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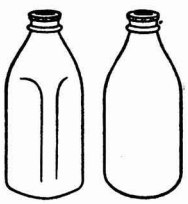

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INFLUENCE OF CRUDE FIBER IN THE RATION ON EFFICIENCY OF FEED UTILIZATION BY DAIRY COWS[1]

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INTRODUCTION

Roughages commonly fed to dairy cows in Hawaii tend to be high in fiber. One of these roughages is "strip cane," and another is "cane tops," both of which are obtained as waste or by-products from sugar plantations. Near Honolulu other roughages are grown as soiling crops (Napier grass, *Pennisetum purpureum*) or obtained in wild state from gulches and other waste areas (koa haole, *Leucaena glauca*). These and other roughages are unusually high in crude fiber. For example, strip cane contains 44 per cent crude fiber, sugar cane tops, 37 per cent, Napier grass, 41 per cent and koa haole, 37 per cent crude fiber when calculated on the dry matter basis. Therefore, in this area, it is very important that an investigation be made as to the correct balance of crude fiber to total digestible nutrients in the rations fed to cattle.

REVIEW OF LITERATURE

Many investigators have worked on various phases of nutrition closely related to the problem mentioned above. Henke (6) reported that the 4 per cent F.C. milk production was 25.4 lb. per cow daily when the average concentrate consumption was 19.8 lb. as compared to 24.1 lb. when concentrate consumption averaged 15.4 lb. Napier grass was fed as the roughage. In another experiment the same author (5) studied pineapple tops as a substitute for Napier grass when the concentrate rations were kept equal. The 4 per cent F.C. milk yield averaged 5.5 per cent higher when pineapple tops were fed. The amount of crude fiber calculated on the percentage of dry matter in the feed was 12.4 per cent as compared to 21.1 per cent when Napier grass was fed. However, the content of digestible protein was slightly higher in the pineapple tops group. At the Massachusetts Station high concentrate feeding also was compared with rather low allowance of concentrates and a maximum amount of roughage (8). On a high concentrate ration the milk yield was 31.7 lb. daily, as compared with 27.7 lb. for the ration containing a smaller amount of concentrates. This experiment, as well as those carried out at the Hawaii Agricultural Experiment Station, was concerned primarily with the economic phase of this type of feeding.

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[1] Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Technical Paper no. 193.

As to the physiological optimum of crude fiber in the rations, relatively little work has been done. Working with fattening lambs, Cox (4) found that when using the proportions 35 : 65, 45 : 55 and 55 : 45 of concentrates to roughage, the best gain in weights always was obtained for the 45 : 55 ratio. The concentrates in this, as in most of the other experiments, consisted of corn; in some of the experiments cottonseed meal also was used. The roughage consisted of alfalfa meal or alfalfa hay and Atlas silage.

Other workers have obtained similar results for growing pigs. In one series of experiments, increasing amounts of oats were used with corn and in another series, increasing amounts of ground wheat straw were used with corn (10). The intake of metabolizable energy and necessary nutrients in the different groups were kept equal; the only difference between groups was the increasing amount of crude fiber or roughage. In the oats-corn series the best gain in the pigs was obtained in the group receiving 75 per cent oats and 25 per cent corn. In the wheat straw meal-corn series, the best result was obtained in the group receiving 7 per cent wheat straw meal and 93 per cent corn.

A number of other experiments have been carried out from time to time dealing with different phases of nutrition closely related to this problem, and Cox has given a review of them (4). He finds, however, that work pointed specifically to determination of the significance of this factor (by Cox named the "physical balance") has been sketchy and has lacked continuity. Therefore, it appears important that investigations of this type be carried out with milking cows and possibly also with beef cattle.

PLAN OF EXPERIMENT

Four lots of three cows each from the station dairy herd were used for the experiment. The cows were of the Holstein-Friesian breed with an age averaging close to 7 yr. The number of lactation days averaged 127 and the daily milk yield per cow, 32.6 lb., with a fat percentage of 3.48. Four per cent F.C. milk averaged 30 lb. daily per cow. The weight of the cows averaged 1,182 lb. at the start of the experiment. From the averages given above, it is evident that variations occurred among individual cows. Therefore, it was necessary to design the experiment in such a way that errors in final results due to variation among cows could be excluded as far as possible.

The four lots of cows were used during a 16-wk. change-over design for four rations containing decreasing amounts of crude fiber. The lots were divided at random after the age, weight, milk production and days since calving were considered. The four rations were designated by the letters A, B, C and D according to the following Latin square scheme, as suggested by Cochran *et al.* (3) :

Period	Cows				Cows				Cows			
	1	2	3	4	5	6	7	8	9	10	11	12
I	A	B	C	D	A	B	C	D	A	B	C	D
II	B	A	D	C	D	C	B	A	C	D	A	B
III	C	D	A	B	B	A	D	C	D	C	B	A
IV	D	C	B	A	C	D	A	B	B	A	D	C

This design means that for each period each one of the four rations is tested on three cows. By the end of the experiment the four rations are tested on all 12 cows.

The composition of the concentrate rations used is given in table 1.

TABLE 1

Concentrate Mixtures

Mixture no.	Cane molasses	Pineapple bran	Soybean oil meal	Meat meal	Fish meal	Salt	Bone meal	Sum
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
15a	250	430	200	50	50	10	10	1000
15b	250	500	130	50	50	10	10	1000
15c	250	540	90	50	50	10	10	1000
15d	250	575	55	50	50	10	10	1000

The chemical composition of these mixtures and also of other feeds used in the experiment is given in table 2.

TABLE 2

Percentage chemical composition of feed and concentrate mixtures

	Moisture	Protein	Ether extract	Crude fiber	Ash	N-free matter	Calculated	
							D.P.	T.D.N.
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Napier grass	75.57	1.28	0.47	9.02	2.98	10.68	0.77	14.61
Molasses	20.30	2.27	.....	.....	11.16	66.26	0.73	59.67
Conc. mixture 15a	12.70	16.08	2.60	8.83	8.69	51.10	11.95	64.93
" " 15b	12.52	12.72	2.53	9.75	8.38	54.10	9.02	65.97
" " 15c	12.71	11.16	2.57	10.34	9.03	54.19	7.29	65.46
" " 15d	13.37	10.46	2.67	10.46	8.61	54.43	6.84	64.34
Soybean oil meal	10.25	43.02	4.16	5.65	5.39	31.53	36.56	79.06

The roughage used consisted of mature Napier grass produced on the station's dairy farm. Before feeding, the grass was always chopped into pieces of 1 to 2 in. in length. The amounts of roughage used in rations A, B, C and D were 60, 40, 20 and 0 lb., respectively.

The calculated composition of the complete rations is presented in table 3. In this example, the average milk production of the cows was 28 lb. daily of 4 per cent F.C. milk.

In planning the rations, the main concern was to obtain, as far as possible, equal amounts of digestible protein and total digestible nutrients in each of the ration for the same level of milk production. Sufficient amounts of carotene and minerals were provided in different rations. The only variable factor was the crude fiber content. The four different concentrate mixtures were composed, one for each ration, to simplify the feeding work. At lower or higher levels of production, some of the mixtures needed supplements of a slight amount of soybean oil meal to raise the protein content to a suitable level. Table 3 shows that nutrients provided in different rations parallel each other as closely as possible. The crude fiber in the rations is slightly lower than the 24, 22, 18 and 14 per cent originally planned for rations A, B, C and D, respectively.

TABLE 3

Composition of rations

Feed	Amount	Dry matter	D.P.	T.D.N.	Nutritive ratio	Ether extract	Crude fiber	Crude fiber of D.M.
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)		(*lb.*)	(*lb.*)	(%)
Ration A								
Napier grass	60.0	14.658	0.462	8.765		0.279	5.412	
Conc. #15a	13.7	11.960	1.637	8.895		0.356	1.210	
Molasses	3.0	2.390	0.022	1.790		.....	.....	
Soybean oil meal	0.3	0.269	0.110	0.237		0.012	0.017	
Sum		29.28	2.231	19.69	1: 9	0.647	6.64	22.7
Requirement[a]			2.134	18.36				
Ration B								
Napier grass	40.0	9.772	0.308	5.844		0.188	3.608	
Conc. #15b	18.8	16.446	1.696	12.402		0.475	1.832	
Molasses	2.0	1.594	0.015	1.193		.....	.....	
Sum		27.81	2.019	19.44	1: 10	0.663	5.44	19.6
Requirement			2.134	18.36				
Ration C								
Napier grass	20.0	4.886	0.154	2.922		0.094	1.804	
Conc. #15c	23.6	20.600	1.720	15.448		0.606	2.441	
Molasses	1.0	0.797	0.007	0.597		.....	.....	
Soybean oil meal	0.3	0.269	0.110	0.237		0.012	0.017	
Sum		26.55	1.991	19.20	1: 10	0.712	4.27	16.1
Requirement			2.134	18.36				
Ration D								
Conc. #15d	28.6	24.776	1.955	18.401		0.763	2.990	
Soybean oil meal	0.3	0.269	0.110	0.237		0.012	0.017	
Sum		25.05	2.065	18.64	1: 9	0.775	3.01	12.0
Requirement			2.134	18.36				

[a] Requirement calculated for cows weighing 1,200 lb.

All concentrate was fed individually. For practical purposes, the roughage was weighed out daily to each lot of three cows. Normally all feed was eaten. In cases where leftovers occurred, these were weighed back and the net consumption of feed recorded.

All ingredients in the concentrate mixtures were sampled and analyzed for dry matter, ash, protein, fat, crude fiber and *N*-free extract. For control, samples also were taken of the concentrate mixtures and analyzed as above for each separate mixture. A sample of the Napier grass used was taken each day and analyzed for dry matter content. The daily samples were composited and chemical analysis as above carried out for each period. Results of the analysis are given in table 2.

All milk produced by the cows was weighed daily. The fat test of the milk was taken during Monday afternoon, Tuesday morning and afternoon and Wednesday morning each week. A Babcock tester was used.

After the milk and fat test for each week was finished, the amount of concentrate to be fed during the week to follow was calculated. The feed intake

was equalized for different groups according to milk production. Morrison's feeding standards for good cows under usual conditions were followed.

Weight of animals was taken before and at the end of each period during three successive daily weighings. As it appeared worthwhile to follow the general well being and physical condition of the animals fed the different rations as closely as possible, pulse, temperature and respiration rate measurements were taken weekly. These readings always were started at 3:30 p.m. before milking of the cows. For comparison of night and day records, data also were assembled in the mornings, readings starting at 4:00 a.m. The morning readings were continued for 5 wk. Temperature was obtained with a so-called veterinary thermometer inserted into the rectum approximately 3 in. and left for 3 min. Respiration rate was determined by number of flank movements per minute. In determining pulse rate, the tips of the fingers were placed on the under side of the tail where the movements per minute of the coccygeal artery were counted.

RESULTS

Generally, the course of the experiment proceeded according to plan. During some of the experimental days, particularly during the last week of June, rather high climatic temperatures apparently influenced the well being and milk production of the cows. This incident did not interfere with the final results of the experiment.

For one cow (no. 175), abnormally low milk production was obtained during the last period of the experiment, particularly during the last week. From 17 lb. of milk daily in the beginning of this period, the yield decreased to 8 lb. and low production then continued until the dry state was reached. Therefore, normal data for this cow during the period mentioned are missing. By use of Snedecor's (13) formula, the missing value was calculated. The original incorrect value is given within parenthesis in table 4 and the calculated value is marked with an asterisk.

During the whole of the experiment, consumption of the different rations used was good. Only for the ration A (including 60 lb. Napier grass daily) a few weigh-backs occurred; of 15,020 lb. of this roughage fed, less than 0.5 per cent of the total was not eaten. In the groups fed 40 and 20 lb. roughage daily, all roughage was consumed. Slight leftovers occasionally occurred during the first week of each period, after the switchover from one ration to another had taken place. All concentrate was eaten, except for ration D, where 7 lb. were left by one cow and 4.5 lb. by another one during the first part of period I.

In order to minimize carry-over effects from one period to a following, the data for the first week in each period are omitted in the following calculations. By this omission the source of error introduced by the leftovers mentioned above also is excluded.

The results in milk yield for the different rations are collected in table 4.

After analysis of variance of the data for milk yield presented in table 4, the analytical results were assembled in table 5. By examination of the mean squares, it is found that results obtained for points 1 to 4 listed in the table are

TABLE 4

4% F. C. milk yield, in pounds, obtained for different rations
(One seventh of the milk production for each period is given in the table)[a]

Period	Group 1 Cows					Group 2 Cows					Group 3 Cows				
	226	289	292	172	Total	277	175	219	176	Total	192	287	259	224	Total
I	A 96	B 97	C 85	D 91	369	A 57	B 88	C 85	D 80	310	A 58	B 67	C 74	D 58	257
II	B 86	A 83	D 83	C 86	338	D 62	C 82	B 65	A 65	274	C 62	D 65	A 62	B 50	239
III	C 82	D 85	A 64	B 65	296	B 51	A 58	D 67	C 59	235	D 60	C 56	B 63	A 45	324
IV	D 75	C 80	B 62	A 55	272	C 43	D 65[b] (36)	A 57	B 39	204	B 50	A 44	D 64	C 50	208
Totals	339	345	294	297	1,275	213	293	274	243	1,023	230	232	263	203	928

Treatment totals:	*Sum*	*Daily av.*	*Sum*	*Daily av.*	*Sum*	*Daily av.*
	A = 298	24.83	A = 237	19.75	A = 209	17.42
	B = 310	25.83	B = 243	20.25	B = 230	19.17
	C = 333	27.75	C = 269	22.42	C = 242	20.17
	D = 334	27.83	D = 274	22.83	D = 247	20.58

[a] The reason that only one seventh of the milk production for each period is given, and not the total sum, is due to the fact that giving the same final result this method decreases the labor needed in successive calculations.

[b] This figure was calculated according to the formula for missing data in a Latin square, as reported by Snedecor (13).

highly significant. The mean square for between rations is 228.25 and the mean square for errors is 21.10. The F-value, therefore, is 228.25/21.10 = 10.82. According to Snedecor's tables (13) for the distribution of F, an F-value of only 5.18 is needed in this case for significance at the 1 per cent point.

It should be mentioned that a test for significance also was carried out with use of the original milk yield value for cow 175 in period 4 without making use of the above-mentioned corrected value. In this case, an F-value of 4.52 was obtained, which still is sufficient for significance close to the 1 per cent point.

TABLE 5

Analysis of variance of 4% fat corrected milk units (lb.)

	Degrees of freedom	Sum of squares	Mean squares
1. Between groups	2	4019.54	2009.77
2. Between cows within groups	9	1925.38	213.93
3. Between periods within groups	9	3324.38	369.38
4. Between rations	3	684.75	228.25
5. Ration × group interactions	6	21.13	3.52
6. Error	17[a]	358.74	21.10
7. Total	46[a]	10333.92	

[a] One degree of freedom subtracted for cow 175.

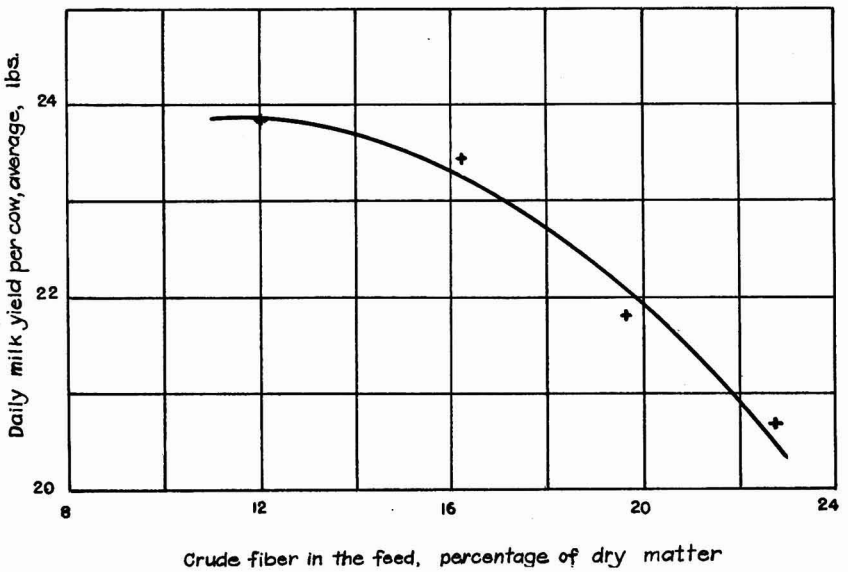
It is clear, therefore, that the amount of crude fiber in the rations tested had a definite influence upon the milk yield. This is graphically illustrated in figure 1, where the average daily milk yields in pounds obtained for the different rations tested are plotted against the crude fiber content of the feed. The curve in the graph is fitted by free hand. It is seen that the yield of milk definitely decreased as soon as the crude fiber content in the feed increased over 16 per cent, calculated on the dry matter basis.

Effect of different rations on weights of cows during experiment. The average body weights for the cows on rations A, B, C and D were, at the start of the first period, 1,177, 1,154, 1,141 and 1,255 lb., respectively; at the start of the second period, 1,182, 1,157, 1,126 and 1,148 lb.; at the start of the third period, 1,196, 1,145, 1,266, 1,006 lb.; at the start of the fourth period, 1,121, 1,237, 1,211, 1,085 lb.; and at the end of the fourth period, 1,234, 1,169, 1,061 and 1,214 lb. The average weight of all cows at the beginning of the experiment was 1,182 lb.

At the end of the experiment the average weight was 1,170 lb. Each figure was based upon weighings taken during 3 successive days. Therefore, it appears that a loss in weight of 12 lb. per cow occurred during the 16 wk. of experiment. This loss is very truly a result of the more concentrated feed for part of the cows and could hardly be ascribed to a real loss in body weight. An example will illustrate this. Cow no. 172 had an initial weight of 1,401 lb. when she started on ration D. After 4 wk. on this concentrated ration her weight was 1,266 lb. From this ration she was turned over in period II to the slightly less concentrated ration C. At the end of this period her weight had increased to 1,291 lb. From this ration she was fed the still less concentrated ration B in period III, and an increase in weight to 1,347 lb. resulted. In the last period she was fed

ration A and this increased her weight to 1,411 lb. at the end of the experiment.

Since these differences in body weight occurring over a short time could not be due to real gain in body tissues, they must be ascribed to different weights of the digestive channel content. According to Sisson and Grossman (12), the stomachs of large cattle have a capacity of 40 to 60 gal. and those of medium size, 30 to 40 gal. This would correspond to about 40 gal. or 150 l. for the cows used in this experiment and illustrates the large feed capacity of the cows.



An examination of the rations used in the experiment listed in table 1 reveals that for a cow milking 28 lb. daily, ration A for 1 day weighed 77 lb.; ration B, 61 lb.; ration C, 45 lb. and ration D, 29 lb. Although these rations contain the same amount of T.D.N. and D.P. and although the difference in dry matter content is not more than 4.23 lb. between rations *A* and *D*, it is obvious that the coarser nature of rations *A* and *B* must affect the weight of the animals, as borne out in the example for cow no. 172. This study illustrates the danger in emphasizing changes in weight occurring in experiments of this type.

Effect of different rations upon pulse rate. In order to study whether the type of feeding had any influence upon the pulse rate of the cows, the afternoon readings were collected in table 6. After analysis of variance of the pulse rates presented in table 6, the analytical results were assembled in table 7. The mean square for between rations is 136.81 and for the error, 30.07. The F-value for between rations, therefore, is 136.81/30.07 = 4.55. In Snedecor's tables for the distribution of F with $n^1 = 3$ and $n^2 = 18$, the value of F for the 5 per cent point is 3.16 and for the 1 per cent point, 5.09. As is seen, the significance comes closer

TABLE 6

Pulse rate of cows at afternoon readings
(Average for last 3 wk. of each period)

Period	Group 1 Cows					Group 2 Cows					Group 3 Cows				
	226	289	292	172	Total	277	175	219	176	Total	192	287	259	274	Total
						Pulse-rate/min.[a]									
I	A 64	B 76	C 69	D 61	270	A 68	B 72	C 64	D 67	271	A 69	B 76	C 68	D 55	268
II	B 65	A 69	D 57	C 74	265	D 78	C 68	B 62	A 64	272	C 70	D 63	A 59	B 75	267
III	C 72	D 60	A 76	B 73	281	B 72	A 80	D 59	C 68	279	D 65	C 67	B 69	A 72	273
IV	D 59	C 61	B 53	A 60	233	C 77	D 71	A 53	B 72	273	B 71	A 64	D 52	C 71	258
Totals	260	266	255	268	1,049	295	291	238	271	1,095	275	270	248	273	1,066

Treatment totals:

Sum	*Daily av.*	*Sum*	*Daily av.*	*Sum*	*Daily av.*
A = 269	67.3	A = 265	66.3	A = 264	66.0
B = 267	66.8	B = 278	69.5	B = 291	72.8
C = 276	69.0	C = 277	69.3	C = 276	69.0
D = 237	59.3	D = 275	68.8	D = 235	58.8

[a] The figures mean average pulse rate for the last 3 wk. of each period.

TABLE 7

Analysis of variance of pulse rate per minute for experimental cows

	Degrees of freedom	Sum of squares	Mean squares
1. Between groups	2	67.63	33.82
2. Between cows within groups	9	652.12	72.46
3. Between periods within groups	9	357.62	39.74
4. Between rations	3	410.42	136.81
5. Ration × group interactions	6	262.20	43.70
6. Error	18	541.26	30.07
7. Total	47	2291.25	

to the 1 than to the 5 per cent point. This means that the rations used in this experiment did influence the pulse rate of the cows.

We are not aware from earlier reports that increase in roughage fed to milk cows would affect the pulse rate, although Thomas (14) recently has reported that feeding 120 to 130 per cent of required T.D.N. to producing cows increases the heart rate. Exercise or carrying a burden increases the heart rate and it appears that the increase in roughage fed to cows is a parallel to that phenomenon.

Respiration rate. The data obtained for respiration rate were treated in the same way as were the data for pulse rate. No significant influences of rations upon respiration rate occurred. The *P*-value obtained for this influence fell between 0.2 and 0.05.

The afternoon respiration rate varied from 24 to 68 per minute, with an average of 37.8 ± 3.32.

Body temperature. Similarly, body temperature reflected no significant influence of types of rations. Variations in afternoon body temperature were from 100.0 to 102.8° F. Average body temperature was 101.3 ± 0.182° F.

Morning readings versus afternoon readings for pulse rate, respiration rate and body temperature. In table 8 the averages of pulse rate, respiration rate and

TABLE 8

Averages of pulse rates, respiration rates and body temperatures

	No. of observations	Mean	Standard error	Lowest value observed	Highest value observed
Morning readings					
Pulse rate/min.	60	72.2	±1.446	52	104
Respiration rate/min.	60	32.4	±1.155	22	66
Body temperature (° F.)	60	101.5	±0.065	100	102.5
Afternoon readings					
Pulse rate/min.	180	66.3	±3.470	48	88
Respiration rate/min.	192	37.8	±3.320	24	68
Body temperature (° F.)	192	101.3	±0.182	100	102.8

body temperature measured at 4 to 5:15 in the mornings as compared to measurements at 3:30 to 4 in the afternoon are given. The readings in the mornings were taken to see whether or not the surrounding air temperature would influ-

ence the characters measured. The lowest air temperature at night was about 15° F. below the highest temperature during the day. Minimum temperature for the nights when pulse rates were taken averaged 70.8° F., whereas maximum temperatures in afternoons of the same days averaged 85° F.

In table 8 an indication is given that the pulse rate decreases with increase in temperature. This is in agreement with results of Kleiber and others, as reported by Brody (2). Due to the lack of sweat glands in species such as cows and pigs, the skin is not cooled by sweating and therefore less blood is sent to the surface when environmental temperature rises. However, the difference in rate for morning and afternoon readings as reported here ($72.2 - 66.3 = 5.9°$ F.) is not sufficient for statistical significance. Neither is there any significant difference between morning and afternoon readings of respiration rate and body temperature.

DISCUSSION

The influence of percentage of crude fiber in the feed upon milk production is clearly demonstrated. A number of experiments have been carried out where the amount of roughage and its influence upon the milk production has been studied. In most cases of this work, the emphasis is put upon the character of roughage as such and less on the fiber content of the feed.

Morrison (9) discusses milk production on roughage alone and also the effect of successive additions of concentrate, and refers to a number of feeding experiments. As the level of concentrate feeding was increased in these experiments, the amount of additional milk secured per pound of concentrate decreased steadily. The decrease in milk per pound of concentrate fed was from 1.3 lb. milk at a high level of roughage to 0.3 lb. at a low level. The highest level of roughage was taken as 11,338 lb. of hay or "hay equivalent" fed per cow per year. The low level was taken as 7,385 lb. of hay or "hay equivalent" per year. However, the milk yields in different groups were not strictly comparable because at the higher levels of concentrates the digestible nutrient intake was about 16 per cent above the standard used, while in the medium levels of concentrate feeding it was only "a trifle" more than advised in the standards used. Probably different results would have been obtained if all the groups had been fed at the same level of total digestible nutrients and protein.

Among workers who have suggested that the feeding of milk cows is regulated according to crude fiber content of the feed, is Axelsson (1). He believes that the amount of crude fiber in the feed given shows an optimum value. Logically such an optimum occurs. As mentioned before, Cox has observed such an optimum in experiments with fattening lambs. The best gains in weight of the lambs were obtained for the 45:65 ratio of concentrate to roughage. In our experiment the best milk production was obtained when the fiber content was decreased to 16 per cent or below (calculated on dry matter basis). All rations with more than 16 per cent fiber resulted in a significantly lower milk production.

An interesting experiment in this field recently has been reported by Huffman and Duncan (7). These workers used 12 cows in 15 trials to study the

effect on milk production after a part of the total digestible nutrients in alfalfa had been replaced by corn. The replacement of a part of the alfalfa hay by corn on an equal total digestible nutrient basis always resulted in an increased production of 4 per cent fat corrected milk. After the change back to an all-alfalfa ration, a definite drop in milk production occurred. Possible explanations for the increased production are discussed in the report mentioned. It is suggested that the corn grain supplies an unidentified factor or factors needed to balance alfalfa hay for milk production.

It should be noted that in all the trials reported (7), a lower level of crude fiber was fed when part of the hay was replaced by corn. For example, in trial no. *1 b* a decrease from 29 per cent to 23 per cent fiber occurred.[2] In trial 2, the decrease was from 31 to 24 per cent fiber. In trial 3, the decrease in fiber was from 32 to 25 per cent and in trial 4, it was from 32 to 19 per cent. Similar results were obtained for the other trials. From the results obtained in our experiment, we believe that this reduction in the level of fiber definitely has influenced the milk production.

In a study of the optimum level of crude fiber in the feed it should be remembered that crude fiber has different chemical composition in different feeds and that its main components, cellulose, lignin and pentosans, may occur in quite different ratios (11). Therefore, it could not be expected that the optimum value for fiber in feeding experiments always will be the same in different feeds. Variations may occur according to the type of roughage used. For the type of fiber occurring in the two main feeds used in this experiment, namely, pineapple bran and Napier grass, the results mentioned in this paper may be expected, however. On the other hand, it appears possible that in a roughage such as clover hay and alfalfa, the optimum level of crude fiber may coincide with another, possibly a higher, level of the fiber.

SUMMARY

A feeding experiment has been carried out with different levels of crude fiber in the feed of dairy cows of Holstein-Friesian breed. Except for fiber, other nutritive factors (digestible protein and T.D.N.) were kept alike in different rations. A sufficient supply of vitamins and minerals also was provided. Four different rations were tried, namely, A with 60 lb. Napier grass, B with 40 lb. Napier grass, C with 20 lb. Napier grass daily per cow and D with no Napier grass. The balance needed of protein and T.D.N. was made up of pineapple bran, soybean oil meal, meat meal, fish meal and molasses. The crude fiber content for different rations were in percentage of dry matter as follows: A, 22.7 per cent; B, 19.6 per cent; C, 16.1 per cent; and D, 12.0 per cent. The effect of the level of crude fiber in the feed upon milk production was highly significant. With increase over 16 per cent in crude fiber content of the feed, a drop in milk production occurred, regardless of the fact that equal amounts of T.D.N. and digestible protein were fed.

The average daily yield of 4 per cent F.C.M. per cow for 12 wk. was 20.6 lb.

[2] Figured on the dry matter basis.

on ration A, 21.7 lb. on ration B, 23.4 lb. on ration C, and 23.8 lb. on ration D. Pulse rates per minute for cows with no roughage were 62, and increased to 69 with roughage. The statistical significance of this influence of rations fell close to the 1 per cent point.

For high milk production, the crude fiber level in the feed should not exceed 16 per cent when calculated on basis of dry matter content of the ration. This holds true for feeds such as mature Napier grass and pineapple bran as major constituents of the rations.

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STUDIES ON KETOSIS IN DAIRY CATTLE. X. THE EFFECT OF A VITAMIN A DEFICIENCY[1]

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In earlier studies, it was observed that the blood plasma carotene and vitamin A of cows exhibiting spontaneous ketosis were quite normal, showing that the ketotic condition was not caused by a vitamin A deficiency. Likewise, these animals did not respond to massive oral doses of vitamin A (1). This latter observation was confirmed by Hayden *et al.* (2).

However, these studies did not answer the question as to whether a vitamin A deficiency in cows would produce ketosis. It appeared possible that the typical symptoms and blood picture associated with ketosis could be produced by a combination of a vitamin A deficiency and fasting immediately postpartum, since fasting during the early postpartal period usually results in a marked hypoglycemia and ketonemia (3), and the symptoms associated with a vitamin A deficiency resemble those frequently observed in ketosis. This report is believed to provide a rather conclusive answer to the above question.

EXPERIMENTAL METHODS

The data reported herein were obtained from an experiment designed to study the influence of quality and quantity of feed upon the incidence of ketosis. In the early part of the study, it was observed that the blood plasma carotene and vitamin A values were lower than was expected; so much so, in fact, that the decision was made to continue the animals on the same diet and study the influence of a vitamin A deficiency on ketosis. The variable factors planned in the beginning were protein, fat, soluble carbohydrate and energy intake. Timothy hay (U. S. no. 2) was used because of its relatively low protein content. Raw soybeans were used as a source of protein and fat and made up 40 per cent of the concentrate ration (K-3) which was fed to all three groups for 4 mo. prepartum and to groups 1 and 2 postpartum. In addition to the soybeans, the concentrate mixture consisted of beet pulp 30 per cent, molasses 20 per cent, crushed barley 5 per cent, ground wheat 3 per cent, steamed bone meal 1 per cent, and iodized salt 1 per cent. Group 3 received concentrate ration K-1 during the postpartal period. It consisted of beet pulp 50 per cent, crushed barley 30 per cent, ground wheat 18 per cent, steamed bone meal 1 per cent, and iodized salt 1 per cent.

Morrison's feeding standards (4) were used throughout the experiment for calculating the total digestible nutrient requirements. The cows were fed rather heavily during the prepartal period to get them in a relatively fat condition. Prior to being placed on experiment, all of the cows had received liberal amounts of corn silage and a good quality of lespedeza hay in addition to a concentrate.

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TABLE 1

Blood plasma carotene and vitamin A of cows in groups 1, 2, and 3

Cow		Vitamin A concentrate fed prepartum		Plasma carotene			Plasma Vitamin A			Remarks
				Day of parturition	5–7 d. postpartum	10–14 d. postpartum	Day of parturition	5–7 d. postpartum	10–14 d. postpartum	
		Days	Total I.U.	(μg./100 ml.)	(μg./100 ml.)	(μg./100 ml.)	(μg./100 ml.)	(μg./100 ml.)	(μg./100 ml.)	
					Group 1					
Becky	H[a]			.....	21.6	27.6	.....	5.7	6.0	
Lou	A			49.2	31.2	29.4	5.2	3.0	4.5	Retained placenta
Dita	H			27.0	33.6	24.0	5.4	3.9	3.9	
Viola	J			96.0	82.2	90.0	8.1	7.5	13.2	Milk fever
Av.				57.4	42.2	42.8	6.2	5.0	6.9	
					Group 2					
Lively	G			30.6	29.4	43.2	4.5	3.3	7.8[b]	Retained placenta
Matilda	H			31.8	26.4	29.4	2.7	4.8	4.5	Retained placenta
Lobelia	A			47.4	47.4	48.0	4.2	4.8	5.1	
Lizzie	G			42.6	37.8	56.4	3.3	5.1	8.1	Milk fever
Av.				38.1	35.3	44.3	3.7	4.5	5.9	
					Group 3					
Katrina	J	52	6,000,000	67.8	53.4	31.8	18.0	11.4	9.9	
Melanie	H	43	4,800,000	36.0	30.6	39.0	10.8	6.0	16.8	Milk fever
Burke	H	21	2,000,000	30.6	51.0	64.2	5.7	8.4	6.0	
Lorna	A	10	1,200,000	39.6	30.6	33.6	6.6	5.4	5.4	Milk fever
Maggie	H	26	2,800,000	30.0	45.6	33.0	8.4	7.2	7.2	Retained placenta
Av.				40.8	42.2	40.3	9.9	7.7	9.1	

[a] Letter denotes breed.

[b] Vitamin A concentrate given *per os* a few days previously; value not included in average.

Beginning approximately 4 mo. prepartum, the cows were changed to timothy hay and the 40 per cent soybean ration. During the fourth and third months prepartum, the cows were fed all they would eat up to 140 per cent of requirements. During the 2-mo. dry period this was increased to 180 per cent.

In the postpartal period, the cows in group 1 received a rather high level of energy intake, whereas the cows in groups 2 and 3 were limited to approximately 50 per cent of their total digestible nutrient requirements for 3 wk. and then put on full feed as rapidly as possible. The hay was fed at the rate of 1.5 lb. per 100 lb. live weight to all cows during the prepartal period and to the cows in group 1 during the postpartal period. The cows in groups 2 and 3, which were maintained on a low level of energy intake postpartum, received 0.8 lb. of hay per 100 lb. weight during the first 3 wk. postpartum and 1.5 lb. thereafter. The concentrate mixture constituted the remainder of the total digestible nutrients. In addition, the cows in group 3 received 400,000 I. U. of a vitamin A concentrate twice per week for varying periods prepartum as indicated in table 1. The dosage was kept relatively low because of the opportunity afforded by this study to determine whether a relatively small intake of vitamin A over short periods would suffice to prevent the injury to the fetus which otherwise was certain to occur. Two additional cows were included in group 2 in the original study, but they calved before the vitamin A analyses were initiated and so are not included in this report.

Blood samples for carotene, vitamin A and glucose determinations were drawn at frequent intervals prepartum, on the day of parturition and twice per week thereafter.

A modification of the procedure of Moore (5) and of Kimble (6) was used for plasma vitamin A and carotene. Somogyi's (7) modification of the Shaffer and Somogyi method was used for blood glucose.

RESULTS

In table 1 the plasma carotene and vitamin A values of 13 cows are shown for the period immediately postpartum. The cows in groups 1 and 2 exhibited marked vitamin A depletion, as will be observed from the plasma vitamin A values. The cows in group 3, which received the vitamin A concentrate, exhibited a somewhat higher level of plasma vitamin A during the postpartal period.

An even better estimation of the degree of vitamin A depletion of these cows may be obtained from the data presented in table 2 on their calves. All of the calves from the cows in groups 1 and 2 showed evidences, usually marked, of vitamin A deficiency. At 5 days of age, only one of the calves from these eight cows was normal in appearance, even though the calves were allowed free access to the colostrum of their dams for the first 3 days. The one calf (Viola's) which was normal in appearance at 5 days, received 500,000 I. U. of vitamin A concentrate on the day of birth. Additional evidence of the degree of vitamin A depletion in these cows was the low level of vitamin A in the colostrum, which was indicated by the relatively small increase in the plasma vitamin A of the calves by the fifth day after birth.

TABLE 2

Blood plasma carotene and vitamin A of calves from cows in groups 1, 2, and 3

Dam of Calf	At birth			5 d. after birth[a]		
	Carotene	Vitamin A	General condition	Carotene	Vitamin A	General condition
	(μg./100 ml.)	(μg./100 ml.)		(μg./100 ml.)	(μg./100 ml.)	
			Group 1			
Becky	.....	.....	Weak, eye hemorrhage	1.8	7.8	Weak, died of scours at 2 wk.
Lou	1.8	2.1	Eye hemorrhage	3.6	3.6	Scours
Dita	0.0	1.2	Eye hemorrhage, paralyzed, rapid respiration	.....	.....	Did not recover from paralysis
Viola	1.8	6.0[b]	Eye hemorrhage	2.4	5.4[c]	Good
Av.	1.2	1.7		2.6	5.7[b]	
			Group 2			
Lively	1.2	2.4	Eye hemorrhage, blind, paralyzed	.....	.....	Killed on 2nd day
Matilda	.....	.....	Twins, born dead	.....	.....	Dead at birth
Lobelia	1.2	3.6	Eye hemorrhage, rapid respiration	2.4	5.4	Weak
Lizzie	2.4	2.7	Eye hemorrhage	8.4	8.7	Scours
Av.	1.6	2.9		2.4	5.4	
			Group 3			
Katrina	0.6	3.0	Good	6.0	14.1	Good
Melanie	0.6	2.4	Good	2.4	14.4	Good
Burke	0.6	1.5	Good	3.6	8.1	Good
Lorna	0.0	1.2	Weak	.....	.....	Died of bacteremia
Maggie	0.6	2.7	Good	1.8	6.6	Good
Av.	0.5	2.2		3.5	10.8	

[a] All calves were allowed to remain with dams for 3 d.

[b] Obtained colostrum before sample was taken
[c] Received 500,000 I.U. of vitamin A *per os* on day of birth } not included in averages.

TABLE 3

Blood glucose level and general condition of cows depleted of vitamin A and on varying levels of energy intake postpartum

Cow	Blood glucose in mg./100 ml.										Comments on symptoms of ketosis
	Days prepartum		Day of parturition	Days postpartum							
	15–13	8–6		3–4	7–8	10–11	13–14	17–18	20–21	23–24	
						Group 1					
Becky	.....	43.6	52.4	34.1	43.6	39.2	36.0	43.4	44.1	45.6	Negative
Lou	39.0	39.9	57.3	29.9	38.6	37.8	33.5	42.6	38.1	40.0	Negative
Dita	.....	45.1	63.5	33.6	42.1	27.9	39.4	30.5	38.6	38.6	Negative
Viola	46.3	45.6	.....	38.1	39.4	41.9	38.6	35.4	37.5	41.9	Negative
Av.	42.7	43.6	57.7	33.9	40.9	36.7	36.9	38.0	39.6	41.5	
						Group 2					
Lively	50.0	46.4	65.6	25.7	38.1	33.8	31.1	35.1	28.1	39.4	See table 4
Matilda	47.3	45.1	62.1	41.0	33.2	38.1	35.8	34.6	30.2	49.1	Negative
Lobelia	41.2	38.0	.....	34.8	32.7	37.8	38.1	34.0	28.5	38.6	Negative
Lizzie	38.1	46.4	71.8	33.3	29.2	25.9	21.4	17.6	20.3	37.0	Negative
Av.	46.7	44.0	66.5	33.7	33.3	33.9	31.6	30.3	26.5	41.0	
						Group 3					
Katrina	41.9	43.5	61.8	44.6	38.3	29.2	44.6	38.1	.....	.....	Negative
Melanie	42.6	36.7	92.6	31.1	22.4	17.0	18.6	35.4	35.9	.....	Negative
Burke	40.3	48.6	88.0	36.7	31.3	21.1	21.9	21.9	25.4	49.4	Negative
Lorna	41.7	47.0	.....	36.2	.....	31.3	27.3	34.6	33.5	38.6	Negative
Maggie	45.4	48.1	67.8	27.8	37.8	27.3	33.2	24.0	26.5	41.3	Negative
Av.	42.4	44.8	62.0	35.3	32.5	31.5	29.1	30.8	30.3	43.1	

The relatively small amount of vitamin A concentrate administered to the cows in group 3 protected four of the five calves from showing symptoms of a vitamin A deficiency at birth. The one calf in this group which showed evidence of a vitamin A deficiency was from the cow Lorna, which did not receive any vitamin A supplement until 10 days before parturition. On the other hand, the feeding of a small amount of vitamin A supplement to the cows Maggie and Burke, beginning with the 26th and 21st days prepartum, was sufficient to prevent the development of any external signs of a vitamin A deficiency in their calves.

An analysis of the hay showed that the cows were receiving approximately 35 μg. of carotene per pound of body weight from this feed. Since the concentrate mixture supplied very little vitamin A, the cows apparently were just at or slightly below their minimum requirements for carotene intake (8). However, the very low blood plasma vitamin A levels of the cows at time of parturition and the marked symptoms of vitamin A deficiency observed in the calves at birth suggested that the vitamin A depletion of the cows was greater than could be explained on the basis of the carotene intake. It was suspected that the high proportion of soybeans in the ration was responsible inasmuch as Hilton *et al.* (9) had observed that the feeding of soybeans to cows depressed the vitamin A content of the butter obtained from these cows. A carefully controlled experiment then was conducted with calves in which it was found (11) that the feeding of soybeans did indeed exert a very marked depressing effect upon both the plasma and liver vitamin A.

The data in tables 1 and 2 show that the cows in groups 1 and 2 were depleted of vitamin A to such an extent that not only were the plasma vitamin A values of the cows extremely low, but most of the calves showed evidences of vitamin A deficiency of considerable severity at birth.

The blood glucose values and observations on possible symptoms of ketosis in these cows during the postpartal period are presented in table 3. The cows in group 1 had blood glucose levels which are in the normal range for well-fed cows during the postpartal period, on the basis of a very large volume of data (unpublished) which has been accumulated in this laboratory. Some decrease usually occurs during this period. No symptoms of ketosis were observed.

The cows in groups 2 and 3 which received a lower level of energy intake postpartum (approximately 50 per cent of requirements) exhibited a lower level of blood glucose than the cows in group 1. However, the decrease in blood glucose was of the same magnitude in the cows in group 3, which received a vitamin A supplement, as in the cows in group 2 which were depleted of vitamin A. No symptoms of ketosis were observed in any of the animals.

One of the cows (Lively) in group 2 showed marked symptoms of a vitamin A deficiency. The blood plasma carotene and vitamin A and the blood glucose values obtained on this cow are presented in some detail in table 4. It will be noted that the plasma vitamin A values were very low immediately prepartum and postpartum. The calf was completely blind and paralyzed at birth. On the fourth, fifth and sixth days following parturition, this cow exhibited night

TABLE 4

The effect of low energy intake postpartum on the blood glucose level of a cow exhibiting a marked vitamin A deficiency

Date	Plasma carotene	Plasma vitamin A	Blood glucose	Per cent of T.D.N. requirements consumed	Remarks
	(μg./100 ml.)	(μg./100 ml.)	(mg./100 ml.)		
7/25	189.6	14.7	41.9	126	
8/8	132.0	18.0	41.6	135	
8/22	90.6	13.5	46.5	136	
8/28	.....	.....	.....	163	Beginning of dry period
9/19	60.6	11.7	46.4	161	
10/14	45.6	9.9	38.3	138	
10/17	46.2	5.1	46.4	151	
10/21	27.0	5.4	44.8	91	
10/22	30.6	4.5	65.6	66	Calved, calf blind and paralyzed, cow retained placenta
10/23	.....	.....	.....	55	Calf died
10/24	.....	.....	.....	55	Placenta removed
10/25	30.0	1.5	25.7	42	
10/26	.....	.....	.....	31	
10/27	.....	.....	.....	55	Cow exhibited slight incoordination and night blindness
10/28	29.4	3.3	25.4	55	Cow exhibited marked incoordination and night blindness
10/29 A.M.	34.8	4.8	38.1	60	Cow exhibited marked incoordination and night blindness
P.M.	.....	.....	.....	.....	1,000,000 I. U. vitamin A *per os*
10/30	30.6	14.1	43.5	57	1,000,000 I. U. vitamin A *per os*, marked incoordination
10/31	.....	.....	.....	45	1,000,000 I. U. vitamin A *per os*, slight improvement
11/1	38.4	16.2	33.8	44	1,000,000 I. U. vitamin A *per os*, definite improvement
11/2	.....	.....	.....	50	1,000,000 I. U. vitamin A *per os*, definite improvement
11/3	.....	.....	.....	52	
11/4	43.2	7.8	31.0	51	1,000,000 I. U. vitamin A *per os*
11/5	.....	.....	.....	53	Still showed some night blindness and slight incoordination
11/6	.....	.....	.....	47	1,000,000 I. U. vitamin A *per os*
11/7	40.8	12.6	35.1	47	Appeared almost normal
11/11	39.0	8.1	28.1	55	Put on full feed
11/13	.....	.....	.....	95	
11/14	23.4	5.1	39.4	95	
11/18	30.0	6.3	42.7	104	

blindness and incoordination. On the sixth day, the incoordination was so marked that the cow had considerable difficulty maintaining her equilibrium. A careful study of the blood glucose values fails to show any evidence that the hypoglycemia of ketosis is associated with a vitamin A deficiency. On the day that the general incoordination of the animal was most severe and might have been assumed to compare with the incoordination often associated with ketosis, the blood glucose value had risen to an almost normal level of 38.1 mg. per cent. With the oral administration of large doses of vitamin A, the cow improved rapidly but the blood glucose again decreased. When the cow was put on "full feed," the blood glucose returned to normal very quickly, indicating that the low postpartal blood glucose was due to a lack of sufficient energy and not to a vitamin A deficiency.

In table 5, similar blood data are presented on a cow which was depleted of vitamin A but was maintained on a relatively high energy intake postpartum. The blood glucose level was quite normal for a cow receiving from 70 to 90 per cent of the required total digestible nutrient intake. No symptoms of ketosis were observed.

DISCUSSION

It is evident from the low levels of blood plasma carotene and vitamin A and the marked signs and symptoms of vitamin A deficiency in one of the cows postpartum and several of the calves at birth, that a very marked depletion of vitamin A was effected in a number of the cows during the parturient period. The fact that no symptoms of ketosis were observed and that there was no apparent relationship between vitamin A depletion and deficiency and the level of blood glucose shows that a vitamin A deficiency *per se* does not produce ketosis in cows. Superimposing fasting upon vitamin A depletion did not change this relationship. While it is preferable to determine blood ketone bodies in such studies, the fact that a hypoglycemia and typical symptoms of ketosis must exist (11) for an adequate diagnosis of ketosis makes it possible to rule out ketosis when such data do not exist. Since the degree of vitamin A depletion effected in this study seldom is observed under field conditions, it appears quite clear that the incidence of ketosis in dairy cattle is much too high to be explained on this basis even if a vitamin A deficiency did produce ketosis. It must be concluded that not only is ketosis in dairy cows, as it occurs under field conditions, not due to a vitamin A deficiency as has been reported by Patton (7) but that a vitamin A deficiency does not produce ketosis in dairy cows.

SUMMARY

Detailed observations were made on 13 cows which were maintained on a low carotene diet for approximately 4 mo. prepartum and 3 wk. postpartum. The vitamin A depletion of these cows was accentuated by the feeding of a concentrate ration containing 40 per cent soybeans which was later shown to exert a marked depressing action on blood plasma and liver vitamin A. Five of the cows received a vitamin A supplement prepartum which resulted in the birth of four normal-appearing calves. The other eight cows dropped calves which

TABLE 5

The blood glucose level of a vitamin A depleted cow on a relatively high energy intake postpartum

Date	Plasma carotene	Plasma vitamin A	Blood glucose	Per cent of T. D. N. requirements consumed	Comments
	(μg./100 ml.)	(μg./100 ml.)	(mg./100 ml.)		
6/27	38.4	7.2	45.1	159	
7/1	16.8	7.5	43.6	158	
7/4	27.0	5.4	63.5	41	Calved, calf exhibited general paralysis, extensive eye
7/5	24.6	4.2	47.5	58	hemorrhage, rapid respiration
7/8	33.6	6.9	33.6	70	Calf given vitamin A supplement
7/11	33.6	3.9	42.1	78	
7/15	24.6	4.5	27.9	80	Respiration of calf normal and eye more clear but paresis not improved
7/18	24.0	3.9	39.4	82	Calf was killed
7/22	27.0	4.8	30.5	89	
7/25	27.0	5.1	38.6	81	Cow exhibited no symptoms of ketosis
7/29	25.8	4.5	38.6	87	
8/1	30.0	6.0	41.6	86	
8/5	28.8	6.3	34.6	89	
8/8	33.6	7.2	47.8	88	

showed marked signs and/or symptoms of a vitamin A deficiency. The blood plasma vitamin A values of these eight cows were extremely low during the postpartal period and one cow exhibited marked symptoms of a vitamin A deficiency. Of the eight cows, four were maintained on a low level of energy intake for 3 wk. postpartum (50 per cent of requirements). The five cows receiving the vitamin A supplement prepartum were on the same low level of energy intake postpartum. In spite of the severe vitamin A depletion and deficiency produced in these cows, none showed symptoms of ketosis and the degree of hypoglycemia produced by partial fasting was as large in the case of the vitamin A-supplemented cows as in the vitamin A-depleted cows. The cows exhibiting a marked vitamin A depletion but receiving higher levels of energy intake postpartum exhibited normal levels of glucose during the postpartal period. None of the cows showed symptoms of ketosis. It is concluded that not only is spontaneous ketosis, as it is observed under field conditions, not due to a vitamin A deficiency but that a vitamin A deficiency *per se* does not produce ketosis in dairy cows in the postpartal period.

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STUDIES ON KETOSIS IN DAIRY CATTLE. XI. LIPIDS, MINERALS AND ASCORBIC ACID IN THE BLOOD OF COWS WITH SPONTANEOUS KETOSIS[1]

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Rather extensive fatty infiltration and degeneration of various organs of cows with ketosis have been observed in studies carried on in this laboratory (10). This suggested that disturbances in fat metabolism may be involved. It appeared advisable, therefore, to determine whether any consistent abnormalities existed in the various blood lipids of cows with ketosis, since little information of this nature was available.

The adrenal cortex of ketotic cows always has shown extensive degeneration (11). Prior to these studies it was shown (9) that an extract of the adrenal cortex was effective in the treatment of ketosis. To obtain additional information on the possible role of the adrenals in the development of ketosis, an investigation of the level of ascorbic acid, sodium and potassium in the blood plasma also was included in this study. In addition, blood chlorides, phosphates and phosphatase activity were determined.

EXPERIMENTAL PROCEDURE

All of the cases reported herein were field cases diagnosed as ketosis by practicing veterinarians. One of the cows studied had had ketosis in previous years and was subjected to study before and during the development of the ketotic condition. In all cases blood glucose and acetone bodies were determined as an aid in diagnosis. Some of these cases were used simultaneously for other investigations which will be reported later. The methods used are as follows: Glucose, Somogyi's (13) modification of the Shaffer and Somogyi procedure used on cadmium sulphate filtrates; acetone bodies, Barnes and Wick (1); ascorbic acid, Mindlin and Butler (5); sodium, a modification of Snell and Snell (12); potassium, Harris (2); chlorides, Whitehorn (14); phosphates, Saarinen's (6) modification of the Kuttner, Cohen and Lichtenstein procedure. This same procedure also was used in determining phosphatase activity.

The method used for blood lipids is a modification of one developed by one of the authors (7, 8). A modification of Bloor's method, developed by Katsura and associates (3, 4), was used for a comparison in developing the shorter method used in this study.

In this study instead of separating the phospholipids by precipitation, they were calculated from the difference between the amount of total lipids and the other lipids that were extracted separately. This separation is based upon the heavy hydration of phospholipids in certain pH areas where phospholipids are not extractable from aqueous emulsion with ether and petroleum ether.

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For extraction the plasma pH is changed with a buffer solution, which liberates lipids from lipoprotein combinations so that all lipids except the phospholipids are extractable as such with low-boiling dry solvents. One method for complete fractionation of the plasma lipids based upon this procedure was published earlier by Saarinen (6, 7). In the present study instead of using this more accurate alcohol ether extraction, the total lipids were extracted from an alkaline aqueous emulsion with dry solvents after dehydrating the phospholipids with alcohol. This procedure is described in some detail.

The extraction of the total lipids (*A*). Measure 2 ml. of mixed plasma into a 25-ml. glass-stoppered graduate cylinder. Wash the pipette into the cylinder with 2 ml. of distilled water. Add 0.25 ml. of concentrated ammonium hydroxide. After mixing, add 2 ml. of redistilled alcohol (95 per cent) and mix again. Add 10 ml. of redistilled ethyl ether. Tighten the stopper with distilled water and shake vigorously by hand for 1 min. Loosen the stopper to release the pressure and allow to stand approximately 30 min. Repeat the shaking four times, allowing the mixture to stand 5 min. between shakings. Then add 10 ml. of petroleum ether and repeat the shaking four times as above. Wash the stopper with distilled water and let the cylinder stand over night or for at least 6 hr. The extract, in which 10 ml. are equivalent to 1 ml. of plasma, contains all the plasma lipids except free fatty acids but these normally are present only in negligible amounts.

The extraction of plasma lipids other than phospholipids (*B*). Measure 4 ml. of plasma into a 50-ml. glass-stoppered graduate cylinder and wash the pipette into the cylinder with 4 ml. of distilled water. Add 0.80 ml. of acetate buffer stock solution (one volume 1 *N* sodium hydroxide plus two volumes of 1 *N* acetic acid), followed by 20 ml. of redistilled ethyl ether. Stopper with moistened glass stopper and shake carefully for 30 sec. Loosen the stopper to release the pressure, stopper again and shake vigorously for 2 min. Then let stand for 20 to 30 min. After standing, shake four times, shaking for 2 min. each time and allowing to stand for 5 min. between shakings. After this extraction, 20 ml. of petroleum ether is added and the shaking is repeated four times, shaking for 1 min. each time and allowing to stand for 5 min. between shakings.

This extract, in which 10 ml. is equivalent to 1 ml. of plasma, contains all of the plasma lipids except the phospholipids.

The total amount of lipids in each extract is determined oxidometrically using the technique of Katsura *et al.*, modified as explained later. The same technique also is used for precipitating free cholesterol. The cholesterol determinations, however, are made colorimetrically, using a modification of Urbach as given by Zeiss (15).

Oxidometric determination of lipids in extracts A and B. Duplicate 5 ml. quantities of each extract (equivalent to 0.5 ml. plasma) are measured into acid-washed Erlenmeyer flasks of 50-ml. volume and evaporated to dryness over a boiling water bath. Traces of ammonia and acetic acid are driven off by blowing air into the flasks. Then the flasks are placed in a drying oven at

100° C. for 10 min. and air again is blown through the flasks several times. After cooling, 3 ml. of an oxidation mixture consisting of four parts of Nicloux silver dichromate-sulfuric acid solution and one part of 1 *N* aqueous potassium dichromate solution are added to each flask by means of an Ostwald pipette with a sharp tip. Two blank determinations are made simultaneously using the same procedure. The flasks are stoppered and placed over a boiling water bath for exactly 30 min. During this time, the flasks are rotated gently until all of the lipid material is dissolved, as will be indicated by a uniform adherence of the oxidation mixture on the surface of the flask. The flasks next are placed in an oven at 100 to 101° C. for exactly 1 hr., being rotated gently at 15-min. intervals and allowed to cool. After cooling, 25 ml. of distilled water are added to each flask. Just before titration, 2 ml. of 40 per cent slightly alkaline potassium iodide solution are added. The liberated iodine is titrated with a 0.05 *N* sodium thiosulfate solution, using soluble starch (1 per cent in saturated KCl solution) as indicator. The blank minus the actual titration value reveals the amount of chromic acid required for the oxidation of the lipids in extracts A and B.

Determination of total cholesterol. For the determination of total cholesterol, 5 ml. of each extract (A and B) are used for duplicates. If the extractions are carried out properly, the cholesterol will be extracted quantitatively in both cases. Thus, the cholesterol determination also is used to check on the quantitativeness of the extractions, particularly for extraction B which requires greater care.

Five ml. of each extract is measured into a 50-ml. glass-stoppered Erlenmeyer flask, evaporated over a steam bath and dried in an oven as described previously. After cooling, 5 ml. of chloroform and 1 ml. of acetic anhydride are added and mixed. Just before warming, 1 ml. of a solution consisting of nine volumes of acetic anhydride and one volume of concentrated sulfuric acid is added and mixed by rotating. The flasks immediately are placed in the dark in a covered water bath at 38° C. for exactly 15 min. The flasks are cooled and readings are made with a suitable photometer at a wave length of 660 mμ. For calculation a standard curve is established with pure cholesterol. The results are calculated on the assumption that 90 per cent of the value for blood cholesterol is due to true cholesterol (7, pp. 41 and 125).

Ester cholesterol. For each duplicate, 5 ml. of extract B are placed into a 50-ml. Erlenmeyer flask and evaporated to dryness over a water bath and the acetic acid is removed by passing a stream of air into the flask. The residue is redissolved with 5 ml. of acetone, followed by the addition of 2.5 ml. of 0.2 per cent digitonin solution in 95 per cent alcohol and the addition of 0.5 ml. of distilled water. After mixing, the flasks are placed on a water bath in contact with steam. The temperature of the water bath is maintained at 50 to 60° C. for 15 min., after which it gradually is increased to boiling. After evaporation the residue is dried by passing a stream of air slowly through the flask. The cholesterol esters are extracted with ether in a warm flask using three repeated extractions and 3 to 4 ml. of ether for each extraction. The portions are fil-

tered into another glass-stoppered Erlenmeyer flask through a fat-free filter and then evaporated to dryness. After cooling, 5 ml. of chloroform and 1 ml. of acetic anhydride are added and mixed. The remainder of the procedure is the same as for total cholesterol. When a limited number of determinations are being made, time may be saved by extracting the cholesterol esters from the dried residue directly with chloroform.

Acidometric titration of non-volatile free acids in ether-petroleum ether extract. Twenty ml. of extract B are measured into a 50-ml. Erlenmeyer flask for each duplicate and evaporated to dryness as before when the samples are prepared for oxidation of the lipids. This removes the acetic acid present in the extract. After cooling, 10 ml. of a benzene-alcohol mixture (1:1) containing 0.02 per cent phenolphthalein are added and the free acids are titrated with freshly diluted carbonate free 0.01 *N* potassium hydroxide solution using a microburette with 0.01 ml. divisions.

Calculations. Both total and ester cholesterol are calculated using a standard factor and the extinction value (L = 2-log G) as a basis. When the amount needed for complete oxidation of total cholesterol in plasma (3.92 ml. of 0.1 *N* oxidant per 1 mg. of cholesterol) is subtracted from the titration value B (blank minus titration), the difference reveals the amount of oxidant used by the fatty acids in cholesterol and glycerol esters. This divided by the reduction constant of blood fatty acids (3.60 ml. of 0.1 *N* oxidant per 1 mg. of fatty acids) gives the amount of fatty acids in milligrams. The difference between titration values A and B shows the amount used by phospholipids. This value divided by the constant 2.82 gives the amount of phospholipids in milligrams.

If 5 ml. of extract (equivalent to 0.5 ml. plasma) are used for every determination and titrations are made with 0.05 *N* thiosulfate, the calculations of the lipid fractions would be as follows:

(a) Total cholesterol in mg. per cent determined colorimetrically,
(b) Ester cholesterol in mg. per cent determined colorimetrically,
(c) Phospholipids in mg. per cent. = $(A-B)/2.82 \times 100$,
(d) Fatty acids in cholesterol and glycerol esters and as free-fatty acids in mg. per cent = $(B-3.92a)/3.60 \times 100$,
(e) Cholesterol ester fatty acids in mg. per cent = 0.646b,
(f) Cholesterol esters in mg. per cent = 1.62b or $0.972(b+e)$,
(g) Glycerol esters + free fatty acids in mg. per cent = $d-e$,
(h) Total lipids in mg. per cent = $a+c+d$.

RESULTS

Blood plasma lipids. The blood plasma lipid values of cows with ketosis are presented in table 1. Because a number of the cows exhibited complications of various kinds in addition to the ketosis, an attempt was made to group the complicated and uncomplicated cases separately. On the basis of the present knowledge of ketosis in cows, such a differentiation appears advisable. Histopathological studies of cows with ketosis revealed that many cases exhibit some kind of complication other than that which is secondary to the ketotic condition.

TABLE 1

Blood plasma lipids of ketotic cows

Date	Cow	Blood glucose	Blood acetone bodies	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Fatty acids in		Free acids	Days with ketosis and comments
									Choles-terol esters	Glycerol esters and as free		
		(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*m. equiv./* 100 *ml.*)	
						A. Apparently uncomplicated ketosis						
3/24/48	Hall[a]	56.2	10.0	.....	.....	62.3	52.4	9.4	34.1	12.6	.....	20 d., glucose adm.
3/25/48	Hall	37.5	10.9	.....	.....	66.6	48.2	18.4	31.3	6.0	.....	21 d.
3/24/48	Downs I	41.9	13.4	.....	.....	110.9	90.5	20.4	58.8	21.2	.....	7 d., recovering
3/24/48	Downs II	35.1	.....	.....	.....	105.1	82.6	22.5	53.7	6.3	.....	7 d., recovering
3/25/48	Flegel	39.4	20.5	.....	.....	98.2	78.7	19.5	51.2	3.8	.....	6 d., recovering
7/ 9/48	Hermosa[a]	53.1	3.4	269.5	92.9	101.6	97.4	4.2	63.3	11.7	0.08	Prepartum
7/13/48	Hermosa[a]	50.3	5.4	226.0	80.1	80.9	67.7	13.2	43.7	21.3	0.10	Day of parturition
7/22/48	Hermosa[a]	40.4	6.4	238.1	100.0	85.0	56.8	28.2	36.1	17.0	0.11	Postpartum
7/28/A.M.	Hermosa[a]	33.3	14.4	283.2	109.2	116.2	90.5	25.7	57.8	0.0	0.09	1 d., early ketosis
P.M.	Hermosa	23.8	15.5	254.8	79.4	117.1	86.9	30.2	56.4	1.9	0.09	1 d., early ketosis
8/ 2/48	Hermosa[a]	41.7	2.5	286.3	120.6	109.0	103.0	6.0	56.7	0.0	0.11	Recovering
8/ 6/48	Hermosa[a]	38.1	6.0	352.3	150.4	129.1	95.5	33.6	62.0	10.8	0.15	Recovering
7/28/48	Inez	20.5	31.1	245.8	66.0	118.8	101.1	17.7	61.0	0.0	0.10	4 d., glucose adm.
8/ 2/48	Inez[a]	36.7	3.3	233.4	76.6	96.7	80.4	16.3	52.2	7.8	0.12	Recovering, glucose adm.
8/ 6/48	Inez[a]	34.2	5.2	372.7	175.2	125.3	101.3	24.0	65.8	6.4	0.12	Recovering, glucose adm.
8/16/48	Beltsville	22.3	45.0	291.9	104.3	137.6	103.7	33.9	50.0	0.0	0.07	1 d.
8/21/48	Beltsville[a]	31.6	26.8	271.5	96.5	119.5	96.2	23.3	55.5	0.0	0.05	5 d.
7/ 8/49	King	21.6	15.0	340.7	146.1	99.6	65.2	34.4	42.3	52.7	0.19	10 d., responded to glucose adm.
Av. during ketosis		.....	.....	283.3	99.0	106.7	82.1	24.6	50.6	21.0	0.11	

TABLE 1 (*Continued*)

Date	Cow	Blood glucose	Blood acetone bodies	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Choles-terol esters	Glycerol esters and as free	Free acids	Days with ketosis and comments
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*m. equiv./ 100 ml.*)	
						B. Complicated ketosis						
3/ 3/48	Hoffman	18.9	40.2	.....	.....	66.3	52.0	14.3	41.3	42.9	.....	15 d., ruptured hypophysis
3/ 5/48	Hoffman[a]	28.9	30.9	.....	.....	72.3	55.3	17.0	45.9	44.4	.....	17 d.
3/ 3/48	Thom[a]	23.5	33.7	.....	.....	.....	.....	.....	.....	.....	.....	9 d., glucose adm.
3/ 9/48	Thom	56.2	8.3	.....	.....	61.4	47.2	14.2	30.7	41.0	.....	15 d., pneumonia
3/30/48	Cunningham	24.4	25.1	.....	.....	143.5	111.6	31.9	72.5	20.3	.....	2 d., uterus inflamed
4/ 6/48	Burdette[a]	39.4	5.0	.....	.....	82.6	66.6	16.0	43.6	33.3	0.30	11 d., glucose administration
4/ 7/48	Burdette	41.2	8.1	.....	.....	76.6	63.7	12.9	41.4	35.8	0.28	12 d., atrophied hypophysis
2/ 7/49	Mullinix	33.7	38.9	135.5	22.7	66.1	41.0	25.1	26.7	20.0	0.21	13 d., ilium inflamed, ulcerated
3/16/49	Thomas	19.3	56.8	270.6	92.2	91.7	74.4	17.3	48.4	38.3	0.13	4 d., abomasum & duodenum slightly inflamed
3/30/49	Enterprise	22.1	46.2	314.5	92.9	125.5	102.2	23.3	66.4	29.7	0.20	16 d., excess glucose pumped in rumen
4/28/49	Sherman[a]	17.8	12.8	281.3	130.5	86.9	75.4	11.5	49.0	14.9	0.13	4 d., severe inflammation of abomasum & intestines
5/ 3/49	Sherman	22.9	24.0	307.6	125.3	96.7	74.9	21.8	48.6	37.0	0.18	
Av. during ketosis		.....	.....	257.1	83.3	91.0	70.9	20.1	47.0	33.1	0.20	

[a] Not included in average.

TABLE 2

Blood plasma lipids of cows in early and late stages of ketosis

Date	Cow	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Fatty acids in			Free acids
							Choles-terol esters	Glycerol esters and as free	Total	
		(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*m. equiv./* 100 *ml.*)
				A. Early stage of ketosis (1–4 d.)						
7/28/48	Inez	245.8	66.0	118.8	101.1	17.7	61.0	0.0	61.0	0.10
7/28/48	Hermosa	269.0	94.3	116.7	88.5	28.0	57.1	1.0	58.0	0.09
8/16/48	Beltsville 382	291.9	104.3	137.6	103.7	33.9	50.0	0.0	50.0	0.07
3/16/48	Thomas	270.6	92.2	91.7	74.4	17.3	48.4	38.3	87.7	0.13
	Av.	269.3	89.2	116.2	91.9	24.2	54.1	9.9	64.0	0.10
				B. Medium stage of ketosis (6–7 d.)						
3/24/48	Downs I	.....	.....	110.9	90.5	20.4	58.3	21.2	79.5	
3/24/48	Downs II	.....	.....	105.1	82.6	22.5	53.7	6.3	60.0	
3/25/48	Flegel	.....	.....	98.2	78.7	19.5	51.2	3.8	55.0	
	Av.			104.7	83.9	20.8	54.4	10.4	64.8	
				C. Late stage of ketosis (10–21 d.)						
3/25/48	Hall	.....	.....	66.6	48.2	18.4	31.3	6.0	36.3	.....
7/ 8/49	King	340.7	146.1	99.6	65.2	34.4	42.3	52.7	95.0	0.19
3/ 3/48	Hoffman	.....	.....	66.3	52.0	14.3	41.3	42.9	84.2	.....
2/ 7/49	Mullinix	135.5	22.7	66.1	41.0	25.1	26.7	20.0	46.7	0.21
	Av.	238.1	84.4	74.7	51.6	23.1	35.4	30.4	65.6	0.20

Several cases listed as complicated would have been classified as uncomplicated if the animals had not been slaughtered. It appears, therefore, that at least some of the cows listed here in the uncomplicated group probably represent complicated cases. For example, the cow Hermosa, which appeared to be an excellent example of an early case of spontaneous ketosis, exhibited lipofibroma when posted 1 yr. later. This cow had exhibited ketosis postpartum for 3 consecutive yr. and for this reason the blood picture was followed closely immediately prepartum and postpartum. As will be observed from the blood glucose and acetone body values, blood samples were drawn from this cow before and during the development of ketosis and after recovery. In some of the cases, the blood samples were drawn after the animals had been treated with glucose so that the previous diagnosis of the veterinarian had to be used. Consequently, the blood sugar was higher and the acetone bodies lower than would have been the case before treatment. A number of these cows were slaughtered for more extensive biochemical and histo-pathological studies. Complications frequently were observed in the postmortem examinations. Blood samples frequently were drawn from the same cow in different stages of ketosis.

In computing the averages shown in table 1, the data from a single sample of blood drawn during the height of ketosis was used. Considerable variation was observed in the lipid values. All of the lipid values except the free fatty acid equivalent appeared to be somewhat lower than was to be expected. The free fatty acid equivalent was proportionally high in both complicated and uncomplicated ketosis but was observed to be highest in the cows with complicated ketosis.

These differences appeared to be due to the stage of ketosis, since the complicated cases did not respond to treatment readily and, therefore, often represented later stages of this condition. To determine whether the differences were due primarily to the stage of ketosis, some of the animals with either no complications or less severe complications were grouped into early, medium and late stages of ketosis. None of the cows grouped as early cases had exhibited signs or symptoms of ketosis for more than 4 days. Those classified as medium and late stages had exhibited signs and/or symptoms for 6 to 7 days and 10 to 21 days, respectively. As will be noted in table 2, most of the lipid fractions showed a decrease when the animals exhibited ketosis over a longer period of time. However, the free cholesterol did not appear to change appreciably, whereas the neutral fat and free fatty acid fractions usually increased.

Blood minerals, ascorbic acid and hematocrit values. The data on these substances are presented in table 3 for cows which exhibited ketosis of either a complicated or uncomplicated nature. The inorganic phosphorus varied widely with some values below normal. The values for blood plasma sodium, potassium and chlorine were within the normal range in most cases. The plasma ascorbic acid values also varied widely. Some of the values were low, but since the majority of the values were within the normal range it does not appear that there is any specific relationship between the blood plasma ascorbic acid and

ketosis in cows. The red cell volume usually was high, undoubtedly due to dehydration.

Because some of the inorganic phosphorus values were low, the phosphates were analysed more completely in the latter part of the study. Unfortunately, four of the five cows reported in this study (table 4) represented complicated cases. Three of the plasma acid-soluble inorganic phosphorus values were somewhat low and three of the values for serum phosphatase activity were distinctly low.

DISCUSSION

In general, the values for the various blood lipids, minerals and ascorbic acid of ketotic cows reported in this paper do not deviate markedly from normal values in the early stage of lactation. However, most of the blood lipid values were lower than was to be expected, with the exception of neutral fat and free fatty acids, which increased with the duration of the ketotic condition. Ordinarily, the blood plasma phospholipids and cholesterol esters decrease at parturition and then gradually increase during the second and third week postpartum. Cows with ketosis do not appear to exhibit this normal increase. In the later stages of ketosis, these values are even lower than in the early stages.

The blood mineral values of the ketotic cows were more nearly normal than the blood lipids. The fact that plasma sodium and potassium remained normal indicates that if the adrenal cortex is involved in ketosis, the factor regulating plasma sodium and potassium is not affected. Since the blood serum phosphatase activity was fairly low, it is possible that some abnormalities may exist in metabolic processes where phosphorus is involved. Several normal values for inorganic phosphorus in blood plasma indicate that a phosphorus deficiency is not associated with ketosis.

Since cows with ketosis usually exhibit inappetance, some of the alterations observed in the blood picture may be associated with fasting rather than with ketosis as such. Since little information is available on the effect of fasting in the early postpartal period upon the blood substances studied, such a study appears to be necessary before any further conclusions can be drawn.

SUMMARY

In a study with 18 cows diagnosed as having ketosis, an analysis was made of various blood and blood plasma substances. Plasma phospholipids and cholesterol ester fractions were somewhat low, particularly in the later stages of ketosis. Free cholesterol in the plasma was relatively normal. The amount of free ether-petroleum ether soluble non-volatile acids in plasma determined by acidometric titration was relatively high in the later stages of ketosis. The neutral fat fraction was relatively low in the early stages of ketosis and normal or high in the later stages.

Marked variations were observed in the plasma ascorbic acid values. The serum phosphatase activity was relatively low. The plasma acid-soluble phosphorus values, both inorganic and organic, were sometimes low but were normal

TABLE 3

Hematocrit values, ascorbic acid and minerals in the blood of cows with ketosis

Date	Cow	Blood glucose	Blood acetone bodies	Red cell volume	Plasma ascorbic acid	Plasma Na	Plasma K	Blood Cl	Plasma inorg. P
		(*mg. %*)	(*mg. %*)	(%)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)
				A. Apparently uncomplicated ketosis					
4/20/46	Downey	21.3	32.2	.....	.....	345	19.4	.....	.....
4/16/47	Pelagie	21.6	36.2	.....	.....	307	17.3	.....	.....
3/24/48	Hall	56.2	10.0	36.7	0.062	.....	22.1	307	3.0
3/25/48	Hall	37.5	10.9	35.7	0.063	248	16.8	294	3.3
3/24/48	Downs I	41.9	13.4	36.8	0.175	261	19.8	307	5.5
3/24/48	Downs II	35.1	.....	31.4	0.062	281	22.0	314	3.0
3/25/48	Flegel	39.4	20.5	31.7	0.573	248	20.5	269	6.3
	[a]Av.			34.0	0.218	282	19.8	298	4.2
				B. Complicated ketosis					
3/ 3/48	Hoffman	18.9	40.2	.....	0.549	.....	.....	307	4.1
3/ 5/48	Hoffman	28.9	30.9	.....	0.940	366	.....	310	3.4
3/ 9/48	Thom	56.2	8.3	28.2	0.130	318	18.4	319	.....
3/30/48	Cunningham	24.4	25.1	41.6	0.113	292	15.6	294	4.6
4/ 6/48	Burdette	39.4	5.0	30.0	0.388	252	13.2	248	8.1
4/ 7/48	Burdette	41.2	8.1	34.6	0.275	292	10.1	168	6.4
2/ 7/49	Mullinix	33.7	38.9	34.5	0.338	.....	.....	285	2.7
3/16/49	Thomas	19.3	56.8	.....	0.975	.....	.....	300.5	3.5
3/30/49	Enterprise	22.1	46.2	.....	0.360	.....	.....	275	2.7
	[a]Av.			34.2	0.427	312	15.2	284	4.1

[a] The value for each cow used for calculating the group averages was the average of all individual values.

TABLE 4

Blood phosphates and phosphatase values of cows with ketosis

Date	Cow	Blood glucose	Blood acetone bodies	Blood acid-soluble P			Plasma acid-soluble P			Phosphatase activity	Remarks
				Inorg.	Org.	Total	Inorg.	Org.	Total		
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*units/ 100 ml.*)	
8/16/48	Beltsville 328	22.3	45.0	.....	.....	.....	6.86	0.82	7.68	.....	Apparently uncomplicated ketosis
2/ 7/49	Mullinix	33.7	38.9	2.50	4.30	6.80	2.71	0.83	3.54	1.57	Complicated ketosis
3/16/49	Thomas	19.3	56.8	3.68	2.45	6.12	3.54	1.31	4.85	1.05	Complicated ketosis
3/30/49	Enterprise	22.1	46.2	2.55	3.61	6.16	2.66	1.12	3.78	1.55	
4/28/49	Sherman	17.8	12.8	1.85	5.34	7.19	2.40	1.89	4.29	1.99	Complicated ketosis
	Av.	.....	.....	2.65	3.93	6.57	3.63	1.19	4.83	1.54	

in most cases. Plasma sodium and potassium were normal and the blood chloride values also were in the normal range. Additional data on the effect of fasting and other factors secondary to ketosis are needed before the data can be properly evaluated.

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STUDIES ON KETOSIS IN DAIRY CATTLE. XII. BLOOD LIPIDS, PHOSPHATES AND PHOSPHATASE ACTIVITY OF COWS ON DIFFERENT LEVELS OF FEED INTAKE POSTPARTUM[1]

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A study of cows with ketosis (1) showed some alterations in the blood plasma lipids, phosphates and serum phosphatase activity. These alterations were more distinct in cases where cows had exhibited a ketotic condition for 10 days or more. Since these cows also exhibited inanition, which usually is associated with bovine ketosis, it was difficult to decide whether the alterations were due to the ketotic condition as such or to inanition.

For purposes of comparison, a study was made of the effects of inanition during the postpartal period while the researches on ketotic cows were still in progress.

EXPERIMENTAL PROCEDURE

At the time that this study was initiated rather extensive investigations were in progress in this laboratory relative to the effect of the quality and quantity of feed on the postpartal metabolism of the cow. The blood samples used in this study were drawn from these same animals.

For 6 mo. prior to parturition the cows all were fed rather heavily on low (10 per cent), medium (14 per cent) and high protein (23 per cent) rations. Following parturition half of the cows were fed liberally. The energy intake of the other half was limited to 35 per cent of Morrison's feeding standards for 8 to 15 days postpartum. In order to avoid repetition, the exact details relative to the feeding of these animals will be presented in a later paper together with the results of the studies for which these experiments originally were designed.

The chemical procedures used in this study are the same as those listed in the preceeding paper (1).

RESULTS

Postpartal blood plasma lipid values from cows on different levels of protein and energy intake are presented in tables 1 and 2. The data shown in table 1 are from cows which received 70 per cent of Morrison's feeding standards for total digestible nutrients for the first week postpartum and 80 per cent during the second week postpartum. The level of protein in the ration did not exert any apparent effect upon the blood lipid values. When the postpartal plasma lipid values for the individual cows are subjected to a careful examination, it will be observed that at this level of feed intake there was a gradual increase in total lipids, phospholipids, total cholesterol, ester cholesterol and cholesterol esters, independent of the protein intake. Free cholesterol did not change appre-

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TABLE 1

Blood plasma lipids of cows on high plane of nutrition postpartum

Date	Cow	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Fatty acids in		Free acids	Remarks
							Choles-terol esters	Glycerol esters and as free		
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*m. equiv./ 100 ml.*)	
					A. High protein ration					
8/ 4 48	Esmeralda	296.3	132.6	112.6	83.3	29.3	51.1	0.0	0.06	Prepartum
8/24 48	Esmeralda	.....	.....	103.7	79.9	23.8	.....	.....	0.07	Prepartum
8/27 48	Esmeralda	240.6	79.7	104.4	87.4	16.7	56.8	13.2	0.11	1 d. postpartum
9/17 48	Esmeralda	363.4	134.7	154.3	135.6	18.7	74.4	0.0	.....	22 d. postpartum
1/ 3 49	Ruby	302.2	.....	111.6	96.2	15.4	.....	.....	0.20	16 d. postpartum
1/17 49	Ruby	498.1	263.8	191.0	124.3	66.7	43.3	0.0	.....	30 d. postpartum
Av. 16 d. postpartum (Ruby)		302.2	.....	111.6	96.2	15.7	.....	.....	0.20	
					B. Medium protein ration					
5/12 48	Bonita	250.0	76.6	82.3	.....	.....		91.1	0.10	Prepartum
5/19 48	Bonita	243.8	80.9	87.4	.....	.....		75.5	0.08	Prepartum
5/20 48	Bonita	.....	.....	90.4	.....	.....		111.0	0.11	Day of parturition
5/26 48	Bonita	262.9	82.3	58.6	.....	.....		122.0	0.15	6 d. postpartum
6/ 2 48	Bonita	311.0	109.9	67.2	.....	.....		133.9	0.10	13 d. postpartum
7/22 48	Faith	336.2	148.9	122.9	84.5	38.4	54.9	9.5	0.05	Prepartum
7/28 48	Faith	306.2	122.7	129.1	100.3	28.8	54.4	0.0	0.04	Prepartum
8/11 48	Faith	281.8	78.9	114.0	96.7	17.3	62.9	26.0	0.18	4 d. postpartum
8/21 48	Faith	361.7	120.4	159.6	131.8	27.8	81.7	0.0	0.08	14 d. postpartum
Av. 13–14 d. postpartum		336.4	115.2	113.4	131.8	27.8	81.7	0.0	0.09	
					C. Low protein ration					
8/11 48	Acacia	210.3	37.6	95.5	76.1	19.4	49.4	27.8	0.06	Prepartum
8/27 48	Acacia	295.2	116.3	120.0	105.8	14.2	58.9	0.0	0.10	5 d. postpartum
9/ 3 48	Acacia	334.0	123.4	120.1	114.0	16.1	74.1	6.4	0.06	12 d. postpartum
9/17 48	Acacia	355.4	90.1	184.8	174.5	10.3	80.5	0.0	0.06	26 d. postpartum
1/ 3 49	Pomona	243.5	.....	85.7	68.4	17.3	.....	.....	0.15	3 d. postpartum
1/17 49	Pomona	375.9	117.5	171.8	133.2	38.6	86.6	0.0	.....	20 d. postpartum
Av. 12 d. postpartum (Acacia)		334.0	123.4	130.1	114.0	16.1	74.1	6.4	0.06	
Av. of all three groups 12–16 d. postpartum		324.2	119.8	118.4	114.0	19.8	77.9	3.2	0.13	

TABLE 2

Blood plasma lipids of cows on low plane of nutrition postpartum

Date	Cow	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Fatty acids in		Free acids	Remarks
							Choles-terol esters	Glycerol esters and as free		
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*m. equiv./ 100 ml.*)	
					A. High protein ration					
6/ 9 48	Martha	286.7	87.9	93.8	.....	.....	.....	.....	0.09	Prepartum
6/22 48	Martha	238.7	74.5	89.8	68.7	21.1	45.6	28.8	0.14	9 d. postpartum
6/25 48	Martha	255.2	92.2	86.9	69.7	17.2	45.3	30.8	0.12	12 d. postpartum
6/28 48	Martha	247.9	86.5	80.9	68.7	12.2	60.3	20.2	0.13	15 d. postpartum
7/28 48	Beth	211.7	59.6	93.8	74.2	19.6	48.2	10.1	0.06	Prepartum
8/19 48	Beth	199.8	56.7	77.0	66.1	10.9	43.0	23.1	0.12	Prepartum
8/23 48	Beth	212.6	81.5	73.9	63.0	10.9	41.0	16.2	0.10	3 d. postpartum
8/31 48	Beth	239.4	74.5	93.8	81.1	12.7	52.7	18.4	0.18	11 d. postpartum
1/ 3 49	Lizzie	148.1	.....	88.1	69.2	18.9	.....	.....	0.13	11 d. postpartum
3/10 49	Elinor	250.0	56.7	102.2	88.1	14.1	57.3	33.8	0.05	Prepartum
4/ 1 49	Elinor	233.1	71.6	68.7	55.9	12.8	85.0	7.8	0.19	10 d. postpartum
Av. 8–15 d. postpartum[a]		216.9	76.8	84.1	68.8	15.3	62.7	17.6	0.15	
					B. Medium protein ration					
5/ 5 48	Valencia	198.4	66.7	72.2	.....	.....	59.5		0.13	Day of parturition
5/ 8 48	Valencia	233.2	54.6	88.0	.....	.....	90.6		0.18	3 d. postpartum
5/12 48	Valencia	236.4	73.8	69.8	.....	.....	92.8		0.11	7 d. postpartum
5/14 48	Valencia	250.9	90.8	90.6	.....	.....	69.5		0.13	9 d. postpartum
5/17 48	Valencia	263.4	85.1	92.2	.....	.....	86.1		0.13	12 d. postpartum
5/19 48	Valencia	241.4	80.9	95.5	.....	.....	65.0		0.12	14 d. postpartum
7/13 48	Adventuress	260.5	76.6	91.7	77.5	14.2	50.3	41.9	0.07	Prepartum
7/22 48	Adventuress	208.4	59.6	72.7	64.2	8.5	41.7	34.4	0.06	Prepartum
7/28 48	Adventuress	174.7	60.3	77.5	59.3	18.2	38.5	58.7	0.04	Prepartum
8/ 2 48	Adventuress	179.2	71.6	67.0	57.2	9.8	37.2	3.4	0.08	1 d. postpartum
8/ 9 48	Adventuress	205.8	56.0	78.7	62.8	15.9	40.8	30.3	0.14	8 d. postpartum
8/13 48	Adventuress	231.0	57.4	89.8	67.7	22.1	57.5	26.3	0.20	12 d. postpartum
8/16 48	Adventuress	259.2	75.2	100.1	82.6	17.5	53.7	30.2	0.19	15 d. postpartum

TABLE 2 (*continued*)

Blood plasma lipids of cows on high plane of nutrition postpartum

Date	Cow	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Fatty acids in		Free acids	Remarks
							Choles-terol esters	Glycerol esters and as free		
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*m. equiv./ 100 ml.*)	
9/13 48	Bounty	248.8	73.0	94.1	82.8	11.3	53.8	27.9	0.14	1 d. postpartum
9/17 48	Bounty	297.8	120.6	117.2	110.2	7.0	60.0	0.0	0.10	5 d. postpartum
9/23 48	Bounty	309.4	111.3	116.4	98.2	18.2	63.8	17.9	0.15	11 d. postpartum
Av. 8–15 d. postpartum[a]		264.4	86.6	99.6	84.6	18.4	53.9	26.2	0.15	
				C. Low protein ration						
6/ 9 48	Bunny	279.0	87.2	101.5	.....	.....	.....	90.3	0.10	Prepartum
6/22 48	Bunny	312.0	127.0	100.9	89.8	11.1	56.9	27.5	0.08	Prepartum
6/28 48	Bunny	239.9	83.0	88.6	71.3	17.3	46.3	22.0	0.09	1 d. postpartum
7/ 1 48	Bunny	254.6	129.8	89.8	73.0	16.8	35.0	0.0	0.14	4 d. postpartum
7/ 6 48	Bunny	254.1	120.6	87.4	47.2	40.2	30.7	15.4	0.10	9 d. postpartum
7/ 9 48	Bunny	210.1	61.0	70.8	57.0	13.8	37.0	41.3	0.15	12 d. postpartum
7/12 48	Bunny	248.1	98.6	70.6	69.7	0.9	45.3	33.6	0.09	15 d. postpartum
8/ 4 48	Remembrance	276.2	114.9	99.1	91.7	7.4	59.6	2.6	0.06	Prepartum
8/31 48	Remembrance	229.6	71.6	100.8	75.4	25.4	49.0	8.2	0.10	1 d. postpartum
9/ 3 48	Remembrance	259.0	92.2	92.4	79.7	12.7	51.8	22.6	0.13	4 d. postpartum
9/ 7 48	Remembrance	214.2	72.3	70.2	57.7	12.5	37.5	34.2	0.17	8 d. postpartum
2/18 49	Melanie	322.3	85.8	117.1	98.2	18.9	98.2	21.2	0.05	Prepartum
2/25 49	Melanie	285.7	24.1	146.6	130.3	16.3	84.6	30.4	0.08	Prepartum
3/ 2 49	Melanie	313.1	87.2	110.9	91.2	19.7	59.3	55.7	0.10	Prepartum
3/21 49	Melanie	222.7	47.5	71.3	63.3	8.0	41.1	62.8	0.15	9 d. postpartum
3/23 49	Melanie	226.6	53.9	106.6	86.9	19.7	56.5	9.6	0.20	11 d. postpartum
Av. 8–15 d. postpartum		225.4	72.1	78.5	63.6	14.9	41.3	33.5	0.14	
Av. of all three 8–15 d. postpartum		235.6	78.5	87.4	72.3	16.2	52.6	25.8	0.15	

[a] The value for each cow used for calculating the group averages was the average of all values between 8 and 15 d. postpartum.

TABLE 3

Blood plasma lipids in early and late stages of ketosis and the effect of postpartum fasting on plasma lipids

	No. of cows	Total plasma lipids	Plasma phospho-lipids	Total plasma choles-terol	Plasma ester choles-terol	Plasma free choles-terol	Fatty acids in			Free acids
							Choles-terol esters	Glycerol esters and as free	Total	
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*m. equiv./ 100 ml.*)
Early stage of ketosis (1–4 d.)[a]	2–4	269.3	89.2	116.2	91.9	24.2	54.1	9.9	64.0	0.10
Later stage of ketosis from 6th day on[a]	2–6	238.1	84.4	87.6	65.4	22.1	43.5	21.8	65.3	0.20
Cows on high plane of nutrition postpartum	4	324.2	119.8	118.4	114.0	19.8	77.9	3.2	81.1	0.13
Cows on low plane of nutrition postpartum	10	235.6	78.5	87.4	72.3	16.2	52.6	25.8	78.4	0.15
Cow starved for 8 d. postpartum	1	184.8	24.5	88.1	61.3	26.8	39.6	32.6	72.2	0.44

[a] Data from (1)

ciably. There are insufficient values for free acids to draw any precise conclusions. However, it will be noted that the free acids are lower before than after parturition. Also, when the values for the cows on a low plane of nutrition postpartum (table 2) are compared to those on a higher plane of nutrition (table 1), the free acids usually were higher after the cows had been on a low energy intake for several days.

TABLE 4

Plasma phosphate and phosphatase values of cows on high and low planes nutrition postpartum

Date	Cow	Plasma acid-soluble P			Phosphatase activity	Remarks
		Inorg.	Org.	Total acid-soluble		
		(*mg. %*)	(*mg. %*)	(*mg. %*)	(*units/ 100 ml.*)	
		A. Cows on high plane of nutrition postpartum				
9/ 3 48	Esmeralda	5.91	2.12	8.03		High protein feeding
9/17 48	Esmeralda	6.42	0.95	7.37		High protein feeding
12/ 3 48	Peggy	2.94	0.71	3.65		Medium protein feeding
12/ 4 48	Peggy	3.59	1.05	4.64		Medium protein feeding
2/11 49	Peggy	.....	.....	.....	3.07	Medium protein feeding
8/27 48	Acacia	3.91	1.58	5.49		Low protein feeding
9/ 3 48	Acacia	6.76	1.82	8.58		Low protein feeding
Av. of individual averages		4.92	1.37	6.29	3.07	
		B. Cows on low plane of nutrition postpartum				
8/25 48	Beth	6.03	0.77	6.80		High protein feeding
8/31 48	Beth	4.20	0.86	5.06		High protein feeding
3/28 49	Elinor	.....	.....	.....	3.07	High protein feeding
4/ 1 49	Elinor	4.58	0.92	5.50	1.92	High protein feeding
9/17 48	Bounty	4.46	1.18	5.64		Medium protein feeding
9/20 48	Bounty	4.56	0.69	5.25		Medium protein feeding
8/31 48	Remembrance	4.88	1.92	6.80		Low protein feeding
9/ 9 48	Remembrance	4.05	0.72	4.77		Low protein feeding
3/21 49	Melanie	1.87	0.43	2.30	1.58	Low protein feeding
3/23 49	Melanie	3.59	0.83	4.32	1.41	Low protein feeding
3/28 49	Melanie	.....	.....	.....	2.22	Low protein feeding
Av. of individual averages		4.28	0.92	5.20	2.11	

The data in table 2 are from cows which received 35 per cent of Morrison's feeding standards for total digestible nutrients for from 8 to 15 days postpartum. The difference in the protein intake did not appear to influence the blood lipids. However, several of the lipid fractions were altered by the low energy intake. Whereas several of the plasma lipid fractions of the cows on the higher energy intake (table 1) increased markedly in the early postpartal period, these same fractions either decreased or exhibited but a slight increase when the energy intake was maintained at a low level (table 2). This picture is similar to the results obtained with cows in early and late stages of ketosis (1). For purposes of comparison, a summary is given in table 3 of the data in table 1 and 2 together

with a summary of the data previously presented on ketotic cows (1). Cows receiving 70 to 80 per cent of Morrison's feeding standards for total digestible nutrients presented a blood plasma lipid picture very similar to that of cows in the early stages of ketosis. The cows on the lower plane of nutrition postpartum showed a blood plasma lipid picture very similar to that found in cows which had exhibited ketosis for some period of time. Apparently, the alterations in blood plasma lipids observed in cows with ketosis are due to the inanition which is associated with ketosis, rather than to ketosis as such. For purposes of comparison a Guernsey cow was fasted completely for 8 days beginning 3 wk. postpartum. At the beginning of the fasting period the blood plasma lipid picture was as follows: total lipids, 278.4 mg. per cent; plasma phospholipids, 95.0 mg. per cent; cholesterol-glycerol ester plus free fatty acid fraction, 183.4 mg. per cent; and free acids, 0.12 milliequivalents per 100 ml. of plasma. These values appear to be about normal for a cow in this stage of lactation. The plasma lipid values after 8 days of fasting are shown in table 3. Complete fasting produced blood lipid changes of a similar nature but of a greater magnitude than was noticed in ketotic cows.

In table 4, some data are presented on blood plasma acid soluble phosphates of cows on high and low levels of energy intake postpartum. Fasting appeared to have little or no effect on the plasma acid soluble phosphates. These data are quite similar to those observed in cows with ketosis (1).

Values are presented on serum phosphatase activity of two cows which were partially fasted postpartum. These values were somewhat below normal during the fasting period, which indicates that the low values observed in ketotic cows (1) may have been due to inanition.

SUMMARY

To determine whether some of the alterations previously observed in the blood lipids and phosphatase values of cows with ketosis are due to ketosis *per se* or secondarily to the inanition associated with ketosis, 16 cows were used in a study of the effect of different levels of protein and energy intake postpartum. The postpartal plasma lipid values of cows receiving 70 to 80 per cent of their total digestible nutrient requirements were similar to those of cows in the early stages of ketosis. The postpartal plasma lipid values of cows receiving only 35 per cent of their total digestible nutrient requirements were similar to those of cows in the later stages of ketosis. Complete fasting for 8 days produced alterations of the same nature but of greater magnitude.

ACKNOWLEDGMENT

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STUDIES ON KETOSIS IN DAIRY CATTLE. XIII. LIPIDS AND ASCORBIC ACID IN THE LIVER AND ADRENALS OF COWS WITH SPONTANEOUS AND FASTING KETOSIS[1]

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The few reports available (1, 2, 3, 6, 9) relative to the condition of the liver of cows and ewes with ketosis deal primarily with the pathology of this organ. In these reports a fatty liver always has been observed to be a part of the ketotic syndrome so that it has been assumed rather generally that the livers of ketotic ruminants always are fatty. It also has been shown by Groenewald *et al.* (1) with ewes and by Shaw *et al.* (8) on cows, that the adrenals tend to be fatty. The adrenal also was implicated when an extract from it was found to promote recovery of cows with ketosis (Shaw, 7).

It was deemed advisable to conduct further studies to determine whether these abnormalities are associated with the early development of ketosis or are secondary to the inanition associated with ketosis.

EXPERIMENTAL PROCEDURE

Studies were conducted on cows exhibiting spontaneous ketosis and on cows which had been fed at either a medium or low plane of nutrition postpartum. The feeding and management of the experimental cows was discussed rather briefly in a previous report (5). The blood glucose and acetone bodies were determined in all cases but will be reported elsewhere in connection with other studies. The methods used were similar to those discussed in a previous communication with the exception that the lipids were extracted from the ground tissue by repeated extraction with a warm alcohol-ether mixture (2:1) and purified by resolving in petroleum ether. Ascorbic acid was extracted from macerated tissue with 2.5 per cent metaphosphoric acid. Liver samples were obtained by biopsy and also after slaughter.

RESULTS

Liver lipid and ascorbic acid values of cows with both uncomplicated and complicated ketosis are presented in table 1. When compared with normal cows in mid-lactation, the total liver fat values will be observed to be high in most cases. The total cholesterol of the liver of the ketotic cows was much higher than that of normal cows in mid-lactation, the increase being due mainly to the ester cholesterol fraction. The free cholesterol fraction which represents the main form of cholesterol in the liver of normal cows is proportionally low in cows with ketosis. The ascorbic acid values vary widely, with some of the values being relatively low.

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TABLE 1

Total fat, cholesterol and ascorbic acid in the liver of cows with "spontaneous" ketosis

Date	Cow	Blood glucose	Blood acetone bodies	Total liver fat	Liver cholesterol			Liver ascorbic acid	Days with ketosis and comments
					Ester cholesterol	Free cholesterol	Total cholesterol		
		(*mg.* %)	(*mg.* %)	(%)	(*mg.* %)	(*mg.* %)	(*mg.* %)	(*mg.* %)	
				A. Apparently uncomplicated ketosis					
3/25/48	Hall	37.5	10.9	22.3	393.4	99.9	493.3	36.3	21 d., glucose administered
7/28/48	Inez	20.5	31.1	9.1	136.6	77.6	214.2	.....	4 d., glucose administered
7/28/48	Hermosa	23.8	15.5	4.0	167.3	0.0	167.3	.....	1 d., early ketosis
8/17/48	Beltsville (328)	22.3	45.0	6.9	213.0	82.6	295.6	.....	2 d., early ketosis
7/ 9/49	King	21.6	15.0	23.3	329.0	113.6	442.6	.....	11 d., glucose administered
	Av.			13.1	247.9	74.7	322.6	36.3	
				B. Complicated ketosis					
3/ 9/48	Hoffman	28.9	30.9	23.6	305.2	115.6	420.8	42.4	21 d., ruptured hypophysis
3/12/48	Thom	56.2	8.3	27.0	491.0	81.8	572.8	10.1	21 d., pneumonia
3/30/48	Cunningham	24.4	25.1	13.1	93.4	203.3	296.7	30.7	2 d., uterus inflamed
4/ 8/48	Burdette	41.2	8.1	11.2	72.4	193.2	265.6	29.1	13 d., atrophied hypophysis
2/ 7/49	Mullinix	33.7	38.9	16.7	308.0	43.0	351.0	16.2	13 d., ilium inflamed, ulcerated
3/17/49	Thomas	19.3	56.8	9.5	195.7	61.7	257.4	16.9	5 d., abomasum and duodenum slightly inflamed
5/ 3/49	Sherman	22.9	24.0	8.4	289.0	24.8	313.8	25.3	9 d., severe inflammation of abomasum and intestines
	Av.			15.8	250.7	103.3	354.0	24.4	
				C. Normal cows in mid-lactation					
6/29/48	Roma	.....	.....	4.0	57.7	137.3	195.0	.....	
7/ 6/48	Roma	.....	.....	2.8	37.4	180.8	218.2	.....	
6/29/48	Belladonna	.....	.....	2.2	25.7	111.3	137.0	.....	
7/ 6/48	Belladonna	.....	.....	1.8	35.8	108.4	144.2	.....	
	Av.			2.7	39.2	134.5	173.6	.....	

The data on the liver lipids were grouped according to the stage of ketosis as shown in table 2. These data show, contrary to the general belief, that liver

TABLE 2

Total fat and cholesterol in the livers of cows in early and in late stages of ketosis

Date	Cow	Total liver fat	Liver cholesterol		
			Ester cholesterol	Free cholesterol	Total cholesterol
		(%)	(*mg.* %)	(*mg.* %)	(*mg.* %)
		A. Early stage of ketosis (from 1–5 d.)			
7/28/48	Inez	9.1	136.6	77.6	214.2
7/28/48	Hermosa	4.0	167.3	0.0	167.3
8/17/48	Beltsville (328)	6.9	213.0	82.6	295.6
3/17/49	Thomas	9.5	195.7	61.7	257.4
	Av.	7.4	178.2	55.5	233.8
		B. Later stage of ketosis (from 11–21 d.)			
3/25/48	Hall	22.3	393.4	99.9	493.3
7/ 8/49	King	23.3	329.0	113.6	442.6
3/ 9/48	Hoffman	23.6	305.2	115.6	420.8
2/ 7/49	Mullinix	16.7	308.0	43.0	351.0
	Av.	21.5	333.9	93.0	426.9

fat may be normal or only slightly increased in the early stages of ketosis. The data on the liver fat of the cow, Hermosa, are of particular interest. A sample of liver was taken by liver biopsy on the first day that any signs or symptoms of ketosis were observed. The blood glucose showed a sharp drop on this day and the first increase was noticed in the blood acetone bodies. The liver fat was only 4 per cent which is quite low for this stage of lactation. The total liver fat of the cow Beltsville 328, which also was a very early case of ketosis, was only 6.9 per cent. In later stages of ketosis the total liver fat always was high. The increase in liver cholesterol, especially in the ester fraction, clearly is associated with the stage of ketosis, since there was a marked elevation in the later stages of ketosis.

The postpartal liver lipid values of normal cows on different levels of protein and energy intake during the postpartal period are shown in tables 3 and 4. For purposes of comparison, prepartal values also were determined on these cows. Table 4 represents cows on a low level of energy intake postpartum and table 3 includes cows on a higher level of energy intake during the postpartal period.

As will be observed in table 3, the postpartal liver fat and cholesterol values were somewhat higher than before parturition or in mid-lactation (table 1). At this higher level of energy intake, the level of protein intake did not appear to influence the liver lipids.

The data in table 4 are in rather sharp contrast to those in table 3. A low level of energy intake postpartum increased the total liver fat markedly as well as the liver cholesterol, especially the ester cholesterol fraction. In case of fast-

TABLE 3

Fat and cholesterol in the livers of cows on a high plane (70–80%) of nutrition postpartum

Date	Cow	Total liver	Liver cholesterol			Remarks
			Ester cholesterol	Free cholesterol	Total cholesterol	
		(%)	(*mg.* %)	(*mg.* %)	(*mg.* %)	
			A. High protein feeding			
8/20/48	Esmeralda	2.3	93.5	.....	.....	Prepartum
9/ 9/48	Esmeralda	5.5	182.8	64.4	247.2	14 d. postpartum
12/18/48	Rubye	6.8	102.1	356.8	458.9	Day of parturition
1/ 3/49	Rubye	5.4	36.3	253.9	290.2	16 d. postpartum
4/ 6/49	Anxiety	4.1	.....	.....	.....	Prepartum
4/20/49	Anxiety	6.7	.....	.....	.....	12 d. postpartum
4/29/49	Virginia	9.7	.....	.....	.....	13 d. postpartum
5/ 4/49	Canary	3.1	.....	.....	212.5	Prepartum
6/ 3/49	Canary	4.2	392.7	259.3	652.0	14 d. postpartum
Av. of individual av. (12–16 d. postpartum)		5.8	203.7	192.5	376.1	
			B. Medium protein feeding			
5/14/48	Bonita	3.5	.....	.....	247.0	Prepartum
5/27/48	Bonita	9.2	.....	.....	227.0	7 d. postpartum
6/ 3/48	Bonita	5.8	.....	.....	276.0	10 d. postpartum
8/ 7/48	Faith	8.1	93.2	168.2	261.4	Day of parturition
8/20/48	Faith	6.5	237.8	.....	.....	13 d. postpartum
Av. of individul av. (10–13 d. postpartum)		6.7	237.8	.....	276.0	
			C. Low protein feeding			
8/13/48	Acacia	4.5	121.0	58.9	179.9	Prepartum
9/ 3/48	Acacia	6.0	.....	.....	.....	12 d. postpartum
12/21/48	Pomona	5.1	103.2	138.1	241.3	Prepartum
1/13/49	Pomona	5.9	67.0	371.0	438.0	13 d. postpartum
4/ 6/49	Charm	3.8	.....	.....	.....	Day of parturition
4/20/49	Charm	8.2	.....	.....	.....	14 d. postpartum
4/ 8/49	Hilda	3.9	.....	.....	.....	Prepartum
4/29/49	Hilda	7.1	.....	.....	416.7	12 d. postpartum
Av. of individul av. (12–14 d. postpartum)		6.8	67.0	371.0	427.2	
Av. of all groups (10–16 d. postpartum)		6.7	169.5	281.8	359.8	

ing, the level of protein also appears to have exerted an effect, the total liver fat and the ester cholesterol usually being higher when the protein intake was limited.

For purposes of comparison the liver lipid values in early and in later stages of ketosis are presented in table 5, together with those of cows on low and higher levels of energy intake postpartum. The total liver fat and ester cholesterol values are quite similar when these values for cows in the early stages of ketosis are compared to the postpartal values of cows on a 70 to 80 per cent plane of nutrition. Likewise, these values were increased both in the later stages of ketosis and on the lower level of nutrition postpartum. In the later stages of ketosis the total liver fat, total liver cholesterol and ester cholesterol

TABLE 4

Total fat and cholesterol in the liver of cows with fasting ketosis

Date	Cow	Blood glucose	Blood acetone bodies	Total liver	Liver cholesterol			Remarks and days fasted postpartum
					Ester cholesterol	Free cholesterol	Total cholesterol	
		(*mg. %*)	(*mg. %*)	(%)	(*mg. %*)	(*mg. %*)	(*mg. %*)	
				A. High protein feeding				
6/ 9/48	Martha	50.8	4.6	5.1	.....	.....	.....	Prepartum
6/20/48	Martha	41.0	9.4	4.2	.....	.....	.....	7 d. postpartum
8/13/48	Beth	47.8	3.0	4.1	121.3	62.9	184.2	Prepartum
8/24/48	Beth	37.8	7.4	9.6	168.4	66.7	235.1	4 d. postpartum
8/31/48	Beth	16.8	24.6	15.8	196.7	7.4	204.1	11 d. postpartum
12/21/48	Lizzie	48.4	3.7	4.7	36.3	163.4	199.7	Prepartum
1/ 3/49	Lizzie	24.1	18.0	11.1	126.7	99.0	225.7	11 d. postpartum
3/11/49	Elinor	46.8	3.0	4.1	112.8	140.9	253.7	Prepartum
4/ 1/49	Elinor	12.2	23.7	8.3	123.8	7.0	130.8	10 d. postpartum
4/29/49	Barbara	49.5	.....	3.0	98.7	63.9	162.6	Prepartum
5/13/49	Barbara	11.9	.....	13.9	88.6	107.1	195.7	Day of parturition
Av. of individual av. (10–11 d. postpartum)				11.7	149.1	37.8	186.9	
				B. Medium protein feeding				
4/27/48	Valencia	44.3	3.8	4.3	164.2	.....	248.0	Prepartum
5/14/48	Valencia	26.5	9.3	16.4	.....	.....	516.0	9 d. postpartum
5/19/48	Valencia	26.8	8.2	13.4	.....	.....	265.0	14 d. postpartum
5/20/48	Valencia	.....	.....	12.5	.....	.....	265.8	15 d. postpartum
7/29/48	Adventuress	42.7	1.9	2.6	22.5	94.4	116.9	Prepartum
8/10/48	Adventuress	20.2	25.3	15.3	201.0	76.4	277.4	9 d. postpartum
8/16/48	Adventuress	33.9	20.5	17.3	183.7	99.7	283.4	15 d. postpartum
9/ 9/48	Bounty	50.5	2.7	3.1	59.6	136.5	196.1	Prepartum
9/13/48	Bounty	46.1	1.5	4.7	198.1	32.1	230.2	1 d. postpartum
9/23/48	Bounty	20.7	23.6	.....	129.6	179.6	309.2	11 d. postpartum
9/25/48	Bounty	41.4	10.6	6.6	66.8	163.7	230.5	Full-fed
Av. of individual av. (9–15 d. postpartum)				15.2	160.9	133.8	312.8	

TABLE 4 (*continued*)

Date	Cow	Blood glucose	Blood acetone bodies	Total liver	Liver cholesterol			Remarks and days fasted postpartum
					Ester cholesterol	Free cholesterol	Total cholesterol	
		(*mg.* %)	(*mg.* %)	(%)	(*mg.* %)	(*mg.* %)	(*mg.* %)	
				C. Low protein feeding				
6/ 9/48	Bunny	47.1	3.1	4.0	.....	.....	209.4	Prepartum
7/ 9/48	Bunny	35.8	17.2	16.2	168.8	116.1	284.9	12 d. postpartum
7/12/48	Bunny	33.4	17.5	12.7	232.0	28.7	260.7	15 d. postpartum
8/20/48	Remembrance	43.5	1.0	1.8	106.3	.....	.....	Prepartum
8/31/48	Remembrance	47.4	4.1	6.5	77.6	166.5	244.1	1 d. postpartum
9/ 9/48	Remembrance	27.2	21.6	21.4	232.7	98.7	331.4	10 d. postpartum
2/25/49	Melanie	51.6	3.4	4.7	53.6	146.0	199.6	Prepartum
3/23/49	Melanie	25.1	15.6	28.4	434.5	80.9	515.4	11 d. postpartum
4/20/49	Melanie	35.6	.....	12.4	325.1	160.8	485.9	Full-fed
Av. of individual av. (10–15 d. postpartum)				21.4	239.2	84.0	373.2	
Av. of all groups (9–15 d. postpartum)				16.1	199.7	85.2	290.9	

in the liver were somewhat higher than was the case when the cows were on a 30 per cent level of nutrition postpartum.

Table 5 also includes liver lipid values for a cow fasted for 8 days beginning 3 wk. postpartum. At the beginning of the fasting period, the total liver fat was 5.5 per cent. After 8 days of fasting it had increased to 36.6 per cent. Both free and total cholesterol values were of about the same magnitude as in the later stages of ketosis. The ester cholesterol showed about the same increase as was observed in the later stages of ketosis. Free cholesterol had decreased to about the same extent as was observed in ketosis and as the result of lowered energy intake postpartum. The increase in liver fat was in all cases due almost entirely to an increase in the neutral fat fraction.

TABLE 5

Comparison of liver lipid values in early and later stage of ketosis and on low and higher levels of energy intake for 9–16 days postpartum

	No. of animals	Total liver fat	Liver cholesterol		
			Ester cholesterol	Free cholesterol	Total cholesterol
		(%)	(*mg.* %)	(*mg.* %)	(*mg.* %)
Cows with early ketosis (1–5 d.)	4	7.4	178.2	55.5	233.8
Cows with late ketosis (11–21 d.)	4	21.5	333.9	93.0	426.9
Cows on higher plane of nutrition postpartum	11	6.4	169.5	281.8	359.8
Cows on lower plane of nutrition postpartum	9	16.1	199.7	85.2	290.9
Cows fasted for 8 d. postpartum	1	36.6	287.7	95.8	383.5

A similar comparison to that presented in tables 1 to 5 was made on the adrenals. These data are shown in table 6. The total fat of the adrenals of ketotic cows was higher than that of normal cows and usually higher than that of cows partially fasted postpartum. Four such values in the later stages of ketosis were higher than that of a cow in the earlier stage. The ester cholesterol showed the opposite picture. Partial fasting appeared to increase the adrenal fat to some extent, but not as much as in the case of some of the cows with ketosis. Complete fasting for 8 days resulted in a total adrenal fat of 13.8 per cent, which is higher than most of the observations made on ketotic cows. The free cholesterol value was higher than any previously observed. The adrenals of cows with either spontaneous ketosis or fasting ketosis exhibited low ester cholesterol and high free cholesterol.

The adrenal ascorbic acid values of cows with ketosis were somewhat low (table 6); however, the adrenal ascorbic acid content of a normal cow on a low energy intake postpartum also was low and the adrenal ascorbic acid content of the cow fasted completely was the lowest observed.

DISCUSSION

These data are believed to be the first to demonstrate that the liver lipids of cows in the early stages of spontaneous ketosis may be relatively normal and that the extremely fatty livers of these cows are associated with the later stages

TABLE 6

Total fat, cholesterol and ascorbic acid in the adrenals of cows with "spontaneous" ketosis and of cows on varying levels of energy intake postpartum

Date	Cow	Blood glucose	Blood acetone bodies	Total adrenal fat	Adrenal cholesterol			Adrenal ascorbic acid	Comments
					Ester cholesterol	Free cholesterol	Total cholesterol		
		(*mg. %*)	(*mg. %*)	(%)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	
				A. Apparently uncomplicated ketosis					
3/25/48	Hall	37.5	10.9	5.9	54.5	204.9	259.4	66.6	Glucose administered
7/ 9/48	King	21.6	15.0	8.6	164.8	222.8	387.6	.....	Glucose administered
	Av.			7.3	109.7	213.9	323.5	66.6	
				B. Complicated ketosis					
3/ 9/48	Hoffman	28.9	30.9	7.6	170.5	217.3	387.8	64.4	
3/12/48	Thom	56.2	8.3	4.9	56.0	211.1	267.1	106.0	Glucose administered
3/30/48	Cunningham	24.4	25.1	14.0	62.3	257.6	319.9	110.0	
4/ 8/48	Burdette	41.2	8.1	11.2	70.3	309.1	379.4	62.2	Glucose administered
2/ 7/49	Mullinix	33.7	38.9	6.9	160.3	143.7	304.0	85.9	
3/17/49	Thomas	19.3	56.8	4.8	46.8	232.6	279.4	90.4	
	Av.			8.2	94.3	228.6	322.9	86.5	
				C. Early stage of ketosis (5 d.)					
2/17/49	Thomas			4.8	46.8	232.6	279.4	90.4	
				D. Later stage of ketosis (10–21 d.)					
3/25/48	Hall			5.9	54.5	204.9	259.4	66.6	
7/ 8/48	King			8.6	164.8	222.8	387.6	.....	
3/ 9/48	Hoffman			7.6	170.5	217.3	387.8	64.4	
2/ 7/49	Mullinix			6.9	160.3	143.7	304.0	85.9	
	Av.			7.3	137.5	197.2	334.7	72.3	

TABLE 6—(*Continued*)

Total fat, cholesterol and ascorbic acid in the adrenals of cows with "spontaneous" ketosis and of cows on varying levels of energy intake postpartum

Date	Cow	Blood glucose	Blood acetone bodies	Total adrenal fat	Adrenal cholesterol			Adrenal ascorbic acid	Comments
					Ester cholesterol	Free cholesterol	Total cholesterol		
		(*mg. %*)	(*mg. %*)	(%)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	
					E. Normal cows				
4/ 8/48	Eskay 1			3.4	94.0	223.4	317.4	149.5	Mid-lactation
12/21/49	Eskay 2			3.4	178.7	135.8	314.5	.....	Later stage of lactation
12/21/49	Eskay 3			3.2	297.2	95.8	393.0	.....	Later stage of lactation
12/21/49	Eskay 4			3.1	208.0	123.3	331.3	.....	Later stage of lactation
12/21/49	Eskay 5			2.7	159.0	200.7	359.7	.....	Later stage of lactation
12/21/49	Eskay 6			3.1	226.5	98.9	325.3	.....	Later stage of lactation
	Av.			3.2	193.9	146.3	340.2	149.5	
					F. Cows on low plane of nutrition postpartum				
5/20/48	Valencia			6.2	50.2	277.5	327.7	92.0	Medium protein feeding
7/12/48	Bunny			4.4	190.3	98.7	289.0	.....	Low protein feeding
	Av.			5.3	120.3	188.1	308.4	92.0	
					G. Cow fasted for 8 d. postpartum				
2/ 1/50	Lizzie			13.8	82.9	502.4	585.3	56.7	

of ketosis. It appears, therefore, that a fatty liver is not a primary cause of ketosis in cows. The data on the effect of fasting during the postpartal period suggest that the fatty liver associated with ketosis in cows is due to inanition. The same is true regarding the increase in the total cholesterol and ester cholesterol in the liver. The fatty adrenals observed in ketotic cows also appears to be due, mainly, to fasting. In both liver and adrenals the increase in fat was due, primarily, to neutral fat. However, fasting resulted in a decrease of free cholesterol and an increase of ester cholesterol in the liver and an increase in free cholesterol of the adrenals. The adrenals of the cows with ketosis were enlarged and flabby but contained more dry matter than was found in the adrenals of normal cows. The dry matter content of the adrenals was determined in the last three cases of ketosis studied and varied from 22.3 to 28.9 per cent. The adrenals taken from five normal cows showed a lower and rather constant dry matter content varying only from 20.5 to 21.3 per cent. The adrenal gland of the cow fasted completely for 8 days contained 24.6 per cent dry matter and 13.8 per cent fat but was smaller and firmer than the adrenals of the cows with ketosis. The results of a histological study of these glands will be reported elsewhere.

CONCLUSIONS

Liver lipids were determined on 12 cows with spontaneous ketosis, 24 normal cows on various levels of protein and energy intake postpartum and on two normal cows in mid-lactation. Similar studies were made on the adrenals of nine cows with ketosis, two normal cows which were partially fasted postpartum, one normal cow which was fasted completely for 8 days postpartum and one normal cow in mid-lactation.

The results show that, contrary to general belief, the fat content of the liver often presents normal postpartal values in the early stages of ketosis. The fatty liver appears in the later stages of ketosis. This effect was reproduced by fasting postpartum, and indicates that the fatty liver associated with spontaneous ketosis is due for the most part to inanition. The total cholesterol, and especially the ester cholesterol fraction followed the same pattern. It is concluded that a fatty liver is not a predisposing factor in the development of most cases of spontaneous ketosis.

Postpartum-fasted cows which received a low protein ration, both before and after parturition, exhibited livers with a higher fat content than cows on a high protein ration.

The high fat content of the adrenals of ketotic cows also was reproduced by fasting. The free cholesterol increased and the ester cholesterol decreased in both fasting and spontaneous ketosis, which is opposite to the change observed in livers.

Some of the ascorbic acid values of both the liver and adrenals of cows with ketosis were somewhat low. This also was reproduced by fasting.

The flabbiness of the adrenals observed in ketotic cows was not reproduced by fasting and could not be explained on the basis of the water or fat content of these glands.

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STUDIES OF HEATED MILK. II. ACETOL AND RELATED COMPOUNDS[1]

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It has been reported previously that the heating of skim milk evidences the formation of carbonyl compounds, and further, that these compounds can be removed from the milk for further study by such procedures as steam distillation or ether extraction (11). Since these compounds are heat generated in the milk, and, as such, represent end products of the chemical changes taking place, it was considered expedient to accomplish their identification insofar as possible.

EXPERIMENTAL

Acetol. Forty pounds of condensed skim milk (29 per cent total solids) were autoclaved in a sealed, stainless steel can at 122° C. for 3 hr. and then cooled to room temperature. The contents of the can were filtered, the curd discarded and the brown whey-filtrate retained for steam distillation. Steam distillation was accomplished using conventional apparatus, mineral oil being employed as an anti-foaming agent. The distillate was collected for a period of 45 min. after which time the distillate was transferred to a separatory funnel and extracted five times with an equal volume of ethyl ether. The ether layers were combined, set aside for other investigations and attention given to the extracted distillate. It was found to give the following reactions: an orange precipitate with 2,4-dinitrophenylhydrazine reagent, iodoform with iodine-potassium iodide reagent and a brownish-red color when submitted to the nitroprusside test, this color becoming a stable greenish-blue upon acidification of the reaction mixture.

Sufficient of the 2,4-dinitrophenylhydrazone (2,4-DNPH) for purification and recrystallization was prepared by adding 0.5 g. of the reagent in 10 ml. of concentrated H_2SO_4 to 750 ml. of extracted distillate. After 4 hr. the precipitated reaction product was recovered by filtration. It was found to be slightly soluble in alcohol and to give a purple color when treated with dilute alcoholic sodium hydroxide. Strain (13), among others, has indicated that the bis 2,4-DNPH's of glyoxal and diacetyl exhibit a dark blue color on treatment with alcoholic alkali. The product was recrystallized from nitrobenzene and after two such treatments no melting point increase could be effected, the final melting point being 296–297° C. with decomposition.

A search of the literature revealed methylglyoxal as forming a 2,4-DNPH of this melting point, the reported values being 296–297, 297, 298 (2) and 299–300 (7). The carbon, hydrogen and nitrogen analyses of the derivative from

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extracted distillate were observed to compare well with those of the bis 2,4-DNPH of methylglyoxal: Calculated for $C_{15}H_{12}O_8N_8$, carbon 41.67 per cent, hydrogen 2.80 per cent and nitrogen 25.92 per cent; found, carbon 41.82 per cent, hydrogen 3.21 per cent, nitrogen 25.67 per cent.

The above data, together with the qualitative reactions given by the extracted distillate, strongly suggested that acetol or methylglyoxal might be the compound in question. Both of these compounds give the same 2,4-DNPH and similar results with many qualitative tests (12). The authentic 2,4-DNPH of methylglyoxal was made and observed to melt at 296–297° C., both alone and when intimately mixed with the derivative prepared from the heated milk distillate.

With the identity of the derivative established, it remained to ascertain whether the parent compound was acetol or methylglyoxal. Both the steam distillate of heated milk and the ethyl ether extractable material from heated milk gave positive results in the test for acetol developed by Baudisch and Deuel (1). This test depends upon the reaction of acetol with *o*-aminobenzaldehyde to give 3-hydroxyquinaldine, which melts at 265° C. and gives a brilliant blue fluorescence in dilute aqueous solution when exposed to ultraviolet light. This reaction is not given by methylglyoxal (1). In conducting the tests for acetol in heated milk, the 3-hydroxyquinaldine was isolated and identified by melting point, as well as by its blue fluorescence.

Acetaldehyde. For the most part, the carbonyl compounds of heated milk appear to be present in quantities too small to permit direct study. However, the conversion of these compounds into their 2,4-DNPH's afforded a practical approach to the problem, providing some suitable method could be developed for separating and purifying the mixed derivatives. With this aim in mind, chromatographic separation of the derivatives prepared from the ether extract of heated milk was attempted. These experiments were largely unsuccessful. However, it seems salient, in view of recent findings (10), to report the isolation of the 2,4-DNPH of acetaldehyde in one instance. The pure crystalline derivative was found to melt at 166–167° C. and to give elemental analyses which compared very well with those calculated for the 2,4-DNPH of acetaldehyde, melting point, 168° C. (9). The mixed melting point of the unknown and an authentic derivative showed no depression (166–167° C.).

Acetic acid. During certain phases of this investigation, the presence of acetic acid in ethyl ether extract residues from heated milk was fairly obvious by odor alone. Since the presence of this compound in heated milk does not appear to have been reported previously, a concerted effort was made to confirm the fact. Acetic acid was isolated and identified both from the steam distillate and the ether extract of heated milk. For the sake of brevity, only an account of the steam distillate isolation will be presented.

Concentration of the acids present in 2 l. of steam distillate, collected from 50 lb. of autoclaved skim milk (29 per cent total solids), prepared as previously described, was effected as follows: The pH of the distillate was adjusted to the phenolphthalein end point with sodium carbonate, after which treatment the distillate was extracted four times with equal volumes of ethyl ether. The ex-

tracted distillate was concentrated under vacuum to a volume of 75 to 100 ml. This concentrate was acidified to liberate the acids and then extracted five times with 100-ml. volumes of ethyl ether. The combined ether layers were dried and the solvent removed by evaporation in a water bath at 50° C. The pungently acidic residue (3 g.) was distilled and yielded three fractions boiling as follows: 99.5–100, 100–110 and 110–115° C. Distillation was stopped at 115° C., since it had been noted previously that the temperature rose very rapidly thereafter and that decomposition of the residue occurred. The two lower-boiling fractions were observed, by qualitative tests, to contain appreciable quantities of formic acid. The fraction boiling 110–115° C., of about 0.5 g. in weight, had a strong, distinct odor of acetic acid and contained no formic acid, as evidenced by a very slow reaction with $KMnO_4$ reagent. A *p*-nitrobenzyl ester derivative, melting point 77–78° C., was prepared from this fraction. This derivative showed no depression in melting point on admixture with *p*-nitrobenzylacetate, the accepted melting point for which is 78° C. (8). In additional experiments, the presence of acetic acid was further confirmed by observation of refractive index, n_D^{23} 1.372; the preparation of the *p*-bromphenacyl ester, melting point 84–85° C. (8) and conversion of the acetic acid to ethyl acetate.

Control experiments. Ten pounds of condensed skim milk, processed in the same manner as the condensed milks used for autoclaving, were steam distilled for a period of 45 min. Periodic tests on the distillate indicated an absence of carbonyl compounds during the first 25 min., after which time a very slight positive reaction with 2,4-dinitrophenylhydrazine reagent could be noted. The absence of these compounds at the start of the treatment would appear to be the critical point. With the autoclaved milks, the first 5 to 10 min. of distillation gave the greatest yield of carbonyl compounds, after which time they were continuously evolved at a lower rate. The distillate from the non-autoclaved milk exhibited no distinct acidity. Thus, the chemical differences in the steam distillates of autoclaved and non-autoclaved condensed skim milks were amply demonstrated for the purposes of this investigation.

DISCUSSION

The results of this investigation have established acetol as one of the compounds produced in milk by heat. However, the possibility that methylglyoxal was copresent has not been precluded by this study.

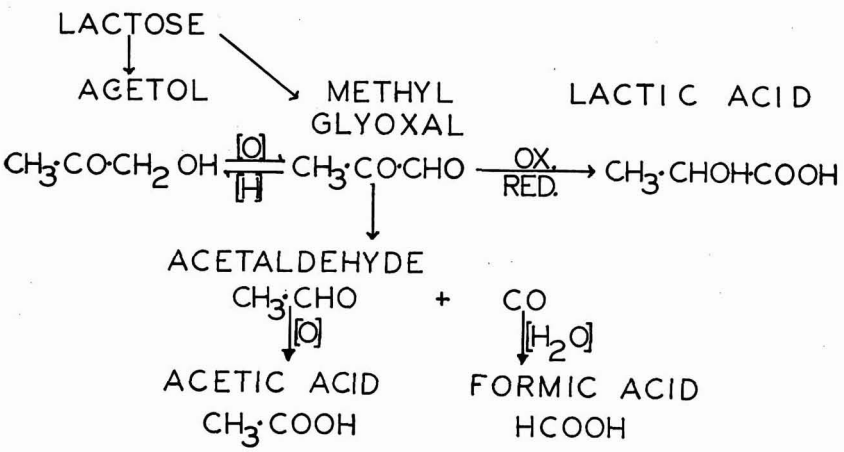
From earlier literature in the field of carbohydrate chemistry (1, 4), it might have been anticipated that acetol or methylglyoxal is formed in milk as a result of heating. Merely heating aqueous sugar solutions has been reported to produce small quantities of the above compounds (3). However, the matter of distinguishing between acetol and methylglyoxal when present at great dilution poses a difficult problem. This subject has been reviewed thoroughly and greatly clarified in a recent paper by Sattler and Zerban (12).

Although data in the literature concerning the physical and chemical properties of methylglyoxal are scarce, observations concerning the acetol-methylglyoxal relationship in these experiments indicated that the latter is present in traces,

if at all. Methylglyoxal is reported to commence boiling at 72° C. (7). No such compound was detected in this study. On the other hand, the ether extract fraction giving a positive Baudisch and Deuel test for acetol, was observed to be relatively stable and to boil in the vicinity of 140° C. These properties are in keeping with those of acetol. Sattler and Zerban (12) have observed a ratio of roughly 500 parts of acetol to one part of methylglyoxal in their study of this matter. It seems unlikely that methylglyoxal, an unstable, highly reactive compound, would exist in free form, in heated milk (122° C.) for any appreciable length of time.

The mechanism of acetol and methylglyoxal formation from sugars has been reviewed (3, 12). While the interrelationship of the compounds reported in this paper is a matter of conjecture, the following scheme is presented as accounting in a logical manner for the formation, in part at least, of certain of these and other compounds known to be produced in autoclaved milk.

Gould (5, 6) has demonstrated the presence of lactic and formic acids in heated milk. The present paper gives consideration to the other compounds concerned in the above scheme.



The presence of acetaldehyde in heated milk has not been firmly established in this investigation, since it was isolated only once in the form of its 2,4-DNPH derivative. However, in view of recent findings by Mohammad *et al.* (10), it seems worthy to note the presumptive presence of this compound in heated milk. The aforementioned group has observed that the rate of browning of protein-acetaldehyde systems is about 35 times as fast as that observed with protein-glucose systems under comparable conditions.

SUMMARY AND CONCLUSIONS

This study has demonstrated the presence of acetol and acetic acid in autoclaved condensed skim milk. The results of adequate control experiments preclude that these compounds are present in significant amounts in unheated

milk. Presumptive but not conclusive evidence that acetaldehyde is formed during the heating of milk also was obtained.

The relationship of acetol to methylglyoxal and certain other compounds known to be present in heated milk is discussed.

A wide variety of carbonyl compounds were observed to be formed during the prolonged heat treatment of milk. These compounds, for the most part, have not yet been identified.

ACKNOWLEDGMENTS

The authors are indebted to the Microchemical Division of Hoffmann-La Roche, Inc., for the performance of quantitative elemental microanalyses in connection with this research.

The *o*-aminobenzaldehyde used in this study was located through the courtesy of Louis Sattler of the Chemistry Department, Brooklyn College.

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ABSTRACTS OF LITERATURE

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BOOK REVIEW

460. Elements of Dairying. 2nd ed. T. M. OLSON. The Macmillan Co., New York, N. Y. 708 pp. 1950.

This book, originally printed in 1938, is designed to serve as a text for use of college students in a first course in dairying. In the revised edition, the material is discussed under the three main headings of dairy cattle, dairy products and dairy farming. Needed new information dealing with the selection of individual dairy cattle, maintaining a profitable herd, a cropping system for dairy farms, prevention and care of diseases affecting dairy cattle, nutrition deficiencies, dairy farm buildings (including equipment) and plans for acquiring a dairy farm have been added by the author. In the appendices new material has been added, including a list and history of the dairy organizations and up-to-date score cards on dairy products and dairy cattle. The tables dealing with nutrition of dairy cows have been shortened with some elimination and other tables covering growth of dairy cattle have been added. At the end of each chapter there are questions on the important points covered. The book is well organized, illustrated and indexed. C. Y. Cannon

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

461. The treatment of chronic bovine mastitis with aureomycin. R. A. PACKER, Iowa State College, Ames. Vet. Med., **45,** 5: 199–201. May, 1950.

Aureomycin was used for treatment of 91 quarters with chronic staphylococcic infections. Except for 1 animal, all of the animals treated were from 2 herds. Diagnosis of the infection was made by isolation and identification of the causitive organism. Of 70 quarters infected with *S. aureus* treated with 1 200-mg. dose of aureomycin hydrochloride, 34.3% were considered freed of the organisms. *S. aureus* was eliminated in 68.5% of 35 quarters by the administration of 2 injections of aureomycin. The drug had little or no effect on 2 cases of *E. coli* infection or 12 cases of chronic streptococcic mastitis. The aureomycin was incorporated in an ointment base and dispensed in collapsible tubes. The drug was injected from these tubes directly in the teat canal after the regular milking period. The conclusions were that aureomycin is of definite value in the treatment of chronic bovine staphylococcic mastitis. B. B. Morgan

462. Report on subtilin and bacitracin as possible treatment for bovine mastitis. J. O. HEISHMAN, U. S. D. A., Beltsville, Md. Am. J. Vet. Research, **11,** 39: 206–210. Apr., 1950.

The bactericidal action of subtilin and bacitracin was tested against several strains each of *Str. agalactiae, Str. uberis* and hemolytic staphylococci on blood agar plates. *Str. agalactiae* was the most resistant to these antibiotics. A combination of bacitracin and penicillin was more effective than either one alone in preventing growth of these 3 groups of organisms. The *in vitro* tests indicated that these antibiotics should be effective against such organisms in mastitis cases. Infusions of subtilin, bacitracin and penicillin-bacitracin combination both in single and double doses were made into udders of cows known to be infected. Improvement in appearance of milk and clinical condition followed, and the udder tolerated the substances without noticeable disturbance of milk production. No significant permanent elimination of infection occurred, however, so these antibiotics were judged to be no better than others now generally available.
E. W. Swanson

463. Pathogenesis of bovine mastitis. II. The pathologic alterations in twenty-five glands. G. R. SPENCER and S. H. MCNUTT. Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **11,** 39: 188–198. Apr., 1950.

Examination of the gross and microscopic changes produced by mastitis infection in 18 udders from herds in which complete antemortem history and examination were available and 7

udders without antemortem information was made. Typical cases were described in detail. Nineteen of 43 quarters with no clinical history of mastitis were eliminating streptococci, and 13 of these showed varying areas of focal mastitis. More detailed examination may have revealed similar conditions in the remaining 6. Of 24 quarters having neither clinical nor bacteriological evidence of mastitis, 4 had foci of inflammation similar to those found in streptococcic mastitis. Palpation was found to be a poor method of diagnosing fibrosis. Firm areas in the gland were most often interstitial edema and retained secretions. Atrophy and fibrosis were most often found together. The inflammatory foci were characterized by a blocking of the small ducts with fibrin, leucocytes and organisms with a consequent distension of the alveoli with secretion and edema in the interstitial tissues. Following stasis of secretion, large numbers of organisms developed in the milk areas and the epithelium was destroyed. The principal site of the inflammatory changes was the ventral portion of the gland. Long standing cases had extensive areas of atrophy and fibrosis with a thickening and roughening of the large duct and cistern walls. These changes were readily detectable by gross inspection of the dissected gland. The indications were that inflammation was caused by and accompanied infection and aided the progress of infection by hindering the normal milk flow. A difference in susceptibility of cows is postulated on the basis of the wide variation in pathological changes which were poorly correlated with the duration of infection. E. W. Swanson

464. The Brucella abortus ring test. M. H. Roepke, K. G. Paterson, F. C. Driver, L. B. Clausen, L. Olson and J. E. Wentworth. Minn. Agr. Expt. Sta., St. Paul. Am. J. Vet., Research, **11,** 39: 199–205. Apr., 1950.

The ring test, conducted by mixing 1 drop of stained *Br. abortus* antigen with 1 ml. of milk and noting after about 1 hr. the amount of the dye in the cream layer and skimmilk layers, has been widely used in Denmark in a brucellosis control program. The application of the test in Minn. herds under an area control plan was investigated in 9 counties. Since most of the herds marketed cream, an adaptation of the test for cream samples was developed. Most of the tests were taken before the country-wide blood tests. The blood and ring tests agreed in 96.2% of 8,469 herds. The ring test was 68% efficient in locating infected herds; however, 65% of the infected herds not located did not have an infected cow in production so the ring test was 88% efficient for infected herds in which the infected animals were producing. False positives from the ring test were attributed to contamination of the milk weighing vat, frozen milk and imperfect technique. The test is proposed as a helpful adjunct to the blood test on the area control plan. E. W. Swanson

465. Persistence of Brucella abortus infection in cattle. C. A. Manthei and R. W. Carter, U. S. D. A., Beltsville, Md. Am. J. Vet. Research, **11,** 39: 173–180. Apr., 1950.

Presence of *Br. abortus* infection was detected by inoculation of guinea pigs and by cultural means in several groups of naturally and artificially infected cows over periods of 2 yr. Bacteremia was compared in unvaccinated, strain 19-vaccinated and strain 45/20-vaccinated cows. It was lowest in strain 19-vaccinated and highest in strain 45/20-vaccinated. Peak bacteremia was reached at 2 wk. Highest levels of bacteremia were accompanied by high abortion rate and high persistence of the infection. Groups of 24 naturally-infected and 38 artificially-infected cows were followed for successive pregnancies up to the 9th. Eight animals in each group ceased shedding *Br. abortus*. Recoveries of *Br. abortus* from uterine material, colostrum and blood and the number of abortions revealed no detectable difference between the course of natural and artificially produced infections. One group of 18 cows was artificially infected with a virulent strain of *Br. abortus*. The distribution of the organism was followed therein for 2 pregnancies, followed by autopsy of 15 cows and examination of the lymph glands for *Br. abortus*. Udder infection occurred in 17 of these cows and persisted in 16. The supramammary lymph gland was the commonest site of infection. Genital infection was erratic but was most persistent in a repeat-breeder cow. *Br. abortus* was found in 1 cow for 97 wk. and in another for 101 wk. *Br. abortus* was not found in spleens, livers, kidneys, ovaries, vaginas, bile, urine or mesenteric or iliocecal lymph glands from this group of cows. E. W. Swanson

466. Q fever studies in southern California. IX. Isolation of Q fever organisms from parturient placentas of naturally infected dairy cows. L. Luoto and R. J. Huebner, Nat'l. Inst. of Health, Bethesda, Md. Pub. Health Reports, **65,** 16: 541–544. Apr. 21, 1950.

The authors tested placental tissue of 33 serologically positive cows and found that 13 (39%) contained *C. burnetii*. Some placental tissues were infectious for guinea pigs after diluting as high as 1-100,000,000. They also found that *C. burnetii* was encountered more often during first parturitions than in subsequent ones.

The authors were unable to demonstrate the presence of *C. burnetii* in the placentas of 4 serologically negative cows. D. D. Deane

467. Anthrax in livestock during 1949 and incidence of the disease from 1945 to 1949. C. D. STEIN, B. A. I., Washington, D. C. Vet. Med., **45,** 5: 205–208. May, 1950.

A survey made in 1949 showed that 93 anthrax outbreaks were reported from 16 states with a loss of 773 animals. The outbreaks were sporadic and occurred primarily in cattle. Of the 93 outbreaks, 56 occurred in California, Louisiana and Texas. During the 5-yr. period from 1945–1949, 597 outbreaks involving 7,909 livestock in 32 states were reported. Abattoirs under Federal meat inspection during this 5-yr. period condemned 38 cattle for anthrax. Twenty cases of anthrax occurred in man, 15 in agricultural workers and 5 in veterinarians. The conclusions were that anthrax was of considerable importance and that every effort should be made to prevent its occurrence.

B. B. Morgan

468. Bovine endometritis—A review of literature to 1947, with special reference to the catarrhal type of the disease. F. L. M. DAWSON, Ministry of Agriculture and Fisheries, Weybridge, England. Brit. Vet. J., **106,** 3: 104–106. Mar., 1950.

A brief chronological review of the literature on bovine endometritis is given. The dates of the periodicals consulted ranged from 1843 to 1947. The paper is divided into several sections: (a) first controversial period: 1900–1924, (b) rise of "Nielsenism": 1925–1935, (c) discovery of reproductive hormones: 1935 onward, (d) bacteriological aspects and (e) clinical research methods.

B. B. Morgan

469. Studies with johnin and tuberculin intradermal tests in cattle naturally infected with Mycobacterium paratuberculosis (Johne's disease). D. SIKES and A. H. GROTH. Louisiana State Univ., Baton Rouge. Am. J. Vet. Research, **11,** 39: 181–187. Apr., 1950.

Intradermal injections of johnin and tuberculin were made at 6-mo. intervals from 1940–1944 in a herd of 400 cattle. A summary of the Johne's disease-positive cattle showed that reactions to johnin from the candal fold site were not as sensitive as from a previously unused neck site, being 9.5 and 77.6%, respectively. Reading at 48 hr. gave about 50% more positive responses than reading at 72 hr. Although the herd was presumed to be free of tuberculosis, 1.8% gave positive reactions to tuberculin at the candal fold site and 11.8% gave positive reactions at the neck site. The sensitivity of Johne's disease-infected cattle to tuberculin persisted for many months.

E. W. Swanson

Also see abs. no. 482.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

470. Report on trials of the Alfa buttermaking process. G. L. HILLS, L. BALLARD, F. WILKINSON, M. THOMAS and L. R. HUNTER, Australian Dairy Produce Board, Melbourne. (Mimeoprint) 1949 (?).

This preliminary report covers a number of trials in which the Alfa process of continuous buttermaking is compared with conventional churning (in wooden churns) by using divided lots of the same creams under controlled conditions in an Australian butter factory. The average score of the freshly made butter was practically the same for both processes, but occasionally tallowy flavors would develop in the Alfa butter due to Cu contamination from accessory equipment. The microbiological quality of the Alfa butter was superior to that of the churned butter, as shown by consistently lower total counts and almost complete absence of yeast and coliform contamination. The moisture in the Alfa butter was better dispersed than in the churned butter. Moisture distribution and other physical properties of the Alfa butter could be controlled by adjustment of the brine temperatures in the transmutator unit. The Alfa butter was shiny in appearance and had better spreadability at low temperature than churned butter. It also showed greater tendency to oil off when standing at 86° F. for 3 hr.

Composition control in the Alfa process is accomplished by the continuous addition of salt and water (or skim milk); under good conditions water and salt contents could be adjusted within ±0.10% of the desired figure. The Alfa process shows an advantage in overrun of 1.1% (based on fat loss and composition), but a cost study indicated that its manufacturing cost was 0.3–0.4¢/lb. than that of churned butter. However, this finding was distorted by the fact that the Alfa unit was not operated at full capacity.

The Alfa process as applied in this study is suitable only for sweet cream, since neutralized cream would cause excessive sludge deposition in the separator and interfere with the composition control. Other aspects of practicability and operation of the equipment are discussed.

V. H. Nielsen

471. Comparison of several methods for determining the butterfat content of sour cream. C. B.

LANE and R. L. FRANCE, Breakstone Bros., Inc. Laboratories, Walton, N. Y. Milk Plant Monthly **39,** 4: 38–39, 71. Apr., 1950.

Sour cream was tested by the Babcock, Mojonnier and Roese-Gottlieb methods. Samples were obtained from vat pasteurizers after homogenization and standardization had been completed. The Babcock procedure used 30%, 18 g., sealed, long-necked, 0.2% graduated bottles, into which 18 g. of sample were weighed. Fourteen to 17 ml. of sulfuric acid were added in 3 portions, after which 5–10 ml. of water at 60° C. were added. Centrifuging and reading of the tempered samples were according to the recommended Babcock testing procedure for cream. An average of the results obtained for 106 trials showed the Babcock procedure to be 0.30% below the Mojonnier method and 0.09% lower than the Roese-Gottlieb method. The Roese-Gottlieb method averaged 0.21% lower than the Mojonnier method.

J. A. Meiser, Jr.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

472. A new type of bacterial spoilage in Canadian process cheese. E. G. HOOD and J. F. BOWEN, Science Service, Dept. of Agr., Ottawa. Sci. Agr., **30,** 1: 38–42. Jan., 1950.

Two widely separated outbreaks of bacterial spoilage of process cheese food were investigated. The defective packages were badly swelled, contained gas holes and possessed a very obnoxious, putrefactive odor. Non-fat dry milk solids were used in the blends and the defect became apparent in samples held at 100° F. for 1–4 d. or in retail stores at summer temperatures in 1–2 wk.

An organism corresponding closely to *Clostridium sporogenes* was found to be responsible and was present in half the samples of non-fat dry milk solids used in the cheese food blends. Experimental batches made up with varying amounts of non-fat dry milk solids, with 2% casein digest and with 1-yr.-old cheese developed the defect when inoculated with the organism and incubated 5 d. at 98° F.

O. R. Irvine

Also see abs. no. 484, 486, 496.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

473. Browning and the fluorescence of evaporated milk. N. P. TARASSUK and H. D. SIMONSON, Div. of Dairy Ind., Univ. of Cal., Davis. Food Technol., **4,** 3: 88–92. 1950.

A method was developed for measuring the fluorescence of evaporated milk after the proteins were digested with pancreatin. A marked increase in fluorescence occurs during heat sterilization of evaporated milk ,and the chemical changes responsible for the increase in fluorescence continue during storage. Browning and fluorescence develop simultaneously during sterilization of evaporated milk, and their formation proceeds at a parallel rate, but the browning and fluorescent materials are not necessarily identical. Both browning and fluorescence show a close relationship to CO_2 production. The data indicate that the fluorescing and browning materials are associated with the milk protein and become soluble only when the protein is hydrolyzed. Fluorescence studies denote that high-temperature, short-time sterilization will reduce the brown discoloration and cooked flavor of evaporated milk and thereby produce a superior quality product.

E. R. Garrison

474. The "browning reaction" in dried milk powder. J. B. MOSTER and R. A. CHAPMAN, Macdonald College, Quebec. Can. J. Research, Sec. F, **27,** 11: 429–434. Nov., 1949.

Heated and stored dried whole milk powders showed a marked loss of amino nitrogen, as determined by the Van Slyke volumetric method, when compared with fresh powder. No such loss was observed when the formol titration was used. Titration curves (from pH 6.0–11.0) of the powders suggested a mechanism for the protein-sugar condensation. The heating of synthetic mixtures of amino acids and lactose resulted in intense browning, accompanied by a loss of amino nitrogen when a large excess of lactose was employed (1: 13), but no loss occurred when equal parts of sugar and amino acid were present.

O. R. Irvine

475. Mechanical cow lowers milk price in Whitehorse. L. HARRINGTON. Food in Canada, **9,** 12: 26, 28, 30. Dec., 1949.

Details are given of the reconstituting process by which non-fat dry milk solids, butter and water are combined to make a 4.2% fat milk. Sixty-five lb. of water, 6.25 lb. of powder and 3.25 lb. of butter are mixed at 100–112° F., after which the batch is pasteurized at 145° F. for 30 min. It then is homogenized, cooled and packaged in paraffined containers. Introduction of the product resulted in a lowering of the price of milk from 85–30¢/qt. in this Yukon community.

O. R. Irvine

476. Special milk powders for manufacture of milk chocolate. H. A. HOLLENDER (Abstract of a thesis for Doctor of Philosophy degree at the

University of Wisconsin). K. E. LANGWILL. Confectioner J., **75:** 44–47. Nov., 1949.

This thesis treats the subject of milk lipolysis and its effect on "milk" flavor of milk chocolate. Milk powders were prepared under varying temperatures and time treatment relationships with percentages of sugar from 0–10%. Powders then were incorporated into an experimental milk chocolate formula, heated and ground in a mortar to a plastic mass containing no visible particles of sugar or milk powders. Relation of free fatty acid to "milk" flavor was observed at 15 intervals. "Milk" flavor has a definite relationship to free fatty acids. Milk chocolate having the most desirable flavor has the highest free fatty acid content and the lowest pH. The lipase activity of the raw whole milk powders is accelerated by increased temperature of storage. This phenomena is not observed in milk chocolate containing milk powders prepared from properly forewarmed milk. Milk powders prepared with sugar seem to be conducive to best "milk" flavor. Tables are given to substantiate results. T. A. Eggers

477. Preparacion y conservation de la mezcla lactea Escudero con leche acidofila y leche bifida. (Preparation and preservation of Escudero's milk mixture with acidophilus and bifidus milk.) S. SORIANO and A. M. DE SORIANO. Rev. Asoc. argentina dietol., **6,** 23: 235–242. July, Aug., Sept., 1948.

A study was made of the keeping quality of Escudero's milk mixture (cereal water, milk, lactose, cream) used for feeding children. This mixture, when submitted to summer room temperatures (25° C.), became unacceptable within 24 hr. because of its high bacterial content. Increasing the acidity to 3.5% by the addition of lactic acid reduced the microflora development, and the product was acceptable for 48 hr. Acidification could be accomplished by adding lactic acid or milk fermented with *Lactobacillus acidophilus* or *L. bifidus*. An initial acidity as high as 3.5% was not necessary if the milk mixture was sterilized before inoculation with the pure cultures. The use of pure cultures had the disadvantage of requiring a bacteriologist. L. S. Olsen

478. Concentrated milk food product and process of preparing same. W. H. HOECKER and B. W. HAMMER. (Assignors to Golden State Co.) U. S. Patent 2,501,445. 8 claims. March 21, 1950. Official Gaz. U. S. Pat. Office, **632,** 3: 871. 1950.

The fat/milk solids-not-fat ratio of milk is adjusted to 0.5–1.75/1 by the addition of butterfat. After concentrating to 40–70% total solids and pasteurizing in the range of 150–190° F. for 0.5–30 min., the product is homogenized while hot at a pressure in the range of 500–3500 lb./in.^{2}. This product is free of cooked flavor, keeps well at refrigerator temperatures, is easily spreadable, has a uniform and smooth texture and is resistant to changes in viscosity at temperatures encountered during storage and use. R. Whitaker

479. De mogelijkheid van het verwerken van weibloem in brood. (On the possibility of using whey flour in the baking of bread.) (English summary.) H. HEERES and E. A. M. MEYKNECHT, State Dairy Organization, The Hague, Holland. Neth. Milk and Dairy J., **4,** 1: 54–79. Jan.–Mar., 1950.

A number of experiments in a commercial bakery were made to test the possibility of using whey flour in the baking of bread. Substitution of part of the wheat flour by whey flour caused a smaller loaf volume; in using 2%, a volume decrease of 2.7% was obtained which could not yet be considered significant. Baking flour consisted of: I. 80% of wheat flour of foreign origin + 20% of flour of home grown wheat. II. 90% of mixture I + 10% of potato flour. In case II with 2% whey flour, 4.2% decrease in volume was found. This tendency to diminish the volume of the loaf was the only objection to the use of 2% whey flour, as other properties remained the same or were slightly better. Difference in processing or fat content of the whey gave the same result. Lactose, a lactalbumin preparation, casein and dried skim milk (roller and spray) all caused a greater decrease in volume. Use of 2% whey flour in bread would take away a good part of the whey surplus. It would be good from a nutritional standpoint, as minerals and vitamins would be supplied which are deficient in plain normal bread. A. F. Tamsma

480. Concentration of albumin from whey. G. J. STREZYNSKI. (Assignor to DeLaval Separator Co.) U. S. Patent 2,500,101. 6 claims. March 7, 1950. Official Gaz. U. S. Pat. Office, **632,** 1: 289. 1950.

Whey is fed into the bowl of a centrifuge where an albumin-rich portion collects on the peripheral wall and a lactose-rich, albumin-free portion is removed from the central area. The albumin-rich portion, containing some lactose, leaves the bowl through ports, is collected and diluted with water and is directed back into the bowl through a channel ending at the ports. By proper balancing of the diluted albumin against the whey intake, a lactose-free albumin can be recovered from the ports. R. Whitaker

481. Method of stabilizing dried starch sirup. T. Nordenskjöld and E. A. Jönsson. U. S. Patent, 2,501,406. 1 claim. March 21, 1950. Official Gaz. U. S. Pat. Office, **632,** 3: 860. 1950.

To produce a dehydrated hydrolyzed starch product, free from hygroscopicity, the sirup is spray dried with milk. R. Whitaker

Also see abs. 503.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

482. Effects on acid production by lactic starters of various "drugs" in milk from mastitis-treated cows. W. A. KRIENKE, Fla. Agr. Expt. Sta., Gainesville. Milk Plant Monthly, **39,** 4: 32, 36–37. Apr., 1950.

The addition of 5.0 ml. of a 25% solution of sulfamethazine to 100 ml. of "drug-free" milk almost completely inhibited acid production when inoculated with 1% active buttermilk culture. One ml. and 0.1 ml. additions of the "drug" resulted in a developed acidity of 0.35% and 0.46%, respectively, as compared to 0.71% for the control. Thus, milk from 1 treated cow would render the milk from more than 80 untreated cows unfit for fermented dairy products.

The addition of 0.005 mg. of aureomycin hydrochloride to 1 ml. of "drug-free" milk greatly retarded the production of lactic acid in starters. However, reducing the concentration to 0.00005 mg. allowed a nearly normal acid production. A single infusion of 200 mg. of aureomycin hydrochloride thus would inhibit acid production in 1,000 lb. milk and retard it greatly in 1,400 lb. milk.

Milk from aureomycin-treated cows contained sufficient amounts of the "drug" after 12 milkings to retard acid production considerably. When 1% of the milk from the 1st milking was combined with 99% of the "drug-free" milk, acid production was completely inhibited. Mixtures containing 10% of milk from treated cows and 90% of milk from untreated cows did not favor acid production until the 6th milking.

The use of penicillinase as an inactivator of penicillin in milk was not practical since the cost of the enzyme necessary to permit normal acid development exceeded the cost of the milk.

J. A. Meiser, Jr.

483. Effect of concentration and reaction (pH) on the germicidal activity of chloramine-T. G. R. WEBER, Pub. Health Service, Cincinnati, O. Pub. Health Reports, **65,** 15: 503–512. Apr. 14, 1950.

The pH of a chloramine-T compound greatly affects its germicidal activity. Those compounds with a pH higher than approx. 7.5 were found to be too slow for practical use where short exposure periods were used. Increasing the concentration of the chloramine-T compound from 50–1,500 ppm. did not reduce the killing time sufficiently to equal that of even the more alkaline hypochlorites at 50 ppm. concentration. A chloramine-T compound in a concentration of 250 ppm. with a pH of not more than 7.0 or a concentration of 500–1,000 ppm. at a pH of not over 7.5 appeared to have a germicidal action, in the absence of organic matter, as rapid as that of the slower (alkaline) hypochlorites at 50 ppm. The author reported that commercial chloramine-T products do not as a rule have a pH as low as 7.0–7.5 and concluded that, while chloramine-T compounds appear to have a limited value where rapid germicidal action is needed, they may be the sterilizer of choice under conditions where long exposure periods are necessary. D. D. Deane

484. Syrningsvanskeligheder foraarsaget af Bakteriofager. (Starter failures due to bacteriophage.) A. J. OVERBY, Danish Royal Vet. & Agr. College Dairy Laboratory. Maelkeritidende, **62,** 47–48. 1949.

For the first time, bacteriophage active against multiple-strain starters has been demonstrated in Denmark. The phage did not survive a temperature of 85° C. (185.0° F.) for 5 min. or a temperature of 88° C. (190.4° F.) for several seconds. The bacteriophage was active against 11 of 14 isolated single cultures of lactic streptococci from "slow" starter.

One strain of the bacteriophage did not pass through a Seitz filter. Two electron micrographs showed that the head of the bacteriophage had a diameter of 0.15μ and the tail had a length of 0.30μ.

In a creamery that had experienced difficulty with slow starter, it was thought that bacteriophage from the air entered the starter and cream-ripening vats. After thoroughly sanitizing all equipment and atomizing a 5–10% hypochlorite solution in the various manufacturing rooms, no more difficulty was experienced.

The literature review contains 51 references.

G. W. Wilster

485. Estimation of lipase in dairy products. II. An extraction-titration method for the estimation of bacterial lipase. D. J. LUBERT, L. M. SMITH and H. R. THORNTON, Univ. of Alberta. Can. J. Research, Sec. F, **27,** 12: 491–498. Dec., 1949.

In testing for bacterial lipase a loopful of the culture is inoculated into 10 ml. of sterile skim

milk and incubated for 24–48 hr. The lipase then is estimated on 2 ml. of this culture using a procedure similar to that of abstract 494. Ether-soluble acids carried into the reaction medium do not interfere with the measurements and ether-soluble acids are not produced from protein or lactose during the test. The main test organism was *P. fluorescens.* O. R. Irvine

486. Estimation of lipase in dairy products. III. Lipase activity in cultures of micro-organisms and in cheese. D. J. LUBERT, L. M. SMITH and H. R. THORNTON. Can. J. Research, Sec. F, **27,** 12: 499–503. Dec., 1949.

The lipolytic activity of a number of micro-organisms was determined by the method of abstract 485. No organism produced a bacterial lipase having an activity optimum on the acid side of neutrality. No lipase activity at approx. pH 5.0 was demonstrated in 20 samples of commercial cheddar cheese or in 1 sample of blue veined cheese by this method, or by the method of Peterson *et al.* (J. Dairy Sci., 31, 1: 31–38. 1948). Weak lipolytic activity was found in 1 sample of blue cheese by the extraction-titration method. One sample of cheddar displayed no lipolytic activity when tested at pH 8.50.
O. R. Irvine

487. Estimation of lipase in dairy products. IV. Lipolytic activity of Pseudomonas fluorescens. D. J. LUBERT, L. M. SMITH and H. R. THORNTON. Can. J. Research, Sec. F, **27,** 12: 504–509. Dec., 1949.

Lipolytic activity of a strain of *P. fluorescens* was greatest when the reaction medium was at approx. pH 8.9 at the start of a reaction period and when the reaction was carried out at approx. 42° C. The lipase hydrolyzes tricaproin and tricaprylin less readily than tributyrin. $CaCl_2$ inhibited activity. Lipolytic activity was greater in nutrient broth-base medium than in skim milk, but the former gelled when ether was added. Lipolytic activity and fluorescence were not related. O. R. Irvine

488. Preservation of foods with antibiotics. I. The complimentary action of subtilin and mild heat. A. A. ANDERSEN and H. D. MICHENER. Western Regional Research Lab., Albany 6, Cal. Food Technol., **4,** 5: 188–189. May, 1950.

This new principle in food preservation is based on the destruction of enzymes and microorganisms with subtilin and mild heat. Some of the non-spore-forming bacteria, particularly the Gram-negative ones, are resistant to subtilin but sensitive to heat, while the heat-resistant organisms, such as clostridia and thermophiles, are extremely sensitive to subtilin with mild heat. Peas, asparagus, corn, green beans, peeled potatoes, tomato juice and milk have been preserved from microbial spoilage by this method of treatment. Experiments with peas, asparagus and corn were described to illustrate the process and its effectiveness in food preservation. In general, the addition of 10 or 20 ppm. of subtilin prevented spoilage when these foods were sealed in no. 1 cans, and the cans heated in boiling water for 10 or 20 min. and then stored at 77 and 122° F. All of the control cans (without subtilin but heated) spoiled during storage. The possible physiological effects of continued use of foods containing subtilin and other antibiotics has not been determined, and additional information on this subject is needed before safe use can be made of this principle in preserving foods. E. R. Garrison

489. Contribucion al conocimiento de las bacterias lipoliticas de la manteca. (Contribution to the identification of the lipolytic bacteria of fat.) A. M. SORIANO. Rev. Asoc. argentina dietol., **6,** 24: 284–292. Oct., Nov., Dec., 1948.

One hundred and three samples of fats acquired in the stores in the city of Buenos Aires were studied microbiologically with respect to the content of lipolytic bacteria capable of producing rancidity. The bacteria were isolated using Turner's differential plating medium for lipase-producing bacteria and the bacteria then were identified. Of the samples examined, 52.4% were contaminated with lipolytic bacteria belonging to 8 species, 5 of which belonged to the genus *Pseudomonas.* Fifteen samples contained no lipolytic bacteria.

The species encountered and the frequency with which they occurred in the samples examined were: *Pseudomonas traslucida* 33.85%, *Ps. incognita* 30.18%, *Ps. fluorescens* 28.30%, *Ps. arguata* 6.14%, *Ps. mira* 3.07%, *Bacillus effusus* 15%, *Achromobacter superficiale* 1.88% and *Achr. fomosum* 1.88%. L. S. Olsen

490. Salt tolerance in the genus Aerobacter. I. O. FODA and R. H. VAUGHN, Div. of Food Technology, Univ. of Cal., Berkeley. Food Technol., **4,** 5: 182–188. May, 1950.

Fifty-two cultures of coliform bacteria were isolated from olive brines by direct plating on Levine's E.M.B. agar after enrichment of the brines in glucose broth containing 10% salt. These cultures were identified as *Aerobacter aerogenes,* but differed from the common types of this species in their appearance on E.M.B. agar and from all other coliform bacteria tested because of their striking resistance to NaCl. In-

creased tolerance to salt, which extended up to 14.5% NaCl with some cultures, was obtained by periodically transferring the cultures to glucose broth with increasing salt concentrations. The additional resistance gained through acclimatization was adaptive and was readily lost when the bacteria were returned to a salt-free environment.
E. R. Garrison

491. Partial purification of a factor essential for growth of Leuconostoc citrovorum. J. C. Keresztesy and M. Silverman, Nat. Institute of Health, Bethesda, Md. J. Biol. Chem., **183,** 2: 473–479. Apr., 1950.

Concentration of an acid-labile factor required for the growth of *Leuconostoc citrovorum* (ATCC 8081) was achieved by norit adsorption of liver extract (fraction S) and butanol extraction of the concentrated eluates. In media lacking folic acid the concentrates containing the citrovorum factor promoted the growth of *Streptococcus lactis* R (*S. faecalis* R) and *Lactobacillus casei.* The citrovorum factor stored at room temperature for 24 hr. in 0.1*N* HCl lost 90–100% of its activity for *L. citrovorum,* but only 41–48% of its activity for *S. lactis* R. A similar treatment of folic acid failed to alter its activity. The implication of these results are discussed; however, further purification of the citrovorum factor is necessary before a satisfactory interpretation can be offered.
H. J. Peppler

492. Vitamin B_{12} and "citrovorum factor" in the nutrition of Lactobacillus leichmannii and Leuconostoc citrovorum. T. H. Jukes, H. P. Broquist and E. L. R. Stokstad, Lederle Lab., Pearl River, N. Y. Arch. Biochem., **26,** 1: 157–159. Mar., 1950.

Chromatographic and cultural studies provided an indication that the "citrovorum factor" (CF) is a compound which contains folic acid. The attending observations further suggest that certain precursors are converted to B_{12} (reaction A), which in turn participates in the conversion of other precursors into the desoxyribosides of guanine, adenine, hypoxanthine and cytosine. Also, folic acid is converted to CF (reaction B), which in turn participates in the reversible conversion of thymidine to the desoxyribosides. Previous findings established that vitamin B_{12} or the desoxyribosides of either guanine, hypoxanthine, adenine, cytosine or thymine promote the growth of *Lactobacillus leichmannii* 313, while CF or thymidine, but not the other desoxyribosides or vitamin B_{12}, permitted the growth of *Leuconostoc citrovorum* 8081 in a purified culture medium. Thus, *L. leichmannii* may accomplish step *B* in the above scheme, but not step *A*, while *L. citrovorum* would be able to carry out step *A* but not step *B*. The scheme is given further support by the discovery that *L. citrovorum* produced vitamin B_{12} activity in purified media, and *L. leichmannii* synthesized CF.
H. J. Peppler

493. Utilization of optical isomers of methionine and formylmethionine by some lactobacilli. J. R. Spies and D. C. Chambers, U.S.D.A., Washington, D. C. J. Biol. Chem., **183,** 2: 709–712. Apr., 1950.

The relative degrees of utilization of pure optical isomers of methionine and formylmethionine in a defined medium by *Lactobacillus arabinosus* 17–5, *Leuconostoc mesenteroides* P-60 and *Streptococcus faecalis* R were determined by an acidimetric method. None of the bacteria utilized D-methionine or formyl-D-methionine at levels of 6γ or 10γ/ml. medium. Only *S. faecalis* utilized L-methionine and its formyl derivative; growth with the latter was slightly better than it was with free L-methionine. Pyridoxine was found ineffective in promoting the utilization of D-methionine by *L. arabinosus.*
H. J. Peppler

Also see abs. no. 461, 462, 472.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

494. Estimation of lipase in dairy products. I. An extraction-titration method for the estimation of milk lipase. L. M. Smith, D. J. Lubert and H. R. Thornton, Univ. of Alberta. Can J. Research, Sec. F, **27,** 12: 483–490. Dec., 1949.

Milk lipase is determined by allowing 2 ml. of the skim milk to react on 0.6 ml. of tributyrin for 30 min. at 37° C. at pH 8.8 in the presence of borate buffer. The reaction is stopped by adding phosphoric acid and reducing the temperature, after which the reaction medium is extracted with ethyl ether. An aliquot of the ether layer then is titrated. The result of a blank determination is deducted from this value. Such factors as extraction efficiency, substrate concentration, pH, temperature and length of reaction period were examined and are discussed.
O. R. Irvine

495. Detecting alien fats. W. H. Martin, W. D. Ritz and C. H. Whitnah, Kansas State College, Manhattan. Ice Cream Field, **58,** 6: 20, 23, 24, 57, 58, 59, 61. Dec., 1949.

See abs. 75.

496. New and improved methods of extracting fat from cheese, fresh curd and milk for fat acidity determination. J. F. Bowen, E. G. Hood

and C. A. GIBSON, Dominion Dept. of Agr., Ottawa. Sci. Agr., **29,** 11: 551–552. Nov., 1949.

To secure samples of fat from cheddar cheese, approx. 250 g. are ground in a Waring blendor and heated in the dry state on a boiling water bath until "oiled off." In separating fat from fresh curd, approx. 800 g. are finely ground in suitably-sized portions in the blendor. To each portion 500 ml. of 90° C. water is added and thoroughly mixed, after which all portions are combined and held at 0° C. until a fat layer has formed. This fat layer then is churned, clarified by centrifuging and filtered. Samples of fat from milk are obtained by churning the fat, after which it is melted, centrifuged and filtered. The "acid degree" of these fat samples then is determined by titrating a 10-g. portion in boiling 95% neutral ethanol with 0.1 *N* NaOH, using phenolphthalien as indicator. O. R. Irvine

497. Rate of destruction of reduced ascorbic acid in riboflavin-fortified pasteurized milk. A. D. HOLMES. Mass. Agr. Expt. Sta., Amherst. Food Technol., **4,** 3: 92–93. 1950.

The milk used in this study was produced by a 70-cow herd composed of 5 breeds and was pasteurized at 143° F. for 30 min. in stainless steel vats. Riboflavin was added to 19 weekly samples of the freshly pasteurized milk in amounts of 0.0, 4.0 and 8.0 mg./l., and the milk stored in the dark at 10° C. Ascorbic acid determinations were made on the samples after 0, 24, 48, 72, and 96 hr. of storage. Additions of riboflavin did not increase rate of loss of reduced ascorbic acid in pasteurized milk. Samples fortified with 0, 4 and 8 mg. of riboflavin/l. showed an average loss of 77, 73 and 69%, respectively, of the original amounts of reduced ascorbic acid after 96 hr. of storage. E. R. Garrison

498. Effects of borate and other ions on the alkaline phosphatase of bovine milk and intestinal mucosa. C. A. ZITTLE and E. S. DELLA MONICA, Eastern Regional Research Lab., Philadelphia, Pa. Arch. Biochem., **26,** 1: 112–122. Mar., 1950.

The inhibitory effect of borate and other anions on alkaline phosphatase prepared from cow's milk and calf intestinal mucosa was studied in ethanolamine-HCl buffer containing sodium phenylphosphate; the phenol liberated was determined with the reagent of Follin and Ciocalteu. Both milk and mucosa phosphatases were inhibited competitively by sodium tetraborate, apparently of the anionic type, while the inhibition of milk phosphatase by ethanolamine was found to be of the noncompetitive (cationic) type. Milk phosphatase closely resembles kidney and bone phosphatases and is distinguished from the intestinal mucosa enzyme by its higher pH optimum, lower enzyme-substrate constant (K_s) at pH 9.6, greater inhibition by cations and lesser interference by anions. The inhibitory effects of the anions phosphate, pyrophosphate, carbonate and arsenate on the alkaline phosphatases are given for comparison with the data obtained with tetraborate. H. J. Peppler

499. Effects of glutamic acid, lysine and certain inorganic ions on bovine alkaline phosphatases. C. A. ZITTLE and E. S. DELLA MONICA, Eastern Regional Research Lab., Philadelphia, Pa. Arch. Biochem., **26,** 1: 135–143. Mar., 1950.

Earlier studies (*ibid.,* **26,** 1: 112–122) of attempts to distinguish between 2 types of alkaline phosphatases by determining the relative effects of anions and cations have been extended to include observations on the effects of lysine, glutamic acid, carbonate and the ammonium ion. Milk phosphatase was inhibited to a greater extent by lysine and the ammonium ion than was the intestinal mucosa phosphatase; the latter enzyme was inhibited more strongly by glutamic acid and carbonate ion. Low substrate concentrations of lysine stimulated milk phosphatase. The results further the suggestion that there are 2 types of alkaline phosphatases, the intestinal enzyme and the milk enzyme, the latter appearing to be similar to the phosphatases of bone and kidney. H. J. Peppler

500. The properties of the enzyme-substrate compounds of lactoperoxidase. BRITTON CHANCE, Medical Nobel Inst., Stockholm. J. Am. Chem. Soc., **72,** 4: 1577–1583. Apr., 1950.

Although the mechanisms of action of lactoperoxidase and horse-radish peroxidase appear to be identical, the oxidations of the milk enzyme proceed at a much faster rate than those of the plant enzyme. In spite of the differences between their hemes and proteins, both enzymes exhibit similarities in the formation of primary peroxide complexes, alkyl hydrogen peroxides and the oxidation of pyrogallol and ascorbic acid.

H. J. Peppler

Also see abs. no. 471, 473, 474, 523.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

501. Waste prevention in the dairy industry. Task Committee on Dairy Waste Disposal. Milk Dealer, **39,** 6: 51–52, 104–106. Mar., 1950.

The more common causes and methods of eliminating excessive waste losses in dairy plants are: (a) Leakage and drippage, such as the constant and continual loss of milk from improperly

assembled or fitted equipment. (b) Overflow, which can be greatly reduced if not completely eliminated by careful attention and by the use of liquid level control devices. (c) Spillage, largely due to careless handling. (d) Freezing-on, which can be minimized with adequate refrigerant controls and proper operation. (e) Willful waste. Perhaps the largest volume of milk solids entering the drainage system is put there more or less willfully or get there because no effort is made to save them. (f) Residual waste, the total losses from which may reach amazing proportions unless care is taken to allow time for proper drainage. (g) Separators, the open type of which produces large quantities of foam, causing loss of milk solids. C. J. Babcock

502. Waste prevention in the dairy industry. Task Committee on Dairy Waste Disposal. Milk Dealer, **39,** 7: 47–48, 56–63. Apr., 1950.

The simplest device for measuring the flow of waste is a standard 90° V-notch sharp-crested weir located in a weir box and equipped with either a hook gauge or water level recorder. Instructions and detailed drawings for the construction and use of a weir are given. C. J. Babcock

503. Method of drying protein. E. ERICKSON. (Assignor to Hercules Powder Co.) U. S. Patent 2,502,134. 2 claims. March 28, 1950. Official Gaz. U. S. Pat. Office, **632,** 4: 1166. 1950.

Casein or other protein is dried on a perforated belt, by passing heated air countercurrently through the belt in a series of tunnel compartments. The moisture-laden air is reheated before entering the 1st compartment to cause the curd to adhere to the belt on its immediate entrance to the drying tunnel.
R. Whitaker

504. A new approach to plant planning. G. R. JOHNSON, Pace Associates, Chicago, Ill. Ice Cream Rev., **39,** 9: 48, 56, 60. Apr., 1950.

Success in the dairy processing field demands the use of a plant and facilities designed to meet, (a) present production requirements, (b) probable future expansion of the business without seriously disrupting operations and (c) high standards of efficiency and flexibility of operation.

Formulation of any expansion program should be based upon careful study and analyses of all factors involved. The probable cost of expanding an existing plant and facilities should be carefully weighed against the cost of a new plant In making such comparisons, maintenance and operational costs should be carefully studied in addition to the initial investment. In 1 instance cited, savings of $20,000 in plant costs and $75,000 in operational costs over a 10-yr. period would have resulted from building a new plant rather than enlarging the old one.

A careful study of all factors will enable the plant owner embarking on a building program to do so with confidence, for his decisions will be based upon facts and not guesswork.
W. J. Caulfield

505. Automatic ventilation of common storages. J. H. L. TRUSCOTT, E. W. FRANKLIN and JOY GILLIAT, Ontario Agr. College, Guelph. Sci. Agr., **29,** 11: 497–511. Nov., 1949.

Equipment is illustrated and described which has performed satisfactorily in maintaining uniform temperatures in unrefrigerated storages during the period Oct. 4–Apr. 19, at levels of 32 and 40° F. The storage rooms are equipped with constantly-running fans to ensure air circulation within the rooms. Cooling is accomplished by drawing air through a duct at floor level past an automatic shutter. Warmer air is expelled from the room at ceiling level past an automatic shutter by a fan connected to a differential thermostat and operates when the outside air temperature is below that within the storage. This fan also is cut off thermostatically when the room temperature falls to the desired level. A thermostatically controlled heat source also is connected to the air circulation system and may be used if necessary. The specially-designed differential thermostat is described and the performance of the equipment is related to weather records for the district. O. R. Irvine

Also see abs. no. 470.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

506. Receiving milk by tanker pick-up system. H. G. MOJONNIER, Mojonnier Bros. Co., Chicago, Ill. Milk Dealer, **39,** 6: 47, 107–108. Mar., 1950.

Edisto Farms Dairy of Columbia, S. C., has inaugurated a tanker pick-up system which promises to improve quality and at the same time bring other labor and product saving advantages inherent in bulk handling methods. The milk is cooled to 38° F. in stainless steel insulated refrigerated producer's tanks on the farm. A milk pump with piping for transferring the milk is carried on the tanker. At each farm the amount of milk in the producer's tank is ascertained by measuring the depth of the milk with a stainless steel ruled measuring stick. At 2 farms the pick-up is made only every other day but there

is no significant difference in bacterial count as a result of 2 d. holding on the farm. A charge of 20¢/cwt. is made for the tanker pick-up service. As the milk is transported in bulk in a well insulated tank there is only about 1° F. rise in temperature during transportation. The system is a labor saver as the handling of both full and empty cans is eliminated. C. J. Babcock

507. Lowering costs through efficient plant management. H. A. RUEHE, Univ. of Ill., Urbana. Milk Plant Monthly, **39,** 4: 64, 66–67, 74. Apr., 1950.

Efficient plant management requires a continuous inspection of the following business phases: (a) physical plant, (b) procurement, (c) processing and (d) sales. Sound judgement based on the above findings is the difference between a reasonable profit and complete failure.
J. A. Meiser, Jr.

508. Know your profit per line. F. MERISH. Milk Plant Monthly, **39,** 4: 52–54, 56. Apr., 1950.

Indirect expenses, which include office expenses, advertising, delivery and other administrative or commercial outlay, must be prorated to the various lines in a plant if a company wishes to determine the profit or loss per line. Advantages of this system are: (a) provides experience figures for setting up prices, (b) determines if sales volume per line is ample to cover overhead, (c) gauges efficiency of operations and (d) obtains a true picture of yearly profits.
J. A. Meiser, Jr.

509. Automatic sales accounting. E. D. PAULSON, Menzie Dairy Co., McKeesport, Pa. Milk Dealer, **39,** 7: 44–45, 64–66. Apr., 1950.

The advantages of punched card accounting system are: (a) Reports are more easily obtained on time. (b) Special reports can be prepared more easily through the use of the punched cards, because once the information is recorded in punched form, a variety of reports can be printed other than the routine ones. (c) Routemen are relieved for more productive work since the machines do their "paper work." (d) Reports are automatically printed by machines and, since they are on a standard form, they are much easier to read. (e) More comprehensive reports may be obtained just as easily as all routine reports and without additional expense. (f) It has reduced the cost of forms. (g) Due to the flexibility of equipment used, other applications also may be performed. The farmer's payroll also is prepared. This includes checks, check registers and other product reports. C. J. Babcock

510. Boosting sales of by-products. T. KNIGHT. Milk Plant Monthly, **39,** 4: 92–93. Apr., 1950.

Each product handled by the plant was rated according to the point system. Utilizing the previous month's sales as the base period, each routeman was given a base number for each by-product sold. The object of the contest was to meet this quota or better it. For men who sold 60–70% of their quota, an award of $2.50/mo. was given. Those reaching 90% or better received $5.00/mo. To insure added sales, the quota was changed each month, taking into account the seasonal demand for individual by-products. In addition to the above plan, incentive programs were incorporated to promote milk sales and reduce route returns. This latter contest paid cash bonuses of $5.00 and $10.00 for the leading routemen.
J. A. Meiser, Jr.

511. Outlook for ice cream and dairy products. R. C. HIBBEN, IAICM, Washington, D. C. Ice Cream Trade J., **46,** 5: 56. May, 1950.

The weather, buying power, quality of product and merchandising effort are factors which will influence ice cream sales in 1950. For the first time in its history the majority of ice cream manufacturers engaged in a merchandising program concentrating their sales effort on a single flavor, cherry-vanilla.

The fluid milk industry is faced with a problem of disposing of an increased supply of milk resulting from a record production. Intensified sales training programs, now engaged in by many milk companies, are producing results.

Increased production of butter will necessitate greater sales effort. Such programs now are under way. Prospects are for increased imports of cheese and decreased exports; this may result in lower prices for cheese. The dry milk industry is faced with the problem of doubling domestic sales; failure to do so may result in a demoralized market. The outlook for the evaporated milk industry for 1950 is for a stabilized demand throughout the year.

Also see abs. no. 501, 504.
W. H. Martin

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

512. Stability of carotene in alfalfa meal. Effect of antioxidants. C. R. THOMPSON. Western Regional Research Laboratory, Albany, Cal. Ind. Eng. Chem., **42,** 5: 922–925. May, 1950.

The conditions of exposure of finely divided alfalfa meal to oxygen are severe and most edible antioxidants lack sufficient activity to afford the

necessary protection. Structure of the compound appeared to be correlated with antioxidant activity. 2,5-disubstituted hydroquinones, p-substituted phenylenediamines, and derivatives of 2,2,4-trimethyl-1,2-dihydroquinoline were the most active compounds tested. Vegetable oils plus acetone were suitable solvents. The addition of increasing amounts of antioxidants gave increased stability but approached a limit above which additional amounts gave no effect. B. H. Webb

513. Pastures studies XXIX and XXX. Investigations on the lignin fractions of pasture herbage and of the feces of ruminants. I. The lignin fraction of pasture herbage. II. The lignin fraction of the feces of ruminants. F. J. SOWDEN and W. A. DELONG, Macdonald College, Quebec. Sci. Agr., **29,** 9: 409–417, 418–423. Sept., 1949.

Finely ground samples of herbage collected at several periods during 1942 and 1943 were air dried and finely ground. Lignin then was determined by the standard (Manning-DeLong) and Crampton-Maynard methods. The results indicated that widely different amounts of lignin were isolated and that the fractions differed in purity, as indicated by nitrogen and methoxyl content. Absorption spectra on 3 samples of forage lignin when compared to that of wood lignin confirmed the presence of impurities in both types of fractions. The ratio of clover to grasses in immature herbage may influence the nature of the fractions isolated.

Samples of the above herbage were fed to a steer in 1942 and to sheep in 1943 and samples of the feces were collected and analysed for lignin. Oven drying resulted in higher apparent lignin content in the isolates than did freezing and extracting before drying. Lignin content on the 1942 samples isolated by the standard method ranged from 14.85–16.32% and by the C-M method from 23.92–26.69%. Nitrogen and methoxyl values showed the C-M fractions to be less pure. Spectrographic analysis and solubility values in sulphite solution indicated that both fractions were about 50% pure relative to wood lignin. The data, however, suggest that lignin is not demethoxylated in its passage through the animal.

The study reveals the need for more accurate methods of lignin analysis before this means can be used as an accurate index of digestibility of herbage. O. R. Irvine

514. Roller crusher for drying hay. J. W. WHITE and W. KALBFLEISCH, Experimental Farms Service, Ottawa. Sci. Agr., **30,** 3: 119–124. Mar., 1950.

A crusher consisting of 2 spring-loaded steel rolls, 6 in. in diam. and 5 ft. long and driven by an auxiliary 45 h.p. engine is used to hasten the drying of hay in the swath. On early-cut hay, drying time was reduced from 2–3 d. to 1 d. by crushing. O. R. Irvine

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

515. Examination of bull semen with the ordinary and phase contrast microscopes. P. G. D. MORRIS, Royal (Dick) Veterinary College, Edinburgh, Scotland. Brit. Vet. J., **106,** 3: 85–93. Mar., 1950.

Observations were made on the semen of bulls with the ordinary and phase contrast microscopes. Using the phase microscope reduced the risk of artefacts which may appear in stained preparations. An attempt was made to classify certain morphological variations of the anterior portion of the head beneath the galea capitis as seen with the phase microscope. Three types were classified: (a) sperm with a dark zone under the anterior portion of the membrane of the galea capitis and separated from the nuclear substance by a narrow light zone, (b) sperm showing a diffuse grey zone below the anterior portion of the limiting membrane and (c) sperm showing a clear zone between the membrane of the galea capitis and the extremity of the nucleus. Ten good photomicrographs illustrate the paper.

B. B. Morgan

516. The effect of homogenization, pasteurization and lyophilization on egg yolk-sodium citrate diluents for bull semen. J. B. HERRICK. Iowa State College, Ames. Am. J. Vet. Research, **11,** 39: 159–160. Apr., 1950.

Egg yolk for diluting semen was prepared by homogenizing followed by lyophilizing egg yolk, sodium citrate (3%) and sulfanilamide (0.3%). This mixture was reconstituted at the rate of 3 parts yolk to 5 parts distilled water and filtered through cheese cloth. Survival time of bull sperm in the reconstituted egg yolk diluent was equal to that in diluent made with fresh egg yolk. Refrigeration of the lyophilized product for 30 d. at 40° F. was without effect. Pasteurization of the prepared diluent was not harmful and produced a product which could be stored refrigerated without development of contamination and without precipitation of yolk material.

E. W. Swanson

517. A study of size inheritance in the house mouse. I. The effect of milk source. L. BUTLER and J. D. METRAKOS, McGill Univ. Can. J. Research, Sec. D. **28,** 1: 16–34. Feb., 1950.

Three strains of mice were used to study the effect of fostering on the growth pattern of the mouse. The strains used breed true for size and have been designated as "large," "small" and "intermediate." The 14-d. mean weight of mice that received milk from "large" strain mothers is significantly different from those that received milk from either the "small" or the "intermediate" strain mothers. Although these differences tend to remain, they are not statistically significant at 140 d. The significance of these results are discussed in relation to the arithmetic and geometric concepts of polygenic growth. O. R. Irvine

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

518. Suckling device for calf feeders. P. O. STEVENS. (Assignor to Mutual Products Co.) U. S. Patent 2,501,146. 9 claims. March 21, 1950. Official Gaz. U. S. Pat. Office, **632,** 3: 790. 1950.

A pail of liquid calf feed, held in a slightly tilted position by a rack, is provided with a tube leading from the lowest corner of the pail to a nipple held in a horizontal position over the rack. Suction provided by the suckling calf draws the feed from the pail to the nipple. R. Whitaker

519. Cattle stanchion. H. A. DUMFORD. U. S. Patent 2,499,819. 15 claims. March 7, 1950. Official Gaz. U. S. Pat. Office, **632,** 1: 217. 1950.

A U-shaped stanchion is hinged on the bottom and attached to the floor by means of a chain; the top is attached to a bar but is arranged for easy opening and closing. The bar is attached to an upper support or to the ceiling by means of a centrally located swivel which permits rotary movement of the stanchion. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

520. The suspending power and viscosity of carrageenin. R. C. ROSE and W. H. COOK, Natl. Research Laboratories, Ottawa. Can. J. Research, Sec. F, **27,** 9: 323–336. Sept., 1949.

Commercial and laboratory prepared samples of carrageenin were heated in milk at 70° C. for 20 min., cooled rapidly to 10° C. and stored at 5–10° C. for 24 hr., when viscosity determinations were made. Suspending power varied from sample to sample but was closely related to viscosity ($r = 0.98$). The high viscosity of cold milk containing as little as 0.04% carrageenin appears to be due to the formation of a casein-carrageenin gel which is heat sensitive. Viscosity-concentration curves for whole milk and skimmilk were almost identical. That for dialysed milk was similar. The behavior of carrageenin in 0–0.5 *N* solutions of NaCl, $CaCl_2$ and KCl also was studied. The correlation coefficient between suspending power in milk and viscosity of 0.05 *N* NaCl was 0.91, suggesting that the latter could be used to predict the former. O. R. Irvine

521. Seaweed extracts as a food thickening. R. C. ROSE, Natl. Research Laboratories, Ottawa, Can. Food in Canada, **9,** 11: 9–11. Nov., 1949.

Agar, sodium alginate and carrageenin are food and beverage thickeners derived from 3 types of seaweeds. Canada has ample quantities of alginate- and carrageenin-bearing seaweeds which are harvested along the coasts of the Maritime provinces.

Carrageenin is a hot water extract of *Chondrus crispus,* known commonly as Irish moss or carrageen. The extract is filtered, concentrated and dried. It thickens foods by gelling and by reacting with milk protein. The addition of potassium salts increases the gelling temperature of the solution and the strength of the resulting gel. The stabilizing effect of small amounts of carrageenin in chocolate milk recently has been shown to be due to a gelling action on the milk proteins.

O. R. Irvine

522. Stabilizers and emulsifiers in ice cream. F. E. POTTER and D. H. WILLIAMS, U.S.D.A., Washington, D. C. Ice Cream Rev., **33,** 9: 148–151. Apr., 1950.

Stabilizers aid in producing smooth texture in ice cream hydration, formation of a gel structure throughout the mix or reaction with certain milk constituents to form substances that take up water as water of hydration. In selecting a stabilizer, ease of incorporation into the mix, effect on mix viscosity, type of body produced in ice cream, ability of the stabilizer to retard ice crystal growth, quantity required to stabilize the mix and cost must be considered. Pertinent data with respect to 12 different stabilizing agents for ice cream are summarized by the authors.

Emulsifiers are ester combinations of long-chain fatty acids with a higher alcohol, such as glycerol or sorbitol. Emulsifiers may be classified into 3 groups which are: (a) a mixture of monoglycerides and diglycerides, (b) esters of fatty acids and sorbitol or other higher alcohols and (c) polyoxyalkylene derivatives of group *b.* The chemical structure of each group of emulsifiers is presented. Emulsifiers aid in promoting dispersion of the fat. They tend to orient themselves at the fat-water interface in the mix, thereby reducing interfacial surface tension and retarding clumping of fat globules. Emulsifiers do not re-

place stabilizers but provide a supplementary effect which results in a drier ice cream and possibly a smoother texture. Use of emulsifiers in ice cream has not been ruled on as yet by regulatory officials. W. J. Caulfield

523. Shrinkage of ice cream as affected by the state of milk proteins. N. P. TARASSUK and J. T. HUTTON, Univ. of Cal., Davis. Ice Cream Trade J., **46,** 5: 44. May, 1950.

Shrinkage was determined by subjecting pint samples of ice cream, which had been stored at −10° F. for 48–72 hr. and tempered at 2° F. for 3 d., to a vacuum of 230 mm. of mercury for 2 min. and then replacing them in the cabinet at 2° F. for 5 d.; the volume of water required to fill the space evacuated by the ice cream was determined. Surface tension, viscosity, pH, titratable acidity and protein stability were determined, the latter by the temperature of coagulation on addition of 0.30 ml. of 2% $CaCl_2$ to a 5-ml. portion of mix.

A modified Hull spectrophotometric test for tyrosine was used to determine the effect of incipient hydrolysis of proteins of the mix on shrinkage of ice cream. The higher concentration of milk solids in ice cream, as compared to milk, necessitated the addition of 20 ml. of 0.72 *N* trichloroacetic acid, in place of 10 ml. for the precipitation of proteins. Upon addition of the phenol reagent, the filtrate becomes cloudy and requires refiltration before making spectrophotometric color determinations. Abut 80% of the blue color developed in the test was attributed to tyrosine and 20% to tryptophane. Results were expressed in "tyrosine units." A unit is 1 mg. of tyrosine/1. of sample, or its equivalent.

A direct relationship between shrinkage and overrun throughout the range of 90–130%, was found to exist. Ice cream containing emulsifiers of the Span and Tween series contained smaller air cells and was more susceptible to shrinkage. Addition of diglycol laurate, a surface tension lowering agent, resulted in increased shrinkage due to the presence of free fatty acids. No correlation was found between the use of previously frozen mix ingredients (cream and condensed skim milk) and shrinkage.

Shrinkage susceptibility is markedly influenced by differences in milk from individual cows, possibly due to inherent breed characteristics. Wide differences in pH and protein stability observed in individual milks could not be correlated with shrinkage. However, the correlation between shrinkage and tyrosine value is outstanding. Aging of mixes resulted in a definite and consistent increase in shrinkage.

The addition to the mix of a hydrolysate prepared from acid-precipitated casein at the rate of 0.1% (calculated as dry unhydrolized casein) and the mix allowed to stand overnight increased shrinkage. These tests also indicated that products other than tyrosine were responsible for shrinkage.

Heat denaturization of lactalbumin and globulin were studied. As the heat treatment of the mix was raised, shrinkage increased. Whey proteins added to the mix to replace the proteins precipitated by heat markedly decreased shrinkage. The undenatured globulin fraction of whey protein was a factor in reducing shrinkage; addition of lactalbumin appears to increase shrinkage.
W. H. Martin

524. Soft ice cream and your business. W. A. JOSEPHSON, Sou. Dairies, Inc., Birmingham, Ala. Ice Cream Field, **55,** 3: 74–76. Mar., 1950.

A too "rich" and "eggy" flavor, coupled with poor sanitation, are the reasons given for discontinued success of the old "custard" type soft ice cream.

The new soft ice cream industry is credited with starting in southern Illinois and northern Missouri and has made rapid gains on the Pacific Coast. The so-called soft ice cream machines are based on the principle of extruding ice cream, continuously or intermittently, at a temperature of about 16–19° F.

A survey in Los Angeles County, Cal., reported that 36–40% of the ice cream sold is soft. Soft ice cream outlets in the county, on the average, sold 5 times the gallonage per store as did the competing conventional outlets. Owners of these stores are drawn from nearly all walks of life and the patrons represent a cross section of American life.

The success of these stores is due to guidance from franchise and equipment people in getting started. Beyond that they depend on the following: (a) Soft ice cream is good. (b) Soft ice cream usually is a low-fat, high-solids product which is not too rich. (c) The value of low overrun is recognized, 50–55% usually being taken. (d) These stores generally are operated under sanitary conditions. (e) They nearly always employ the drive-in principle. (f) The operation is kept simple and as a result is profitable.

Soft ice cream in California, it is felt, has decreased the sale of hard ice cream, whereas in other localities this effect is not so prominent. The ice cream industry should consider better merchandizing methods, improved sanitation, as well as the possible use of so-called "converts" which will convert small portions of hard ice cream into soft ice cream, or still another con-

verter which will extrude soft ice cream from a can of hard ice cream in a cabinet. W. C. Cole

525. A pint of sundaes. Anonymous. Ice Cream Trade J., **46,** 5: 30, 32, 68. May, 1950.

A new type of combination package including a pint of ice cream and a transparent plastic "bag" of sundae topping in 1 convenient carton has been introduced by a number of ice cream manufacturers in the midwestern markets. The plastic bag which contains the proper amount of topping for 4 or 5 servings withstands subzero temperatures. Sponsors of the combination package believe that convenience, economy and the desire of the consumer for sundaes will result in increased sales of ice cream. W. H. Martin

526. Pre-cut ice cream cakes on "production line" basis. Anonymous. Ice Cream Trade J., **46,** 3: 64–65. Mar., 1950.

Redi-kut ice cream cake is made in a special 2.5-qt. mold consisting of 20 individual segments. The segmented mold may be filled direct from the freezer. The top of the mold then is clamped down. In the top of the mold and separate from it is a disk holding 20 metal spikes which become imbedded in the ice cream. After the mold containing the ice cream is passed through a brine tank, it is plunged into hot water, the top of the mold is removed and the segments of the cake adhere to the disc containing the spikes. The segments then are pushed by a lower movable disc onto the base of a cardboard cake-dispensing unit. A steel ring brings the segments together and a 0.5-in.-high flexible card-board strip stapled to 1 edge of the cake-dispensing unit is closed with a clasp holding the cake in shape for decorating. The decorated cake is ready for delivery or storage in the hardening room.

W. H. Martin

527. Ice cream bars go 'round. A. K. Velan, Velan Eng. Co., Montreal, Can. Ice Cream Field, **55,** 3: 78–79. Mar., 1950.

A description is given of a rotating machine designed to automatically freeze, chocolate coat and wrap ice cream bars. It was developed in Denmark and Switzerland and is known as the RIA system. W. C. Cole

528. Ice cream cup. A. A. Heyman. (Assignor to Maryland Baking Co.) U. S. Patent 2,501,939. 4 claims. March 28, 1950. Official Gaz. U. S. Pat. Office, **632,** 4: 1117. 1950.

A crisp pastry cup with tapering sides for nesting and a flared top portion having an internal notched ring to provide an anchorage for the ball of ice cream is described. R. Whitaker

529. Package-filling spout for ice cream machines. R. J. H. Lane. U. S. Patent, 2,502,329. 1 claim. March 28, 1950. Official Gaz. U. S. Pat. Office, **632,** 4: 1216. 1950.

This device, easily attached to an ice cream freezer, has a sliding valve which, when lifted, allows the ice cream to pass through a suitably shaped opening into the package. R. Whitaker

530. Ice cream. C. F. Koerver. (Assignor to the Borden Co.) U. S. Patent 2,500,315. 6 claims. March 14, 1950. Official Gaz. U. S. Pat. Office, **632,** 2: 457. 1950.

The ratio of lactose to mineral salts normally present in ice cream mix is increased 10% by addition of lactose to improve the flavor, impart an additional refreshing sensation when eaten and overcome "slickness," especially in high-fat ice cream. R. Whitaker

531. Frozen purees from citrus fruits. E. A. Bevens, Bur. of Agr. and Ind. Chem., Pasadena, Cal. Ice Cream Field, **58,** 6: 26, 62, 63. Dec., 1949.

Experiments conducted by the Laboratory of Fruit and Vegetable Chemistry in Los Angeles in 1947 and since show that satisfactory frozen citrus purees can be prepared. Sound, mature fruit is washed with a good detergent and then rinsed well with cold water. Next, the stem end is cut off and other dark specks are removed; in the case of Navel oranges the "navel" end should be cut off. Next, the fruit is quartered or crushed and finally reduced to a puree by passage thru a mechanically driven screwing device with minimum incorporation of air. Screen sizes of 0.027 and 0.033 are preferable when purees are intended for use in sherbets, ices, pies and beverages, but larger sizes are better where the purees are to be used for marmalades, jams or toppings.

The yield of puree from whole fruit is about 50–60%; 0.65–0.75% peel oil is recommended. To control the oil content it may be necessary to pass part of the fruit thru an abrasive machine before it is quartered or crushed in order to remove most of the oil sacs.

One part of sugar is added to 5 parts of puree. This mixture then is placed in containers and the contents frozen in an air blast at sub-zero temperatures. The containers are stored at 0–10° F. Lacquered or enameled cans are recommended for high-acid purees. Purees can be kept satisfactorily for more than a year.

Navel orange purees can be stored for several months without bitter flavors developing but upon prolonged storage, the purees gel. This problem now is being studied.

Orange and lemon purees have been used successfully in commercial milk sherbets and water ices. Sherbets with 2.5% butterfat were considered better than water ices. It is recommended that 14–18 oz. of 5: 1 orange puree and 1.5 oz. of citric acid (50% solution) be added/gal. of sherbet mix. W. C. Cole

532. Report on apple ice cream. J. C. HENING and C. S. PEDERSON. N. Y. State Agr. Expt. Sta., Geneva, N. Y. Ice Cream Field, **55,** 4: 62, 64, 65. Apr., 1950.

A new type apple juice and the use of apple juice concentrate in ice cream is reported. Ice cream made with this concentrate appeared like vanilla ice cream but had a strong true apple flavor. The success of apple ice cream depends upon the preparation of the juice and concentrate.

McIntosh apple juice was prepared by the ascorbic acid method of Pederson (1947) and Holgata, *et al.* (1948). The concentrate was prepared by the freezing concentration described by Pederson and Beattie (1947). The ascorbic acid inhibits the action of oxidizing enzymes during extraction, deaeration and pasteurization. Pasteurization was accomplished at 165–175° F. for 20 sec., with cooling in 30-lb. enamel-lined cans. Concentration to 3.6: 1 was accomplished by slow freezing to the desired degree and then removing the ice by centrifuging. McIntosh apples will yield 60–65% juice it is claimed.

This McIntosh juice concentrate was used in ice cream to the extent of 24%. Baldwin concentrate blended with McIntosh 1: 4 gave a good product but other concentrates were too acid. McIntosh concentrate was the best product tried. W. C. Cole

533. A new flavor gets nationwide promotion. Anonymous. Ice Cream Trade J., **46,** 4: 32, 92. Apr., 1950.

Chocolate almond ice cream has been introduced by the Borden Co. The almonds are chocolate coated and then injected into vanilla ice cream in the same manner as cherries or other fruits. Nationwide promotion has been placed behind the new flavor with full page advertisements appearing in several of the leading magazines. W. H. Martin

534. Bulk ice cream in the profit picture. W. D. DOBSON, Carnation Co., Los Angeles, Cal. Ice Cream Trade J., **46,** 4: 38–39, 84. Apr., 1950.

On the west coast there has been an increase in the sale of packaged ice cream, resulting in a decline in bulk sales as a percentage of total sales. Dealers have not pushed hand-packed ice cream. The sale of soft ice cream also has cut into the sales of bulk ice cream. To cope with this situation, Carnation Co. has been holding dealer meetings for the purpose of teaching them to dip bulk ice cream and to make attractive fountain items. Dealers have been shown that a gross margin of 33 1/3% will result in increased sales and a greater net profit than resulted when a 43% gross was taken. Other dealer helps in the form of point of sale advertising, proper location of display cabinets and properly trained personnel should be provided as a means of increasing bulk sales. W. H. Martin

535. Ice cream production is down three per cent from 1948. Anonymous. Ice Cream Field, **55,** 3: 66, 67. Mar., 1950.

The Bureau of Agricultural Economics of the U.S.D.A. estimates that the 1949 ice cream production was 553,705,000 gal. for the U. S. This amounted to a 3% reduction as compared to 1948. The largest percentage decrease occurred in New Jersey, whereas the southern states, as a group, showed the greatest decrease. Washington State showed a gain of 8% over 1948, which was the greatest increase shown by any 1 state. Sherbet production showed a 17% increase over 1948. Tabulated gallonages are given for the U. S. by months for 1949 and for states for 1948 and 1949. W. C. Cole

536. What will the profits picture be in 1950? L. C. ANDERSEN, General Ice Cream Corp. Ice Cream Trade J., **46,** 3: 28–29, 103. Mar., 1950.

Profits will be satisfactory in 1950 if ice ceram manufacturers will refrain from giving unnecessary service and not offer items on which a profit cannot be made. Costs are likely to be up in 1950 because of increases in labor costs, increases in taxes and higher replacement costs, coupled with the possibility of reduced volume of sales. Costs on each item offered should be determined for the purpose of deciding whether or not the item should be sold. In figuring the cost of an item, material cost, manufacturing expense and truck and cabinet cost on basis of space occupied by the particular item should be considered. Greater operating efficiency and sales efforts may help to reduce costs and improve the profit picture. W. H. Martin

537. Soda fountain operation. III. Menu. A. C. DRAPER, Rexall Drug Co. Ice Cream Field, **54,** 6: 40, 42, 44, 46, 48, 50, 51. Dec. 1949.

The author stresses the importance of planning the menu for a fountain before the fountain is planned. Location is important in deciding upon

the menu, but some items in the menu usually will increase the sale of others. If a fountain in a drug store sells ice cream items only, it will do an average of 5–10% of the store's business. Adding sandwiches may increase this to 12–15% and adding hot food can increase it to 20–30%. Adding hot food or any other service at a fountain necessitates planning for the problems that accompany such additions. In tabular form the author recommends the proportions of various items to use in various sized fountains. A discussion of costs, expenses and profits is given, and data reported in tables and charts serve as guides in determining these values. Examples in making such calculations are included. W. C. Cole

538. Soda fountain operation. XII. Approach to layout. A. C. DRAPER, Rexall Drug Co. Ice Cream Field, **55,** 3: 22, 24, 53–55. Mar., 1950.

This is the concluding article in the series. The author outlines the considerations in deciding upon placement of fountain in store, type and shape of equipment and dimensions of equipment used. Drawings of the most common types of layouts are shown and the advantages and disadvantages of each type discussed. W. C. Cole

539. Impulse buying of ice cream. V. M. RABUFFO. Ice Cream Trade J., **46,** 5: 28–29, 92. May, 1950.

A study of consumer buying habits in super markets in 7 cities indicated that 59.1% of ice cream purchases were completely unplanned. This fact shows the need for major emphasis on the point of sale suggestion for buying ice cream. The industry should concentrate on the points that will help influence the sale of ice cream when the customer enters the store. Some of the tools and devices which may be used include point-of sale-posters, a lighted super structure over the ice cream cabinet, the location of the cabinet in a strategic place, accessibility of packages, attractive package design and insulated bags to protect the ice cream while in transit to the home.

W. H. Martin

540. Selling ice cream through small town grocery store. P. B. PERSON, Knerr Dairy Co., Fargo, N. D. Ice Cream Trade J., **46,** 4: 44–45, 100. Apr., 1950.

The Knerr Dairy of Fargo, N. D., has been successful in building up its volume of ice cream sales through the use of newspaper and radio advertising to help its many small dealers in rural towns to sell more ice cream. Spot radio announcements and co-sponsored athletic events on the radio and advertisements in small town newspapers and at the movie houses are some of the things which have been used as sales builders.

W. H. Martin

Also see abs. no. 511.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

541. Will fat-free milk ruin your market? W. L. FOUST, Warren, O. Milk Dealer, **39,** 6: 138–141. Mar., 1950.

The sale of fat-free milk will not ruin regular milk sales unless it is sold as a cheap product; best results are achieved when the price is not more than 1¢ under the price of regular milk. A survey showed that 32% was sold as a baby food on doctors' orders, 18% was being used by women during pregnancy because of its high calcium content and low fat, 36% was being used by persons on reducing diets and the remaining 14% was being used by the lower income groups because of the slight economic advantage. Members of the medical profession point out that about 20% of the people today should be using this type product since it is protein they need most, not fat; therefore, it is reasonable to assume that about 20% of bottled milk sales could be sold in this manner. If the value of skimmilk and cream is utilized by selling more low-fat milk at a reasonable price, then butter can be sold at a comparative price of oleo and still make money. There is a definite place in the market for this product and if the price is kept up so that a profit is made and not sold as an economy package, new markets can be captured. There has been a greater consumer acceptance with a low-fat, high-solids milk than with a purely fat-free milk.

C. J. Babcock

542. A proposed new method of evaluating milk. W. W. FASSETT, Sacramento, Cal. Milk Dealer, **39,** 6: 45, 90–96. Mar., 1950.

The Jacobson theory that for every increase of 0.1% in fat test an increase of 0.04% SNF occurs is cited. This theory is based on averages of 100,000 tests. According to this theory, 3% milk has an SNF content of 8.27%. Therefore, for each pound of fat in 3% milk there would be 2.75 lb. of SNF. Since each increase of 0.1% in fat means an increase of 0.04% SNF, then 5% milk would contain 9.07% SNF. The advantages of evaluating milk by the SNF-fat combination are as follows: (a) All types of milk would sell on an equal basis as far as both fat and SNF are concerned. (b) It would equalize the purchasing power of plants in localities which are receiving milk from milksheds on which varying types of milk are produced. (c) It would make it pos-

sible to pay full value for all types of milk instead of underpaying on 1 type to make up for over payment on others. The following objections have been raised: (a) Some milk is claimed to have a higher vitamin content than others. (b) Some milk has a color intensity which is desirable for trade and for which some plants are willing to pay a premium. (c) Some milk is claimed to be more digestible than others. C. J. Babcock

543. Container with pouring throat and connecting dispensing opening. G. C. RIED and S. S. JACOBS. (Assignors to American Can Co.) U. S. Patent 2,499,416. 5 claims. March 7, 1950. Official Gaz. U. S. Pat. Office, **632,** 1: 113. 1950.

An improved pouring opening for paper containers for milk and other liquids, consisting of a cover-all flap to protect the pouring lip and the corner of the container molded in a rounded manner to facilitate pouring is described.

R. Whitaker

544. Single closure for bottles. C. H. KREBS. (Assignor to Standard Cap and Seal Corp.) U. S. Patent 2,501,849. 7 claims. March 28, 1950. Official Gaz. U. S. Pat. Office, **632,** 4: 1093. 1950.

This cap for milk bottles comprising metal foil, laminated on the outside to paper and on the inside to machine glazed paper, extends over and protects the entire pouring lip. By having the glazed surface of the inner layer next to the foil and the rough side next to the bottle, any difference in pressure between the exterior and interior of the bottle is equalized, but the product does not leak, as this layer is gas permeable and liquid impermeable. R. Whitaker

545. Supplementing fluid cream with frozen cream. H. V. ATHERTON, Univ. of Vt., Burlington. Milk Dealer, **39,** 6: 157–158. Mar., 1950.

Preliminary results in the use of frozen cream to produce a 40% cream which will whip and which can be standardized down to 18% to produce a satisfactory coffee cream indicate that cream should be frozen and stored with a 40% fat content rather than as 50% cream, as is practiced for the ice cream industry. Best results are obtained by combining fresh cream and frozen cream on a 50-50 basis, heating the mixture to 140° F. or higher and then homogenizing at 100 lb./in.^{2}, single stage. The resulting mixture appears to be entirely satisfactory for commercial usage. C. J. Babcock

546. Undersøgelser over Piskningen af mager Fløde. (Research on the whipping of cream having a low fat content.) A. J. OVERBY, Royal Vet. & Agr. Dairy Laboratory. Maelkeritidende, **59,** 43, 44, 45. 1946.

In Nov., 1940, a Danish regulation provided that cream to be sold retail must not contain more than 20% fat. On Feb. 10, 1943, the rule was changed to 15% in cream sold retail. Consumers were not able to whip 15% cream satisfactorily. It had been made unlawful, in 1925, to add any whipping aid to cream.

A good whipped cream must have a fine aroma, flavor and appearance. The foam must have a certain firmness and be of a definite volume, while no wheying off should occur after standing for a time.

The volume increases with an increase in the fat percentage until an optimum fat percentage is reached for stabilizing the foam, which is formed only from the liquid phase of the cream; therefore, less foam will be formed when the fat percentage is high.

Cream contains more foam substance (Skumstof), which is not composed of casein and albumin, but is the "membraneslime" that surrounds the fat globules. During whipping, many small foam lamellae, the walls of which must be strong, serve to hold the foam firm. The fat in satisfactory whipped cream should be present in small aggregations; large fat aggregates are undesirable as they cannot find a place on the lamella walls. The Danish experiments confirmed Hening's and Dahlberg's findings that it was possible to increase the viscosity and improve the whipping property of low-testing cream by reheating it. Cream of 15% fat was pasteurized and cooled to 2° C. (35.6° F.) and held at this temperature for 3 hr. The cream was slowly heated to 28–29° C., held at this temperature for 0.5 hr, cooled to 2° C. (35.6° F.) and left at this temperature until the following day.

Dalberg's and Hening's findings that superior whipping property and greater viscosity of cream resulted when the milk was separated at 5° C., as compared with separating at 50° C. were confirmed. Cream obtained from milk that had been frozen had a poor whipping property.

For economical and technical reasons it might prove of benefit to reheat cream having a high fat content, cool it and then standardize with cold skimmilk to the desired fat content. Reheating gives the best results when cream having a high fat percentage is used. When cream of a higher fat content for whipping purposes comes into general use again, the method of reheating the cream for increasing its whipping properties would have definite significance. By use of the heat treatment method, marketing 20% cream that has as good a whipping property as 30% cream is possible.

Eleven tables and 2 illustrations are given in the article. There are 23 references. G. H. Wilster

547. 3-day-a-week retail delivery. W. HOLM, Sec., Columbus Milk Distributors Assn., Columbus, O. Milk Dealer, **39,** 7: 146–151. Apr., 1950.

Going from every-other-day delivery to 3-d.-a-week delivery eliminates Sunday delivery. The advantages of eliminating Sunday delivery are lower labor costs, fewer relief problems, employees like it, 52 less operating days for the plant and consumers like it. C. J. Babcock

Also see abs. no. 475, 497, 506.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

548. The excretion of steriod hormones concerned with controlling reproductive processes in animals. H. H. COLE, Univ. of Cal, Davis. Am. J. Vet. Research, **11,** 39: 161–165. Apr., 1950.

This paper is a review citing 80 references concerning the excretion of estrogens, androgens and progesterone. A critical discussion is presented of the observations concerning estrogen excretion by the cow in urine and feces and androgen excretion in cattle feces. E. W. Swanson

Also see abs. no. 498, 499.

SANITATION AND CLEANING

K. G. WECKEL, SECTION EDITOR

549. Modern materials and methods for dairy sanitation. L. L. LITTLE, E. F. Drew and Co., Inc., Boonton, N. J. Milk Plant Monthly, **39,** 4: 42–44, 46. Apr., 1950.

Although physical force is largely responsible for removal of soil from dairy equipment, scrubbing can be reduced greatly by compounding cleaners for specific jobs. Protein removal by chemical action of alkali cleaners can be facilitated by use of wetting agents. Grease films which necessitate saponification are removed more readily after emulsification. Mineral deposits usually are removed by acid cleaners; these deposits can be prevented by alkali cleaners in combination with wetting agents. Although mildly alkaline all-purpose cleaners are used for cleaning equipment in dairy plants, different methods for applying these compounds must be used for the varied pieces of equipment. These methods are vat solution, solution pail, dry powder, solution spray and circulation. J. A. Meiser, Jr.

550. Het verband tussen stalinspectie en melkkwaliteit. (The relationship between the judging of farm conditions and the quality of the milk.) (English summary.) H. HEERES, State Dairy Organization, The Hague, Holland. Neth. Milk and Dairy J., **4,** 1: 10–20. Jan.–Mar., 1950.

The relationship was determined from studies of 10,200 farms producing market milk in the west part of Holland in the milk year 1948–1949. The result of judging of farm conditions was expressed as 8 for the best and 1 for the poorest. The quality of the milk was determined by methylene blue test and sediment test and given 3 for the best and 1 for the poorest quality. Yearly figures from 52 determinations varied between 52×3 and 52×1. A correlation coefficient of -0.4 ± 0.009 was found, using the individual figures. In calculating the average quality figures for the 8 judging classes and employing these 8 figures, the correlation coefficient increased to -0.995.

On the average, a close relationship exists; however, in single cases other influences cause complications, making it impossible to calculate one factor from the other for individual cases with a proper degree of certainty. Under these circumstances, it is advisable to give attention to both factors. A. F. Tamsma

Also see abs. no. 483.