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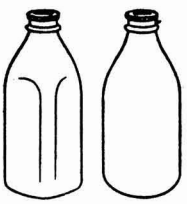

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COMPARISON OF METHODS OF ESTIMATING MILK AND FAT PRODUCTION IN DAIRY COWS

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Production records of milk and fat constitute an important and necessary part of the program of herd improvement in dairy cows. In Illinois there are at present about 1,123,000 dairy cows of milking age, of which less than 4 per cent are being tested for production. This is too small a number to afford a satisfactory basis for any widespread improvement.

Cost may be a limiting factor in restricting the scope of testing. This is suggested by the fact that in Illinois in 1936 there were 29 herds, including 326 cows, on Advanced Registry test. The numbers on this type of test had increased by 1945 to 48 herds and 652 cows. In 1936 there were in Illinois 27 herds, including 619 cows, in Herd Improvement Registry testing. By 1945, this number had increased to 165 herds including 3,586 cows. In 1936 there were 23,812 cows tested in Illinois in Dairy Herd Improvement Associations. This figure rose to 44,282 in 1941. The number declined seriously during the war and is not yet back to the 1941 peak. Table 1 gives the comparative costs of the three different types of testing.

TABLE 1

Approximate cost per year in Illinois for testing by three different types of tests. 1947[a]

Type of test	Cost/cow	Cost/herd	Av. no. cows /herd
A. R.	$22.60	$293.80	13
H. I. R.	8.65	181.65	21
D. H. I. A.	3.65	84.00	23

[a] Derived from Illinois testing fee scales for 1947.

This investigation was carried out to devise a method of testing that will lessen the amount of time and labor required on the part of the tester and the cow owner, and so to spread testing efforts over a wider area.

The semi-official test, the test used in this study for the standard or measure of reliability of other tests, either now in use or proposed, was adopted by the various breed associations as a sufficiently accurate method of measuring produc-

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tion. It departed from the method of the official test by the device of using only a 2-day test period each month together with daily milk weights. Later the test period was reduced to 1 day per month. Later still the bi-monthly 1-day test period was adopted as optional with the producer. The results obtained by this departure from the strictly official method of supervision of tests encourages the belief that some further departure from the present methods of testing may yield sufficiently satisfactory results to merit consideration.

REVIEW OF LITERATURE

A 1-day test every 30 days has yielded results within 2 per cent of those obtained by testing every day (6). Later it was found (7) that a 1-day bi-monthly test varied only 3.8 per cent from the daily test. Bi-monthly tests (8) were found to be practically as accurate as monthly tests, having a coefficient of correlation of 0.97 with the standard error of estimate within the limit of significance.

In methods of testing involving less frequent test periods, a high correlation was observed between a 1-day test taken during the fifth month (1, 3) and the monthly method of testing. The correlation between the 7-day test (12), taken at the beginning of the lactation period, and the yearly test was low.

The monthly decrease in production was found to be very uniform in all breeds (10), the production in the twelfth month being approximately 50 per cent that of the best month.

Comparing the monthly to the bi-monthly method of testing. McDowell (7), working with 70 C.T.A. records, found that the monthly and bimonthly test varied on the average 2.91 and 3.80 per cent, respectively, from the actual. The greatest variation for the C.T.A. method was 3.8 per cent, as compared to 12.5 per cent for the bi-monthly method. Dairymen estimating production in 48 cases erred by differences of from 1 to 63 per cent with an average error of 28.0 per cent from the C.T.A. record.

Gifford (4) found that 69.0 per cent of more than 100 Advanced Registry Holstein and Guernsey records were within the standard error of estimate comparing the monthly with the bi-monthly test plan, 95.0 per cent were within two times the standard error of estimate and 99.17 per cent were within three times the standard error of estimate, the standard error of estimate in each case being sufficiently small to make this finding significant. The coefficients of correlation between monthly and bi-monthly testing were high. He concluded that the bi-monthly testing is a satisfactory method of estimating production.

McKellip and Seath (8), in comparing bi-monthly to monthly records, found that the coefficient of correlation was over 0.97, and the standard error of estimate within the limit of significance. They concluded that bi-monthly tests when used with daily milk weights were practically as accurate as monthly records made by centering the tests and not using daily milk weights.

Gifford (4), using 841 records divided into four groups according to various production levels, found coefficients of correlation ranging from 0.956 to 0.997

between the different groups, and between assorted pairs of the various groups, differences in the coefficients ranging from 0.001 to 0.015. He concluded that bi-monthly testing is satisfactory.

An analysis of 500 Jersey records (2) comparing the monthly to the bi-monthly plan with 250 of the records made under 2-day supervision and 250 under 1-day supervision showed that 258 of the bi-monthly records exceeded the monthly records and 242 were lower. The average variation for all records was 7.21 lb. of fat.

Predicting yearly yields from short time records. Petersen (9) comparing the 1- and 2-day tests on 35 Jerseys, found that all but five cows varied less than 2 per cent and only one cow varied as much as 3 per cent.

Cannon *et al.* (1) found that, based on a single 1-day test and using 400 Advanced Registry records and 1,289 Dairy Herd Improvement Association records, prediction factors for production were determined which showed a high correlation between estimated yield and actual yield. It was found that a 1-day test taken during the fifth month gave the most dependable prediction of yield.

Yapp (12), studying the reliability of the 7-day test, showed that the correlation between the 7-day test and the yearly test was not high and concluded that the 7-day test is not a satisfactory criterion of semi-official production.

Gaines (3) found that a 7-day test made shortly after calving did not give a true indication of the whole lactation but that such a test conducted 60 days or more after calving was more accurate. He found that a 7-day test conducted during the fifth month most nearly indicated the lactation record.

Turner (10) found that the monthly decrease in production is very uniform in all breeds, the production in the twelfth month being approximately 50 per cent of that of the best month. He concluded that the principal causes for variation are nutrition and temperature.

EXPERIMENTAL PROCEDURE

The milk and butterfat records made on the five major dairy breeds kept in the herd at the Illinois Station were used as a source of data for this study. Since 1932 all of the cows in the various breeds have been kept under as nearly the same environmental conditions as possible, thus minimizing one important variable. All records of less than 305 days were discarded. All records of more than 305 days that showed plain indications of abnormalities, such as abortion, also were discarded. (The records included only the first 305 days of the lactation period.) This was called the semi-official record, since the test was made by the semi-official method using a 1-day supervision period. The semi-official record for 305 days then was used as the standard against which the other methods developed in this study were measured. Six hundred and eighty-four records meeting these conditions were found and these form the basis of the material used in this study.

Different methods studied. Since the semi-official test was used as the standard and since it seemed desirable to make as direct comparisons as possible, it

TABLE 2

Variation from semi-official, or actual, of four different methods of estimating production of fat-corrected milk

Breeds	Total no. of cows		Method I Bi-monthly test				Method II Tests taken 2nd 6th and 10th mo.				Method III Tests taken 2nd and 10th mo.				Method IV Tests taken 4th and 8th mo.			
			Cows varying from actual records															
			0–2.0 %	2.1–5.0%	5.1–10.0 %	Above 10.1 %	0–2.0 %	2.1–5.0%	5.1–10.0 %	Above 10.1 %	0–2.0 %	2.1–5.0%	5.1–10.0 %	Above 10.1 %	0–2.0 %	2.1–5.0%	5.1–10.0 %	Above 10.1 %
All	684	%	43.27	29.54	20.03	7.16	42.40	28.95	20.91	7.74	33.76	27.33	26.50	12.41	28.65	27.92	28.95	14.47
		No.	296	202	137	49	290	198	143	53	231	187	182	84	196	191	198	99
Ayrshire	44	%	31.81	31.81	15.95	20.43	29.54	31.83	9.09	29.54	18.18	18.18	31.82	31.82	18.18	25.00	40.91	15.91
		No.	14	14	7	9	13	14	4	13	8	8	14	14	8	11	18	7
Brown Swiss	89	%	39.33	39.33	16.85	4.49	41.57	34.83	17.98	5.62	31.46	38.71	29.21	5.62	31.46	26.97	26.97	14.60
		No.	35	35	15	4	37	31	16	5	28	30	26	5	28	24	24	13
Guernsey	87	%	35.63	27.58	27.58	9.21	29.88	36.78	26.44	6.90	36.78	21.84	25.29	16.09	21.84	25.29	33.33	19.54
		No.	31	24	24	8	26	32	23	6	32	19	22	14	19	22	29	17
Holstein	382	%	48.69	27.48	18.32	5.51	47.12	26.70	20.16	6.02	36.13	28.01	25.92	9.94	31.68	28.80	28.01	11.51
		No.	186	105	70	21	180	102	77	23	138	107	99	38	121	110	107	44
Jersey	82	%	36.58	29.27	25.61	8.54	41.46	24.39	26.83	7.32	30.49	28.05	25.61	15.85	24.39	29.27	24.39	21.95
		No.	30	24	21	7	34	20	22	6	25	23	21	13	20	24	20	18

was decided to use different months from these same records as a basis for computation of the new methods.

The methods used in this study for estimating production of milk and butterfat were as follows: Method I, wherin the tests are taken every other month (bimonthly); method II, wherein the tests are taken on the second, sixth and tenth months; method III, wherein the tests are taken on the second and tenth months; and method IV, wherein the tests are taken on the fourth and eighth months. The formulas used for estimating yields for the various methods were as follows[1]: Method I $- Y = (61m_1) + (61m_3) + (61m_5) + (61m_7) + (61m_9)$; method II $- Y = (102m_2) + (102m_6) + (101m_{10})$; method III $- Y = (153m_2) + (152m_{10})$; Method IV $- Y = (153m_4) + (152m_8)$.

TABLE 3

Comparison of the accuracy of five different methods of testing (684 records of fat-corrected milk, all breeds)

	Semi-official	Method I	Method II	Method III	Method IV
Av. of plus parameters (*lb.*)	15,573	15,199	15,327	15,531	15,197
Av. of minus parameters (*lb.*)	8,215	7,707	7,797	7,891	7,569
Records above plus parameters (%)	12.0	12.3	11.5	12.4	11.3
Records below minus parameters (%)	9.5	8.5	9.4	9.9	7.4
Variation of plus parameters from semi-official parameters (%)	0.0	2.5	1.8	0.3	2.5
Variation of minus parameters from semi-official parameters (%)	0.0	6.2	5.1	3.9	7.8

In no case was any test used that was taken earlier than the first full month of lactation. Except for the estimate on the standard or semi-official yield, none of these methods can be considered as being based on the centering method of testing. However, since estimates I, II, III and IV were derived from different testing periods of the semi-official records, which were centered, they do derive some of whatever benifits may accrue to the centering method, and to that extent are exempt from any criticism that may arise from the use of different systems.

Various other groups of test periods within the standard records used were tried. The fact that none of these combinations was as acurate in estimating the standard yield as the four groups selected caused them to be discarded. These four methods were compared to the standard method for their accuracy in estimating milk, butterfat and FCM yields. Their standard deviations, coefficients of correlation, coefficients of variation and coefficients of regression were determined as measures of comparison to the standard method.

[1] Y = yield for 305 days; m indicates test for month in which test was made.

As has been noted previously (2, 4, 7), the bi-monthly method of testing has been found to be as accurate for all practical purposes as testing only once a month. This study shows that bi-monthly testing yielded results with a high degree of dependability when the 684 records included in the study were compared on the bi-monthly basis with the same records considered on the monthly

TABLE 4

Percentage of error groups for four different types of tests, showing variation from semi-official test in terms of fat-corrected milk

Breed	Type of test	*Cows varying from mean S.O.*				
		1–5%	1–10%	1–15%	1–20%	More than 20%
		(%)	(%)	(%)	(%)	(%)
Ayrshire	S.O.	20	34	39	66	34
	I	20	30	45	64	36
	II	20	30	45	70	30
	III	11	27	45	60	40
	IV	9	34	45	63	37
Brown Swiss	S.O.	11–	31	52	63	37
	I	13	30	46	64	36
	II	17	34	43	62	38
	III	13	29	47	60	40
	IV	10	20	45	60	40
Guernsey	S.O.	17	48	66	77	23
	I	20	42	61	76	24
	II	24	39	64	75	25
	III	22	40	58	70	30
	IV	25	39	56	77	23
Jersey	S.O.	20	41	51	73	27
	I	21	41	59	77	23
	II	21	38	54	77	23
	III	20	35	51	68	32
	IV	23	43	61	74	26
Holstein-Friesian	S.O.	16	35	52	68	32
	I	21	33	50	68	32
	II	21	34	48	67	33
	III	17	31	49	65	35
	IV	19	35	52	67	33
All breeds	S.O.	14	28	44	56	44
	I	13	27	43	54	46
	II	13	27	41	53	47
	III	13	28	41	52	48
	IV	14	27	41	54	46

basis. Only 49 of these records, or 7.16 per cent, varied more than 10 per cent from the standard. Reference to table 2 shows that when testing by method II, the increase in variation above 10 per cent from the actual was only 0.58 per cent. In method III, the increase was 5.25 per cent. In method IV, the increase was only 7.31 per cent. In other words, bi-monthly testing was 92.84 per cent dependable; testing three times per lactation was 92.26 per cent depend-

TABLE 5

Accuracy of milk yields for four different types of testing compared to the semi-official test (av. fat-corrected milk)

Breed	S.O.[1]	I	Error	No.	S.O.	II	Error	No.	S.O.	III	Error	No.	S.O.	IV	Error	No.
	(*lb.*)	(*lb.*)	(%)		(*lb.*)	(*lb.*)	(%)		(*lb.*)	(*lb.*)	(%)		(*lb.*)	(*lb.*)	(%)	
Ayr. +	11,513	11,671	+1.4	5	10,200	10,436	+2.3	10	10,472	10,973	+4.9	14	11,557	12,060	+4.4	9
Ayr. −	10,168	9,441	−7.1	39	10,357	9,431	−7.2	34	10,249	9,014	−12.0	30	10,003	9,236	−7.7	35
All Ayr.	10,321	9,694	−6.1	44	10,321	9,660	−6.4	44	10,321	9,725	−5.8	44	10,321	9,814	−4.9	44
B.S. +	13,494	13,732	+1.8	16	12,786	13,122	+2.6	25	12,874	13,371	+3.9	34	13,151	13,613	+3.5	22
B.S. −	11,718	11,206	−4.4	73	11,744	11,217	−4.5	64	11,519	10,897	−5.4	55	11,671	10,873	−6.8	67
All B.S.	12,037	11,660	−3.1	89	12,037	11,876	−1.3	89	12,037	11,842	−1.6	89	12,037	11,550	−4.0	89
Guer. +	8,986	9,105	+1.3	12	9,257	9,522	+2.9	27	9,465	9,962	+5.3	41	8,706	8,891	+2.1	11
Guer. −	9,528	9,019	−5.3	76	9,542	9,004	−5.6	61	9,445	8,946	−5.3	47	9,561	8,833	−7.6	77
All Guer.	9,454	9,031	−4.5	88	9,454	9,163	−3.1	88	9,454	9,419	−0.4	88	9,454	8,840	−6.5	88
Jer. +	9,191	9,453	+2.9	16	9,004	9,247	+2.7	28	9,091	9,520	+4.7	33	8,711	9,146	+5.0	15
Jer. −	8,975	8,485	−5.5	66	9,023	8,499	−5.8	54	8,967	8,383	−6.5	49	9,085	8,438	−7.1	67
All Jer.	9,017	8,674	−3.8	82	9,017	8,754	−2.9	82	9,017	8,630	−4.3	82	9,017	8,568	−5.0	82
H.F. +	13,180	13,292	−0.8	82	13,248	13,532	+2.1	124	13,356	13,951	+4.5	180	13,238	13,799	+4.2	100
H.F. −	13,238	12,622	−4.7	299	13,214	12,546	−5.1	257	13,108	12,343	−5.8	201	13,221	12,334	−6.7	281
All H.F.	13,225	12,766	−3.5	381	13,225	12,867	−2.7	381	13,225	13,102	−0.9	381	13,225	12,719	−3.8	381
All +	12,383	12,431	+0.4	131	11,993	12,273	+2.3	214	12,174	12,722	+4.5	302	12,379	12,845	+3.8	157
All −	11,802	11,222	−5.0	553	11,849	11,215	−5.4	470	11,673	10,947	−6.2	382	11,750	10,936	−6.9	527
All	11,894	11,453	−3.7	684	11,894	11,562	−2.8	684	11,894	11,711	−1.5	684	11,894	11,383	−4.3	684

[1] S.O. = semiofficial

able; testing the first and last months was 87.59 per cent dependable; and testing the fourth and eighth months was 85.52 per cent dependable.

Table 3 shows the average parameters about the means for fat-corrected milk for all breeds and by individual breeds for the five different methods of testing. The variation for the different methods is slight enough to lack significance. The per cent of variates for all breeds found outside the parameters varies from 11.3 to 12.4 for those above the plus parameters, being 12 per cent for monthly testing; and from 7.4 to 9.9 per cent for those below the minus parameters, being 9.5 per cent for monthly testing. Comparing the four methods of testing directly with semi-official testing, the average plus parameters range from 0.3 per cent to 2.5 per cent lower than those for semi-official testing and the average minus parameters range from 3.9 per cent to 7.8 per cent below. This shows a high degree of dependability for testing less frequently than once a month.

Table 4 shows the per cent of records varying from the mean for the five different methods of testing. According to the results shown here, there is no significant difference between the five different methods of testing insofar as the number of individual records varying from the mean is concerned. In other words, testing once a month or as few times as twice during the lactation makes no difference in the number of records varying from the mean as measured at any particular point. The table shows a check at 1 to 5 per cent, at 1 to 10 per cent, 1 to 15 per cent and at 1 to 20 per cent.

Measured in terms of the means for FCM, as shown in table 5, the average error for all breeds is – 3.7 per cent; for method II, the error is – 2.8 per cent; for method III, – 1.5 per cent; and for method IV, – 4.3 per cent.

CONCLUSIONS

Testing every other month is slightly less accurate than testing once a month but is sufficiently accurate for practical application.

Testing three times during the lactation (method III) was only slightly less accurate than testing once a month, there being only 0.58 per cent more variation from the standard than that found in bi-monthly testing.

Testing only twice during the lactation gave sufficiently accurate results, compared to the standard monthly test, to recommend this type of testing under conditions where more frequent tests are difficult to obtain.

Testing three times during the lactation, when the tests are taken on the second, sixth and tenth months, is a sufficiently accurate method to merit a consideration of its adoption as a means toward lowering the cost of testing. Any plan that will increase the number of cows with records will accelerate the improvement of our dairy herds.

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THE BACTERIAL COUNT, TYRAMINE CONTENT AND QUALITY SCORE OF COMMERCIAL AMERICAN CHEDDAR AND STIRRED CURD CHEESE MADE WITH *STREPTOCOCCUS FAECALIS* STARTER[1]

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The present investigation was concerned with the use under practical plant conditions of a special strain of *Streptococcus faecalis* that showed promise as a starter for developing flavor in Cheddar and stirred curd cheese (1).

EXPERIMENTAL PROCEDURE

The experimental lots of cheese were made in 3 consecutive days in September, 1947, in a large commercial cheese plant. Three vats of milk were made into cheese on the first day and 4 vats on the next 2 days. One control batch of cheese was made each day with 1.2 per cent of the starter ordinarily used by this cheese company and hereafter referred to as "commercial starter," with no change in the regular procedure used in the plant. As this starter had been developed by one of the large commercial cheese companies, on the second and third days a second control batch of cheese was made using the regular procedure with 1.2 per cent of a good active Hansen lactic starter. The third batch of cheese was made by the regular procedure and the milk was inoculated with 0.8 per cent of Hansen lactic and 1.0 per cent of *S. faecalis* starters. The fourth batch of cheese also was made with 0.8 per cent of Hansen lactic and 1.0 per cent of *S. faecalis* starters, but the stirred curd process was used. All starters were allowed to grow for 1 hour in the pasteurized milk at 86° F. before adding rennet.

The milk was produced in one of the mid-western states on partially dried-out pastures and with some supplementary roughage feeding. Each vat contained 10,000 lb. of milk pasteurized at 162° F. for 70 seconds. The character of the milk was not constant from vat to vat. The fat test varied from 3.5 to 4.0 per cent and the acidity at the time of adding rennet, which was 1 hour after adding the starter, varied from 0.171 to 0.182, expressed as percentage of lactic acid.

The first three batches of cheese were made according to the usual procedure on a definite time schedule of 4 hours and 45 minutes from the addition of rennet to the salting of the curd. The acidity of the whey at the time of salting the curd varied from 0.50 to 0.65 per cent. The fourth batch of cheese was made by the stirred curd process which was identical to the Cheddar process until

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after the whey was drained. The curd was hand and mechanically stirred for 20 minutes. The acidity then was 0.25 or 0.26 per cent and the curd was salted. The total time from the addition of rennet to the salting of the curd was 2 hours and 45 minutes.

During the week of manufacture the cheese was shipped to a cheese storage where it was held at 55 to 60° F. until shipped by refrigerated freight to Ithaca, New York. It arrived within 1 month and then was cured at 50 and at 60° F.

Analyses were made for the usual chemical composition and for tyramine, soluble protein, volatile acids and pH when the cheese was received and after curing for 4 months. The total bacterial count was determined by the standard plate method using tryptone glucose beef extract milk agar and incubating 48 hours at 32° C. The cheese also was plated on sodium azide-penicillin agar and the approximate percentage of enterococci was determined by fishing 10 colonies for identification. This agar contained tryptone, yeast extract, glucose and 0.01 per cent sodium azide to which 100 Oxford units of penicillin per liter were added at the time of pouring the plates. The incubation time was 48 hours at 32° C. The methods have been described previously. The cheese was scored after curing for 2, 4 and 6 months at 50° F., but the scoring was omitted for 6 months at 60° F. as the cheese was overcured and lower in flavor quality. The intensity of the Cheddar flavor was given as mild ±, medium ± and sharp ±. To save space, only the average scores are presented and the intensities were averaged by giving numbers for intensities ranging from 1 to 9.

RESULTS

Sufficient of the manufacturing details were presented under experimental procedure to make it unnecessary to give additional data. The composition of the samples of fresh cheese (table 1) was very uniform, except that the salt content varied more than desired. The moisture contents were sufficiently low to permit good curing for long periods. None of the data on acidity, volatile fatty acids and soluble nitrogen will be presented as they do not assist in evaluating the results.

The standard plate counts of the pasteurized milk in the vats before adding starter varied from 3,000 to 22,000 per ml., and the enterococci-lactobacilli count on azide-penicillin agar varied from 0 to 200 per ml. The bacterial count with azide-penicillin agar on the Hansen starter was known to be 0, whereas the *S. faecalis* starter was 100 per cent enterococci. The commercial starter showed a standard plate count of 760 million per ml. and no growth on azide-penicillin agar.

The first analyses of the cheese for bacteria and tyramine were made 1 month after manufacture (table 2). The Hansen starter cheese showed average total bacterial counts of 34,200,000 per g., yet even in this young cheese the count on azide-penicillin agar was 8,300,000. The cheese showed no enterococci and no tyramine, so one might expect very slow curing cheese (2). However, when this cheese was 4 months old at 50° F. the total counts had increased, and the

TABLE 1

The composition of the experimental Cheddar and stirred curd cheese

Cheese[a] No.	Identity	Moisture	Fat	Salt	Protein	pH
		(%)	(%)	(%)	(%)	
919–4	Commercial starter	36.9	33.2	1.63	23.93	5.19
919–5	LDK starters[b]	36.6	32.6	1.18	23.14	5.14
919–6	LDK stirred	36.0	34.2	1.79	24.19	5.32
920–7	Hansen starter	35.6	33.6	1.69	23.84	5.28
920–8	Commercial starter	35.8	34.2	2.12	25.06	5.29
920–6	LDK starters	36.7	33.2	1.50	23.86	5.08
920–5	LDK stirred	35.9	35.8	2.14	23.13	5.23
921–7	Hansen starter	35.6	33.6	1.53	23.96	5.24
921–8	Commercial starter	36.6	32.8	2.09	24.64	5.39
921–6	LDK starters	37.4	32.8	1.41	24.53	5.09
921–5	LDK stirred	35.9	32.8	1.88	23.87	5.18

[a] The data for the cheese made with Hansen starter are averages of 2 lots of cheese; all other data are averages of 3 lots of cheese.

[b] L starter was Hansen's lactic starter. DK starter was *Streptococcus faecalis.*

azide-penicillin count was 44,800,000 per g., of which 2,200,000 were enterococci. The tyramine content had increased to 102 γ per g. This cheese cured at 60° F. for 4 months gave much lower bacterial counts, but the enterococci count of 3,200,000 was higher. The tyramine content averaged 218 γ per g. so that after curing for 4 months a medium flavor intensity would be expected.

TABLE 2

The average bacterial counts and the tyramine content of Cheddar and stirred curd cheese made on 3 consecutive days

Cheese identity	Bacterial counts (millions omitted)			Tyramine
	Standard agar plate count	Azide-penicillin agar		
		Total count	Enterococci count	
				(γ/g.)
		Cheese 1 mo. old		
Hansen starter[a]	34.2	8.3	0	0
Commercial starter	55.9	15.5	1.1	49
LDK starters	1,373.0	635.0	590.6	65
LDK stirred	785.0	383.3	383.3	99
	Cheese 4 mo. old—last 3 mo. at 45–50° F.			
Hansen starter	53.0	44.8	2.2	102
Commercial starter	63.7	48.7	13.1	290
LDK starters	547.7	475.3	394.5	354
LDK stirred	431.7	183.7	141.4	345
	Cheese 4 mo. old—last 3 mo. at 55–60° F.			
Hansen starter	19.9	6.4	3.2	218
Commercial starter	19.7	1.4	0.3	440
LDK starters	412.7	378.3	367.3	536
LDK stirred	317.7	203.3	183.0	593

[a] The data for the cheese made with Hansen starter are averages of 2 lots of cheese; all other data are averages of 3 lots of cheese.

The cheese made with commercial starter gave bacterial counts similar to that made with Hansen starter, except that enterococci always were found in the early stages of ripening and 49 γ of tyramine per g. were present in the cheese when 1 month old. After curing for 4 months, the cheese held at 50° F. contained 290 γ tyramine per g. and at 60° F. the amount had increased to 440 γ. This quantity of tyramine was unexpectedly high for cheese made from pasteurized milk of low bacterial count.

As found previously in cheese made in the laboratory (3), the addition of both Hansen lactic and *S. faecalis* starters produced cheese with very high bacterial counts which survived well during curing. After 1 month the total bacterial content of the cheese was about half *S. faecalis* and this percentage was

TABLE 3

The flavor and body scores of Cheddar and stirred curd cheese made on 3 consecutive days

Cheese identity[b]	Flavor score and rating				Body score	
	Score		Intensity rating[a]		50° F.	60° F.
	50° F.	60° F.	50° F.	60° F.		
			Cheese 2 mo. old			
Hansen starter	40.0	40.3	2.2 mi +	3.4 mi +	29.1	29.3
Commercial starter	39.6	40.0	2.1 mi +	3.3 mi +	28.7	28.9
LDK starters	40.5	40.7	3.2 mi +	3.8 mi +	29.2	29.2
LDK stirred	40.4	40.6	2.8 mi +	3.5 mi +	28.4	28.2
			Cheese 4 mo. old			
Hansen starter	40.1	40.2	3.4 mi +	6.7 med +	29.0	29.0
Commercial starter	40.0	39.9	3.7 mi +	6.9 med +	29.0	28.9
LDK starters	41.2	40.9	4.7 med −	7.5 sh −	29.0	28.9
LDK stirred	40.4	40.4	3.5 mi +	6.9 med +	28.2	28.3
			Cheese 6 mo. old			
Hansen starter	40.0		5.2 med +		28.6	
Commercial starter	39.0		5.7 med +		29.0	
LDK starters	40.4		6.2 med +		28.9	
LDK stirred	40.7		5.9 med +		28.4	

[a] The numbers for flavor intensity are averages based upon numbers given for flavor intensity terms as 1 mild −, 2 mild, 3 mild +, etc., up to 9 sharp +.

[b] The data for the cheese made with Hansen starter are averages of 2 lots of cheese; all other data are averages of 3 lots of cheese.

much higher after 4 months. Tyramine production in this cheese was rather rapid, but only slightly more rapid than in cheese made with the commercial starter.

All of the cheese developed flavor rapidly for a product made from pasteurized milk and differences in Cheddar flavor intensities were slight (table 3). However, the regular Cheddar cheese made with both Hansen lactic starter and *S. faecalis* starter (LDK starters) tended to be slightly higher in flavor than the other samples. Both the Cheddar and stirred curd cheese made with lactic and *S. faecalis* starters tended to be highest in flavor scores but the differences were very slight. This slightly higher score for the *S. faecalis* cheese was illustrated by the detailed data not given in table 3. The four judges scored 11

samples of cheese below 40 and none of these samples contained the *S. faecalis* starter. It is interesting to note that the commercial starter produced rapidly-curing cheese of good flavor, but this cheese tended to develop less desirable flavor as aging progressed. This is indicated in the 39 score for the cheese cured 6 months at 50° F. The scores were not taken for all of the cheese ripened at 60° F. for 6 months, and no data are given for this cheese in the table as the cheese was overcured, but the cheese with commercial starter scored 3 points below the *S. faecalis* cheese.

The body of the cheese was scored uniformly, irrespective of the type of starter, but the stirred curd cheese received a lower score due to the open texture.

On several occasions in the laboratory, cheese was made by the stirred curd method from pasteurized milk inoculated with both lactic and *S. faecalis* starters. In all trials, the stirred curd cheese developed normal acidity even though the acid in the whey from the curd was low at the time of salting. *S. faecalis* grew in the salt concentration present in Cheddar and stirred curd cheese and developed acid while the cheese was in the press. In the present plant trials with stirred curd cheese, no time was given for acid development in the curd after drawing the whey. The salt was added to the curd when the whey tested 0.25 per cent acid. The pH of the cheese was normal when taken from the press and the cheese developed normal Cheddar flavor.

DISCUSSION

The commercial Cheddar cheese made by the usual factory workers from pasteurized milk with both lactic and *S. faecalis* starters developed Cheddar flavor of intensity and quality as expected. It was fast-curing cheese of excellent quality. The unexpected part of this study was that the cheese made without *S. faecalis* starter developed both flavor and tyramine almost as rapidly as the cheese to which *S. faecalis* had been added.

It is evident that the pasteurized milk contained small numbers of tyramine-producing bacteria which grew rapidly in the cheese. All samples of cheese gave counts in the millions per gram on azide-penicillin agar when only 1 month old, but some cheese showed no enterococci among the 10 colonies selected per plate. It is assumed that these other bacteria were lactobacilli that grew in the azide-penicillin agar. The cheese made with Hansen starter developed tyramine only as a result of natural inoculation in the milk and the quantities produced were substantial. The bacteria involved may have been solely enterococci, but, if such is the case, relatively small numbers of a few million per gram were ample to produce the tyramine.

The cheese made with commercial starter gave the same approximate bacterial counts as with Hansen starter, but the tyramine content of the cheese was appreciably higher and almost identical to the tyramine content of *S. faecalis* cheese. This problem has not been studied intensively, even though the evidence appears to indicate the presence of tyramine-producing bacteria in the commercial starter. The noteworthy fact is that the cheese to which *S. faecalis*

starter was added contained great numbers of this organism but only slightly increased amounts of tyramine. Hence, it must be concluded that only a few million of *S. faecalis* bacteria per gram of cheese are needed to convert the available tyrosine into tyramine or else other bacteria are able to produce this chemical reaction. That other bacteria may be involved is indicated by the higher tyramine content of cheese made with commercial starter as compared with Hansen starter. Limited trials to isolate from the cheese other bacteria that produced tyramine were unsuccessful, but a direct inoculation in the tyrosine broth with the cheese made without *S. faecalis* starter showed it to produce tyramine rapidly. This may have been due to bacteria other than the enterococci.

It should be evident, therefore, that the addition of *S. faecalis* starter to pasteurized milk will not increase substantially tyramine and flavor production if the natural bacterial population in the milk is ample to produce rapidly curing cheese of excellent flavor. If the cheese tends to cure slowly to relatively flat flavors then *S. faecalis* starter should increase the rate of curing and the quality of the flavor. It seems reasonable to assume that the use of *S. faecalis* starter should give rather uniformly rapid curing with excellent flavors under the widest variety of conditions, but the improvement should be most pronounced in milk of best quality where the infection with tyramine-producing bacteria is the least. The results clearly substantiate the observation that tyramine and flavor production increase together in cheese.

The usual commercial lactic starter may or may not develop sufficient acid in the presence of salt. The ability of *S. faecalis* to develop acid rapidly after salting the curd is very important in several regards. It permits the addition of salt to cheese curd containing insufficient acid with assurance that the correct acidity will develop in the cheese during pressing. This should be an aid in manufacturing Cheddar cheese of more uniform acid content and consequently more uniformly good quality, as slow acid development sometimes has been a cause of poor quality cheese. It also should be a boon to the manufacture of stirred curd. Matting of cheese curd was introduced to permit lactic acid bacteria to produce sufficient acid to control curing properly and to reduce the tendency toward undesirable fermentations, such as gas production. The use of better quality milk properly pasteurized will eliminate undesirable fermentations in the vat and *S. faecalis* starter will assure proper acidity, even after salting. Hence, the time of stirring the curd was reduced to that needed to control moisture, and the making process was reduced by 2 hours and the cured cheese was good typical Cheddar.

CONCLUSIONS

The control cheese made from pasteurized cheese milk with commercial lactic starter contained 290 γ tyramine per g. and gave counts of 13 million enterococci per gram after curing for 4 months at 50° F. The flavor scored 40 and was rated as mild + in intensity. This control cheese cured well.

The addition of *Streptococcus faecalis* starter to the pasteurized milk produced cheese which, after curing for 4 months at 50° F., contained 354 γ tyra-

mine per g. and gave counts of 394 million enterococci per gram. The flavor score was 41.2 and the intensity of flavor was medium –. Similar trends in the data were obtained for cheese ripened at 60° F. Apparently cheese made from pasteurized milk which naturally contained bacteria that ripened the cheese especially well, was improved slightly or not at all by the use of *S. faecalis* starter in the milk.

Cheese of good Cheddar flavor was made by the stirred curd process with *S. faecalis* starter, as much of the desired lactic acid was produced in the cheese after the salting of the curd. The stirred curd method saved 2 hours in the manufacturing process.

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THE EFFECT OF THE INTERVAL BETWEEN WASHING OF THE UDDER AND ATTACHMENT OF MILKING MACHINES UPON THE MILK PRODUCTION OF DAIRY COWS[1]

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During recent years considerable emphasis has been placed upon the importance of rapid milking of dairy cows. This method of milking not only saves time but also is believed to increase milk production and to decrease the incidence of mastitis. It has been observed that many dairymen do not attach milking machines during the recommended period of time after washing a cow's udder. A number of reports (1, 2, 3, 4, 5, 7, 9, 11) has indicated that any delay in removing the milk from a cow following washing of the udder with warm water results in a decrease in milk production.

Data on a series of six trials are presented in this report. These trials were conducted under carefully controlled conditions in an effort to determine the effects of regularly delayed milking, following washing of a cow's udder, upon milk production.

EXPERIMENTAL PROCEDURE

All cows used in these trials were grade Holsteins from the Pennsylvania State College research herds. All cows were milked twice daily. Their udders were washed carefully with water at 125 to 130° F. for approximately 20 seconds. Two or three streams of fore-milk were removed from each teat into a strip cup immediately after washing the udders. Individual interval timers were used for each cow. Machine stripping was followed throughout. All cows were fed and managed similarly and all cows in each trial were housed in the same stable. They were located at random throughout the barn so as to avoid any positional effects. Thus the only known variation was the elapsed time between washing the udders and attachment of milking machines.

In the first trial 30 cows with an average daily milk production of 52 lb. were divided into five groups as nearly alike as possible on the basis of daily milk and milk fat production, stage of gestation and stage of lactation. Milking machines were attached 2, 4, 6, 8 and 10 minutes after the washing was started. This trial was conducted for 35 days at each of the two milkings per day.

It was necessary, because of the need for cows for other experiments on pasture management, to discontinue 15 of the cows on the first trial after 35 days. Accordingly, 15 cows remaining in the 2-, 6- and 10-minute groups of the first trial were continued for an additional 35 days. The data covering the 70 days

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with the latter group of 15 cows is referred to as the second trial. The cows used in the second trial were carried for 70 days with elapsed times of 2, 6 and 10 minutes between washing and attachment of machines at each milking.

In the third trial, 16 cows with an average daily production of 40 lb. per day were divided into four similar groups. In this trial the time intervals were 2, 5, 10 and 20 minutes and the trial was conducted for 90 days.

In the fourth trial, 12 cows with an average daily milk production of 45 lb. were divided into four similar groups. In this trial, the cows all were fed on the basis of 110 per cent of the Morrison (8) standards for good cows under usual conditions. The hay and silage fed were identical in amount for all cows on the trial. The daily grain ration was changed every 2 weeks on the basis of milk and milk fat production for 5 days previous and also on the basis of body weight at the beginning of each 2-week period. This trial was conducted for a period of 84 days.

TABLE 1

The effect of delayed milking following washing of cows' udders upon milk production (first trial)[a]

Days	Milk production with time intervals of:				
	2 min.	4 min.	6 min.	8 min.	10 min.
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
−5 thru −1	50.4	56.3	50.8	51.6	52.7
1 thru 5	49.8	55.2	50.5	49.4	51.0
31 thru 35	49.2	52.4	49.3	46.9	50.8
Decrease	1.2	3.9	1.5	4.7	1.9

[a] Six cows were used per group. Data are expressed in terms of mean daily milk production.

In the fifth and sixth trials, experiments were designed to compare the production of halves of udders, in which whole udders were delayed 12 minutes but each half was milked simultaneously in a quarter-section milker,[2] to production of halves of udders of cows milked 2 and 12 minutes, respectively, following the beginning of washing. These two trials were designed to explain the discrepancies between previous results published by Knodt *et al.* (6) and those of Ward and Smith (11).

Analyses of variance were conducted according to the methods of Snedecor (10) wherever applicable.

RESULTS

A summary of the data obtained in the first trial will be found in table 1. Where recommended procedure was followed, as in the 2-minute group, there was a mean daily decline of 1.2 lb. of milk during the 35-day period. In those groups in which there was a delay of 4, 6, 8 or 10 minutes prior to the attachment of machines following the beginning of washing, the respective mean declines were 3.9, 1.5, 4.7 and 1.9 lb. of milk per cow per day. There were no

[2] The authors wish to express their appreciation to the De Laval Separator Co., New York, N. Y., for the equipment used in these trials.

observable immediate declines or variations in milk production at the beginning of or during this trial. An analysis of variance proved there was no statistically significant effect of treatment in this trial as far as milk production was concerned.

Fifteen cows from the first trial were continued for an additional 35 days. During the first trial, these cows were in the 2-, 6- and 10-minute groups and were continued for an additional 35 days, making a total of 70 days in what is referred to as the second trial (table 2). These three groups were comparable

TABLE 2

The effect of delayed milking following washing of cows' udders upon milk production (second trial)[a]

Days	Milk production with time intervals of:		
	2 min.	6 min.	10 min.
	(*lb.*)	(*lb.*)	(*lb.*)
−5 thru −1	54.7	52.2	55.5
1 thru 5	54.3	50.8	54.0
66 thru 70	41.8	41.4	45.6
Decrease	12.9	10.8	9.9

[a] Five cows were used per treatment. Data are expressed in terms of mean daily milk production.

in milk production, stage of lactation and stage of gestation. During the course of this trial there was a decline of 12.9, 10.8 and 9.9 lb. of milk per cow per day for the 2-, 6- and 10-minute groups, respectively. An analysis of variance proved that the differences in milk production shown in table 2 were not significant.

TABLE 3

The effect of delayed milking following washing of cows' udders upon milk production (third trial)[a]

Days	Milk production with time intervals of:			
	2 min.	5 min.	10 min.	20 min.
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
−5 thru −7				
0	40.2	37.0	41.1	40.8
1 thru 10	40.4	37.7	41.4	39.1
81 thru 90	36.8	29.4	31.7	29.2
Decrease	3.4	7.6	9.4	11.6

[a] Four cows were used per treatment. Data are expressed in terms of mean daily milk production.

The data for the third trial are presented in table 3. The time intervals between the beginning of the washing of the cows' udders and the attachment of the machines were 2, 5, 10 and 20 minutes, respectively. During the 90-day period during which this trial was conducted, declines of 3.4, 7.6, 9.4 and 11.6 lb. per cow per day were found for the 2-, 5-, 10- and 20-minute groups, respectively. While the differences were quite large between the mean declines in

daily milk production of the various groups, actually these differences were not statistically significant. There was considerable variation in the response of various cows. One cow in the 5-minute group declined 17.7 lb. per day and two cows in the 10-minute group declined 20.8 and 16.3 lb. per day, respectively, but two others decreased only 1.4 and 1.1 lb. per day, each, during the 90 days of this trial. Apparently these decreases were not the result of the delay in milking but, rather, were due to a lack of persistency of the cows involved.

As a result of the variable responses observed in the third trial as compared to the other two, a fourth trial was conducted. These data are presented in table 4. In this experiment, as in trial 3, two cows that were lacking in per-

TABLE 4

The effect of delayed milking following washing of cows' udders upon milk production (fourth trial)[a]

Days	Milk production with time intervals of:			
	2 min.	5 min.	10 min.	20 min.
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
−7 thru −1	44.4	44.3	45.4	45.1
1 thru 7	42.9	43.4	39.5	42.9
78 thru 84	22.8	37.9	42.3	42.8
Decrease	21.6	6.4	3.1	2.3

[a] Three cows were used per treatment. Data are expressed in terms of mean daily milk production.

sistency unavoidably were placed in the 2-minute group. One of these declined 22.5 and the other 38.0 lb. per day during the course of this trial. Production of the other cow in the 2-minute group declined only 0.5 lb. per day during the 84-day experimental period. In those groups in which milking was delayed 5, 10 and 20 minutes there were mean daily decreases of 6.4, 3.1 and 2.3 lb. of milk respectively. The decreases in production of the latter three groups are considered equal to or less than the normal decline in production to be expected in such a period of time.

All cows in trials 5 and 6 were milked for 5 days with a delay of 2 minutes between the beginning of washing of the udders and attachment of the machines. The production of each half was determined on each of the twice-daily milkings. It will be observed in table 5 that in those cows in which one-half of the udder was delayed 2 minutes and the other half 12 minutes that the mean daily milk production per half declined 0.1 and 3.3 lb., respectively. However, when milking was delayed 12 minutes on both halves, the mean daily declines were only 0.6 and 0.3 lb., respectively.

While a reversal trial is open to question because of the apparent carry-over effects of previous treatment, the same groups of cows immediately were milked for a period of 5 days involving a 2-minute delay as presented in trial 6. The left halves of the udders of the cows of group A did not recover in milk production during the 5-day control period at the beginning of trial 6 (table 6).

TABLE 5

The effect of delayed milking following washing of cows' udders upon milk production (fifth trial)[a]

Group	A		B	
Half of udder	R. H.	L. H.	R. H.	L. H.
Delay (*min.*)	2	2	2	2
Days	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
1	27.0	27.6	26.1	28.5
2	27.2	27.9	26.4	28.4
3	27.7	27.7	26.5	28.8
4	28.3	28.4	27.1	28.1
5	27.9	29.3	27.1	28.0
Av.	27.6	28.0	26.6	28.3
Delay (*min.*)	2	12	12	12
1	27.5	23.2	26.7	28.0
2	27.8	20.6	26.0	29.2
3	27.9	19.7	25.9	28.5
4	27.1	20.0	25.9	27.5
5	27.4	20.0	25.8	27.0
Av.	27.5	24.7	26.0	28.0
Decrease	0.1	3.3	0.6	0.3

[a] Each datum represents mean total daily milk production per half-udder for four cows.

TABLE 6

The effect of delayed milking following washing of cows' udders upon milk production (sixth trial)[a]

Group	A		B	
Half of udder	R. H.	L. H.	R. H.	L. H.
Delay (*min.*)	2	2	2	2
Days	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
1	28.2	26.2	25.8	27.2
2	28.9	26.0	26.4	28.0
3	28.7	26.1	26.5	28.4
4	28.6	26.4	26.7	27.9
5	28.1	26.8	27.1	28.0
Av.	28.5	26.3	26.5	27.9
Delay (min.)	12	12	2	12
1	27.3	26.8	25.8	26.8
2	26.4	25.9	26.4	26.8
3	27.0	25.4	26.5	25.5
4	26.9	25.9	26.7	25.4
5	28.9	27.5	27.1	25.9
Av.	27.3	26.3	25.3	26.0
Decrease	1.2	0.0	1.2	1.9

[a] Each datum represents mean total daily milk production per half-udder for four cows.

Following this period, the groups were reversed and these data are presented in table 6. The greatest decline in mean daily milk production also occurred in those halves delayed in milking for 12 minutes of cows in which the other half was delayed 2 minutes in milking following preparation. In trial 6 in those cows where both groups of halves of the udders were delayed 12 minutes prior to milking, the change in mean daily milk production was 0.0 lb. and only equal to that of those half-udders delayed for 2 minutes on the other group of cows in this trial, respectively. Analysis of variance of these data indicates that the declines in production were not significant where entire udders were delayed for 12 minutes. However, the declines in mean daily milk production were highly significant when delays of 12 minutes were incurred and compared to the other halves of the udders that had been milked after a 2-minute delay. This raises a question relative to attempting to draw conclusions from half-udders or from quarters in experiments of this type.

In only exceptional cases did the cows fail to let down their milk to the stimulation of preparation. The incidence of this was not related to treatment.

DISCUSSION

The series of six trials reported was conducted in an effort to obtain additional data emphasizing the importance of rapid milking following the washing of cows' udders. These data indicate that an interval of up to 20 minutes between the beginning of washing of a cow's udder and attachment of milking machines does not affect milk production. Apparently the cows were able to condition themselves immediately to a change in procedure from a 1- to 2-minute routine prior to these trials.

These data are in disagreement with the results obtained by Miller and Petersen (7) and Ward and Smith (11). The mean levels of production of the cows studied by the above authors were considerably below those reported in this publication, indicating that they may have been nearer to the end of the lactation period. In addition, the work of Ward and Smith (11) may have been confounded by the effects of milking one half of the udder upon production of the delayed half as indicated by the data presented in this report. Further, their study was conducted upon only five cows as compared to 66 used in the trials in these experiments. The variations between cows are extremely large and also may have been a factor.

The data presented show no marked declines in milk production during the first week to 10 days in any of the groups of cows used in the first four trials. Conclusions from these data, however, should not affect in any way the routine followed in good milking procedures. A method should be followed in which the cows' udders are washed 1 to 2 minutes prior to the attachment of machines because of efficiency of labor and production of clean milk. Occasional cows may lie down in their stalls if delays of longer than 2 minutes are incurred following washing. Their udders then will have to be washed again to produce clean milk.

SUMMARY

Regular delays up to 20 minutes from the beginning of washing cows' udders until the attachment of milking machines apparently did not decrease milk production under the conditions of these trials. Routine fast milking procedures, however, should emphasize the importance of attaching machines 1 to 2 minutes after washing because of labor efficiency and production of clean milk. Data are presented which emphasize the importance of using entire udders in drawing conclusions in work of this type.

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THE EFFECT OF THE ASCORBIC ACID CONTENT OF FLUID MILK UPON THE KEEPING QUALITY OF ITS DRIED PRODUCT

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Milk freshly drawn from the udder contains an average of 22 to 23 mg. per l. of ascorbic acid, present entirely in the reduced form, according to Knight *et al.* (4) and Kon and Watson (5, 6). As soon as milk is drawn, however, it is exposed in a greater or less degree to the oxygen of the air, to light and perhaps to contaminating metals. These all serve, according to Kon and Watson (6) and Whitnah *et al.* (7), to reduce the amount of ascorbic acid until, after a 24-hour period, only a small fraction of the original amount may be left.

Since ascorbic acid is oxidized rather easily, it is possible that it plays an important role as a retarder of oxidative deterioration. While considerable work has been reported on the effect of ascorbic acid added to fluid milk, reports of the effect of ascorbic acid added to fluid milk on the keeping quality of its dried product are conflicting (1, 2); therefore, it was decided to add ascorbic acid in dilute water solution to fluid milk and determine the effect of the addition on the keeping quality of the dried product.

EXPERIMENTAL PROCEDURE

All fluid milks used were mixed samples from the same herd and, while considered to be of average to good quality, were not selected milks. Four groups of dried whole milk were prepared from different herd milks. In preparing each group a large quantity of herd milk was standardized at 3.3 per cent fat and divided into four equal lots. One lot was used as a control and was concentrated and spray-dried without the addition of ascorbic acid. Increasing amounts of ascorbic acid in dilute aqueous solution were added to the remaining lots. To the second, third and fourth lots, 5, 10 and 20 mg. per l., respectively, were added. These additions were equivalent to increases of approximately 25, 50 and 100 per cent, respectively, based on a normal estimated ascorbic acid concentration of 20 mg. per l. in fresh raw milk several hours old. All four lots were preheated to 170° F. for 30 minutes, concentrated in a vacuum pan and spray-dried. The dry milk was collected in 10-gallon milk cans which were closed tightly and held overnight at 34° F. The following morning the cans and contents were allowed to come to room temperature and the milk was weighed into small tin cans holding approximately 70 g. each. The cans were punctured and evacuated under 2 mm. pressure for 1 hour, the vacuum released with nitrogen and the holes soldered. The cans of dry milk were placed in storage at 98.6° F. Sample cans were removed at 2-week intervals and the milk reconstituted and judged for flavor. Moisture in the dry milks of group

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1 ranged from 2.5 to 3 per cent, of groups 2 and 4, from 1.6 to 1.8 per cent and of group 3, 1.3 per cent. Fat content was approximately 26.7 percent in all samples.

RESULTS

Table 1 shows the apparent ascorbic acid content (all those substances which reduce 2-6, dichlorophenolindophenol) of the fluid milk in the control sample

TABLE 1

The apparent ascorbic acid content of the control samples of raw milk and their dried products as soon as prepared

Group no.	Raw milk	Reconstituted dried milk
	(*mg./l.*)	(*mg./l.*)
1	15.6	15.1
2	15.6	10.4
3	15.0	4.6
4	14.8	4.2

of each group and the concentration in the freshly prepared dried whole milk when reconstituted. While there is no appreciable variation in the concentration of apparent ascorbic acid in the fluid milk of each group, there is a marked difference in the concentration of apparent ascorbic acid in the reconstituted dried milk. Milks seem to vary in the degree of retention of apparent ascorbic acid content during processing.

A comparison of the data in tables 1 and 2 shows that those samples which retained the greatest amount of apparent ascorbic acid during processing have the best keeping quality. To what extent this destructive action accompanying heating can be used as a criterion of the keeping quality of the dried product is being determined.

TABLE 2

The effect of increasing the ascorbic acid content of fluid milk before heat treatment on the keeping quality of its dried product

Ascorbic acid added	Keeping quality of dried product at 37° C.			
	Group 1	Group 2	Group 3	Group 4
(*mg./l.*)	(*d.*)	(*d.*)	(*d.*)	(*d.*)
0	364	213	38	70
5	405	395	68	140
10	419	443	144	182
20	600	456	181	295

The data in table 2 are based on the time required for the stored milk to become so tallowy as to be inedible. These data indicate that ascorbic acid added to the fluid milk before preheating increases the keeping quality of its dried product. In every series of dry milks, the larger the amount added, the better the keeping quality. However, no recommendation can be made on the

basis of these experiments as to just how much ascorbic acid should be added to keep the dried milk edible for a given number of days.

The keeping quality of the dried milk in group 1 to which 20 mg. per l. of ascorbic acid were added was exceptional. This dried milk sample never did become tallowy. It finally was judged inedible because of other flavors that developed from long storage at 37° C. The concentration of apparent ascorbic acid of this milk never dropped below 16 mg. per l. and after storage for 20 months was greater than immediately after drying.

Figure 1 shows the change in the apparent ascorbic acid of this milk and also of a milk with poor keeping quality with no added ascorbic acid. A study of

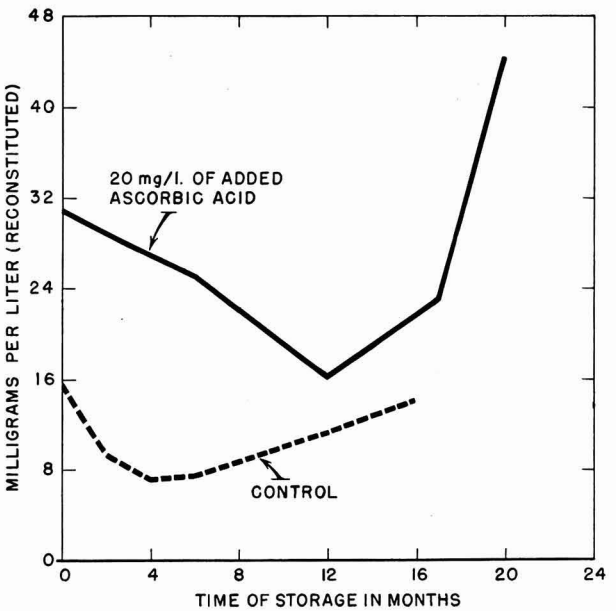

FIG. 1. The change in apparent ascorbic acid during storage at 37° C. (98.6° F.)

this figure indicates that reducing substances are formed in storage and that in a dried milk with good keeping quality the apparent ascorbic acid, while dropping to a certain minimum value, seems afterwards to increase until there appears to be more apparent ascorbic acid present than in the dried milk freshly made. On the other hand, in the milk marked "control," the concentration of apparent ascorbic acid drops to a lower value than does that of the milk of good keeping quality, which apparently allows the oxidation to proceed and produce tallowiness and other off flavors.

The results in figure 1 illustrate the changes in reducing capacity in two dried milks which differ greatly in their keeping quality. It would seem that

to retard oxidation materially a dried milk must have an initial reducing capacity of such magnitude that even after a loss in this characteristic a sufficient reducing action remains to prevent the onset of oxidation until the product can form its own reducing substances. If a product does not possess a high reducing capacity initially, the loss in this property makes possible the early onset of oxidative reactions and results in a product of low keeping quality.

Since it had been suggested by Guthrie (3) that the complete destruction of ascorbic acid in milk prior to its pasteurization might inhibit the development of the tallowy flavor, several lots of dry milk from fluid milk in which the ascorbic acid had been oxidized with hydrogen peroxide were prepared to find out what effect the complete absence of reduced ascorbic acid would have on the keeping quality of the dry milk. Three pairs of dry milks were prepared, using the same heat treatment and storage temperatures as with those described in table 1. To one fluid milk of each pair just enough dilute hydrogen peroxide was added carefully to oxidize the ascorbic acid completely. The results shown in table 3 indicate that the dried products from the milks to which hydrogen peroxide was added became tallowy more rapidly than those where no hydrogen peroxide was added.

TABLE 3

The effect of oxidizing the ascorbic acid in the fluid milk with hydrogen peroxide on the keeping quality of its dried product

Pair no.	Time to become inedible at 37° C. (98.6° F.)	
	Normal ascorbic acid	Ascorbic acid oxidized by H_2O_2
	(*d.*)	(*d.*)
1	308	239
2	231	189
3	266	224

CONCLUSIONS

1. Ascorbic acid added to milk before drying increases the keeping quality of its dried product.

2. Oxidation of ascorbic acid in the fluid milk with hydrogen peroxide does not increase the keeping quality of its dried product.

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THE EFFECT OF A PERIOD OF NON-MILKING ON THE LEUCOCYTE COUNT OF MILK

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This paper reports an experiment on the effect of interrupted milking upon the leucocyte count of milk. A parallel study on the effect of interrupted milking upon milk production and the vitamin A and carotene content of milk will be reported elsewhere.

METHODS

Cows in the middle of their lactation periods and free from mastitis (2) were used, six as experimental animals and two as controls. During the experiment they were on a controlled ration and, except as noted below, were milked at 12-hour intervals. Representative samples of milk were taken from each cow at each milking for 3 days. Then for 10 days the six experimental cows were not milked but the two control animals were milked as usual. After 10 days, normal milking schedules were resumed on the experimental cows and milk samples were collected at each milking for 3 days. The milk samples from the first 3 days are referred to as the "pre" samples and those from the last 3 days as the "post" samples.

Milk films were prepared from each sample, defatted in xylol and stained with Newman-Lampert stain (1). Sixty microscopic fields were examined on all "pre" milk films and the average number of leucocytes determined as described in "Standard Methods" (1). The "post" milk films were treated similarly except that in most cases fewer microscopic fields were examined because of the higher level of leucocytes.

For statistical analysis the leucocyte counts were converted to logarithms. The difference between the logarithms of the "pre" and "post" counts of the milk from the experimental animals was compared with the corresponding difference from the milk of the controls by an analysis of variance.

RESULTS AND DISCUSSION

The leucocyte count of the milk from the control cows varied little throughout the entire experiment but that from the experimental animals increased significantly after the period of non-milking. The geometric mean leucocyte count of the "pre" samples of the experimental animals was approximately 100,000 leucocytes per ml., and that of the "post" samples 4,860,000 leucocytes per ml. The individual counts and records of milk production at each milking are given in table 1. The corresponding leucocyte counts of the controls averaged 180,000 leucocytes per ml. during the "pre" period and 300,000 leucocytes per ml. during the "post" period, which may be compared with the average count of the control animals for the entire 16 days of 200,000 leucocytes per ml.

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TABLE 1

Leucocyte counts on "pre and post interruption" milk samples and milk production records of six experimental cows and two control cows

Cow	Pre sample						Post sample					
	1st d.		2nd d.		3rd d.		1st d.		2nd d.		3rd d.	
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
						Leucocyte counts (*millions/ml. of milk*)						
A	0.13	0.16	0.11	0.17	0.96	0.95	4.98	4.86	4.38	3.72	4.14	2.98
B	0.14	0.01	< 0.01	0.01	0.05	0.08	8.40	5.64	2.91	3.88	3.60	2.46
C	0.01	0.04	0.02	0.04	0.02	0.10	9.72	8.40	6.06	5.94	6.48	3.00
D	0.73	0.89	0.21	0.46	0.83	0.26	11.34	6.72	6.84	5.16	3.66	5.40
E	0.06	0.07	.....[a]	0.32	0.15	0.21	3.60	2.52	3.96	3.48	2.82	1.86
F	0.15	0.07	0.07	0.13	0.06	0.08	15.00	17.10	12.18	7.44	4.28	4.80
Control G	0.27	0.31	0.26	0.28	0.22	0.33	0.49	0.48	0.40	0.35	1.15	0.35
Control H	0.09	0.08	0.05	0.30	0.30	0.04	0.10	0.08	0.26	0.24	0.36	0.40
						Milk production (*lb.*)						
A	7.7	6.1	8.2	5.7	7.3	6.2	1.7	0.5	0.5	0.9	0.8	1.2
B	12.6	10.6	13.0	10.1	12.6	9.5	2.6	0.6	1.6	1.3	1.8	1.3
C	11.2	8.9	11.3	8.2	10.4	7.7	2.4	0.6	0.8	0.8	1.1	1.1
D	9.5	8.1	9.6	7.8	8.5	7.6	0.9	0.5	0.5	0.6	1.1	0.9
E	9.2	8.6	10.7	8.3	10.8	8.8	3.9	1.9	2.1	2.3	3.2	3.1
F	8.9	7.0	8.9	7.1	8.2	7.0	1.4	0.5	0.7	0.7	0.7	0.7
Control G	13.6	11.6	14.7	10.7	13.6	10.5	14.3	10.9	11.7	14.3	12.6	10.9
Control H	9.6	9.1	9.3	8.8	9.3	7.6	9.5	6.1	10.4	9.2	6.8	9.3

[a] No sample.

The higher leucocyte counts of the "post" samples as compared with the "pre" samples is evident from table 1. No marked difference occurred in the leucocyte counts of the milk from the control cows. An analysis of variance of the differences between the "pre" and "post" log counts showed that the increased count in the experimental animals was significantly greater ($P < 0.02$) than the corresponding difference in the samples from the control cows.

Since the only known variable between the experimental and control cows was the milking schedule, the increased leucocyte counts of the "post" samples from the experimental animals may be attributed to the 10-day interruption in milking. The ratio in logarithms of the relative increase in milk from the experimental cows to that from the control cows was 1.439 ± 0.433. In this experiment non-milking decreased milk production 85 per cent and increased the leucocyte count about 27-fold. The confidence limits of the true proportional increase in leucocyte count varied from a factor of 2.4 to a factor of 316 at odds of 19 in 20.

SUMMARY

Interruption in the milking of normal cows in the middle of their lactation cycles increased the leucocyte count of the milk about 27-fold.

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THE EFFECT OF *STREPTOCOCCUS LACTIS* AND COLIFORM ORGANISMS ON THE SOLUBLE NITROGEN OF MILK

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The results of various investigators have shown that many strains of *Streptococcus lactis* are capable of bringing about protein degradation in milk. Early investigations concerning proteolysis of *S. lactis* as measured by soluble nitrogen, by amino nitrogen (determined by the Van Slyke procedure, formol titration or the nitrogen fraction soluble in phosphotungstic acid) and by ammonia determinations, were reviewed by Hammer and Patil (4). In most of these early investigations trials were carried out after periods of incubation ranging from 1 week to 4 months, using milk to which amounts of calcium carbonate sufficient to neutralize all, or nearly all, of the acid that could be produced had been added. Hammer and Patil (4) incubated several strains of *S. lactis* in milk and determined nitrogen soluble in acetic acid and Van Slyke amino nitrogen after periods of incubation ranging from that required for coagulation to 9 days. They demonstrated that a definite proteolysis is evident in many freshly coagulated cultures.

Very little work has been published on proteolysis by *Escherichia coli* and *Aerobacter aerogenes*. Kendall *et al.* (5) reported that *E. coli* had little action upon the protein constituents of milk, as shown by measurements of ammonia content. Spitzer *et al.* (8) found that at 15 days *E. coli* had produced no ammonia but had produced detectable amino acids and peptones, while *A. aerogenes* had produced detectable ammonia and no detectable amino acids or peptones. They used Folin's aeration method for ammonia and precipitation with zinc sulfate and phosphotungstic acid for peptone and amino acid determinations. Berger *et al.* (2) demonstrated the production of peptidases by *E. coli* growing in a peptone glucose medium. Staffe (9) found that growth of *E. coli* in milk at 30° C. causes an increase in the nitrogen soluble in methyl alcohol. These increases were detectible at 1 week, and the nitrogen soluble in methyl alcohol continued to increase throughout the 7-week experimental period. Taylor (10) grew *E. coli communis* in milk for 5 months, after which he coagulated the casein with acid and filtered the mixture. Because of the large amount of precipitate obtained from the whey when phosphotungstic acid was added and the negative Biuret test on the filtrate, he concluded that casein was digested mainly to proteoses and peptones with the formation of only a small percentage of amino acids.

EXPERIMENTAL

In this work proteolysis by *S. lactis, E. coli* and *A. aerogenes* growing separately and in combination at 30° C. in skim milk in the absence of calcium car-

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bonate for periods up to 15 days was studied. Nitrogen soluble and insoluble in trichloracetic acid, total nitrogen, titratable acidity, plate count and differential count (where two or more organisms were concerned) were determined.

All organisms used were isolated from samples of raw milk and raw cream just prior to the beginning of the study. Only active strains were used. All strains of *S. lactis* were typical members of the species as characterized by Sherman (7). *E. coli* and *A. aerogenes* were typical according to *Bergey's Manual of Determinative Bacteriology* (3). The stock cultures were transferred once a week, using sterile litmus milk. Cultures were transferred every 24 hours for two or three transfers prior to each trial. One-tenth of 1 per cent inoculum was used in all cases. For trials in which two or more different organisms were used, cultures 20 to 24 hours old were mixed in equal quantities; after the mixture had been shaken thoroughly, the desired inoculum was withdrawn and added to the test bottles of milk. Cultures were incubated at 30° C. to obtain a high rate of change in the substratum without inhibiting the growth of one or more types of organisms used. Data on uninoculated control samples are not included since spore-forming organisms caused rapid decomposition of such samples; however, the presence of acid-producing bacteria prevented the growth of significant numbers of spore-forming organisms. This was evidenced by the absence of any significant number of colonies of spore-forming organisms on the plates incubated for total bacterial count and the absence of abnormal fermentation in the inoculated cultures.

Milk for the trials consisted of fresh skim milk which was heated at 185° F. for 20 minutes to destroy as many undesirable organism types as possible without subjecting the milk constituents to the changes that would accompany sterilization in the autoclave. Skim milk was chosen because the absence of fat facilitated accurate sampling and analysis. Four-ounce screw top bottles were used as containers, and a separate bottle containing 100 ml. of milk was used for each day of testing. At the end of the incubation period the loss in weight, due to evaporation, was made up by adding sterile distilled water to each bottle. With trials using only *S. lactis,* caps were screwed down tightly; in trials where *E. coli* and *A. aerogenes* were involved, caps were left loose to permit the escape of gases.

Coagulated samples were blended for 5 minutes in a sterile Waring blender with a semi-micro attachment and then transferred to a 250-ml. Erlenmeyer flask. Foam was dispersed by adding 8 to 10 drops of n-octyl alcohol to each sample in which coliform organisms had grown. This latter practice was not employed in trials with *S. lactis,* and foam undoubtedly was responsible for the occasional variation in total nitrogen values. Extreme care was used to agitate the undiluted and the diluted samples thoroughly just prior to pipetting, since there was some tendency for precipitation in coagulated samples, even after they had been blended for 5 minutes. Blending for longer periods did not overcome this tendency. A 50-g. quantity of blended sample was weighed into a 100 ml. volumetric flask and the remaineder of the volume was made up with

distilled water. A 10 ml. quantity of this solution, representing 5 g. of sample, was used for each nitrogen determination. Nitrogen fractions insoluble in trichloroacetic acid were precipitated by the method developed for cheese serum by Lane and Hammer (6), except that in this work 10 ml. portions of the diluted sample were used, while Lane and Hammer (6) used 1 ml. of cheese serum. All nitrogen determinations were made by the Kjeldahl procedure. Duplicate determinations were made in all cases.

Samples for total bacterial counts and/or differential count were taken directly from the blender in case of coagulated samples and from the sample bottle when blending was unnecessary because the culture had not coagulated. Total counts were made on standard milk agar and differential counts were made on violet red bile agar, according to *Standard Methods for the Examination of Dairy Products* (1), except that a temperature of 30° C. was used for incubation of plates for total counts.

RESULTS

Determinations were run on four strains of *S. lactis;* one strain of *E. coli;* one strain of *A. aerogenes;* and a combination of two strains of *S. lactis, E. coli* and *A. aerogenes.*

TABLE 1

Proteolysis by Streptococcus lactis in heated skim milk (av. of duplicate determinations)

Culture	Age	Titratable acidity	Plate count	Sol. N
	(*d.*)	(%)	(*/ml.*)	(*% of total N*)
	0	0.16	300	7.6
	1	0.77	3,400 M[a]	12.9
C5	3	0.85	1,200 M	13.9
	7	0.86	1,600	15.5
	15	0.88	1,200	16.3
	0	0.14	50	6.4
	1	0.74	2,200 M	8.5
C5	3	0.79	1,000 M	10.4
	7	0.83	310 T[b]	11.8
	15	0.88	60 T	12.7
	0	0.16	300	7.6
	1	0.73	2,400 M	10.3
C3	3	0.82	500 T	11.3
	7	0.83	3,200	12.5
	15	0.87	250	13.0
	0	0.15	190	9.3
	1	0.80	2,500 M	14.4
M17	3	0.86	67 M	16.0
	7	0.86	200 T	17.6
	15	0.89	85 T	19.0

[a] M = million
[b] T = thousand

In table 1 are presented representative results showing increases in soluble nitrogen caused by *S. lactis.* In all instances the production of soluble nitrogen by the four strains of *S. lactis* tested was fairly rapid during the first day or so

when the population was at a maximum and acid production was at the most rapid rate. Later when acid production occurred very slowly or not at all and the population of viable cells was declining rapidly, the rate of production of soluble nitrogen also declined, although there was a gradual increase in the soluble nitrogen throughout the full period of experimentation. Variations in soluble nitrogen production were evident between the various strains of *S. lactis* employed and between the different trials with the same strain. The variations between different strains and variations found in three trials with one strain (C5) were of about the same magnitude.

TABLE 2

Proteolysis by Escherichia coli and Aerobacter aerogencs in heated skim milk (av. of duplicate determinations)

Culture	Age	Titratable acidity	Plate count	Coliform count	Sol. N
	(*d.*)	(%)	(*/ml.*)	(*/ml.*)	(*% of total N*)
	0	0.17	180	.....	6.7
	1	0.41	.....	1,200 M[a]	5.9
E. coli	3	0.63	1,000 M	880 M	7.0
	7	0.70	.....	400 M	7.4
	15	1.14	.....	< 100	21.5
	0	0.17	220	.....	6.8
	1	0.42	.....	2,200 M	5.8
A. aerogenes	3	0.42	.....	2,800 M	5.7
	7	0.46	.....	600 M	6.4
	15	1.00	5,300	5	26.1

[a] M = million

Representative data on *E. coli* and *A. aerogenes* (table 2) show these organisms caused a decrease in the soluble nitrogen during the first day or two when the populations were at the maxima. As the numbers of viable cells decreased these deficits gradually were overcome, and between the seventh and fifteenth days both organisms caused a marked increase in soluble nitrogen. The increase in soluble nitrogen was higher at 15 days for this strain of *A. aerogenes* than for the strain of *E. coli* used. The increase in soluble nitrogen was slightly greater during a second trial with *A. aerogenes,* while *E. coli* caused about the same increase in soluble nitrogen during each of two trials.

When *S. lactis* C6, *S. lactis* M17, *E. coli* and *A. aerogenes* were grown together in approximately equal quantities in skim milk, in no case was a rapid increase in soluble nitrogen caused by the combination of organisms (table 3). However, there was a gradual and definite rise in the soluble nitrogen in all cases, the increase being intermediate between that of the pure cultures grown singly during the early stages and more like that of *S. lactis* alone in the later stages, where the greater proteolysis typical of these gram-negative bacteria was held in check.

In one trial using the combination of organisms, extra bottles of sample were neutralized to 0.27 per cent titratable acidity with sodium hydroxide at 7 and

TABLE 3

Proteolysis by a mixture of Streptococcus lactis C5, S. lactis M17, Escherichia coli and Aerobacter aerogenes in heated skim milk (av. of duplicate determinations)

Age	Titratable acidity	Standard plate count	Coliform count	Sol. N
(*d.*)	(%)	(*/ml.*)	(*/ml.*)	(*% of total N*)
0	0.16	160	.....	6.2
1	0.80	3,400 M[a]	400 M	6.7
3	0.97	600 M	20 M	8.6
7	0.85	240 M	1,300 T	11.1
15	0.94	> 300 T[b]	40	15.6

[a] M = million
[b] T = thousand

15 days, after which soluble nitrogen determinations were made. With the neutralized samples, the values for per cent soluble nitrogen were 11.0 and 16.6, which are not appreciably different from the values 10.7 and 16.2 which were obtained with samples which were not neutralized. Thus it seems that partial neutralization of the lactic acid formed did not influence significantly the values for protein degradation as obtained in this study.

DICCUSSION

The greatest increase in soluble nitrogen is associated with the period of rapid proliferation and acid production in the case of *S. lactis;* comparatively little increase in soluble nitrogen occurs during the period when the viable bacterial population is declining and the cells may be presumed to be undergoing some autolysis. Apparently *S. lactis* frees nitrogenous degradation products more rapidly than they can be utilized during the period of active growth. During the period of decline in population, the proteolytic enzyme system active earlier apparently becomes comparatively inactive, either because of the reduction in metabolic activity of the cells or because the reaction of the medium is not suitable for the continued action of the enzyme system concerned.

The decrease in soluble nitrogen associated with the period of maximum proliferation of *E. coli* and *A. aerogenes* indicates that utilization of the simpler nitrogenous materials is at a rate greater than that at which the cellular enzymes are acting to free such nitrogenous materials. Since the rate of increase of soluble nitrogen immediately after a maximum population has been reached continues to be slow, the early activity of the proteolytic enzymes of these organisms quite possibly is at a very low level. The considerable increases in soluble nitrogen after the cultures have been held for more than 7 days and the viable populations have declined to very low levels indicate the probability that the major proteolytic activity of these organisms is associated with cell autolysis and the consequent release of proteolytic enzymes.

The behavior of the mixed cultures during the first 7 days seems to indicate that the coliform bacteria utilize the soluble nitrogen products formed by *S. lactis* during the early stages of growth. During the period from 7 to 15 days the

presence of the *S. lactis* cells seems to retard quite markedly the proteolytic activity found to be associated with pure cultures of the coliform bacteria during this period. The lower population of coliform bacteria in the mixed culture, together with titratable acidity values slightly higher during the first 7 days of growth than would be found in a pure culture of either *E. coli* or *A. aerogenes*, seem to account for this retardation.

The increase in proteolysis which *S. lactis* alone will cause possibly is enough to demonstrate why results of some of the tests used for proteolysis in dairy products have not correlated well with other evaluations of the quality of the product. The demonstration that one organism can influence considerably the protein degradation by another organism also serves as a note of caution in the use of tests for proteolysis when mixed cultures of microorganisms are involved, as is the case in so many dairy products. These studies seem to provide additional evidence of the necessity for further studies of the influence of various microorganisms upon the enzymatic activities of other members of a mixed population.

SUMMARY

S. lactis organisms caused a rapid increase in soluble nitrogen during the first day or two, followed by a small and gradual increase which continued for at least 15 days.

When *E. coli* and *A. aerogenes* were grown alone they caused a deficit in soluble nitrogen during the first few days of growth. This deficit was overcome later, and these organisms caused marked increases in soluble nitrogen between 7 and 15 days.

Mixed cultures containing *S. lactis* and coliform organisms caused gradual increases in soluble nitrogen, increases which were somewhat between the results of the component organisms, except that the soluble nitrogen values at 15 days never were as high as those for the coliform organisms alone and were of about the same magnitude as those for *S. lactis*.

ACKNOWLEDGMENT

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THE NUTRITIVE VALUE OF TIMOTHY HAY AT DIFFERENT STAGES OF MATURITY AS COMPARED WITH SECOND CUTTING CLOVER HAY[1]

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It generally is recognized that certain factors such as color, stage of maturity of the plant, and texture are indicative of the feeding value of a roughage. However, little work appears to have been done to actually measure quantitatively such relationships by means of balance experiments with animals, although considerable chemical work as quoted by Huffman (6) has been carried out. Ritzman and associates, as quoted by Prince *et al.* (8), determined the digestibility of the protein and of the energy of timothy hay cut at three different stages of maturity and found that the earliest cut hay was far superior in digestibility of both protein and energy as compared to the late cut.

Newlander (7), in his review of research carried out by different agricultural experiment stations and the U. S. Department of Agriculture to determine when the hay crop should be cut, gives an excellent summary of results obtained by feeding trials. Hodgson *et al.* (5) carried out feeding trials with dairy cattle and sheep to study and compare the value of home-grown roughages, both as hays and as silages. Bohstedt (2) classified hays according to quality and determined carotene, fiber and protein content in each type of hay. Dawson *et al.* (4) determined the yield, chemical composition and feeding value for milk production of alfalfa hay cut at three stages of maturity by means of feeding trials with dairy cows.

EXPERIMENTAL

The timothy hays studied in this experiment were cut in the same field at three different stages of maturity and were cured in the field. They were compared to a second cutting mow-cured hay containing approximately 90 per cent red and ladino clovers and 10 per cent grasses. All were harvested during the summer of 1947. The timothy cut on June 21 is referred to as early timothy. It was about 16 to 18 inches in height when cut and about 10 per cent of the heads were showing. The timothy cut on July 5 is referred to as medium timothy. It was in the early bloom stage. The timothy cut on July 25 is referred to as late timothy. It was in the seed stage and many leaves were dead and brown in color. The clover hay was cut on September 9 and was approximately one-half in bloom. The hays were grown on fertile soil and the growth was moderately heavy.

The animals used to measure the nutritive value of these hays were purebred

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dairy heifers weighing between 725 and 750 lb. at the beginning of the experiment. There were two Guernseys, one Jersey and one Ayrshire. Since 6 kg. of hay per head per day were found to be consumed completely, this amount was fed each animal in two feeds as the sole ration.

The nitrogen and energy balances were carried out between April 1 and June 12, 1948, according to the schedule given in table 1. It was planned so that each kind of hay would be fed to three different animals but to only one at a time. However, due to the refusal of the other animals to eat adequate amounts of late timothy after having been fed the other hays, it was necessary to use heifer 4 for a second balance with this hay.

TABLE 1

Schedule of balance experiments

Heifer no.	First balance	Second balance	Third balance
1	Mow-cured clover	Early timothy	Medium timothy
2	Early timothy	Mow-cured clover	(Refused late timothy)
3	Medium timothy	Mow-cured clover	Early timothy
4	Late timothy	Medium timothy	Late timothy

Although the procedure and methods do not differ materially from those used in previous research reported from this laboratory (1), a few modifications in the procedure necessitate a brief description.

Feed. The hays were chopped, mixed, sampled for analysis and weighed into separate single-feed portions before the start of the experimental period. A plain salt block was kept before each animal at all times. A preliminary feeding period of about 10 days was allowed before each of the first series of digestion balances. The length of this period later was reduced to 5 days, since all animals were on all-roughage rations.

The collection periods were from 14 to 16 days in duration. That portion of any feed refused by an animal was removed from the feed box, dried in an oven at about 55° C. and compounded. At the end of the period the total refuse was weighed at room temperature and sampled for analysis. Gross energy determinations were made on both feed and refuse by means of the bomb calorimeter. Nitrogen determinations also were made on both feed and refuse. The nitrogen and the energy in the refuse were subtracted from the amount fed in order to determine the amount consumed.

Excreta. The feces and urine were collected separately by means of the automatic collection devices developed in this laboratory. These collection devices are essentially the same as those used in this laboratory for many years (9) with the exception of minor changes in the chute. This was shortened materially in order to reduce the error due to evaporation and contamination. Both feces and urine were weighed and sampled daily. Composite samples of feces were kept at a temperature of approximately −16° C., while the composite samples of urine were stored at a temperature slightly above freezing. At the end of the collection period the feces samples were thawed and mixed

thoroughly and an aliquot was taken in triplicate for both moisture and nitrogen determinations. The remainder of the sample was dried at a temperature of about 55 to 60° C., ground in a Wiley mill and analyzed for moisture and for gross energy. The urine samples were allowed to come to a temperature of about 20° C., and their specific gravity determined; they were analyzed in triplicate for both nitrogen and gross energy. The urine samples used for the gross energy determinations were dried in the capsules under vacuum.

Metabolism measurements. At the end of each collection period, at least two 12-hour metabolism measurements were made by means of the open circuit respiration chamber which has been used in this laboratory for the past quarter cenrtuy. Benedict *et al.* (1) have described this apparatus. Periodic gas recovery checks were made with the apparatus to test its accuracy. The apparatus for the analysis of the chamber air for carbon dioxide, oxygen and for the direct determination of the methane was the most recent type of the Carpenter modification (3) of the Haldane apparatus.

RESULTS AND DISCUSSION

The protein and energy contents of the hays are shown in table 2, while the nitrogen and energy balances are shown in table 3 and 4 and the metabolizable energy values in table 5.

TABLE 2

Composition of hays as fed

Hay	Moisture	Protein	Gross energy
	(%)	(%)	(*Cal./g. D. M.*)
Mow-dried clover	11.06	13.837	4.3895
Early timothy	10.15	7.900	4.4368
Medium timothy	9.39	6.106	4.3647
Late timothy	9.89	4.500	4.4194

The timothy hays cut at three stages of maturity show considerable variation in protein content. The late timothy contains less than one-third the protein content of the legume hay. The digestible protein picture is even more striking. The clover hay had twice as much digestible protein as the early timothy, three times more than the medium timothy and seven times more than the late timothy. The early timothy, however, gave almost as high a positive balance of protein as the clover hay because half of the gross intake of the protein in the clover hay experiments was lost in the urine. Even the late timothy supplied enough protein for maintenance. The digestibility of the protein in the timothy hay diminished appreciably as the plant advanced in maturity. The percentage digestibility decreased from 55.4 in the early timothy to 30.2 in the late timothy.

The gross energy content of all four hays studied was essentially the same. While the differences in percentage digestibility

$$\left(\frac{\text{gross energy of feed} - \text{energy in feces}}{\text{gross energy of feed}} \times 100\right)$$

TABLE 3

Nitrogen balances

Roughage	Clover hay			Early timothy			Medium timothy			Late timothy	
Heifer no.	1	2	3	2	1	3	3	4	1	4	4
Weight (*lb.*)	756	756	708	726	776	734	743	738	811	751	751
Intake (*g.*)	131.857	132.190	132.382	74.155	75.168	75.543	54.431	57.937	57.841	40.938	42.095
Outgo (*g.*)											
Feces	47.280	47.912	48.673	33.003	33.043	34.295	29.894	33.042	29.771	28.705	29.244
Urine	71.302	65.760	66.273	31.500	19.327	32.532	20.823	21.839	26.057	13.870	10.722
Total	118.582	113.672	114.946	64.503	52.370	66.827	50.717	54.881	55.828	42.575	39.966
Balance (*g.*)	+13.275	+18.518	+17.436	+9.652	+22.798	+8.716	+3.714	+3.056	+2.013	−1.637	+2.129
Digestibility (%)	64.1	63.8	63.2	55.5	56.0	54.6	45.1	43.0	48.5	29.9	30.5
Av. digestibility[a]		63.7			55.4			45.5		30.2	

[a] All differences between roughages are significant at the 1% level.

TABLE 4

Energy balances

Roughage	Clover hay			Early timothy			Medium timothy			Late timothy	
Heifer no.	1	2	3	2	1	3	3	4	1	4	4
Weight (*lb.*)	756	756	708	726	776	734	743	738	811	751	751
Intake (*Cal.*)	23,289	23,302	23,304	23,421	23,768	23,810	21,661	23,473	23,650	22,800	23,483
Outgo (*Cal.*)											
Feces	9,643	8,522	9,402	8,357	8,524	8,338	9,726	10,496	10,418	11,705	11,638
Urine	1,454	1,164	1,091	1,473	1,045	1,003	692	696	651	526	567
Methance	1,995	1,715	1,559	1,706	2,034	1,813	1,355	1,709	1,623	1,328	1,437
Heat production	10,276	11,890	11,186	10,342	11,672	11,463	10,828	10,708	10,386	10,235	9,997
Total	23,368	23,291	23,479	21,878	23,445	22,617	22,601	23,609	23,543	23,794	23,639
Balance	−79	+11	−175	+1,543	+323	+1,193	−940	−136	+107	−994	−156
Digestibility (%)	59.2	63.4	58.6	64.3	64.1	65.0	55.1	55.3	55.9	48.8	50.4
Av. digestibility[a]		60.4			64.5			55.4		49.6	

[a] Differences between the clover and the early timothy is not significant. The difference between the clover and the medium timothy is significant at the 5% level, while all other differences are significant at the 1% level.

TABLE 5

Metabolizable energy

Roughage	Heifer no.	Dry matter consumed	Gross energy	Metabolizable energy	Metabolizability	Metabolizable energy per g. dry matter[a]	
		(*g.*)	(*Cal.*)	(*Cal.*)	(%)	(*Cal.*)	
Clover hay	1	5,306	23,289	10,197	43.8	1.922	2.079
Clover hay	2	5,308	23,302	11,901	51.1	2.242	
Clover hay	3	5,308	23,304	11,011	47.2	2.074	
Early timothy	2	5,267	23,421	11,885	50.7	2.257	2.286
Early timothy	1	5,351	23,768	11,995	50.5	2.242	
Early timothy	3	5,366	23,810	12,656	53.1	2.359	
Medium timothy	3	4,967	21,661	9,888	45.6	1.991	1.992
Medium timothy	4	5,377	23,473	10,572	45.0	1.966	
Medium timothy	1	5,418	23,650	10,943	46.3	2.020	
Late timothy	4	5,158	22,800	9,241	40.5	1.792	1.822
Late timothy	4	5,313	23,483	9,841	41.9	1.852	

[a] Differences between clover and early timothy and between clover and medium timothy are not significant. Differences between early and medium timothy are significant at the 1% level and between medium timothy and late timothy at the 5% level.

were not quite as pronounced as in the case of the protein, the energy in the late timothy was 15 per cent less digestible than that in the early timothy. The early timothy hay was superior to the clover hay when the metabolizable energy values were compared. The medium timothy had much less metabolizable energy per gram of dry matter than the early timothy or the clover, while the late timothy ranked a poor fourth.

The palatability of the hays differed quite markedly. The animals cleaned up the clover and early timothy hays quite readily, but refused some of the medium timothy. In the case of the late timothy, the animals that had received the clover or the early timothy, or even the medium timothy, refused to eat very much of this poor hay. Only one animal, number 4, could be induced to eat it, probably because she had not been fed anything better than medium timothy previously.

SUMMARY AND CONCLUSIONS

The relative nutritive value of timothy hay cut at three different stages of maturity and a second-cutting mow-cured clover hay was determined by means of eleven protein and energy digestion balance experiments with dairy heifers. The early-, medium- and late-cut timothy hays contained 57.1, 44.1 and 32.5 per cent, respectively, as much protein as the second cutting clover. However, the gross energy values for all of the hays were essentially the same.

The digestibility of the protein decreased markedly from the clover hay through the different timothy hays. The values for the early, medium and late timothy hays were 87.0, 71.4 and 47.4 per cent, respectively that of the clover hay. These same hays furnished only 49.7, 31.5 and 15.4 per cent, respectively, as much digestible protein as was furnished by the clover hay.

The early-cut timothy hay was superior to the other hays with respect to

metabolizable energy. When compared to the early-cut timothy, the clover, medium timothy and late timothy contained 90.9, 87.1 and 79.7 per cent, respectively, as much metabolizable energy.

These results show that early-cut timothy may be a better source of energy than good legume hay for dairy cattle, but not of digestible protein. However, practically the same amount of nitrogen was stored from the early-cut timothy as from the clover under the conditions of this experiment. Early-cut timothy hay may furnish up to 3.2 times as much digestible protein and 1.25 times as much metabolizable energy as late-cut timothy hay.

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COTTONSEED OILS IN PURIFIED AND SKIM MILK DIETS FOR CALVES[1]

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Early attempts to utilize fats other than that of milk in the diets of young calves have been reviewed by Gullickson *et al.* (5) and by Savage and McCay (7). The results have been variable and in many cases unsuccessful. Though work in recent years has shown certain fats to be more suitable than others, the reasons for the undesirability of some fats are not understood.

Gullickson *et al.* (5) observed better growth when calves received butterfat, lard or tallow than when they received liquid vegetable fats. These results suggest that a high degree of unsaturation might decrease utilization of fat and possibly other nutrients by calves. More recent studies by Jacobson and Cannon (6) and Wiese *et al.* (9) also permit this interpretation. Jacobson and Cannon (6) found that calves grew well with 3 per cent hydrogenated soybean oil in the diet. These authors also observed that calves grew fairly satisfactorily with 2 per cent crude soybean oil but not with 3 per cent. Thus it appears that there is a level above which certain unsaturated fats disrupt metabolism. Several workers have reported that calves fed liquid vegetable fats develop scours (2, 5, 6, 9). Apparently there is appreciable absorption of corn oil and cottonseed oil because calves fed these fats had higher levels of blood fat than calves receiving milk fat (5).

Observations made in the studies reported here include growth rates of calves fed purified diets as compared to whole milk, the occurrence of fatty livers in calves fed purified diets, some comparisons of cottonseed oils and butterfat for growth, and comparison of blood plasma fat levels of calves when their respective diets contain butterfat and hydrogenated cottonseed oil.

EXPERIMENTAL PROCEDURE

Animals used in these experiments were grade, purebred or crossbred dairy calves. Breed was disregarded for assignment to rations. Calves were started on the experimental rations at 2 to 4 days of age, after they had received colostrum. They were isolated in individual pens bedded with wood shavings or dehydrated sugar cane pulp. The calves were kept muzzled, but this was not wholly effective in preventing consumption of bedding. The diets were fed from nipple pails at a rate of 10 lb. daily per 100 lb. of body weight. The calves were weighed and feed allowances were adjusted weekly.

The studies reported herein were made in two trials. In trial I the diets used were (a) whole Ayrshire milk averaging 4 per cent butterfat, (b) a purified

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diet similar to that described by Wiese *et al.* (9) with 3.5 per cent refined cottonseed oil and (c) the same as diet *b* except that the oil was hydrogenated.

The purified diets differed from those used by Wiese *et al.* (9) in the following ways: (a) commercial casein[2] replaced vitamin-free casein; (b) calves received 0.5 minim wheat germ oil every other day in the vitamin A and D capsules but in addition each pound of milk contained 1 mg. of *a*-tocopherol and (c) no folic acid or biotin was added.

Trial II was a randomized block design with three diets and 15 calves. These diets were (a) whole Aryshire milk, (b) 10 per cent edible, spray-dried nonfat milk solids plus 3.5 per cent butter oil and (c) the same as diet *b* except that the fat was hydrogenated cottonseed oil.

The fat was emulsified by homogenization in all diets except the whole milk. All calves received capsules containing 30,000 I. U. of vitamin A and 1,000 units of vitamin D every other day. The sources of vitamins A and D were fish liver oil and irradiated ergosterol. Two batches of each type of cottonseed oil were used. The nonhydrogenated cottonseed oils had iodine values of 108.5 and 115.0. The hydrogenated fats had iodine values of 64.6 and 63.6 and Wiley melting points of 34.1 and 32.2° C.

TABLE 1

Daily gain and choline and lipide content of livers of calves in trial I

Diet	No. of calves	Av. time on diet	Av. daily gain	Av. liver lipides (dry basis)	Av. liver choline (dry basis)	Av. liver choline (dry, lipide-free basis)
		(*d.*)	(*lb.*)	(%)	(*mg./g.*)	(*mg./g.*)
Whole milk	4	51.5	1.0[a]	7.0	13.7	14.8
Purified basal + hydrogenated cottonseed oil	4	44.8	0.54	20.5	14.0	17.7
Purified basal + nonhydrogenated cottonseed oil	5	36.2	0.11	16.1	16.9	20.2
5% least significant difference				8.9	3.6	3.7

[a] One calf sacrificed at 12 d. of age not included in this average.

All blood samples were drawn from the jugular vein. Allen's method (1) was used for the determination of plasma fat. Liver lipides were measured as the ether extract of the dried liver. Engel's method (3) was used for the determination of choline in the dry liver samples. Choline chloride was used as a standard in the determinations and all choline values are expressed as amounts of choline chloride.

RESULTS

Hydrogenated vs. nonhydrogenated cottonseed oil in purified diets. The average daily gain of calves in trial I (table 1) show that whole milk gave the best growth. Though whole milk contained on the average 0.5 per cent more

[2] "New Process" casein purchased from Sheffield Farms Co., Inc., 524 West 57th Street, New York, N. Y.

fat than the purified diets, this additional fat would not seem to account for the additional growth. Calves which received the purified diet with nonhydrogenated refined cottonseed oil made practically no growth, became emaciated and died in 5 to 9 weeks. These calves developed severe scours after receiving the nonhydrogenated oil about 1 week. The purified diet containing hydrogenated cottonseed oil allowed intermediate growth.

Incidence of fatty livers from purified diets. Observations on the amount of lipides in the livers of animals of trial I indicated that the purified diets containing cottonseed oil caused fatty livers (table 1). Analyses of the livers of

TABLE 2

Lipides in dried livers of calves in trial II

Diet	Lipide content in individual livers					Av.	Av. adjusted[a]
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Whole milk	5.26	5.78	4.32	4.09	2.82	4.45	4.45
Nonfat milk solids + butter oil	3.54	8.86	3.51	5.46	19.12	8.10	5.19
Nonfat milk solids + hydrogenated cottonseed oil	5.82	6.60	4.09	5.65	6.13	5.66	5.66
5% least significant difference						5.73	1.87

[a] A value of 4.62 substituted for 19.12 in the case of the animal on the butter oil diet.

the calves in trial II, in which some calves had received hydrogenated cottonseed oil in reconstituted nonfat milk solids, showed (table 2) that the cottonseed oil alone was not responsible for the fatty livers. The purified diets used in these trials apparently were responsible for the fatty livers.

TABLE 3

Average blood plasma fat and daily gain of calves in trial II

Diet	Av. plasma fat at:			Av. daily gain
	2 wk.	4 wk.	6 wk.	
	(*mg./100 ml.*)	(*mg./100 ml.*)	(*mg./100 ml.*)	(*lb.*)
Whole Ayrshire milk	156.1	164.0	180.2	.75
Nonfat milk solids + butter oil	125.8	148.3	145.9	.90
Nonfat milk solids + hydrogenated cottonseed oil	91.3	64.9	70.2	.70
5% least significant difference	57.6	57.6	57.6	.34

Blood plasma fat levels. Table 3 shows a summary of plasma fat of calves in trial II at 2, 4 and 6 weeks. The diet consisting of reconstituted nonfat milk solids and 3.5 per cent butter oil supported lower levels of plasma fat than did whole milk, but the difference was not significant. The additional 0.5 per cent fat in the whole milk might account for this difference. When hydrogenated cottonseed oil replaced the butter oil, significantly lower blood fat levels oc-

curred than on the whole milk diet at 2, 4 and 6 weeks or on the butter oil diet at 4 and 6 weeks.

The average daily gains of calves fed the different diets were not significantly different.

Liver choline. The differences in choline content of livers of calves receiving the different diets in trial I were not significant when compared on a dry weight basis (table 1). However, when the comparisons were made on a fat-free dry basis, the differences were accentuated to the extent that the average amount of choline in the livers of calves receiving whole milk was significantly greater than those receiving the purified diet with nonhydrogenated cottonseed oil.

The differences in choline content of livers of calves receiving the respective diets in trial II were not statistically significant (table 4).

TABLE 4

Choline in dried livers of calves in trial II

Diet	Choline in individual livers					Av.
	(*mg./g.*)	(*mg./g.*)	(*mg./g.*)	(*mg./g.*)	(*mg./g.*)	(*mg./g.*)
Whole milk	13.6	11.4	11.5	11.7	10.8	11.8
Nonfat milk solids + butter oil	13.5	14.3	11.7	11.2	12.5	12.6
Nonfat milk solids + hydrogenated cottonseed oil	14.0	12.8	12.5	11.6	11.5	12.5
5% least significant difference						1.1

DISCUSSION

The results obtained with hydrogenated and nonhydrogenated cottonseed oil agree with those of other workers (5, 6). Purified diets containing 3.5 per cent nonhydrogenated cottonseed oil were unsatisfactory. As Jacobson and Cannon (6) found to be the case with soybean oil, partial hydrogenation allowed calves to tolerate cottonseed oil in these studies.

The purified or synthetic diets used in these experiments did not produce as good growth as whole milk. The milk contained 4 per cent fat compared to only 3.5 per cent for the purified diets. It is not believed, however, that the additional fat in the whole milk played any important part in the results obtained. It is interesting that calves fed the more highly saturated fat in reconstituted nonfat milk solids had lower levels of plasma fat than calves receiving butter oil in the reconstituted nonfat milk solids. The hydrogenated cottonseed oil in skim milk produced growth comparable to butter oil in nonfat milk solids and to whole milk.

The cause of the high lipide content of livers of calves fed the purified diets is not known. However, since reconstituted nonfat milk solids containing hydrogenated cottonseed oil did not cause fatty liver, it seems that the purified diet was responsible. The question arises as to whether or not the purified diet contained adequate choline. Ten lb. of this diet contained 1 g. of choline chloride. Ten lb. of whole milk would contain somewhat less than this amount (4, 8); therefore a choline deficiency is not suspected.

The choline contents of the livers of the calves in these experiments were lower than has been reported previously (4, 8). The amounts of choline in the livers of calves receiving whole milk were lower than for calves which received the purified diets. The differences were not statistically significant on the dry basis, but a true difference between the whole milk diet and the purified diet containing nonhydrogenated cottonseed oil was indicated when the comparison was made on a fat-free dry basis. Inasmuch as greater differences in choline content of livers were indicated when the comparison was made on a fat-free dry basis than when on a dry basis, the question arises as to which is the proper comparison. However, if the choline content of liver is stated on a dry basis, the choline content could increase without becoming apparent if the fat content increased accordingly. Therefore it would seem to be desirable to make choline comparisons of livers on a fat-free basis when the fat content of the liver varied widely, unless total liver choline values were available.

SUMMARY

1. Calves did not grow so well when fed purified diets as when receiving whole milk. Although growing relatively poorly, the calves appeared normal when fed the purified diet containing 3.5 per cent hydrogenated cottonseed oil. They grew very poorly on such a diet containing 3.5 per cent nonhydrogenated refined cottonseed oil and died in a few weeks.

2. Calves which received the purified diets developed fatty livers whether the oil was hydrogenated or not. Since the calves receiving hydrogenated cottonseed oil with reconstituted nonfat milk solids had normal liver lipide values, it is apparent that the purified diet was concerned in the production of fatty livers.

3. The calves which received the hydrogenated cottonseed oil had lower blood plasma fat values than calves which received butterfat.

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THE AMINO ACID COMPOSITION OF BOVINE COLOSTRUM AND MILK[1, 2]

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A review of the literature on the amino acid composition of various biological materials (3) reveals that comparatively few data are available for whole bovine milk and practically none for colostrum. However, a few workers (4, 6, 7, 13) have carried out microbiological determinations of amino acids in skim milk powder, processed milk, purified milk proteins and some of the colostral proteins, whereas others (2, 4, 10, 16) have determined some amino acid values for milk proteins by chemical methods. Other papers, in which isolated data are given for one or more of the essential amino acids, have been reviewed by Snell (14) and Schweigert and Snell (12). Although the amino acid content of milk may be computed from the amino acid composition and the relative proportions of the various proteins occurring in it, direct determination of the amino acids in the hydrolyzate would be more desirable for routine work. In this connection Hodson and Krueger (7) determined microbiologically the essential amino acid content of fresh cows' milk. Results for a single sample of milk also have been given by Stokes *et al.* (15). The object of the present investigation was to study how the essential amino acids in colostrum and milk vary with the stage of lactation.

EXPERIMENTAL PROCEDURE

Samples of colostrum and milk were obtained from five Jersey cows and one Holstein cow from the College dairy herd maintained under winter feeding conditions. Two samples of colostrum were secured for analysis from each of the Jersey cows. The first sample was taken within 1 hour after calving and the second represented a 24-hour composite sample. Three-day composite samples of milk were taken for analysis at the 60th and 90th days of lactation. The colostrum and milk production records of the above cows were kept as a matter

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of routine procedure as well as the amount of colostrum and milk ingested by the calves during their colostrum and milk feeding periods. From these records and the percentage composition of some of the amino acids found in colostrum and milk, an approximation was made of the amount of the various amino acids ingested per day from colostrum and from milk.

The amino acid determinations were carried out microbiologically using *Lactobacillus arabinosis, Streptococcus faecalis* and *Leuconostoc mesenteroides.* The media used in the various determinations were essentially the same as those described by Sauberlich and Baumann (11) with the exception of those used for isoleucine and methionine, which were prepared according to the method of Kuiken *et al.* (8) and Lyman *et al.* (9). The hydrolyzates for the determination of leucine, isoleucine, valine, phenylalanine, arginine, histidine, threonine, methionine and lysine were prepared according to the method of Stokes *et al.* (15). Ten ml. of colostrum or 20 ml. of milk were taken for the digestion, the hydrolyzates finally were made up to 100 ml. and preserved with a few drops of toluene and stored in the refrigerator. For the tryptophan assay the enzymatic digestion procedure of Wooley and Sebrell (17) was followed. Five ml. of colostrum or 10 ml. of milk were used for the enzymatic determination. The digest was made up finally to 100 ml. and preserved as above. In all cases, the assays were run after the proper dilutions had been made.

In order to determine whether or not the direct hydrolysis of colostrum or milk would result in the destruction of any of the amino acids, parallel hydrolyzates of casein and of casein plus lactose were prepared and assayed for 9 amino acids. The amount of lactose used was twice the weight of the casein. To investigate this point further, the amino acid composition was determined on two samples of fresh colostrum and one sample of milk and on the precipitated proteins prepared from each colostrum and milk. The total proteins were prepared by the method of Block and Bolling (3).

The reliability and reproducibility of the various amino acid determinations used in this work were ascertained by making parallel acid hydrolyzates from the same sample of fresh unpasteurized market milk and from colostrum obtained from a first-calf Holstein cow immediately following parturition.

The total nitrogen in the colostrum and milk was determined according to the method recommended by the A. O. A. C. (1). The factor of 6.38 was used to convert nitrogen to protein.

RESULTS

Before adopting the procedure of direct acid hydrolysis of colostrum and milk, it was desirable to determine whether or not any destruction occurred in the amino acids of casein when casein was digested with 6*N* hydrochloric acid in the presence of lactose. The results obtained by the acid hydrolysis of casein, with and without lactose, are shown in table 1. By comparing the amounts of the various amino acids found in the casein by this procedure, it can be seen that the degree of destruction due to the formation of sugar-amino acid

complexes did not exceed the usual error encountered in microbiological assays. From these results it may be assumed that the amino acid composition of both albumin and globulin will remain unchanged under similar treatments. The destruction probably would be significant in the case of cystine. Therefore, the method of direct hydrolysis with 6*N* hydrochloric acid was followed in subsequent analyses.

TABLE 1

A comparative study of the amino acid composition of the acid hydrolyzates of casein, with and without added lactose, and of colostrum and milk and their corresponding proteins[a]

Amino acid	Casein[b] without lactose	Casein with lactose	K9 colostrum	K9 protein	K12 colostrum	K12 protein	K15 milk	K15 protein
Leucine	8.11	7.89	7.27	7.36	7.52	7.35	8.64	8.55
Isoleucine	5.75	5.78	5.28	4.90	4.34	4.82	6.98	5.74
Valine	6.60	6.60	6.72	7.51	7.08	7.52	7.36	6.28
Phenylalanine	4.70	4.78	4.42	4.42	4.26	4.41	4.86	4.86
Arginine	3.79	3.41	4.64	4.90	4.77	4.61	3.64	3.51
Histidine	2.89	2.76	2.01	2.22	2.26	2.44	2.45	2.66
Threonine	4.21	4.12	6.38	6.92	6.97	7.25	4.11	4.73
Methionine	2.74	2.65	1.68	1.59	1.66	1.82	2.18	2.26
Lysine	7.07	7.13	6.62	6.79	6.98	7.05	7.75	7.96
Protein (%)	94.72	94.72	20.28	91.43	12.99	95.99	2.80	96.16

[a] All values are expressed as percentage of total protein. The colostrum and milk samples were obtained from Jersey cows.
[b] Vitamin-free Labco casein.

The next question that arose was that of the reproducibility of the method of microbiological assay with milk and colostrum hydrolyzates so that the determinations could be made on a routine basis. To verify this point, five aliquots from a sample of unpasteurized market milk and two aliquots from a sample of colostrum were subjected to acid hydrolysis. The results of the amino acid determinations on their hydrolyzates are presented in table 2. The

TABLE 2

Variations in the amino acid content of five individual hydrolyzates of one milk sample and two of colostrum[a]

Amino acid	Fresh, unpasteurized milk						Colostrum		
	Hydrolyzate no.						Hydrolyzate no.		
	1	2	3	4	5	Av.	1	2	Av.
Leucine	8.31	8.71	8.97	8.36	8.14	8.50	7.28	7.27	7.28
Isoleucine	5.89	5.72	5.53	5.67	5.57	5.68	4.61	4.45	4.53
Valine	6.83	6.72	6.66	6.69	6.66	6.71	7.96	7.94	7.95
Phenylalanine	4.51	4.57	4.51	4.51	4.41	4.50	4.33	4.35	4.34
Arginine	3.28	3.05	2.96	3.05	3.13	3.09	4.28	4.24	4.26
Histidine	2.66	2.64	2.69	2.69	2.73	2.68	2.48	2.44	2.46
Threonine	4.83	4.80	4.61	4.73	4.92	4.78	1.95	2.02	1.99
Methionine	2.25	2.24	2.32	2.28	2.32	2.28	1.64	1.68	1.66
Lysine	7.45	7.57	7.33	7.17	7.26	7.36	6.81	7.00	6.91

[a] The milk sample was fresh, unpasteurized market milk (N = 0.505%) and the colostrum sample was obtained immediately after parturition from a first-calf Holstein cow (N = 2.399%). All values are expressed as percentage of total protein.

maximum variation in any of the amino acids from aliquot to aliquot was within experimental error, both in case of the milk and colostrum. Based on these findings, the microbiological method was used for subsequent determinations.

In table 3 are presented the average and range of concentrations of ten amino acids in colostrum and milk samples obtained from five Jersey cows. The average protein content of the colostra for the first- and 24-hr. samples and the milk samples obtained on the 60th and 90th days of lactation were 14.0, 10.3, 3.5 and 3.6 per cent, respectively. Inasmuch as the protein content of the colostra taken within 1 hr. after parturition is higher than that of the 24-hr. sample, the amino acid content of the first colostrum also is considerably higher when expressed as per cent of the undried sample. However, when the amino acid composition is expressed as per cent of the protein, these differences become negligible and there is considerable overlapping in the range of values.

Individual variations were appreciably large but these were ascribed to the individuality of the cows rather than to partial destruction in hydrolysis or to errors in assay. The fact that some of the data in tables 1 and 2 differ from those in table 3 can be explained on the above basis, also on breed differences and on the plane of nutrition. Insofar as the colostrum data are concerned, the data in table 1 were obtained from the two cows of the Jersey breed, the data in table 2 were obtained from a first-calf Holstein cow, whereas the data in table 3 are the average and range of values obtained from 5 other Jersey cows. With few exceptions, the range of values presented in table 3 will include the individual values presented in tables 1 and 2. The milk data presented in table 1 were obtained from an individual Holstein cow, the data in table 2 were obtained from a market milk sample and the data in table 3 were obtained from Jersey cows at a definite stage of lactation. The cows producing the market milk undoubtedly were on various planes of nutrition, whereas all of the cows in the College herd were on the same plane of nutrition. All of the colostra and milk data, however, are considered to be significant.

The data presented in table 1 for casein do not differ markedly from those obtained for milk (tables 1, 2 and 3). Some differences are evident because milk contains some albumin and globulin in addition to the casein.

On the average, the proteins of colostrum contain more valine, arginine, threonine and tryptophan than normal milk but less leucine, isoleucine, phenylalanine and methionine. The amounts of histidine and lysine appear to be similar in both milk and colostrum proteins. Inasmuch as the globulin content of colostrum is very high in comparison to that of milk, a difference in the amino acid composition of these two biological materials is to be expected. A comparison of the amino acid composition of the protein of bovine colostrum (table 3) with that of human colostrum (5) reveals that the protein in bovine colostrum is higher in threonine, leucine, isoleucine and valine. When the amino acid values are expressed as per cent of the sample, there is a general tendency for all of the amino acids to decline in concentration both in colostrum and milk with

TABLE 3

Amino acid content of bovine colostrum and milk of five Jersey cows
(All values expressed as % of the undried sample and of the protein)

Amino acid	Time after parturition							
	1 hr.		24 hr.		60 d.		90 d.	
	% of sample	% of protein	% of sample	% of protein	% of sample	% of protein	% of sample	% of protein
Leucine	1.36[a]	9.71	0.90	8.83	0.36	10.29	0.36	10.04
	1.20–1.46[b]	9.32–10.07	0.62–1.31	7.68–9.57	0.32–0.39	8.27–11.29	0.34–0.38	9.16–10.84
Isoleucine	0.81	5.85	0.63	6.28	0.25	6.96	0.25	7.05
	0.75–0.87	5.22– 6.30	0.51–0.77	5.77–7.85	0.21–0.29	6.62– 7.65	0.24–0.27	6.33– 7.77
Valine	1.23	8.78	0.78	8.30	0.26	7.30	0.26	7.45
	1.05–1.35	8.48– 9.22	0.44–1.15	8.08–8.67	0.24–0.27	7.07– 7.43	0.25–0.28	6.97– 7.97
Phenylalanine	0.64	4.63	0.47	4.49	0.19	5.30	0.16	4.50
	0.57–0.70	4.24– 5.03	0.32–0.57	4.13–4.98	0.16–0.22	4.88– 5.78	0.15–0.17	4.15– 4.82
Arginine	0.74	5.31	0.51	5.04	0.13	3.78	0.14	3.97
	0.58–0.86	4.68– 6.21	0.31–0.59	4.22–5.65	0.12–0.14	3.66– 3.93	0.13–0.16	3.56– 4.50
Histidine	0.38	2.76	0.29	2.87	0.12	2.81	0.11	3.17
	0.34–0.45	2.34– 3.22	0.21–0.34	2.47–3.29	0.11–0.13	2.75– 2.90	0.10–0.13	2.79– 3.88
Threonine	0.97	6.93	0.61	5.96	0.17	4.96	0.16	4.53
	0.80–1.15	6.48– 7.35	0.37–0.75	5.43–6.69	0.13–0.22	3.99– 5.70	0.15–0.17	4.28– 4.85
Tryptophan	0.25	1.81	0.24	2.29	0.05	1.46	0.06	1.70
	0.20–0.34	1.32– 2.48	0.14–0.32	2.16–2.37	0.04–0.06	1.40– 1.69	0.05–0.08	1.54– 1.82
Methionine	0.24	1.70	0.17	1.70	0 08	2.35	0.07	1.80
	0.20–0.28	1.49– 2.02	0.11–0.23	1.45–1.84	0.07–0.09	2.31– 2.40	0.06–0.09	1.66– 1.95
Lysine	1.06	7.56	0 72	7.15	0.27	7.79	0.24	6.75
	0 90–1.14	6.38– 7.81	0.52–0.89	6.45–8.04	0.25–0.31	7.44– 8.06	0.23–0.25	6.35– 7.35

[a] Average value.
[b] Range of values.

the progress of lactation as the result of parallel diminution in the protein content.

A comparison of the amino acid composition of the milk on the 60th and 90th days of lactation reveals no significant difference except that histidine appears to increase and phenylalanine, methionine and lysine appear to decrease with the progress of lactation. The leucine, isoleucine, phenylalanine and arginine content of the market milk sample is significantly lower than that found for Jersey milk (table 3) on the 60th day of lactation. All of the other amino acids, however, seem to be comparable. A comparison of the values reported in this paper for Jersey milk with those published by Hodson and Krueger (7) reveals no significant difference with the exception of histidine, which the latter authors found to be lower.

The amount of the various amino acids secreted in the total volume of colostrum and milk at various stages of lactation and the amount of these constituents ingested by the calves during early life were calculated. Data on the output, as computed from a knowledge of the protein and amino acid contents of the colostrum and milk, are presented in table 4. The cow evidently secretes

TABLE 4

Average daily output of amino acids in the colostrum and milk of five Jersey cows and the average amounts supplied to their calves on the first day and the 60th day of lactation

Amino acid	Output			Intake	
	1st d.	60th d.	90th d.	1st d.	60th d.
	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)
Leucine	90.7	39.2	32.0	14.4	14.7
Isoleucine	62.9	26.7	22.5	10.0	12.2
Valine	78.5	27.9	23.7	12.5	10.7
Phenylalanine	47.6	20.3	14.3	7.6	7.1
Arginine	51.5	14.8	12.6	8.2	5.7
Histidine	28.9	10.9	10.1	4.6	4.4
Threonine	61.2	15.0	14.4	9.7	6.9
Tryptophan	23.7	5.5	5.4	3.8	2.3
Methionine	17.3	9.0	5.7	2.8	3.0
Lysine	72.4	29.8	21.7	11.5	10.6

large quantities of the various amino acids during the first 24 hr. postpartum. The output of amino acids by the 60th day of lactation shows a marked drop over that of the first day of lactation and a further small but consistent decline by the 90th day. The data on the average daily intake of amino acids by the Jersey calves on the first and 60th days of lactation are shown in the same table. These data have been calculated on the basis that the calves ingested 1.6 kg. of colostrum during the first day postpartum and 4.1 kg. of milk on the 60th day. During the first few days of life the calf ingests relatively large quantities of amino acids. By the 60th day, the total amount of amino acids obtained from milk alone did not differ markedly from that on the first day. It should be pointed out, however, that the calves were consuming appreciable amounts of dry feed at this age in addition to the milk, so the amino acid intake reported

in this paper only represents that obtained from the milk and ordinarily would be less than the total daily intake.

SUMMARY

The concentration of ten amino acids in bovine colostrum and milk has been determined.

The colostrum collected within 1 hr. after parturition is higher in total protein than the 24-hr. composite sample and thus contained larger amounts of the ten amino acids. The amino acid composition of the colostrum, based on total proteins, is similar.

No essential difference was obtained in the amino acid composition of the proteins of milk collected at the 60th and 90th days of lactation.

Data on the output of ten amino acids in colostrum and milk have been calculated and the approximate ingestion of these amino acids by the calf has been computed.

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THE EFFECTS OF NORDIHYDROGUAIARETIC ACID, SALT AND TEMPERATURE OF STORAGE ON THE STABILITY OF FAT AND FAT-SOLUBLE VITAMINS IN CREAM AND BUTTER[1]

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In a recent paper (2) evidence was presented to show that the oxidized flavors in fresh milk are not associated with deterioration of fat but with the unstable lipids which are mostly a part of the stabilizing fat globule membrane. It has been pointed out, however, that the fat itself also may undergo deterioration in the presence of ascorbic acid, resulting in the development of metallic to fishy flavors and losses in vitamins A and E and carotenoids. The susceptibility of fat to this type of deterioration, as determined by the re-emulsification test (2), depends on the type of product, the temperature of pasteurization and the conditions of storage. Thus, from the biochemical point of view, at least two reactions which produce the oxidized flavors may be stimulated by the addition of ascorbic acid to milk products containing unstable fat.

Furthermore, storage tests on cream and butter (2) also have indicated that the activity of an unknown plasma factor is responsible for the sensitization of fat to the foregoing type of deterioration and that its activity is reduced to a safe minimum only in butter churned from cream pasteurized at 76.6° C.

In this connection it also is of interest to note that most of the samples of fat obtained from market butters were found unstable by the re-emulsification test (4). Although some of the butters contained added salt, there nevertheless was a possibility that the stability of fat was affected prematurely by the conditions of storage and to a lesser degree by the salt itself. A well-known factor, such as the exposure of butter to light, together with the transitional storage of butter at and above freezing temperatures, could have been the contributing factor.

Both frozen cream and storage butter are used in the preparation of foods containing ascorbic acid and possibly other substances capable of interaction with the unstable lipids. This may result in the development of the objectionable flavors and losses in the fat-soluble vitamins. Consequently, studies were conducted to learn whether the activity of the plasma factor could be restrained by the addition of a fat soluble anti-oxidant to cream.

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EXPERIMENTAL PROCEDURE

Five lots of cream and two lots of butter were prepared from mixed morning milk obtained from the Cornell University herd. Two portions of milk were depleted of their total vitamin C content by adding 0.03 ml. of 30 per cent H_2O_2 per l. of milk and then pasteurized at 82.2 and 87.8° C., for 30 and 10 minutes, respectively; two portions of milk containing 20 mg. of ascorbic acid per l. were pasteurized at the same temperatures and retained as the controls. Nordihydroguaiaretic acid (NDGA) anti-oxidant[2] was added as a propylene glycol solution at the rate of 0.005 per cent of the milk fat to a third portion of a control milk containing ascorbic acid, prior to its pasteurization at 82.2° C. Creams then were obtained from these samples.

The butter samples were churned from cream separated from milk pasteurized at 76.6° C. for 30 minutes. One part of butter was retained as a control, while the other had 2 per cent salt added.[3]

The samples of cream were held in tightly sealed glass containers protected from the light. The butter samples were shaped in cylindrical forms and wrapped either directly in two thicknesses of tin foil (unsalted butter) or in two thicknesses of parchment paper first and then in tin foil (salted butter). The cream and butter then were held at –17.7 to –16.1° C. for 15, 40, 76, 107, 137, 168, 198, 229 and 247 days. At the end of these periods of time, three samples from each lot of cream and butter were transferred to an incubator, the temperature of which was maintained at 0 to 1° C. and kept there for an additional 10, 20 and 30 days prior to the stability test. The cream and butter samples then were scored for flavors, and the stability of fat obtained from these products was determined by the re-emulsification test (2). Throughout the duration of this test, the samples were protected from light. They were scored for flavors; then the gravity cream was churned, and the butter obtained was centrifuged and the fat was analyzed for its fat-soluble vitamin content. Vitamins A and E and the carotenoid content of the fat were determined using Koehn and Sherman (1) and Quaife (8) methods, respectively.

RESULTS

Data on the effects of H_2O_2 treatment and the anti-oxidant activity of NDGA in cream pasteurized at 82.2° C. are presented in table 1 and figures 1, 2, 3, 4, 5, 6 and 7. Related data on cream pasteurized at 87.8° C. are not included because this temperature of pasteurization only slightly improved the stability of fat over that in cream pasteurized at 82.2° C.

The flavor scores on the graphs (figures 1 to 7) are shown by broken and solid lines which indicate the points of the development of strong metallic to fishy flavors in reconstituted milks (re-emulsification test) containing ascorbic acid alone (broken line) and together with copper (solid line) during storage for 48 hours at 0 to 5° C. These lines occasionally are preceded by short dotted lines indicating low intensity metallic-to-fishy flavors.

[2] Supplied by the Nordigard Corporation, Chicago, Illinois.

[3] 2232 B & A, sodium chloride crystal.

TABLE 1

The effect of the elimination of vitamin C in cream, of the anti-oxidant properties of nordihydroguaiaretic acid in cream containing ascorbic acid and of 2% salt added to butter on the development of oxidized flavors.

Treatment and pasteurization	Flavor scores[a] of cream[b] and its buttermilk after storage at −17.7 to −16.1° C. and then at 0 to 1° C. for						
	0 d.	10 d.		20 d.		30 d.	
	cream	cream	buttermilk	cream	buttermilk	cream	buttermilk
	(*d.*)						
82.2° C. control	15	–	?	2	4	4	4
Ascorbic acid	40	–	1	1	2	3	4
	76	–	1	1	1	4	2
	107	–	4	4	3	4	3
	137	–	1	3	1	2	4
	168	–	3	4	1	4	3
	198	1	1	3	4	4	3
	229	2	4	4	4	4	4
	247	2	4	4	4	4	4

Samples of cream separated from NDGA- and H_2O_2 -treated portions of milk, and the samples of salted and unsalted butter did not develop any objectionable flavors and retained their sweetness throughout the duration of the experiments.

[a] – indicates no oxidized flavors detected; numbers 1 to 4 indicate increasing intensity of oxidized flavors detected.

[b] 56 per cent fat.

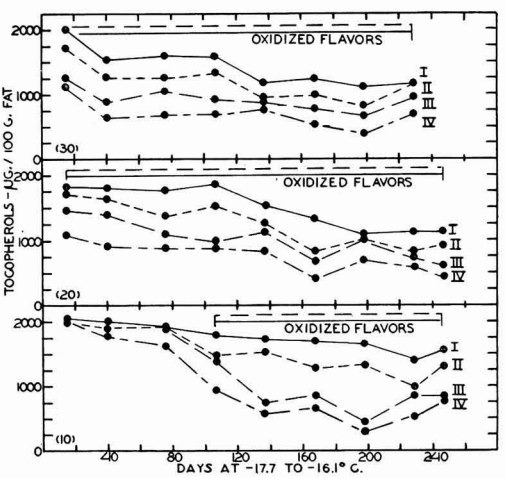

FIG. 1. The effects of holding of cream with ascorbic acid and no added anti-oxidant for 15 to 247 days at −17.7 to −16.1° C. and then for the additional 10, 20 and 30 days at 0 to 1° C. upon the development of oxidized flavors and the stability of tocopherols, as determined by the re-emulsification test. I, fat prior to re-emulsification test; II, fat from reconstituted milks containing 0.1 mg. of copper per l.; III, 20 mg. of ascorbic acid per l.; and IV, 0.1 mg. copper and 20 mg. of ascorbic acid per l.

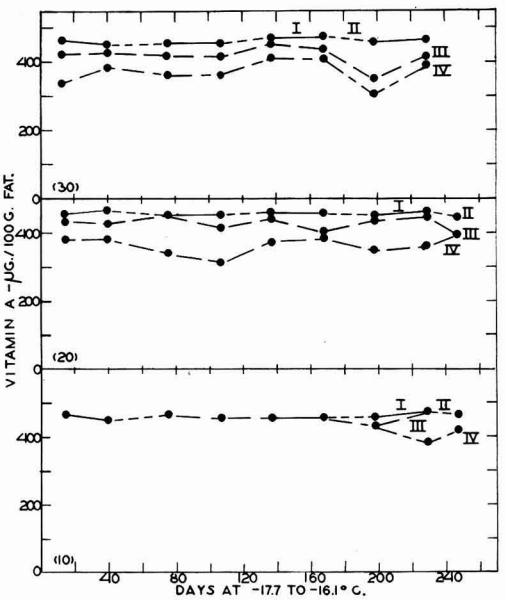

FIG. 2. The stability of vitamin A in cream with ascorbic acid and no added anti-oxidant as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

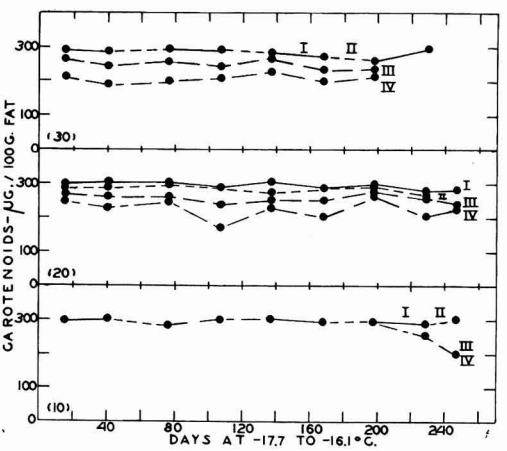

FIG. 3. The stability of carotenoids in cream with ascorbic acid and no added anti-oxidant as determined by the re-emulsification test. See figure 1 for the identification of I, II, III and IV.

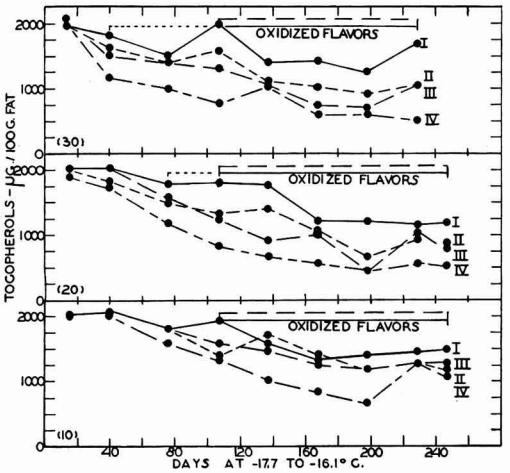

FIG. 4. The effects of holding of cream depleted of the total vitamin C content for 15 to 247 days at −17.7 to −16.1° C. and then for the additional 10, 20 and 30 days at 0 to 1° C. upon the development of oxidized flavors and the stability of tocopherols as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

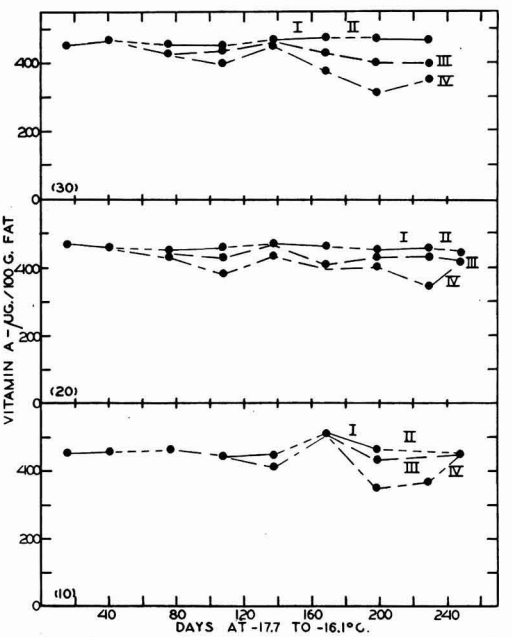

FIG. 5. The stability of vitamin A in cream depleted of the total vitamin C content as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

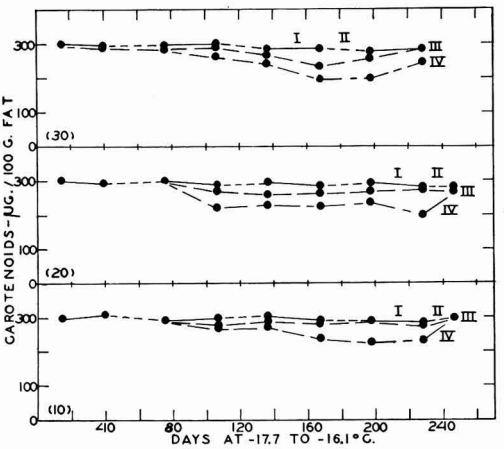

FIG. 6. The stability of carotenoids in cream depleted of the total vitamin C content as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

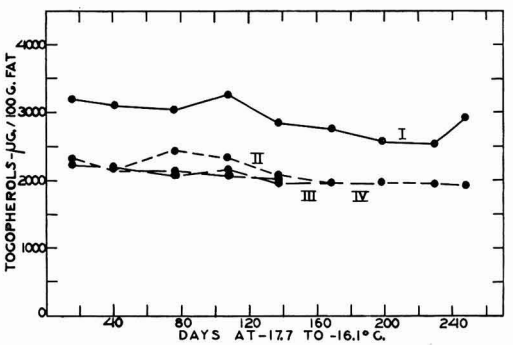

FIG. 7. The effects of holding of cream with ascorbic acid and added NDGA anti-oxidant for 15 to 247 days at −17.7 to −16.1° C. and then for the additional 10, 20 and 30 days at 0 to 1° C. upon the apparent increase of the tocopherol content of the fat, and the stability of tocopherols as determined by the re-emulsification test. See figure 1 for identification of I, II, III and IV.

The data of table 1 show that the depletion of cream of the total vitamin C content resulted in the prevention of oxidized flavors even when the cream was held at 0 to 1° C. after its transfer from sub-zero temperatures at the end of 15 up to 247 days of storage. The oxidized flavors were promoted in the cream separated from milk containing ascorbic acid. Their appearance and intensity varied, however, with the time of storage of cream at sub-zero, and then at 0 to 1° C. These results again show as before (2) that ascorbic acid plays an important part in the reaction which produces the oxidized flavors in cream.

The flavor scores in table 1 also revealed that NDGA anti-oxidant was as ef-

fective in the prevention of the oxidized flavors as the depletion of cream of the total vitamin C content by the rapid oxidative method, thus confirming the findings of Stull *et al.* (6). However, data in table 1 and figures 1 and 4 show that, although the elimination of vitamin C in cream by hydrogen peroxide treatment has prevented the development of oxidized flavors, the stability of fat, as determined by the re-emulsification test, was improved but slightly over that of the fat in the corresponding control cream. In this case the fat resisted deterioration for the additional 20 and 10 days only when the creams were held at 0 to 1° C. after their transfer from sub-zero temperatures at the end of storage for 15 and 40 days, respectively. As in the preceding studies (2), the susceptibility of fat to deterioration in the presence of ascorbic acid added to reconstituted milk manifested itself by the development of metallic-to-fishy flavors and losses in vitamins A and E and carotenoid content of the fat.

The data in figures 1, 2, 3, 4, 5 and 6 show that the promotion of oxidized flavors in the reconstituted milks containing ascorbic acid apparently was dependent on the stability of tocopherols and that the destruction of vitamin A and carotenoids follows that of the tocopherols. The oxidized flavors were not induced by copper alone in the reconstituted milk containing unstable fat. It caused, however, some losses in the tocopherol content of the fat. The latter was reduced appreciably when ascorbic acid acted as a catalyst alone and together with copper.

In contrast to the effects described above, the addition of NDGA to milk containing ascorbic acid resulted in the stabilization of cream against oxidized flavors and also in an apparent increase of the tocopherol content of the fat (fig. 7) and the stabilization of fat against deterioration in the presence of ascorbic acid. In this case neither the organoleptic changes nor the losses in the fat-soluble vitamins were detected, either prior to or after the re-emulsification test when applied to the fat from storage cream during the experimental trial of 277 days. Approximately 5 per cent of added NDGA was recovered in the fat churned from storage cream. This was verified by the Quaife (8) method of analysis for vitamin E, using NDGA glycol stock solution as a control. The re-emulsification test apparently removed the anti-oxidant from the fat, as evidenced by 20 to 40 per cent losses in the values for the vitamin E content of the fat.

Finally, it is of interest to point out that the addition of 2 per cent salt to butter did not affect the stability of fat stored up to 247 days at – 17.7 to – 16.1° C. and then for the additional 30 days at 0 to 1° C. The stability of fat held in the form of butter could be attributed to the elimination of the plasma factor described in the introductory part of this paper and to the protection of butter against the effects of the exposures to light during the storage.

DISCUSSION

The primary consideration in the selection of nordihydroguaiaretic acid (NDGA) anti-oxidant as a stabilizing agent for the fat was its solubility in fat

as described by Lundberg *et al.* (5) in their studies on the anti-oxidant properties of the compound. Although the effectiveness of this anti-oxidant in frozen cream was demonstrated by Stull *et al.* (6), it remained to be seen whether the inhibition of oxidized flavors in the cream was brought about by the stabilization of fat or plasma phases or both. There was good reason to believe (3) that the fat soluble anti-oxidant might extend a protective influence not only to the fat but also to the unstable lipids which are a part of the fat globule stabilizing membrane and that the water-soluble anti-oxidant may not stabilize the fat by virtue of the fact that it is not soluble in fat.

The data in figure 7 were conclusive in showing that nordihydroguaiaretic acid added to milk prior to pasteurization and separation of cream had penetrated the fat phase of the cream. This was evident from the apparent increase in the tocopherol content of the fat from approximately 2,000 to 3,200 γ per 100 g. of fat. Consequently, the stabilization of cream against the oxidized flavors associated with deterioration of the unstable lipids and that of the fat against the reaction responsible for the development of the metallic-to-fishy flavors and losses in the fat-soluble vitamins could be attributed to the anti-oxidant activity centered in the fat phase of the cream. The addition of NDGA to milk prevents the sensitization of the fat in cream to deterioration by a plasma factor. It is not possible to state at the present time whether the activity of the plasma factor also could be restrained by NDGA added to cream pasteurized at 61.6° C. The observations of Stull *et al.* (7) concerning the promotion of oxidized flavors in samples of cream containing both copper and NDGA, but which were pasteurized at 65° C. suggest the possibility that the anti-oxidant might not stabilize the fat in cream heated to 61.6° C. However, the addition of the anti-oxidant from glycerol solution or water suspension to cream after the pasteurization treatment (7) makes it rather doubtful whether enough time was allowed for the diffusion of NDGA into the fat phase of the cream prior to the solidification of the fat.

Since the stability of fat in butter was not as yet affected by the salt, sensitization effect of the plasma factor probably could not be attributed to the plasma salts only. Possibly substances other than salts might have been responsible for the sensitization of fat to deterioration as determined by the re-emulsification test.

It is not known whether the oxidized flavors in the reconstituted milks were associated with the formation of the new compounds resulting from the interaction of ascorbic acid and the fat-soluble vitamins, or those from the interaction of ascorbic acid and the unstable fat. The data in table 1 and figures 4, 5 and 6 (I) show, however, that in spite of the fact that the tocopherol content of the fat in cream depleted of the total vitamin C content diminished progressively with the time of storage at sub-zero and 0 to 1° C., the cream did not develop the oxidized flavors and the vitamin A and carotenoid content of the fat were not affected.

The storage tests on cream and butter, as carried on in this study, were quite

similar to the storage conditions which may be encountered in the commercial and domestic handling of these products. The data suggest that the environmental conditions adopted for this study could be applied successfully in measuring the effectiveness of various anti-oxidants.

CONCLUSIONS

Nordihydroguaiaretic acid (NDGA), added to milk at the rate of 0.005 per cent of the fat, prior to pasteurization at 82.2° C. for 30 minutes and subsequent separation, was effective in preventing oxidized flavors in cream and in stabilizing the fat and fat-soluble vitamins when the cream was held 30 days at 0 to 1° C. following storage for 15 to 247 days at −17.7 to −16.1° C.

Nordihydroguaiaretic acid caused an apparent increase in the tocopherol content of winter fat from approximately 2,000 to 3,200 γ per 100 g. of fat, indicating a possibility that the anti-oxidant activity centered in the fat phase of the cream was largely responsible for the stabilization of cream.

Depletion of the total vitamin C content of cream resulted in the prevention of the oxidized flavors for 247 plus 30 days at indicated temperatures and the fat became unstable after 30 days at 0 to 1° C. following storage for 40 days at sub-zero temperatures. In contrast to this, control cream containing ascorbic acid developed oxidized flavors during storage at both sub-zero and 0 to 1° C., and the fat became unstable after 20 days at 0 to 1° C. following storage for 15 days at sub-zero temperatures.

The fat in butter containing 2 per cent of added salt retained its stability for at least 247 days at sub-zero temperatures and then for the additional 30 days at 0 to 1° C.

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OCCURRENCE IN BLOOD PLASMA FROM CERTAIN DAIRY CALVES OF FACTORS THAT INTERFERE WITH THE COLOR REACTIONS OF ACTIVATED GLYCEROL DICHLOROHYDRIN WITH VITAMIN A AND CAROTENOIDS[1]

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The introduction of a new chemical method of assaying blood for vitamin A presents the problem of adaptation to different species and to the same species under different dietary conditions. Activated glycerol dichlorohydrin (GDH), recently introduced by Sobel and Werbin (10, 11) as a colorimetric reagent that has certain advantages over the usual antimony trichloride reagent, has been used successfully for the measurements of vitamin A and carotene in the blood serum of human subjects (9) and of vitamin A in blood plasma of dairy cows (12).

Abnormally low vitamin A values occasionally were observed in the plasma of certain calves in the Iowa State College dairy herd when the Kimble procedure (5) was used for extracting vitamin A and carotenoids and GDH was employed as the colorimetric reagent. In view of factors affecting the vitamin A color reaction with antimony trichloride (2, 3, 6, 7, 8), it was postulated that the aforementioned low values were due to the presence of some substance or substances that interfere with the color reaction with GDH. Although this difficulty has not been reported for blood plasma, Wall and Kelley (13) found that petroleum ether extracts of fortified poultry mashes contained substances that suppress the GDH-vitamin A color development.

The primary objective of this study was to modify the recommended GDH procedure to overcome the difficulties occasionally encountered in vitamin A analysis of blood plasma from dairy calves.

EXPERIMENTAL

Analytical methods. Since saponification of blood serum has been used as a means of counteracting the effects of inhibitors of the antimony trichloride-vitamin A reaction (1, 8), the logical step was to compare saponification and nonsaponification using GDH as the colorimetric reagent for vitamin A and carotene. The detailed methods, employing the Beckman spectrophotometer for colorimetric readings, were as follows:

a. Nonsaponification method. The method of extracting calf blood plasma was essentially the same as that of Kimble (5). Nine ml. of blood plasma[2] were pipetted into a 50-ml. glass-stoppered centrifuge tube to which were added 9 ml. of 95 per cent ethanol and 21.6 ml. of redistilled Skellysolve A. The tube was stoppered and shaken by end-over-end inversion at the rate of 100 times per

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[2] Since samples of calf plasma often contain relatively small quantities of vitamin A and carotene, the quantity was increased to 9 ml. to improve the accuracy of the analyses.

minute for 10 minutes. Subsequently, the tube was centrifuged at low speed for approximately 30 seconds to obtain a clear supernatant layer of Skellysolve A extract, 18 ml. of which then were transferred to another 50-ml. centrifuge tube.

The solvent was evaporated by warming the tube in a water bath at 45 to 55° C.; when most of the solvent had been removed, a stream of nitrogen was passed over the extract until evaporation was completed. The tube was stoppered and cooled to room temperature after which 1.0 ml. of redistilled U.S.P. chloroform (dried over anhydrous Na_2SO_4) was added to dissolve the dried extract. Four ml. of GDH were added and mixed thoroughly with the chloroform solution.

Approximately 3 ml. of the resulting solution were poured into a 1-cm. Beckman Corex cell for the determination of the optical density of the colors resulting from the interactions of the vitamin A and the carotenoids with GDH. The optical density measurements were made in a Beckman model DU spectrophotometer with the slit width set at 0.065 mm. Two minutes after the addition of GDH, readings were made at a wave length of 555 mμ for vitamin A and 4 minutes after the addition, at 950 mμ for carotenoids. The instrument was set at 100 per cent transmission, using a solution containing 4 ml. of GDH and 1 ml. of chloroform.

The Beckman spectrophotometer was standardized at 555 mμ using U.S.P. Vitamin A Reference Standard (crystalline vitamin A acetate in cottonseed oil) and at 555 and 950 mμ, using crystalline β-carotene[3] according to the method of Sobel and Snow (9).

The carotenoid determination at 830 mμ with GDH is reported to be equally as sensitive as the usual method of determination in the Skellysolve A extract at 440 mμ (9). Preliminary observations revealed that the sensitivity of the measurement of carotene with GDH as the colorimetric reagent steadily increased between 800 and 1000 mμ. In view of these findings and in order to increase the sensitivity of carotene measurements in calf blood plasma, a wave length of 950 mμ was selected. Carotenoid values in calf plasma measured at 440 mμ in Skellysolve A extract were in agreement with those measured at 950 mμ using GDH as the clorimetric reagent, but the latter procedure is more convenient when the Beckman spectrophotometer is employed.

b. Saponification method. This procedure was essentially the same as the foregoing except that 9 ml. of freshly prepared 1 *N* KOH in 95 per cent ethanol were substituted for 9 ml. of 95 per cent ethanol. Although the use of 1 *N* KOH in 90 per cent ethanol has been recommended for saponification of human serum (1, 9), preliminary observations indicated that 1 *N* KOH in 95 per cent ethanol was a satisfactory solution for saponification of calf plasma. After the addition of the alcoholic KOH, the tube was stoppered and the contents were mixed thoroughly. The stopper was loosened and the tube was placed into a 60° C. water bath for 20 minutes. The plasma thus saponified was cooled to room temperature and 21.6 ml. of redistilled Skellysolve A were added. These steps in the saponification were essentially the same as those described by Sobel and Snow (9). Subsequent steps were the same as described in the nonsaponification

[3] Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

method. No significant differences were observed when aldehyde-free 95 per cent alcohol was substituted for U.S.P. alcohol in the saponification procedure. Blank runs were negative.

Validity of procedures. Internal standards were used as a means of estimating the accuracy of the respective analytical procedures and of ascertaining whether the difficulty was due to inefficient extraction or to the presence of a suppressing agent. These standards were prepared at frequent intervals by dissolving, respectively, known quantities of U.S.P. Vitamin A Reference Standard and crystalline β-carotene in redistilled U.S.P. chloroform. One ml. of standard solution was substituted for the chloroform in the foregoing procedures. The dried extracts of both saponified and nonsaponified plasma samples were dissolved in the internal standard solution and then analyzed for the total vitamin A and carotenoid content.

Blood samples. Venous blood samples were drawn from calves of various ages and on several different diets. The plasma was separated by centrifugation of oxalated blood, and most analyses were made within 48 hours after collection.

RESULTS AND DISCUSSION

Effect of color suppression on accuracy of the procedures. The results from the use of vitamin A internal standards in the analysis of calf plasma are summarized in table 1. The recovery of added vitamin A in the nonsaponified plasma

TABLE 1

Recovery by the GDH method of known amounts of vitamin A added to extracts of nonsaponified and of saponified blood plasma from calves

Calf no.	Nonsaponified			Saponified		
	Original	Internal standard		Original	Internal standard	
		Added	Recovered		Added	Recovered
	(γ/100 ml.)		(%)	(γ/100 ml.)		(%)
3129	21.2	62.8	56.4	21.0	62.8	99.4
3140	5.8	32.4	16.7	25.1	32.4	95.4
3142	21.0	62.8	60.2	21.8	62.8	99.2
3148	20.3	30.1	94.7	21.8	30.1	100.7
3151[a]	0.8	68.0	0.6	17.2	68.0	89.9
3151[b]	0.1	33.9	10.6	15.7	33.9	96.2
3152	14.5	33.8	85.8	16.4	33.8	93.8
3156	1.3	32.7	15.9	8.4	32.7	87.3
3160	18.6	32.4	79.3	20.7	32.4	105.6
3167	9.7	31.4	65.6	13.0	31.4	91.4
3168	1.0	31.4	9.2	8.5	31.4	90.1
Pooled[c]	13.1	23.9	68.2	14.9	23.9	89.1

[a] Sample collected 1/13/49.
[b] Sample collected 1/18/49.
[c] Samples from several calves.

ranged from 0.6 to 94.7 per cent while that in the saponified samples varied from 87.3 to 105.6 per cent. As a matter of convenience, the quantities of vitamin A added were not standardized; this, however, does not vitiate the comparative results from nonsaponification and saponification. In all instances, the recovery of

added internal standards was better from the latter than from the former on the same sample of plasma, thus indicating that the abnormally low values occasionally noted in calf blood plasma probably were due more to suppressing agents than to incomplete extraction of vitamin A.

Preliminary experiments indicated that the recovery of β-carotene added to the extracts of saponified blood plasma was better than from nonsaponified plasma. It is possible that the initial level of carotenoids and/or dietary factors other than the carotenoid intake may be involved.

In accord with observations from the use of antimony trichloride reagent (8), color development with GDH in certain nonsaponified samples was slow. The maximum absorptions at 555 mμ and at 950 mμ were not attained until 4 to 8 minutes and 8 to 10 minutes, respectively, after addition of the GDH. These absorption maxima after the protracted times were not so great as those obtained when the samples were saponified and measured at the prescribed periods, 2 and 4 minutes, respectively. Apparently substances having a depressing effect on the rate as well as on the extent of color development with vitamin A and with carotene were present.

Although the chemical nature of the inhibitory constituents of the blood plasma of calves has not been determined, these factors may be the same as or similar to the lipidelike substances (8) interfering with the antimony trichloride-vitamin A color reaction.

The wide range of recovery of added vitamin A indicates considerable variability in the quantity of inhibitors present. In half of the samples, recoveries of internal standards from extracts of saponified plasmas were less than 95 per cent, suggesting that this treatment did not counteract or remove completely the color inhibitors. It is possible that the time and/or temperature employed in saponification may be adjusted to overcome completely the color suppression. The consistently higher percentage recoveries affected by the present saponification procedure render it preferable to the usual nonsaponification method.

Factors possibly related to the suppression of color development. Among several factors that may be related to the presence of factors interfering with color development, diet seemed to warrant consideration. With this in view several dietary groups were studied. The analytical results presented in table 2 indicate that the greatest degree of suppression of the vitamin A color reaction was in calves of group I, which received whole milk daily and 100,000 I.U. of either carotene or vitamin A per 100 lb. body weight at bi-weekly intervals. Saponification effected an increase not only in vitamin A values but also in the carotenoid concentrations, the former being more marked than the latter. The color inhibition varied with different individuals; calf 3151 consistently had lower vitamin A levels than other calves when the plasma was not saponified. In another experiment blood samples were drawn from this subject at 3-hour intervals over a 15-hour period following the administration of 100,000 I.U. of natural ester vitamin A per 100 lb. body weight. When the nonsaponification procedure was used, no vitamin A was detected in the blood plasma, but when

saponification was employed, the vitamin A levels in the sequential collections were 17.2, 18.5, 22.7, 29.2, 23.1 and 19.3 γ per 100 ml. plasma.

The diets of the calves in group II included hay, hence the high carotenoid levels. These values were affected to a greater extent by saponification than

TABLE 2

Vitamin A and carotenoids, as determined by the GDH method, in nonsaponified and in saponified samples of blood plasma from calves receiving various diets

Dietary group	Calf no.	Vitamin A		Carotenoids	
		Nonsaponified	Saponified	Nonsaponified	Saponified
		(γ/100 ml.)			
I	3151[a]	0.0	14.5	15.1	18.0
Whole milk with occasional vitamin A or carotene supplement	3151[a]	0.1	15.7	11.2	13.5
	3151[a]	0.8	17.2	15.0	19.4
	3156	1.3	8.4	8.7	10.9
	3167	9.7	13.0	10.5	12.5
	3168	1.0	8.5	9.4	12.4
II	3129	21.2	21.0	75.3	80.1
Reconstituted buttermilk, hay and concentrate mixture	3140	5.8	25.1	88.9	113.2
	3142	21.0	21.8	94.0	108.8
	3148	20.3	21.8	35.4	38.6
	3152	14.5	16.4	32.3	40.5
	3160	18.6	20.7	54.7	64.8
III					
Concentrate mixture plus daily supplement of 5,000 I.U. carotene/100 lb. body wt.	2991	5.2	5.1	37.8	39.1
	2994	7.2	8.2	21.3	21.2
	3007[b]	10.2	9.9	46.5	45.3
	3007[b]	7.8	7.8	35.1	33.3
	3007[b]	5.1	5.5	31.8	34.9
IV					
Concentrate mixture plus daily supplement of 10,000 I.U. vitamin A/100 lb. body wt.	2991[b]	35.2	35.5	21.2	22.8
	2991[b]	45.4	42.5	13.6	14.2
	2994[b]	44.3	50.4	21.3	20.3
	2994[b]	62.4	65.3	17.3	16.4

[a] Three samples drawn over a 6-week period.
[b] Samples taken at weekly intervals.

were those for vitamin A. Calf 3140, however, was a notable exception, thus further emphasizing the role of individuality.

The differences in vitamin A and in carotenoid levels found by the two chemical methods were insignificant in groups III and IV, thus indicating little or no action of inhibitors on color development. Although the experimental subjects of these two groups were older than those of the other groups, it is improbable that age *per se* was related to group differences in color suppression.

The foregoing results suggest that dietary constituents may be important factors relating to the presence of inhibitory substances in calf blood plasma. Inasmuch as milk was common to all calves in which the color suppression was noted, it seems possible that this dietary constituent may be an etiological factor. However, in view of the limited observations and the possible interrelationship of other factors, the present data can be considered merely as indicative.

The data from group I suggest the possibility that occasional administration of massive amounts of certain types of concentrates of vitamin A and/or carotene intensifies the suppression effect.

If diet affects the presence or activity of inhibitors, this may account for some of the reported irregularities of vitamin A levels in calf blood. Jacobson and Thomas (4) found a decided increase in the plasma vitamin A level when calves that had received massive amounts of vitamin A daily were placed on a deficiency diet.

It should be emphasized that individual metabolic idiosyncrasies seem to play a more pronounced role than other factors that have been considered. The results presented herein point to the need for further exploration of the factors affecting the apparent concentrations of vitamin A and carotene in the blood of calves.

SUMMARY

Samples of blood plasma from calves were analyzed for vitamin A and carotenoids by saponification and by nonsaponification methods using activated glycerol dichlorohydrin (GDH) as the colorimetric reagent.

The accuracy of analysis, as measured by the use of internal standards, was increased by saponification of plasma.

Low vitamin A values occasionally found by the nonsaponification method are attributed primarily to the presence of inhibitors of color reactions.

Color suppressing factors apparently contributed to the low carotenoid levels found in some nonsaponified samples of calf blood plasma.

Although a marked variability among individual calves was noted, there appears to be a possible relationship between diet and the presence of color inhibitors in blood plasma.

Since there is no known method of predicting the presence of substances that suppress the color reactions, it is recommended that all calf blood plasma samples be analyzed by the saponification method when GDH is used as the colorimetric reagent.

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The authors wish to express their appreciation for the helpful suggestions given by Dr. S. W. Fox, Iowa State College, in the investigation, and Dr. D. B. Parrish, Kansas State College, in the preparation of the manuscript.

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ABSTRACTS OF LITERATURE

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BOOK REVIEWS

487. Farm work simplification. LAWRENCE M. VAUGHAN and LOWELL S. HARDIN. John Wiley and Sons, Inc, New York, N. Y. 145 pp. $2.80. 1949.

This book deals with the simplification of practically all phases of farm work. It is broad in its scope and sets forth the principles of effective work from the standpoint of labor, machines, organization and the human factors involved in the simplification of the usual farm tasks. Several pages are devoted exclusively to time- and labor-saving on dairy chores and specific examples with reference to milking operations, barn arrangement, feeding practices, etc., that will reduce labor are set forth.

The book is divided into two parts. Part I discusses the place of work simplification in farming with respect to savings that can be made, the principles of effective work and how the results may be applied. Part II is devoted to methods and plans that may be used in making an analysis of any operation from the standpoint of developing time- and labor-saving methods. Such items as product analysis, man analysis, man and machine analysis and body motions are stressed. Suggestions for teaching and training individuals in work simplification are stressed with emphasis on the fact that research and education go together.

Throughout the book are found examples of labor savings for specific jobs that well may be adapted to most farms seeking to improve their efficiency. Since dairy farming involves not only feed and care of cattle but crop production, hay harvesting, transportation, etc., this book is well worthwhile for the practical operator, the plant fieldman, extension workers, research economists and the teacher of agriculture.

H. A. Herman

488. Animal breeding. LAURENCE M. WINTERS. 4th ed. John Wiley and Sons, Inc., New York, N. Y.; Chapman and Hall, Ltd., London. 404 pp. $4.50. 1948.

Keeping pace with the progress in animal breeding through the years since the first edition of this book in 1925, the present fourth edition again has been expanded and changed. Numerous additions have been made to include many recent developments and more detail in this well-known book. The opening chapter has been expanded and now contains an introduction to the problem of animal improvement by breeding. Among the several reorganizational changes has been that of making gametogenesis serve as the connecting link between the chapter on "The Reproductive Organs" and the chapter on "The Physical Basis of Heredity". The order of presentation of much of the applied material has been changed to follow the more fundamental sections of the book. New chapters entitled "The Effectiveness of Selection" and "Building Superior Germ Plasma" have been added. The author cites many examples from his own extensive experience in breeding research, as well as from the research of others, to illustrate the application of the fundamental principles of breeding to practical animal improvement. N. L. VanDemark

489. Milchwirtschaftliche Patentberichte. (Patents concerning the dairy field.) M. SCHULZ. Volkswirtschaftlicher Verlag Dr. Anton Fehr, Kempten-Allgäu, Germany. 336 pp. 1947.

This book is a compilation of patent reports concerning the whole field of dairy manufacturing processes, both German and foreign, covering the patents for the years 1935 to 1945. I. Peters

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

490. Q fever. Experimental Q fever in cattle. E. J. BELL, R. R. PARKER, and H. G. STOENNER, U. S. P. H., Hamilton, Mont. Am. J. Pub. Health, **39,** 4: 478–484. Apr., 1949.

Q fever infection was produced in lactating cows by inoculating the udder with massive doses of a yolk sac culture of *Coxiella burnetii.* The organisms were present in the milk for a long

period of time following injection via the teat canal and in one instance were found for over 200 d. in milk from a quarter inoculated via the glandular tissue. Inoculation of the udder produced an acute mastitis with a marked systemic reaction of brief duration. Recovery from the acute phase was spontaneous with the major symptoms disappearing in about 8 d. Various tissues obtained from these cows sacrificed at intervals up to 63 d. after injection were infectious to guinea pigs. The rickettsiae were recovered from the feces of calves feeding on infected milk. Less success was found in attempting to infect heifers by other routes, such as intranasal, intravenous, by way of the alimentary tract and by way of the vaginal tract. Attempts also were made to transmit Q fever to cattle by infected ticks of the species *Otobius megnini* Duges, but no definite results were reported. D. D. Deane

491. A rapid ring test for brucellosis in fresh milk. G. C. Van Drimmelen, Institute of Onderstepoort, Pretoria, S. Africa. J. S. African Vet. Med. Assoc., **19,** 4: 130–134. Dec., 1948.

A colored antigen test for milk samples in the diagnosis of bovine brucellosis is described. The antigen used in the test is stained with haematoxylin. A well-mixed sample of milk is placed in a small test tube and one drop of the antigen is added and mixed well. The sample then is incubated at 37° C. for 50 min. A ring of violet-colored bacilli in the cream layer, with a white milk layer underneath, indicates a positive test. When a white cream layer covers milk which has a violet tinge, the results indicate a negative test. A method for preparing the stain and antigen is outlined. The author reported that the test was in wide use in Denmark and that it could be used to advantage in survey work. K. M. Dunn

492. Pathogenesis of bovine mastitis. I. The relation of age to streptococcic infection. G. R. Spencer and M. E. Kraft. Univ. of Wisconsin, Madison. Am. J. Vet. Research, **10,** 35: 115–118. Apr., 1949.

The incidence of streptococcic infection is reported according to age for 12 herds in which chemotherapy had not been practiced. Two herds considered to have good sanitary milking management showed a progressive increase from 5.26 to 35% during the first four lactation periods, followed by a decline. Ten herds with poor milking management showed an increase from 60.61 to 72.73% during the first five lactations, followed by a sharp increase. Data from one herd showed that the incidence of infection was also linear during the first lactation. Large herds and poor sanitary practices were associated with high infection incidence. Evidence indicates that degree of exposure outweighs aging of the udder as a cause of increased infection with successive lactations. E. W. Swanson

Also see abs. no. 532.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

493. Globular and free fat in butter. I. A method for counting and measuring the fat globules in butter and application to the working process. N. King. Netherlands Milk Dairy J., **1,** 1: 19–32. Jan., 1947.

The counting technic developed is as follows: approximately 10 mg. of butter accurately weighed were diluted with 200–500 mg. of butter oil. A very cautious mixing procedure was followed under carefully controlled temperature conditions. A measured quantity of diluted butter was put on a special slide and examined in polarized light by means of a simple mountable polarizer and analyser. The amount of globular fat decreased in the course of working. This decrease was in the number of fat globules rather than a change in the average size. The disappearance of "unbound" moisture was parallel at first with the destruction of fat globules. However, in the last stages of working, the disappearance of the "unbound" moisture was not attended with a destruction of the fat globules. W. M. Roberts

494. Globular and free fat in butter. II. Some applications of the dilution method for determining the globular fat in butter. N. King. Netherlands Milk Dairy J., **1,** 2: 115–117. Apr., 1947.

Application of the dilution method for counting and measuring the fat globules showed that whey butter contained a low percentage of globular fat and that the fat globules were distorted. There was scarcely any difference between the butter from the usual wooden and the metal churn. Also, the fat globules in butter prepared by the Fritz continuous process are deformed appreciably by the strong agitation of the beating wings of this machine. W. M. Roberts

495. Butter workmanship. L. C. Thomsen. Univ. of Wisconsin, Madison. Natl. Butter Cheese J., **40,** 5: 34–35, 58. May, 1949.

The body and texture of butter are influenced greatly by the physical state of the butterfat in the cream and butter. Butterfat exists as free fat —fats with low melting points usually in the liquid state—and as globular fat. The quantity of free fat may be as low as 10 to 15% at the start of the working process and 70 to 75% at the end. A high ratio of free fat to globular fat is respon-

sible for stickiness, leakiness and oiliness in butter. Crumbliness, brittleness and graininess are due to a low free fat-globular fat ratio.

Pasteurization of cream by direct steam injection and churning at high speed mutilate the fat globules, thereby causing an increase in free fat. Low temperature churning and low temperature wash water have an opposite effect. Control of the free fat-globular fat ratio results in control of of the body and texture of butter.

H. E. Calbert

496. Experiments on the packing and storage of butter. VI. The effect on keeping quality of butter of exposure to light during manufacture. C. R. BARNICOAT, Dairy Research Inst., (N. Z.), Palmerston North. New Zealand J. Sci. Technol., **29A,** 4: 185–191. Dec., 1947.

Results with 36 well-manufactured butters (of low Cu and Fe content) churned from pasteurized sweet cream and exposed before working to different light intensities for various periods indicated that interior diffuse daylight, not exceeding 50 foot candles and for not over 2-hr. duration, had no deleterious effects on the product.

W. C. Frazier

497. Experiments on the packing and storage of butter. VII. The effect of "free" air in butter. C. R. BARNICOAT, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., **29A,** 4: 193–197. Dec., 1947.

"Free" air content of 16 samples of factory butter varied from 4.9 to 6.7 per cent. Reduction of the air content or working under a high vacuum did not alter the keeping quality but did change appearance and physical characteristics.

W. C. Frazier

498. Experiments on the packing and storage of butter. VIII. The effect of certain added substances on the storage-life of butter. C. R. BARNICOAT, Dairy Research Inst. (N. Z.), Palmerston North. New Zealand J. Sci. Technol., **29A,** 4: 199–205. Dec., 1947.

The following substances were added either singly or in combination: (a) dairy salt, (b) borates, (c) citrates, (d) phosphates, (e) oat flour and (f) $NaHCO_3$ to an alkaline pH. Except with citrates, the results under the conditions employed were discouraging in that the storage life was not lengthened and even seemed to be shortened in some instances. W. C. Frazier

499. Butter packaging. J. M. NESBITT. Can. Dairy Ice Cream J., **28,** 2: 56–60. Feb., 1949.

A good packaging material for print butter should have the following characteristics: (a) impermeability to water vapor, gases, odors and light; (b) close adherence to the surface of the butter to prevent evaporation and loss of weight; (c) grease-proof, odorless and tasteless material; (d) high tensile strength when damp or wet; (e) adequate protection against mold growth on the surface of the butter; (f) easy adaptation to automatic packaging machines and (g) attractive appearance and reasonable cost. The article gives the advantages and disadvantages of aluminum foil, vegetable parchment and fiber boxes.

H. Pyenson

500. A cream improvement program. C. H. P. KILLICK. Can. Dairy Ice Cream J., **28,** 2: 40–43, 90. Feb., 1949.

The reasons for the improvement in quality of cream in Manitoba during the past 25 yr. are: (a) the closing of cream-buying stations and (b) the establishment of a compulsory cream grading service with fixed standards and legal minimum price differentials. A price differential between grades of cream is essential as an incentive for improved quality, but if fixed, exerts less effect as the price for butterfat rises. It also was found that the price level for butterfat appears to affect quality. The improvement in quality has reduced off-flavored, old and sour cream to less than 2% and improved butter quality from 70% first grade to 94% first grade. H. Pyenson

501. Four-day grading and its effect on cream quality. H. W. GREGORY, Purdue Univ., Lafayette, Ind. Natl. Butter Cheese J., **40,** 5: 38, 40. May, 1949.

Successful cream grading programs have one or more definite factors required in grading to supplement the senses of taste and smell. Delivery within 4 d. after production serves as a means of insuring a better quality cream. The 4-d. plan of cream grading has brought about an improvement in Indiana butter manufactured from cream purchased under this plan. H. E. Calbert

Also see abs. no. 519, 533, 534, 535, 568.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

502. Lipolytic bacteria, a cause of rancidity in cheddar cheese. E. G. HOOD, C. A. GIBSON and J. F. BOWEN. Can. Dairy Ice Cream J., **28,** 2: 27–30, 88. Feb., 1949.

An analysis of the results of 16 experimental vats of cheddar cheese made from milk with the addition of varying percentages of lipolytic bacteria gave consistently lower flavor scores and consistently higher acid values than uninoculated vats. Normal uninoculated vats had an average

acid value of 1.0 while inoculated vats had acid values ranging from 1.8 to 16.0 after storage for 10 d. The defects encountered in grading, namely "not clean", "slightly rancid", and "rancid", were the same as those found in commercial grading. The extent to which flavor defects developed and acid degree values increased was related directly to the percentage of lipolytic organisms added to the cheese milk supply. The low flavor scores and the high acid degree values of the experimental cheese were thought to be due to lipolysis or a chemical break-down of the fat as a result of the action of lipolytic bacteria. The presence of lipolytic bacteria suggests that these organisms may be a cause of the sporadic or scattered occurrence of rancid flavor in commercial cheddar cheese. H. Pyenson

503. Further studies on rheological properties of cheese during manufacture and ripening. Part I. MARGARET BARON. Natl. Inst. for Research in Dairying, Shinfield, Nr. Reading. Dairy Ind., **14,** 2: 146–151. Feb., 1949.

Investigations were carried out to determine the relationship between the physical, chemical and bacteriological properties of milk, curd and cheese. The pH of the soft-pitched curd was low in comparison with the rest, with one exception. The streptococci in these curds contained a high proportion of strains capable of producing slight gas in litmus milk. During ripening at 58° F., the cheese firmed up rapidly during the first 14 d., after which they softened somewhat at 28 d. and then gradually increased in firmness at a steadily declining rate until about 130 d. old, after which the body remained constant. The firmness of a cheese throughout its life was related directly to its firmness at pitching.

A highly significant relationship was found between the weight and firmness of green cheese. The heavier the cheese, the softer it was, but this relationship did not exist as the cheese aged.

A detailed study of variation of body throughout a single cheese was carried out on some 28 cheeses. At the top or bottom of the cheese the periphery was slightly firmer than the center.

G. H. Watrous, Jr.

504. Further studies on rheological properties of cheese during manufacture and ripening. Part II. Statistical results. MARGARET BARON. Dairy Ind., **14,** 3: 255–263. Mar., 1949.

This report contains a statistical analysis of the work reported in part I of this study. The effects of variations in manufacture on the properties of green cheese were seen most clearly at 14 d. Milk with high fat content gave rise to soft young cheese, and the reverse held true. Milk with high S.N.F. produced a firm, tough cheese when ripe. High casein content alone did not make cheese firmer than normal.

The firmness at cutting influenced the elasticity of the cheese; curd cut when soft resulted in cheese of high elasticity. The firmness at pitching mainly determined the firmness of the finished cheese; a curd pitched firm was found to cause a firm and dry cheese. The acidity at milling affected the elasticity and possibly the firmness of the cheese; high acidity produced a soft cheese with very little springiness. G. H. Watrous, Jr.

505. Extraneous matter in cheese. D. C. BECKETT. Can. Dairy Ice Cream J., **28,** 3: 96–98. Mar., 1949.

For the third year in succession, extraneous matter tests on cheddar cheese have been conducted in the Dairy School Laboratories at Kemptville during the months of June, July and August. During the 1948 season, a total of 1,407 samples were received for testing. The percentage of acceptable samples was 26.15 in 1943, 65.00 in 1946, 57.72 in 1947 and 86.86 in 1948.

H. Pyenson

506. De stremkrachtbepaling volgens de methode van Van Dam. (The rennet test according to the direct method of Van Dam.) English summary. H. MULDER and L. RADEMA. Netherlands Milk and Dairy J., **1,** 2: 128–133. Apr., 1947.

The parallelism found by Van Dam between the peptic and the coagulating activity of rennets does not exist if the rennets contain very different amounts of pepsin. In order to estimate the curdling power of a rennet, it is recommended to estimate first the strength of a chymosin solution according to the method of Van Dam. Then the curdling power of the rennet of unknown strength can be estimated by means of comparative tests. Details of the method are given.

W. M. Roberts

507. Package and packaging material therefor. D. B. ANDREWS. (Assigned to Marathon Corp.) U. S. Patent 2,467,875. 6 claims. Apr. 19, 1949. Official Gaz. U. S. Pat. Office, **621,** 3: 889. 1949.

A block of cheese is wrapped tightly in a 2-layer wrapper with a tearing element imbedded in the coating. The wrapper may be removed easily by pulling a tab which causes the material to tear in a predetermined manner. R. Whitaker

508. Cheese-cutting device. H. A. OLANDER. U. S. Patent 2,468,229. 8 claims. Apr. 26, 1949. Official Gaz. U. S. Pat. Office, **621,** 4: 1132. 1949.

A block of cheese may be sliced in thin layers by this device which consists of a holder in which

the cheese is placed and a wire cutter which is pulled horizontally through the cheese. The cutter is positioned by a tortuous slot at both ends of the holder, the cutter being moved along said slot from one level to another to produce uniform slices. R. Whitaker

Also see abs. no. 513, 520.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

509. Method of making low-ash crude lactose. E. F. ALMY and M. E. HULL. (Assigned to M & R Dietetic Laboratories, Inc.) U. S. Patent 2,467,453. 4 claims. Apr. 19, 1949. Official Gaz. U. S. Pat. Office, **621,** 3: 781. 1949.

To sweet whey having a pH of 5.4 or higher is added sodium tetraphosphate in an amount sufficient to prevent heat coaguluation and hydration of the protein. The whey then is condensed to a total solids content suitable for lactose crystallization. R. Whitaker

510. The manufacture of cultured buttermilk from non-fat dry milk solids. R. N. COSTILOW, M. L. SPECK and W. M. ROBERTS, N. Carolina State College, Raleigh. Milk Plant Monthly, **38,** 4: 36–41. Apr., 1949.

Cultured buttermilk of good quality may be prepared from reconstituted non-fat dry milk solids. The suggested procedure for its manufacture involves pasteurization at 180° F. for 30 min., which sterilizes the reconstituted milk solids, yet does not impart a pronounced cooked flavor to the finished product. A slight preference exists for a 10% total solids content, since cultured buttermilk containing 8% solids was too thin, whereas the 11% product was too viscous. A resulting acidity, calculated as lactic acid, of 0.85 to 0.90% was found to be ideal from the consumer viewpoint. Following the above recommendations does not necessarily insure a premium product, since the flavor of the finished product cannot be better than the quality of the non-fat dry milk solids used in the preparation of the cultured buttremilk. J. A. Meiser, Jr.

511. The manufacture, use and storage of dehydrated sweetened condensed milk. A. T. MUSSETT and W. H. MARTIN. Can. Dairy Ice Cream J., **28,** 2: 68–74. Feb., 1949.

See abs. 88, p. A19.

512. Plastic cream—its production and uses. R. J. SPIERS. Can. Dairy Ice Cream J., **28,** 4: 42, 46. Apr., 1949.

See abs. 87, p. A19.

Also see abs. no. 524, 525, 526, 545.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

513. The effect of penicillin on the acid-producing ability of starters. E. G. HOOD and H. K. KATZNELSON. Can. Dairy Ice Cream J., **28,** 3: 32–33. Mar., 1949.

Where occasional mastitis-infected animals are treated with penicillin, the problem of inhibition of starter activity in the milk is not likely to present itself, owing to the dilution of the penicillin in the pooled milk supply. Its inhibitory effect can be a source of considerable concern to cheesemakers in areas where extensive use of the antibiotic is being made. It is suggested that the milk obtained from cows during the 3-d. penicillin treatment and for 1 d. thereafter, be used for purposes other than cheesemaking. A less costly method would be to inactivate the penicillin in the milk at the cheese factory using the enzyme penicillinase at the rate of 0.02 mg./100 ml. milk. H. Pyenson

514. Air express shipment of milk samples for bacteriological analysis. Anonymous. Milk Plant Monthly, **38,** 4: 70–72. Apr., 1949.

Since many operators lack the ability to analyze microscopic counts correctly, a service whereby milk samples designated for bacteriological examination are flown by plane to a central laboratory was established. Shipping containers consist of an aluminum box filled with perforated airfoam rubber for receiving the sample bottles. Sterilized bottles containing formaldehyde are expressed to the dairy, the samples taken and returned by air express to the central laboratory. Since reports can be mailed out the following day, the time lag is not objectionable. J. A. Meiser, Jr,

515. Molds in the dairy plant. S. G. KNIGHT, Univ. of Wisconsin, Madison. Milk Plant Monthly, **38,** 4: 82–83. Apr., 1949.

Molds grow on a wide variety of materials under many different conditions. This fact necessitates the control of these simple plants if one wishes to produce products that do not possess a "musty" or rancid flavor. Although growth may occur anywhere in the plants, molds, like roaches, readily grow in inaccessible damp areas which are not cleaned properly.

Sanitation of wooden equipment involves constant and thorough cleaning followed by complete drying in dust-free air. Heavily infected equipment may be treated with chlorine solutions, hot

water or steam. Wrapping materials for dairy products may be freed of mold growth by soaking for 20 to 30 sec. in a 2 to 3% salt solution heated to 200° F. Also, 375 p.p.m. chlorine solutions and 10% calcium or sodium propionate solutions retard mold growth on wrapping materials.

Walls and shelves should be washed with alkaline phosphate solutions followed by a rinse with 1,000 p.p.m. chlorine solutions or 400 to 600 p.p.m. solutions of quaternary ammonium salts. Other methods for controlling mold growth on walls involve the use of a 4% borax solution, a 2% acetic acid solution, paints containing fungicides or ultraviolet lights. J. A. Meiser, Jr.

Also see abs. no. 502.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

516. Preliminary investigations of the determination of fully-saturated glycerides in New Zealand butterfat. I. TING, Fats Research Lab., Wellington, N. Z. New Zealand J. Sci. Technol., **29A,** 5: 240–246. Feb., 1948.

Existing methods for determination of the fully-saturated glycerides in butterfat were found unsatisfactory. Higher yields of these glycerides were obtained by the author than by other workers. W. C. Frazier

517. De dispersiteit van karnemelkvet en het centrifugeren van karnemelk. (The distribution of buttermilk fat and the separation of buttermilk.) English summary. H. MULDER. Netherlands Milk Dairy J., **1,** 1: 57–67. Jan., 1947.

From the data in the literature covered by this review, the fat in buttermilk can be divided into fat from the original milk fat globules, groups of globules and butter granules, colloidal fat and phospholipids. On comparing the methods of Rahn, Sandelin and Sirks for estimating the size distribution of fat globules in buttermilk, the method of Sirks appeared to be most accurate because it involves the measuring and counting of all fat globules. Some of the original milk fat globules and a relatively large quantity of colloidal fat remained in the buttermilk after centrifuging. W. M. Roberts

518. A general formula for the treatment of correction problems in the butyrometric determination of fat. TH. BROUWER. Netherlands Milk Dairy J., **1,** 2: 98–109. Apr., 1947.

A formula was derived for correcting the butyrometric determination of fat so that it will be comparable to the gravimetric method. The formula is:

$$C = B\left(\frac{100\ SK}{Q} - 1\right) + \frac{100\ MK}{Q} + \frac{100\ R}{Q}.$$

C = correction expressed in %; B = fat content read on the butyrometer; S = volume of one scale unit expressed in ml.; M = volume of the meniscus expressed in ml.; Q = weight of the quantity of substance taken in the operation, expressed in g.; K and R = constants. W. M. Roberts

519. The determination of diacetyl in butter. P. C. DEN HERDER. Netherlands Milk Dairy J., **1,** 2: 110–113. Apr., 1947.

By using a specially constructed apparatus, the method of Prill and Hammer for the determination of diacetyl in butter was modified by substituting a CO_2-stream for the steam distillation. W. M. Roberts

520. Olika faktorers inverkan pá löpekoaglets elastiska egenskaper. (Different factors affecting the elastic properties of rennet coagulum.) E. HANSSON, G. SJÖSTRÖM and E. G. SAMUELSSON, Kam. Bact. Inst., Alnarpsinstitutets Mejeriavdelning. Svenska Mejeritidningen, **41,** 5: 45–48. Jan., 1949.

The elasticity of the coagulum changes in direct proportion to the amount of rennet when this varies within the range of 3 to 20 parts of rennet to 10,000 parts of milk. Since the pH in milk diminishes with the addition of lactic acid, the elasticity of rennet increases in proportion to the change in the pH. The addition of $CaCl_2$ to milk causes an increase in the elasticity of the coagulum. This increase is greater than that brought about by the changes in the pH of milk. The elasticity increases constantly when the rennet temperature is between 25 and 30° C. Between 35 and 45° C. the increase in elasticity was not constant.

A "Konsistometer" is described. The elasticity is represented by the formula: $E = \frac{\varphi}{\delta} - 1$, where T = (in minutes) between the addition of rennet and measuring the elasticity; k = concentration of rennet in parts per 10,000 parts milk; φ = torsion angle measured in cm. on balance scales; and δ = opposite torsion angle for the cylinder. G. H. Wilster

521. Tocopherols (vitamin E) in milk: their chemical determination and occurrence in human milk. MARY LOUISE QUAIFE, Distillation Products, Inc., Rochester, N. Y. J. Biol. Chem., **169,** 3: 513–514. Aug., 1947.

A method previously applied to the estimation of vitamin E in blood plasma has been adopted for milk. Because of the large proportion of low molecular weight triglycerides in butterfat, the molecular distillation technic is not applicable to milk. The method used employs solvent extraction and colorimetric assay. Values for winter cow's milk are quoted at from 17 to 30 γ/g. of butterfat, as compared with a group of summer milks with a mean value of 42 γ/g. of fat. Fifteen samples of human milk, collected within 1 wk. postpartum, gave values of from 76 to 1,800 γ/g. of fat, with 12 of the 15 samples exceeding 200 γ. Four composite samples of human milk, from 14 mothers in their first eighth month of lactation, showed tocopherol levels of from 37 to 58 γ/g. of fat. A. O. Call

522. Instability of milk due to an increased activity of calcium ions. L. SEEKLES and W. TH. G. M. SMEETS. Netherlands Milk Dairy J., **1,** 1: 7–18. Jan., 1947.

Typical and non-typical cases of the "Utrecht-abnormality (instability) of milk" are due to its increased calcium ion activity. The exact cause of this condition is not known but it may be due to mineral regulating processes in the animal body and factors which affect them. Typical cases often could be corrected successfully by oral or subcutaneous administration of sodium citrate. The instability of milk decreases as the pH is increased. Also, the condition was corrected by adding sodium citrate, a mixture of monopotassium and disodium phosphates, potassium oxalate or sodium flouride to the milk.
W. M. Roberts

523. The condition of casein in milk. P. VAN-DER BURG. Netherlands Milk Dairy J., **1,** 2: 69–77. Apr., 1947,

It was stated that casein occurs in milk as a salt of Ca; this salt keeps some colloidal Ca and PO_4 in suspension. Ca combines with the casein in an amount equivalent to the organic phosphate. From formaldehyde titration values obtained after heating milk, as well as after the addition of oxalate, it is concluded that the positive amino groups of lysine take part in the binding of the negative phosphate groups. This leaves only the negative carboxyl groups for the colloidal calcium.
W. M. Roberts

524. Method of treating casein products. J. G. WEELDENBURG. (Assigned to American Enka Corp.) U. S. Patent 2,468,531. 7 claims. Apr. 26, 1949. Official Gaz. U. S. Pat. Office, **621,** 4: 1208. 1949.

Filaments of casein are made acid-resistant, the wet strength is increased and the swelling value reduced by reacting the casein with a solution of dimethylol and the monomethylol derivative of mono- and di- substituted phenols, then removing the excess solution, drying and heating.
R. Whitaker

525. Method of purifying casein. R. J. BLOCK and H. W. HOWARD. (Assigned to the Borden Co.) U. S. Patent 2,468,730. 6 claims. May 3, 1949. Official Gaz. U. S. Pat. Office, **622,** 1: 125. 1949.

Casein in solution above pH 6 is coagulated by adjusting the pH to between 4 and 6 and, after filtering, is suspended in water. Sulphur dioxide gas is added to about pH 1.9 to produce a colloidal sol. The purified casein then is reprecipitated by adjusting the pH to between 4 and 6 and finally dried. R. Whitaker

526. Method of producing egg substitutes. T. W. LINDEWALD and S. GRUBEN. (Assigned to Svenska Mjölkprodukter Aktiebolag.) U. S. Patent 2,468,677. 6 claims. Apr. 26, 1949. Official Gaz. U. S. Pat. Office, **621,** 4: 1246. 1949.

A substitute for egg albumin is made by drying skim milk treated with rennet at pH 6 to 7 in the presence of a substance capable of removing all of the dissociated calcium. R. Whitaker

527. A study of whey proteins from the milk of various animals. H. F. DEUTSCH, Univ. of Wisconsin, Madison. J. Biol. Chem., **169,** 2: 437–448. July, 1947.

Milk serum proteins, obtained in most cases by removal of the casein from the skim milk by precipitation with rennin, were dialyzed, frozen and dried in vacuo. Electrophoretic mobility measurements, as well as sedimentation analyses (using an oil turbine Svedberg ultracentrifuge), then were made. The samples of milk tested were from the cow (no breed given), goat, pig (two breeds), human, sheep and horse. For each species samples included various days postpartum. Tables giving the results and also electrophoretic boundaries as well as sedimentation diagrams are included. Each species shows marked difference in the electrophoretic and sedimentation patterns of the whey proteins. The patterns change with the transition from colostrum to normal milk.
A. O. Call

528. Composition of Percheron mares' colostrum. A. D. HOLMES, A. F. SPELLMAN and R. T. WETHERBEE, Mass. Agr. Expt. Sta., Amherst. J. Nutrition, **37,** 3: 385–392. Mar., 1949.

Thirty samples of colostrum were collected for the first 6 days of lactation from normal purebred Percheron mares. The initial samples of colostrum were not collected at the same interval following parturition. This possibly accounts for some of the wide variation of components in the first samples. Protein and magnesium content averaged higher in the first samples than in later samples. The amounts of ascorbic acid, phosphorus and potassium were lower in the initial samples than in later samples. Average values for individual mares ranged as follows: water, 86.3 to 88.1%; protein, 3.7 to 5.4%; ascorbic acid, 47 to 66 mg./1.; P, 75 to 86 mg./100 g.; K, 80 to 101 mg./100 g.; and Mg, 13 to 16 mg./100 g.
R. K. Waugh

529. Interaction of homologous alkyl sulfates wih bovine serum albumin. F. KARUSH and M. SONNENBERG, N. Y. Univ. College of Medicine. J. Am. Chem. Soc., **71,** 4: 1369–1376. Apr., 1949.

A colorimetric method for determining low concentrations ($10^{-5}M$) of homologous alkyl sulfates (dodecyl, decyl and octyl sulfates) was developed in order to study the reversible binding of these compounds by bovine serum albumin. Employing the method of equilibrium dialysis, experiments were conducted at room temperature (25–28° C.) and at low temperature (1–2° C.). The values of the thermodynamic functions $\Delta F°$, $\Delta H°$ and $\Delta S°$, for the binding process have been calculated. The binding of the alkyl sulfates is considered to be largely or wholly an entropy effect. However, no decision can be made as to the extent to which these changes are associated with the release of water molecules bound to protein and anion and/or structural changes of the protein. The binding of organic ions to serum albumin probably involves the electrostatic interaction between a positively charged group of the protein and the negative group of the anion. All of the positive groups on the protein are not available for binding. Results of this study show that only 14 groups are involved in binding, although the analytical data for bovine serum albumin indicate that at pH 6.1 the arginine and lysine residues alone account for 84 such groups. Considering the possibility that a variation of the intrinsic association constants of the binding sites would account for the shape of the binding curves observed, a quantitative formulation has been proposed. The new theory describes the heterogeneity associated with differences among the sites on the same molecule. H. J. Peppler

530. Studies on proteins from bovine colostrum. I. Electrophoretic studies on the blood serum proteins of colostrum-free calves and of calves fed colostrum at various ages. R. G. HANSEN and P. H. PHILLIPS, Univ. of Wisconsin, Madison. J. Biol. Chem., **171,** 1: 223–227. Nov., 1947.

The blood serum proteins of calves were measured electrophoretically before and after they had ingested colostrum and at various ages. When colostrum was fed within 24 hr. after birth there was a marked increase in the γ-globulin in the blood serum, but when colostrum was fed after the calves were 24 hr. old there was no such increase of this blood serum fraction. The same effect and the same limitation as to age were found when a water-soluble globulin was isolated from colostrum, added to warm skim milk and fed the calves. When calves were not given colostrum within 24 hr. after birth, it required about 8 wk. for their serum blood protein fractions to approach normal values.
A. O. Call

531. Studies on proteins from bovine colostrum. II. Some amino acid analyses of a purified colostrum pseudo-globulin. R. G. HANSEN, R. L. POTTER, and P. H. PHILLIPS, Univ. of Wisconsin, Madison. J. Biol. Chem., **171,** 1: 229–232. Nov., 1947.

A water-soluble fraction of bovine colostrum referred to as pseudoglobulin was isolated and purified by repeated precipitation and solubilization. Sedimentation analyses, using a Svedberg oil turbine ultracentrifuge, and also electrophoretic analyses indicated that the fraction was homogeneous. Determinations were made for fourteen different amino acids and the percentages are given in a table compared with reported values for the same amino acids as found in human γ-globulin. In general, the colostrum globulin is similar in amino acid content to the human γ-globulin except that it shows a somewhat higher level of proline and isoleucine and the lysine value is somewhat lower than that reported for human γ-globulin. A. O. Call

532. Biophysical studies of blood plasma proteins. IX. Separation and properties of the immune globulins of the sera of hyperimmunized cows. E. L. HESS and H. F. DEUTSCH, Univ. of Wisconsin. J. Am. Chem. Soc., **71,** 4: 1376–1381. Apr., 1949.

Ethanol fractionation has been applied to the recovery of the antibody-enriched fractions of hyperimmune sera prepared by inoculating 2 Holstein cows with Newcastle virus in whole egg embryo and with viable *Brucella abortus* suspensions at 4-d. intervals over a 3-mo. period. *B. abortus* agglutinins and bactericidins and Newcastle virus hemagglutination inhibiting and neutralizing antibodies were assayed. Increases in the

content of the serum γ-globulins were followed by electrophoretic analysis. The major portion of the antibodies was precipitated from the diluted serum at pH 7.6 and 18% ethanol, yielding more than 90% of the γ-globulins present in the original serum. Antibodies of *B. abortus* differed in solubility; the bactericidins were comparatively more soluble than the agglutinins. Subfractionation of the γ-globulins revealed the presence of the neutralizing antibodies for Newcastle virus in both the γ_1- and γ_2-globulin fractions; the hemagglutination inhibiting antibodies appeared to remain largely in the γ_1-globulin fraction. The concentration of neutralizing antibodies for Newcastle virus and bactericidins and agglutinins for *B. abortus* was distinctly lower in the γ_2-globulin fraction. Electrophoretic spreading experiments and solubility studies indicated that the γ-globulin fractions are electrically inhomogeneous. Their sedimentation behavior, however, revealed an essentially monodisperse system. H. J. Peppler

Also see abs. no. 498, 506, 538.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

533. Butter manufacturing process. H. C. Horneman, R. V. Hussong, S. N. Quam and B. W. Hammer. (Assigned to Sugar Creek Creamery Co. and Cherry Burrell Corp.) U. S. Patent 2,466,894. 16 claims. Apr. 12, 1949. Official Gaz. U. S. Pat. Office, **621,** 2: 494. 1949.

Butter is produced continuously from cream through the following series of steps: preheating, filtering, pasteurizing by a steam-vacuum treatment, centrifuging to produce milk fat, blending the fat with water and salt in a mixing chamber, cooling to stabilize the emulsion and finally working into butter. R. Whitaker

534. Butter manufacturing means and method. H. C. Horneman, R. V. Hussong, S. N. Quam, and B. W. Hammer. (Assigned to Sugar Creek Creamery Co. and Cherry Burrell Corp.) U. S. Patent 2,466,895. 18 claims. April 12, 1949. Official Gaz. U. S. Pat. Office, **621,** 2: 494. 1949.

Essentially the same as abs. no. 533 covering patent 2,466,894 with details given for controlling the crystallization of the blended milk fat, water and salt to yield a butter of good texture by means of a freezer-type cooler and a tubular texture developer equipped with restricted orifices. R. Whitaker

535. Dairy Process. H. C. Horneman, R. V. Hussong, S. N. Quam and B. W. Hammer. (Assigned to Sugar Creek Creamery Co. and Cherry Burrell Corp.) U. S. Patent 2,466,896. 12 claims. Apr. 12, 1949. Official Gaz. U. S. Pat. Office, **621,** 2: 494. 1949.

A dairy product, having many of the characteristics of butter, is produced by the same sequence of steps as described in abstract no. 533 covering U. S. patent 2,466,894. R. Whitaker

536. Practical ammonia refrigeration for ice cream and milk plants. Clyde H. Minster, Greenbrier Dairy Products Co., Beckley, W. Va. Ice Cream Rev., **32,** 9: 44, 94, 96, 98, 100, 102, 104, 106. Apr., 1949.

In determining the refrigeration load, the author suggests the use of a flow sheet depicting each operation involved in processing of the various products of the plant. The diagrammatic flow sheet presented with the article should serve as a useful guide in the preparation of such a chart.

The refrigeration load in the typical milk plant is mostly the cooling of products and product containers. Sweet water cooling with an ice bank hold-over system is suggested as an economical method for cooling raw milk since this system permits storage of refrigeration in the form of ice which in turn will reduce the size of compressor required. Further economies can be effected in the refrigeration load if the raw milk which is to be separated can be by-passed around the raw milk cooler directly to the preheater. The full use of regeneration whenever possible in cooling milk following pasteurization will conserve both heat and refrigeration. Milk or milk products placed in warm containers frequently will show an appreciable temperature rise which is detrimental to its quality. The cooling of the containers constitutes a portion of the refrigeration load which frequently has been overlooked. To date no satisfactory method has been devised for adequate precooling of milk bottles prior to filling. The temperature of storage rooms for bottled milk should not be permitted to rise above 33° F. In addition to temperature control, good air circulation is essential in the storage room.

The refrigeration load for the ice cream plant involves: (a) cooling and storage of mix, (b) partial freezing in the freezer, (c) hardening and storage of the ice cream and (d) freezing of novelties with low temperature brine. Direct expansion ammonia systems are best adapted for the freezing and hardening of ice cream. The freezing of novelties is carried out with a low temperature brine which in turn is cooled to the desired temperature with ammonia coils. Mix cooling can be carried out with either sweet water, brine or direct expansion. W. J. Caulfield

537. Ammonia equipment in ice cream plants. V. C. Patterson. Can. Dairy Ice Cream J., **28,** 4: 72–76. Apr., 1949.

See abs. 454, p. A94.

Also see abs. no. 554, 555, 573, 574, 575.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

538. De bepaling van het vetgehalte en de involed van systematische fouten op de verliescijfers der vetbalans. (The estimation of the fat content and the influence of systematic errors on the figures of loss in the balance-sheet for fat.) English summary. A. F. TAMSMA, H. J. J. JANSSEN, and S. A. H. PASSENIER. Netherlands Milk Dairy J., **1,** 2: 78–97. Apr., 1947.

In checking the amount of butterfat lost in dairy plant operations, the reliability of the figures has been found to depend to some extent upon the methods used for routine butterfat determinations. Analytical errors accounted for about 2% fat loss when the Gerber test was used as the basic method. Either a test must be found which is sufficiently accurate and will give a fat loss of 0% in the ideal case or a correction factor should be used. The Weibull method is suggested as being very satisfactory. If the Gerber test is used, the correction factor would have to amount to 2%, i.e., the Gerber fat content must be multiplied by 0.98. W. M. Roberts

539. Modern trends in self-service and package merchandising of ice cream. H. H. ROBBINS, Paraffined Carton Research Council. Ice Cream Trade J., **45,** 4: 54, 55, 116–118. Apr., 1949.

Merchandising fundamentals that will make for increased sales include quality of product, proper packaging, display and merchandising and advertising. The tremendous growth of self-service type of merchandising in food stores has resulted in the necessity for placing more emphasis on packages and impressing brand names on the consumer's mind.

From 1938 to 1947 groceries handling ice cream have increased from 22 to 68%. New household refrigerators with low-temperature storage compartments provide ample space for storage of packaged ice cream. Low-temperature storage boxes for home and farm use make ice cream available to many people who have had it only occasionally. New automatic packaging machinery has reduced packaging costs. With facilities for storage of ice cream in the home, consumers are purchasing in larger amounts and new outlets make ice cream more readily available. Dealers are becoming more interested in selling packaged ice cream because it has eliminated dipping losses. Improved quality and the introduction of the higher quality, low-overrun ice cream has increased home consumption. Open display cabinets and mobile cabinets have increased sales of packaged ice cream. Aggressive merchandising by drug stores is increasing the sale of ice cream not only at the fountain but also for carry-home use. W. H. Martin

540. Carnation hits hard for more sales. ANONYMOUS. Ice Cream Field, **53,** 3: 46–48, 50–51. Mar., 1949.

This is a condensation of the Feb. issue of *The Mixer,* a monthly publication of the Carnation Co., Los Angeles, Calif. Charts and statistics are given to stress the advantages of ice cream in drug stores and markets. The Rexall survey (1929) is quoted as saying that "For every $1.00 the fountain brings in, the store volume in other departments increases $0.93." The advantages of rapid turn-over are emphasized by charts. Drug stores are urged to consider shorter gross profit on packaged ice cream in order to increase sales volume. It is suggested that a "take home" cabinet, separate from the fountain, serves as an additional display; furthermore, this method of selling ice cream is less costly than selling at the fountain. The Hotel Gazette (1943) is quoted as finding that America's favorite dish is ice cream. W. C. Cole

541. How to increase per capita sales. M. L. FINNEBURCH, Liquid Carbonic Corp., Chicago, Ill. Ice Cream Field, **53,** 3: 44, 60, 62, 64, 65. Mar., 1949.

To emphasize the importance of retail outlets for ice cream, the following statistics are given: Forty million Americans eat away from home every day. In drug stores 43% of the multiple sales start at the soda fountain, 25% of the average store volume comes from the soda fountain, 31% of the store gross profits come from the soda fountain and, on the average, the soda fountain shows a net profit of 14%. The author emphasizes the increased profits resulting from increased sales. He classes the soda fountain as a "food and refreshment factory", claiming it is the "Personality" department and, because of this, has a distinct advantage from the sales point of view. W. C. Cole

542. "One-stop-saver" plan. V. M. RABUFFO. Ice Cream Trade J., **45,** 4: 44–46, 108–110. Apr., 1949.

Stores using the plan must phone orders to the company during the day prior to delivery; the minimum order is for 5 gal. of ice cream or 12 dozen novelties at one delivery and the store personnel must take the ice cream from the truck at the curb and place it in the cabinet in the store. The price is based on the quantity delivered, ranging from $1.46 per gal. for 5 to 9 gal., to $1.37 per gal. for 50 gal. or more at one delivery. W. H. Martin

543. Prices drop. Ice Cream Trade J., **45,** 4: 40–42, 97–104. Apr., 1949.

Wholesale prices of ice cream have been reduced from 8 to 25 cents per gal. in most of the major markets throughout the United states during the past 2 mo. These reductions have been possible as the result of lower prices for milk and milk products used in the manufacture of ice cream. It is the hope of the industry leaders that these price reductions will be passed on from the dealers to the consumers and that increased sales will result. W. H. Martin

544. A double incentive plan. T. KNIGHT. Milk Plant Monthly, **38,** 4: 50–51. Apr., 1949.

Providing a routeman's outstanding bills are lower than in the preceding month, he receives $1.50 for each new quart of business secured. This bonus is reduced to $1.00 if his outstanding bills exceed those of the preceding month. Accurate recording and prompt posting of this collection data by the sales manager make this plan an effective method of promoting sales and speeding up collections. J. A. Meiser, Jr.

545. Cost problems of the dry milk industry. D. BUTZ and E. F. KOLLER, Univ. of Minnesota, St. Paul. Natl. Butter Cheese J., **40,** 5: 36–37, 60. May, 1949.

The costs of manufacturing 1 lb. of milk powder by the spray and roller methods in 1947 were 4.4 and 3.7 cents, respectively. Labor, packaging supplies and fuel compose 0.75 of the manufacturing cost. Manufacturing costs were 1 cent/lb. lower in spray-drying plants producing over 6 million lb. of powder annually than in plants producing less than this amount. Indications for 1948 show that labor and fuel costs increased. The estimated average manufacturing cost in 1948 will be 0.5 cent/lb. higher than in 1947.

Seasonality of products is a big factor in powder manufacturing costs. Labor and equipment are designed to handle milk flow during peak periods, resulting in less efficient use of labor and equipment at other times of the year. Diversity of plant operations may be an answer to this problem. The introduction of more labor-saving equipment and methods would cut manufacturing costs. More effective utilization of fuel should be studied. H. E. Calbert

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

546. Effect of vitamin A supplements upon the state of vitamin A in blood serum of the dairy cow and in blood serum and liver of its neonatal calf. D. B. PARRISH, G. H. WISE, and J. S. HUGHES, Kansas Agr. Expt. Sta., Manhattan. J. Biol. Chem., **172,** 2: 355–365. Feb., 1948.

Sixteen pregnant cows (5 Holstein, 3 Ayrshire, 4 Guernsey and 4 Jersey) were divided into two groups about 4 wk. before parturition, one group being given supplements of vitamin A in the form of the alcohol and the other vitamin A in the form of the ester. Before this supplementation their blood serum averaged 34 γ total vitamin A/100 ml., of which 10% was in the form of the ester. Following daily oral doses of 500,000 I. U., and later 1 million, of both the alcohol and the ester, the total vitamin A level of each group increased to an average of 45 γ/100 ml. of serum, of which 24% was in the form of the ester. At parturition the supplementations were discontinued, resulting in a decrease to 28 γ/100 ml., of which 9% was in the ester form. There was no significant difference between the groups receiving the two different forms of vitamin A.

Fifteen newborn male calves (6 Ayrshire, 4 Holstein, 3 Guernsey and 2 Jersey), of which 6 were from the dams on the experiment above, comprised a group used to determine the level of vitamin A in their blood serum and livers. With one exception, they were 4 d. or less of age. The blood serum of 10 of the calves showed vitamin A values of from 5 to 22 γ/100 ml. Of the total vitamin A in their blood serum, 2 calves showed more than 60% of it to be in the form of the ester and the other 8 had a fourth or less of their serum vitamin A in the ester form. Fourteen of the group were sacrificed for vitamin A assays of their livers. Although the total vitamin A of their livers varied widely, depending upon the supplement of their dams, in all cases more than 70% was present in the ester form. A. O. Call

547. The lactation response as limited by feeds produced under two systems of soil fertilization. K. A. KENDALL, W. B. NEVENS and O. R. OVERMAN, Univ. of Illinois, Urbana. J. Nutrition, **36,** 5: 625–637. Nov., 1948.

The effect of fertilizer treatment of soils on the nutritive value of rations consisting of lespedeza hay and wheat grain and of soybean hay and wheat grain were appraised through reproduction and lactation responses of rabbits. All plots on which crops were grown for this study received 4 tons of raw rock phosphate, 2.25 tons of kainit and 1,000 lb. of muriate of potash over a period of 12 yr. In addition, one series of plots was treated with 12 tons of limestone over this same period.

The lespedeza and wheat ration from the soil fertilized with PK + Ca appeared to be slightly more palatable than the same ration from soil

which did not receive Ca. Rabbits receiving the rations from soil receiving only PK averaged 6.43 young per litter while those fed rations from soils receiving PK + Ca averaged 7.75 young. Average daily weights, body length and gastrointestinal contents, and deposition of dry matter, total nitrogen and ash were greater for young fed on milk from mothers which received rations from soils fertilized with PK + Ca than those fed on milk from mothers which received rations from soils treated only with PK. R. K. Waugh

548. The placental and mammary transfer of tocopherols (vitamin E) in sheep, goats and swine. F. Whiting and J. K. Loosli, Cornell Univ., Ithaca. J. Nutrition, **36,** 6: 721–726. Dec., 1948.

The effects of supplementing prepartum rations with tocopherols on the tocopherol content in livers and blood plasma of newborn, and on the amount of tocopherol in colostrum were studied. Half the animals received rations supplemented with 80 mg. of γ-tocopherol per 100 lb. of body weight daily until parturition. The other half served as controls. Samples from the newborn were taken before they had received colostrum and the colostrum was sampled before the young were suckled.

The supplementation of the mothers' prepartum ration slightly increased the tocopherol content of livers of new born. This increase was not statistically significant. The tocopherol content of blood plasma of lambs and kids was increased significantly by the prepartum supplementation, but no increase was observed with newborn pigs. Supplementation resulted in a two-fold increase in tocopherol content of colostrum in all species. Colostrum contained 3 to 4 times as much tocopherol as milk from the same species 4 d. later.
R. K. Waugh

549. The effect of mixed tocopherols on milk and butterfat production of the dairy cow. P. H. Phillips, J. Kastelic and E. B. Hart, Univ. of Wisconsin, Madison. J. Nutrition, **36,** 6: 695–701. Dec., 1948.

A farm herd of Holstein cattle was used to study the effect upon per cent butterfat, total fat and milk production of supplementing rations with 1 g. of mixed tocopherols per cow daily. H.I.R. monthly tests and milk records were used for the production data. In trial I 7 cows were used as controls and 8 other cows received daily 21.3 g. of a preparation which furnished 1 g. of mixed tocopherols. The tocopherols were added to the evening feed. In trial II 7 cows which had received tocopherols and 4 cows which had served as controls in trial I were fed a grain ration with which the tocopherols had been mixed at the time of grinding the grain. Neither method of feeding the mixed tocopherols had any effect on per cent butterfat, total butterfat or milk production.
R. K. Waugh

Also see abs. no. 530.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

550. The amino acid composition of bovine semen. B. C. Ray Sarkar, R. W. Luecke, and C. W. Duncan, Michigan State College, East Lansing. J. Biol. Chem., **171,** 2: 463–465. Dec., 1947.

A composite of 149 semen samples from 40 different bulls representing Holstein, Guernsey and Jersey breeds was separated into sperm and seminal plasma by centrifugation. Each fraction then was dried and analyzed microbiologically for eleven different amino acids. The results are presented in a table. The amino acid compositions of sperm and seminal plasma are quite similar, except that arginine and leucine are higher in sperm while tryptophan is higher in the plasma. Nitrogen values of 17.61 and 12.05%, respectively, for the sperm and seminal plasma on a moisture-, fat- and ash-free basis are reported. A. O. Call

Also see abs. no. 488, 552.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

551. Breed type and production records in Jersey cattle in New Zealand A. H. Ward and O. M. Castle, New Zealand Dairy Board. New Zealand J. Sci. Technol., **29A,** 4: 175–183. Dec., 1947.

Records on cows are classified according to New Zealand standards into: very highly commended, highly commended, commended and commended for superior progeny.
W. C. Frazier

552. Average length of gestation period in dairy cattle in New Zealand. A. H. Ward and O. M. Castle, New Zealand Dairy Board. New Zealand J. Sci. Technol., **29A,** 4: 171–173. Dec., 1947.

The length of the gestation period for 2,255 cows conceiving within a 6-mo. period was summarized. The average length of gestation period for single calves was 283 ± 11.3 d. Some 85 cows had a period of less than 267 d and 87 a period of greater than 299 d. The gestation period for single births is approximately 4 d. longer than for twin births. Significant differences between

average gestation periods in different sections of New Zealand were observed W. C. Frazier

553. Method of making milker nipples. C. A. THOMAS. (Assigned to Babson Bros. Co.) U. S. Patent 2,463,920. 7 claims. Mar. 8, 1949. Official Gaz. U. S. Pat. Office, **620,** 2: 582. 1949.

On the type of milker which has the milk-collecting vessel directly beneath the cow, the vacuum automatically is cut off when the teat cup drops off. This is accomplished by having the flexible hose to the teat cup slip over a rigid tube cut at an angle of about 22.5° with the flat side facing upward. When the teat cup hangs down, the wall of the flexible tube covers the flat end of the rigid tube, thus preventing loss of vacuum.
R. Whitaker

554. Automatic shut-off mechanism for milking machines. M. K. EADES. U. S. Patent 2,466,841. 8 claims. Apr. 12, 1949. Official Gaz. U. S. Pat. Office, **621,** 2: 479. 1949.

A float-operated valve automatically stops the milking machine from operating when the flow of milk ceases. R. Whitaker

555. Milking apparatus. A. C. WEIBY. (Assigned to Solar Corp.) U. S. Patent 2,467,512. 2 claims. Apr. 19, 1949. Official Gaz. U. S. Pat. Office, **621,** 3: 796. 1949.

To provide the pulsating vacuum required for a milking machine, a rotor periodically connects the pulsing line with the suction source and periodically connects the pulsing line with the atmosphere. R. Whitaker

556. Strip cup. C. A. THOMAS. (Assigned to Babson Bros. Co.) U. S. Patent 2,467,949. 3 claims. Apr. 19, 1949. Official Gaz. U. S. Pat. Office, **621,** 3: 908. 1949.

This strip cup has a cover in the shape of a shallow funnel. Milk from the udder is directed on the surface, where it spreads out to form a film and then drains down into the cup below through the center opening. R. Whitaker

Also see abs. no. 487.

ICE CREAM

C. D. DAHLE, SECTION EDITOR

557. Defects in ice cream. C. D. DAHLE, Pennsylvania State College. Ice Cream Trade J., **45,** 4: 48, 49, 110, 111. Apr., 1949.

The causes and remedy for common flavor defects are discussed. These include old material, unclean, cooked, neutralizer, salty, sour, oxidized and rancid flavors. Body defects discussed include sogginess, fluffy, gummy and crumbly. Texture defects include coarse, buttery, sandy and poor melt-down characteristics.

Shrinkage is discussed in detail, including the effects of continuous freezers, dry ice, paper containers, storage temperatures, overrun, types of sweeteners, stabilizers, emulsifying agents, drynes of ice cream, free-fatty acids, protein stability, composition of the mix, types of dairy products and seasons of the year. Shrinkage theoreticaliy is attributed to the escape of air from the air cells by diffusion or collapse of the air cell wall due to pressure from within or without the cell. Wall strength is important in retaining air within the cell. Shrinkage may be attributed to some of the fundamental factors which have a definite effect on the behavior of milk solids during certain seasons of the year. W. H. Martin

558. Significance and control of coliform in ice cream making. G. W. SHADWICK. Can. Dairy Ice Cream J., **28,** 3: 74–78. Mar., 1949.

See abs. 441, p. A92.

559. Production and pasteurization of ice cream mix by H.T.S.T. method. C. M. MINTHORN. Can. Dairy Ice Cream J., **28,** 2: 76–78, 90. Feb., 1949.

See abs. no. 466, p. A98

560. Controlling viscosity of chocolate ice cream mixes. C. D. DAHLE, W. R. DAVEY and W. D. SWOPE. Can. Dairy Ice Cream J., **28,** 4: 58–64. Apr., 1949.

See abs. 471, p. A99.

561. Ice cream cup. A. A. HEYMAN. (Assigned to Maryland Baking Co.) U. S. Patent 2,462,497. 2 claims. Feb. 22, 1949. Official Gaz. U. S. Pat. Office **619,** 4: 1074. 1949.

A flat-bottomed ice cream cone is described which has a series of protruding arms of pastry around the inner periphery and also upward from the bottom which impart the following features to the cone: the portion of ice cream is supported on top of the cone, suggesting overfilling and preventing accidental spilling, the cups may be nested without breaking the nesting rings and the ice cream may be nearly all consumed before the cup need be eaten. R. Whitaker

562. Prospectus for packages. W. D. KELLOGG, Container Corp. of America, Chicago, Ill. Ice Cream Field, **53,** 3: 75. Mar., 1949.

Packaged ice cream provides a means whereby the industry may attain its announced goal of a billion gal. of ice cream per yr. Education, up-

grading, reduction in distribution costs and better merchandising are steps listed as necessary if this goal is to be attained. Dispensing ice cream in packages is more sanitary and more economical and permits better merchandising through more attractive designs; it is necessary in self-service stores.

Making a factory-filled package comparable in quality to "hand-dipped" ice cream is essential if packaged ice cream is to be accepted universally. The need of proper carry-out insulated bags as well as adequate home refrigerator storage are stressed as a means of convincing consumers they should maintain a supply of ice cream in their homes. W. C. Cole.

Also see abs. no. 536, 537, 539, 540, 541, 542, 543.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

563. One-piece paper container. J. Norden. U. S. Patent 2,468,306. 4 claims. Apr. 26, 1949. Official Gaz. U. S. Pat. Office, **621,** 4: 1152. 1949.

A square-shaped paper bottle suitable for holding liquids like milk is constructed from one long strip of paper which is wound spiral-like with the edges overlapping and terminating at the mouth or open end. R. Whitaker

564. Milk can inverting fork truck. T. A. Gleason. U. S. Patent 2,468,326. 5 claims. Apr. 26, 1949. Official Gaz. U. S. Pat. Office, **621,** 4: 1157. 1949

This attachment for the front of a small truck consists of fork-shaped arms which are employed to lift cans of milk upward and then inverting same over a tank or other receptacle.

R. Whitaker

565. Fat-free milk. K. G. Weckel. Can. Dairy Ice Cream J., **28,** 3: 80, 86, 101. Mar., 1949.

See abs. 391, p. A81.

566. Problems in the operation of city milk plants. L. W. Hoyt. Can. Dairy Ice Cream J., **28,** 3: 57, 58, 100. Mar., 1949.

See abs. 235, p. A52.

Also see abs. no. 522, 523, 536, 544, 569, 571, 572.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

567. Reproduction and lactation studies with rats fed natural and purified rations. G. M. Maruyama and P. H. Phillips, Univ. of Wisconsin, Madison. J. Nutrition, **36,** 5: 613–623. Nov., 1948.

The adequacy of a ration consisting of corn, soybean oil meal, dehydrated alfalfa leaf meal, minerals and supplements of most of the known vitamins was tested for reproduction and lactation in rats in experiment I. The rats were fed the basal ration for 3 wk. prior to mating. The importance of standardizing these and other factors for reproduction and lactation studies is emphasized. Supplementing the diet with 0.5% L(+)-lysine and 0.3% DL-methionine improved reproduction and lactation, the percentage of rats born that were weaned increasing from 46 to 72%. When folic acid was omitted from the basal ration only 34% of the young born were weaned.

In experiment II a purified ration consisting of sucrose, casein, corn oil, minerals and vitamins was employed, in addition to the basal ration of natural materials used in experiment I. In this trial omitting the folic acid from the diets reduced the weaning percentage from 29 to 0 for the basal made of natural materials and from 59 to 10 for the purified.

Lysine, methionine and folic acid apparently are essential for good reproduction and lactation in the female rat. R. K. Waugh

Also see abs. no. 531, 547, 548.

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

568. Research on a growth-promoting factor in summer butter. A. Kentie. Netherlands Milk Dairy J., **1,** 2: 118–127. Apr., 1947.

The fatty acids of summer butter contain a growth-promoting factor for rats. The factor can be adsorbed on Fuller's earth but loses its growth-promoting properties upon complete hydrogenation. W. M. Roberts

569. De voedingswaarde van melk. (Nutritive vlaue of milk.) English summary. Chr. Engel. Netherlands Milk Dairy J., **1,** 1: 43–56. Jan., 1947.

Data have been collected from recent literature about the amino acids, fatty acids, vitamins and mineral components of cow's milk and human milk. W. M. Roberts

Also see abs. no. 521.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

570. Residual arsenic and strychnine in the tissues of drug-treated cattle. W. E. Ham, E. A.

KLINE and M. E. ENSMINGER, State College of Washington, Pullman. Am. J. Vet. Research, **10,** 35: 150–153. Apr., 1949.

Arsenic trioxide and nux vomica were fed in the grain ration to groups of cattle for 120 d. in 1946 and 201 d. in 1947. Average daily intakes were 0.357 g. As_2O_3 and 5.69 g. nux vomica. Biopsy and slaughter tissues analyzed at the end of the trial showed levels of arsenic approaching or exceeding the maximum allowed by Pure Food and Drug regulations. Tissues analyzed after depletion periods of 20 and 41 d. contained about the same amount of arsenic as found in untreated control animals. Strychnine was not found in the tissues or organs at any of the sampling periods. Effects of the drugs upon blood glucose, ascorbic acid, carotene, vitamin A, calcium, phosphate and cell count were not significant.

E. W. Swanson

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

571. The fieldman's role in producing quality milk. H. A. BOLAND, Galliker Dairy Co., Johnstown, Pa. Milk Plant Monthly, **38,** 4: 42–45. Apr., 1949.

Analysis of laboratory reports and personal contact with producers and fieldmen shows the major causes of bacteriological problems in relations to the dairy farm to be: (a) improper care of mechanical coolers, (b) improper care of milking machines, (c) confusion as to the proper use of washing compounds and chemical sterilizers, (d) failure on the part of fieldmen to locate the cause of trouble and (e) fieldmen improperly equipped to do the job.

Fieldmen must locate trouble spots quickly and then insure that the producer possesses the necessary tools and is using them properly and effectively in erradicating this defect. Also, the fieldmen should possess tools which allow him to perform his duties with a minimum of guesswork. These tools properly used and built into a compact kit will take much out of the guesswork in maintaining clean milk production.

J. A. Meiser, Jr.

572. Cleaner dairy equipment. M. C. JAMIESON and W. G. MCLEOD. Can. Dairy Ice Cream J., **28,** 4: 27–31. Apr., 1949.

Results of a new type of sanitary program conducted among milk producers in Manitoba are discussed. The "Seeing is Believing" test (the Jamieson Kit) is applicable for producers as well as manufacturers. It provides a measure of sanitation on the farm and in the plant. The program of education conducted in this study has proven that producer education in sanitation is needed and appreciated and that improvement is possible. H. Pyenson

573. Can washers. H. P. FAUST. Can. Dairy Ice Cream J., **28,** 4: 78–80. Apr., 1949.

A rotary can washer usually answers the cleaning problem where a rate of four cans a minute is adequate and the total number of cans is not large. The increase in labor cost has made the straightway can washers more common. The rate of washing cans should be integrated with the weighing, cooling and storage of milk. Other questions to be considered are: (a) adequacy of water supply, (b) hardness of the water, (c) boiler capacity for peak loads, (d) adequate size of water and steam mains to prevent pressure drops when other equipment is operated and (e) proper electrical lines and voltage maintenance. The prewash rinse should be provided with means of raising the water temperature, particularly in winter; in the washing positions a large volume of water at relative low pressures is necessary; only a small rinse pump should be necessary; a small volume of water at a relatively high temperature is desirable in the sterile rinse position; and a limited volume of steam is needed in the steam sterilization position. H. Pyenson

574. Can-washing operation and maintenance. C. A. ABELE. Can Dairy Ice Cream J., **28,** 4: 82–84, 90. Apr., 1949.

Provision of a mechanically efficient can-washer, charged with an effective washing compound, does not assume the delivery of completely cleaned milk cans at the discharge end of the washer. The third factor essential to satisfactory can-washing results is intelligent operation. The most effective method of determining the effectiveness of the nozzle jets is to run a cut-out can through the washer, with the side panels removed, before the start or after the clean-up following each day's run. The maintenance of wash solution concentration is very important.

H. Pyenson

575. How polyphosphates improve can washing. A. H. RAZEE. Can. Dairy Ice Cream J., **28,** 4: 86–90. Apr., 1949.

Polyphosphates soften the hardest water, thereby preventing the precipitation of detergent materials. They also eliminate the formation of film and scale on the equipment and on the cans being washed. In alkaline solution, the polyphosphates are specific solvents for denatured proteins, such as are found in milkstone. These polyphosphates can be used to treat rinse water so that even upon dilution, the residual alkali

will not precipitate. In the field of dairy sanitation, the polyphosphates have been acclaimed as one of the most significant developments in detergency. H. Pyenson

576. Chemical sterilizers in the dairy industry. C. K. JOHNS. Can. Dairy Ice Cream J., **28,** 3: 29–31. Mar., 1949.

The following suggestions are given in regard to the use of chemical sterilizers: (a) have equipment surfaces adequately cleaned, (b) prepare sterilizing solution according to directions, preferably in hot water, (c) hypochlorite is best applied immediately before using the equipment (quaternaries may be used following wash-up), (d) hypochlorite solutions above 5% strength are unstable and lose available chlorine and (e) crystalline hypochlorite should be kept tightly covered, as exposure to the air causes a decrease in strength. H. Pyenson