Dams'—Milk production	0.8743
—Fat production	0.8679
Sire indices—Milk production	0.9199
—Fat production	0.9345

DISCUSSION

A sharp distinction should be drawn between the use of records in the proving of sires and their use in measuring the life-time production of individual animals or even in comparing, individually, mother and daughter production records. In the proving of sires, the average production of the acceptable number of dams used in sire proving is taken as being representa-

TABLE 3

Coefficients of variation of first records and the average of all records for use in proving sires

	Milk production		Fat production	
	First records	All records	First records	All records
Daughters' production				
Mean (pounds)	8960	8912	369	363
Standard deviation (pounds)	957	897	40	43
Coefficient of varietion (%)	10.68	10.06	10.84	11.84
Dams' production				
Mean (pounds)	8971	8835	365	356
Standard deviation (pounds)	982	847	41	37
Coefficient of variation (%)	10.95	9.59	11.23	10.39
Sire index				
Mean (pounds)	9091	8992	374	372
Standard deviation (pounds)	1808	1887	78	85
Coefficient of variation (%)	19.88	20.98	20.85	22.84

tive of the production possibilities of the total field of inheritance represented in the group of dams. On this field the sire is used, and the average production of the daughters resulting compared to the average production of the dams gives the basis upon which the sire may be evaluated when dam-daughter comparisons are used. This being the case it should make no

difference how few or how many records are available for the several animals involved, or how many or how few of these records are used, provided, that regardless of the number of records used they give, with insignificant variations, the same results. On the other hand, the average of all records may have a definite use in making individual comparisons and in indexing individual females.

The averages of the dam-daughter comparisons for the 169 sires included in the study show that the average based on first lactations of daughters is 8960 pounds of milk and 369 pounds of butterfat, while the average based on the averages of all records is 8912 pounds of milk and 363 pounds of butterfat—a difference of 48 pounds of milk and 6 pounds of butterfat. In the case of the dams involved in these comparisons the average of their first lactations is 8971 pounds milk and 365 pounds butterfat while the average based on the averages of all records is 8835 pounds of milk and 356 pounds of butterfat—a difference of 136 pounds of milk and 9 pounds of butterfat. These differences amount to only 0.5 per cent for milk and 1.65 per cent for butterfat in the case of the daughters and 1.54 per cent for milk and 2.53 per cent of the fat for dams, with the average of all records taken as a base.

The equal-parent indices calculated from the dam-daughter comparisons by both methods are 9091 pounds of milk and 374 pounds of butterfat when calculated on first dam-daughter records, and 8992 pounds of milk and 372 pounds of butterfat when calculated by the use of the averages of all records. Here again differences are only 99 pounds of milk and 2 pounds of butterfat, and if the statement—"If the difference between the average butterfat production of the dams and that of the daughters is less than 25 pounds, the increase or decrease should not be considered as significant" (2)—is accepted as a practical rule to follow in determining significant differences, then the differences secured from the averages of all these indices are not significant. In fact, for 98.2 per cent of the sires the differences for their daughters between the two methods was less than 10 per cent for milk, and for 98.8 per cent of the sires the difference for fat for the daughters was less than 10 per cent. For the dams 92.3 per cent and 88.2 per cent did not exceed a 10 per cent difference for milk and fat. It should be remembered that if the first records were taken as a base the averages of all records would show the same ranges, and for this reason the correlation coefficients give a better picture of the relationship of the two types of calculations. Table 2 shows the correlation coefficients for daughters' records, dams' records and sire indices when calculated on first records and on the average of all records.

One of the most frequently heard objections to the use of first records only in the proving of sires is that the method is unfair to the sire whose daughters are slow-maturing and come into maximum production slowly. To determine whether or not the supposed slow-maturing factor is deserving of consideration when adopting a method for proving sires, a check was

made of the per cent differences in the indices contained in table 1. It is of interest to note that in more than 50 per cent of the cases the indices based on first records for either milk or butterfat production are higher than those based on the average of all records. In the case of the daughters' and dams' records the averages of the first records are higher than the averages of all records in 66 per cent and 61 per cent respectively for milk and 68 per cent and 69 per cent respectively for butterfat. The data in table 1 show that in no case is there a difference of as much as a minus 10 per cent between the average production of the daughters of any sire calculated by the two methods. There is a difference of minus 10 per cent or more between the indices for milk calculated by the two methods for only 11 of the 169 bulls included in this study. These 11 bulls have been investigated further and in 9 of the 11 cases satisfactory evidence was found that eliminated a slow-maturity characteristic as the reason for the difference between the average of the first records and the average of all the records for these bulls. The reasons for the variations and the number of sires affected are as follows:

(a) Daughters calving for the first time at such an early age that they could not make what would be considered normal records. (9) One sire.

(b) Daughters bred back so quickly after calving that the effect of pregnancy had a great effect upon the first lactation. One sire.

(c) The average of all of the records of the dams was much lower than the average of the first records while the averages of the daughters' records did not vary appreciably. This made the index based on the average of all records much higher. Four sires.

(d) A change in herd management which was reflected in the records. Two sires.

(e) Heavy culling of daughters after the completion of their first lactations. Those retained raised the average through the influence of subsequent lactations. One sire.

It was not possible to draw conclusions on the other two bulls from the available information. It is apparent, however, from the data available for this study that the claim of slow-maturity as a characteristic of the daughters of some bulls is not worthy of consideration in the development of a plan for reporting dam-daughter comparisons.

In recent years there has been an increased rapidity in reporting dam-daughter comparisons by breed registry organizations and the Bureau of Dairy Industry. As these organizations adopt more modern methods of recording and reporting sire data the rapidity of this service will be increased to the place where there will be only the first records of daughters to report in an increasingly large number of cases. Under such conditions why include the average of all the dams' records? Lush and Straus (7) have shown in their study involving heritability of butterfat production that in their dam-daughter comparisons the daughters had an average of less

than two lactations while the dams had an average of over three lactations. Furthermore, in cases where there are records in addition to the first record for some of the daughters of a bull, and when some of the poorer daughters have been culled, the use of the averages of all records gives a distorted value since the addition of good records to the average without a similar addition of an equal number of poor records for the poorer culled cows would raise the figure which is to represent the sires average transmitting ability. According to Perry (8) "This question has particular reference to the dams since most bulls are proved by the 2-year-old lactation record of the daughters." In fact, the use of unselected first records for daughters of a sire will give the fairest picture of the transmitting ability of the sire; and while some selection will have occurred among the dams of the daughters of the sires, the effect of this will be reduced to a minimum under a continuous herd testing program.

It is generally agreed that environmental factors exert a smaller influence on the first lactation of a cow than they do on any subsequent lactation. Kendrick (5) states that, "Variations found in lactation records may be largely attributed to six general causes: 1) Differences in management and feeding; 2) differences in length of lactation periods; 3) differences in number of times cow is milked each day; 4) differences in the degree of influence of gestation; 5) differences in ages; and 6) differences in inheritance of cows." If we were to add to Kendrick's list, differences due to disease and injury the field would be well covered. In the case of each of these causes, with the exceptions of frequency of milking and inheritance a smaller influence is exerted on the first lactation than on any other lactation. This is substantiated in the exhaustive study of the Penshurst herd made by Heizer (3), since he found, from the use of the first record, the average of all records, and the best record that "The Herd Test mature equivalent calculated on the first record seems to have a little higher prediction value than any of the other records utilized in this study."

Any dam-daughter reporting agency, whether it may be a state or federal agency or a breed registry organization is limited in the amount of service it can render by the funds available for this activity. Reporting dam-daughter comparisons on the basis of first records eliminates a large amount of the labor necessary in reporting dam-daughter comparisons where averages of all records must be calculated. Furthermore, the coefficients of variation presented in table 3 show that statistically there is little difference in the variability of the two methods. This indicates that both methods are equally reliable and the use of first records could be chosen with no sacrifice of accuracy. The labor saved by using first records only could well be used in a further expansion of the program or in related activities.

SUMMARY

A comparison was made of first dam-daughter 305-day mature equivalent records, and the averages of all records on a similar basis, in reporting dam-daughter comparisons for dairy sires. A comparison of these data for 169 Ayrshire sires and 3388 dam-daughter pairs shows there is only a very small and insignificant difference in the results obtained by the two methods. The averages of dams' and daughters' records and the averages of sire indices calculated by the use of both types of comparisons show that the first records on a mature equivalent basis average slightly higher than the averages of all records on the same basis. It is suggested that a real saving can be made in the labor required to report dam-daughter comparisons by using first records only in dam-daughter comparisons.

ACKNOWLEDGMENTS

The authors are indebted to Professor E. J. Wellhausen of the Department of Agronomy and Genetics for advice in the statistical treatment of the data. The authors also acknowledge the assistance of Mr. Donald B. Trombly and his staff of the Division of Records of the Ayrshire Breeders' Association for their help in assembling the data.

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ANNOUNCEMENT

Report of the Curricula Committee of the American Dairy Science Association

The final report of the Curricula Committee of the American Dairy Science Association has been compiled and submitted to the Board of Directors by Professor H. P. Davis. This final report has been mimeographed for distribution in the office of the Secretary of the Association and copies may be obtained by writing to Professor R. B. Stoltz, Secretary-Treasurer of the American Dairy Science Association, Ohio State University, Columbus, Ohio.

Those interested in the teaching of college students in dairying and those in administrative positions who are responsible for the establishment of policy regarding educational objectives and the building of curricula will be interested in this report which represents the combined thinking of approximately 100 members of the Association who have actively participated in the work of this committee over the past five years.

The report contains recommended curricula for both *dairy production* and *dairy manufacturing* majors; giving minimum course requirements and suggested electives. The report also contains some background discussion outlining educational objectives and the academic approach.

The Editor

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ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
Columbus, Ohio

MILK AND MILK PRODUCTS

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JOURNALS

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Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEWS

432. Annual Review of Biochemistry, Volume 12. Annual Reviews, Inc., Stanford University P. O., California. 24 sections, 704 pages, including author and subject index; $5.00.

This twelfth volume of Annual Reviews is prepared in a manner similar to that employed for previous issues, wherein certain subjects in the broad field of biochemistry are reviewed at the request of an editorial board. These reviews are both compilation of the work reported on the subject, and brief interpretation of its significance. The volume is distinctly a tool of the scientific worker. Of the 24 sections, several are of immediate value to the research worker in the dairy industry field. In Biological Oxidations and Reductions, is included a resumé of recent developments of the systems involving nicotinamide, thiamin, and flavin enzymes, plus enzyme functions attributed to other vitamins as pantothenic acid and tocopherol. In Proteolytic Enzymes, is included reference to recent studies on pepsin and rennin. The section The Steroids includes review of the bile acids and sterols. In two sections on the Chemistry of Proteins and Amino Acids is reviewed the work on protein behavior, structure and physical-chemical constants. Of current interest in the field of dairy nutrition is the chapter, Fat Metabolism, which includes discussion of the work on intestinal absorption, digestibility, excretion and nutritive value of fats. In separate sections is covered Protein, and Carbohydrate Metabolism, and the Chemistry of Carbohydrate, and Lipins. In Mineral Nutrition is reviewed the work on utilizability and influence of the various minerals. The Chemistry of the Hormones is a review of the protein hormones, with a brief review of the steroid hormones. The Water Soluble Vitamins, and Fat Soluble Vitamins, contain complete review of the more recent work on assay, stability and function of the various factors. The section, Nutrition, is an appraisal of the dietary requirement levels. Other chapters in the volume are: Animal Pigments, Synthetic Drugs, Photosynthesis, Mineral Nutrition of Plants, Carbon Dioxide Assimilation in Heterotrophic Organisms, Biochemistry of Organisms, including growth factors, vitamin synthesis, and antibacterial agents, The Electron Microscope in Biology, Viruses and Microchemistry.

K.G.W.

433. Refrigerating Data Book, 1942. Fifth Edition. American Society of Refrigerating Engineers. 1943.

This volume is practically a revision of the 1939 book, Volume I. The 1941 edition was published as Volume II and was devoted to applications

of refrigeration. This fifth volume comprises five parts covering in Part I, Refrigerating Cycles; Part II, Fundamental Data; Part III, Industrial Systems; Part IV, Domestic-Commercial System; Part V, Air Conditioning Systems. While fundamentals in refrigeration remain the same, newer treatment will be found to have been given many of the subjects in the 1942 Data Book, and more comprehensive tabular material will be noted.

L.M.D.

BREEDING

434. Seasonal Variation in the Semen of Bulls. RALPH W. PHILLIPS, BRADFORD KNAPP, JR., LOUIS C. HEEMSTRA AND ORSON N. EATON, Beltsville, Md. Amer. Jour. Vet. Res., *4*, No. 11: 115. April, 1943.

Studies were made on semen from three beef type and three milking type shorthorns at two week intervals throughout the year. Observations were made on motility, volume, number per cc., total number of sperm, proportions of abnormal heads, necks, middle pieces, tails and total abnormal spermatozoa.

Differences between breeds were significant only in volume and total sperm; between bulls in all but volume, and between seasons in number per cc., total sperm, and proportion of abnormal heads, necks, middle pieces and tails. Increase of abnormal tails occurred in winter; of others, in summer.

Examination of results of 1,135 matings on normal cows over a period of eight years revealed the highest percentage of fertile matings in April and the lowest in August, corresponding roughly to differences in semen quality.

S.A.F.

435. Artificial Insemination of Dairy Cows. E. J. PERRY AND J. W. BARTLETT. N. J. Agr. Col. Ext. Bul. 235. 20 pages, illus. April, 1943.

A brief account of the history, possibilities, limitations and organization procedure of artificial insemination associations, together with detailed directions for management of the bulls used, and the technique of semen collection and of the insemination of the cow.

11 figures.

J.G.A.

436. Conception Rate in Dairy Cattle by Artificial Insemination at Various Stages of Estrus. G. W. TRIMBERGER AND H. P. DAVIS. Nebr. Agr. Expt. Sta. Res. Bul. 129. 14 pages. April, 1943.

A breeding experiment with 295 dairy cows and heifers of the Jersey, Guernsey, Ayrshire, and Holstein breeds was conducted in the University of Nebraska dairy herd. The females were given artificial services at various stages of estrus to determine the effect of time of service upon conception. The breeding results, expressed as percentages of conception from one

insemination in the females bred at various stages of estrus, were as follows: start of estrus, 44.0; middle of estrus, 82.5; middle of estrus and rebred in 24 hours, 84.0; end of estrus, 75.0; six hours after estrus ended, 62.5; 12 hours after estrus ended, 32.0; 18 hours after estrus ended, 28.0; 24 hours after estrus ended, 12.0; 36 hours after estrus ended, 8.0; and 48 hours after estrus ended, none conceived. During the 35 months of this experiment, 194 females were bred by artificial insemination in the routine breeding in the university herd, and 123 or 63.4% of the females conceived at the first insemination. This large group can be used as a normal standard for a basis of comparison.

Although the individuals were closely watched and observed very frequently, 22% of the females in which conception failed to take place did not return in heat at the next expected period. Silent heat periods were frequently observed among these females but no intermittent heat periods were found.

The results obtained indicate the potential benefits in higher percentage of conception in females bred during the middle of estrus, toward the end of estrus, and those bred in full heat and rebred in 24 hours. The latter is usually not very practical, especially in artificial breeding rings, but there are occasions when it may be desirable to follow this procedure. The results also show that a good rate of conception is obtained in females bred as late as six hours after the end of estrus. The low rates of conception in experimental groups bred long after the end of estrus indicate that it is seldom practical to breed females later than six hours after estrus ends. Statistical treatment of the data verified these conclusions.

4 tables; 43 literature references. J.G.A.

BUTTER

437. Water Supplies of Butter Manufacturing Plants. R. T. Cosley, H. F. Long and B. W. Hammer. Iowa Agr. Expt. Sta. Res. Bul. 319. May, 1943.

The water supplies of 70 butter plants in Iowa were collected for detailed study over a period of 18 months. The examination included tests for total bacterial count, Escherichia-Aerobacter organisms, proteolytic and lypolytic organisms, *Pseudomonas putrefaciens,* and butter keeping quality tests.

It was found that more than half of the water supplies were satisfactory. Some supplies meet public health requirements but contained butter spoilage organisms. Some municipal water supplies were in this category. Some supplies varied in quality from time to time. When coliform organisms were present in the wash water they were found in 1-ml. samples of unsalted butter, usually in 0.1-ml. samples of unsalted butter, and sometimes were detected in salted butter. The use of tryptose lauryl-sulfate broth gave

fewer false presumptive coliform tests than standard lactose broth. The wash water with highest bacterial counts generally gave greatest butter deterioration but there were some exceptions. Some Pseudomonas species were isolated. It was found that water storage tanks were often the source of contamination. Chlorination of water supplies is recommended.

Standards for water for butter plants are proposed to include (1) compliance with U. S. Public Health Service Standards and (2) low bacterial counts and freedom from organisms causing butter spoilage.

A.C.D.

CHEESE

438. Use of Rennet Paste in Making Romano Type Cheese. C. A. PHILLIPS, Univ. Cal., Davis, Cal. Natl. Butter and Cheese Jour., *34,* No. 6: 13. June, 1943.

Domestically produced rennet paste is a semi-solid brownish or grayish colored product prepared from dried stomachs of lamb or kids and sometimes calves. These may or may not contain some milk. The odor is like rennet although one sample had a slight spicy odor. It keeps well at 33° F. It is diluted with water and filtered through cloth before using. Seventy-three 20-pound cheese were made over a two-year period. Part skim milk ripened one hour with *S. lactic* starter was coagulated with 8 oz. of paste per 1000 lbs. of milk. The curd was finely cut in 30 minutes and heated to 104° F. for 90 minutes. Salt to give 1.1% in the cheese was added to the curd; salting continued for 3 weeks at 60° F. until 5.5 to 6% was incorporated. Cheese were treated with cottonseed oil and black pepper each month for one year at 50° F. Yields approximated 8 pounds. Rennet paste, unlike rennet extract, gives the typical sharp, piquant flavor of Italian Romano. This domestic cheese, like the Italian product, contains 40% fat in the dry matter and 33 to 39% water. W.V.P.

439. Sampling Semi-Hard Cheeses. J. C. MARQUARDT, N. Y. Agr. Expt. Sta., Geneva, N. Y. Natl. Butter and Cheese Jour., *34,* No. 4: 16. April, 1943.

The simplest and most satisfactory way to sample a semi-hard cheese is to slice it into two parts. A method which does not ruin the cheese for sale is to remove a disc-like area from the surface with a knife blade 4 inches long by $\frac{1}{4}$ inch wide. Inserting the knife to the center of the cheese and then lifting indicates firmness; if the cheese does not drop off it is too firm, if it cannot be lifted it is too soft. Size and temperature must be considered. Holding cheese at room temperature for a few weeks indicates "shelf-life." W.V.P.

440. Oregon's Practical Licensing Law. G. H. WILSTER, Oreg. State Col., Corvallis, Oreg. Natl. Butter and Cheese Jour., *34*, No. 3: 14. March, 1943.

Oregon for 2½ years has been licensing cheesemakers and buttermakers under an improved, popular and successful system which is based on written, oral and practical tests. The examining board may later include psychological tests. Successful candidates must demonstrate a knowledge of principles of dairy sanitation, practical manufacture, and factory tests. W.V.P.

CHEMISTRY

441. Determination of Nicotinic Acid. Modifications in the Microbiological Method. W. A. KREHL, F. M. STRONG AND C. A. ELVEHJEM, Col. of Agr., Univ. Wis., Madison. Jour. Indus. and Engin. Chem., Analyt. Ed., *15*, No. 7: 471. July, 1943.

Certain modifications in the Snell-Wright microbiological assay for nicotinic acid are described. Changes are proposed in the basal medium and a different procedure for growing the necessary inoculum is described. The variations have resulted in nearly doubling the response of the bacteria to quantitities of nicotinic acid in the range 0.04 to 0.3 microgram, and have produced a linear standard curve. Among the results given for various food materials, skim milk powder, by the 72-hr. assay, contained 8.2 micrograms of nicotinic acid per gram. The assay, modified as recommended, is capable of a higher degree of consistency and uniformity than was usually obtained with the original method. B.H.W.

442. Adsorption of Riboflavin by Lactose. Influence of Concentration. ABRAHAM LEVITON, Bur. Dairy Indus., U. S. Dept. Agr., Washington, D. C. Jour. Indus. and Engin. Chem., Indus. Ed., *35*, No. 5: 589. May, 1943.

Lactose selectively adsorbs riboflavin as it crystallizes from whey concentrates. The relation between degree of adsorption and initial riboflavin concentration under conditions of complete crystallization is linear. A minimum concentration of riboflavin exists below which no adsorption occurs. Under conditions of incomplete crystallization the value of the riboflavin concentration below which no adsorption occurs is displaced toward lower and lower values as the degree of supersaturation with respect to lactose is lowered, and reaches a final minimum value of 2.5 micrograms per ml. It has been found practical to prepare lactose containing as high as 300 micrograms riboflavin per gram. Much higher concentrations may be realized, but the rate of crystallization under the conditions required for the preparation of the more concentrated adsorbates becomes exceedingly

slow. Concomitantly, the lactose crystals change from a characteristic tomahawk to a thin platy form. Riboflavin exerts a definite retarding action on the rate of crystallization of lactose, an effect which becomes more and more pronounced as the riboflavin concentration is increased, and as the degree of supersaturation with respect to lactose is decreased. B.H.W.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

443. Control of Bacteria in Dry Milk. P. S. PRICKET, Mead-Johnson & Co. Natl. Butter and Cheese Jour. **Part I. Effect of Processing,** Vol. *34,* No. 6: 16. June, 1943. **Part II. Meeting Standards,** Vol. *34,* No. 7. July, 1943.

Part I. Bacteria must have moisture, food, favorable temperatures and time to grow. In plant practice, the separator may contribute bacteria; thermophilic bacteria may grow while milk is in hot wells and vacuum pan; condensing water may contaminate milk; storage tanks and homogenizer offer favorable growth conditions; and air-borne contamination may enter the spray drier. Spray drying is not as effective in destroying bacteria as the atmospheric roll process. Clean, careful packing of powder is essential.

Part II. Standards of the American Dry Milk Institute are 15,000, 50,000 and 100,000 per cc. of reconstituted milk for Extra, Standard and Third grades, respectively. To meet these standards the raw milk should be of low bacterial content as determined by methylene blue reduction test, Breed microscopic count or plate count. Proper plant sanitation requires an efficient can washer, safe water supply, sterilization of equipment, elimination of dead ends and pockets in pipelines and equipment and careful cleaning of pumps. Laboratory help should locate time and source of contamination. Thermophilic organisms in dry milk may cause gastro-enteritis in infants and undesirable chemical changes in milk constituents which injure the product for commerical use. Incubation of bacterial plates at 55° C. for 48 hours is recommended to detect thermophiles. A trained, interested, intelligent and cooperative personnel is highly desirable in laboratory and production work to produce clean, dry milk. W.V.P.

444. Keep 'Em Spraying. HANS EDEL, Maple Island Farm Creamery, Stillwater, Minn. Natl. Butter and Cheese Jour., *34,* No. 5: 16. May, 1943.

This practical article indicates methods by which quality of product and efficiency of production are maintained by one company making spray process dry milk. Details discussed emphasize the value of high quality milk; the advantage of precondensing to 35 to 40% solids, the use of proper temperatures of preheating; the necessity of cooling of surplus concentrate to be dried while the pan is being cleaned; the need of checking homogenizer

packings, valves, by-pass connections, strainers, etc., the good results of keeping temperatures of 140°–160° in the drying chamber, and the necessity of strict cleanliness of milk handling and drying equipment as well as air filters and flue bags. Detailed records of milk quality, concentration, temperatures and pressures and laboratory analyses help improve efficiency. W.V.P.

445. The Dry Milk Industry. MERRILL O. MAUGHAN, Amer. Dry Milk Inst., Chicago, Ill. Natl. Butter and Cheese Jour., *34,* No. 3: 10. March, 1943.

Despite the derogatory name "dry skim milk" the dry milk industry has increased its output 30 times over production 25 years ago and this trend will continue for the war's duration. Beyond the war the future looks good for dehydrated foods with a few exceptions. Skimmilk must be conserved by drying for the markets being developed. All branches of the dairy industry are interdependent; perhaps milk should be rationed to plants and all farmers paid the same figure. Quality must be guarded, research and educational work carried on, production of milk encouraged, and sound business practices observed. W.V.P.

446. A Method of Smoothing Drier Drums. ANONYMOUS. Natl. Butter and Cheese Jour., *34,* No. 3: 24. March, 1943.

A wet-sanding method of cleaning and smoothing drier drums keeps drums in working condition without dismantling. A sanding bar which is made of ground level channel iron $1\frac{1}{2}'' \times 1\frac{1}{2}'' \times \frac{3}{16}''$ as long as the drum, a piece of hardwood to fit the channel iron, Scotch masking tape and 6″ wide strips of special sanding cloth, is fastened to the knife blade and tightened against the revolving drum with the knife adjusters. W.V.P.

DISEASE

447. An Epic Investigation of Streptococcal Fever. C. S. LEETE, Prin. Milk Sanit., State Dept. of Health, Albany, N. Y. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 91. 1942.

The milkborne outbreak of streptococcal fever that was investigated consisted of 23 cases. Twenty of the cases were employees of a dairy company and three were carpenters repairing the plant. Twenty-two of the cases drank raw milk and one ate green cheddar cheese curd made from the raw milk.

The plant received 123,000 pounds of milk produced by 6,069 cows in 331 herds and the milk was delivered in 2,029 cans. Loop smears of milk from each cow were stained with Newman's No. 2 stain. Samples with long chain streptococci or cell counts of 500,000 or more were considered suspicious. On

this basis 23 herds and 1931 animals were suspicious. These cows were physically examined and each quarter tested with brom-thymol-blue. The suspicious herds were now reduced to 55 with 150 suspicious cows. Samples of milk from the 150 cows were sent to the laboratory for detailed examination. One quarter from one cow gave milk containing hemolytic streptococci, Lancefield group A. This quarter was positive to strip cup and brom-thymol-blue, and the teat had been injured and a teat dilator used just prior to the outbreak. A person with a throat infected with the organism involved had treated the injured teat. A.C.D.

448. Gramicidin and Tyrothricin Therapy in Chronic Streptococcic Mastitis. RALPH B. LITTLE, Dept. Anim. and Plant Path., Rockefeller Inst. for Med. Res., Princeton, N. J. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 107. 1942.

The crude alcoholic extract of cultures of *Bacillus brevis* is known as tyrothricin. It may be separated by crystallization into tyrocidin and gramicidin, the latter being germicidal only to gram-positive microorganisms. The solution may be diluted with water or mineral oil before injection into the milk cistern.

Most investigators agree that gramicidin and tyrothricin treatment of bovine mastitis has definite merit and is more effective that Entozon, acriflavine, trypaflavin, or Novoxil. The treatment should be given by a veterinarian and the herd carefully managed for the control of mastitis. A.C.D.

449. An Anaerogenic Variant of *Salmonella enteritidis* (Gaertner) Isolated from an Enzootic Among Domestic and Wild Rats. ROBERT A. BRUCE AND HARRY E. DASCOMB, Univ. Rochester, School of Medicine and Dentistry, Rochester, N. Y. Jour. Infect. Dis., *72,* No. 2: 157–159. Mar.–Apr., 1943.

Nine strains of an anaerogenic variant of *S. enteritidis* were isolated from rats in an enzootic in the rat colony and in wild rats. This variant was agglutinated to a titer of 1: 10,240 with homologous anti-serum, whereas agglutination with anti-*Eberthella typhosa* serum was only 1: 80. When the homologous serum was tested, it agglutinated each of two laboratory strains of *S. enteritidis* strains with a titer of 1: 5, 120; *E. typhosa,* 1: 80; and *S. schottmulleri,* 1: 20. The antigenic structure of the variant was IX, XII: g, m (Kaufmann). The authors emphasize that the routine identification of "non-lactose fermenters" by fermentation reactions alone, without serological procedures, is not adequate. J.F.C.

450. The Action of Tyrothricin on Fecal Streptococci in Vitro and in Vivo. ENID C. RODANICHE AND WALTER LINCOLN PALMER, Dept.

of Medicine, Univ. Chicago. Jour. Infect. Dis., *72*, No. 2: 154–156. Mar.–Apr., 1943.

Thirty recently isolated fecal strains of streptococci were tested, together with 5 strains of throat streptococci included for the purposes of comparison. One-tenth ml. of a suspension containing approximately 4,000,000 per ml. of the test organism was inoculated into each of 8 tubes of veal infusion broth. Tyrothricin solution was added to each tube in amounts graded to give final concentrations ranging from 0.0001 to 0.2 mg. per ml. The tubes were incubated 24 and 48 hours. In tubes showing visible growth the concentration of tyrothricin was considered inactive. Transfers of 0.1 ml. were made from tubes showing no growth into fresh tubes of broth, and after 24 and 48 hours incubation transfers were made from these secondary tubes that showed no growth. When no growth occurred in subculture, the concentration of tyrothricin in the original tube was considered bactericidal, but when growth did occur in subculture the concentration was considered only bacteriostatic. The fecal strains appeared to be no more resistant to the action of the tyrothricin than did the throat strains. There were rather wide differences in resistance between strains within each group that could not be explained on the basis of cultural or morphological differences. Tyrothricin added to the diets of white mice in a concentration of 2% of the diet and fed *ad lib.* to the mice caused a marked reduction in the number of streptococci that could be isolated from rectal swabs or from the feces. This inhibition of streptococci in the intestines was more easily demonstrated when 3% of sulfasuxidine was added to the diet to inhibit the coliform organisms. J.F.C.

451. Studies on Vitamin A. Vitamin A and Total Lipid of the Serum in Pneumonia. HUGH W. JOSEPHS, Johns Hopkins Univ., Baltimore, Md. Amer. Jour. Dis. Children, *65*, No. 5: 712–727. 1943.

The vitamin A, carotene, and total lipid of the blood serum are reduced in pneumonia. The reduction of these three elements exhibited no regularity, except that the longer the duration of the illness the fewer the values that approached normal. Vitamin A values fell most rapidly of the three elements studied.

During convalescence the serum lipids of children over two years of age tended to rise to a point well above normal and to remain there for a considerable period of time, but in children under two years of age there was little tendency for such a rise unless vitamin A supplement was given.

The rise in serum carotene in four to five days after the injection of a carotene supplement was begun was related to the average lipid level during this period. Also there appeared to be a definite correlation between lipid levels and the post-absorptive rise of vitamin A in the blood serum.

The abnormal rise in serum lipids in older children and in younger children receiving vitamin A during convalescence was compared with a similar lipid rise in rats given excessive doses of vitamin A. R.K.W.

452. Determination of the Number of *Brucella abortus* Organisms Required to Infect Guinea Pigs and Cattle. L. S. HUTCHINGS AND I. F. HUDDLESON, East Lansing, Mich. Amer. Jour. Vet. Res., *4*, No. 11: 155. April, 1943.

Strains of *Br. abortus* were suspended in a broth containing 0.1% tryptose and 0.5% sodium chloride in distilled water and standardized to pH 6.9 after sterilization. Suspensions were standardized by a photelometer and checked by plating. Ten organisms of different strains of *Br. abortus* and *Br. suis* were infective to guinea pigs and showed increased virulence on continued passage. B. A. I. strain 19 was infective to guinea pigs in a dose of approximately five million organisms but not of 900,000 organisms.

Approximately five million *Br. abortus* organisms of a recently isolated culture infected susceptible pregnant heifers when deposited in the conjunctival sac. S.A.F.

453. The Distribution of *Trichomonas foetus* in the Preputial Cavity of Infected Bulls. DATUS M. HAMMOND AND DAVID E. BARTLETT, Beltsville, Md. Amer. Jour. Vet. Res., *4*, No. 11: 143. April, 1943.

A total of 15 examinations were made of the penis and prepuce of three artificially and one naturally infected bulls to determine the distribution of *Trichomonas foetus*. Light scrapings were made of definite areas and in eight cases the urethra was flushed.

"Trichomonads were usually found in highest numbers on the portion of the glans penis not including the galea glandis, with relatively lower numbers scattered over the remainder of the penis and prepuce. Variations in general level of numbers and in distribution of trichomonads were noted both in different individuals and in the same individual at different times. All flushings from the lower urethra were negative.

"The findings with respect to distribution of trichomonads in the preputial cavity indicate that the accuracy of diagnosis in bulls will be improved by making certain that in obtaining samples material is collected from the surface of the glans penis and adjacent prepuce. In the application of treatment this area should receive special attention." S.A.F.

454. The Effect of Chemotherapeutic Agents on the Normal Bovine Mammary Gland: 1. The Effect of Novoxil. W. G. ANDBERG AND F. J. WEIRETHER, St. Paul, Minn. Amer. Jour. Vet. Res., *4*, No 11: 134. April, 1943.

"In a series of three experiments a total of 18 apparently normal quarters of eight cows were infused with Novoxil to determine its effects upon apparently normal lactating mammary glands.

"The infusions produced a condition simulating acute mastitis. The glands became enlarged, tender, and firm; the milk became abnormal in consistency; and the percentages of the milk chlorides increased and those of the lactose decreased. This was accompanied by an increase in polymorphonuclear leucocytes in the milk.

"Five of the six cows slaughtered four weeks after infusion showed macroscopic evidence of the irritation localized in the cistern in the form of numerous small, raised, reddish, granular foci, some of which were covered with milk clots. Fibrotic nodules also developed in this region. In four of the 16 infused quarters, palpable thickenings were noted in the teat canals in the preinfusion period. In one such quarter, a fibrotic nodule developed within three weeks after infusion, resulting in occlusion of the milk flow.

"Two cows passed bloody urine one day after infusion. One of the cows had a history of previous urinary disturbance. However, a subsequent negative uranalysis on two cows following infusion suggested that Novoxil possibly stimulated a previous urinary infection but did not initiate such a condition.

"Novoxil used in the first experiment was kept at room temperature for approximately one year. When shaken, it was difficult to incorporate the silver particles into the oil and the suspension was stable for only a short time. Fresh preparations of Novoxil, used in the next two experiments, suspended easily and settled slowly.

"No apparent permanent changes in the udder were noted macroscopically nor microscopically in two cows four and seventeen months after infusion with Novoxil (one year of age)." S.A.F.

FEEDS AND FEEDING

455. Annual Lespedeza for Florida Pastures. J. D. Warner and R. E. Blaser. Fla. Agr. Expt. Sta. Bul. 375. 22 pages, illus. Sept., 1942.

This bulletin discusses varieties of lespedeza, their adaptation to Florida soils, and their fertilizer requirements. Practical suggestions for the establishment of lespedeza pastures are included. J.G.A.

456. Feeding Value of Whey. W. V. Price, G. Bohstedt and I. W. Rupel, Univ. Wis., Madison. Natl. Butter and Cheese Jour., *34*, No. 6: 64. June, 1943.

This is a summary of timely information which indicates that the half of the total milk solids present in whey include valuable carbohydrates,

proteins and minerals, as well as liberal amounts of vitamins B_1, B_2 and C. Specific instructions are given for feeding whey to calves and pigs. Whey can be used as a substitute for mineral acid or molasses in making grass silage. W.V.P.

457. Supplementing and Improving Dairy Pastures. W. B. NEVENS. Ill. Agr. Col. Ext. Cir. 553. 8 pages, illus. March, 1943.

This circular contains (1) recommendations for emergency pasture crops as supplements to bluegrass, and (2) general recommendations for pasture management. J.G.A.

458. Comparison of Molasses-Alfalfa Silage and Phosphoric Acid-Alfalfa Silage as Feeds for the Milking Cow. W. A. KING. N. J. Agr. Expt. Sta. Bul. 704. 35 pages. March, 1943.

Feeding trials, physiological studies, digestion experiments, and protein and mineral balance studies with 21, 15, 16, and 12 cows, respectively, showed that: As a preservative for alfalfa silage, molasses proved to be superior to phosphoric acid. This was indicated by greater economy in milk production on the molasses-alfalfa silage; by digestive disturbances and physiological changes in the blood and urine when phosphoric acid was used; and by a large number of negative phosphorus balances in spite of larger phosphorus intake.

Molasses-alfalfa silage proved to be very palatable and economical. Both kinds replaced satisfactorily all the corn silage and part of the hay in the rations of these cows.

Apparent digestibility was about the same regardless of preservative used.

A large part of the phosphorus in the phosphoric acid silage was apparently unavailable to the cows.

The protein of the alfalfa silages proved to be adequate for milk production without the addition of protein concentrates in the grain ration.

16 tables; 44 literature references. J.G.A.

459. Growing More Feed. G. H. AHLGREN. N. J. Agr. Expt. Sta. Cir. 453. 8 pages. Jan., 1943.

General recommendations for growing more corn, small grains, legumes, and grasses. A schedule of seeding rates and dates for various crops under New Jersey conditions is appended. J.G.A.

FOOD VALUE OF DAIRY PRODUCTS

460. The Future Role of Milk in Nutrition. GILBERT DALLDORF, Dir. of Labs., Grasslands Hosp., Valhalla, N. Y. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 101. 1942.

It has been very difficult to determine the vitamin requirements of man and it is known that these requirements vary for different animals. Medical evidence does not show a general vitamin shortage problem. However, milk is a very good carrier for thiamin yet the preservation of this vitamin in wheat during milling would be preferable. "There is a harmony in well-balanced diets which defies duplication by artificial means." Our foodstuffs are naturally varied enough to permit good diets without substituting proprietary preparations for food. A.C.D.

461. Influence of Minor Dietary Changes on Frequency of Infant's Stools. Study of the Effect of Varying the Content of Lactose, Milk Fat, and Thiamin. IRVIN J. WOLMAN AND SYDNEY BOROWSKY, Univ. Pa. Hosp., Philadelphia, Pa. Amer. Jour. Dis. Children, *65*, No. 6: 827. June, 1943.

A total of 249 normal newborn infants were divided into four experimental groups. They were fed variations of a modified milk diet to deter-

Composition of Diets Fed

Modified milk mixture	Lactose	Corn syrup	Milk fat	Thiamine
No.	*per cent*	*per cent*	*per cent*	*U.S.P. units per quart*
1	8.2	0.0	2.4	65 – 85
2	6.6	1.6	2.4	65 – 85
3	8.2	0.0	2.4	35 – 55
4*	7.4	0.0	2.8	65 – 85

* Control.

mine effects of minor changes in diet, without alterations in the caloric value, upon the frequency of stools.

Results indicate that average stool frequency can be increased during the first few months of life by any three of the changes studied:

(1) Increasing lactose from 7.4 to 8.4 per cent and decreasing milk fat in isocaloric amount as in diet 1.

(2) Decreasing milk fat and increasing carbohydrate where 1.6 per cent corn syrup replaced an equal amount of lactose as in diet 2.

(3) Using a diet with the same composition as diet 1; except lacking in supplementary thiamine.

The stool frequency using diet 3 was slightly less than using diet 1; a thiamine hypovitaminosis may have occurred when diet 3 was fed. Thiamine is necessary for proper tonus and motor function of the gastrointestinal tract and a deficiency of thiamine may have resulted in a hypomotility. R.K.W.

462. **Ascorbic Acid Content of Cow's Milk During Four Successive Lactation Periods.** A. D. HOLMES AND FRANCIS TRIPP, E. L. Patch Co., Boston, Mass., E. A. WOELFFER, H. P. Hood and Sons, Boston, AND G. H. SATTERFIELD, Univ. North Carolina, Raleigh. Food Res., *8*, No. 3: 237. May–June, 1943.

In a study of the ascorbic acid content of the milk produced by a grade Guernsey cow during her third, fourth, fifth and sixth lactation periods, the daily volumes of milk produced were 7.97, 16.94, 11.0 and 19.73 liters, respectively. The ascorbic acid content averaged 26.93, 22.10, 21.78 and 21.89 mg. per liter, respectively, while the total daily production of ascorbic acid averaged 214, 372, 239, and 432 mg., respectively. The authors conclude that advancing age did not diminish the animal's ability to produce milk rich in ascorbic acid even though she was over eleven years old at the conclusion of the study. F.J.D.

463. **Fortification of Oil, Fat, and Flour. Stability of Added Carotene and Effect of Antioxidants.** P. W. MORGAL, L. W. BYERS AND E. J. MILLER, Mich. Agr. Expt. Sta., East Lansing, Mich. Jour. Indus. and Engin. Chem., Indus. Ed., *35*, No. 7: 794. July, 1943.

The stability of crystalline carotene in Crisco, lard, oleomargarine, white flour, soybean meal and soybean flour, both with and without added antioxidants, has been determined. The stability of a carotene concentrate used for comparison and prepared from alfalfa-leaf meal was superior in most preparations to the crystalline carotene. The stability of carotene was greatly influenced by the presence or absence of antioxidants contained in the solvent. In solvents free of antioxidants, the addition of antioxidants had a strong stabilizing effect. An accelerated decomposition test for carotene is described. B.H.W.

464. **The Contribution of Non-Fat Milk Solids to the Nutritive Value of Wheat Breads.** H. H. MITCHELL, T. S. HAMILTON AND J. B. SHIELDS, Animal Nutr. Div., Univ. of Ill., Urbana, Ill. Jour. Nutr., *25*, No. 6: 585–603. June, 1943.

Nine breads varying in their contents of non-fat milk solids, "enriched" nutrients and white or whole wheat flour were studied using young rats as test animals. The incorporation of non-fat milk solids in white bread to the extent of 6% of the flour improved the growth promoting and bone calcifying values of bread much more than its enrichment with thiamine, nicotinic acid and iron. Enriched white bread containing 6% of dried skim milk is definitely superior in growth-promoting value and hemoglobin production to enriched white bread supplemented to an equivalent extent with dicalcium phosphate and riboflavin. It was also equal to whole wheat bread in

growth-promoting value and hemoglobin production and is distinctly superior to it in the promotion of bone calcification.

Skim milk solids is a better supplement to white flour for growth than the residue of the wheat berry discarded in its milling, though a combination of the two supplements is better than either alone. C.F.H.

465. Health and Vitamin D. F. H. PLETCHER, formerly with Lab. Dept., Borden's Farm Products Co., Brooklyn, N. Y. Milk Dealer, *32*, No. 9: 34–48. June, 1943.

An exhaustive resume of study of rickets from earliest records and detailed work of Mellanby, Huldschinsky, McCollum and Hess and Steenbock is reviewed. Since 1919 a complete understanding of the cause and prevention of rickets and the role of Vitamin D has been unfolded. Dr. T. A. Palm of London in 1890 contributed much to the discovery of the effect of sunlight in the deposition of mineral within the body.

The coordination of effect of diet, sunlight and differentiation of functional characteristics of Vitamins A and D in the metabolism of growing organisms was finally evolved. Interesting incidents in research of different investigators is given in the establishment of a distinct entity to Vitamin D. The importance of milk in supplying Vitamin D to the diet is traced. C.S.T.

466. Health and Vitamin A. F. H. PLETCHER, formerly with Lab. Dept., Borden's Farm Products Co., Brooklyn, N. Y. Milk Dealer, *32*, No. 8: 35–46. May, 1943.

The author exhaustively reviews early views and works on nutrition and shows the vital relationship of dairy products in the field of nutrition and health as the vendor of a food which carries the fifth component of an adequate diet so essential to health and life. The work of Atwater, Babcock, Hart, Humphrey, McCollum and Steenbock is reviewed and the discovery of Vitamin A is graphically traced. Within the last 10 years its importance in the human diet has been recognized and tribute to early investigators is paid. The relationship between carotene as a precursor of Vitamin A and of Vitamin A is shown and a general discussion of the role both play in nutrition is shown. Vitamin A's relationship to vision, to night-blindness, to metaplasia and to human tissue is portrayed. Vitamin A requirements in normal daily diets for proper health balance and in regard to use of dairy products is shown. C.S.T.

ICE CREAM

467. Egg Solids in the Ice Cream Mix. B. I. MASUROVSKY, Res. Editor, Ice Cream Trade Jour., New York City. Ice Cream Trade Jour., *39*, No. 7: 34. July, 1943.

The author recommends the use of egg yolk or whole eggs for ice cream of low milk solids content inasmuch as egg solids, particularly the yolk solids, are exceptionally good hydrophilic, water-binding colloids. Dry egg yolk to the extent of between 0.5% and 1.0% is suggested to improve the body and texture characteristics of ice cream. The same quantity is recommended as a valuable ingredient in the sherbet mix, and it is suggested that the mix be heated to 155° F. for 30 minutes and, if possible, homogenized when egg products are used. F.J.D.

468. Ice Cream Body Building with Limited Milk Solids. P. H. TRACY AND HARRY PYENSON, Univ. Ill., Urbana. Ice Cream Trade Jour., *39*, No. 7: 12. July, 1943.

A comparison of several cereal "body builders" for wartime ice cream revealed that all of them affect the flavor adversely, with wheat flour being the least noticeable. They all improve body, with oat flour being most effective. All the products tried increase whipping time, and acidity and lower the freezing point. Some brands of wheat flour were found to be superior to others. The amount of various flours possibly of use is limited by the flavor effect, soybean imparting more flavor than wheat or oat flour. Ice creams containing less than about 18% milk solids and more than 3% wheat flour are hard to whip and have a sticky body as well as a cereal flavor. High heat treatment of flours prior to adding to the mix increase their effectiveness. The moisture content of most of the wheat flours ranged from 10 to 13%. Soy flour was lowest at 7.1%. F.J.D.

MILK

469. Milk Distribution in War Time. TOM G. STITTS, Food Distrib. Admin., U. S. Dept. Agr., Washington, D. C. Internatl. Assoc. Milk Dealers Assoc. Bul., *35*, No. 27: 395–398. May, 1943.

There are two main reasons for the adjustments being brought about in the fluid milk business. The first is the need to maintain a stable economy and the second to conserve vital resources.

Fluid milk alone accounts for eleven per cent of the food budget in the cost of living index. When retail milk prices were frozen along with other retail food prices, efforts were made to keep the farmer producing in the face of higher costs by means of subsidies which were later discontinued. Also an effort was made to cut down on services not absolutely necessary not only to reduce the cost of handling milk but also to conserve on strategic materials as manpower, trucks, tires and gasoline. As an example, every other day delivery can effect delivery mileage reductions of 40%. Because milk is high on the list of food products which must continue to be marketed no matter how long the war lasts, it is important that equipment be con-

served so that certain minimum service will always be possible. Elimination of luxury services will aid greatly in assuring this minimum of necessary service to the end of the war. E.F.G.

470. Cost of Handling Fluid Milk by Retail Food Stores in the Alameda County and San Francisco Marketing Areas. JOHN MARSHALL, JR., Calif. Dept. Agr., San Francisco, Calif. Internatl. Assoc. Milk Dealers Assoc. Bul., *35*, No. 26: 375–392. April, 1943.

Time studies were made on 26 retail stores judged to be representive of 126 stores in Alameda County and 145 stores in San Francisco area for which operating statements already had been obtained and analyzed. On the basis of time studies made in the store and an allocation of expense to milk and other items sold the following facts were revealed.

The total operating expenses of the 15 Alameda County stores amounted to 11.26% of sales while the mark-up needed to provide a profit of 2% on sales was 16%. The 11 stores in the San Francisco area showed total operating expenses 11.90% of sales and to include the 2% profit on sales required a 16.17% mark-up.

The average order value of fresh milk customers at the Alameda stores was 82 cents for 5.87 items while the average customer of the stores spent only 64 cents for 4.53 items. In the San Francisco stores milk customers spent 90 cents for 6.55 items and the average of all customers was 69 cents for 5.26 items. Both studies showed that milk buying customers spend more on each trip to the store than the average customer and that milk customers are more profitable and tend to increase the total volume of the entire business.

The cost of handling fluid milk is less than the average cost of handling all items. The cost of handling milk in fibre bottles through a grocery store is less than the same milk in glass and warrants a narrower retail grocery margin for milk in fibre bottles. E.F.G.

471. Milking Machines, Materials and Men. LAWRENCE BEVILLE, International Harvester Co., Buffalo, N. Y. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 189. 1942.

In the State of New York there are 1,361,600 dairy cows and 49% or 667,200 are milked by hand. A hand milker can milk 8 cows per hour and by machine he milks 20. This allows a saving of 100,080 man hours per day if all cows were milked by machine. Milking by machine can be done well without causing infection.

The problem of good washing of milking machines is still with us but definite progress is being made in developing better methods and getting them into use. A.C.D.

472. War Time Milk Transportation Problems. CHARLES H. MILBURN, Standard Brands, Inc., New York City. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 81. 1942.

The Metropolitan area of New York City consumes each day some 27,700 tons of food of which 6,306 tons (6,000,000 quarts) is milk. The problem is to bring this food, especially the milk, into New York City even though the city is bombed. A committee has worked out a program and organization to report damaged plants and roads to a central office. The central office will advise deputies who will stop all milk trucks in transit that were to travel over such faulty roads or plants and will reroute them where the milk can be handled. A.C.D.

473. Symposium on Causes and Reasons for Rejected Milk. Part I. R. L. FURNA, N. Y. City Dept. of Health, Chateaugay, N. Y. 16th Ann. Rept. N. Y. State Assoc. of Milk Sanit., page 121. 1942.

The milk sanitarians from the milk supply of New York cities began regular deck inspection work in 1937. That year 1,062,497 cans of milk were inspected and 20,397 cans or 1.9% rejected. The three principal causes of rejection were (1) improper cooling, (2) dirty utensils, and (3) mastitis. In 1941 there were 1,341,679 cans of milk inspected and 1.43% rejected. The cooling of milk had been improved but there were more dirty utensils and mastitis, probably due to poorer help and more milking machines. Milk is also sometimes rejected for excessive sediment and odors from barn feed, and weeds.

There is a definite tendency toward greater rejections from milk hauled over longer routes.

The odor of milk is a better criterion of quality than the taste. The strainer dipper detects flakes, and the sediment test shows foreign matter. In rejecting the milk it is desirable to give exact reasons for doing so. Since odor is dependent upon so much it is necessary to have experience before actually grading milk. Bad milk including high bacterial counts and mastitis can be nearly always detected by odor.

Part 2

W. H. MANSON, New York City Dept. of Health, Millerton, N. Y.

The problem of producing poor milk when viewed from the personal standpoint is due to: (1) lax plant operation and dairy control by company representatives, (2) poor herd management on the part of the dairymen with limited professional advice, (3) lack of satisfactory cooling, washing and sterilizing facilities, and (4) the dairymen in the business of producing milk who are not adapted to the job.

Part 3

E. R. McHALE, New York City Dept. of Health.

Attention was directed to the failure to provide refrigeration or protection to milk against warming up on the trucks used to haul milk. A.C.D.

474. Protecting and Maintaining Milk Supplies in Wartime. SOL PINCUS, Deputy Commr., Dept. of Health, New York City. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 71. 1942.

The program to protect and maintain the milk supply of New York City during Wartime is built around the following objectives: "1. Blackout protection of the plants in case of night air attack. 2. Protection of the milk supply and plants against possible sabotage. 3. Provision for emergency pasteurization in case some of our plants were disabled by enemy action."

A.C.D.

475. Milk Filtration with Cotton Media. HERBERT L. DAVIS, Johnson and Johnson Co., New Brunswick, N. J. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 43. 1942.

The filtration of milk through cotton on the farm is an aid to clean milk production used even in certified dairies. Tests have shown cotton will remove particles down to 44 microns in size.

Strainers may be classified into three types, namely, (1) those that protect the cotton so well that plain cotton will not wash, (2) those that need gauze to protect the upper surface against washing, and (3) those that need gauze on both sides to prevent washing.

The most thorough removal of sediment is secured by cotton disks and such disks cannot be reused. A.C.D.

476. Precision Timing of High-Temperature, Short-Time Pasteurizers. A. C. FAY AND JOSEPH FRASER, H. P. Hood and Sons, Boston, Mass. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 21. 1942.

Several procedures have been developed to accurately determine the holding time in high-temperature pasteurization. It is very important that this time must not vary a few seconds from that desired.

One method is to connect an alemite gun in place of the thermometer at the outlet of the final heater section. The pasteurizer is operated with water, the dye injected, and the time determined with a stop watch for the dye to appear at the end of the holding tube.

Another method is dependent upon changing the electrical conductance of the milk by injecting a saturated salt solution into the milk by an alemite gun attached at the outlet of the final heater section. The change in electrical conductance can then be determined at the outlet of the holding tube by

an ammeter. The time may be secured by the stop watch or by an automatic clock stopped by the increased electrical conductance. Either method is accurate if properly manipulated. Since the final heating with the Electropure Pasteurizer varies with conductance the regulator bulb of this machine must be disconnected and placed in water about pasteurization temperature to keep the flow diversion valve open. As a final method the holding time may be calculated by the ratio of capacity to the volume of the holding tube. A.C.D.

477. Current Methods of Measuring Foam. SYDNEY ROSS, Dept. of Chem., Stanford Univ., Calif. Jour. Indus. and Engin. Chem., Analyt. Ed., *15*, No. 5 : 329. May, 1943.

Foam may be measured by static or by dynamic methods. Static methods are those in which measurements are made after the foam has been produced with no reference to the manner of its production. Static methods consist of forming a foam and observing the rate at which liquid drains from it, beginning observations after the cessation of foam formation. In dynamic methods the rate of collapse of the foam bubbles and films is measured, the rate of drainage being considered indirectly as it affects the rate of collapse of the films. The chief static and dynamic methods currently used to measure foaminess of liquids have been described and examined. B.H.W.

478. What Is the Correct Way of Running Sediment Tests? MILT KING, Sediment Testing Supply Co., Chicago, Illinois. Natl. Butter and Cheese Jour., *34*, No. 7 : 15. July, 1943.

A pint of milk is sucked up into the tester from the bottom of the can as the inlet of the tester is drawn across the can bottom. W.V.P.

479. The War and Quality. MILTON H. BUTTON, Dir., Wis. Dept. Agr., Madison, Wis. Natl. Butter and Cheese Jour., *34*, No. 5 : 14. May, 1943.

Milk quality will be maintained despite labor, equipment and machinery shortages. Scarcity of milk products for civilian use might tend to lower standards of quality but rationing should offset this trend. W.V.P.

480. Filtering Milk on the Farm. K. G. WECKEL, Univ. of Wis., Madison, Wis. Natl. Butter and Cheese Jour., *34*, No. 3 : 12. March, 1943.

Filtration of milk is accepted in production of certified milk and by health ordinances. A good filter should be rugged, simply made, easily cleaned and sterilized, and should provide close fitting parts to hold the filter disk tightly. Failure in filtration can be caused by breaking fibers of filter when milk is poured over it, by "bumping" the strainer to hurry the process

and by a poorly fitting disk-holding device. The type of filter disk should be selected to fit the strainer design; poor strainers generally require the most expensive disk for efficient action. Rate of filtration varies and decreases with cold milk, high-fat milk, mastitis, and fine mesh filters.

Filtering does not prevent solution of some extraneous material; it does not improve the methylene blue test; nor change milk composition nor subsequent acid development. Filtering calls the farmer's attention to improper production practices. W.V.P.

PHYSIOLOGY

481. Formation in Vitro of Highly Active Thyroproteins, Their Biologic Assay and Practical Use. E. P. REINEKE AND C. W. TURNER. Mo. Agr. Expt. Sta. Res. Bul. 355. 88 pages. Nov., 1942.

A detailed technical dissertation on methods for assay of thyroidal substances, methods for preparation of iodinated proteins, and their physiological effects. From the viewpoint of dairy science the most interesting finding was "that artificial thyroproteins will stimulate increased milk production when fed, for short periods, to goats in declining lactation. Increases were noted in the milk yield, milk fat percentage, and yield of butterfat of lactating cows after feeding artificial thyroproteins for a three-day period."

17 figures, 23 tables, 5 pages of literature citations. J.G.A.

482. The Relation of the Endocrine System to the Regulation of Calcium Metabolism. I. L. CAMPBELL AND C. W. TURNER. Mo. Agr. Expt. Sta. Res. Bul. 352. 134 pages.

The outstanding findings in this comprehensive study, the summary alone of which occupies three pages, are that "there is little doubt that the parathyroid hormone is the most important endocrine factor in the immediate regulation of calcium metabolism in both mammals and birds." "The results of this study provide strong evidence that the parathyroids are independent of a controlling anterior pituitary hormone." "Feeding experiments have shown convincingly that gross enlargement of the glands occurs under conditions which tend to lower the calcium content of the blood. It seems quite probable that the parathyroid glands are sensitive to slight changes in the level of physiologically available calcium in the blood, and that the output of hormone is adjusted accordingly to preserve an equilibrium between the demand for and supply of calcium to the tissues." "Until some convincing evidence of the mediation of some other organ is forthcoming, it seems unnecessary to postulate any but a direct secretion under conditions tending to lower the level of physiologically available calcium in the blood."

17 figures, 35 tables, 13 pages of literature references. J.G.A.

MISCELLANEOUS

483. More Results with an Acid Detergent but in a Standard Can Washer. F. M. SCALES, Sealtest Lab., Sheffield Farms Co., New York City. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 175. 1942.

In further research on the washing of milk cans with acid detergent an installation was made using a standard can washer. Cans were washed using two different alkaline washing powders of modern formulae and an acid detergent designed by the author and sold as Mikro San. Some 276 cans were washed daily for 12 days using each detergent. The results showed that from the standpoint of sterility, cleanliness, and odor the acid detergent proved superior. The pH of the acid washing solution was 6.3.

A.C.D.

484. A Discussion and Demonstration of the Effect of Alkaline Detergents on Water Hardness. C. W. RINK, Diamond Alkali Co., Pittsburgh, Pa. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 163. 1942.

After a brief discussion of water hardness as an ever present problem in washing dairy equipment, the speaker demonstrated a test for suitability of a washing powder for a given water supply. The test consists of adding one teaspoon of the powder to one quart of water. After dissolving the powder some of the solution should be diluted 1–10 with water. Both solutions should remain clear and not become milky. The ideal washing powder to cleanse thoroughly and leave no film or scale should contain silicates, silicated complex phosphates, and wetting agents. A.C.D.

485. The Dairy Industry under a Total War Economy. C. E. BEARDSLEE, Dairy Prod. Sect., Food Branch, War Prod. Board, Washington, D. C. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 55. 1942.

The dairy industry has the tremendous production goal of 120,000,000,000 pounds of milk for 1942 and this quantity may be exceeded. Shortage of critical materials is a serious problem both in the dairy industry and in many industries servicing the dairy industry. The program in Washington endeavors to conserve these critical materials for a long war without too seriously handicapping this essential food industry. A.C.D.

486. Ways and Means of Cooperating in the War Effort by Prolonging the Life of Milk Handling Equipment. C. W. WEBER, State Dept. of Health, Albany, N. Y. 16th Ann. Rpt. N. Y. State Assoc. of Milk Sanit., page 9. 1942.

The life of milk handling equipment should be prolonged by every possible means. The time that equipment may be used is lengthened by reducing friction, shock, and corrosion to a minimum. Each of the points is discussed and various methods suggested for holding them to a minimum.

A.C.D.

487. Preventive Motor Maintenance for the Milk Dealer. G. D. BOWNE, Mgr., Food Indus., application and engin., AND W. W. MCCULLOUGH, Maintenance Engin., Westinghouse Elec. & Mfg. Co., East Pittsburgh, Pa. Milk Dealer, *32*, No. 9: 25–27–74–76. June, 1943.

Proper motor maintenance in a milk plant where most of individual units are operated by individual motor is always important, but under wartime conditions both regular and preventive measures to secure continuous operation are vitally important. Systematic inspection, analysis of equipment, and detailed records should be established and enforced.

The authors suggested (1) once a week check on oil level bearings and rings and of insulation; (2) once a month check on brush holders, brushes and shunts; (3) once a year check of air gap, insulation resistance, line voltage, change grease and check renewal parts for motors and (4) every 2 years dismantle and completely overhaul all motors. Motor bearings, sleeve bearings and ball bearings in all parts of all motors should be periodically checked for wear, alignment, speed, oil flow and supply, excessive temperatures and general overhaul. Two charts for general and specific examination of all motors and motor parts are given to maintain proper efficiency of all motors in the milk plant. C.S.T.

488. Check Up on Your Refrigerating Equipment. L. C. THOMSEN, Univ. Wisconsin, Madison. Natl. Butter and Cheese Jour., *34*, No. 7: 8. July, 1943.

This practical discussion of operating principles of mechanical refrigeration machinery shows how to determine rated capacity and states "any given machine should be operated at the maximum possible suction temperature (pressure) which will still give the desired temperature to the product to be refrigerated." Optimum suction pressure during operation may not be attained if the surface area of the expansion unit is too small or if the velocity of air, brine, water or dairy product over the expansion surface is too low or if the refrigerant moves too slowly, or if the efficiency of the heat exchanging surface is impaired by oil, scale or frost. When high discharge pressures are the result of faulty operating conditions, power is wasted; such pressures may be caused by non-condensable gases, shortage of water, or high initial water temperatures. Two graphs show for ammonia compressors the relationship of suction pressure to output and to power consumption and the relation of discharge pressures to output and to power consumption. W.V.P.

489. Salvaging Fat from the Babcock Test. A. W. BIENDARRA, Fairmont Creamery Co., Green Bay, Wis. Natl. Butter and Cheese Jour., *34*, No. 6: 9. June, 1943.

After reading cream tests, the large end of a 9-cc. pipette is inserted into the necks of bottles to the bottom of the fat column; the tip is closed with a finger and the pipette and fat withdrawn. Twelve ounces of fat have been salvaged from 55 tests; the cumulative savings of fat are impressive.

W.V.P.

490. A Wartime Dairy Barn. G. L. EDICK. U. S. Dept. Agr. Leaflet 232. 8 pages, Illus. April, 1943.

Contains sketches, illustrations, and suggestions for dairy barns designed to use a minimum of metal and other critical materials.

8 figures. J.G.A.

491. Is Your Piping Installed Properly? C. T. BAKER, Consulting Engin., Atlanta, Ga. Natl. Butter and Cheese Jour., *34*, No. 3: 17. March, 1943.

Improper pipe layout may cause high power bills, loss in capacity and failures in pipe installation. Specific cases are described to show that pipes and valves of improper size, especially those connected to pumps, may cause unnecessary trouble. W.V.P.

492. Countercurrent Multitubular Heat Exchangers. Comparison of Design Methods. M. G. LARIAN, Mich. State Col., East Lansing, Mich. Jour. Indus. and Engin. Chem., Indus. Ed., *35*, No. 8: 840. Aug., 1943.

The tubular heat exchanger is a common piece of equipment in chemical plants. Heat exchanger design and methods for calculating the heat transfer area of countercurrent tubular heat exchangers is discussed. Of five methods of calculation only one is entirely correct, but the calculations for this are lengthy. The other methods are simpler and often fairly accurate. Some methods for predicting film coefficients are briefly discussed.

B.H.W.

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