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VITAMIN E IN THE NUTRITION OF CATTLE. I. EFFECT OF FEEDING VITAMIN E POOR RATIONS ON REPRODUCTION, HEALTH, MILK PRODUCTION, AND GROWTH.[1,2]

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Reproductive failures in cattle constitute a problem of great economic importance to cattle breeders. Ever since Evans and Bishop (7) established that rats require vitamin E for successful reproduction, the possible relationship of this factor to breeding troubles in cattle has been a subject of much interest to livestock breeders. No attempt will be made to present a comprehensive review of the extensive literature concerning vitamin E in its relation to reproduction in cattle. This recently has been done in an excellent review by Asdell (1). The information available, however, indicates that it has not been established that cattle and other ruminants need vitamin E for successful reproduction. It is significant that most of the studies reporting improvement in reproducing ability following vitamin E administration were conducted over relatively short periods with cattle on normal rations (3, 11, 16). Frequently the treatments administered followed or occurred simultaneously with other forms of therapy. Cases where standard diets for cattle are deficient in vitamin E and would be improved by addition of this factor likewise must be viewed with some skepticism, for all feedstuffs in such rations have seen shown to be comparatively rich in this vitamin (5). This fact also reduces any possible therapeutic value of the much smaller doses provided in the wheat germ oil, whether it is injected or fed.

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2 The authors are indebted to Merck & Company, Inc., for supplying all synthetic alpha tocopherol that was fed.

3 Division of Dairy Husbandry.

4 The experiment was planned and started by the senior author. Later Dr. L. S. Palmer, Chief, Division of Agricultural Biochemistry, was associated actively with the chemical and rat studies conducted in connection with the project. Dr. Palmer died in March, 1944.

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9 Division of Agricultural Biochemistry.

Various other effects not directly related to fertility and reproduction have been attributed to vitamin E in the nutrition of ruminants. Willman *et al.* (28) reported success in treating a characteristic muscular syndrome of lambs, known as "stiff-lamb" disease with wheat germ oil, *alpha*-tocopherol or mixed tocopherols. Recently it was shown by Gullickson and Calverley (9) that cattle fed vitamin E-deficient rations may die from heart failures. Harris *et al.* (13) reported that when dairy cows are fed a daily supplement of 1 g. of mixed natural tocopherols the fat output is increased by about 25 per cent; this contention, however, has not been confirmed by others (10, 24, 27).

The present experiment was designed to determine whether vitamin E is needed by cattle for successful reproduction. The plan followed was to feed all animals throughout their lives on rations containing liberal amounts of all known essential nutritional factors except vitamin E. The relationship of vitamin E to growth, health and milk production of cattle also is considered.

EXPERIMENTAL PROCEDURE

Selection of feedstuffs. Before any cattle were started on the experiment, suitable feeds low or completely lacking in vitamin E had to be found. This was accomplished by conducting rat bioassays on a great variety of feedstuffs such as commonly are fed to cattle. A modification of the bioassay method of Palmer (22) was followed. Sexually mature female rats that had been fed normal rations throughout the period of growth and their first gestation were placed on the basal vitamin E-free ration immediately after the birth of their litters. After weaning at 21 to 25 days the young were reared to sexual maturity on the basal vitamin E-free ration modified so as to contain such percentage of the feedstuff tested as was likely to be present, on the dry matter basis, when fed in maximum amounts to cattle on experiment. Each feedstuff was tested in this manner and some were tested again in combination with others to determine their additive effect, if any, on reproduction. If the product was highly indigestible for rats, a benzene extract of it was made and this then was incorporated at high levels in the basal vitamin E-free ration which was fed during the gestation period of the vitamin E-deficient rats. The rats were mated when about 90 days old and thus received the test food for periods of 9 to 10 weeks. Wheat germ oil of known potency was employed for the positive controls, a total of 3.5 to 4.0 ml. being incorporated, in the basal ration over a period of 6 to 7 weeks, corresponding to the minimum period the test feeds were consumed. At the start of the experiment test rats were allowed to litter normally, but later, to permit better control, the animals were sacrificed on the 21st day of pregnancy and the living and dead young and the resorptions were counted *in utero*. A less complete study was made with male rats of the ability of some of the products to prevent the characteristic testicular degeneration. The results of these tests provide confirmatory evidence of vitamin E content.

Table 1 lists the feeds which gave less than 100 per cent live litter efficiency when tested. The feedstuffs commonly fed to cattle were found to be relatively

rich in vitamin E, as shown by the following comparatively small amounts needed to give 100 per cent live litter efficiency: alfalfa hay, 114 g.; prairie hay, 135 g.; reed canary grass hay, 147 g.; oat hulls, 132 g.; wheat straw, 131 g.; corn bran, 133 g.; wheat gluten, 40 g.; and fish meal, 59 g. These results are in agreement with those reported by Cabell and Ellis (5).

After the ingredients in the ration of the cattle had been selected, tests were made on all new lots of feed acquired. If vitamin E was indicated in any of them or in the ration in which they were included, the feedstuff responsible either was discarded or was subjected to such treatment as had been found to inactivate the vitamin E present. Thus the vitamin E present in some lots of rice straw was inactivated subsequently by heat treatment at 90 to 100° C. for

TABLE 1

A comparison of the vitamin E activity of various feedstuffs

Feedstuff tested	Average amount consumed	No. of female rats used	No. of complete resorptions	No. of live litters	Live litters
	(*g.*)				(%)
Brewers' dried grains, heated 2×	310	6	5	1	16.7
Brewers' dried grains, heated 3×	414	10	10	0	0
Corn starch	150	6	6	0	0
Corn gluten meal	123	24	13	11	45.8
Distillers' grains, solvent extracted	191	6	6	0	0
Dry skim milk	483	10	10	0	0
Meat scraps	121	7	4	3	42.9
Molasses beet pulp	360	10	6	4	40.0
Plain beet pulp	112	15	4	11	73.3
Polished rice	367	8	7	1	12.5
Polished rice, heat treated	730	8	8	0	0
Potato meal	426	10	7	3	30.0
Rice straw	240	8	6	2	25.0
Rice straw, heat treated	797	11	11	0	0

1 week and all the polished rice fed was heat treated at a temperature of about 100° C. in a seed-house drying tower for at least 2 weeks. Similarly, any vitamin E in brewers' dried grains was inactivated by tripling the heating time in the drying drums at the brewery. The vitamin E found occasionally in lots of dry skim milk was destroyed by adding about 10 per cent of lard and allowing the mixture to stand at room temperature for several months or until the fat became rancid.

The chemical composition of each feed was determined by official A.O.A.C. methods (2). Calcium and phosphorus content was determined by the method developed by Morris *et al.* (19).

Cattle used. Fifteen calves of mixed breeding, consisting of nine females and six males, were in the original group. Two of these, E430, a male, and E438, a female, were used as positive control animals. During the progress of the experiment a few calves other than decendants of the original group were added. Two of these, E607 and E615, also were used as positive controls. A tocopherol supplement also was fed to E559 beginning about 3 weeks before her

TABLE 2

Data relating to the cattle used in the experiment

Herd no.	Breed	Birth date	Sex	Generation	Herd no. of		Age when removed from experiment			Remarks
					dam	sire	(*Date*)	(*Yr.*)	(*Mo.*)	
E348[a]	Gr. Hol.	6–14–39	F	P_1	Unknown	Unknown	6-13-43	4	0	Discontinued. Died suddenly 6-29-43
E380	Gr. Hol.	5–11–40	F	"	"	"	5-10-45	5	0	Died suddenly
E383	Gr. Hol.	6–23–40	F	"	"	"	2- 1-44	3	7	Died suddenly
E384	Gr. Hol.	7– 1–40	F	"	"	"	4-10-43	2	9	Died suddenly
E390	Gr. Hol.	8–22–40	F	"	"	"	4- 7-44	3	7	Died suddenly
E394	Guern.	12–25–40	M	"	"	"	3- 8-43	2	2	Slaughtered
E396	Jersey	1– 3–41	M	"	"	"	3- 8-43	2	2	Slaughtered
E397	Jersey	2– 6–41	M	"	"	"	2- 3-44	3	0	Sold for slaughter
E398	Jersey	5– 7–41	M	"	"	"	3-15-43	1	10	Slaughtered
E401	Gr. Hol.	8–14–41	F	"	"	"	1-28-44	2	5	Discontinued
E403	Gr. Jer.	9–22–41	F	"	"	"	8-13-45	3–	11	Died suddenly
E405	Gr. Hol.	9–23–41	F	"	"	"	1-28-44	2	4	Discontinued
E408	Gr. S. Horn	9–28–41	F	"	"	"	1-28-44	2	4	Discontinued
E413	Gr. Hol.	10– 5–41	F	"	"	"	1-28-44	2	4	Discontinued
E430[b]	Guern.	12–24–41	M	"	"	"	4-21-44	2	4	Slaughtered
E438[b]	Gr. Hol.	2–12–42	F	"	"	"	3-21-45	3	1	Slaughtered
E480	Gr. Jer.	3–30–43	F	F_1	E390	E397	8-22-45	2	5	Died suddenly
E481	Gr. Jer.	4–12–43	F	"	E380	"	6-26-45	2	2	Died suddenly
E482	Gr. Jer.	4–12–43	M	"	E383	"	10-15-45	2	6	Died suddenly
E490	Gr. Jer.	10– 8–43	M	"	E405	E398	10-10-43	2 days		Died
E496	Gr. Jer.	10–30–43	F	"	E401	E397	3- 8-44	0	3	Accidental death
E507	Gr. Jer.	1– 6–44	F	"	E413	"	11- 1-45	1	10	Died suddenly
E511	Gr. Jer.	1–17–44	F	"	E408	"	7-27-44	0	6	Broke leg—slaughtered
E516	Gr. Jer.	2–21–44	M	"	E403	"	3-21-45	1	1	Slaughtered
E541	Gr. Jer.	7– 8–44	F	"	E380	"	4- 4-46	1	9	Died suddenly
E558	Gr. Jer.	5– 8–45	F	F_2	E507	E482	7- 8-47	2	2	Died suddenly
E559[c]	Gr. Jer.	5– 9–45	F	F_1	E380	"	3-15-49	3	10	Discontinued
E562	Gr. Jer.	6–23–45	F	F_2	E481	"	6-28-45	0	0	Died—indigestion
E573	Gr. Jer.	11–27–45	F	F_2	E541	"	10-19-47	1	11	Died suddenly
E585	Gr. Hol.	4–15–46	M	P_1	Unknown	Unknown	12-18-47	1	8	Sold—difficult to handle
E595	Gr. Hol.	6–25–46	F	"	"	"	3-15-49	2	9	Discontinued
E607[b]	Gr. Hol.	7–14–46	F	"	"	"	3-15-49	2	8	Discontinued
E612	Gr. S. Horn	5– 1–46	F	"	"	"	3-15-49	2	7	Discontinued
E615[b]	Gr. Hereford	8–12–46	F	"	"	"	3-15-49	2	6	Discontinued
E639	Gr. Jer.	10–17–47	M	F_3	E573	E585	11-30-48	1	1	Sold for slaughter
E641	Gr. Jer.	10–24–47	F	F_2	E559	"	11-30-48	1	1	Sold for slaughter

[a] Placed on experiment 6–3–42.
[b] Control animal fed synthetic *alpha* tocopherol once weekly at rate of 0.4 g./100 lb. weight.
[c] Fed mixed tocopherols, 2 g. daily after 10–3–47.

first calving and until she was discontinued on experiment about 18 months later. Data relating to the cattle used in the experiment are presented in table 2.

Feeding and Management. The ration fed was planned to provide adequate amounts of all known essential nutrients excepting vitamin E. It was made up of some of the feedstuffs listed in table 1, along with a few others that earlier tests had shown to be relatively free of vitamin E. The ration consisted of rice straw as the sole roughage and a concentrate mixture which was designed to be palatable and to provide the nutrients needed. A mixture typical of those fed contained 25 per cent polished rice, 30 per cent brewers' dried grains, 18 per cent distillers' grains (solvent extracted), 11 per cent corn starch, 9 per cent dry skim milk, 4 per cent rendered lard, 2 per cent steamed bone meal and 1 per cent iodized salt. Delsterol (2000D) was added as a source of vitamin D, at the rate of 0.6 g. per lb. of concentrate mixture.

Steamed bone meal and iodized salt were available free choice to all animals. A vitamin A concentrate (potency approximately 8000 I.U.) was fed once daily to each animal at the rate of approximately 10,000 units (I.U.) per 100 lb. of weight (14). It was fed directly to calves, but for older animals, it was mixed with the grain at time of feeding. Table 3 presents the results of several vitamin E tests made on the rations fed.

TABLE 3

Vitamin E tests of various rations fed to cattle

Ration no.	Female rats					Male rats			
	No. of rats used	Av. amt. eaten by time mated	No. of complete resorptions	No. of live litters	Live litter efficiency	No. of rats used	Days fed test ration	Testes, % of normal weight	Av. stage of testicular degeneration[a]
		(*g.*)			(%)				
1	10	1135	10	0	0	10	100	55.8	4.35
2	9	979	9	0	0	10	100	59.4	4.00
3	9	984	9	0	0	10	100	69.5	3.25
4	7	987	7	0	0	10	100	61.6	4.10

[a] Stages of degeneration range from 0.0 to 5.0 for the most advanced stage.

All calves were fed whole milk until about 3 weeks old, followed by fresh skim milk to about 6 months of age. The whole milk fed to the calves born in the herd was produced by cows receiving vitamin E-poor rations. Rice straw was fed *ad libitum* along with enough concentrates to provide the protein and energy required according to Morrison (20).

The positive control animals, E430, E438, E607 and E615, were fed according to the same plan as all the others except that a vitamin E-rich supplement was added to their diet. For E430 and E438 this consisted of the addition, once weekly, of sufficient synthetic *alpha*-tocopherol, dissolved at 2 per cent level in partially hydrogenated vegetable oil (Crisco), to provide approximately 0.4 g. *alpha*-tocopherol per 100 lb. weight of animal. E438 was fed at approximately twice this level after she calved. For E607 and E615 the tocopherol concen-

trate[10] was incorporated in the grain mixture in such amounts as to assure an intake of about 5 mg. per kg. of body weight per day. E559 was fed a concentrate[10] providing about 2 g. of mixed tocopherols daily from 3 weeks before her first calving until discontinued on experiment about 18 months later.

Tests made on feces from cattle fed vitamin E-poor rations, as well as on those from similar animals fed normal rations, indicated that vitamin E is not synthesized within the digestive tract. Ten female rats that consumed dried feces in amounts equivalent to from 60 to 620 g. of fresh feces obtained from cattle fed vitamin E-poor rations all showed complete resorption of fetuses during pregnancy and 10 males reared from weaning to 100 days of age on a ration containing 50 per cent on dry matter basis of fresh feces showed complete degeneration of the germinal epithelium. Three of ten females consuming 73 to 700 g. of feces from cattle fed normal rations gave birth to normal litters.

The calcium and inorganic phosphate content of the blood of each animal was determined at monthly intervals for over 3 years during the years 1941 through 1944. These analyses showed all animals to be in a state of luxus nutrition as far as these elements were concerned; the inorganic phosphorus of the plasma seldom was below 6.0 mg. per cent and frequently exceeded 9.0 mg. per cent. Following 1944, blood analyses were made at less frequent intervals. Invariably all values were well within the normal range as indicated by various investigators (12, 15, 21).

While on experiment the cattle were kept isolated. They were turned outdoors for exercise in a vegetation-free lot whenever weather permitted. The exercise lot was divided into two parts permitting segregation of animals whenever desired. Calves were kept in individual pens in the barn until large enough to be stanchioned. Shavings and waste rice straw were used for bedding.

Reproduction. The cattle were observed daily, especially for manifestations indicating development and functioning of the organs of reproduction. Sexual development and behavior among the bulls was tested by permitting them to mingle with females showing estrum. Beginning when about 6 months old, all males receiving the vitamin E-poor ration invariably showed marked libido during such contact. Semen specimens obtained intermittently by use of artificial vagina and rectal massage of ampula were studied for normality and fertility. Spermatogenesis was not affected, as examination and tests showed that all ejaculates obtained were normal in sperm activity, morphology and longevity.

Studies of the sexual development and activity in females included frequent observations for both physical and psychological signs of estrum, as well as rectal examinations of the uterus and ovaries for evidence of ovulation. These showed that the estrus cycle with all its characteristic and continuous changes including ovulation occurred regularly and in a normal manner starting when the heifers were 7 to 9 months old. Females exhibiting estrum invariably showed a strong desire to mount or ride other animals in the herd.

Breeding and calving records were kept for all animals. A summary of those

[10] "Myvadry" prepared by Distillation Products, Inc., Rochester, N. Y.

relating to the females is presented in table 4. The data show that the reproducing ability of the cattle was not affected adversely by feeding the vitamin E-poor ration continuously through three generations.

The data in table 5 show that a total of only 30 services was required to produce 25 pregnancies in the 19 females of breeding age that were fed the vitamin

TABLE 4

Data relating to the breeding ability of cows

Herd no.	Cows						Calves			
	Times bred	No. of calving	Calving date	Age at calving		Length of gestation	Herd no.	Sex	Birth weight	Physical condition
				(*yr.*)	(*mo.*)	(*d.*)			(*lb.*)	
E348[a]	1	1	6-10-43.	3	3	275	E483	F	69	Very good
E380	1	1	4-12-43	2	11	279	E481	F	75	Very good
	2	2	7- 8-44	4	2	278	E541	F	77	Very good
	1	3	5- 9-45	5	0	277	E559	F	75	Very good
		Cow died	5-10-45.							
E383	1	1	4-12-43	2	10	283	E482	M	97	Excellent
	2	Cow died	2- 2-44.	Pregnant	186	days.				
E384	2	Cow died	4-10-43.	Pregnant	246	days.				
E390	1	1	3-30-43	2	7	281	E480	F	95	Excellent
	1	Cow died	4- 7-44.	Pregnant	240	days.				
E401[b]	1	1	10-30-43	2	3	279	E496	F	85	Excellent
E403	1	1	2-21-44	2	5	284	E516	M	67	Excellent
	1	Cow died	8-13-45.	Pregnant	248	days.				
E405[b]	1	1	10- 8-43	2	1	281	E490	M	78	Good
E408[b]	3	1	1-17-44	2	4	285	E511	F	74	Excellent
E413[b]	1	1	1- 6-44	2	3	270	E507	F	55	Good
E438[c]	1	1	3-23-44	2	1	279	E522	F	72	Good
	2	Cow slaughtered	3-21-45.	Pregnant	180	days.				
E480	1	1	10-30-44	1	7	286	none	F	calf smothered at birth	
	1	Cow died	8-22-45.	Pregnant	225	days.				
E481	1	1	6-23-45	2	2	279	E562	F	47	Fair
E507	1	1	5- 8-45	1	4	279	E558	F	55	Good
		Cow died	10-31-45							
E541	1	1	11-27-45	1	5	281	E573	F	78	Very good
		Cow died	4- 4-46							
E558	1	Cow died	7- 8-47.	Pregnant	245	days.				
E559[d]	1	1	10-24-47	2	5	286	E641	F	80	Very good
	2[e]	2	2-12-49			277	E682	F	80	Very good
E573	1	1	10-17-47	1	11	285	E639	M	61	Good
		Cow died	10-19-47							
E595	1	1	7-25-48	2	1	275	E672	M	87	Good
E607[c]	1	1	9- 5-48	2	2	279	E674	F	72	Good
E612[c]	1	1	2-26-49	2	7	276	E683	M	96	Good
E615[c]	1	1	8-11-48	2	0	280	E673	M	80	Good

[a] Started on experiment 6-3-42 and removed from experiment 6-12-43.
[b] Removed from experiment 1-28-44.
[c] Positive control animal.
[d] Fed 2 g. mixed tocopherols daily after 10-3-47.
[e] Bred to bull fed normal ration.

E-poor rations. An average of only 1.2 services were required per conception. The 6 bulls used had an average breeding efficiency of 83.3 per cent. All F_1 and F_2 generation heifers conceived on the first service. All of the positive control heifers also conceived on the first service for their first calving, but E438 and E559 each required two services for the second pregnancy.

All heifers dropped their first calf when about 2 years old. Two F_1 generation heifers were less than 18 months old when they calved. One F_1 generation bull mated successfully when only about 10 months old, thus indicating that the vitamin E-deficient ration did not delay sexual maturity. One cow (E380) gave birth to three calves within a period of 25 months, with only 10 months between the last two parturitions. There were no abortions during the experiment. The length of all gestation periods were within the normal range (17). All calves were normal in size and vigor at birth. Fetal membranes invariably were expelled within several hours after calving occurred. No abnormalities were found in the reproductive organs of animals that died or were slaughtered.

Growth and physical condition. All animals were weighed at birth or when placed on experiment, and subsequently at 30-day intervals. Weights of experimental animals at various ages and the normal weights (25) at comparable ages of cattle of the several breeds represented are presented in table 6. These com-

TABLE 5

Breeding ability of bulls fed vitamin E-deficient rations as indicated by number of services required per conception when mated to cows fed similar rations

Bull	Services	Conceptions	Services per conception	Breeding efficiency
	(*no.*)	(*no.*)	(*av.*)	(%)
E394	1	1	1.00	100.0
E397	16	11	1.45	68.8
E398	1	1	1.00	100.0
E482	7	7	1.00	100.0
E585	2	2	1.00	100.0
Pure bred Guernsey[a]	3	3	1.00	100.0
Total or av.	30	25	1.20	83.3

[a] Temporarily fed vitamin E-deficient ration.

parisons, although not entirely satisfactory because of the mixed inheritance of the experimental animals, show that some of the cattle in the original group were below normal weight for their breed but that their descendants invariably were considerably above it.

The cattle displayed few abnormalities in action or appearance. The hocks of one F_2 generation heifer (E558) were swollen slightly at birth, a condition which persisted throughout her entire life. The swellings remained soft and were most prominent anteriorly, extending several inches above and below the joint.

All animals, after several months on the experimental diet, invariably exhibited some degree of "tongue lolling," a phenomenon not infrequently observed in cattle fed rations deficient in some essential factor or factors. A marked desire to lick fence or stall boards also was evinced. Feeding of a mineral mixture containing cobalt, manganese, copper, iron and magnesium for several months had no visible effect in alleviating the symptoms noted above or in improving the physical condition of the cattle.

Death losses. Thirteen out of the 28 animals fed the vitamin E-poor rations

for 1 year or more died suddenly at ages ranging from 21 months to 5 years. The daughter of E480 smothered at birth. Two others, E490 and E562, died soon after they were born, due to improper care by an inexperienced caretaker. E496 died as the result of an accident and E511 was slaughtered after she broke a leg falling on slippery pavement.

TABLE 6

Weight in lb. of cattle at birth and at various intervals up to 720 days of age with breed normal[a] weights

Herd no.	Breed	Sex	Age (d.)					
			Birth	30	180	360	540	720
E380	Grade Holstein	F	71	76	234	507	732	960
E383	Grade Holstein	F	90	105	328	578	815	1,022
E384	Grade Holstein	F	70	77	223	425	700	903
E390	Grade Holstein	F	88	90	316	532	840	1,014
E401	Grade Holstein	F	84	94	314	585	837	1,079
E405	Grade Holstein	F	85	99	309	544	773	973
E408	Grade Short Horn	F	75	113	301	564	845	1,049
E413	Grade Holstein	F	82	97	334	504	726	1,010
E438[b]	Grade Holstein	F	75	99	257	589	828	1,022
E595	Grade Holstein	F	95	133	345	580	725	990
E607[b]	Grade Holstein	F	84	135	325	536	740	966
E612	Grade Short Horn	F	95	131	335	481	705	935
Normal weight, Holstein		F	90	112	355	632	845	1,069
E403	Grade Jersey Short Horn	F	50	61	205	403	616	781
E480	Grade Jersey	F	95	106	347	628	892	851
E481	Grade Jersey	F	75	88	262	541	766	1,003
E507	Grade Jersey	F	60	91	383	629	746	.....
E511	Grade Jersey	F	74	104	354	.....	.....	.....
E558	Grade Jersey	F	55	83	290	499	694	874
E559	Grade Jersey	F	75	113	354	723	916	1,066
E573	Grade Jersey	F	78	101	337	594	803	884[c]
E615[b]	Grade Hereford	F	68	83	298	483	705	865
E641	Grade Jersey	F	79	117	309	568	.....	.....
Normal weight, Jersey		F	53	67	243	450	601	733
E585	Grade Holstein	M	83	125	390	781	1,190	.....
Normal weight, Holstein		M	94	125	399	741	1,176	1,438
E396	Jersey	M	43	.....	264	588	824	1,027
E397	Jersey	M	62	.....	282	558	744	1,022
E398	Jersey	M	64	82	281	573	806	970[d]
E482	Grade Jersey	M	97	127	414	756	1,011	1,258
E516	Grade Jersey	M	67	95	342	708	.....	.....
E639	Grade Jersey	M	62	77	267	545	.....	.....
Normal weight, Jersey		M	60	78	282	531	745	969
E394	Guernsey	M	58	78	240	546	765	945
E430[b]	Guernsey	M	76	81	261	543	799	1,039
Normal weight, Guernsey		M	71	87	291	609	.....	.....

[a] Mo. Agr. Expt. Sta. Bull. 336. 1934.
[b] Control animal.
[c] Weight at 707 d. of age.
[d] Weight at 662 d. of age.

Unlike any of the other animals, E348 was fed the vitamin E-poor ration for only about 1 year starting when she was about 2 years old. She was bred and conceived on the first service to E394 about 3 months after she was started on the experiment. The gestation period which followed was uneventful and she appeared to be in excellent physical condition at time of parturition. Two days after giving birth to a normal heifer calf she was discontinued on experiment and placed on a normal ration consisting of alfalfa hay, corn silage and ground grains. During the first few days after changing to the new ration her feed consumption was normal, but after that she refused all feed except an occasional sip of water. She died on the 19th day after calving. The post-mortem examination failed to reveal the cause of her death. It is possible that the extreme change in ration upset the established microflora in the digestive tract to such an extent as to prove fatal to the cow.

The other 12 animals which died suddenly displayed few premonitory symptoms of their impending death. Several collapsed while standing in their stanchions consuming their rations. Most of them showed some loss of appetite for a month or more before their death, with loss in weight or decrease in rate of gain. One heifer (E558) developed a condition of profuse salivation with almost complete anorexia several weeks before her death. Several days before her death, she became too weak to stand. The only male which died suddenly was E482. He was being used as herd sire at the time of his death and was about 30 months old. His weight declined from a maximum of 1,344 lb. on June 16 to 1,275 lb. at the time of his death 4 months later. He had the habit of scattering his feed making it difficult to obtain an accurate record of the amount consumed.

In no case did gross post mortem examinations reveal pathologic changes sufficiently severe to indicate the specific cause of death. Slight hemorrhages were found in the brain of some of the cattle and in others they were apparent on the bowels and occasionally on the heart and pancreas. These sudden deaths, as was shown by Gullickson and Calverley (9), probably resulted from cardiac injury induced by restricting them to a ration deficient in vitamin E. This phase of the study will be considered more fully in a later report.

Milk and fat production. Records were kept of the milk and fat production of each cow. The milk produced at each milking of every cow was weighed and on one day each month, the fat content of a composite of the milk of each cow was determined by the Babcock method.

Of the cows fed vitamin E-poor rations, only E380 completed two lactation periods and only two others completed one. Three cows died and another was discontinued on experiment before completing the first lactation period. The control animal E438 was slaughtered for the purpose of obtaining body fat for chemical and biological tests after she had milked 388 days. The other control animals, E607 and E615, were discontinued after milking about 7 months. The cow E559, which was fed a tocopherol supplement from 3 weeks before her first calving and until discontinued on experiment about 18 months later, experienced two normal calvings and completed one lactation period of 383 days. The milk and fat production of each animal is reported in table 7.

TABLE 7

Milk and butterfat production of cows on experiment

Herd no.	Age at calving		Milking period	Milk	Fat	
	(*yr.*)	(*mo.*)	(*d.*)	(*lb.*)	(%)	(*lb.*)
E380	2	11	277	6904.1	3.04	209.9
	4	2	232	6764.1	3.20	216.5
E383[a]	2	10	289	8186.9	2.93	239.9
E390	2	7	317	10135.3	2.85	288.9
E403	2	5	447	9028.8	3.25	293.4
E438[b]	2	1	358	9078.9	3.31	300.5
E480[a]	1	7	290	7868.2	3.92	308.4
E507[a]	1	4	171	3332.6	3.94	131.3
E559[c]	2	5	383	6764.3	3.38	228.6
E595	2	1	234[d]	4714.7	3.14	148.0
E607[b]	2	2	191[d]	4186.9	2.52	105.5
E615[b]	2	0	216[d]	3028.1	3.11	94.2

[a] Died suddenly before end of lactation period.
[b] Positive control animal.
[c] Fed mixed tocopherols, 2 g. daily during entire lactation period.
[d] Experiment discontinued before completing lactation period.

DISCUSSION

Although the feeding of vitamin E-poor rations apparently did not affect the ability of cattle to reproduce, it nevertheless is significant that out of the dozen or more animals which died suddenly from the characteristic heart ailment described by Gullickson and Calverley (9), six had been pregnant 6 to 8 months at the time death occurred, three died within 3 days and one (E348) 19 days after calving. However, pregnancy and parturition probably were only indirectly the cause of these deaths. A critical vitamin E shortage would be expected to develop during the last several months of the gestation period when the requirement for various essential nutritive factors has been shown to increase markedly (6). In rats during the corresponding period degenerative changes occur, not in the deficient mother but in the developing embryo, which eventually succumbs and is resorbed (8). Careful studies by Mason and Bryan (18) have shown that placental transmission of vitamin E in the rat is very small. It is possible that in the bovine it may be greater, resulting in sacrifice of the mother instead of the developing fetus. Whiting and Loosli (26), in experiments with sheep, goats and swine, found that adding tocopherols to the prepartum ration produced a highly significant increase in the tocopherol content of the blood plasma of the lambs and kids but no increase was observed in the pigs. Palmer *et al.* (23) have shown that deficiencies of protein and various minerals affect the cow more seriously than her unborn calf. The increased burden imposed during the stress of calving and initiation of lactation are other factors that would be expected to affect the already injured heart and the welfare of the cow during this period. Brody *et al.* (4) found that gestation increases heat production in cattle about 40 per cent above the non-gestating level during the last one-third of the gestation period, and cows lactating heavily produce about twice as much heat under normal feeding conditions as when not milking. Pulse

rate, respiration rate and ventilation rate were found to parallel the course of heat production.

It is possible that if the rations fed had been completely vitamin E-free, few if any of the females involved would have survived their first gestation period. That neither pregnancy nor lactation is essential to the development of the lethal condition, however, is suggested by the deaths of bull E482 and several of the non-pregnant females.

There is no indication that the vitamin E-deficient ration fed retarded the growth of cattle except insofar as it appeared to reduce feed consumption in a few cases. Neither did rate of milk and butterfat production appear to be affected adversely, for, as is indicated in table 7, the quantity and fat content of the milk produced by the control cows, including E559, are not markedly different from that of cows fed vitamin E-poor rations. Recently it was shown by several workers (10, 24, 27) that feeding of mixed tocopherols to cows on normal rations has little or no effect on the quantity and fat content of milk produced.

SUMMARY AND CONCLUSIONS

A study was made to determine the role of vitamin E in the nutrition of cattle and especially as it relates to reproduction. A total of 30 animals of mixed breeding (8 males and 22 females), including those in the original group and their second, third and fourth generation descendants, were fed throughout their lives on rations providing adequate amounts of all nutritive factors known to be essential except vitamin E. The rations fed were incapable of supporting reproduction in rats when fed in liberal amounts. Four positive control animals, one bull and three cows, were fed exactly like the others except that each of them was fed a supplement providing either *alpha*-tocopherol or mixed tocopherols. Organs of reproduction developed normally in animals of both sexes. Spermatogenesis in males was not interfered with and all ejaculates studied were normal as to volume, sperm activity, morphology and longevity. The estrus cycle in its various phases, including ovulation, occurred regularly starting when heifers were about 7 months old.

A total of 30 services produced 25 conceptions in the cattle fed vitamin E-poor rations, but only 19 of these terminated in normal parturitions, since the other six cows died suddenly 1 to 3 months before they were due to calve. All calves born were normal in size and vigor at birth. There were no abortions and fetal membranes invariably were expelled within several hours after the calves were born. The average breeding efficiency of the five bulls used was 83.3 per cent.

One bull about 30 months old and 12 females ranging in age from 21 months to 5 years died suddenly, apparently from cardiac failure. Six of the females had been pregnant 6 to 8 months when death occurred and three died within 3 days and one 19 days after calving. Gross post mortem examinations failed to reveal pathologic changes sufficiently marked in any of them to indicate a specific cause of death.

Vitamin E does not appear to be required by cattle for successful reproduction, but long-continued feeding of rations containing very much less vitamin E than is present normally under practical conditions is likely to prove fatal to such aimals. Feeding rations in practice as deficient as those fed in this experiment is exceedingly unlikely as all feeds commonly fed to cattle are relatively rich in vitamin E.

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THE RATE OF PHOSPHATASE INACTIVATION IN MILK[1]

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The phosphatase test over a period of years has proved to be an excellent method of determining whether holder-processed milk is pasteurized properly. Its wide acceptance by industry and health authorities is based on research and the ease and speed with which the results are obtained. In England, where Kay and Graham (10) first introduced the test in 1933, milk must be heated to and held at 145° F for 30 minutes to be considered pasteurized. In addition, the enzyme must be inactivated completely. In areas where the pasteurization temperature may be 142 or 143° F with a 30-minute holding period and a partial inactivation of the enzyme is acceptable, the interpretation of the results may vary considerably. Evidence of this is noted readily in the several modifications of the phosphatase test and standards which are recognized and used (1).

Kay and Graham (10, 11) and other workers (5, 7, 8, 9, 15, 16) have recognized that the time required to heat the milk to pasteurization temperature may affect the results of the test. This is especially true when higher temperatures of pasteurization are used, as in the flash process. The inactivation effect of the preheating time on flash-pasteurized milk may be great enough to indicate proper pasteurization, even though the milk was not held at the required temperatures for the necessary time. With the exception of a recently published study (8) no data were found in the literature indicating the exclusive effect of preheating time. Herschdorfer (6) published experimental data to show that an inverse relationship exists between the rate of cooling and the rate of phosphatase inactivation in milk.

The purpose of this study is to determine the rate of phosphatase inactivation in milk with a minimum influence of either preheating or cooling time.

REVIEW OF LITERATURE

Much interest has developed in the type of reaction involved when phosphatase is inactivated by heat in milk. Unfortunately, whether heat inactivates phosphatase in milk at a monomolecular rate has not been established definitely to everyone's satisfaction. Sanders and Sager (14, 15) have reported that a straight line results when the logarithms of the times of heating are plotted against the corresponding temperatures. Hetrick and Tracy (7) reported that semilogarithmic relationship between temperature and time over the temperature range of 143 to

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185° F. was found to exist for the thermal destruction of phosphatase. On the other hand, Holland and Dahlberg (9) show a curve which appears to be a straight line until temperatures around 170° F. are attained, and then a hook may be observed. Basic information on this subject is imperative and could be used to predict the effect of a higher temperature on the inactivation of phosphatase as compared to a lower temperature if a semilogarithmic relationship between temperature and time were established and expressed in units independent of the pasteurization process and phosphatase test employed.

METHODS

Fresh whole milk from several different sources was used. Samples (approximately 20 ml.) were sucked through a copper coil into an attached U tube by means of a pump. The copper tubing was 25 feet long and had an inside diameter of 0.125 inches. The coil and U tube were maintained in a thermostatically-controlled water bath at a predetermined temperature within a range of $\pm$ 0.1° C. A thermocouple was used to determine the temperature of the milk as it entered the glass tube and during the holding period. It required 7 seconds ($\pm$ 1 second) for a unit of milk to travel through the coil. Samples were pipetted into test tubes held in ice water at the end of a holding period which was accurate to within $\pm$ 3 seconds. The (Rapid) Laboratory Phosphatase Test developed by the New York City Health Department (1) was used, together with a Pfaltz and Bauer fluorophotometer, Model B, with a 660-mμ filter, to determine the degree of phosphatase inactivation. Photometer units were converted to parts per million of phenol by reference to a prepared standard phenol curve (12). Time-temperature conditions that produced color equal to 0.5 p.p.m. of phenol were selected as end points because the data were reproducible under these circumstances. Ball's method (2) was used to interpret the data and to compare them with the results of other workers (4, 7, 8, 14).

EXPERIMENTAL

Fresh samples of milk were kept at approximately 5° C. until poured into a test tube (15 $\times$ 150 mm.) which was attached immediately to the copper coil. The laboratory equipment is shown in figure 1.

Because of the relatively large capacity of the vacuum pump, milk flowed from the test tube and through the coil instantly after the pump was started and the system was closed. It always was necessary to maintain samples for varying periods at a predetermined temperature to determine the holding time required to inactivate the enzyme to the desired degree. After this point was located samples in duplicate were held for those times necessary to inactivate phosphatase to the desired degree and slightly under and over this point. In this manner the end-point of 0.5 p.p.m. ($\pm$ 0.4) of phenol was checked not only by duplicate samples but also by its relative position in a series of samples for each degree of temperature from 60 to 71° C., inclusive. The results of this study are shown in table 1. These data were used to locate the curve in figure 2. The curve indicates that the rate of phosphatase inactivation in milk is a mono-

molecular reaction, at least from a practical view point, over the range of temperature considered. Ball's mathematical technic was used to interpret the data. In this method, experimental data are presented in graph form on semi-logarithmic paper. The vertical scale represents the logarithm of the time in minutes and the abscissa represents degrees of temperature. The standard symbol for designating the slope value of the curve is Z, expressed as degrees of temperature obtained from one logarithmic cycle on semi-logarithmic paper. The Z value for the curve in figure 2 is 4.9° C. When Ball's method was used to interpret the published data of several workers (4, 7, 9, 14), Z values of 4.60, 4.95, 4.50, and 4.85° C., respectively, were obtained.

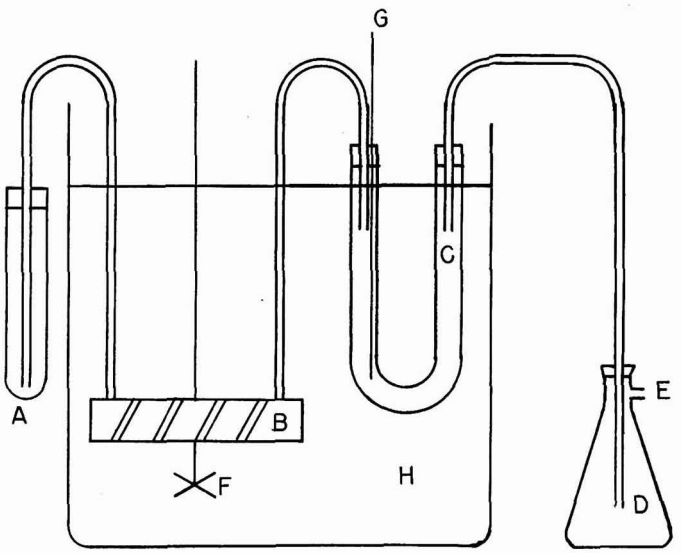

FIG. 1. Laboratory Pasteurizer. A– Test tube containing milk sample. B– Pasteurizer coil, 25 ft. long, 0.125 in. internal diameter (copper). C– U Tube. D– Trap in suction line. E– Line to suction pump. F– Electric water agitator. G– Thermocouple, iron-constantan. H– Constant temperature water bath.

DISCUSSION

It is difficult to compare much of the published information about the heat inactivation of phosphatase because experimental methods and units used to express the results are different in many of the reported studies. Several workers (3, 4, 8, 9, 11, 13, 15, 16) have recognized these variables.

After the present study was started data were reported (7, 14) to indicate that the rate of phosphatase inactivation was a straight line reaction over the temperature range usually employed for pasteurizing milk when the preheating and cooling times were extremely short. Calculated Z values, based on these data, were found to be 4.95° C. and 4.85° C., respectively. Our own data indicate

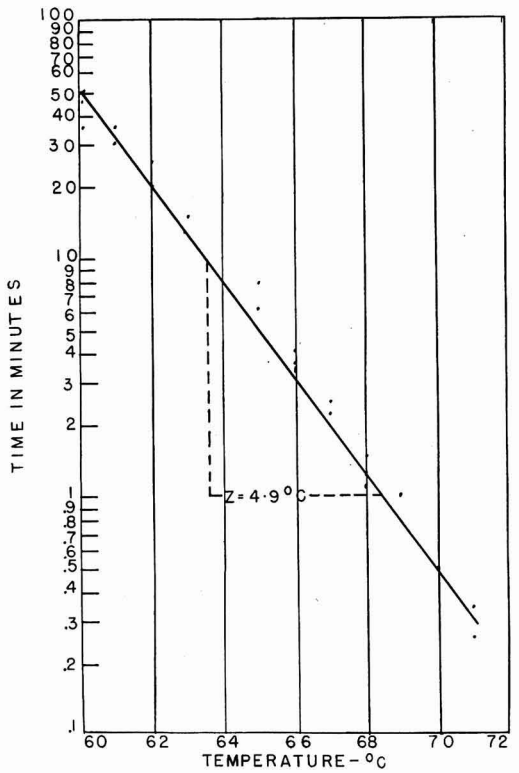

FIG. 2. The rate of phosphatase inactivation in milk.

a Z value of 4.9° C. The high degree of agreement of the Z values based on data from three different laboratories indicates that this method of expressing the effect of heat on phosphatase may be of practical value. When other data (4, 9) were used, the Z values were found to be 4.6° C. and 4.5° C., respectively. These figures show the effect of preheating time on the slope of the curve.

TABLE 1

Effect of time and temperature on the inactivation of phosphatase in milk

Temperature	Holding time	Phenol[a]	Temperature	Holding time	Phenol[a]
(°C.)	(Min.)	(p.p.m.)	(°C.)	(Min.)	(p.p.m.)
60	40	0.35	66	3.50	0.84
61	35	0.17	67	2.50	0.53
62	21	0.33	68	1.25	0.39
63	15	0.47	69	1.00	0.78
64	7	0.64	70	0.50	0.55
65	7	0.54	71	0.30	0.30

[a] Av. of at least three samples, each run in duplicate.

The significance of the variations in Z values for two or more curves with a common point is that the lowest one implies the highest relative inactivation rate for a high temperature as compared to that of a low temperature. It should be emphasized that the Z value is an expression of the inactivation rate and is not to be misconstrued as an intensity factor or lethal value. Equal Z values indicate that the reactions proceed at the same rate; that is, the curves are parallel. However, two phenomena may have the same Z value but one may take a more intense heat treatment than the other to accomplish destruction. This is illustrated in the data presented by Sanders and Sager (15).

It is impossible to say what difference between slope values is commercially significant in a milk plant at present. The time-temperature curve of phosphatase inactivation under commercial conditions will be influenced by the preheating and cooling times and will have a slope value characteristic for the conditions under which the inactivation is accomplished. These effects are not only applicable to the enzyme phosphatase but also to bacteria. Commercial experience shows that the effect of holder- and flash-pasteurization conditions may be practically equal for destroying pathogens ordinarily found in milk but unequal for thermoduric bacteria. This means that the Z values of the thermal death time curves for pathogens are lower than those for thermoduric bacteria.

CONCLUSIONS

1. Ball's methods (2) may be used as a basis for comparing the effect of heat on the rate of phosphatase inactivation in milk pasteurized by different methods.

2. The Z value for the rate of phosphatase inactivation in milk in this study is 4.9° C.

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A STUDY OF THE DIACETYL IN CHEESE. I. DIACETYL CONTENT AND FLAVOR OF CHEDDAR CHEESE[1]

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Diacetyl is a prominent element in the flavor of many food products. This is true particularly of food products that are produced by a fermentation process.

Prill *et al.* (9) have discussed the various properties of diacetyl as well as its higher homologues. In their work the general chemical structure of the *alpha*-dicarbonyl compounds was presented with particular reference to the contribution of diacetyl and its homologues to butter flavor and aroma. While the influence of the diacetyl content on the flavor and aroma of butter and starter cultures has been investigated to a considerable extent, little work has been done to determine the diacetyl content of cheese. Both Crocker (2) and Davies *et al.* (6) have indicated that diacetyl could be a possible constituent of cheese aroma. In listing the various flavors of cheese, Davis (7) referred to the buttery aroma and suggested that the substance responsible was diacetyl. Davies *et al.* (6) attempted to determine the effect of adding diacetyl to the curd in making cheese. They reported that when diacetyl was added in the concentration of 1.7 p.p.m. to the curd, a cheese was produced that had a stinging effect on the tongue.

In 1941, Csiszár and Bakos (3) and Csiszár *et al.* (4, 5) conducted studies in Hungary on the diacetyl content of such types as Emmenthal, Ovári, Trappist, Edam, Roquefort, Camembert and Romadour. These investigators reported that the aroma and quality in cheese were better when the cheese was made with a culture which was highly active in acetylmethylcarbinol-diacetyl formation. The diacetyl content of the various types of cheese as reported by Csiszár *et al.* (4) ranged from none to as much as 1 mg. per 100 g. of cheese. This study was undertaken with these objectives: (a) to determine whether diacetyl is a component of cheese; (b) if diacetyl is present, to determine to what extent; and (c) to determine whether there is a correlation between cheese flavor and the amount of diacetyl present in the cheese.

METHODS

The colorimetric method of Prill and Hammer (10) seemed to offer the best means of determining the relatively small diacetyl content of cheese. This method was very sensitive and could be adapted easily to routine analysis. To adapt this method to the determination of diacetyl in cheese, alterations which involved the preparation of the sample for analysis, a slight modification of the distillation apparatus and measurement of the concentration of ammono-ferrous dimethylglyoximate by means of a Beckman spectrophotometer were made.

Preparation of the cheese sample. Cheese, unlike butter cultures, cultured milks and butter, does not lend itself readily to steam distillation. Upon heating,

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the cheese becomes a plastic mass with a tendency to retain any volatile components ordinarily removed by steam distillation of a liquid. Even after grinding, all of the diacetyl that is present cannot be removed from the cheese unless the sample is converted into a liquid form.

Consistent results can be obtained if the sample of cheese is treated in the following manner: A given weight of ground cheese (usually 200 g.) plus an equal weight of distilled water are placed in a Waring blendor. The blendor is run at low speed for 2 min., followed by mixing at high speed for 3 min. Such treatment produces, for all practical purposes, a homogeneous suspension, as the data presented in table 1 indicate. The differences between the theoretical solids

TABLE 1

Solids measurements of cheese-water mixtures as a criterion of homogeneity

Sample no.	Cheese	Solids/100 g. cheese-water mixture[a]		Difference	Cheese equivalent to the difference
		Measured	Calculated		
	(*g.*)	(*g.*)	(*g.*)	(*g.*)	(*g.*)
1	65.05	32.90	32.53	+0.37	0.57
2	65.50	32.60	32.75	−0.15	0.29
3	64.95	32.80	32.47	+0.33	0.51
4	65.40	33.00	32.70	+0.30	0.46
5	64.60	32.80	32.35	−0.45	0.68

[a] Solids content determined by *Official Methods* (1).

content of the cheese-water mixture, and the measured solids content were converted by calculation to their equivalent weights of cheese. The standard deviation of these differences was calculated and found to be equivalent to ± 0.38 g. of cheese. The maximum difference rarely should exceed three times the standard deviation or approximately ± 1.14 g. of cheese when a 200 g. aliquot of the cheese-water mixture is used to represent 100 g. of cheese.

Subsequent analyses indicated that only rarely would the diacetyl content of cheese exceed 0.1 mg. per 100 g. Therefore, the error in the amount of diacetyl measured by using this procedure would not exceed ± 0.001 mg. This amount of diacetyl is so small that it would not affect materially the final results of the determination.

Modification of distillation apparatus. The distillation apparatus used for the determination of diacetyl in cheese was essentially the same as described by Prill and Hammer (10). However, instead of using glass discs in the reflux-fractionating column, the columns were packed with small pieces of 2 mm. glass tubing, about 5 to 10 mm. in length.

A simple steam generator was made to insure a constant source of uncontaminated steam. This generator was constructed in the following manner: Eleven ft. of nichrome wire with an approximate total resistance of 24 ohms was wound in the form of a coil and placed in the bottom of a 3-l. round-bottomed flask. Each end of the wire was connected to a copper lead, sealed in glass tubing that passed through the rubber stopper. The other end of the copper lead was con-

nected to a rheostat connected to the 110-volt, A.C. outlet to control the flow of steam. A glass funnel with a section of rubber tubing and a pinchcock in the stem was passed through the rubber stopper. This provided a means of introducing distilled water into the flask. A suitable outlet for the steam and a glass steam trap to collect the condensate also were provided. A steam generator of this design furnished an ample supply of steam for all distillations.

Dimethylglyoxime was used as the diacetyl standard as originally proposed by Prill and Hammer (10).

Spectrophotometric measurement of color. By measuring the intensity of the rose-red color complex with the Beckman spectrophotometer only one or two standards were needed for each group of determinations, rather than the range of standards mentioned in the original procedure.

The absorption spectrum was established by measuring the transmittance for a particular concentration of the diacetyl standard at various wave lengths. The results were plotted in the form of a curve relating optical density to wave length. For these determinations a Beckman spectrophotometer (model DU) and 1.000 cm. Corex cells were used. A nominal band width of 1 to 2 mμ. was used throughout the absorption spectrum determinations. The optical density was read directly from the spectrophotometer.

Solutions containing the equivalent of 0.02, 0.05 and 0.10 mg. of diacetyl per ml. were made from the diacetyl standard. The colored ammono-ferrous dimethylglyoximate derivative was prepared from each of these solutions according to the procedure of Prill and Hammer (10). A blank determination on all reagents was prepared at the same time. The optical density of these solutions was measured at various wave lengths ranging from 500 to 560 mμ. Maximum absorption was obtained at 530 mμ. Within the range of concentrations tested, Beer's law is applicable for this determination.

The accuracy of this modification of the Prill and Hammer method for the determination of diacetyl in Cheddar cheese was determined by means of recovery tests. To samples of each cheese, which had been analyzed previously for diacetyl by the modified method, known quantities of purified diacetyl were added. The total diacetyl content of these cheese-diacetyl mixtures then was determined by the modified method. The results of these recovery tests are shown in table 2. The percentages of recovery ranged from 95 to 102.3 per cent. The method appeared to be of sufficient sensitivity to measure the diacetyl content of cheese.

This modified colorimetric procedure was used for the diacetyl determinations of all lots of cheese. Duplicate samples of each cheese were analyzed. Samples were obtained by grinding either the whole cheese or a wedge of the cheese as described in *Official Methods* (1).

EXPERIMENTAL RESULTS

Twenty-eight lots of Cheddar cheese and eight lots of other varieties of cheese were selected for this investigation. The majority of the lots of Cheddar cheese had been manufactured at the Dairy Department of the University of Wisconsin. The rest of the lots were manufactured at various plants within the state of Wis-

consin. Typical cheese was selected so that it would resemble the average cheese found on the commercial market. Usually little was known concerning the making process of any particular lot of cheese. Fourteen lots of the Cheddar cheese had been manufactured from pasteurized milk, the remainder from raw milk. The cheese ranged in age from 1 to 48 months. Each lot of Cheddar cheese was examined by a judging panel composed of from one to three competent cheese judges. Comments upon the flavor of each lot of cheese were made by each judge. The flavor criticisms used were the standard comments usually employed in evaluating the quality of a cheese on the basis of flavor (8).

TABLE 2

Recovery tests

Test no.	Diacetyl in cheese	Diacetyl added	Diacetyl recovered	Recovery
	(*mg.*)	(*mg.*)	(*mg.*)	(%)
1	0.026	0.009	0.034	97.25
2	0.026	0.018	0.045	102.30
3	0.026	0.027	0.052	98.20
4	0.026	0.045	0.069	97.20
5	0 026	0.090	0.111	95.70
6	0.035	0.017	0.050	96.30
7	0.035	0.034	0.068	98.75
8	0.035	0.051	0.084	97.80
9	0.035	0.068	0.100	97.23
10	0.035	0.085	0.114	95.00

Other varieties of cheese included in this investigation with the diacetyl content of each lot (expressed as mg. per 100 g.) were as follows: Swiss (0.036), Brick (0.017), Limburger (0.021), Camembert (0.008), Blue Mold (0.030), Edam (0.043), Port Salut (0.074) and Cottage (0.029).

To illustrate the variations in the diacetyl content of the different lots of Cheddar cheese, the 28 lots were classified according to the diacetyl content. The results of this grouping are shown in table 3. The majority of all lots contained less than 0.05 mg. per 100 g. of cheese.

TABLE 3

Diacetyl in cheddar cheese[a]

Diacetyl content	No. of lots	% of lots
(*mg./ g. of cheese*)		
0.000–0.049	16	57.2
0.050–0.099	5	17.8
0.100–0.149	2	7.2
0.150 and over	5	17.8

[a] The diacetyl content of the Cheddar cheese ranged from 0.016 to 0.335 mg./100 g.

Flavor characteristics were used to divide the 28 lots of Cheddar cheese into two groups. One group of "Excellent Flavor" consisted of lots which had no adverse flavor criticisms. The other group contained all lots that had adverse flavor criticisms. These adverse flavor criticisms included anything from a very

slight off-flavor to a definitely objectionable flavor. This latter group was called the group with "All Other Flavors." Each of these two flavor groups then was divided into two sub-groups based on the diacetyl content of the lot. One sub-group contained all lots with less than 0.05 mg. of diacetyl per 100 g. of cheese. The other sub-group consisted of all lots with more than this amount of diacetyl.

The number of lots in each sub-group then was expressed as the per cent of the total lots in that flavor group. Seventy-nine per cent of the lots in the group with "Excellent Flavor" had a diacetyl content of less than 0.05 mg. per 100 mg. In the group with "All Other Flavors" 63 per cent of the lots had more than this amount of diacetyl.

DISCUSSION

The experimental data have shown that diacetyl was present in cheese both when it had an excellent flavor and when it was criticized adversely for flavor. The majority of the lots of cheese with an excellent flavor had a diacetyl content of less than 0.05 mg. per 100 g. of cheese. On the other hand, most of the lots of cheese with adverse flavor criticisms had a larger diacetyl content than this. The production of diacetyl probably was associated with other biological activities that produced substances that detract from the flavor of a cheese. The presence of these substances, and not the diacetyl content, probably was responsible for the adverse criticisms of the cheese flavor. The data show that it was possible to have more than 0.05 mg. of diacetyl per 100 g. of cheese without the production of the "flavor detracting" substances. Probably there is a maximum diacetyl content which, if exceeded, will cause adverse flavor criticisms. Frequently cheese judges identify the flavor as "acidy" when the cheese is normal in hydrogen ion concentration and has no acid defects in body. Crocker (2) described the flavor of diacetyl as "pseudo-sour." It is highly probable that this "pseudo-sour" flavor of diacetyl gives the impression of "acidy" flavor in a cheese that shows no other signs of high acidity.

Diacetyl seems to be an essential part of the flavor complex of Cheddar cheese. In amounts smaller than 0.05 mg. per 100 g. of cheese it is associated with excellent flavor qualities, but amounts in excess of this appear to be associated with adverse criticisms.

SUMMARY

Diacetyl is a prominent element in the flavor of many food products. To determine its presence in cheese and to make quantitative measurements of it, the colorimetric method described by Prill and Hammer (10) was modified. The color of the ammono-ferrous dimethylglyoximate formed in this procedure was measured spectrophotometrically.

Diacetyl was found in all lots and kinds of cheese examined. The majority of the lots contained less than 0.05 mg. of diacetyl per 100 g. of cheese. The diacetyl content of Cheddar cheese ranged from 0.016 mg. to 0.335 mg. per 100 g. of cheese.

A small quantity of diacetyl probably contributes to the typical flavor of Cheddar cheese. The majority of the lots of Cheddar cheese with an excellent

flavor had a diacetyl content of less than 0.05 mg. per 100 g. Larger amounts of diacetyl than this frequently appear to be associated with flavor defects.

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A STUDY OF THE DIACETYL IN CHEESE
II. THE CHANGES IN DIACETYL CONTENT OF CHEDDAR CHEESE DURING MANUFACTURING AND CURING[1]

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A previous study has shown that diacetyl was present in all lots of Cheddar cheese analysed (3). In many of the lots the amount of diacetyl present in the cheese was much greater than could be accounted for by the diacetyl content of the original milk and starter culture from which the cheese had been made. Since the diacetyl content of Cheddar cheese has been related to the flavor of the cheese and appears to be an element of cheese flavor, a further study of the diacetyl in Cheddar cheese seems advisable.

The purpose of this investigation was to measure the development of diacetyl during the manufacturing and early stages of curing of both pasteurized and raw-milk Cheddar cheese.

METHODS

Manufacturing procedures. Six lots of Cheddar cheese were made on six different days. Three lots were manufactured from milk pasteurized at 144° F. for 30 minutes. The other three lots were made from raw milk which had a reduction time of 4 hr. on the standard methylene blue test (1). The method of Price (11) was followed for all making operations.

Two lots of identical milk were made into Cheddar cheese, the milk being pasteurized for one lot and raw for the other. These lots were used to study the diacetyl content of cheese during the early stages of curing. The curd was placed in square hoops, pressed, cut into 2-lb. blocks, then pressed again overnight. The blocks of cheese were removed from the press the following morning and placed on shelves in the curing room which was maintained at 60 to 62° F. and at a relative humidity of 80 to 85 per cent. After 2 days, when the surfaces were sufficiently dry, each block of cheese was dipped into paraffin and packed in a wooden box. Each wooden box contained 10 of the 2-lb. blocks of cheese. The boxes were covered to minimize drying of the cheese and were stored at an average temperature of 45° F.

Sampling procedure. In the six lots used to study the development of diacetyl during the making process, the diacetyl content of the starter and of the milk was measured. Samples were removed for diacetyl determinations at setting, cutting, dipping (curd and whey) and milling (curd and whey).

Blocks of the two lots of cheese used to study the diacetyl content during the early stages of curing were ground and duplicate aliquot samples were prepared from each block.

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The diacetyl content of all samples was determined by the modified colorimetric procedure (3).

EXPERIMENTAL RESULTS

Development of diacetyl during the manufacturing procedure. The diacetyl measurements at the various steps during the making of the 6 lots of Cheddar cheese are shown in table 1. The diacetyl contents of the three lots

TABLE 1

Diacetyl measurements during the making of six lots of Cheddar cheese

Material	Diacetyl					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
			(*mg./100 g.*)			
Milk	0.002	0.002	0.002	0.003	0.002	0.002
Starter	0.037	0.085	0.115	0.040	0.037	0.070
Milk at setting	0.009	0.012	0.015	0.005	0.006	0.007
Curd before cutting	0.010	0.013	0.017	0.005	0.007	0.008
Dipping						
Curd	0.034	0.042	0.021	0.024	0.037	0.031
Whey	0.007	0.015	0.015	0.007	0.008	0.010
Milling						
Curd	0.035	0.047	.....	0.014	0.030	0.058
Whey	.....	0.023	0.022	0.024	.....	0.030

of raw milk at setting were lower than those of the three lots of pasteurized milk. This also was true of the measurements made of the diacetyl in the curd just prior to cutting.

Lots 2 and 4 were used as typical lots to calculate the quantity of diacetyl produced during the making of pasteurized-milk and raw-milk Cheddar cheese. The amount of diacetyl present at each step during the making procedure was calculated as the total milligrams of diacetyl present in either the weight of milk in the vat or the combined weights of curd and whey.

The quantity of diacetyl produced during the making of lot 2, a pasteurized-milk type of Cheddar cheese, was as follows: from the start of the making and up to the time of setting, 19.44 mg.; from setting to cutting, only 2.15 mg.; from cutting to dipping, 12.50 mg.; and from dipping until milling, 11.90 mg. The total increase of diacetyl was 45.99 mg.

A total of 39.16 mg. of diacetyl was produced during the making of lot 4, a raw-milk type of Cheddar cheese. Of this total, 1.42 mg. was produced prior to setting; none from setting to cutting; 9.50 mg. from cutting to dipping; and 28.74 mg. from dipping to milling.

Diacetyl content during the early stages of curing. The diacetyl content of a sample from a block of cheese representing each vat lot was determined at pressing and at 3, 18, 28, 34 and 45 days after pressing.

The gradual decrease in the diacetyl content of each type of Cheddar cheese during the early stages of curing is shown in table 2.

DISCUSSION

It is very likely that the diacetyl which is in cheese originates in a manner comparable to its formation in starter cultures. In starter cultures diacetyl is formed by bacteria acting upon the lactose and citrates. Van Beynum and Pette (13) have described the formation of the diacetyl from the citrates.

Many investigators have shown that diacetyl is a constituent of the desirable flavor and aroma in butter cultures (8). The organisms responsible for the production of this desirable flavor and aroma are *Streptococcus lactis, Leuconostoc dextranicum* (*Streptococcus paracitrovorus*) and *Leuconostoc citrovorum* (*Streptococcus citrovorus*) (7). Wilster and Price (15) have pointed out that these same organisms always are present in a good cheese starter as used in this country. It is primarily through their action that the lactose and citrates of the milk might be converted into fermentation products such as diacetyl in the cheese.

TABLE 2

Diacetyl content of Cheddar cheese during early stages of curing

Age	Diacetyl content	
	Pasteurized-milk type	Raw-milk type
(*d.*)	(*mg./100 g.*)	(*mg./100 g.*)
0	0.046	0.035
3	0.042	0.032
18	0.033	0.017
28	0.025	0.021
34	0.025	0.016
45	0.027	0.017

Many workers have shown that aeration and increased acidities favor the production of diacetyl in starter cultures. Prill and Hammer (12) noted that aeration of ripening starter cultures by shaking produced significant increases in the diacetyl, while the lack of aeration caused decreases in the diacetyl. They also noted that lowering the pH of the starter culture tended to increase the diacetyl content. Hedrick and Hammer (9) pointed out that an increased diacetyl content could be obtained in ripening cream by development of higher acidities and use of agitation. Increases up to several hundred per cent in the diacetyl content of starter cultures were noted by Brewer *et al.* (2) when air was bubbled through the starter culture. Cox (4) studied the effect of acidity on the production of diacetyl by betacocci in milk. His investigations were in the pH ranges of 5.5 to 4.4. He found that the rate of growth of the organisms was slower progressively with decreasing pH, but at least as much diacetyl was formed eventually at the low pH values as at high pH values. The results of Cox's investigations showed that there was an alteration in the metabolism of the organisms, the diacetyl producing power per unit cell in-

creasing with decreasing pH. According to van Beynum and Pette (13), the diacetyl was produced from citric acid only when the medium was acid and the conditions aerobic.

The same factors as discussed above also influence the rate of diacetyl production during the making of Cheddar cheese. The experimental data of this study with Cheddar cheese show that while the milk and starter are being stirred, prior to the time of setting, there is an increase in diacetyl. This stirring or agitation provides a means of aeration that would be expected to favor diacetyl production. Little diacetyl is produced between the time of setting and the time of cutting. During this period the material in the vat is quiescent, and the aeration that occurs at other times during the making procedure is lacking. Also, the pH at this time is much higher than it is later in the making procedure. This combination of factors does not encourage diacetyl production. From the time of cutting until the time of dipping, the material in the vat is stirred continuously. This aeration favors the production of diacetyl. From the time of dipping until milling diacetyl production is favored by the rapidly decreasing pH in the curd and whey.

In the six lots of cheese used in this investigation, the rate of diacetyl production during the early steps of the making procedure appears to be slower in the raw-milk than in the pasteurized-milk Cheddar cheese. The nature of the experimental procedure does not establish this as an actual fact. The trend is most interesting and might be investigated further because it suggests the action or influence of: (a) either substances or microorganisms in the raw milk that slow down or retard the production of diacetyl by the "aroma-formers" until later in the making procedure, (b) lack of oxygen or a lower oxidation-reduction potential in the raw milk that prevents or slows down the formation of diacetyl.

The microorganisms responsible for the formation of diacetyl in starter cultures probably are present during the making of cheese. Factors that influence the formation of diacetyl in starter cultures undoubtedly also influence its formation in cheese. Therefore, the diacetyl formed during the making of Cheddar cheese probably comes from the same source and is produced in the same manner as the diacetyl produced in starter cultures.

A gradual decline in the diacetyl content of both the pasteurized-milk and raw-milk Cheddar cheese was noted during the early stages of curing. This parallels observations which have been made by several investigators on the decline in the diacetyl content of butter during storage. According to Langton (10), the gradual disappearance of diacetyl in butter is caused by volatilization. On the other hand, many investigators, including Elliker (6), have indicated that some of the diacetyl in butter is reduced to acetylmethylcarbinol and 2,3-butylene glycol by the action of microorganisms. Virtanen and Kontio (14) added known amounts of diacetyl to samples of milk; the milk then was inoculated with cultures of microorganisms that had been isolated from butter. A decline in the diacetyl content of the milk samples was caused by the action of the microorganisms.

These factors responsible for the decline in the diacetyl content of butter during storage also may explain the decline of diacetyl in cheese. Carbon dioxide and moisture are lost in large amounts by volatilization from the surfaces of the cheese in the early stages of curing. This action would be expected to remove volatile substances like diacetyl from the cheese. The changes in the oxidation-reduction potential of the cheese during curing (5) may cause the diacetyl to undergo chemical changes. Undoubtedly, the microorganisms present in the cheese during this period are responsible in part for the decline in diacetyl. The decline of the diacetyl content in the cheese probably can be attributed to a combination of these physical, chemical and biological influences.

SUMMARY

Diacetyl is produced throughout the manufacturing process of Cheddar cheese except during the interval between setting and cutting. Agitation and increased acidity were factors associated with the increase in diacetyl content.

The diacetyl in Cheddar cheese probably is produced by the action of microorganisms upon the citrates in a manner similar to its production in starter cultures.

A gradual decrease in the amount of diacetyl in both raw-milk and pasteurized-milk Cheddar cheese was noted during the early stages of curing. This decrease probably is caused by volatilization of the diacetyl and by its reduction to acetylmethylcarbinol and 2,3-butylene glycol as a result of chemical reaction or the action of microorganisms.

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COBALT TOLERANCE IN YOUNG DAIRY CATTLE[1]

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Cobalt deficiency has been reported to be rather widespread in the United States and Canada, as well as in various other parts of the world. In spite of the fact that numerous publications which deal with various phases of this deficiency are available, little information on the tolerance of ruminants to the feeding of this element has been found. Josland (5) reported from New Zealand in 1937 that when four ewes were drenched daily for 7 months with 1 mg. of cobalt (from the sulfate) per 200 g. body weight, one developed a polycythemia within 3 months, two became mildly anemic and one was not affected. No toxic effects were observed. In 1945, Geyer *et al.* (4) reported that the tolerance of the bovine to cobalt is high and that feeding as much as 50 mg. per day did not produce polycythemia. Ely *et al.* (2) reported in 1946 that there are relatively wide variations in the tolerance of individual animals to high doses of cobalt salts. A detrimental effect on the appetite was reported for animals which received excessive cobalt orally, while excessive intravenous injections produced rapid respiration, incoordination, lacrimation, salivation, defecation and urine leakage within 1 to 5 minutes after injection. Very recently Ely *et al.* (3) reported that equivalent amounts of cobalt fed as the sulfate, chloride or carbonate were equally toxic to the dairy calf if fed in amounts in excess of 40 mg. daily per 100 lb. of body weight.

After Keener *et al.* (6) reported in 1944 that cobalt deficiency existed in New Hampshire, further results of these workers as well as the experiences of farmers, veterinarians, and feed dealers showed that the deficiency was widespread in that state. Since it was obvious that the feeding of supplemental cobalt would become a common practice, first-hand information on the tolerance of the bovine to cobalt seemed necessary. The experiment reported here, a preliminary report (7) of which was given in 1947, was undertaken for that purpose.

EXPERIMENTAL PROCEDURE

This study was started in September, 1944, and the last animals were removed from the experiment on January 1, 1947. All animals were either purebred or high grade Holsteins. Those calves which were placed on the experiment at an early age were changed from whole milk to reconstituted skim milk at 1 to 2 weeks of age and received this until 6 to 10 weeks of age. They were fed a concentrate mixture consisting of 3 parts of ground corn, 3 parts of ground oats, 3 parts of

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wheat bran, 1 part of soybean oil meal and 1 per cent salt throughout the time they were on experiment. The roughage fed was a grass hay of average quality. Water was furnished twice per day in individual pails to prevent the control animals from receiving cobalt. Cobaltous sulfate (C. P.) was used as the source of cobalt. It was fed to the first group of animals once per day in the drinking water. All later animals were given cobalt twice a day in the same manner except

TABLE 1

Summary of level and duration of cobalt feeding and the weight and health of calves

Animal	Sex	Age at beginning	Time on experiment	Cobalt level	Body weight		General condition
					Beginning	Gain	
		(wk.)	*(wk.)*	*(mg./d./100 lb. body wt.)*	*(lb.)*	*(lb./d.)*	
				GROUP I			
43	M	14	28	100[a]	228	2.14	Normal
			13	400[a]	648	1.71	Indications of hyperchromemia
			16	1000[a]	804	0.87	Rough coat, lacked muscular coordination, improved some at end of period. Hyperchromemia
44	F	12	28	25[a]	181	1.65	Normal
			13	200[a]	505	1.90	Normal
			16	500[a]	678	1.03	Normal
			14	none	794	0.84	Normal
			4	2000[a]	876	− 0.96	Off feed at intervals, hyperchromemia
			21	none	849	1.30	Returned to normal after few wk.
45	F	4	31	none	102	1.71	Normal
				GROUP II			
77	M	2	10	none	90	1.26	Normal
			11	none	178	1.31	Normal
			20	none	279	1.41	Normal
			13	none	476	1.47	Normal
			7	none	610	1.77	Normal
78	M	1	9	none	86	1.30	Normal
			11	50	168	1.04	Indications of hyperchromemia
			20	10	248	1.54	Normal
			13	50	464	1.64	Normal
			7	100	613	1.41	Indications of hyperchromemia
79	M	1	7	none	86	1.02	Normal
			11	90	136	0.97	Indications of hyperchromemia
			20	30	211	1.47	Normal
			13	30	417	1.88	Normal
			7	75	588	0.77	Indications of hyperchromemia

[a] Total cobalt fed daily.

in those instances where the amount was so large that the animals would not drink the water containing it. In this case it was given as a drench twice a day. Calves were kept in individual pens. Weights, heights at withers and chest circumferences were determined weekly. Hemoglobin determinations were made periodically on each animal throughout the experiment. Packed red cell volume determinations were made on all but the first group. The animals in groups II and

IV were slaughtered at the end of the experiment so tissues could be taken for cobalt analysis.

RESULTS

A summary of the rates of cobalt feeding and the results obtained with each animal is given in table 1. The animals in group I were used in preliminary studies and cobalt was not fed according to body weight. However, cobalt was

TABLE 1—(*Continued*)

Animal	Sex	Age at beginning	Time on experiment	Cobalt level	Body weight		General condition
					Beginning	Gain	
		(*wk.*)	(*wk.*)	(*mg./d./100 lb. body wt.*)	(*lb.*)	(*lb./d.*)	
				GROUP III			
80	F	40 (est.)	14	none	473	1.40	Normal
			23	30	610	1.42	Normal
			13	30	838	1.43	Normal
			7	75	968	0.16	Normal
81	F	42 (est.)	13	none	498	1.23	Normal
			23	30	610	1.27	Normal
			13	50	815	1.32	Indications of hyperchromemia
			7	100	935	0.12	Slight hyperchromemia
				GROUP IV			
82	M	2	4	none	92	1.50	Normal
			11	none	134	1.05	Normal
			20	none	215	1.60	Normal
			13	none	439	1.86	Normal
			7	none	608	0.75	Normal
83	M	1	4	none	81	1.11	Normal
			11	50	112	0.75	Indications of hyperchromemia
			20	10	170	1.48	Normal
			13	50	378	1.46	Normal
			6	100	511	−0.21	Listless, poor appetite, unsteady gait, hyperchromemia
			1	none	502	−2.43	Some improvement in condition
84	M	2	4	none	127	1.07	Normal
			11	90	157	0.82	Slight hyperchromemia
			20	30	220	1.51	Returned toward normal
			13	30	431	1.26	Normal
			7	75	546	1.04	Listless, slight hyperchromemia

fed to all other animals in proportion to body weight. With the remaining calves, there were three groups based on age and period on the experiment. In both groups II and IV, a negative control was maintained. Group III consisted of two animals placed on the experiment at an older age.

Figure 1 shows the effect of the level of cobalt intake on the hemoglobin content of the blood. Since the values obtained for packed red cell volume parallel rather closely those obtained for hemoglobin, the packed red cell volume values are not given.

Because of differences in the response of various animals, it was not possible to determine accurately the maximum rate of cobalt feeding which would not produce deleterious effect. However, cobalt in the form of cobaltous sulfate did not produce any apparent harmful effects until fed at a rate approaching 50 mg.

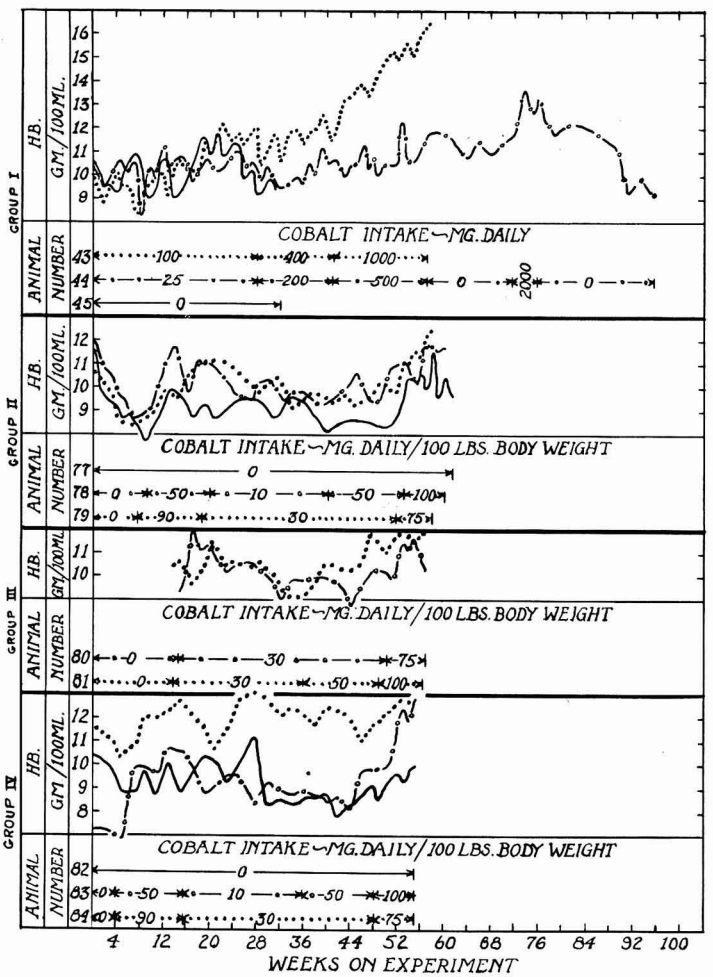

FIG. 1. Curves showing hemoglobin levels and rates of cobalt feeding for the various calves.

daily per 100 lb. body weight for a period of many weeks. With number 84, the hemoglobin and packed red cell volume levels appeared to be a little higher than normal, if compared to values obtained from the other calves. However, if one

considers hemoglobin values of 10.9 ± 0.86 g. per 100 ml. for cows and 12.8 ± 0.8 for bulls as given by McCay (8) to be normal for these animals, neither number 84 nor any of the other animals could be considered to show hyperchromemia except at the highest levels of cobalt intake. In determining the earliest symptoms of excess cobalt consumption, hemoglobin and packed red cell volume changes for each period were given as much consideration as the actual hemoglobin and packed red cell volume levels.

When cobalt levels of 50 mg. daily per 100 lb. of body weight were fed for periods of many weeks, there usually was an increase in hemoglobin and packed red cell volume. This increase was almost imperceptible in some cases and very marked in others. The relationship between the level of cobalt intake and the increase in body weight and height at withers was in line with the conclusions drawn from the blood studies. No depressing effect on growth was noted on any animal which received less than 50 mg. of cobalt per 100 lb. of body weight per

TABLE 2

Cobalt content of kidney and liver tissue

Animal no.	Sample[a]	Cobalt content of:	
		Kidney	Liver
		(γ/*g. dry wt.*)[b]	(γ/*g. dry wt.*)[b]
77 (control)	a	0.26	0.49
	b	0.31	0.44
78	a	2.4	2.1
	b	2.5	3.3
79	a	1.9	7.1
	b	2.4	7.0
82 (control)	a	0.41	0.85
	b	0.33	0.61
83	a	4.9	.....
	b	5.4	4.4
84	a	4.2	15.4
	b	2.2	4.3

[a] Taken from different parts of organ.
[b] Av. of duplicates in most cases.

day. However, there was a depressing action in some cases when cobalt was fed at higher levels and particularly when more marked effects were noted in the blood and externally. With the most severe external symptoms, a loss of weight was observed.

Definite external effects of excessive cobalt consumption were observed only on a few animals which had been fed at rates approaching at least 100 mg. of cobalt per 100 lb. of body weight per day for many weeks. One animal showed some effect in 4 weeks when fed a little over twice this amount. These external effects were rough hair coat, listlessness, depressed appetite, decreased water consumption and lack of muscular coordination. In fact, they appeared to be in general almost the same as for cobalt deficiency. However, the blood picture was quite the opposite.

The effect of feeding relatively large amounts of cobalt over considerable periods of time on the storage of cobalt in the tissues of the liver and kidneys is shown in table 2. Although the cobalt content of these tissues from the animals fed cobalt was several times as high as those from the controls, it is still relatively low when one considers the amounts of cobalt fed and the fact that the results were expressed on the basis of dry weight. Since, according to the data of Comar and Davis (1) orally administered cobalt is stored to a much greater extent in the liver and kidney than in most other organs of the body, it would appear that there is little possibility that excessive concentrations of cobalt will be stored in the various organs of the body when the animal is fed this element at the usual rates.

The results obtained in this experiment indicate considerable variation in the response of different animals to high rates of cobalt feeding. The rates of feeding generally followed allow a wide margin of safety. The practice usually followed by feed manufacturers in this area of supplementing with approximately 2 g. of cobalt sulphate or equivalent per ton of mixed feed generally furnishes less than 1 per cent of the tolerance level determined in this experiment.

SUMMARY

1. An experiment was carried out with growing Holstein dairy cattle to determine the amount of cobalt they can consume continuously with safety over a considerable period of time.

2. There was considerable individual variation in the tolerance level.

3. The oral consumption of a small excess of cobalt sulfate produced an increase in hemoglobin and packed red cell volume.

4. The oral consumption of a greater excess of cobalt sulfate resulted in loss of appetite, decreased water consumption, rough hair coat and a lack of muscular coordination, as well as an increase in hemoglobin and packed red cell volume.

5. High levels of cobalt as fed during this experiment increased the cobalt content of kidney and liver tissues to several times that of the controls, but in light of the levels fed and the duration of the feeding, these accumulations are considered to be low.

6. Growing dairy animals as maintained on this experiment appeared to be able to consume daily up to approximately 50 mg. of cobalt per 100 lb. of body weight from cobaltous sulfate for many weeks without definite harmful effects.

7. The levels of cobalt generally added to concentrate rations by feed manufacturers and those generally recommended for inclusion in mineral mixtures appear to afford a wide margin of safety.

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FINAL CREAM QUALITY RESULTING FROM KEEPING DELIVERIES SEGREGATED VERSUS MIXING AS PRACTICED IN BUYING STATIONS[1, 2]

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Segregation of farm-separated cream according to grades in the buying station generally is considered to be conducive to higher average quality at the creamery than when different grades of cream are mixed at the time of purchase. This tenet is supported by the dairy laws of various states which specify that lots of first and second grade cream are not to be mixed in the buying stations. Manhart (3), in discussing the requirements of the 4-day plan of cream grading, stated that the mixing of undergrade cream with good cream in the buying station was detrimental to the program.

In some areas, however, relatively little grading or segregation is practiced in the station and cream is mixed indiscriminately as a matter of convenience in handling and shipping. Commonplace as is the recommendation to segregate cream, no published data have been found on the merit of the practice under field conditions. If segregation on a grade basis is an important factor in cream quality, then some system of segregation of individual deliveries of cream should enhance quality further. The study herein reported was initiated to appraise the value of such individual segregation as one of the steps in marketing good quality cream and also to estimate the effect of indiscriminate mixing as practiced in many buying stations.

METHODS

The study was carried on over a period of 11 months from April, 1947, to February, 1948, so that any seasonal influences would be included. All cream used in the study was obtained from deliveries by producers to cream stations under normal operating conditions and generally represented the quality range of cream so marketed in Kansas. Due to the practical difficulties involved, the segregation trials were conducted on small lots of cream under controlled laboratory conditions simulating those encountered in practice.

Sampling procedure. Cream stations were visited on days when receipts were expected to be relatively large, so that the samples obtained would represent a larger proportion of the cream marketed. Three samples were taken directly from the well-stirred cream of each delivery and placed in clean, dry, 6-oz. sample jars. The ladle used for sampling was rinsed in warm water and then in a hypochlorite solution (200 to 300 p.p.m.) after sampling each delivery to minimize contamination from one lot of cream to the next. One sample from

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each delivery was placed immediately in ice water. The other two samples were held at station temperature until all samples for the day were obtained.

After sampling, the cream delivered by producers was dumped into 10-gallon cans in the usual manner followed in buying stations where segregation on a grade basis is not practiced. As each 10-gallon can was filled, a record was made of the individual deliveries contained. Samples then were taken from the full cans in the same manner as from the separate lots of cream delivered by producers, one being placed in ice water. Thus the samples from the 10-gallon cans contained mixed cream representing deliveries from several patrons. At the end of the buying day all cream samples were returned to the laboratory. The iced set of samples was held iced until analyzed, usually a 16- to 24-hour period. The other two sets of samples were placed at different holding temperatures.

Holding temperatures. In view of the fact that much cream receives little cooling after it has been purchased in the station, consideration was given to the effect of holding conditions on the comparative quality of segregated and mixed cream. A temperature of 80° F. was considered to be an approximate average at which cream often is held during hot weather. As representative of conditions that might be attained where effective cooling is provided, 50° F. was selected. These two temperatures were used in the investigation for holding the cream. It should be noted that holding at the different controlled temperatures started at the end of the buying day to simulate different handling practices that might be applied at that time. The procedure generally conforms to practical conditions since in those stations where cooling facilities are available they frequently are not employed until the end of the buying day. Since the length of time between the purchase of cream at the station and churning at the creamery sometimes extends to 2 days, this period was used for holding the cream samples at the above temperatures.

Examination of samples. To indicate the quality of the cream when received at stations, examinations were made on the iced samples representing both individual deliveries and 10-gallon cans of cream as mixed in stations. As a further indication of average quality, tests were made on a composite sample prepared from the individual samples, representing all cream obtained at a station.

To compare the effect of segregating versus mixing cream, at the end of the holding period in each trial, a composite sample was made of all 10-gallon can samples obtained at the station. This was compared with a proportional composite taken from all individual samples. As a further measure of the effect of segregation, in all but the first three trials, composite samples were prepared from the individual samples representing each 10-gallon can of cream. These composites then were compared with the corresponding 10-gallon can samples.

Quality determinations. Quality was determined on the basis of organoleptic tests supported by titratable acidity, formol titration and mold content. Cream samples were scored for flavor according to the method common in the butter industry where the numerical score is based on the estimated commercial

grade of butter that should be obtained. Such flavor scores were used in preference to grades so that smaller differences in quality could be designated. Samples were scored by two experienced judges working independently. Acidity determinations were made by titrating 9 g. of cream plus 9 ml. of distilled water with 0.1 *N* NaOH, using phenolphthalein as the indicator. Formol titrations were made by the method used by Martin *et al.* (4) except that 2 ml. of formalin were used instead of 10 ml. The mold content of cream was determined by the Parsons modification (5) of the Wildman methylene blue-borax procedure (6). The mold pads were scored according to a modification of the American Butter Institute tentative mold standard for cream, with the following numerical values being assigned to each grade.

American Butter Institute mold standard:	Good	Fair	Poor	Very Poor
Corresponding scores used in this study:	1 2 3	4 5 6	7 8 9	10 11 12

Mold tests were made on the segregated and mixed cream only after holding.

RESULTS

During the 11-month period of the study 12 different trials were conducted involving cream deliveries from 182 producers delivered at 7 different cream buying stations. The stations were located in five towns in four Kansas counties. The number of deliveries sampled per station contact averaged slightly more than 15 and ranged from 7 to 21.

The quality of the cream obtained at the station before holding as represented by composite samples of the day's purchase in each trial is shown in table 1. The range in flavor scores of individual deliveries of cream is included. Also shown is the comparative quality of segregated and mixed cream as represented by composite samples prepared after holding the cream by each method for 2 days at 50° F. After holding there was little difference between the composite quality of the segregated cream and the mixed cream as indicated by flavor score, titratable acidity, formol titration and mold content. The average change in quality was a reduction of 0.7 in flavor score for both the segregated and mixed cream, with increases in acidity of 0.12 and 0.14 per cent, respectively. In some instances part of these changes probably were initiated during the period in the stations. Although there were inconsistencies in the formol titrations, the average values for both the segregated and mixed cream were near that of the cream before holding. The average mold content was about equal for both holding methods.

After being held 2 days at 80° F., the average composite quality of the segregated cream was little different from the average composite quality of the mixed cream (table 2). However, several trials showed an advantage for the segregated cream. As would be expected, deterioration was more extensive at 80° F. than at 50° F. The average quality change involved a reduction in flavor score of 1.6 for the segregated cream and 1.8 for the mixed cream, with increases in acidity of 0.36 and 0.42, respectively. Although the formol titrations again showed variations, the average increases were almost equal, being 0.56 and 0.62

TABLE 1

Effect on composite quality of holding cream as mixed in cream stations versus segregating and holding separately cream from each delivery

Trial no.	Mo.	No. of deliveries	Range of flavor score	Composite quality before holding			Composite quality after holding 2 d. at 50° F.							
							Flavor Score		Titratable acidity		Formol titration		Mold score	
				Flavor score	Titratable acidity	Formol titration	Segregated	Mixed	Segregated	Mixed	Segregated	Mixed	Segregated	Mixed
					(%)	(*ml.*)			(%)	(%)	(*ml.*)	(*ml.*)		
1	April	18	88.5–92.5	91	.....	.....	90	90	0.73	0.72	.....	.....	3	3
2	April	9	89.5–92	91	0.59	.....	90.5	90.5	0.68	0.74	.....	.....	2	2
3	May	18	90 –93	91	0.59	.....	91	91	0.67	0.68	2.4	2.6	6	6
4	May	17	89 –92	90.5	0.59	2.5	90.5	90.5	0.69	0.69	2.8	2.8	4	4
5	June	21	89 –92	91	0.60	2.0	89	89	0.66	0.70	3.0	3.0	4	4
6	June	7	88 –91	90	0.68	3.1	90	90	0.75	0.74	2.4	2.7	4	3
7	July	15	89 –91.5	90	0.89	2.9	89	89	1.08	1.11	2.9	3.3	4	7
8	Aug.	9	89 –93	91.5	0.53	2.4	90	90.5	0.77	0.75	2.7	2.9	5	6
9	Sept.	20	88 –91	90	0.83	3.0	90	89.5	0.92	1.11	2.8	2.8	6	5
10	Sept.	19	89 –93	91.5	0.64	2.9	90.5	90	0.75	0.83	2.2	1.8	5	6
11	Dec.	14	89.5–92	91.5	0.41	2.4	91	91	0.55	0.60	2.5	2.5	.....	.....
12	Feb.	15	90.5–93	91.5	0.45	2.0	91	91	0.62	0.54	2.1	2.0	1	1
Summary		182	88 –93	90.9	0.62	2.58	90.2	90.2	0.74	0.76	2.58	2.64	4.0	4.2

TABLE 2

Effect on composite quality of holding cream as mixed in cream stations versus segregating and holding separately cream from each delivery

Trial no.	Mo.	No. of deliveries	Range of flavor score	Composite quality before holding			Composite quality after holding 2 d. at 80° F.							
							Flavor score		Titratable acidity		Formol titration		Mold score	
				Flavor score	Titratable acidity	Formol titration	Segregated	Mixed	Segregated	Mixed	Segregated	Mixed	Segregated	Mixed
					(%)	(*ml.*)			(%)	(%)	(*ml.*)	(*ml.*)		
1	April	18	88.5–92.5	91	.....	.....	89.5	89	0.79	0.82	.....	.....	5	5
2	April	9	89.5–92	91	0.59	.....	90	90	0.82	0.82	.....	.....	4	5
3	May	18	90 –93	91	0.59	.....	90	89	0.88	0.84	2.8	3.2	5	5
4	May	17	89 –92	90.5	0.59	2.5	89.5	89	0.84	0.90	2.6	2.6	6	5
5	June	21	89 –92	91	0.60	2.0	88.5	88.5	0.84	0.88	3.5	3.5	6	4
6	June	7	88 –91	90	0.68	3.1	89	89	0.96	1.01	2.9	3.2	4	4
7	July	15	89 –91.5	90	0.89	2.9	89	89	1.44	1.57	3.7	3.7	7	5
8	Aug.	9	89 –93	91.5	0.53	2.4	90	89.5	1.16	1.24	2.7	2.9	6	7
9	Sept.	20	88 –91	90	0.83	3.0	88	88	1.36	1.65	4.2	4.5	8	6
10	Sept.	19	89 –93	91.5	0.64	2.9	89	89	1.25	1.34	3.5	3.4	6	5
11	Dec.	14	89.5–92	91.5	0.41	2.4	90	90	0.71	0.72	2.8	2.7	.....	.....
12	Feb.	15	90.5–93	91.5	0.45	2.0	89.5	89	0.75	0.70	2.7	2.3	5	7
Summary		182	88 –93	90.9	0.62	2.58	89.3	89.1	0.98	1.04	3.14	3.20	5.6	5.3

for the segregated and mixed cream, respectively. There was no practical difference in the average mold content.

Trials 4 to 9 inclusive (tables 1 and 2) generally reflect the quality situation existing during the warmer months. Although there were exceptions, as would be expected, the average quality of the cream at the time of purchase was lower during the summer months than during the remainder of the year.

During the study it frequently was evident that the higher quality cream deteriorated a greater proportionate amount than did the lower quality cream. Although this characteristic sometimes is recognized, it often receives little consideration in actual commercial operations. To determine the influence of quality on the extent of deterioration in the segregated and mixed cream the data from trials showing comparisons on a 10-gallon can basis were grouped according to flavor scores of the 10-gallon lots of cream. Such cream scoring 91 and over when purchased comprised the better group and cream scoring under 91 represented the poorer group. These data are summarized in table 3 for both of the holding temperatures and with the average results on all cream included for comparison. The average quality of the better cream is indicated by a flavor score of 91.4, an acidity of 0.52 and a formol titration of 2.41. The poorer quality cream had an average flavor score of 90.1, with acidity and formol titrations of 0.79 and 3.08, respectively.

With the better cream very little difference in quality resulted from segregation or mixing. With the poorer cream the differences were somewhat greater, particularly as shown by acidities and formol titrations of the samples held at 80° F. However, in the quality range involved, the differences appear to be of little practical importance. At 80° F. the better cream showed more deterioration in quality than the poorer cream. The average flavor score of the better cream dropped 2.1 points in both the segregated and mixed cream, compared to corresponding changes of 1.4 and 1.6 in the poorer cream. Although the actual increase in acidity was less, the proportionate increase was greater. At 50° F. the drop in quality was slightly greater for the better cream, but the difference was too small to be of practical significance. Although the better cream deteriorated more at 80° F. than the poorer cream, its average quality after holding 2 days generally was the same as that of the poorer cream held at 50° F.

DISCUSSION

The cream used in the study was obtained under practical conditions and generally represented the quality marketed through cream stations in Kansas. With such cream the principal cause of low quality is deterioration. Although the investigation was conducted on small lots of cream rather than on commercial quantities, the results on segregation and mixing of cream show the comparable effects of these treatments and indicate what might be expected under commercial conditions.

During the study formol titrations on some samples failed to show a consistent relationship to other quality measures. This characteristic has been noted

TABLE 3

Influence of cream quality on extent of deterioration during 2 days holding

Class of cream	No. of deliveries	Range of flavor score	Av. quality before holding			Holding temp.	Average quality after holding							
							Flavor score		Titratable acidity		Formol titration		Mold score	
			Flavor score	Titratable acidity	Formol titration		Segregated	Mixed	Segregated	Mixed	Segregated	Mixed	Segregated	Mixed
				(%)	(*ml.*)	(°*F.*)			(%)	(%)	(*ml.*)	(*ml.*)		
All cream[a]	182	88–93	90.9	0.62	2.58	50	90.2	90.2	0.74	0.76	2.58	2.64	4.0	4.2
						80	89.3	89.1	0.98	1.04	3.14	3.20	5.6	5.3
Better cream	77	89–93	91.4	0.52	2.41	50	90.5	90.4	0.64	0.66	2.53	2.60	3.6	4.5
						80	89.3	89.3	0.87	0.92	3.00	3.08	5.4	4.9
Poorer cream	58	88–90.5	90.1	0.79	3.08	50	89.5	89.2	0.87	0.93	2.99	3.12	4.8	4.6
						80	88.7	88.5	1.19	1.33	3.34	3.74	5.4	5.6

[a] From summaries of tables 1 and 2.

by other workers (2, 4). Conversion of results to a fat-free basis did not give greater uniformity. Inconsistencies were more frequent on composite samples. Nevertheless the over-all average changes in formol titrations generally were in agreement with other quality changes and thereby supported the other measures of quality.

Mold tests also showed some inconsistencies between samples. However, this is not unusual when small lots are involved (1). The over-all average of results was in general accordance with the cream quality. With the recognized influence of such factors as proportionate surface area and size of delivery, it is difficult to predict the influence on mold content of the segregation of individual deliveries under commercial conditions.

The effect on final composite quality of segregating cream from different deliveries to cream stations was of doubtful importance. Under commercial conditions it would appear that any advantages in quality that might be gained would not be sufficient to justify the practical difficulties involved. Where flavor defects, due to plants or foreign materials, are a relatively minor problem, and where cream of reject quality is not involved, it also is doubtful that the manner of mixing cream in the cream station has any important practical effect on the average quality. However, segregation on a grade basis may provide more opportunity for selection at the creamery if temperature control is maintained.

The fact that the better cream showed a greater amount of deterioration than the poorer cream when held at a relatively high temperature is in accordance with general observations under practical conditions as well as with available information on the growth rates of bacteria. The bacterial development in the good cream at the time of purchase probably is in the logarithmic growth phase where changes are potentially rapid, while in the poorer cream activity has reached a peak and further population changes generally are slower. Although the better cream may remain higher in quality than the poorer cream for some time, it undergoes greater deterioration unless holding temperatures are relatively low. Obviously high quality cream requires better care after purchase than low quality cream if further changes are to be kept at a minimum. In commercial practice this situation would seem to merit greater attention. With any improvement in the quality of cream marketed by the producer also must come improvement in subsequent handling if the gains so made are not to be nullified.

Although the study was concerned mainly with the comparative effects of segregation and mixing, the results re-emphasize the importance of temperature in quality changes. It is evident that such practices as segregation or mixing of cream are of minor significance compared with effective temperature control.

CONCLUSIONS

There was little practical difference between the average quality of cream held segregated and cream held as mixed in buying stations when holding conditions involved temperatures of 50 or 80° F. for 2 days following purchase. Most differences were slightly in favor of the segregated cream. Segregation generally had a greater effect on the poorer quality cream. It is considered that

any contribution to quality that might be gained through segregation of deliveries under commercial conditions would be insufficient to justify the practical difficulties involved.

The better quality cream deteriorated proportionately more after purchase than the poorer quality cream when the holding temperature was 80° F. This emphasizes that any improvement in the quality of cream marketed by producers must be accompanied by a corresponding improvement in subsequent holding conditions.

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BACTERIOLOGICAL STUDIES OF BOVINE SEMEN. I. NUMBERS OF BACTERIA AND THE RELATION TO FERTILITY[1]

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During the past decade the problems associated with the presence of bacteria in bull semen used for artificial breeding have received increased attention. Special emphasis has been placed on the use of various antibacterial agents, including penicillin, streptomycin and various sulfonamides, for controlling the bacteria which always are found in the semen of bulls (2, 3, 5, 8, 9, 11, 13). The effect upon fertility of the addition of either sulfanilamide or penicillin to diluted bull semen used for routine artificial breeding also has been investigated. Salisbury and Knodt (15) reported that sulfanilamide in the diluter gave a significant increase in fertility. Almquist (1) found that penicillin markedly improved the fertility of semen from certain relatively infertile bulls although an earlier report by Almquist *et al.* (4) showed that it did not increase the fertility of semen from bulls of relatively high breeding efficiency. However, there is need for more fundamental information on the numbers of bacteria commonly found in bull semen, particularly the relationship of bacteria to the fertility of bull semen used for artificial breeding. Such information would be of particular value in determining the possible role of bacteria in fertility and in interpreting the results of fertility tests involving antibacterial substances.

Gunsalus *et al.* (10) found that the logarithmic average of plate counts for 15 ejaculates from 12 bulls used for artificial breeding was 22,000 bacteria per ml. Twenty-eight ejaculates from seven bulls used for natural service gave a somewhat higher average logarithmic plate count of 225,000 per ml. Plate counts for the 43 ejaculates collected from 19 bulls by means of the artificial vagina ranged from 1,000 to 22 million bacteria per ml. Other workers (3, 5, 7, 14) have reported bacterial counts of semen collected with the artificial vagina ranging from 100 to 960,000 organisms per ml.

The present paper deals with the numbers of bacteria commonly encountered in bull semen and the relationship between the average numbers of bacteria in semen and the fertility level of bulls used for routine artificial breeding. A study of the incidence of specific types of bacteria in bull semen and their relation to fertility also has been completed and will be reported in a subsequent publication.

METHODS

The semen samples used in these studies were collected by means of the artificial vagina from Guernsey, Holstein and Jersey bulls located at the Western

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Pennsylvania Artificial Breeding Cooperative, Clarion.[2] Routine collection technics were followed in obtaining the samples and with few exceptions a different sterile artificial vagina was employed for the collection of each ejaculate. Usually two or more ejaculates were collected in succession from the same bull on each collection day. Occasionally when the first ejaculate was of sufficient volume and of satisfactory quality for insemination purposes, additional ejaculates were not collected. Although strict asepsis was not practiced in the handling of the semen samples, reasonable precautions were taken to prevent excessive bacterial contamination. The rubber innerliners of the artificial vagina were disinfected by immersion in 70 per cent alcohol and were allowed to air-dry prior to use. All glassware contacted by the semen was sterilized previously by dry heat in an oven at not less than 170° C. for at least 1 hour.

Since it was impractical to conduct the routine bacterial examinations at the headquarters of the Cooperative it was necessary to ship the undiluted semen samples to the laboratory at State College, a distance of about 105 miles. Subsamples of 1.5 ml. were taken from each ejaculate immediately after collection and placed in test tubes. These samples were cooled gradually to about 5° C. and then packaged for shipment. The test tubes were placed in a refrigerated cardboard shipping carton (12) and shipment then was made by special delivery parcel post. Upon arrival at the laboratory the temperature of each shipment was determined by inserting a precooled thermometer into a tube of water placed alongside the semen samples at the time of packaging.

The bacteriological examination was initiated within 1 hour after the samples arrived at the laboratory. The examination consisted of the inoculation of veal infusion agar, containing 4 per cent defibrinated bovine blood, with suitable dilutions of semen to determine the approximate numbers of living bacteria per ml. of semen. Sterile distilled water was used as the dilution medium and dilutions of 1:10, 1:100, 1:1,000 and 1:10,000 were employed to obtain countable plates. In the early phases of the study the inoculated media were incubated at 37° C. for both 48 and 96 hours. Based on the results reported below, incubation for 48 hours was discontinued and all subsequent counts were made after 96 hours of incubation. As suggested by the American Public Health Association (6) all plate counts were adjusted to the first two significant figures and represent the number of colonies of bacteria that would have developed on the agar if an entire ml. of semen had been used.

RESULTS

Effect of short time, low temperature storage upon growth of bacteria in bull semen. Since shipment of the semen samples required about 24 to 32 hours at a temperature of approximately 5° C., it was necessary to conduct a preliminary study to determine the effect of short time, low temperature storage on the bacterial plate count of undiluted semen. Portions of four ejaculates from three bulls of the College dairy herd were plated within 30 minutes after collection and

[2] The authors wish to thank G. W. Thompson, Manager, for his valuable assistance in this study.

after 4, 12 and 32 hours of storage at 5° C. There were no significant differences in the plate counts of semen stored under these conditions.

Further studies were conducted to ascertain the reliability of plate counts of semen samples packaged and shipped by the Cooperative in the manner previously described. Plate counts on portions of seven ejaculates of semen plated immediately after collection at the Cooperative averaged 100,000 organisms per ml. Other portions of the same ejaculates plated at the College following routine shipment averaged 130,000 per ml. As a result of the slight difference obtained it is believed that the following data are representative of the bacterial content of freshly collected semen.

Effect of incubation time upon plate counts. In order to test the reliability of counts obtained following incubation for 48 and 96 hours at 37° C., a series of plates representing 54 ejaculates from 21 bulls were counted after 48 hours of incubation and recounted after a total incubation period of 96 hours. The average plate counts increased from 53,000 to 92,000 bacteria per ml. as the result of the additional 48 hours of incubation. Although increases were noted in the average plate counts of semen from all bulls after 96 hours of incubation, average plate counts of semen from only 4 of the 21 bulls showed an increase of more than five times the counts obtained after 48 hours. Bacterial growth obtained during the additional 48-hour incubation period consisted essentially of slow-growing diphtheroids apparently common to nearly all samples of bull semen. Similar observations were reported by Gunsalus *et al.* (10), who selected an incubation period of 96 hours to facilitate counting when slow-growing diphtheroids were present. Due to the relatively slight increases in plate counts, it is believed for the purposes of this study that the 48-hour counts are equally as significant as the 96-hour counts. However, the longer period of incubation is somewhat more reliable and an incubation period of 96 hours now is routine at this station for studies of this nature.

Bacterial plate counts of bull semen. Information on the numbers of bacteria commonly encountered in semen from bulls used in artificial breeding was obtained from plate counts of 202 ejaculates from 36 bulls. With the exception of six recently acquired bulls, none of them had been used in natural service for 6 months prior to the initiation of these studies. Table 1 shows that the arithmetic mean plate count of the 202 ejaculates was 200,000 organisms per ml. An extremely wide range of from less than 100 to more than 3 million per ml. was found. Marked differences were noted between samples from different bulls and between various ejaculates from the same bull. As indicated by the wide range of the average counts, a maximum difference of about 3,000-fold was observed between bulls. Differences between ejaculates from the same bull were as great as 2,000-fold.

Comparison of plate counts of first and second ejaculates. It has been reported (15, 16) that when several ejaculates are taken in succession, there are fewer bacteria in second than first ejaculates. In the present study the average plate count of 91 first ejaculates from 32 bulls was 220,000 per ml. as compared

to an average count of 130,000 per ml. for the same number of second ejaculates. In the case of 11 of the 32 bulls, the average counts of second ejaculates were greater than those of first ejaculates. The differences between first and second ejaculates were neither sufficiently large nor consistent enough to be considered significant.

Relationship between the plate counts of semen and the fertility level of bulls. Fertility data for 33 bulls were collected for the period from July 1 to November

TABLE 1

Bacterial plate counts of semen from 36 bulls used in artificial breeding

Bull	No. of ejaculates	Plate count/ml.	
		Av.	Range
G– 1	4	1,200,000	400,000–2,300,000
G– 2	5	910,000	160,000–3,300,000
G– 3	6	20,000	2,200– 64,000
G– 4	9	19,000	75– 120,000
G– 5	15	320,000	2,200–1,000,000
G– 6	16	260,000	65–2,600,000
G– 7	4	7,900	2,600– 16,000
G– 8	2	3,900	2,600– 5,300
G– 9	3	1,500,000	1,000,000–2,100,000
G–10	2	4,900	50– 9,800
G–11	4	19,000	190– 50,000
G–12	5	74,000	550– 280,000
G–13	12	6,700	650– 36,000
G–14	9	36,000	600– 800,000
G–15	4	9,300	900– 120,000
G–16	2	56,000	6,600– 12,000
G–17	12	54,000	320– 270,000
H– 1	2	110,000	26,000– 190,000
H– 2	7	290,000	450–1,900,000
H– 3	5	60,000	1,100– 270,000
H– 4	4	49,000	9,100– 140,000
H– 5	2	2,600	1,200– 4,100
H– 6	1	570,000	
H– 7	4	300,000	2,100– 900,000
H– 8	1	19,000	
H– 9	2	600	100– 1,200
H–10	18	240,000	180–2,000,000
H–11	2	13,000	9,800– 17,000
H–12	2	3,400	2,200– 4,700
H–13	4	79,000	4,400– 200,000
H–14	14	140,000	4,700–1,300,000
H–15	8	46,000	640– 160,000
H–16	3	12,000	1,400– 2,600
J– 1	1	170,000	
J– 2	6	6,300	250– 210,000
J– 3	2	360,000	80,000– 650,000
Summary	202	200,000	50–3,300,000

1, 1947, based on the percentages of first and second service cows which did not return to service 90 to 120 days following the last insemination. The 33 bulls represented various levels of fertility as shown by the wide range of from 34 to 82 per cent 90- to 120-day non-returns. While the fertility data represent all ejaculates used for inseminations during the 4-month period, a few of the ejaculates were not available for bacterial examination.

The bulls were grouped on the basis of their general level of fertility and table 2 shows the averages as well as the ranges of the plate counts at the various fertility levels. Note that the lowest average plate count is associated with the low level of fertility; conversely, the highest average plate count was obtained from bulls at the high level of fertility. However, the differences appeared to be of insufficient magnitude to indicate any significant relationship between fertility and the plate count of semen. Nevertheless, the possibility still exists that the fertilizing capacity of any particular sample of semen may be affected by the number of bacteria present.

TABLE 2

Relationship of the bacterial plate count of semen to level of fertility

Level of fertility	No. of bulls	No. of ejaculates	Plate count/ml.	
			Av.	Range
High (66–82)[a]	10	47	290,000	200–3,300,000
Medium (56–65)	12	47	200,000	65–2,600,000
Low (34–55)	11	93	140,000	75–2,000,000
All levels	33	187	190,000	65–3,300,000

[a] Per cent 90- to 120-day non-returns.

Relationship between the plate counts of semen and the age of bulls. A comparison of the plate counts of the semen samples from bulls of various ages was made and the data are presented in table 3. The 36 bulls were divided into five groups based on their ages as of July 1, 1947. Note that the lowest numbers of bacteria were found in semen from the bulls in the 6- to 7- and 8- to 9-year-old age groups, while the greatest numbers of bacteria were found in semen from the oldest group of bulls. However, the average count for the latter group was influenced markedly by the large numbers of bacteria contained in semen from one bull. The average plate count of the four ejaculates from this bull was 1,200,000 per ml. Since differences in plate counts of semen between age groups were not marked, there appears to be no important relationship between the age of bulls and the average plate count of semen.

TABLE 3

Relationship of the bacterial plate count of semen to age of bulls

Age (yr.)	No. of bulls	No. of ejaculates	Plate count/ml.	
			Av.	Range
1–3	10	35	220,000	75–3,000,000
4–5	13	75	240,000	50–3,300,000
6–7	6	40	84,000	640–1,300,000
8–9	4	29	210,000	65–2,600,000
10 and over	3	23	400,000	2,200–2,300,000
All ages	36	202	200,000	50–3,300,000

SUMMARY

1. Wide variations were found in the bacterial plate counts of semen from various bulls and in the counts of semen collected at different times from the same animal. The plate count on 202 ejaculates from 36 bulls ranged from less than 100 to more than 3 million organisms per ml., with an average of 200,000 per ml.

2. No significant differences were observed in the plate counts of first and second ejaculates collected in succession from the same bull. Plate counts for 91 paired ejaculates showed 220,000 bacteria per ml. for first ejaculates and 130,000 per ml. for second ejaculates.

3. There was no apparent relationship between the average plate counts of semen and the general fertility level of bulls.

4. There was no important relationship between the age of bulls and the average plate count of semen.

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THE EFFECT OF FEEDING ALFALFA HAY CONTAINING DDT RESIDUE ON THE DDT CONTENT OF COW'S MILK[1,2]

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Insecticides containing DDT now are being used on various crops grown as feed for milking cows. The relationship between the amount of DDT residue on the crop when fed, or the amount of DDT ingested by the cows, and the amount of DDT that may appear in the milk is not too well known. It is possible that enough DDT may be secreted in the milk to make it detrimental to consumers, especially if consumption of such milk is continued over a long period of time, since Kunze *et al.* (3) have reported that as little as 5 p.p.m. of DDT in the diet of the rat for 4 to 6 months will produce histopathological alterations of the liver.

Carter *et al.* (1) fed pea vine silage to milking cows at the rate of 3 lb. per 100 lb. of body weight. The silage contained 2.7 to 5.4 γ of DDT per g. on a fresh basis and 7.7 to 18.7 γ on a dry weight basis. The daily intake of DDT per cow was approximately 44 to 88 mg. The DDT content of the milk was less than 0.5 γ per g.

Wilson *et al.* (5) found 15 γ of DDT per g. in the milk from cows fed pea vine silage that provided an intake of about 1.5 g. of DDT per day per 1,000 lb. of body weight. These same investigators also found 44 γ per g. in the milk from a cow that received 24 g. of DDT per day.

This report gives the results of recent studies showing the concentration of DDT in the milk from cows fed alfalfa hay that had been treated with DDT under field conditions.

EXPERIMENTAL PROCEDURE

In August, 1947, a field of alfalfa, from which the third cutting was to be taken, was treated with different amounts of DDT by means of an aerosol machine. Part of the field was treated with 0.6 lb. of DDT per acre, the rate usually recommended for control of the potato leafhopper, and harvested 20 days later. The hay from this part of the field was designated as "Light DDT Hay." Another part of the field was treated with 2.4 lb. of DDT per acre, about four times the amount required for control of the leafhopper, and harvested 14 days later. This hay was designated as "Heavy DDT Hay." Both lots of hay were harvested in the quarter- to half-bloom stage, but both lots were of poor quality because they were rained on in the field and had to be cured on a barn hay-finisher.

The Heavy DDT Hay was fed to three cows (two Holsteins and a crossbred cow). In addition to the hay, all three cows received corn silage at the rate

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of 2 lb. per 100 lb. live weight daily, along with enough grain for normal production. The two Holstein cows received the DDT hay at the rate of 1 lb. per 100 lb. live weight daily. One Holstein (no. 1638) received the hay for 34 days before she calved and for 128 days afterwards, after which the hay was discontinued and she was turned on pasture. The other Holstein (no. 1666) had been fresh for 32 days when the DDT hay first was added to her ration; she received this hay for 111 days and thereafter received untreated hay. The crossbred cow (X-47) received the DDT hay at the rate of 0.5 lb. per 100 lb. live weight daily. She had been fresh for 17 days before the hay was added to her ration; she received the DDT hay for 110 days and then was turned on pasture.

The Light DDT Hay was few to one crossbred cow (X-16). It was fed at the rate of 1.5 lb. per 100 lb. of live weight daily, along with corn silage at the same rate and enough grain for normal production. This cow had been fresh for 32 days when the DDT hay was substituted for untreated hay in her ration. She received the DDT hay for 98 days and then was turned on pasture.

The amount of all feed fed and refused was weighed accurately each day. A small portion of the treated hays was saved each day and composite samples were analyzed for DDT each month. Accurate records were kept of the daily milk production. A 2-day composite sample of milk was saved every 10-day period and analyzed for DDT. Because of the poor quality of the hay, the cows did not consume the quantities desired. However, since accurate weights were kept of the amount of hay fed and refused, the actual intake of hay was determined readily and the approximate DDT intake thus could be calculated.

The amount of DDT on the hay was calculated from the determinations of total organic chlorine in the residue, following the procedure of Carter and Hubanks (2). Approximately 300- to 500-g. samples of hay were extracted with benzene for 30 minutes in a tumbling apparatus. Aliquots of the benzene solution then were evaporated on the steam bath, and the residue was taken up in isopropanol and refluxed with metallic sodium for 1 hr. The chloride in solution then was determined by titration with standard silver nitrate, using an electrometric titrimeter.

The amount of DDT in the milk was determined by the colorimetric method of Schechter *et al.* (4), as modified for use with milk samples. Samples of 50 ml. were extracted with a mixture of 75 per cent ethyl ether and 25 per cent petroleum ether. This ether extract then was evaporated and the butterfat containing the DDT was processed by the recommended procedure. It was found that two or more treatments with the sulfuric acid-sodium sulfate reagent, as well as two or more treatments with the concentrated sulfuric acid-fuming sulfuric acid reagent, were of value in the separation of the DDT from the butterfat.

RESULTS AND DISCUSSION

As shown in table 1, the colostrum milk from cow no. 1638 contained 14 γ of DDT per g. The maximum amount of DDT in the milk was 10.1 γ per g. This was equivalent to 259.1 γ per g. of butterfat, or 94,597 γ per lb. of butter containing 80.5 per cent butterfat. The average daily intake of DDT for the entire

feeding period was 553 mg., while the average daily output during the milking period was 165 mg. DDT was detectable in the milk for 160 days after the DDT-treated hay was removed from the ration.

As shown in table 2, cow no. 1666 began secreting DDT in her milk after 3 days on DDT hay, and the average for the first 10-day period was 3.2 γ of DDT

TABLE 1

Average daily intake of alfalfa hay and DDT for cow no. 1638 and output of DDT in her milk, by 10-d. periods

Start of each 10-d. period	Hay intake	DDT in hay	DDT intake	Milk produced	DDT in milk	DDT output in milk	DDT in fat
	(*lb.*)	(γ/*g.*)	(*mg.*)	(*lb.*)	(γ/*g.*)	(*mg.*)	(γ/*g.*)
11– 6–47[a]	11.7	121.0	642.2	0.0			
11–16–47	10.1	121.0	554.3	0.0			
11–26–47	10.5	121.0	576.3	0.0			
12– 6–47[b]	14.5	121.0	795.8		14.4[c]		
12–16–47	13.2	121.0	724.5	56.4	8.9	227.7	222.6
12–26–47	12.4	121.0	680.6	57.8	10.1	264.8	259.1
1– 5–48	12.6	74.2	424.1	56.5	7.4	189.7	217.8
1–15–48	12.9	74.2	434.2	53.4	6.9	167.1	174.6
1–25–48	8.8	74.2	296.2	53.2	9.0	217.2	250.0
2– 4–48	8.2	134.5	500.3	50.6	6.7	153.8	186.2
2–14–48	10.0	134.5	610.1	48.4	6.8	149.3	186.1
2–24–48	8.9	134.5	543.0	46.4	4.0	84.2	105.3
3– 5–48	8.2	128.4	477.6	45.0	6.0	122.5	166.7
3–15–48	8.1	128.4	471.8	44.0	8.4	167.7	221.2
3–25–48	8.9	128.4	518.4	41.1	6.7	124.9	191.3
4– 4–48	10.4	128.4	605.7	45.3	6.4	112.3	149.7
4–14–48[d]				46.8	2.9	61.6	82.9
4–24–48				46.3	1.6	33.6	49.6
5– 4–48				44.1	0.9	18.0	24.3
5–14–48				40.7	0.5	9.2	13.3
5–24–48				41.8	0.3	5.7	9.0
6– 3–48				37.4	0.2	3.4	4.0
6–13–48				36.2	0.3	4.9	7.9
6–23–48				34.6	0.2	3.1	6.8
7– 3–48				37.3	0.2	3.4	5.7
7–13–48				29.0	0.2	2.6	5.3
7–23–48				30.4	0.3	4.1	8.5
8– 2–48				31.1	0.1	1.4	2.9
8–12–48				32.6	0.2	3.0	5.1
8–22–48				23.9	0.1	1.1	2.3
9– 1–48				23.4	0.2	2.1	5.5
9–11–48				24.1	0.1	1.1	3.0
9–21–48				19.2	0.1	0.9	2.6
10– 1–48				22.2	Trace		
10–11–48				16.1	0.0		

[a] Started on Heavy DDT Hay November 4, 1947, at rate of 1 lb. per 100 lb. of live weight.
[b] Calved December 8, 1947. Average weight 1,300 lb.
[c] In colostrum milk.
[d] DDT hay discontinued and cow turned on pasture 0.5 day per d. for 3 d.; on pasture continuously after April 17, 1948.

per g. of milk. The highest value for any 10-day period was 9.7 γ per g. of milk. The average daily intake of DDT for the 111-day feeding period was 727 mg. and the average daily output was 136 mg. DDT was detectable in the milk for 170 days after the DDT hay was removed from the ration.

While the amount of DDT in the milk of both cows (no. 1666 and 1638) was about the same at the time the treated hay was removed from the ration, the concentration of DDT in the milk of no. 1638 decreased more rapidly than that in the milk of no. 1666, although the milk from both cows showed the presence of DDT for about the same length of time. Cow no. 1638 was turned on pasture, whereas cow no. 1666 was kept on dry feed; this might account for the difference in rate of decrease in DDT concentration.

TABLE 2

Average daily intake of alfalfa hay and DDT for cow no. 1666 and output of DDT in her milk, by 10-d. periods

Start of each 10-d. period	Hay intake	DDT in hay	DDT intake	Milk produced	DDT in milk	DDT output in milk	DDT in fat
	(*lb.*)	(γ/*g.*)	(*mg.*)	(*lb.*)	(γ/*g.*)	(*mg.*)	(γ/*g.*)
12–26–47[a]	11.7	121.0	642.2	58.4	3.2	84.8	69.6
1– 5–48	13.5	74.2	454.4	58.6	3.8	101.0	86.4
1–15–48	14.8	74.2	498.1	57.2	4.9	127.1	111.4
1–25–48	13.5	74.2	454.4	55.8	8.7	220.2	185.0
2– 4–48	14.5	134.5	884.6	52.4	6.7	159.2	152.2
2–14–48	14.8	134.5	902.9	48.8	6.3	139.5	146.5
2–24–48	14.7	134.5	896.8	43.1	4.5	88.0	99.0
3– 5–48	14.6	128.4	850.3	42.0	7.9	150.5	175.6
3–15–48	13.4	128.4	780.4	41.3	9.7	181.7	215.5
3–25–48	13.8	128.4	803.7	36.5	6.9	114.2	148.3
4– 4–48	14.3	128.4	832.9	38.1	7.2	124.4	159.9
4–14–48[b]				30.4	6.2	85.5	118.1
4–24–48				34.4	2.4	37.4	51.0
5– 4–48				30.3	2.4	33.1	50.2
5–14–48				28.8	1.2	15.7	25.3
5–24–48				30.9	0.5	7.0	10.2
6– 3–48				26.8	0.6	7.2	16.0
6–13–48				28.6	1.0	13.0	20.0
6–23–48				24.5	0.7	7.8	14.3
7– 3–48				24.4	0.4	4.4	7.0
7–13–48				24.2	0.3	3.3	6.4
7–23–48				26.6	0.5	6.0	10.2
8– 2–48				22.4	0.3	3.0	6.0
8–12–48				23.9	0.4	4.3	8.4
8–22–48				19.6	0.5	4.4	9.7
9– 1–48				22.3	0.4	1.0	2.1
9–11–48				22.4	0.3	3.0	6.2
9–21–48				21.4	0.3	2.9	6.5
10– 1–48				17.5	Trace		
10–11–48				16.5	Trace		

[a] Calved November 24, 1947; started on Heavy DDT Hay on December 26, 1947, at rate of 1 lb. per 100 lb. of live weight. Av. weight 1,475 lb.

[b] Changed to hay containing no DDT on April 15, 1948.

Cow X-47 (table 3) received a smaller allowance of Heavy DDT Hay for 110 days, and therefore a smaller intake of DDT, than the other two cows. She also showed a smaller quantity of DDT in her milk, the highest value being 3.0 γ per g. The average daily intake of DDT was 303 mg. and the average daily output was 51 mg. No DDT was detectable in the milk of this cow after she was on pasture for 40 days.

TABLE 3

Average daily intake of alfalfa hay and DDT for cow X-47 and output of DDT in her milk, by 10-d. periods

Start of each 10-d. period	Hay intake	DDT in hay	DDT intake	Milk produced	DDT in milk	DDT output in milk	DDT in fat
	(*lb.*)	(γ/*g.*)	(*mg.*)	(*lb.*)	(γ/*g.*)	(*mg.*)	(γ/*g.*)
12–26–47[a]	6.1	121.0	334.8	55.2	1.4	35.0	23.7
1– 5–48	4.7	74.2	157.4	57.1	0.4	10.4	7.4
1–15–48	5.7	74.2	188.8	60.3	2.3	62.9	47.9
1–25–48	4.6	74.2	154.8	55.3	2.8	70.3	58.4
2– 4–48	5.7	134.5	347.8	54.8	3.2	79.5	65.3
2–14–48	6.0	134.5	366.1	50.7	2.7	62.1	52.9
2–24–48	6.0	134.5	366.1	48.2	1.3	28.4	28.9
3– 5–48	5.9	128.4	343.6	46.4	1.7	35.8	31.2
3–15–48	6.0	128.4	349.4	46.3	2.9	61.0	60.5
3–25–48	5.9	128.4	343.6	44.5	3.0	60.6	65.2
4– 4–48	6.1	128.4	355.3	45.1	2.5	51.1	50.5
4–14–48[b]				45.8	1.3	27.0	26.0
4–24–48				43.6	0.4	7.9	8.0
5– 4–48				35.1	0.0	.....	.....
5–14–48				32.7	0.1	1.4	2.0
5–24–48				34.3	0.0		
6– 3–48				30.1	0.0		
6–13–48				25.3			

[a] Calved December 9, 1947, started on Heavy DDT Hay, at the rate of 0.5 lb. per 100 lb. of live weight on December 26, 1947. Average weight 1,175 lb.

[b] Turned on pasture April 14, 1948, and DDT hay discontinued.

TABLE 4

Average daily intake of alfalfa hay and DDT for cow X-16 and output of DDT in her milk, by 10-d. periods

Start of each 10-d. period	Hay intake	DDT in hay	DDT intake	Milk produced	DDT in milk	DDT output in milk	DDT in fat
	(*lb.*)	(γ/*g.*)	(*mg.*)	(*lb.*)	(γ/*g.*)	(*mg.*)	(γ/*g.*)
12–26–47[a]	8.8	4.4	17.6	26.9	0.0	0.0	
1– 5–48	12.1	11.2	61.5	27.9	0.2	2.5	4.7
1–15–48	14.2	11.2	72.1	27.0	0.6	7.3	14.2
1–25–48	14.6	11.2	74.2	26.9	0.4	4.9	9.3
2– 4–48	14.7	13.7	91.4	25.5	0.2	2.3	4.4
2–14–48	14.5	13.7	90.1	23.3	0.3	3.2	5.9
2–24–48	14.7	13.7	91.4	22.1	0.55	5.5	12.8
3– 5–48	14.9	30.5	197.0	21.6	0.8	7.8	19.0
3–15–48	14.9	30.5	197.0	21.1	0.9	8.6	21.1
3–25–48	14.2	30.5	196.5	20.9	0.6	5.7	14.3
4– 4–48[b]				22.3	0.3	3.0	6.7
4–14–48				24.2	0.4	4.4	8.4
4–24–48				19.1	0.1	0.9	2.3
5– 4–48				20.5	0.0	0.0	
5–14–48				17.7	0.0	0.0	
5–24–48				18.0	0.0	0.0	
6– 3–48				14.6	0.0		

[a] Calved November 24, 1947, started on Light DDT Hay December 26, 1947, at rate of 1.5 lb. per 100 lb. of live weight.

[b] Turned on pasture April 2, 1948; DDT hay discontinued.

Cow X-16 (table 4) had a much smaller DDT intake than the three cows that were fed the Heavy DDT Hay, and also showed the lowest concentration of DDT in the milk. The highest concentration of DDT in her milk was 0.9 γ per g. The average daily intake of DDT was 109 mg. and the average daily output was 4.8 mg. DDT was no longer detectable in her milk 30 days after the DDT hay was removed from her ration and she was turned on pasture.

A cursory examination of the data indicated the possiblity of a relationship between the DDT intake, the DDT concentration in the milk and the total output of DDT in the milk. Table 5 shows that the four cows in this experiment secreted from 5 to 30 per cent of the DDT intake into the milk. Because of differences in the length of the feeding period and in total milk production, no good correlation between DDT intake and output could be expected. However, there was some correlation between the intake of DDT and the concentration of DDT in the milk. Similar data reported by Carter *et al.* (1) and Wilson

TABLE 5

Average daily intake of DDT from the feed and output in milk

Cow no.	Interval DDT hay was fed	Av. daily intake	Av. concentration in milk	Av. daily output in milk	% intake in milk	
					During DDT feeding	After DDT feeding
	(*d.*)	(*mg.*)	(γ/*g.*)	(*mg.*)	(%)	(%)
			Present experiment			
1666	111	727	6.4	136	18.6	1.8
1638	162	553	7.3	165	29.8[a]	3.0
X-47	110	303	2.2	51	16.8	1.1
X-16	98	109	0.5	5	4.8	0.6
			Other experiments			
Carter (1)	53	44–88[b]	0.5			
Wilson (4)	141	1500[b]	15.0			
Wilson (4)	150	24000[c]	44.0			

[a] The output in the milk was 22.4% when the total intake and output are considered, since this cow received DDT during the previous dry period.
[b] Fed pea vine silage.
[c] Fed crystalline DDT.

et al. (5), which also are shown in table 5, seem to show a similar relationship. In order to get a better correlation between these various factors, however, the hay samples in the present experiment should have been analyzed more frequently during the feeding period.

Considerable DDT appeared in the milk from the three cows fed hay from a field treated with 2.4 lb. of DDT per acre. This rate of applying DDT was at least four times as heavy as normally would be required for control of the potato leafhopper in alfalfa. A much smaller quantity of DDT appeared in the milk from the cow that received the hay from a field that was treated with only 0.6 lb. of DDT per acre, about the recommended rate of application. The DDT appeared in the milk after the cows were on DDT for only a few days, and in one case DDT was present after only 3 days on DDT hay.

The fact that these hays were damaged rather badly by rain may have had considerable effect on the results. Hays similarly treated but cured without rain might have been consumed in larger quantities, thereby increasing the intake of DDT and the output of DDT in the milk. These results indicate that care should be taken not to apply more DDT than actually is needed for insect control; otherwise, if the crop is fed to milking cows, considerable DDT will appear in the milk.

Other studies made in connection with this experiment indicate that weathering is helpful in reducing the amount of DDT on the forage at cutting time; therefore, it seems probable that if minimum effective dosages are applied about midway in the development of the crop at least 21 to 25 days prior to cutting, the DDT residue hazard will be reduced greatly.

SUMMARY

1. Alfalfa treated with 2.4 lb. of DDT per acre, in the form of an aerosol, and fed to cows at the rate of 1 lb. of hay per day per 100 lb. of body weight produced milk containing up to 10.1 γ of DDT per g. or 259.1 γ per g. of butterfat. The daily intake of DDT was as high as 903 mg. and the output in the milk was as high as 265 mg.

2. Alfalfa treated with 0.6 lb. of DDT per acre and fed to cows at the rate of 1.5 lb. of hay per 100 lb. of body weight produced milk containing up to 0.9 γ of DDT per g.

3. The output of DDT in the milk varied from 5 to 30 per cent of the intake. The DDT appeared in the milk after a very few days of feeding, and in one case was present in appreciable quantities after 3 days of feeding.

4. After the feeding of DDT hay was discontinued, DDT was detected in the milk for 160 to 170 days when large quantities of DDT had been fed and for only 30 to 40 days when small quantities had been fed.

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LOSS OF REDUCED ASCORBIC ACID FROM LACTOSE-ENRICHED MILK[1]

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In a study of the rate of disappearance of reduced ascorbic acid from samples of commercial milk, Hand (4) observed a decrease from 19 to 7 mg. per l. for milk stored 6 days at 1° C. Kon and Watson (9), Holmes and Jones (6), Buruiana (1), Krauss (10), Diemair and Fresenius (2) and others have noted the rapid loss of ascorbic acid from milk, particularly when the milk was exposed to light. Holmes and Jones (8) determined the permanency of synthetic ascorbic acid added to milk and found the same trend, but a less rapid loss of reduced ascorbic acid than that reported by Gunsalus and Hand (3). However, in a study of the stability of reduced ascorbic acid in mares' milk (mares' milk may contain from five to ten times as much ascorbic acid as cows' milk), Holmes and Jones (7) obtained data which show that the rate of loss of reduced ascorbic acid from mares' milk was only a fraction of the rate of loss from cows' milk when both were stored in commercial glass milk bottles in the dark at 10° C. Naturally, a question arose concerning the factor or factors that caused the reduced ascorbic acid of mares' milk to be more stable than that of cows' milk. Linton (11), Hildebrandt (5) and others have reported that mares' milk contains much more lactose than cows' milk. Since for decades it has been a common practice in the preparation of modified milk formulas for infant feeding to add lactose to cows' milk to approximate the quantity in human milk, it seemed desirable to determine whether the addition of lactose to cows' milk would decrease the rate of disappearance of reduced ascorbic acid from cows' milk to that observed for mares' milk.

EXPERIMENTAL PROCEDURE

In this study the samples of commercial pasteurized cows' milk were stored in the dark at 10° C. in a home-size electric refrigerator. Each week when the samples were prepared, all the milk was mixed thoroughly in a single container to insure identical milk for the three series of samples. Fifteen g. per l. of analytical reagent grade of *alpha*-lactose were added to one series of samples, 30 g. per l. were added to a second series and no lactose was added to a third series of samples which served as controls. For 15 weeks in the period from April to August, one sample of each series was prepared on Monday morning and each sample was assayed for its reduced ascorbic acid content daily from Monday to Friday, inclusive. During the period of observation the samples were stored in commercial, flint, quart milk bottles. As the aliquots were withdrawn for assay the volume of milk in the bottles decreased and the volume of supernatant atmos-

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phere increased, which is the normal condition for the household use of milk. The reduced ascorbic acid content of the various milk samples was determined by a modification of Sharp's (12) rapid method. Twenty-five ml. of a mixture of 8 per cent acetic acid and 3 per cent metaphosphoric acid was added to 25 ml. of milk and the mixture was diluted with 25 ml. of distilled water and titrated with a standardized solution of sodium 2,6-dichlorbenzenoneindolphenol.

RESULTS AND DISCUSSION

The initial reduced ascorbic acid content of the pasteurized commercial milk used in this study varied from 9.0 mg. per l. to 17.8 mg. and averaged 14.7 mg. per l. After storage for 1 day the amount of reduced ascorbic acid in the 15 samples of each series averaged 8.6 mg. per l. for the controls, 8.3 mg. for the milk with 15 g. of lactose per l. added and 8.1 mg. for that with 30 g. of lactose added. At the end of storage for 2 days, the average values were 5.5 mg. per l. for the control samples, 4.8 mg. for the milk with 15 g. of lactose per l. added and 4.9 mg. for the milk with 30 g. of lactose added. When 3 days had elapsed, the average amounts of reduced ascorbic acid present in the different series of samples were 4.4 mg., 4.0 mg. and 4.1 mg., respectively. At the end of the 96-hour experimental period the same amount of reduced ascorbic acid was found in each of the three series of samples, *i.e.*, 3.8 mg. per l. Judged by the data assembled here, the addition of reagent grade of *alpha*-lactose did not inhibit either the rate or amount of loss of reduced ascorbic acid from commercial cows' milk stored in darkness at 10° C. for 96 hours, a period comparable to the length of time that milk may be stored in partially-filled containers in the home.

These data indicate that the high lactose content of mares' milk is not the primary factor in the slower disappearance of reduced ascorbic acid from mares' than from cows' milk. However, it should be recognized that the lactose in mares' milk in excess of that in cows' milk may not be in the same form as the reagent grade used in this study and hence may react in a manner different from the lactose that was added to the cows' milk.

The rate of decrease in the amount of reduced ascorbic acid present in the different series of milk samples included in this study was slightly higher but very similar to that reported by Hand (4), *i.e.*, 19.0 mg. on the first day, 15.6 mg. on the second day, 10.9 mg. the fourth day and 7.1 mg. of reduced ascorbic acid per l. of milk on the sixth day after milking. Gunsalus and Hand (3) also noted a decrease of reduced ascorbic acid in raw cows' milk during storage for 6 days of from 14.9 mg. to 1.7 mg. per l. or an average daily loss of over 14 per cent as compared with an average daily loss in this study of over 18 per cent. Possibly the amount of ascorbic acid in milk may influence the rate of disappearance of reduced ascorbic acid from milk, for when Holmes and Jones (8) added 75 mg. per l. of ascorbic acid to raw milk, the loss was 7 per cent per day for a 10-day storage, and when 150 mg. per l. was added, the loss for the 10-day period was 3 per cent per day. Both of these losses were less than the 10 per cent loss reported by Hand (4) or the 14.7 per cent loss found by Gunsalus and Hand (3). However, in

this study no significant difference was noted in the loss of ascorbic acid from the control samples and those enriched with 15 or 30 g. of lactose per l.

SUMMARY

There was no significant difference in the rate of loss of reduced ascorbic acid, during storage in darkness for 96 hours at 10° C. of a series of cows' milk samples to which 15 g. per l. of *alpha*-lactose had been added, a series of samples to which 30 g. per l. of lactose was added and a series of control samples. These results lead to the conclusion that, even though the lactose in mares' milk may be in a different form than that used in this study, the high lactose content of mares' milk is not the principal factor in causing the greater stability of reduced ascorbic acid in mares' milk than in cows' milk.

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THE RELATIONSHIP OF PRODUCTION OF HEIFERS MILKED PREPARTUM TO THE COMPOSITION OF COLOSTRUM[1]

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Many heifers and cows have badly swollen and congested udders before freshening. It is thought that such a condition makes it undesirable to put these animals on full feed and to try to bring them into full production until the swelling has subsided. Sometimes the udder becomes so distended that some believe that the gland may be injured permanently. Breeders frequently are concerned about the advisability of milking such animals before parturition.

Turner (5) was of the opinion that prepartum milking for a period of about 10 days has certain advantages. In all cases when cows were milked prepartum the udders were soft and pliable. The colostral character of the milk largely disappeared and the globulin content was reduced to that of normal milk at parturition. From the standpoint of the well-being of the calf, the initiation of milk secretion prior to parturition caused many of the calves to die at an early age. They seemed much more susceptible to *Bacillus* (*Escherichia*) *coli* infection and other calf diseases.

Keyes *et al.* (2) reported that 25 cows and heifers had been milked for a period of 2 to 16 days prepartum. They observed wide variations in the percentage total solids until the seventh day before parturition, when the solids content became more uniform and gradually approached that of normal milk at the time of parturition. The production of milk for the prepartum-milked cows started at about 1 lb. a day and increased to 25 lb. on the day of parturition. The calves had scours and showed signs of general inactivity, a condition which was corrected when 5 ml. of "carotone" (a carotene preparation) was fed daily for 7 days.

Turner (5) suggested that prepartum milking might reduce the incidence of milk fever by a gradual initiation of lactation, a suggestion that was not supported by recent work of Smith and Blosser (4). Eaton *et al.* (1) reported that the first milking after calving from cows which had not been milked prepartum contained approximately 5 times as much carotene and vitamin A, 3 to 4 times as much protein, 0.5 as much lactose, slightly greater amounts of fat and 1.25 times as much ash as the milk obtained from the cows milked prepartum. They concluded that prepartum milking materially alters the composition and lowers the nutritive value of the first milk secreted at the termination of pregnancy.

Because of the possibility of decreasing the inflammation and congestion of the udder at calving by prepartum milking and because of the adverse effect which

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this practice might have on the nutrition of the calf, further study seemed warranted.

EXPERIMENTAL PROCEDURE

During the winter of 1947–1948, 16 first-calf Ayrshire heifers in the station herd were divided into two groups according to their expected freshening dates. It was planned that every other heifer was to be milked prepartum for a period of 2 weeks; however, some of the heifers calved before the expected dates of parturition. The heifers were milked prepartum with machines once daily until production increased to 2 lb., after which time they were milked twice daily and samples were taken for analysis. Accurate records of production were obtained

TABLE 1

The production and composition of colostrum on day of parturition

No. of heifer	Period milked prepartum	Production day before parturition	Total production before parturition	Composition of colostrum day of parturition		
				total N	non-casein N	non-casein N of total N
	(*d.*)	(*lb.*)	(*lb.*)	(*g./100 ml.*)	(*g./100 ml.*)	(%)
Group 1						
792	0	0	0	2.46	1.61	65.4
796	0	0	0	2.42	1.31	54.1
799	0	0	0	2.54	1.52	59.8
800	0	0	0	2.16	1.60	74.1
804	0	0	0	2.54	1.45	57.1
806	0	0	0	1.95	1.06	54.4
811	0	0	0	2.45	0.96	39.2
815	0	0	0	2.17	1.18	54.4
Av.	0	0	0	2.34	1.34	57.3
Group 2						
790	18	10.5	31	1.07	0.38	35.5
791	8	20.1	48	0.67	0.21	31.3
794	14	0.1	2	1.49	0.66	44.3
798	10	24.3	142	0.55	0.11	20.0
802	7	3.9	16	1.35	0.67	49.6
803	3	1.0	2	1.80	1.09	60.6
808	16	16.6	55	0.71	0.14	19.7
810	3	5.1	7	1.41	0.73	51.8
Av.	10	10.2	38	1.13	0.50	39.1

until the time of parturition. The calves remained with the dams for the first 3 days following parturition as was the usual practice. After parturition the udders were milked out twice daily and samples taken for analysis.

Samples of milk or colostrum were analyzed for total nitrogen by the Kjeldahl method. Casein was precipitated and non-casein nitrogen determined directly, whereas casein nitrogen was determined by difference (3).

RESULTS

Data presented in table 1 show the total nitrogen, non-casein nitrogen and the per cent non-casein nitrogen of the total nitrogen in the colostrum of the heifers in both groups on the day of parturition. For the heifers in group 2, which were milked prepartum, production on the day before parturition varied from

0.1 to 24.3 lb. Total production before parturition varied from 2 to 142 lb. The level of production on the day before parturition was dependent upon the extent that the heifers were stimulated into production. The number of days milked prepartum was not necessarily a determining factor. Heifer 794 was milked for 14 days and during this period produced only 2 lb. as compared with 798 which was premilked 10 days and produced 142 lb.

The total nitrogen, the non-casein nitrogen and the per cent non-casein nitrogen of the total nitrogen was much higher on the day of parturition for the heifers in group 1 than for those in group 2. Much greater variations existed in the composition of the milk for the heifers in group 2. The total nitrogen varied from 0.55 to 1.49 g. per 100 ml. and the non-casein nitrogen from 0.11 to 1.09 g. per 100 ml. The per cent non-casein nitrogen of the total nitrogen varied from 20.0 for heifer 798, which had produced a total of 142 lb. before parturition, to 60.6 per cent for heifer 803, which had been milked only 3 days prepartum and had produced only 2 lb. before parturition.

Total nitrogen, non-casein nitrogen and the per cent non-casein nitrogen of the total nitrogen are good criteria to indicate when the colostral period is over and normal milk is being produced. Total nitrogen in the colostrum of the heifers in group 1 not milked prepartum decreased from an average of 2.34 on the day of parturition to 0.70 g. per 100 ml. on the fourth day after calving when normal milk was produced. During the same period the per cent non-casein nitrogen of the total nitrogen decreased from an average of 57.3 to 20.8.

Data presented in table 2 show the relationship between production and the nitrogen content of colostrum of four heifers in group 2 which were milked prepartum. Heifer 798 was milked 10 days before parturition. Production increased rather rapidly and 24.3 lb. were produced on the day before parturition. The total nitrogen and the per cent non-casein nitrogen decreased to the level of normal milk 3 days before parturition. Therefore this heifer's calf did not receive any true colostrum. Prepartum milking of heifer 808 was started 16 days before parturition; however, she did not produce as much as 1 lb. per day until the seventh day before calving, after which her production increased rapidly to 16.5 lb. on the day before calving. Changes in total nitrogen and non-casein nitrogen were very similar to that of 798, except that normal milk was produced only 1 day before calving. Again, this heifer's calf did not receive any true colostrum. Heifer 790 was milked 18 days prepartum. Production increased slowly until the third day before calving, at which time she produced about 5 lb. Production continued to increase so that 10.5 lb. were produced on the day before parturition. Milk of normal composition was produced on the second day after calving. Heifer 802 was milked 7 days prepartum. She produced only 3.9 lb. on the day before parturition. The total nitrogen and the per cent non-casein nitrogen after parturition were similar to those of the heifers in group 1 not milked prepartum.

DISCUSSION

In this study the total nitrogen, casein nitrogen and the non-casein nitrogen have been used to compare the composition of colostrum with that of normal milk.

TABLE 2

Effect of prepartum production on the total nitrogen and per cent non-casein nitrogen in colostrum and milk

Periods milked	Heifer 798			Heifer 808			Heifer 790			Heifer 802		
	Production	Total N	Non-casein N	Production	Total N	Non-casein N	Production	Total N	Non-casein N	Production	Total N	Non-casein N
(*d.*)	(*lb.*)	(*g./100 ml.*)	(%)	(*lb.*)	(*g./100 ml.*)	(%)	(*lb.*)	(*g./100 ml.*)	(%)	(*lb.*)	(*g./100 ml.*)	(%)
Prepartum												
10	4.0	1.88	64.3	0.2			0.3					
9	4.3	1.54	47.4	0.2			0.4					
8	5.7	1.09	41.7	0.4			0.4					
7	6.0	0.82	36.9	1.1			0.6			0.2		
6	11.2	0.82	34.1	1.9	2.31	46.9	1.5	1.87	47.2	1.0		
5	12.0	0.73	25.7	3.4	1.66	49.4	2.2	2.07	47.8	2.1	1.47	58.4
4	14.6	0.69	21.7	5.9	1.38	37.2	3.4	1.80	47.2	2.9	1.62	52.2
3	15.0	0.65	20.5	10.9	1.00	33.1	4.2	1.43	51.6	2.4	1.25	49.9
2	20.2	0.65	22.8	7.0	0.84	24.8	6.6	1.31	43.7	3.0	1.56	57.6
1	24.3	0.62	19.1	16.0	0.71	19.8	10.5	1.07	35.4	3.9	1.22	46.7
0		0.55	20.2		0.71	19.5		0.68	26.0		1.35	49.5
Postpartum												
1		0.61	22.6		0.70	17.3		0.62	20.8		0.82	35.4
2		0.61	17.9		0.70	20.6		0.65	20.5		0.77	35.8
3		0.65	19.8		0.73	17.3		0.60	19.7		0.60	22.8
4		0.65	15.7		0.71	12.7					0.79	20.7
5		0.59	17.1									
6		0.54	19.3									

Years ago Turner (5) presented data showing that colostrum was unusually rich in globulin and albumin and that the total protein was three to four times as high as in normal milk. Because of the large difference in nitrogen content of colostrum and normal milk and because of the ease in the determination of total nitrogen, this constituent is of strategic value in determining the rate of change from the production of colostrum to the production of normal milk. This is true for cows milked prepartum as well as cows milked postpartum.

The data presented show that prepartum milking affects the rate of change from the production of colostrum to that of normal milk as it affects the rate of production. The total nitrogen and the per cent non-casein nitrogen of the milk on the day of parturition were dependent upon the total amount of prepartum colostrum produced and the level of production at parturition. The composition of colostrum produced on the day of parturition was not related necessarily to the number of days heifers were milked prepartum. Some heifers were stimulated into production before parturition by prepartum milking, whereas others were not.

According to Turner (5), the initiation of milk secretion in late pregnancy and postpartum is due to the pituitary hormone lactogen. Following parturition, the secretion of milk is stimulated intensely by the rising secretion of the lactogenic hormone. While the presence of estrogen in increasing amounts just before parturition initiates the intense secretion of the lactogenic hormone, the stimulus of milking and the removal of milk are the factors that maintain the lactation process.

It seems reasonable to suspect that prepartum milking might cause a stimulation in the production of the lactogenic hormone, which in turn stimulates the secretion of the mammary gland. If this is true, it seems that in the case of some animals the manipulation of the udder and teats a few days prior to parturition causes the secretion of the lactogenic hormone, whereas, in the case of other animals, the lactogenic hormone is not secreted in appreciable amounts until after parturition.

As to the effect of prepartum milking on the condition of the udder following parturition and on the nutrition of the calf, more data are needed before any recommendation can be made. Of the eight calves dropped by the heifers in group 2 milked prepartum, only two failed to receive some colostrum. Each calf was given 25 ml. of cod liver oil on the day of birth to provide additional vitamins A and D. The calves were thrifty and made satisfactory growth.

SUMMARY AND CONCLUSIONS

During the fall and winter of 1947–1948, 16 first-lactation Ayrshire heifers were divided into two equal groups upon the basis of expected dates of parturition, every other animal being assigned to each group. Group 1 was handled in the usual way. The heifers in group 2 were milked for a period of 3 to 18 days with an average of 10 days before parturition.

The total production before calving for prepartum-milked heifers varied from 2 to 142 lb. The production on the day before parturition varied from 0.1 to 24.3 lb. with an average of 10.2 lb.

The total nitrogen and the non-casein nitrogen of the milk produced on the day of parturition for the heifers not milked prepartum averaged 2.34 and 1.34 g. per 100 ml., respectively, whereas the milk from the heifers milked prepartum averaged 1.13 and 0.50 g. per 100 ml., respectively. The non-casein nitrogen amounted to 57.3 per cent of the total nitrogen for the heifers not milked prepartum, but only 39.1 per cent for the heifers milked prepartum. The total nitrogen and the non-casein nitrogen in the milk of heifers prepartum-milked on the day of parturition were dependent on the level of production and the total amount produced before parturition.

Heifers producing appreciable quantities of prepartum colostrum produced normal-appearing milk at the time of parturition.

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EFFECT OF RAW SOYBEANS AND SOYBEAN OIL MEAL ON THE VITAMIN A AND CAROTENE CONCENTRATIONS IN THE BLOOD PLASMA AND MILK OF LACTATING COWS[1]

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In a previous trial (6) it was noted that the carotene concentration in the blood plasma of lactating cows was reduced when the cows were fed 9 lb. of ground, raw soybeans daily. When only the oil portion of the beans was used, the reduction in the carotene concentration in the blood plasma was somewhat less than when the whole bean was fed. Because of this difference, it was reasoned that soybean oil meal probably carried some factor or factors affecting the blood plasma carotene.

This trial was designed to learn whether soybean oil meal when fed to dairy cows would cause their blood plasma carotene to fall as was the case when soybeans or soybean oil was fed. Also, the experiment was to recheck the effects of cracked raw soybeans on the carotene in the blood plasma of lactating cows.

EXPERIMENTAL PROCEDURE

Nine Holstein cows which had been on pasture were assigned to three similar experimental groups. The trial was initated with a preliminary period of 2 weeks in which each animal was fed daily 40 lb. of corn silage, and from 16 to 18 lb. of concentrate. The amount of concentrate, based on milk yield, was established at the beginning and remained the same throughout the trial. The concentrate was made up of two parts: (a) 3 lb. of old process linseed oil meal and (b) enough of a basal mixture consisting of 400 lb. ground yellow corn, 400 lb. crushed oats, 200 lb. wheat bran, 12 lb. salt and 12 lb. bone meal to bring the total up to the requirements for the cow.

The preliminary period was followed by an experimental period of 9 weeks. The same ration used in the preliminary period was fed to one group of cows; the second group received a similar concentrate except that 7.2 lb. of expeller process oil meal replaced 4.2 lb. of the basal grain and 3 lb. of linseed oil meal. The third group of cows had 6 lb. of the basal mixture and 3 lb. of linseed oil meal replaced by 9 lb. of raw soybeans, which were ground fresh every 10 days. When the oil is extracted from 9 lb. of soybeans, there are 7.2 lb. of oil meal left. This accounts for the different amounts of soybean products used.

Blood and milk samples were collected weekly at the same hour from each of the experimental cows. The blood samples were analyzed for vitamin A and carotene[2] according to a method previously described (6). The vitamin A and

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[2] A Klett-Summerson photoelectric colorimeter was employed for all the analyses. The instrument was equipped with a narrow band 440 mμ filter for estimating the carotenoids and 550 mμ narrow band filter for estimating the vitamin A.

carotene contents of the milk were determined by a modification of the Boyer *et al.* (2) method. The modification consisted of using activated glycerol dichlorohydrin (G.D.H.) (5) instead of antimony trichloride as the colorimetric reagent for estimating the vitamin A content of the milk. Duplicate fat tests were made on each of the milk samples using the standard Babcock method. The total fat of each milk sample was calculated according to the method of Berl and Peterson (1).

The values for carotene and vitamin A that were found in the blood plasma and milk the second week of the basal period were used as base points from which all changes were measured after the cows were placed on the experimental rations.

The 9-week experimental period was followed by an additional 3 weeks in which all experimental conditions were maintained except that each cow was fed 100,000 units of vitamin A[3] daily by capsule.

RESULTS AND DISCUSSION

The average concentrations of carotene and vitamin A in the blood plasma and milk fat of all groups of cows are presented in table 1. Carotene values of both the blood plasma and milk fat for all groups of cows declined during the first 4 to 6 weeks of the trial. The plasma carotene concentrations of the control group and the group fed soybean oil meal then tended to level off while that of the cows fed raw soybeans continued to drop before becoming relatively constant.

The rapid lowering of the blood plasma carotene concentrations observed in all groups during the first 4 weeks of the trial is to be expected in dairy cows taken off pasture and limited to silage and concentrate. In a previous trial (6) the effect of feeding raw soybeans on the concentration of blood plasma carotene was apparent after feeding the beans 1 week. In this trial the effect of the raw soybeans was not apparent until the fifth week of the experiment. It was not until after the initial rate of decline in the blood plasma carotene of all groups that the "depressing" effect of the raw soybeans became apparent.

The vitamin A of the blood plasma and of the milk fat did not fluctuate nearly so much as the carotene. The vitamin A concentration trends were similar for all rations.

As can be noted from table 1, the feeding of raw soybeans caused the blood plasma carotene concentrations to be depressed to a greater extent than was the case when the cows were fed either the control ration which contained no soybean products or the ration containing soybean oil meal. This depression is similar to that noted in another experiment (6) but the differences are not so large. This variation between the results of the two trials might have been due to the supplemental carotene fed in the first trial.

The differences in the concentrations of carotene in the blood plasma and milk fat obtained from the cows fed the control ration and the cows fed soybean oil meal were small and probably unimportant. In a previous trial (6), the feeding of soybean oil had an effect on the blood plasma carotene intermediate

[3] Fish oil containing 25,000 U.S.P. units of vitamin A per g., obtained from White Laboratory, Newark, N.J.

TABLE 1

Average vitamin A and carotene concentration in the blood plasma and milk fat of cows fed the control, soybean oil meal and raw soybean rations

Wk.	Control				Soybean oil meal				Raw soybeans			
	Milk fat		Plasma		Milk fat		Plasma		Milk fat		Plasma	
	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A
	(γ/*g.*)		(γ/*100 ml.*)		(γ/*g.*)		(γ/*100 ml.*)		(γ/*g.*)		(γ/*100 ml.*)	
	Basal Period (2 wk.)											
1	4.26	6.46	391	32.3	5.92	7.24	492	32.9	5.06	7.52	434	30.5
2	3.93	5.16	326	36.6	5.21	6.62	397	33.5	4.45	6.82	351	38.8
	Experimental period (9 wk.)											
3	3.15	6.04	262	37.7	4.21	7.06	298	32.3	2.73	5.87	264	35.6
4	2.21	5.96	177	35.4	3.62	7.20	262	33.0	1.76	6.50	204	35.2
5	2.18	4.70	138	33.9	3.43	7.11	239	34.8	2.64	6.19	143	36.2
6	2.12	4.90	131	28.5	3.29	7.00	258	26.0	2.66	6.27	128	30.9
7	1.92	5.35	149	30.4	3.40	6.62	259	27.4	1.81	5.76	113	32.7
8	2.79	5.37	165	35.6	3.50	7.58	292	32.0	1.26	5.65	120	34.4
9	2.36	6.43	208	34.7	4.04	7.75	325	31.3	1.45	5.95	153	28.5
10	2.40	7.01	249	41.1	3.47	7.11	347	36.0	1.59	6.27	141	31.8
11	2.91	6.25	240	42.1	3.09	6.85	294	36.8	1.47	5.04	124	32.8
	Vitamin A Supplemental period (3 wk.)											
12	2.52	7.75	229	39.8	2.85	8.13	250	32.0	0.98	7.44	122	31.7
13	2.87	7.09	226	47.0	3.45	7.01	257	42.0	1.64	7.02	125	36.7
14	2.85	8.87	238	39.3	2.85	8.62	265	34.2	1.65	7.41	125	27.0

between that of the raw soybeans and the control ration. Because of this intermediate effect it was supposed that the soybean oil meal would carry portions of the factor or factors in raw soybeans which cause a depression of blood plasma carotene concentrations.

Since the same basal ration was fed with differences only in the protein supplement, it was assumed that all of the cows consumed similar quantities of carotene and vitamin A. The data show that the ration effect was displayed in carotene differences rather than in vitamin A differences. The cows that were fed the control and the soybean oil meal rations showed larger concentrations of carotene in their blood plasma and also excreted more carotene in their milk than the cows fed the soybean ration. Presumably raw soybeans either were causing destruction of carotene in the digestive tract or were interfering with its absorption into the blood stream.

It seems logical to assume that there was little vitamin A *per se* in the rations so that the cows on all rations were maintaining their vitamin A plasma and milk concentrations by converting carotene after it was absorbed into the body. This left unanswered the question as to whether there were differences among the rations in their effects on vitamin A absorption.

The supplementation of the ration of each cow in all groups with 100,000 U.S.P. units of vitamin A during the last 3 weeks of this trial caused the vitamin A content of the blood and milk fat of all cows to rise. Since the rate and direction of change in vitamin A content was approximately the same with all cows, with differences statistically insignificant, it was assumed that these rations did not affect vitamin A metabolism as they did carotene.

The data furnish no conclusive evidence that soybean products may not affect vitamin A absorption. Shaw *et al.* (3) found symptoms of a vitamin A deficiency when cows were fed a ration that was low in carotene and contained raw soybean meal. Possibly the daily intake of 100,000 U.S.P. units of Vitamin A was sufficient to cover any depressing effects of the soybean products on vitamin A, or the quantity of carotenoids present in the gastro-intestinal area of the cow might have exercised a sparing effect. Sherman (4) has shown that xanthophyll fed to rats may effect a sparing action on vitamin A *per se*.

SUMMARY

A reduction of the carotene concentration of the blood plasma and of the milk of lactating cows resulted when raw soybeans were fed.

When soybean oil meal was fed in an amount equivalent to that of the raw beans minus their oil content, the carotene concentrations in the blood plasma and the milk fat were similar to those when the cows were fed the control ration.

Both the control and soybean oil meal groups excreted more carotene in their milk than did the group fed raw soybeans.

Loss of these nutrients from the bodies of the cows fed raw soybeans thus was not the cause of the observed reduction of their blood plasma carotene concentration. Apparently soybeans either interfered with carotene absorption or caused some destruction of it.

No significant differences were observed among the groups of cows in the quantity of vitamin A *per se* in the blood plasma and in the milk during the last 3 weeks of the trial when all the rations were supplemented with 100,000 U.S.P. units of vitamin A daily.

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THE ADAPTATION OF A STANDARD CURVE TO THE TURBIDOMETRIC METHOD OF ASSAY OF HYALURONIDASE IN BULL SEMEN[1]

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The turbidometric method for the assay of the enzyme hyaluronidase has been modified for the assay of semen by Leonard *et al.* (3). The turbidity reducing unit (TRU) has been used in this assay as the measure of hyaluronidase activity. It generally is recognized that the TRU is not an absolute unit of hyaluronidase activity but varies according to the substrate and blood serum proteins used, the pH control, salt concentration, etc. Dorfman and Ott (1) and Warren *et al.* (4) have developed modifications of the turbidometric method which tend to make the TRU more reproducible.

The turbidometric method of assaying bull semen hyaluronidase in terms of weight equivalent of a partly purified preparation of hyaluronidase and a standard curve may have several advantages over the expression of activity in terms of the TRU. In addition to greater facility in calculating activity of hyaluronidase preparations by use of the standard curve, use of a purified preparation of hyaluronidase lends itself to comparative studies among laboratories by the simple expediency of exchanging hyaluronidase preparations. This is in lieu of an international unit of hyaluronidase.

The purpose of this report is to show that bull semen hyaluronidase assays obtained by using several dilutions of seminal plasma correspond closely to a standard curve derived from use of a purified hyaluronidase preparation obtained from bull testes when assayed turbidometrically.

METHODS

Semen for this study was obtained from dairy bulls in a stud at the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex, N. J. Partly purified bull testes hyaluronidase (30 TRU per mg.) was used for the hyaluronidase enzyme standard[2], and a single preparation of highly purified potassium hyaluronate[2] was used as the enzyme substrate.

Blood serum was prepared from the blood of a single cow to have a uniform supply. Relatively large quantities of blood were collected at a time and the serum was bottled in 15 ml. serum botles and kept frozen until needed. No trouble has been experienced, however, in shifting from one lot of blood serum to another, if the serum was aged properly before and after its dilution.

The turbidometric assay for the hyaluronidase was conducted essentially as

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[1] Paper of the journal series, New Jersey Agricultural Experiment Station, Rutgers University–The State University of New Jersey, Department of Dairy Industry.

[2] The potassium hyaluronate and the bull testes hyaluronidase were furnished through the courtesy of Dr. D. Roy McCullagh, Schering Corporation, Bloomfield, N. J.

described by Leonard *et al.* (3) for bull semen and powdered hyaluronidase. Only 5 minutes, however, were allowed for the development of turbidty after the addition of acidified blood serum. A Klett-Summerson photoelectric colorimeter with red filter no. 66 was used for measuring turbidities. Enzyme incubation was carried out in a water bath regulated to 37° C.

RESULTS AND DISCUSSION

Standard bull testes hyaluronidase curve. To obtain a regression of hyaluronidase activity on turbidity readings, ten enzyme dosages (0.05 mg. to 0.5 mg.) were incubated with 0.2 mg. potassium hyaluronate and subsequently acidified blood serum was added to cause turbidity development. Each enzyme dosage determination was made three times (two exceptions) for a total of 28 determinations. The correlation between hyaluronidase dosage in mg. and turbidity readings was -0.985 ± 0.006. A standard curve (fig. 1) was constructed from

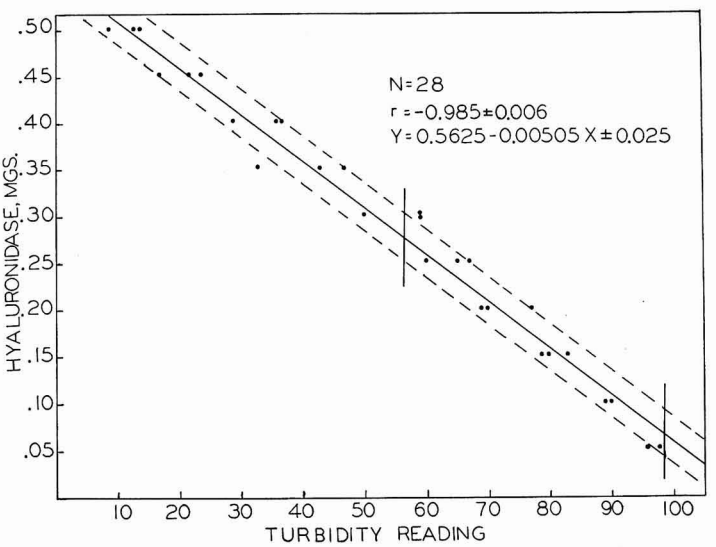

FIG. 1. Regression line obtained by correlating colorimeter turbidity readings with mg. of hyaluronidase. Solid line is the regression line, parallel broken lines are the standard errors of estimate. Vertical solid lines are the turbidity levels of the 0.2 mg. and 0.1 mg. hyaluronate standards.

use of a regression equation obtained by the method of least squares.

Individual data points are shown as well as the standard error of estimate of the regression equation. Solid vertical lines crossing the regression line indicate the turbidity readngs of 99 for the 0.2 mg. hyaluronate standard and 57 for the 0.1 mg. hyaluronate standard. Accordng to this, 1 TRU is equivalent to 0.275 mg. of hyaluronidase. The regression equation, Y (hyaluronidase, mg.) = 0.5625 –

0.00505 X (turbidity reading), was used for converting semen assay turbidity data to mg. equivalent of standard hyaluronidase.

Standard hyaluronidase curve and semen hyaluronidase activity. Semen samples were assayed initially within 1 hour after ejaculation and again after 24 hours of incubation at 37° C. (Johnston and Mixner, 2). Certain standard semen dilution rates were used during assay on each semen sample. In the initial assays, dilution rates 1 : 39, 56 and 100 as well as 1 : 56, 72 and 100 were used. In like manner, dilution rates 1 : 160, 200 and 267 and 1 : 200, 267 and 400 were used for incubated semen. As an example of calculation of semen hyaluronidase potency per ml., if a 1 : 100 dilution of a semen sample gives a turbidity value of 50, then the mg. equivalent of hyaluronidase per ml. of original semen would be 100×0.31 mg. (*mg. equivalent for turbidity value of 50*) = 31 mg. If three different dilution rates are used with a given semen sample, the final mg. equivalent of hyaluronidase should be the same for all three dilution rates.

A total of 76 semen samples was assayed for hyaluronidase equivalent (table 1) using three dilution rates for each sample. Average hyaluronidase activity in terms of mg. per ml. are shown for the samples at the various dilution rates. Analysis of variance revealed that there were no significant differences in potencies exhibited within the four comparisons made at their respective dilution rates.

TABLE 1

Correspondence of semen hyaluronidase assays to standard hyaluronidase assay regression line

No. of semen samples	23			7			17			29		
Type of assay	Initial			Initial			Incubated 24 hr. 37° C.			Incubated 24 hr. 37° C.		
Assay dilution rates 1:	39	56	100	56	72	100	160	200	267	200	267	400
Hyaluronidase activity (*mg. per ml.*)	33.1	33.1	30.6	37.3	35.1	37.0	103.0	98.8	99.8	130.5	127.2	139.8

Turbidity reducing units and mg. of hyaluronidase. One hundred seventeen semen samples were assayed for hyaluronidase, and hyaluronidase activities were calculated both in terms of TRU's and as mg. of standard hyaluronidase equivalent. The coefficient of correlation between these two measures of activity was $+0.954 \pm 0.008$, showing their essential sameness. The derived regression equation indicated that 1 TRU = 0.274 mg. of hyaluronidase, which is nearly identical to the TRU value obtained from data presented in figure 1.

The validity of expressing hyaluronidase potencies in semen in terms of a standard hyaluronidase preparation is shown by the results reported.

SUMMARY

In using the turbidometric assay for hyaluronidase, considerable flexibility in the calculation of hyluronidase potency in semen is achieved by reference to a standard preparation of hyaluronidase. The coefficient of correlation between

mg. of standard hyaluronidase and colorimeter meter reading was -0.985 ± 0.006.

Hyaluronidase assay values of semen in terms of mg. of standard hyaluronidase, obtained by diluting semen at various rates, conformed to the standard regression line of purified hyaluronidase.

A coefficient of correlation of $+0.954 \pm 0.008$ was obtained between turbidity reducing units and mg. of standard hyaluronidase equivalent for 117 semen samples, indicating the essential sameness of the two measures of hyaluronidase potency.

The results indicate the validity of expressing semen hyaluronidase potencies in terms of milligrams of a standard hyaluronidase preparation.

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HYALURONIDASE RELATIONSHIPS IN DAIRY BULL SEMEN[1]

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The possibility of a relationship between the hyaluronidase content and fertilizing capacity of semen has led us to investigate the correlations existing between hyaluronidase levels and other characteristics of dairy bull semen and also to determine whether significant differences in hyaluronidase levels exist among different breeds of bulls and among individual bulls.

Werthessen *et al.* (13) found no change in hyaluronidase concentration in human semen which had been stored for as long as 2 weeks unless putrefaction had occurred. Hechter and Hadidian (1) have shown that rabbit spermatozoa are capable of liberating hyaluronidase, and Johnston and Mixner (3) have reported increases as great as 400 per cent in hyaluronidase concentration in bull semen upon storage at either 5 or 37° C.

Kurzrok *et al.* (4), Werthessen *et al.* (13) and Swyer (12) have reported a direct relationship between sperm concentration and hyaluronidase concentration in human semen, and Sallman and Birkeland (11) have reported a correlation coefficient of + 0.28 between these factors in dairy bull semen. The latter assays for hyaluronidase were conducted within 20 hours after collection of the semen.

METHODS

Semen samples were collected with an artificial vagina from a group of Holstein, Guernsey and Brown Swiss bulls in the stud at the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex. One hundred semen ejaculates were used in obtaining all data except those involving percentages of live sperm, in which case only 50 ejaculates were used.

Seminal hyaluronidase was assayed by the turbidometric method as outlined by Leonard *et al.* (6) except for the use of a standard curve in obtaining hyaluronidase activity (Mixner and Johnston, 8). In this modification, semen hyaluronidase potencies are referred to a standard curve obtained by using a preparation of bull testes hyaluronidase[2] (30 TRU per mg.) and using potassium hyaluronate[2] as the substrate. Semen hyaluronidase concentrations therefore are reported as mg. equivalent of this preparation of hyaluronidase. Turbidities were measured with a Klett-Summerson photoelectric colorimeter with red filter no. 66.

Semen samples were assayed for hyaluronidase initially within 1 hour after ejaculation and again after storage for 24 hours at 37° C. A drop of toluene was added to each sample stored at 37° C. to inhibit bacterial growth. These time

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1 Paper of the journal series, New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey—, Department of Dairy Industry.

2 The bull testes hyaluronidase and potassium hyaluronate were furnished through the courtesy of Dr. D. Roy McCullagh, Schering Corporation, Bloomfield, N. J.

intervals were adhered to rigidly, since Johnston and Mixner (3) have shown that the hyaluronidase level in bull semen changes significantly on storage at either 5 or 37° C.

Undiluted semen. Semen was collected by the use of the artificial vagina in a graduated test tube and the volume read at the time of collection. A photoelectric turbidometric procedure similar to that proposed by Salisbury *et al.* (10) was used in determining sperm concentration. Semen was rated for motility on a 5 to 0 scale (5 = highest motility, 0 = no motility) as proposed by Herman and Swanson (2), using the hanging drop technic and employing a 38° C. microscope stage incubator. Motility initially was rated within 1 hour after ejaculation. Semen then was cooled slowly to 5° C. and rated daily until motility ceased.

Diluted semen. A portion of each semen sample was diluted 1 : 10 with an egg yolk-citrate dilutor containing 3 mg. sulfanilamide per ml. Diluted samples were

TABLE 1

Characterization of semen samples

Semen character	Mean and s.e.	Standard deviation	Range
Undiluted semen			
Initial hyaluronidase (*mg./ml.*)	40.3 ± 1.5	15.4	9–77
24-hr. hyaluronidase (*mg./ml.*)	124.4 ± 4.4	44.3	35–290
Sperm concentration (*millions/ml.*)	1382.8 ± 45.3	452.6	262–2309
Semen volume (*ml.*)	5.2 ± 0.2	2.0	0.8–12.0
Sperm/ejaculate (*billions*)	7.3 ± 0.4	4.0	0.3–23.2
Initial motility	3.4 ± 0.1	1.0	1–5
Duration of "2" motility (*hr.*)	138.8 ± 5.9	58.8	0–276
Total duration of motility (*hr.*)	228.2 ± 10.4	103.8	36–612
Diluted semen			
Total duration of motility (*hr.*)	420.0 ± 13.9	139.4	108–876
Initial live sperm (%)	64.5 ± 1.8	12.5	29–87
Live sperm surviving cold shock (%)	48.8 ± 2.3	16.2	10–83

cooled slowly to 5° C. and motility examinations made as for undiluted samples. The initial percentage of live sperm was determined on freshly diluted semen with a Fast Green FCF-Eosin Y stain as proposed by Mayer *et al.* (7). For the determination of the percentage of live sperm following a cold shock, 0.1 ml. of freshly diluted semen was placed in 0° C. ice water bath for 10 minutes and then stained immediately as before.

RESULTS AND DISCUSSION

The mean values, standard deviations and ranges for each of the semen characters studied are presented in table 1.

The hyaluronidase data were grouped by breeds of bulls and by individual bulls (for summary see table 2). An analysis of variance of the original data indicated that there were no significant breed differences in mg. of hyaluronidase per ml. of seminal plasma assayed initially and also after 24 hours, but that there were highly significant differences in these characters among individual bulls.

When adjustment was made for differences in sperm concentrations of the various ejaculates, using the analysis of covariance technic, highly significant

TABLE 2

Mean hyaluronidase levels in semen initially and at 24 hr., arranged by breeds and bulls

Breed	Bull no.	No. semen samples	Hyaluronidase content of semen	
			Initial assay[a]	24-hr. assay[a]
			(*mg./ml.*)	(*mg./ml.*)
Guernsey	1	8	51.1	155.5
	2	7	51.0	129.9
	3	6	23.7	103.8
	4	8	34.1	112.8
	5	4	54.8	174.5
	Mean		42.9	135.3
Brown Swiss	6	8	50.9	153.3
	7	6	33.5	103.2
	Mean		42.2	128.3
Holstein	8	7	22.0	92.6
	9	9	52.7	180.3
	10	4	51.5	141.3
	11	9	35.0	102.6
	12	5	20.8	67.4
	13	6	45.0	123.2
	14	6	37.3	134.7
	Mean		37.8	120.3

[a] Differences among bulls significant at the 1% level.

differences still were shown among the initial hyaluronidase levels of bulls but not among the 24-hour hyaluronidase levels. This indicates that significant differences do not exist among the average total amounts of hyaluronidase per

TABLE 3

Correlation coefficients obtained between hyaluronidase levels and other semen characters

Semen character	Zero order correlations with:		First order partial correlations independent of sperm concentration with:	
	Initial assay	24-hr. assay	Initial assay	24-hr. assay
Undiluted semen				
Initial hyaluronidase	.....	+ 0.64**	.....	+ 0.48**
Sperm concentration	+ 0.54**	+ 0.70**	.....	.....
Sperm/ejaculate	+ 0.48**	+ 0.54**	+ 0.18	+ 0.18
Semen volume	+ 0.16	+ 0.21*	+ 0.07	+ 0.09
Initial motility	+ 0.04	+ 0.25*	− 0.07	+ 0.11
Duration "2" motility	+ 0.01	+ 0.002	+ 0.03	− 0.02
Total duration motility	+ 0.09	− 0.14	+ 0.01	− 0.03
Diluted semen				
Total duration motility	+ 0.09	+ 0.30**	− 0.06	− 0.12
Initial live sperm (%)	− 0.19	+ 0.16	− 0.30*	− 0.08
Live sperm surviving cold shock (%)	− 0.37**	− 0.16	− 0.38**	− 0.13

* Significant at 5% level.
** Significant at 1% level.

individual sperm among the bulls studied as measured by the 24-hour 37° C. incubation assay.

To determine the relationships existing between hyaluronidase levels (initial and 24-hour assays) and other semen characters, zero order coefficients of correlation were calculated on the original ungrouped data (table 3). Since sperm concentrations showed highly significant correlations of + 0.54 with initial hyaluronidase levels and + 0.70 with 24-hour hyaluronidase levels, first order partial correlations independent of sperm concentration also were derived.

The highly significant coefficients of correlation obtained between sperm concentration and hyaluronidase levels support the observations of Kurzrok *et al.* (4), Werthessen *et al.* (13) and Swyer (12) to this effect. The coefficients of correlation obtained are considerably higher than that obtained (+ 0.28) by Sallman and Birkeland (11) in dairy bulls. The differences may be explained by

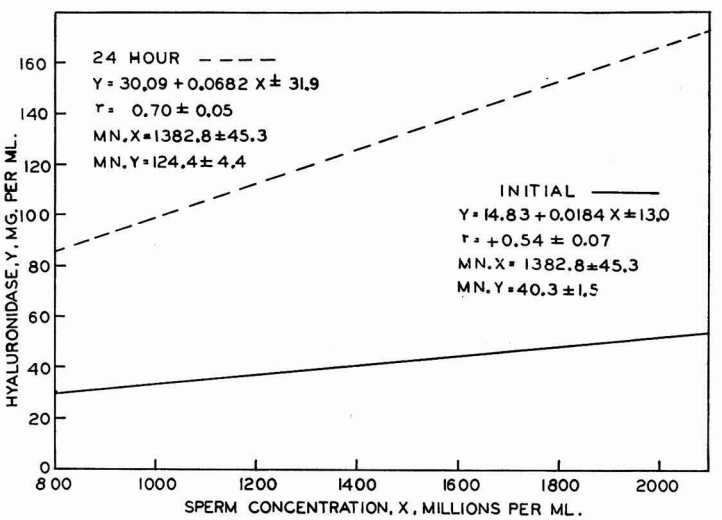

FIG. 1. Regression of hyaluronidase levels both initially and at 24 hours on sperm concentration.

the fact that the assays included in the present analysis were run at fixed intervals after ejaculation while Sallman and Birkeland assayed within a 20-hour period after ejaculation, disregarding the variable effect of semen storage on hyaluronidase levels (Johnston and Mixner, 3).

The regressions of hyaluronidase levels both initially and at 24 hours on sperm concentration are shown graphically in figure 1.

Of the first order partial correlations (independent of the effect of sperm concentration only the correlations of initial hyaluronidase with initial percentage live sperm and with percentage live sperm surviving cold shock have significance. These significant correlations may be explained when the scheme of release of

hyaluronidase from spermatozoa is considered. Johnston and Mixner (3) and Perlman *et al.* (9) have observed that as motility of a semen sample decreases and the sperm die, the hyaluronidase level increases. Since initial percentage live sperm and the percentage of live sperm surviving a cold shock are related inversely to the number of weak, dead and dying spermatozoa, one would expect significant negative correlations between these measures and the initial hyaluronidase levels of semen when they are independent of the effect of sperm concentration.

SUMMARY

One hundred semen samples from 22 dairy bulls (including Guernsey, Brown Swiss and Holstein) were assayed for hyaluronidase within 1 hour after ejaculation and again after incubation for 24 hours at 37° C. Analysis of variance indicated that, although there were no significant differences among the breeds in hyaluronidase levels (either initial or after 24 hours), there were highly significant differences among individual bulls. However, when adjustment was made for the effect of sperm concentration through the analysis of covariance, there were no significant differences among bulls in respect to the 24-hour hyaluronidase levels, whereas the initial hyaluronidase levels still showed highly significant differences.

Correlation coefficients between seminal hyaluronidase levels (both initial and 24-hour assays) and the following semen characteristics were determined: *undiluted semen:* sperm concentration, sperm per ejaculate, semen volume, initial motility, duration of "2" motility and total duration of motility; *diluted semen:* total duration of motility, initial percentage live sperm and percentage live sperm surviving cold shock. Although many of these zero order correlations were significant or highly significant, when first order partial correlations (independent of sperm concentration) were used, only the negative partial correlations of initial hyaluronidase with initial percentage live sperm and with percentage live sperm surviving cold shock retained significance.

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A PROPOSED METHOD FOR THE DETERMINATION OF COLOR OF DRY PRODUCTS OF MILK[1]

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One of the important characteristics by which the consumer judges the acceptability of dry products of milk is the color of the products. Factors such as excessive exposure to heat during processing, high moisture content and prolonged storage time at high temperature are generally known to promote browning. A rapid and reproducible method for estimating the extent of discoloration is desirable from the standpoint of quality control.

In their study of the factors influencing the development of color in evaporated milk, Webb and Holm (5) employed the Munsell color system of visual examination, using permanent color standards prepared from ferric chloride and potassium dichromate. A procedure for extracting color from browned nonfat dry milk solids and dry whey solids using a concentrated solution of trisodium phosphate and sodium chloride was reported by Doob *et al.* (1). Experience with this method indicated that only a small fraction of the color is extracted from browned nonfat dry milk solids. A method involving the measurement of reflectance by means of the Beckman Spectrophotometer recently was reported by Nelson (3) for the determination of color of evaporated milk and related products.

One of the factors influencing the extraction of color from dry products of milk is the rather strong adsorption of the color by casein. Kass and Palmer (2) showed that the adsorption follows Freundlich's equation, $\frac{x}{m} = KC^{\frac{1}{n}}$ where x is the amount of material adsorbed, m is the weight of the adsorbent, C is the equilibrium concentration of the adsorbate and K and n are constants. Since the amount adsorbed depends directly on the concentration of the adsorbent, it is reasonable to believe that by breaking down some of the large casein molecules extraction of the color may be more complete. Hydrolysis of the protein molecules may be accomplished easily at ordinary temperatures by means of proteolytic enzymes with minimum danger of further color production during the hydrolysis. Consequently, when the remaining proteins, proteoses and peptones are precipitated, the filtrate should contain most, if not all, of the undesirable color in addition to the small amount of water-soluble, natural chromogenic materials of milk. The following procedure was developed based upon this principle.

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[1] The subject matter of this paper has been undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors and do not necessarily reflect the views or endorsement of the Department of the Army.

PROCEDURE

Ten g. of the dry product of milk were dispersed in distilled water and made up to a total volume of 100 ml. A 25-ml. portion was transferred to a 125-ml. Erlenmeyer flask containing 1.5 ml. of a 10 per cent trypsin[2] suspension. The sample was incubated for 1 hour at 45° C., after which 1 ml. of 50 per cent trichloroacetic acid and approximately 0.1 g. of Celite Analytical filter-aid (Johns-Manville) were added to the digested mixture. The mixture was filtered and transmission determined on the clear filtrate by means of a Pfaltz and Bauer

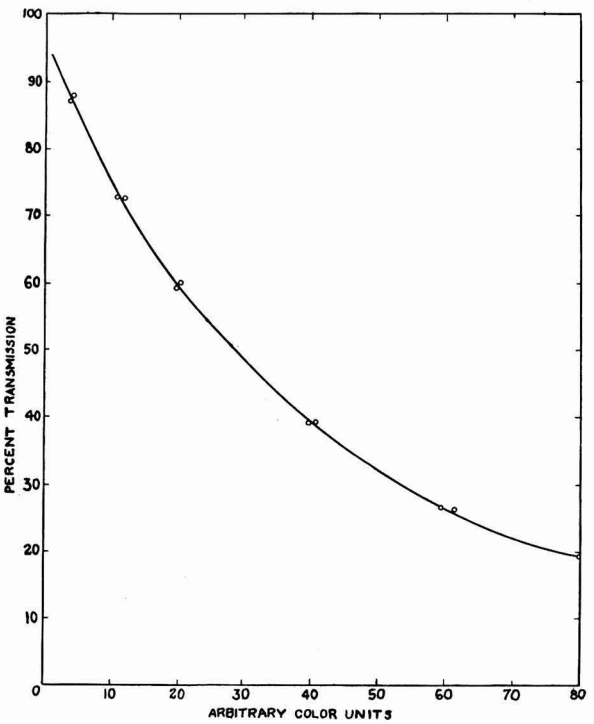

FIG. 1. Standard curve constructed with iodine in 10% potassium iodide solution. 1 mg. iodine per 50 ml. equals 1 color unit.

fluorophotometer with filter no. 485. Correction was made for the small amount of color due to the enzyme by setting the enzyme blank at 100 per cent transmission.

To permit comparison of results among different laboratories it was necessary to standardize the transmission readings against an arbitrary but reproducible standard. After consideration of different colored compounds, it was decided that a solution of iodine in 10 per cent potassium iodide would be the most satis-

[2] Wilson Laboratories, Chicago, Illinois.

factory standard from the standpoints of reproducibility, similarity of color and ease of preparation, the only disadvantage being that the color of this standard does not follow Beer's law in the wave-length region chosen. The standard curve was determined by dissolving 200 mg. of resublimed iodine (Baker's C. P.) in a small volume of freshly prepared 10 per cent potassium iodide solution and diluting to 100 ml. in a volumetric flask with the same potassium iodide solution. Different volumes of this solution were transferred to 50-ml. volumetric flasks and made up to volume with 10 per cent potassium iodide solution. After thorough mixing the transmission readings were determined with a potassium iodide blank set at 100 per cent transmission. The color of a solution containing 1 mg. of iodine per 50 ml. was arbitrarily designated as one color unit. On this basis the standard curve in figure 1 was constructed. Since 25 ml. of the reconstituted sample contained 2.5 g. of the dry product, the number of color units per gram was calculated by dividing the color units obtained from the standard curve by 2.5.

RESULTS AND DISCUSSION

The effect of enzyme concentration was investigated using a sample of nonfat dry milk solids of high moisture content which had been browned on laboratory storage at 37° C. The enzyme concentration was varied from 0 to 400 mg. per 25 ml. of reliquefied sample, and the incubation period was fixed at 1 hour. The result of each determination was corrected for the color due to the enzyme. To estimate the extent of hydrolysis at each enzyme concentration, non-protein nitrogen was determined by the method of Rowland (4).

From the results presented in table 1 it is evident that hydrolysis of the protein greatly facilitates the liberation of color. With the incubation time fixed at 1 hour, increasing the enzyme concentration increases both the color extinction and the non-protein nitrogen. Maximum color liberation appears to have been reached at a trypsin concentration of 150 mg. per 25 ml. of milk. Further increases in enzyme concentration do not seem to have appreciable effects on either color extinction or non-protein nitrogen content.

TABLE 1

The effect of trypsin concentration on the extent of color liberation and protein hydrolysis

Trypsin concentration	Color	Non-protein N
(*mg./25 milk*)	(*extinction*)	(*% of dry milk*)
0	0.135	0.274
25	0.260	1.79
50	0.335	2.41
100	0.378	2.95
150	0.407	3.63
200	0.392	3.74
400	0.385	3.72

The rate of color liberation was determined using a concentration of trypsin of 150 mg. per 25 ml. of a reconstituted sample of badly-browned nonfat dry milk solids. The amount of color extracted was determined at varying incubation

periods up to 5 hours with correction made for the slight decrease in volume resulting from evaporation. Results are plotted in figure 2, along with the corresponding non-protein nitrogen results.

With the enzyme concentration kept constant at 150 mg. per 25 ml. of milk, the color liberated and the non-protein nitrogen value both increase with the time of incubation, rapidly in the first hour and slower thereafter until a maximum and constant value appears to be reached after 3 hours. It is difficult to decide whether the color extracted at this point represents the total color or merely a portion of the total color which is extractable under these conditions. The fact that the precipitate from trichloroacetic acid is colored only slightly indicates that

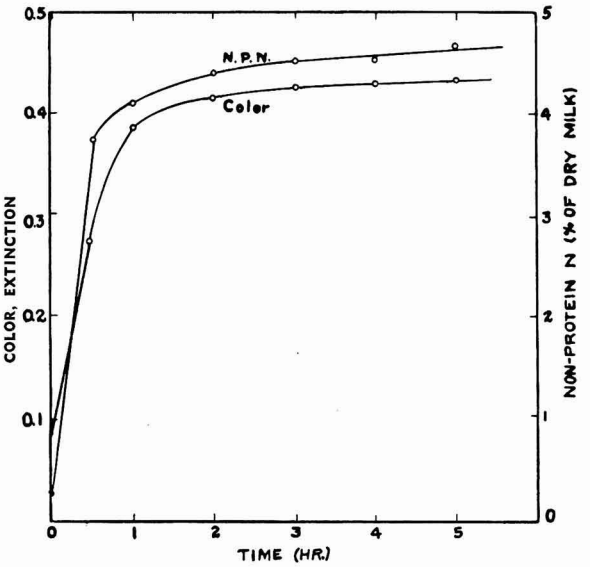

FIG. 2. Rates of color liberation and protein hydrolysis using 150 mg. trypsin per 25 ml. of reconstituted nonfat dry milk solids.

most, if not all, of the color has been extracted. As a 3-hour incubation period would be inconveniently long, a 1-hour period was adopted in the procedure with slight sacrifice in the completeness of color extraction. However, it must be pointed out that the sample used in this experiment is colored much more highly than the worst ones normally encountered, so that the difference in color between 3- and 1-hour incubation ordinarily would be inappreciable. The parallelism which exists between the amount of color extracted and the non-protein nitrogen developed emphasizes the importance of color adsorption by the protein molecules.

As Celite analytical filter-aid was used in the procedure to increase filtration rate and to prevent occasional turbidity, there is a possibility that this material may adsorb a certain part of the liberated color. If appreciable adsorption occurs, the color in the filtrate should decrease with increasing amounts of the filter-

aid used. No appreciable effect on the amount of color extracted was detected when as high as 0.3 g. of filter-aid per sample was used. The use of this material, while not necessary in the cases of nonfat dry milk solids and dry whole milk, is important in some dry whey solids and dry buttermilk solids in order to eliminate turbidity in the filtrate.

TABLE 2

Comparison of the enzyme procedure with the alkaline extraction procedure

Sample		Color extinction using:	
Type	No.	Alkaline extraction	Enzyme
Nonfat dry milk solids	1	0.055	0.125
	2	0.148	0.138
	3	0.060	0.136
	4	0.031	0.150
Dry whey solids	1	0.096	0.081
	2	0.031	0.185
	3	0.172	0.185
	4	0.090	0.130

A comparison of the enzyme procedure with the alkaline extraction procedure of Doob *et al.* (1) for several samples of dry whey solids and browned nonfat dry milk solids was made. The weight of the sample and the volume of liquid used in the enzyme procedure were adjusted to those employed in the alkaline extraction

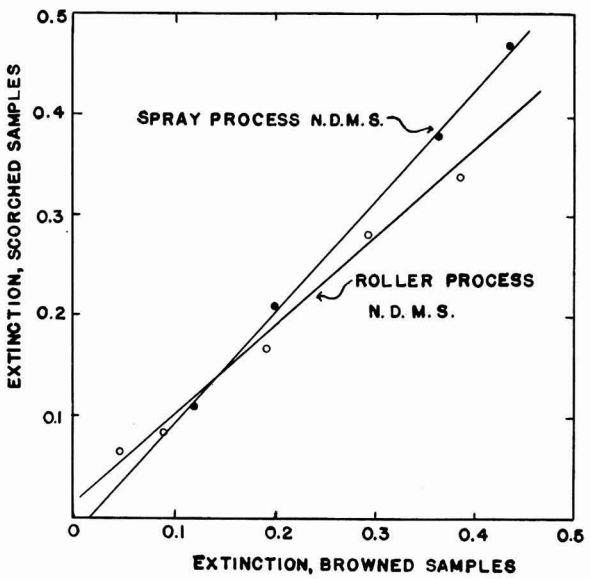

FIG. 3. Relationship between the extractable color of samples containing varying amounts of nonfat dry milk solids scorched at 119° C. and extractable color of visually matched samples similarly prepared from nonfat dry milk solids browned at 37° C. under the influence of high moisture content.

procedure. Data are presented in table 2. It should be noted that, while agreement between the two methods is fair in the case of dry whey solids, the enzyme method is distinctly more efficient in the extraction of color from nonfat dry milk solids. Since dry whey solids normally contain little or no casein, these results again point to the strong influence of casein on color extraction.

The brown discoloration in dry products of milk may arise from storage at ordinary temperature and high moisture content or from scorching in the drying operation. Results of the above experiments show that the present method is applicable to samples browned under storage. To determine whether the method can be applied equally well to products scorched during processing the following series of experiments was performed. A sample of spray process and a sample of roller process nonfat dry milk solids were used. A portion of each was allowed to attain a high moisture content in a humid atmosphere and then browned by storing at 37° C. Another portion of each was scorched in an

TABLE 3

Color of some samples of dry products of milk

Sample		Extinction	Color units
Type	No.		
			(*/g. solids*)
Dry whole milk	1	0.057	0.88
	2	0.054	0.76
	3	0.047	0.60
	4	0.042	0.48
Spray nonfat dry milk solids	1	0.068	1.28
	2	0.100	2.32
	3	0.110	2.56
	4	0.095	2.16
Roller nonfat dry milk solids	1	0.130	3.36
	2	0.100	2.32
	3	0.115	2.76
	4	0.130	3.36
Dry buttermilk solids	1	0.222	7.28
	2	0.390	14.28
	3	0.096	2.20
	4	0.142	4.68

air oven at 119° C. for 3 hours. For the scorched sample of each product a series of mixtures of varying color intensity and known composition were prepared by mixing different amounts of the scorched material with the original product. Another series of mixtures was prepared similarly from the browned product to match the color of each of the scorched mixtures. Matching was done visually. In figure 3 the extractable color of the scorched samples expressed as extinction is plotted against that of the corresponding matched browned sample. With both types of nonfat dry milk solids a straight line of approximately unit slope is obtained. This indicates that the present method works equally well for scorched products and for products browned at ordinary temperatures with high moisture contents. It may be mentioned that the color extinction was found to

be a linear function of the concentration of either the browned or the scorched nonfat dry milk solids in the mixture.

In table 3 the arbitrary color units of some random samples of dry products of milk are presented. Differences between the various products are in part due to differences in the concentration of the natural color materials.

The method is simple and precise and may be applicable to other dairy products.

SUMMARY

Since the chief difficulty associated with the extraction of color from browned dry products of milk is the strong adsorption of the color by the proteins, it has been found that most, if not all, of the color could be liberated by extensive hydrolysis of the milk proteins with trypsin. A simple and reproducible method has been developed which involves measuring photometrically the color of a trichloroacetic acid filtrate of the hydrolyzed mixture and expressing the color intensity in terms of an arbitrary color standard of iodine in potassium iodide solution. The method works equally well for samples experimentally browned under storage with high moisture contents and for samples experimentally scorched at high temperature.

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THE CAROTENE AND VITAMIN A AND PROXIMATE COMPOSITION OF PORTIONS OF THE FIRST MILKING POSTPARTUM[1]

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Under different systems of management, calves receive, immediately after birth, different portions of colostrum which may differ markedly in nutritive value. This was indicated directly by data obtained by Spielman *et al.* (16) on the first milking postpartum from three cows; successive increments increased markedly in the content of fat, carotene and vitamin A. Previously, several workers (7, 14, 18) had noted that in a small percentage of cows, the first milking postpartum contains less carotene and vitamin A than succeeding milkings. They have attributed this to incomplete "let-down" of milk at the first milking. During the lactation period, in general, the first milk drawn contains, under most conditions, appreciably less fat than that obtained at the end of the milking (1, 6), but the other nutrients are believed to remain constant during the milking process. Successive portions of human milk obtained during a single nursing period (10, 19) show a similar increase in fat and also a change in other constituents.

The objectives of this study were to determine the trends which occur in the carotene and vitamin A content and in the proximate constituents of successive 2-lb. increments of the colostrum from the first milking postpartum of dairy cows. Secondarily, these trends have been related to two different experimental systems of management and feeding.

EXPERIMENTAL

Animals. This experiment was conducted on 24 cows of the Ayrshire, Guernsey, Holstein and Jersey breeds in the University of Connecticut herd which calved from January to June, 1948. They represented four experimental groupings which were equalized so far as possible in respect to breed, age, number of previous lactations, length of dry period, health, ancestry and previous dietary history. Group 1-A received the basal ration and was milked postpartum, group 1-B received the basal ration plus 1 million U.S.P. units of vitamin A daily for 30 days prepartum and was milked postpartum, group 2-A received the basal ration and was milked prepartum and group 2-B received the basal ration plus 1 million U.S.P. units of vitamin A daily for 30 days prepartum and was milked prepartum.

For 8 weeks prior to the calculated parturition date, all cows received the same basal ration fed on the basis of liveweight. One lb. of U. S. no. 2 alfalfa

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hay, 3 lb. of well-matured corn silage and 1 lb. of a grain mixture consisting largely of cereal grains and containing approximately 13.5 per cent crude protein was fed per 100 lb. of liveweight. The hay, silage and grain contained on an average 3.87 mg., 1.06 mg. and 0.15 mg. of carotene per pound, respectively, as determined by the method of Moore and Ely (11) as modified by Nelson *et al.* (13). The vitamin A supplement was shark liver oil[2] containing 25 per cent by weight of crude soybean lecithin. This oil contained 54,440 U.S.P. units of vitamin A per g. as assayed spectrophotometrically against the U.S.P. vitamin A reference standard (vitamin A acetate in cottonseed oil). The cows in the prepartum-milked groups were milked twice daily, starting 10 days prior to the calculated date of parturition. They actually were milked for an average of 8.8 days with a standard error of $\pm$ 1.1 days. The newborn calves were not allowed to nurse but were removed immediately to a separate portion of the barn.

Samples. Most cows were milked immediately after calving and in all cases within 4 hours. The first eight cows were milked by hand and the others by machine, but all cows were hand stripped. Samples were taken of each successive 2 lb. of colostrum. All except the last sample represented a minimum of 2 lb. of colostrum, while the maximum amount did not exceed 2.2 lb. Colostrum samples were held at 4° C. pending analysis which, in most cases, was completed within 4 days after collection.

Analyses. The carotene and vitamin A content of the colostrum were determined by the method of Boyer *et al.* (4) using an Evelyn photo-electric macrocolorimeter. The colorimeter was standardized with crystalline carotene in petroleum ether (b. p. 30–60° C.) and with crystalline vitamin A alcohol in chloroform. Specific gravity, protein, fat and ash were determined by the methods of the Association of Official Agricultural Chemists (2). Lactose was determined by the micromethod of Hawk *et al.* (8) with the following modifications. To obtain the protein-free filtrate, 2 ml. each of 5 per cent $ZnSO_4$ and 0.3 *N* $Ba(OH)_2$ as used in Somogyi's blood sugar method (15), were added to 0.5 ml. of colostrum. One ml. of this filtrate plus 2 ml. of Somogyi's copper reagent then were placed in boiling water for 10 minutes. After cooling, 1 ml. of Nelson's arsenous molybdate reagent (12) was added and the volume made to 25 ml. with H_2O. The resulting solution then was read in the Evelyn photoelectric macrocolorimeter against a standard lactose solution which had been treated in the same manner as the protein-free filtrate. All determinations were made and are expressed on a volumetric basis.

The composition of a given nutrient in the successive 2 lb. increments usually followed a linear trend, either positive or negative. Therefore, the magnitude of this trend was determined by least squares to determine the characteristic slope for each nutrient in consecutive samples from each cow. An example of the calculation follows:

[2] This oil was supplied by Mr. Melvin Hochberg of the Nopco Chemical Company, Harrison, New Jersey.

Increment	Amount of colostrum (lb.)	Midpoint of milking interval in % (X)	$X-\overline{X}$ (x)	% Protein (y)	(xy)
1st	2	12.99	−38.5	20.35	−783.475
2nd	2	38.97	−12.5	19.08	−238.500
3rd	2	64.95	13.5	18.31	247.185
4th	1.7	88.98	37.5	17.48	655.500
Total (S)	7.7	205.89	0	.....	−119.290
Mean ($\overline{X}$)	.....	51.47	.....	.....	.....

$$\text{Slope} = b = \frac{S(xy)}{S(x^2)} = \frac{-119.290}{3227.00} = -0.036966$$

$$\text{Effect of Slope } (B^2) = \frac{S^2(xy)}{S(x^2)} = 4.4907 \text{ (in dimensions of } y^2)$$

Calculation of each slope (b) and its effect (B^2) upon the variation of y was determined as above. An analysis of variance was used to test the significance of the combined slope for all treatments and of differences in slope between treatments. Since standard methods of statistical analysis (2) were used, the detailed analysis of variance will not be given.

TABLE 1

Data on trend in constituents of colostrum during first milking postpartum for individual cows

Group	Cow no.	Total colostrum	Denominator for slope $S(x^2)$	Trend on successive increments (X) of milk or slope (b)						
				Specific gravity	Protein	Lactose	Fat	Ash	Carotene	Vitamin A
		(*lb.*)			(*g.* %)	(*g.* %)	(*g.* %)	(*g.* %)	(γ%)	(γ%)
1A	1[a]	20.5	7961.56	−0.1282	−0.022	−0.004	+0.11	−0.0012	+4.1	+ 4.6
	2	14.0	5708.58	−0.1351	−0.017	−0.005	+0.10	−0.0019	+6.5	+ 4.0
	3	7.7	3227.00	−0.2177	−0.037	+0.001	+0.06	−0.0012	+1.4	+ 4.8
	4	16.0	6582.52	−0.1056	+0.004	−0.011	+0.11	−0.0014	+1.1	+ 3.1
	5	16.0	6582.52	−0.1636	−0.002	−0.012	+0.15	−0.0026	+2.4	+ 9.1
	6	16.0	6582.52	−0.0837	−0.015	−0.005	+0.09	−0.0010	+6.3	+ 1.1
	7	16.0	6582.52	−0.1322	−0.009	−0.007	+0.16	−0.0014	+2.2	+ 6.0
1B	8	11.0	5388.16	−0.0502	+0.010	−0.007	+0.03	−0.0001	+1.1	+ 4.8
	9	27.5	11982.16	−0.1429	−0.022	−0.006	+0.11	−0.0018	+3.7	+24.4
	10[a]	22.0	9065.48	−0.1192	−0.017	−0.004	+0.11	−0.0011	+1.3	+10.0
	11	6.0	2217.78	+0.0601	−0.003	+0.003	+0.05	−0.0004	+1.0	+ 7.0
	12	10.0	4000.00	+0.0600	−0.004	+0.002	+0.03	+0.0005	+0.4	+ 3.3
	13	20.0	8250.00	−0.1436	−0.028	−0.006	+0.16	−0.0017	+2.9	+10.5
2A	14	18.0	7423.72	−0.1423	−0.005	−0.008	+0.09	−0.0011	+0.3	+ 0.8
	15	14.0	5708.58	−0.0825	+0.001	−0.003	+0.06	+0.0002	+1.0	+ 1.2
	16	10.5	3816.42	−0.0613	−0.002	−0.010	+0.05	−0.0007	+3.0	+ 1.5
	17[a]	15.4	6898.92	−0.1127	−0.004	−0.006	+0.11	−0.0009	+0.7	+ 1.1
	18	7.2	2046.98	−0.0339	−0.004	+0.004	+0.04	−0.0003	+1.2	+ 0.6
2B	19[a]	20.0	8250.00	−0.1000	−0.002	−0.008	+0.08	−0.0004	+0.6	+ 3.2
	20	13.2	4387.96	−0.0812	−0.000	−0.007	+0.12	−0.0002	+0.6	+ 4.2
	21	20.0	8250.00	−0.0588	+0.016	−0.008	+0.12	+0.0007	+2.8	+10.7
	22	9.8	4080.80	−0.0391	−0.007	0.000	+0.05	−0.0004	+1.6	+ 8.4
	23	13.0	4449.04	−0.0222	+0.003	+0.004	+0.04	+0.0006	+0.4	+ 2.3
	24	9.4	4259.94	−0.0127	−0.007	+0.004	+0.05	+0.0001	+0.3	+ 1.8

[a] Data of these cows plotted in figures 1 to 4.

RESULTS

Data on the trend in the proximate constituents and in carotene and vitamin A for each individual cow are given in table 1. The results from a representative cow from each of the four experimental groups are plotted in figures 1

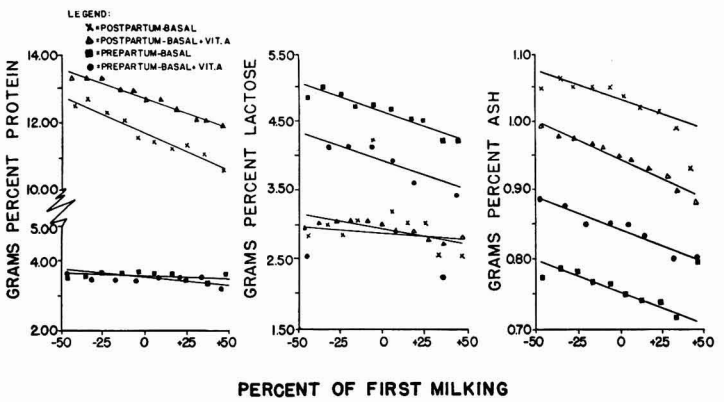

FIG. 1. The changes in the per cent protein, lactose and ash of successive 2-lb. increments of colostrum of the first milking postpartum for individual cows.

through 4 against per cent of total colostrum for each constituent which was determined. With the exception of γ vitamin A per g. of fat, all constituents showed significant trends ($P<0.01$) with successive 2-lb. increments of colostrum drawn.

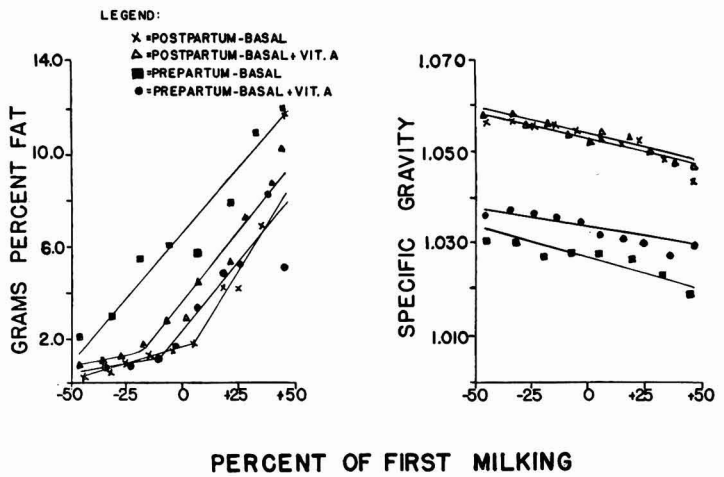

FIG. 2. The changes in the per cent fat and specific gravity of successive 2-lb. increments of colostrum of the first milking postpartum for individual cows.

The protein, lactose and ash (fig. 1) showed a highly significant negative trend ($P < 0.005$). For those cows milked only postpartum, the protein and ash decreased more rapidly ($P < 0.01$) than in cows milked prepartum. The addition of vitamin A had no significant effect on the trends. In terms of absolute amounts the trends in successive increments of colostrum are relatively small.

The fat content rose markedly (fig. 2) with successive increments of colostrum, while specific gravity decreased. Both trends were highly significant ($P < 0.001$). While the decrease in specific gravity was greater ($P < 0.05$) in those cows milked only postpartum as compared to those milked prepartum, the addition of vitamin A had no significant effect. In contrast to protein, lactose and ash, the quantitative changes in the fat content were relatively large.

Both the per cent carotene and vitamin A content (fig. 3) increased markedly

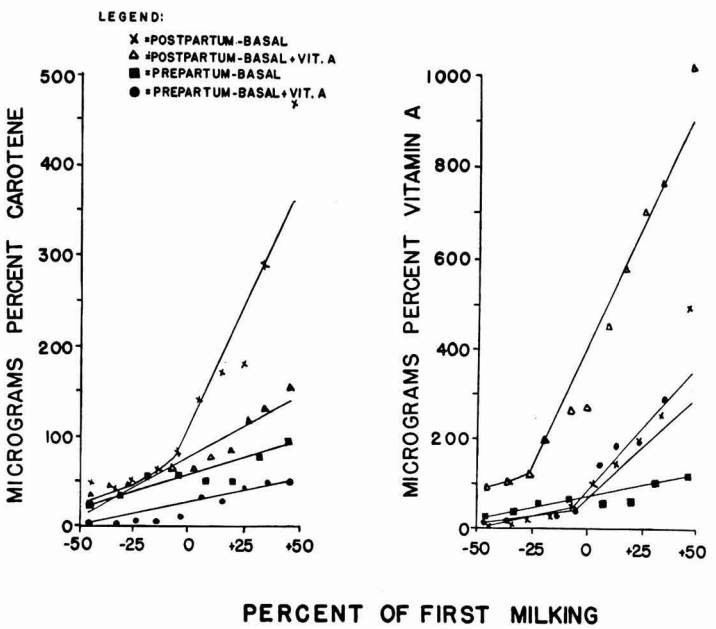

FIG. 3. The changes in the per cent carotene and vitamin A of successive 2-lb. increments of colostrum of the first milking postpartum for individual cows.

with successive increments of colostrum with highly significant trends ($P < 0.001$). Both carotene ($P < 0.025$) and vitamin A ($P < 0.05$) increased at a greater rate with successive increments of colostrum in the cows milked only postpartum than in those cows milked prepartum. The addition of supplementary vitamin A had no significant effect on the trend in per cent carotene. However, the vitamin A content in the colostrum from the cows receiving supple-

mentary vitamin A increased more rapidly ($P<0.05$ for postpartum group and $P<0.025$ for prepartum group) than in that from the cows receiving the basal ration alone. Quantitatively, the changes in both carotene and vitamin A were relatively large.

When the concentration of vitamin A was computed per gram of fat (fig. 4),

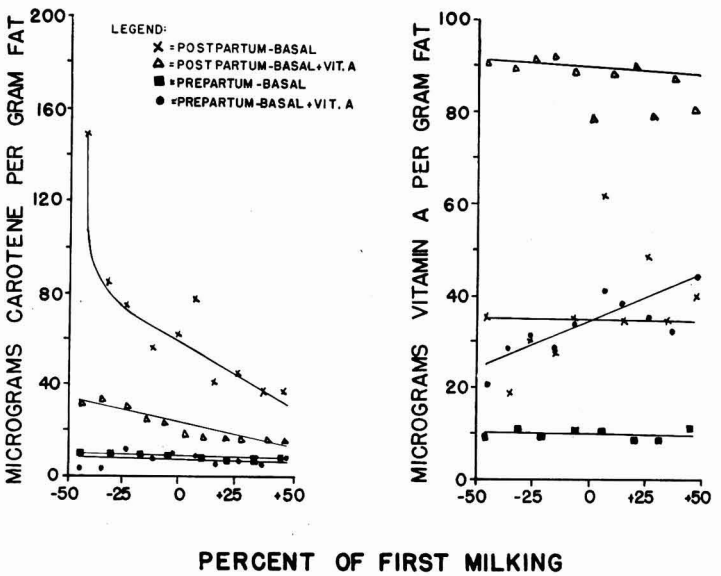

FIG. 4. The changes in the carotene and vitamin A content per g. of fat of successive 2-lb. increments of colostrum of the first milking postpartum for individual cows.

there were no significant trends with successive increments of colostrum, whereas the concentration of carotene per gram of fat showed a decreasing trend ($P< 0.01$) with successive increments. This trend was not affected significantly by either pre- or postpartum milking or by the addition of vitamin A to the basal ration.

DISCUSSION

These data indicate that definite trends do occur in the carotene and vitamin A content and in the proximate constituents in consecutive increments of the colostrum from the first milking postpartum. In cases of udder congestion at parturition, where complete milking may be indicated, milking the cow first and then allowing the calf to nurse would seem to insure optimum vitamin A intake to the calf.

The decrease in the per cent protein, lactose and ash of successive increments of the first milking postpartum is relatively small quantitatively. However, the variation in the various nitrogenous fractions making up the protein contents, especially the globulin fraction, would be of interest. The same also is true of individual minerals making up the per cent ash. Studies (10, 19) on samples of human milk representing various portions of a single nursing period have shown that protein tends to increase slightly, that intermediate portions tend to be higher in lactose than initial or final portions, and that ash tends to follow the same trends in the intermediate portions but is slightly lower at the end than at the beginning.

The increase in the per cent fat with successive increments of colostrum from the first milking postpartum is in agreement with the excellent summary by Espe (6) of similar studies later in lactation. A similar rise in the carotene and vitamin A content has been suggested by other workers (7, 14, 18) although no actual data were given.

The decreasing trend in the carotene content per gram of fat and the relative constancy of the vitamin A content per gram of fat are of interest. The decrease in both the carotene and vitamin A in the blood plasma at the time of parturition, as reported by Sutton *et al.* (17) and others, and the increase in the per cent carotene content of colostrum occurring in cows milked prepartum (17, 5) are suggestive of more active mammary metabolism of these substances. Possibly the levels of plasma carotene and vitamin A at the time of calving are independent of mammary influence but are affected by other factors possibly hormonal (20) in nature.

SUMMARY

The carotene and vitamin A content and the proximate constituents of successive 2-lb. increments of the first milking postpartum have been studied on 24 cows. These represented two managerial groups of which one was milked for an average of 8.8 days prepartum, while the other was milked only postpartum. Some of the cows in each of the above groups received a basal ration only and the others received the same basal ration plus 1 million U.S.P. units of vitamin A daily for 30 days prepartum. The following trends were observed:

1. Per cent protein, lactose and ash and specific gravity showed a significant negative trend with successive increments of colostrum. The per cent of protein and of ash and specific gravity decreased at a significantly greater rate in the cows milked postpartum than in those milked prepartum. Quantitatively the changes were relatively minor.

2. Per cent fat, carotene and vitamin A showed a significant positive trend with successive increments of colostrum. In the cows milked postpartum, the increase in per cent carotene and vitamin A was significantly greater than that found in cows milked prepartum. The addition of supplementary vitamin A had no effect on the trends for carotene but caused a more rapid rate of in-

crease in the percentage content of vitamin A than that observed in cows receiving the basal ration alone.

3. The carotene content per gram of fat showed a significant negative trend with successive increments of colostrum, whereas no significant trends were observed in the content of vitamin A per gram of fat.

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THE COMPARATIVE VALUE OF HIGH AND LOW FAT CONCENTRATES WITH ALFALFA HAY[1]

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In the alfalfa-producing areas where cows, to a great extent, are fed solely on alfalfa hay, milk production is only 50 to 85 per cent of that of cows fed alfalfa hay with grain supplements. The restricted production on alfalfa hay alone is due either to the absence of some nutritive element or elements in the alfalfa hay or to a lack of sufficient total digestible nutrients.

In an effort to determine what was responsible for this subnormal production of cows on alfalfa hay, Smith *et al.* (11) replaced a part of the alfalfa hay with equal amounts of total digestible nutrients in concentrates and noted that the greatest stimulus to milk and butterfat production was obtained when ground soybeans were fed. Since there was a greater stimulus from feeding equal total digestible nutrients in ground soybeans than from soybean meal, the question arose as to whether the stimulus resulted from protein supplementation or from the additional fat supplied.

Evidence of a conflicting nature has been presented in the literature on the effect of a ration high in dietary fat on milk and butterfat production of the dairy cow. Bratton *et al.* (1) found inclusion of raw soybeans in amounts equal to 25 per cent of the grain ration caused a measurable increase in the butterfat test of the milk produced. Williams *et al.* (13) found that cows on an all-soybean ration produced milk with a significantly higher percentage of butterfat than did cows on normal feed.

Loosli *et al.* (4), Maynard and McCay (5) and Maynard *et al.* (6, 7, 8) have conducted many experiments on the influence of dietary fat on milk and butterfat production. These workers concluded that the main effect of a ration high in fat is to increase the amount of milk rather than to influence the fat component of the milk. Monroe and Krauss (9), feeding grain mixtures varying from 2.69 to 4.89 per cent fat, found no differences in the production of milk, butterfat or 4 per cent fat-corrected milk. Gibson and Huffman (2) reported that when soybean oil was added to a basal ration low in fat, an increase in milk production resulted. They also reported a temporary increase in butterfat percentage.

The study reported in this paper was undertaken to investigate further the effect on milk and butterfat production of feeding a supplement high in dietary fat to cows receiving a basal ration of alfalfa hay.

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EXPERIMENTAL PROCEDURE

In this experiment four purebred Holstein cows were used. Two cows, 334 and 346, began the experiment on the high-fat ration and two cows, 341 and 348, on the low-fat ration. The cows were kept in stanchions throughout the experiment with the exception of about 2 hours each day when they were exercised in a cement corral. Special construction of mangers allowed for accurate measurements of feed intakes. Water was available in individual drinking bowls at all times. The cows had free access to bone meal, disodium phosphate and salt in boxes in the paved corral. The daily allowance of good quality alfalfa hay was weighed in canvas bags and fed in equal portions night and morning. Hay refused was weighed back. Ground soybeans or soybean meal was fed in equal portions twice daily. Daily feed intakes were recorded. The cows were weighed at approximately the same time each day.

The amount of alfalfa hay fed was regulated with the requirements of the animal for maintenance and production. Changes from ground soybeans to

TABLE 1

Analysis of feeds used in experiment

Feed	Ash	Moisture	Crude protein	Crude fiber	Ether extract	Nitrogen free extract	Digestible protein	Total digestible nutrients
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Alfalfa hay—1st yr. irrigated hay	7.70	7.79	14.71	30.63	2.44	36.66	10.50	51.11
Alfalfa hay—2nd yr. dry land hay	9.16	8.23	12.48	25.23	2.69	42.06	9.36	52.28
Alfalfa hay—2nd yr. irrigated hay	7.98	6.94	14.23	32.45	2.09	36.31	10.24	51.57
Soybeans	4.13	11.30	35.59	5.79	19.32	23.87	31.67	88.05
Soybean Oil Meal	5.23	13.48	40.38	5.94	4.17	30.80	34.32	76.59

soybean meal, or vice versa, were made abruptly. The amount of supplement varied with the requirements for maintenance and milk production. Total digestible nutrients consumed were greater than the requirements in every 6-week period except one.

The rations were calculated on the basis of average body weight and milk production of the 3 days prior to the beginning of each 6-week period. The mean value of the per cent fat in the dry matter in the high-fat ration was 5.2 per cent, as compared to 2.7 per cent of the low-fat ration.

The cows were milked twice daily by machine. The milk was weighed and aliquot samples were taken at each milking. The milk samples were preserved with corrosive sublimate and butterfat tests were made weekly, using the Babcock method.

The chemical analyses of the feeds used in the experiment are shown in table 1. The average digestion coefficients reported by Morrison (10) were used in calculating the total digestible nutrients and the digestible protein.

Inasmuch as the condition of the cow for a period following calving is known

to influence milk production and, more particularly, the butterfat percentage of the milk and all cows calved again within a year, the stage of lactation for which the data were used was never earlier than the 6th and never later than the 36th week of lactation.

RESULTS

The cows showed no digestive disturbances even though they were changed abruptly from one experimental ration to another. The palatability of the rations seemed to be of a high order, even though the cows ate as much as 10 lb. daily of soybeans or soybean meal. Average daily hay and concentrate consumption, total digestible nutrients required and consumed and average body weights of each individual cow are shown in table 2.

TABLE 2

Average daily hay and concentrate consumption, total digestible nutrients required and consumed and body weights for each cow by periods

Cow	Period	Hay	Concentrate	Total digestible nutrients		Body weight
				required	consumed	
		(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
334	1	40	8 SB[a]	27.5	27.5	1381
	2	40	9 SBM[b]	24.4	28.0	1391
	3	40	7 SB	22.9	27.1	1394
	4	40	6 SBM	19.9	23.1	1417
356	1	35	8 SB	25.2	25.3	1234
	2	35	8 SBM	23.5	24.7	1256
	3	35	7 SB	23.1	24.4	1270
	4	35	6 SB	19.5	23.3	1301
341	1	40	9 SBM	26.4	28.0	1341
	2	40	10 SB	25.0	29.4	1344
	3	36	8 SBM	22.4	25.1	1349
	4	36	4 SB	19.9	22.1	1375
348	1	35	8 SBM	26.2	24.4	1299
	2	35	7 SB	24.0	24.5	1268
	3	35	7 SBM	18.5	24.2	1287
	4	36	5 SB	17.2	23.2	1342

[a] SB = ground soybeans.
[b] SBM = soybean meal.

All four cows followed the expected curve with regard to body weight. In the early stages of their lactations each cow lost some weight, but by the second experimental period they all had begun to gain weight. As the stage of gestation advanced the experimental animals continued to gain weight normally. It may be said that the experimental ration of alfalfa hay with ground soybeans or soybean meal did not alter the expected normal gain in body weight.

The data for milk, 4 per cent fat-corrected milk, per cent butterfat and total butterfat production, as averaged by 6-week periods, are shown in table 3. For the purpose of comparing the two fat levels, the data are summarized by cows and by periods. The results are based on the fact that with three or more experimental periods of equal length, lying within a lactation where milk yield is falling at a constant rate, a comparison of the yields of a given period, with

the average of the yields of periods immediately preceding and succeeding the given period, is justified.

Figures 1 and 2 show the average daily milk production by weeks in relation to the average production of 99 lactations of Holstein cows in the Oregon State College herd (3). The average weekly butterfat test also is given for each cow.

Inasmuch as all the cows usually received more total digestible nutrients

TABLE 3

Daily average butterfat per cent, milk yield, butterfat yield and 4% fat-corrected milk yield of each cow by periods

Cow	Period	Av. daily milk yield	Av. butterfat	Av. daily butterfat yield	Av. daily 4% fat-corrected milk yield
		(*lb.*)	(%)	(*lb.*)	(lb.)
334	HF[a] I	58.7	3.35	1.955	52.9
	LF[b] II	56.5	2.76	1.567	45.4
	HF III	47.2	3.03	1.430	40.3
	LF IV	38.0	2.41	0.918	28.9
	Av. periods I and III	52.9	3.19	1.692	46.6
	Gain on HF	- 3.6	0.43	0.125	1.2
	Av. periods II and IV	47.2	2.58	1.242	37.1
	Gain on HF	0.0	0.45	0.188	3.2
356	HF I	55.2	3.48	1.920	50.9
	LF II	50.8	3.15	1.586	44.3
	HF III	44.4	3.60	1.599	41.7
	LF IV	41.3	3.20	1.326	36.4
	Av. periods I and III	49.8	3.54	1.759	46.3
	Gain on HF	- 1.0	0.39	0.173	2.0
	Av. periods II and IV	46.0	3.17	1.456	40.3
	Gain on HF	- 1.6	0.43	0.143	1.4
341	LF I	69.0	2.2	1.501	50.1
	HF II	61.2	2.6	1.591	48.3
	LF III	49.5	2.3	1.138	36.9
	HF IV	37.3	2.6	0.969	29.5
	Av. periods I and III	59.2	2.25	1.319	43.5
	Gain on HF	2.0	0.35	0.272	4.8
	Av. periods II and IV	49.2	2.6	1.270	38.9
	Gain on HF	- 0.3	0.3	0.132	2.0
348	LF I	55.4	3.6	1.969	51.7
	HF II	47.3	4.0	1.887	47.1
	LF III	43.0	2.9	1.277	36.3
	HF IV	31.0	3.0	0.940	26.5
	Av. periods I and III	49.2	3.25	1.623	44.0
	Gain on HF	- 1.9	0.75	0.264	3.1
	Av. periods II and IV	39.1	3.5	1.413	36.8
	Gain on HF	- 3.9	0.6	0.136	0.5

[a] HF = high fat.
[b] LF = low fat.

than they required, it may be said that any effect of the ration could not be the result of added or a lack of total digestible nutrients. In many periods the total nutrients consumed were far in excess of the required, calculated on the basis of body weight and production. The excess, however, occurred just as frequently on the low-fat as on the high-fat rations.

DISCUSSION

This study shows that good producing cows may produce at a high level on good quality alfalfa hay and supplements of the soybean plant. Production was maintained at the normal level when either ground soybeans or soybean meal was fed with alfalfa hay.

There was no significant difference in the efficiency of the ground soybeans or the soybean meal as supplements to the alfalfa hay. There are indications that the soybean meal was superior for milk production, but the differences were not significant when the Student's t-test is applied (12). Milk production on either the ground soybeans and alfalfa hay or soybean meal and alfalfa hay was significantly greater than milk production on alfalfa hay alone. The level of production was similar to the normal lactation of 99 Holstein cows in the Oregon State College herd (3).

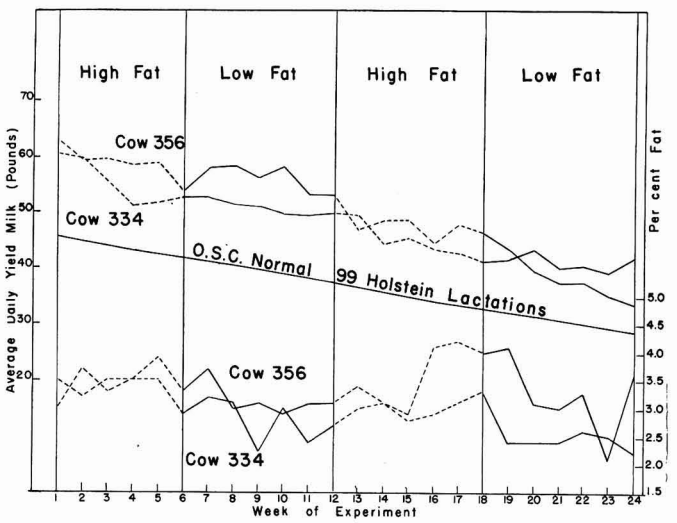

FIG. 1. Average daily milk and butterfat production by weeks for cows beginning experiment on high-fat ration.

The maximum effect of the high-fat ration was demonstrated with butterfat percentage. In every period on the ground soybeans and alfalfa hay there was an increase in the butterfat content of the milk. The percentage increase ranged from 3.4 to 27.5 per cent, which in a statistical analysis is shown to be highly significant. The Student's t-test (12) was used and all four cows showed a significant increase in per cent butterfat when fed the high-fat ration as compared to the butterfat percentage on the low-fat ration.

The influence of fat in the ration when ground soybeans were fed indicated a stimulus to 4 per cent fat-corrected milk and total butterfat production. The

results, however, could not be shown to be statistically significant. In a total of eight comparisons, soybean meal favored milk production in six. Ground soybeans favored per cent butterfat, pounds of butterfat produced and pounds of 4 per cent fat-corrected milk in all of the eight comparisons.

In this experiment considerable attention was given to the individual hay consumption of each of the cows. Loosli *et al.* (4) have presented evidence that increasing the intake of hay of each cow from a level of 1 lb. per 100 lb. of body weight to a level of 1.3 lb. of hay per 100 lb. of body weight reduced the effect of the high-fat ration from what it otherwise might have been at the lower level of hay intake. At Ohio, Monroe and Krauss (9) were unable to obtain significant differences in the comparisons they made where hay was fed *ad libitum.*

Controlled *ad libitum* feeding of U. S. No. 2 alfalfa hay was carried out in the study reported in this paper. As much as 2.5 lb. of hay per 100 lb. of body

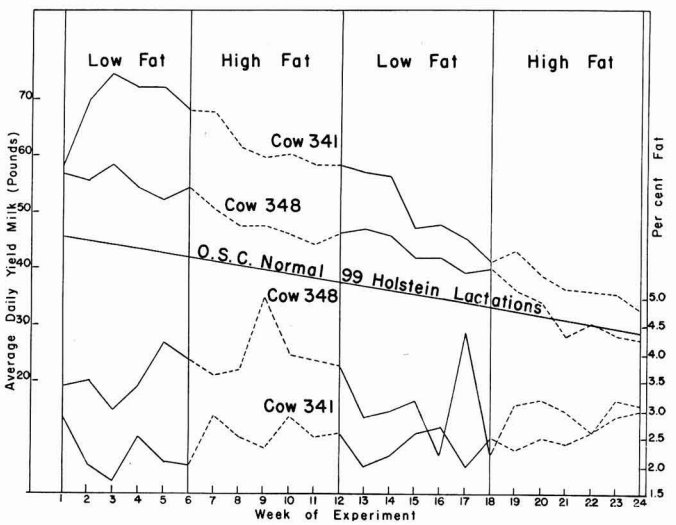

FIG. 2. Average daily milk and butterfat production by weeks for cows beginning experiment on low-fat ration.

weight was fed, although at times some hay was refused. However, the cows did eat well over 2 lb. of hay per 100 lb. of body weight throughout the greater part of the trial. The results for milk production, butterfat production and pounds of fat-corrected milk were not statistically significant for the trial as a whole, but in some instances the individual cows did show an advantage with a high-fat ration, which was significant. These results bear out the suggestion of the Cornell workers (4, 5, 6, 7, 8) that the effect of dietary fat is limited, to some extent, when the roughage consumption of the cows is at a higher level than 1 lb. of hay per 100 lb. of body weight. The results, however, do not corroborate the results of the Cornell workers who noted the greatest effect of

a high-fat ration on milk yield. Rather, this experiment indicates a slight absolute superiority of the low-fat ration for milk production. Fat-corrected milk, however, did show in every period an absolute advantage with a high-fat ration. However, the advantage was statistically significant in the case of only one cow.

In the case of the average daily production of butterfat, highly significant increases were noted in the case of one cow and significant increases in the case of a second cow. Two cows, however, did not show a significant increase, even though an absolute increase was demonstrated.

Excellent milk production for all cows was obtained in spite of a somewhat restricted ration. Some of the cows produced over 500 lb. of butterfat in 305 days and all produced well over 400 lb. It would seem, therefore, that if cows receive an ample supply of good quality alfalfa hay and a supplement containing 2 to 3 per cent dietary fat, milk production would not be significantly greater if a ration containing 4 to 5 per cent dietary fat was fed. The percentage of butterfat in the milk, however, could be expected to increase significantly with a higher per cent of fat in the ration.

SUMMARY

1. Feeding as much as 10 lb. per day of ground soybeans or soybean meal with alfalfa hay caused no digestive disturbances and the palatability of the ration was not reduced noticeably.

2. Alfalfa hay supplemented with ground soybeans or soybean meal allowed milk and butterfat production to be maintained significantly above that expected on alfalfa hay alone.

3. A ration of alfalfa hay and ground soybeans containing 5.2 per cent dietary fat did not increase milk production when compared to a ration of alfalfa hay and soybean meal containing 2.7 per cent dietary fat.

4. Significant increases in the per cent butterfat of the milk produced were obtained when the high-fat ration was fed.

5. An actual, but not significant, increase was noted in total butterfat and in pounds of 4 per cent fat-corrected milk produced.

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LIVABILITY AND FERTILITY OF BOVINE SPERMATOZOA IN DIFFERENT DILUENTS

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Considerable difference of opinion exists as to the relative merits of the available diluents for bovine semen used in artificial breeding. This is evidenced by the report of the first annual meeting of the National Association of Artificial Breeders (4) which indicates that phosphate-yolk, citrate-sulfa-yolk and Phillips' pabulum have been used with varying degrees of success.

Since the development in this country of the phosphate-yolk diluent by Phillips (5) and Phillips and Lardy (6) and the citrate-yolk formula by Salisbury *et al.* (10), the latter formula has been modified by reducing the sodium citrate dihydrate content of the buffer from 3.6 to 2.9 per cent (12) and including in it 0.6 per cent sulfanilamide (2, 9, 11). Also, Swanson (14) has reported 3 per cent sodium citrate to be satisfactory as a buffer when used with egg yolk.

A tablet form of the citrate-sulfanilamide buffer based upon Salisbury's recommendations has been prepared by the Ortho Research Foundation, Raritan, New Jersey. This same organization also has developed a ready-to-use liquid formula for bovine semen containing sodium citrate as a buffer, a sulfonamide, dextrose and other ingredients. A synthetic pabulum has been developed by Phillips and Spitzer (7) and is prepared and distributed commercially by the National Agricultural Supply Co., Fort Atkinson, Wisconsin.

With the exceptions of the early comparisons between phosphate-yolk and citrate-yolk and between citrate-yolk and citrate-sulfanilamide-yolk by Salisbury and Bratton (9), Salisbury (10) and Salisbury and Knodt (11) and the recent report of Hurst and LaMaster (1) in which phosphate-yolk, citrate-yolk and the Ortho liquid were compared, no simultaneous comparisons of the original formulae with their more recent modifications have been reported. Furthermore, the early comparisons between phosphate-yolk and citrate-yolk (10) and between phosphate-yolk and Phillips' pabulum (7) were based on extremely low dilution rates and small numbers of inseminations. The work of Salisbury (8) and Salisbury and Bratton (9) in establishing the practicability of high dilution rates with the citrate diluents has emphasized the need for re-evaluating the present day diluents at these higher rates.

In view of the lack of conclusive evidence of the relative merits of the diluents in use today in terms of spermatozoan livability and fertility at high dilution rates, the experiment reported herein was designed to compare, at dilution rates of 1:200 and using split ejaculates, the following diluents: Phillips' phosphate-yolk (6), Salisbury's citrate-yolk (10), Salisbury's citrate-sulfanilamide-yolk containing 3.6 and 2.9 per cent citrate (9, 11, 12), the Ortho Research Founda-

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tion's buffer tablets and its ready-to-use liquid formula for bovine semen. Phillips' pabulum (7) was not used in these comparisons because unsatisfactory spermatozoan livability was encountered in preliminary storage studies in this laboratory.

EXPERIMENTAL PROCEDURE

The simultaneous comparison of all six diluents was accomplished by use of a 6×6 latin square experimental design. Each semen sample was divided into six portions and each portion diluted at a rate of 1 : 200 in one of the six diluents. All inseminations were made by the regularly employed technicians affiliated with the New York Artificial Breeders' Cooperative, Inc. These technicians were divided at random into six groups. Each technician group received a different diluent during 5 consecutive days of each experimental week. The remaining 2 days of each week were utilized as a change-over period. In order that all technicians would have equal opportunities for using all six diluents, and at the same time minimizing discrimination between diluents when a particular cow was to be inseminated, the experiment was conducted for a period of 6 weeks. At the dilution rates employed, the semen requirements were met by using two bulls per day, a total of ten bulls per week. Since the same ten bulls were used each week, the 6×6 latin square design was replicated ten times. The actual sequence of diluents received by technician groups each week for a single replicate is shown in table 1.

TABLE 1

Basic design of the experiment for 6 weeks using bull 1 (6×6 latin square)

Ejac.	Diluents used by technician groups					
	I	II	III	IV	V	VI
1	PY[a]	2.9 CSAY	3.6 CSAY	OTY	OL	3.6 CY
2	3.6 CSAY[b]	OL	OTY	PY	3.6 CY	2.9 CSAY
3	OL	OTY	3.6 CY	2.9 CSAY	PY	3.6 CSAY
4	3.6 CY[b]	PY	2.9 CSAY	OL	3.6 CSAY	OTY
5	OTY	3.6 CY	PY	3.6 CSAY	2.9 CSAY	OL
6	2.9 CSAY[b]	3.6 CSAY	OL	3.6 CY	OTY	PY

[a] P = phosphate; C = citrate; SA = sulfanilamide; Y = egg yolk; OT = Ortho tablets; OL = Ortho liquid (used directly without egg yolk).
[b] 3.6 and 2.9 indicate percentage concentration of citrate.

The semen used was obtained from Holstein bulls in the active stud of the New York Artificial Breeders' Cooperative, Inc. Semen was collected by means of the artificial vagina and examined for quality according to the routine procedures of this laboratory. Only those semen samples which contained 70 per cent or more motile spermatozoa, 900×10^6 spermatozoa per ml. of fresh semen, and reduced methylene blue in less than 9 minutes were shipped for use in insemination.

The composition of the buffers and diluents used, and the average pH of each, are shown in table 2. All diluents were prepared fresh during the afternoon preceding the morning they were to be shipped.

A 3-ml. portion of each diluted semen sample was stored at 5° C. and

examined daily for the per cent of motile spermatozoa and their relative rate of progressive movement until all the spermatozoa in the sample were dead. These examinations were used to compare the several diluents on the basis of the livability of the spermatozoa in storage.

The fertility of the diluted semen samples was determined from the 60- to 90-day non-returns to first service cows and expressed as the per cent non-

TABLE 2

Composition and pH of each buffer and diluent

	PY[a]	3.6[b] CY	3.6 CSAY	2.9 CSAY	OTY	OL
KH_2PO_4 (*g.*)	2.0	.....	.....	.....	50	.....
$Na_2HPO_4 \cdot 12H_2O$ (*g.*)	20.0	.....	.....	.....	tablets	.....
$Na_3C_6H_5O_7 \cdot 2H_2O$ (*g.*)	.....	36.0	36.0	29.0		.....
Sulfanilamide (*g.*)	.....	.....	6.0	6.0		.....
Water (redistilled over glass) to final vol. (*ml.*)	1000	1000	1000	1000	1000	.....
pH of buffer	7.36	7.41	7.36	7.41	7.20	.....
Ratio of egg yolk to buffer	1:1	1:1	1:1	1:1	1:1	.....
pH of diluent	6.76	6.70	6.72	6.73	6.67	6.52

[a, b] See footnotes for table 1.

returns. The per cent non-returns for each ejaculate × technician group × diluent subclass (table 1) was considered as the experimental unit. These observations were subjected to the analysis of variance (3, 13) for determining statistical significance between the means for the diluents.

RESULTS

In table 3 are shown the percentages of motile spermatozoa at intervals during the first 6 days of storage at 5° C. Reliable estimates of the per cent of

TABLE 3

Livability of spermatozoa in the different diluents during storage at 5° C.

Duration of storage (hr.)	*% motile spermatozoa in different diluents*					
	PY[a]	3.6[b] CY	3.6 CSAY	2.9 CSAY	OTY	OL
3	.....[c]	58	59	58	57	50
24	.....	50	50	51	49	41
48	.....	40	43	46	42	30
72	.....	30	37	41	36	22
96	.....	26	29	31	28	13
144	.....	17	20	24	20	6

[a, b] See footnotes for table 1.
[c] Reliable estimates not possible.

motile spermatozoa in the phosphate-yolk diluent were not possible, but in general spermatozoan livability appeared to be as satisfactory as in the other diluents. From the practical standpoint 3.6 CY, 3.6 CSAY, 2.9 CSAY and OTY were about equal for maintaining the motility of spermatozoa. The per cent

of motile spermatozoa was slightly lower in the OL diluent. However, the immotile spermatozoa were more discernible in the OL than in the yolk diluents and as a consequence the per cent of motile spermatozoa may have been estimated more reliably.

The number of first-service cows inseminated and the mean per cent 60- to 90-day non-returns for these same cows are shown in table 4. From the statistical analyses the mean percentages for non-returns for the 3.6 CSAY, 2.9 CSAY, OT and OL diluents were significantly higher (1 per cent level of probability) than those for the PY and the 3.6 CY diluents. Within the former group the differences between means were not statistically significant.

TABLE 4

Fertility level of semen in different diluents
(based on 60- to 90-day non-returns to 1st service cows)

	Diluents					
	PY[a]	3.6[b] CY	3.6 CSAY	2.9 CSAY	OTY	OL
Total number of 1st services	1945	1847	1843	1924	1843	1810
60- to 90-day non-returns (*mean %*)	50.5	50.5	55.3	56.5	56.4	55.0

[a, b] See footnotes for table 1.

It is of particular interest that all those diluents containing sulfonamides were accompanied by non-return rates that were significantly higher than those containing no sulfonamides. The average increase in non-return rate was approximately 5 percentage units, a value similar to that reported by Salisbury and Knodt (11) when citrate-yolk was compared with citrate-sulfanilamide-yolk.

SUMMARY

By means of a 6×6 latin square design, six bovine semen diluents were compared. Sixty semen samples from ten Holstein bulls were subdivided into six portions and each portion diluted at a rate of 1 : 200 with one of the six diluents.

Based on the per cent 60- to 90-day non-returns to approximately 1850 first service cows per diluent, the mean fertility level for each diluent was as follows: phosphate-yolk, 50.5; 3.6 citrate-yolk, 50.5; 3.6 citrate-sulfanilamide-yolk, 55.3; 2.9 citrate-sulfanilamide-yolk, 56.5; Ortho tablet-yolk, 56.4; and Ortho liquid, 55.0.

The average non-returns for the sulfonamide-containing diluents (3.6 citrate-sulfanilamide-yolk, 2.9 citrate-sulfanilamide-yolk, Ortho tablet-yolk and Ortho liquid) was 5 percentage units higher than for those diluents not containing sulfonamides (3.6 citrate-yolk and phosphate-yolk). This difference was significant at the 1 per cent level of probability.

Livability of spermatozoa during storage at 5° C. was satisfactory for all six diluents.

ACKNOWLEDGEMENTS

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FILLED MILKS FOR DAIRY CALVES. II. COMPARATIVE EFFECTS OF VARIOUS TYPES OF SOYBEAN OILS AND OF BUTTER OIL ON HEALTH, GROWTH AND CERTAIN BLOOD CONSTITUENTS[1]

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Although the economic need for the substitution of relatively inexpensive oils and fats for milk fat in the diet of young calves has been recognized, vegetable oils generally have proved unsatisfactory for this purpose (5). Recent evidence (7) indicates that responses of calves fed a filled milk containing crude soybean oil are characterized by unthriftiness, excessive scouring and high mortality, but reactions of calves receiving hydrogenated soybean oil compare favorably with those of subjects fed milk fat. Since several distinct processing stages are involved in the preparation of hydrogenated soybean oil from the crude product, the relation of the accompanying alterations of the oil to its nutritional value warranted investigation.

The present study was designed to determine the effects of feeding various types of soybean oils to young calves on health and on the levels of certain constituents of the blood.

EXPERIMENTAL PROCEDURE

Twenty young calves, sixteen Holsteins and four Guernseys, were divided into four comparable groups of five animals each. From birth until 4 days of age all calves received mammary secretions from their respective dams. During the subsequent experimental period of 56 days, each calf was restricted to a diet of reconstituted milk supplemented with vitamins A and D and a mineral mixture. The daily dosage of vitamin A was 10,000 I.U. per 100 lb. body weight, whereas that of vitamin D was 1,000 I.U. per calf irrespective of size. A complex mineral mixture employed in a previous experiment (7) was given daily at the rate of 7 g. per 100 lb. body weight to eliminate possible complications from mineral deficiencies.

The distinguishing characteristic of the diets of the respective groups was the kind of oil or fat incorporated in the reconstituted milk. This product consisted of 10 per cent non-fat dry milk solids and 3 per cent oil or fat, dispersed in water by homogenization at 3,000 lb. pressure. On the basis of the source and the type of fat used, the dietary groups were as follows: Group I—milk fat, butter oil; Group II—soybean oil, crude; Group III—soybean oil, refined and bleached; Group IV—soybean oil, hydrogenated and deodorized. The butter oil was prepared by rendering high quality butter. The three soybean oils, representing major sequential stages in standard commercial processing, were from the same batch of oil.[2]

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[2] Supplied by Swift and Co., Chicago, Illinois.

The reconstituted milks, prepared immediately prior to each of the two daily feeding periods, were fed from nipple pails. Adjustments in feeding rates, based on weight changes of calves, were made at weekly intervals. The amount offered to normal calves daily was 1 lb. per 10 lb. body weight. Whenever evidences of digestive disturbances were detected, the amount fed was reduced by 50 per cent. This level was maintained until the health of the calf improved, after which the intake was increased gradually to the standard level.

Several special managemental and prophylactic measures were taken to minimize the number of possible complicating factors. All calves, housed in individual pens bedded with shavings, were muzzled to prevent excessive consumption of shavings and other extraneous materials. In view of the apparent susceptibility of young calves to gastro-enteric infections, "sulfathalidine"[3] was administered at the rate of 6 g. daily per 100 lb. body weight during the first week of the experimental period (12).

Appraisal of the responses of the calves to the experimental treatments involved recorded observations of health, milk intake, weight changes, hemoglobin content of the blood and vitamin A, carotenoid and fat concentrations in the blood plasma. The calves were examined twice daily to detect any abnormalities, particularly diarrhea. The quantities of milk fed and refused at each feeding were measured accurately. At the beginning of the experimental period and at weekly intervals thereafter, calves were weighed and samples of venous blood were collected. The time of collection was 8 to 10 hours after the morning feeding and 20 to 22 hours after administration of vitamins. From each sample of blood, oxalated to prevent coagulation, 0.04 ml. was removed for hemoglobin determinations (9). The remainder was centrifuged within 24 hours to obtain plasma for further analyses. Vitamin A and carotene were determined by procedures described by Squibb *et al.* (13) and the fat by the method reported by Allen (1).

EXPERIMENTAL RESULTS

Health. In view of previous reports of high death losses of calves fed certain vegetable oils (5, 7) the mortality was surprisingly low; only one calf (group II) died during the course of the investigation. Since this animal survived to within 4 days of the termination of the trial, the missing items were supplied by computation (11).

Even though mortality was low, morbidity was high, particularly in groups receiving the crude and the refined soybean oils. The incidence of scours (table 1) was high in all groups during the first week the calves were on the experimental rations. Subsequently, the frequency of scours, though somewhat reduced, was erratic. During the entire trial, the incidence and the severity of the diarrhea were least in the calves fed butter oil, followed in order by the groups receiving, respectively, hydrogenated, refined and crude soybean oils. The differences in the dietary effects were manifested not only in the incidence of scours but also in the accompanying unthriftiness of the calves. Those in the crude and the refined soybean oil groups were thinner and more lethargic than the subjects in the

[3] Provided by Sharp and Dohme, Glenolden, Pa.

other two groups. Differences in the health of animals receiving the butter oil and the hydrogenated soybean oil were not marked, but the degree of improvement in appearance as the feeding trial progressed was greater in the former group than in the latter.

Weight changes. Trends of the mean body weights for each group of calves, as shown in fig. 1, revealed approximately equal gains in calves fed reconstituted milks containing, respectively, butter oil (group I) and hydrogenated soybean oil, (group IV). A similar relationship was observed in calves receiving crude soy-

TABLE 1

Effect of type of oil in reconstituted milk diets of young calves on incidence of scouring

Dietary group	Calf. no.	Periodic (wk.) incidence of scouring[a]								
		1	2	3	4	5	6	7	8	8-wk. period
						(%)				
I (Butter oil)	3043	.....	.....	.....	7.1	.....	.....	7.1	7.1	2.7
	3053	.....	.....	.....	.....	.....	.....	.....	.....	.....
	3056	.....	.....	.....	.....	.....	.....	.....	.....	.....
	3071	14.3	.....	.....	21.4	.....	.....	.....	.....	4 4
	3074	14.3	21.4	.....	.....	.....	.....	.....	.....	4.4
	Av.	5.7	4.3	0	5.7	0	0	1.4	1.4	2.3
II (Crude soybean oil)	3044	.....	.....	.....	.....	7.1	.....	7.1	7.1	3.5
	3052	21.4	35.7	14.3	7.1	.....	.....	.....	28.5	13.2
	3058	28.5	21.4	7.1	14.3	.....	14.3	7.1	21.4	14.2
	3062[b]	21.4	.....	7.1	.....	.....	.....	.....	14.3	5.7
	3067	21.4	21.4	7.1	42.8	21.4	28.5	14.3	.....	19.5
	Av.	18.5	15.7	7.1	12.8	5.7	8.6	5.7	14.3	11.2
III (Refined soybean oil)	3041	14.3	.....	.....	.....	.....	7.1	7.1	.....	3.5
	3047	21.4	21.4	7.1	14.3	21.4	7.1	14.3	28.5	16.8
	3051	.....	.....	.....	.....	14.3	21.4	7.1	21.4	9.7
	3061	50.0	.....	.....	.....	14.3	7.1	14.3	7.1	11.5
	3075	.....	21.4	14.3	7.1	.....	14.3	35.0	14.3	13.3
	Av.	17.1	8.5	4.3	4.3	10.0	11.4	15.6	14.3	11.0
IV (Hydrogenated soybean oil)	3039	21.4	.....	35.0	7.1	.....	.....	.....	7.1	8.0
	3040	21.4	.....	.....	14.3	21.4	.....	.....	.....	6.2
	3048	28.5	14.3	.....	14.3	.....	7.1	7.1	.....	8.9
	3063	21.4	7.1	.....	.....	.....	.....	.....	.....	3.5
	3072	.....	.....	.....	.....	.....	.....	.....	.....	.....
	Av.	18.5	4.3	7.0	7.1	4.3	1.4	1.4	1.4	5.3

[a] Number of times scouring observed for the specific period ÷ number observations × 100.
[b] Died 4 d. prior to termination of experimental period.

bean oil-filled milk (group II) and those fed refined soybean oil-filled milk (group III). Even though weight gains for groups I and IV were greater than those for groups II and III, the differences were not significant statistically. This lack of significance is ascribable, in part, to the marked intra-group variations. The ranges of weight changes, in pounds, for the respective groups were: group I, 18 to 37; group II, −9 to 28; group III, −2 to 34 and group IV, 11 to 49.

Ratios of milk ingested to weight gains. The ratios were erratic, inasmuch as the quantities of milk consumed and the body weight changes within the different groups of calves were highly variable. However, the ratios of milk consumed to weight increases (table 2) for the individual groups revealed, on one extreme, a marked similarity between the groups of calves receiving the butter oil

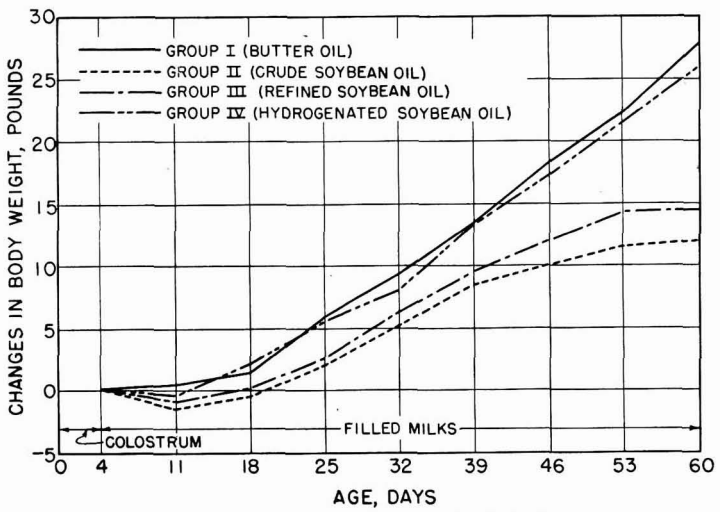

FIG. 1. Effect of type of oil in the reconstituted milk diet of calves on the changes in body weights.

and the hydrogenated soybean oil and, on the other, a close resemblance between the groups of animals given crude and refined soybean oils. The range of ratios, from 1 : 16.1 to 1 : 31.2, is striking.

Blood constituents. a. Hemoglobin of blood. The hemoglobin values presented in table 3 reveal marked variability among individuals but similarity in

TABLE 2

Effect of type of oil in the milk fed to calves on the ratio of quantity ingested to the gain in weight

Group	Oil in diet	Av. milk consumed	Av. wt. gains	Milk/lb. gain
		(*lb.*)	(*lb.*)	(*lb.*)
I	Butter	450.7	28.0	16.1
II	Crude soybean	405.2	13.0	31.2
III	Refined soybean	449.0	14.6	30.8
IV	Hydrogenated soybean	468.8	26.0	18.0

means for the groups. Since only one calf, 3039 of group IV, had hemoglobin levels sufficiently low to be considered in the anemic range, a deficiency of hematopoietic constituents apparently did not contribute to differences in the general health of calves in the various dietary groups. There was, however, in all groups

a slight decline in concentrations from the early stages of the trial to approximately the sixth week.

b. Vitamin A and carotenoids of blood plasma. The data relating to these constituents are shown in tables 4 and 5. If 10 γ of vitamin A per 100 ml. of blood plasma be accepted as the lower level of the normal range of concentrations (3), there were deficient calves in each group (table 4). In accordance with expectations, the extent of the deficiency was least evident in the calves receiving butter oil, inasmuch as this fat contained 5.50 γ of vitamin A and 4.42 γ of caro-

TABLE 3

Concentration of hemoglobin in the blood of calves receiving different oils in the reconstituted milk diets

Dietary group	Calf no.	Concentrations of hemoglobin at 7-d. intervals								
		4	11	18	25	32	39	46	53	60
		(*g./100 ml.*)								
I (Butter oil)	3043	.....	.....	.....	.....	9.8	7.9	9.5	8.8	8.6
	3053	11.3	13.9	13.3	12.6	10.2	11.6	8.6	10.3	9.7
	3056	12.5	11.5	10.5	10.3	10.0	9.5	9.3	8.8	9.5
	3071	16.9	14.2	11.6	11.6	12.1	11.5	12.7	12.1	13.1
	3074	15.0	14.9	13.0	13.2	13.0	11.9	10.8	11.3	11.4
	Av.	13.9	13.6	12.1	11.9	11.3[a]	11.1[a]	10.3[a]	10.6[a]	10.9[a]
II (Crude soybean oil)	3044	.....	.....	.....	9.7	9.7	13.3	12.4	11.3	11.6
	3052	13.7	19.3	15.1	15.0	12.5	12.4	9.9	9.6	9.9
	3058	15.0	14.7	13.9	13.1	11.8	12.9	10.4	11.0	11.3
	3062	13.7	13.3	12.4	10.9	9.9	10.4	11.3	11.6	11.6
	3067	14.5	11.9	13.2	12.3	11.8	10.9	11.0	11.1	9.7
	Av.	14.2	14.8	13.7	12.8[a]	11.5[a]	11.7[a]	10.7[a]	10.8[a]	10.6[a]
III (Refined soybean oil)	3041	.....	.....	.....	.....	12.5	10.2	10.9	11.3	10.6
	3047	15.0	15.0	11.1	14.7	11.6	11.5	11.1	11.6	14.5
	3051	11.9	14.3	15.0	13.1	12.0	12.7	11.1	11.0	10.5
	3061	16.9	16.3	15.5	14.8	12.4	10.7	11.1	11.0	10.1
	3075	13.4	13.3	12.5	12.1	13.4	11.6	9.4	9.7	8.5
	Av.	14.3	14.7	13.5	13.7	12.3[a]	11.6[a]	10.7[a]	10.8[a]	10.9[a]
IV (Hydrogenated soybean oil)	3039	.....	.....	.....	.....	.....	7.9	6.6	9.1	7.1
	3040	.....	.....	.....	.....	.....	8.9	10.0	12.3	10.3
	3048	15.0	18.1	11.2	12.6	12.5	10.9	11.9	11.5	11.6
	3063	13.9	14.5	13.9	12.7	11.2	10.3	11.8	9.6	13.5
	3072	12.0	11.9	10.3	9.8	11.1	9.1	8.8	7.7	9.1
	Av.	13.6	14.8	11.8	11.7	11.6	10.1[a]	10.8[a]	9.6[a]	11.4[a]

[a] Only calves on which hemoglobin measurements were made throughout included in the av.

tene per g., whereas the vegetable oils probably were almost devoid of vitamin A activity. Among the calves fed soybean oils, the vitamin A values were lower in the subjects fed the milks containing the crude and the refined oils than in those receiving the hydrogenated oil. These inter-group differences, however, were not statistically significant.

Throughout the trial, the carotenoid concentrations in the blood plasma also were extremely variable among individual calves (table 5). The highest values

were in plasma from Guernsey calf 3058, receiving crude soybean oil. Mean levels of the carotenoids for the groups of calves fed butter oil and crude soybean oil were similar and higher than for the other groups. The lowest values were observed in group IV (hydrogenated soybean oil). Though the differences between the highest and the lowest group means are great they are not significant statistically, inasmuch as the number of animals in each group was small and the variability great.

TABLE 4

Effect of type of oil in reconstituted milk diets of young calves on concentrations of vitamin A in blood plasma

Dietary group	Calf no.	Vitamin A levels at 7-d. intervals								
		4	11	18	25	32	39	46	53	60
		(γ/*100 ml.*)								
I (Butter oil)	3043	13.0	27.4	23.2	17.4	16.0	16.0	14.5	17.0	15.8
	3053	18.2	14.7	12.7	16.3	11.5	16.7	15.5	15.7	9.5
	3056	16.7	15.0	18.0	18.3	20.1	16.7	10.8	10.7	10.7
	3071	13.6	10.3	14.7	8.1	5.5	7.5	9.6	15.9	15.3
	3074	25.0	28.0	21.7	7.9	10.2	12.4	13.4	13.9	15.5
	Av.	17.3	19.1	18.1	13.6	12.7	13.9	12.8	14.6	13.4
II (Crude soybean oil)	3044	19.9	14.1	12.2	6.5	10.9	7.9	11.1	8.6	8.1
	3052	20.4	13.1	8.9	13.0	10.3	11.4	18.0	11.1	8.8
	3058	11.5	8.0	12.5	13.3	12.2	8.4	6.5	2.0	5.8
	3062	20.0	18.7	10.8	10.0	6.2	2.3	1.9	3.4	4.9[a]
	3067	15.6	11.9	13.8	6.4	4.7	11.7	8.8	5.0	2.8
	Av.	17.5	13.2	11.6	9.8	8.8	8.3	9.3	6.0	6.1
III (Refined soybean oil)	3041	21.1	17.4	10.2	6.8	7.7	9.9	6.0	11.1	13.1
	3047	16.6	6.7	9.5	6.8	7.7	8.0	5.3	8.8	10.0
	3051	15.7	13.0	11.7	10.6	9.6	10.3	9.6	6.5	3.7
	3061	19.3	14.0	25.1	9.3	5.3	3.4	7.6	6.1	6.0
	3075	24.5	14.0	5.2	10.9	6.4	7.1	10.8	7.0	3.8
	Av.	19.4	13.0	12.3	8.9	7.3	7.7	7.9	7.9	7.3
IV (Hydrogenated soybean oil)	3039	22.0	10.1	16.7	14.5	7.5	14.2	12.4	9.4	11.2
	3040	13.3	19.7	13.7	11.2	9.6	12.9	13.8	13.8	8.3
	3048	12.5	5.8	8.6	9.6	9.4	9.5	11.2	8.7	12.3
	3063	22.2	23.3	15.1	15.2	9.2	6.6	2.2	8.8	5.3
	3072	17.1	18.6	15.8	8.5	6.7	12.3	7.7	9.9	6.8
	Av.	17.4	15.5	14.0	11.8	8.5	11.1	9.5	10.1	8.8

[a] Calf died. Item supplied by missing data formula.

c. *Fat concentrations in blood plasma.* Blood plasma fat levels (table 6) decreased in all groups during the first week after changing from colostral secretions to the respective reconstituted milks. Subsequently, the trends were upward, attaining the maximum the third week. The changes thereafter were irregular, particularly in the plasma from calves receiving the soybean oils. The plasma fat content was significantly higher in the groups of calves fed the crude and the refined soybean oils than in those receiving the other oils. During the latter half of the trial, plasma fat levels for the calves receiving butter oil were

over twice as high as those for calves receiving the hydrogenated oil, a difference which was highly significant statistically.

DISCUSSION

In accordance with previous observations (7), the health of calves fed filled milk containing hydrogenated soybean oil was markedly similar to that of calves given a reconstituted milk containing butter oil. In contrast to the relatively good physical condition of animals in these groups, diarrhea, low weight gains and

TABLE 5

Effect of type of oil in reconstituted milk diets of young calves on concentrations of carotenoids in blood plasma

Dietary group	Calf no.	Carotenoids levels at 7-d. intervals								
		4	11	18	25	32	39	46	53	60
		(γ/*100 ml.*)								
I (Butter oil)	3043	21.3	9.8	20.2	19.4	6.8	6.2	9.8	9.8	8.5
	3053	7.9	10.7	14.3	11.7	21.1	23.4	16.8	17.2	14.3
	3056	28.8	13.2	13.6	27.5	21.7	11.5	12.2	7.7	9.8
	3071	3.4	4.0	2.8	5.8	10.2	9.4	9.6	17.1	22.2
	3074	13.2	1.9	3.4	7.7	9.4	12.4	8.1	12.3	15.1
	Av.	14.9	7.9	10.9	14.4	13.8	12.6	11.3	12.8	14.0
II (Crude soybean oil)	3044	18.5	26.2	21.9	13.2	12.3	15.5	16.2	15.8	15.3
	3052	12.2	11.5	5.5	7.0	11.9	11.7	10.7	9.8	3.0
	3058	25.3	13.4	30.3	43.0	41.3	43.7	24.3	23.7	23.7
	3062	12.1	6.8	9.8	10.4	6.4	4.0	2.5	2.6	2.1[a]
	3067	11.3	11.5	10.9	7.7	8.5	9.4	10.7	5.0	6.4
	Av.	15.9	13.9	15.7	16.3	16.1	16.9	12.9	11.4	10.1
III (Refined soybean oil)	3041	19.4	19.4	14.5	9.8	6.2	6.4	5.8	5.5	8.5
	3047	43.0	8.5	11.9	12.6	13.0	10.2	5.7	9.2	7.5
	3051	12.1	10.0	12.3	8.7	13.0	9.8	11.5	6.6	3.4
	3061	22.6	7.2	13.8	8.9	4.3	2.6	0.9	2.5	2.3
	3075	13.4	7.5	4.9	5.5	3.4	7.1	2.5	2.1	0.4
	Av.	22.1	10.5	11.5	9.1	8.0	7.2	5.3	5.2	4.4
IV (Hydrogenated soybean oil)	3039	95.7	23.0	25.2	19.2	9.2	3.6	4.5	3.8	2.6
	3040	20.0	10.9	8.7	5.1	4.2	1.7	2.8	5.3	3.2
	3048	6.6	3.6	1.0	0.8	1.0	3.4	1.5	2.1	5.1
	3063	19.6	7.2	6.0	4.5	1.9	4.7	6.2	0.8	1.9
	3072	24.9	8.7	5.1	3.4	5.3	2.5	7.7	0.0	3.2
	Av.	33.4	10.7	9.2	6.6	4.3	3.2	4.5	2.4	3.2

[a] Calf died. Item supplied by missing data formula.

high morbidity prevailed among subjects fed the crude and the refined soybean oils. These unsatisfactory responses corroborate previous findings (5, 7), except that the mortality was lower and the general health was somewhat better than reported earlier. These apparent discrepancies are tenable, inasmuch as the duration of the experiment reported herein was shorter than that of Gullickson *et al.* (5) and, contrary to the procedure followed by Jacobson *et al.* (7), milk intake by diarrhetic calves was reduced, thus tending to alleviate the undesirable

reactions to the diet. Moreover, it is possible that the soybean oils used in the various investigations were dissimilar in quality and composition.

The largest weight increases were made by calves receiving the butter oil and the hydrogenated soybean oil, but even these gains were substandard (10). This retarded growth evidently was due largely to an inadequate intake of total digestible nutrients, which was approximately 25 per cent less than the recommended allowance (8). In addition to the effect of the low energy content of

TABLE 6

Effect of type of oil in reconstituted milk diets of young calves on concentrations of fat in blood plasma

Dietary group	Calf no.	Fat concentrations at 7-d. intervals								
		4	11	18	25	32	39	46	53	60
		(mg./100 ml.)								
I (Butter oil)	3043	100	58	142	156	114	114	127	66	94
	3053	79	64	98	146	153	159	144	122	124
	3056	113	73	125	152	138	103	118	126	164
	3071	66	23	98	115	108	126	169	179	184
	3074	79	19	117	168	138	98	134	182	157
	Av.	87.4	47.4	116.0	147.4	132.2	120.0	138.4	135.0	144.6
II (Crude soybean oil)	3044	102	136	202	194	217	236	231	219	244
	3052	98	31	34	178	146	185	158	110	69
	3058	39	64	167	294	280	126	231	188	222
	3062	62	66	162	163	167	117	128	139	141[a]
	3067	111	85	164	204	162	191	205	167	157
	Av.	82.4	76.4	145.8	206.6	194.4	171.0	191.0	164.6	166.6
III (Refined soybean oil)	3041	104	118	198	236	244	319	217	213	160
	3047	124	26	105	182	151	141	154	127	177
	3051	142	135	200	377	331	318	239	233	263
	3061	118	56	188	163	226	194	234	233	206
	3075	75	78	154	208	193	174	131	125	121
	Av.	112.6	82.6	169.0	233.2	229.0	229.2	195.0	186.2	185.4
IV (Hydrogenated soybean oil)	3039	114	16	61	44	31	62	51	46	24
	3040	110	28	78	106	128	43	60	64	44
	3048	66	19	24	30	8	34	68	54	76
	3063	126	62	112	95	83	86	89	78	50
	3072	148	28	85	97	73	55	50	60	62
	Av.	112.8	30.6	72.0	74.4	64.6	56.0	63.6	60.4	51.2

[a] Calf died. Item supplied by missing data formula.

the rations, the poor growth of the calves in the crude and the refined soybean oil groups undoubtedly was associated with frequency and severity of scouring.

The small weight gains per unit of milk ingested by calves fed the crude and the refined soybean oils may be attributed primarily to the high incidence of digestive disturbances and the concomitant reduction in milk consumption. The rapid passage of ingesta through the digestive tract during periods of diarrhea probably affected adversely the efficiency of absorption.

The tendency of the hemoglobin values to decline during the early stages of

the experiment is in accord with observations on calves receiving normal diets (15). If the initial hemoglobin decrease is of dietary etiology, the mineral mixture evidently was inadequate to maintain the high concentrations. Since the levels in individual calves were, in most instances, within the normal range, anemia apparently was not a complicating factor.

Inasmuch as differences in blood plasma vitamin A levels among the groups of calves fed the various soybean oils were not marked, this vitamin evidently was not a primary factor in the differences in the response of young calves to these oils. It is significant, however, that blood plasma vitamin A levels of all calves fed the soybean oil rations were extremely low. None of the animals in these three groups consistently maintained values above 10 γ per 100 ml. of blood plasma, the level recommended for adequate vitamin A nutrition (3). These low concentrations indicate that under the conditions of this experiment young calves need a daily vitamin A intake greater than 10,000 I. U. per 100 lb. body weight.

The calves fed butter oil had a daily intake of approximately 14,000 I. U. of vitamin A activity per 100 lb. body weight, yet the plasma vitamin A levels were low. The values for three calves in this group were within the deficiency range during a portion of the experimental period.

In contrast to the findings of Squibb *et al.* (13) in experiments conducted with lactating cows, inclusion of crude soybean oil in the ration of young calves did not depress blood plasma carotenoid levels. Conversely, after the first week, the mean values for the group receiving crude soybean oil were considerably higher than those for the calves fed the refined and the hydrogenated soybean oils and, except for the last 2 weeks, slightly higher than those of the calves receiving butter oil. Although the differences among groups were not significant, the fact that the carotenoid values were high for certain individuals of the crude oil group suggests that the oil supplied a fat-soluble pigment similar to carotene. Comparative spectrophotometric studies of extracts from butter oil and crude soybean oil further indicated similarity of the pigments. No vitamin A activity of the soybean oil pigment, however, was reflected in the blood plasma vitamin A concentrations.

Blood plasma carotenoid and vitamin A levels were reduced when calves scoured, a relationship observed by other investigators (6, 14), but apparently were unaffected by abnormally high body temperatures.

The marked decline in blood plasma fat values of all groups during the first week of the experiment may be attributed in part to the high incidence of scouring, which probably resulted in a low coefficient of absorption during this period. Another factor that might have been involved was the possible reduction in daily fat intake at the time the calves were transferred from colostrum to the experimental rations.

The comparatively high blood plasma fat values for the crude and refined soybean oil groups are similar to observations made by Gullickson *et al.* (5) on older calves fed corn and cottonseed oils. Although there is no obvious reason

for the differences in the fat levels of calves fed the various oils, possible dissimilarities in the rate and the extent of absorption and in the subsequent metabolism of the oils merit consideration.

The characteristics of crude soybean oil causing the deleterious effects on young calves apparently are eliminated in the preparation of the hydrogenated and deodorized oil from the crude product. Since refining and bleaching failed to improve the value of the oil for calf feeding, evidently the free fatty acids, phosphatides, other non-glyceride constituents and pigments removed in the processing did not contribute to the ill effects resulting from feeding crude oil. Therefore, modifications induced by hydrogenation and/or by related processes seemingly enhance the nutritive value of soybean oil.

Graham and Cupps (4) found that hydrogenated herring oil is less toxic than the unhydrogenated product when fed to goats. This observation prompted the suggestion that the arrangement of the double bonds in the oil may be a factor involved in the physiological reaction. Moreover, it is possible that some substance toxic to the young calf may be removed or inactivated during deodorization (2). The obscure nature of the relationships between the physiological reactions of the calf and the physical and chemical characteristics of the dietary fat indicates a need for further investigation.

SUMMARY

Three types of soybean oils, crude, refined and hydrogenated, were compared with butter oil as components of reconstituted milk rations for dairy calves during the period from 4 to 60 days of age. The respective milks, supplemented with a mineral mixture and vitamins A and D, were fed to four comparable groups of calves.

Although there was considerable intra-group variation, the incidence of scouring was lowest for the calves fed butter oil, followed in order by the groups receiving, respectively, hydrogenated, refined and crude soybean oils. Frequent and severe diarrhea was accompanied by unthriftiness and lethargy.

The mean weight gains of the calves fed hydrogenated soybean oil were similar to those of the group receiving butter oil but greater than the mean weight gains of the groups fed the refined and the crude soybean oils, but the differences were not significant statistically.

There were no appreciable differences in the mean hemoglobin levels among the various groups.

Since there were no significant differences in the mean plasma vitamin A values among the groups of calves fed the various soybean oils, the differences in growth and in state of health cannot be attributed to the level of vitamin A intake.

Although the mean carotene levels of the blood plasma of the groups fed the butter oil and the crude soybean oil were considerably higher than those of the other groups, the differences were not significant statistically.

Mean blood plasma fat levels for the calves receiving butter oil were significantly higher than those of the group fed hydrogenated soybean oil but significantly lower than the mean values of the other two groups.

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The authors are grateful to Dr. S. F. Scheidy, Sharp and Dohme, Inc., Glenolden, Pa., for supplying the "sulfathalidine"; to Dr. E. W. Bird, Department of Dairy Industry, Iowa State College, for analyzing the butter oil for vitamin A and carotene; and to Prof. O. Kempthorne, Department of Statistics, Iowa State College, for suggestions in statistical analysis of the data.

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ABSTRACTS OF LITERATURE

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ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

417. Optical method of testing milk for relative uniformity. A. A. McMURRAY. U. S. Patent 2,460,101. 2 claims. Jan. 25, 1949. Official Gaz. U. S. Pat. Office, **618,** 4: 1141. 1949.

The milk from the various udder quarters of cows is tested rapidly for mastitis by this device, which consists of a dark-colored plastic disc suspended in a horizontal position in a cup. Milk from one quarter is filmed evenly over the surface of the disc. A squirt of milk from another quarter then is directed on the disc; if normal, no change is apparent; if mastitic, a difference immediately is noted in the color of the milk blend on dark disc, due to the dilution caused by the watery character of the mastitic milk. Milk from the remaining two quarters is tested in an identical manner. R. Whitaker

BUTTER

O. F. HUNZIKER, SECTION EDITOR

418. Das "Fuer" und "Wider" der verschiedenen Butterungsverfahren. (The pro and con of different buttermaking procedures.) English summary. M. E. SCHULZ AND W. SCHULZ. Die Milchwissenschaft, **3,** 8: 213–224; **3,** 9: 253–259. Aug. and Sept., 1948.

The advantages and disadvantages of buttermaking by the churn, Fritz and Alfa processes were studied in three plants with daily outputs of butter from (a) 7,000, (b) 10,000 and (c) 50,000 l. of milk. The overhead cost declined in plants *a* and *b* from churn to Fritz to Alfa process, whereas in *c* Alfa was highest with churn and Fritz being similar and lower. The smaller floor space required by the Fritz and Alfa processes was of advantage in *b* and *c,* whereas in *a,* with extra floor space available, this advantage was not considered important. Most time was saved in *a* by the churn process, in *b* by the Fritz and Alfa processes and in *c* by the Fritz process. Most advantageous printing of butter was done in *a* with churn butter, in *b* with churn or Alfa butter and in *c* with Alfa butter. No preference in method or saving in personnel was reported in *a* and *b* with any of the methods, whereas the Fritz process was preferred in *c.* Plants *b* and *c* found the churn best for churning sour cream or reworking of butter. All plants favored the churn process for sale of fresh buttermilk. The operation costs were lowest for the churn process and highest for the Alfa process. Fat losses were lowest in all plants with the Fritz and Alfa processes. Moisture control was simplest with the churn and most difficult with the Fritz process. The churn process was given preference for suitability to make sour cream butter and for greatest suitability for salting and coloring of butter, as well as for avoiding body defects at all seasons. The Fritz process ranked second in the last item. Incorporation of air into butter was least in the Alfa and highest in the Fritz process. Oiling-off of butter was least in the churn and highest in the Fritz butter. Working of butter without loss of moisture was best with churn and poorest with Fritz butter. The Fritz process permitted best moisture distribution and easiest cleaning, whereas the churn was poorest. The keeping qualities of both Fritz and Alfa butter were similar and much superior to churn butter. I. Peters.

419. Process for making butter. G. W. SHADWICK. (Assigned to Beatrice Creamery Co.) U. S. Patent 2,463,915. 15 claims. Mar. 8, 1949. Official Gaz. U. S. Pat. Office, **620,** 2: 581. 1949.

Cream is saturated with an inert gas under pressure and then released to atmospheric pressure to form a mass similar to whipped cream. Phase reversal and the consequent production of butter result when the expanded mass is agitated and worked. R. Whitaker

420. Kompletterande provning av kärnäeltare. Medellande nr 167 (ny foljd) fran Statens Maskinprovningar. (Tests on type W45 churn.) E. SAMUELSSON, Alnarp, Åkarp. Mejeritekniska Medd., No. 5–6: 81–83. Dec., 1948

The motor-driven churn, type W45, was found to be very satisfactory when used experimentally for making butter from both sweet and sour

cream. It proved to be especially practical and simple to operate. The observation period was too short for judging the durability of the churn. The churn drum was elevated, thus permitting easy emptying by dropping the butter into a tray placed under the churn. G. H. Wilster

421. Det kugleformende og frie Fedt i Smor, fremstillet efter Fritz-Metoden, i Forhold til nogle Fremstillingsbetingelser. (The influence of some manufacturing data upon the globular and free fat in butter manufactured by the continuous Fritz-process.) English summary. N. KING. Nord. Mejeri-Tid., **13,** 11: 3–6 1947.

The changes in the entrance-temperature of the cream and in the capacity of the Fritz continuous churning machine involve corresponding changes in the percentage of the globular fat and in the number and average volume of the fat globules encountered in the butter manufactured by this method.

The percentage of the globular fat increases at constant temperature with the increasing capacity linearly, as the treatment intensity exerted on the fat globules of the cream diminishes. At constant capacity the percentage of the fat diminishes with the increasing entrance temperature of the cream, as the globules with increasing temperature grow sofer and thefore are destroyed more easily. Linear relations also exist between the number and the average volume of the fat globules on one side and the capacity of the churning machine on the other side. The difference in the capacity and the entrance-temperature also influence the form of the fat globules. Lower temperature and higher capacity minimize deformation of the globules. The globules were regularly round and surrounded by a finely-formed bright birefringent edge (consisting probably of oriented tiny fat crystals). With higher entrance-temperature of the cream and with lower capacity, in contrast, the globules were strongly deformed.

G. H. Wilster

422. Konsistenz der Butter. (The body characteristics of butter.) English summary. W. MOHR AND J. WELLM. Die Milchwissenschaft, **3,** 8: 232–242. Aug., 1948.

Samples of summer and winter butter made by the churn, Fritz and Alfa processes were examined for body characteristics by the following methods: (a) cutting resistance, (b) cone flow point, and (c) plastometer. All three methods were used with equal success. Since the values obtained by the three methods did not fall on the same point on the flow curve of butter, the results cannot be considered as strictly proportional. By means of the above methods it was possible to detect invisible differences in the structure of butter, such as crumbliness, oiliness and layer formation. The three methods offer great possibilities in studying the structural characteristics of butter, since each method expresses thixotropic changes of butter in a different manner. I. Peters

423. Butter slicer. P. H. MORSE. U. S. Patent 2,464,339. 3 claims. Mar. 15, 1949. Official Gaz. U. S. Pat. Office, **620,** 3: 840. 1949.

Butter in block form automatically is advanced forward, step by step, as a slicing knife cuts off rectangular shaped portions of butter suitable for wrapping for retail trade. R. Whitaker

CHEESE

A. C. DAHLBERG, SECTION EDITOR

424. Over de oorzak der gasvorming in korstlose kaas. (On the cause of the gassy fermentation in processed cheese.) English summary. J. W. PETTE AND J. L. LIEBERT, Rijkslandbouwproefstation, Hoorn, Holland. Verslag. Landbouwk, Onderzoek., **54,** 2: 1–23. 1948.

A bacteriological investigation was made of different kinds of gas-fermenting bacteria in blown processed cheese. In hard process cheese (maximum water content, 50–55%) lactate- fermenting butyric acid bacteria (*Clostridium tyrobutyricum*) nearly always caused the blowing; sometimes propionic acid bacteria did so, too. In soft process (maximum water content, 58–63%) the same was true for the butyric acid bacteria; in a few cases putrifying bacteria played a part.

In trying to cause gassy fermentation by inoculating butyric acid bacteria into process cheese, several factors were investigated. If the maximum temperature of processing was below 70° C., blowing occurred, if higher it generally did not occur. Possibly lactic acid bacteria, which can develop after low processing temperatures, cause better anaerobic conditions for the butyric acid bacteria. Processing should be done at so high a temperature that nearly all lactic acid bacteria are killed. Probably lactic acid bacteria only lower the redox-potential. This seems to be very important for the gassy fermentation. High processing temperature may give blowing if the redox-potential is lowered in another way, i.e., by metabolic processes of *Escherichia-Aerobacter* bacteria. This kind of "early blowing" cheese should not be processed together with "late blowing" cheese.

It is not advisable to store at a high temperature a finished product wherein "late blowing" cheese was processed, because this would give a good chance for lactic acid bacteria to develop and thus for butyric acid, also. A. F. Tamsma

425. De invloed van kaliumnitraat op de boterzuurgisting in kaas. (The influence of potassium nitrate on the butyric acid fermentation in cheese.) English summary. E. A. Vos, Rijkslandbouwproefstation, Hoorn, Holland. The Netherlands Milk Dairy J., **2,** 4: 223–245. Oct.–Dec., 1948.

To know more about the influence of KNO_3 on the butyric acid fermentation in cheese, experiments were done with *Clostridium tyrobutyricum* Van Beynum and Pette. In liquid and solid culture media and in Edam cheese the influence of nitrate and nitrite was investigated. In liquid media KNO_3 was reduced, giving per mol. KNO_3 about 3 mol. H_2 less in the fermentation gas. In solid media with 10% gelatin, this reduction mostly did not take place at all, but sometimes was observed to a small extent. In culture media the nitrate could not stop butyric acid fermentation, although nitrite concentrations of 0.007% were effective in liquid media. The nitrite disappeared rather quickly, being unstable in acid organic solution. In cheese nitrate concentrations of 0.03, 0.01, 0.005 and 0.0025% added to the milk stopped the butyric acid fermentation. The spores were not killed but did not develop. This effect was not caused by nitrite, because no more nitrite could be detected in the experimental cheeses than in the control cheeses. Experiments with nitrite used instead of nitrate showed about the same effect in cheese. Although nitrate, and especially nitrite concentrations, decreased rather quickly, they always stopped the development of butyric acid bacteria. This action was ascribed to an increased oxidation-reduction potential in the cheese. The strongly anaerobic butyric acid bacteria cannot develop at the higher potentials, but are not killed. Measurements of the oxidation-reduction potential were carried out, using gold or platinum wires as electrodes. These were driven into the cheese and the hole in the rind of the cheese around the electrode sealed with liquid paraffin. Without nitrate the rH (Clark's rH value) was about 3.7; in case of butyric acid, fermentation values below 1 were found. With nitrate added the figures averaged about 7.5, indicating that this difference might cause the effect. Nitrate and nitrite are supposed to influence the potential via metabolic processes of bacteria. The potential drop in case of butyric acid fermentation is observed some weeks before gas holes develop and before a development of butyric acid bacteria is shown by bacteriological analysis.

A. F. Tamsma

426. Kyllagringsförsök med ost. (Low-temperature storage experiments with cheese.) Anonymous. Svenska Mejeritidningen, **40,** 44: 408–410. Oct., 1948.

Milk production in Sweden is about 50% higher in June and July than in Oct. and Nov. Experimental work was undertaken to determine the most satisfactory conditions for storage of cheese to maintain high quality economically. The study was made on a total of 47,000 kg. cheese. The results were published by K. E. Thomé, T. Bergman and S. Hoff in "Meddeland nr. 22" from Swedish "mejeriförsök." The cheese varieties were herrgards, svecia and gouda. Half the cheese used in the experiments was stored on shelves in the usual manner and half was placed under refrigeration at a low temperature. All the cheese was judged at 2-mo. intervals while stored for 8 mo. Composition, flavor and aroma were noted carefully and recorded. A better quality cheese resulted from low-temperature storage. It was concluded that storage of cheese under refrigeration was a distinct advantage to those in the cheese industry in Sweden.

G. H. Wilster

427. Mekanisk vändning av osten på lagret. (Mechanical turning of cheese on the shelves.) Anonymous. Svenska Mejeritidningen, **40,** 21: 224–225. May, 1948.

For the mechanical turning of cheese, an apparatus developed by G. Jönsson, Malmö Nya Mejeriforening, is used. This consists of two perpendicular side pieces, the same height as the cheese curing room and furnished with wheels or casters. The equipment moves on rails fastened either to the ceiling or laid on the floor. The two side pieces are joined by a center shaft which can be made to move either upward or downward. This center shaft is made with axles and two tongs which can grip the cheese shelves and turn them.

An illustration is given of the numbered shelves and how they are moved and turned mechanically with the cheese upon them. Shelf number 3 with cheese lying flat upon it is moved gently out toward the next row of shelves and then tilted upright, a brace holding the cheese standing on it, as the shelf glides over to shelf no. 16 just across from shelf 3 on the illustration. The cheese now is turned off mechanically onto shelf 16, so the side which was on top now is on the bottom. In like manner all the shelves with the cheese are moved and the cheese turned over mechanically.

Several construction problems remain to be solved before mechanical means for turning cheese can be used widely. G. H. Wilster

Also see abs. no. 429, 443.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

428. Removing ultimate moisture from powdered products. A. C. BEARDSLEE. (Assigned to the Borden Co.) U. S. Patent 2,465,963. 2 claims. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, **620,** 5: 1539. 1949.

Powdered food products, such as milk, having a low final moisture content, first are dried in the conventional way and then further dried by passing through a chamber maintained under a vacuum not exceeding 20 mm. mercury. Heat is applied to the outside of the chamber and air is admitted to facilitate movement of the product and the evaporation of the moisture. After cooling under reduced pressure, the product with a low moisture content is packed in sealed containers.

R. Whitaker

429. The preparation of dried whey-potato mixtures. ALBERT H. STEVENS, U. S. Dept. of Agr., Washington. Natl. Butter Cheese J., **40,** 4: 28–29, 60, 62. Apr., 1949.

A mixture of cheese whey and potatoes, combined in the proper proportions, can be dried successfully. The resulting product is important because of its nutritional value and as a means of utilizing surplus production. The whey-potato mixture can be used in making bread, cakes, wafers, pancakes, doughnuts, dried soups and as an animal feed.

The whey-potato mixture can be dried by either the spray or drum process. The titratable acidity of the whey will influence the amount of potato solids needed to produce a film on the drum drier. For drum-drying, 32.5 lb. of potatoes are used per 100 lb. of cheese whey of acidity not to exceed 0.38%. The potatoes are washed, cooked until soft, ground and combined with preheated (165° F.) whey, agitated for 10 min. and homogenized at 1,500 lb. pressure. Prior to drying, the mixture is preheated to 160° F. It is dried using 50 lb. steam pressure for a maximum drum speed of 12 rpm. or 100 lb. of steam pressure for a maximum drum speed of 20 rpm. The clearance between the rollers should be the same as that used for drying skim milk. H. E. Calbert

430. Manufacture, use and storage of dehydrated, sweetened, condensed skim milk. A. T. MUSSETT AND W. H. MARTIN, Kansas State College, Manhattan. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., **2:** 15–22 Oct., 1948.

See abs. no. 88, p. A19.

431. The Babcock fat test of reconstituted milk. G. M. TROUT, J. R. BRUNNER AND P. S. LUCAS, Michigan Agricultural Experiment Station, East Lansing. Am. Milk Rev., **11,** 3: 48–49. Mar., 1949.

See abs. no. 329, p. A71.
Also see abs. no. 443, 447.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

432. Direct microscopic clump counts of pasteurized milk by carbolated Newman-Lampert no. 2, and the acid- and water-free methylene blue staining procedures. B. S. LEVINE AND L. A. BLACK, U. S. Pub. Health Service, Cincinnati, O. J. Milk and Food Technol., **12,** 2: 69–74, 83. Mar.–Apr., 1949.

The effect of laboratory pasteurization of raw milk on direct microscopic stains was studied. Carbolated Newman-Lambert no. 2 and acid- and water-free methylene blue stains were used. Two lots of 25 milk samples were studied microscopically after the samples were held at 4° C. for 24 hr.

Post-pasteurization counts appeared to be the greatest with carbolated methylene blue stain. The pasteurized samples held in the refrigerator for 24 hr. reduced this loss by 12% by logarithmic average and 15% by arithmetic average, using Newman-Lampert stain. The count was less on the pasteurized milk than when using the carbolated stain. Acid- and water-free stain showed a diminution in bacterial counts on post-pasteurized samples and the counts were lowest after storing the samples in the refrigerator for 24 hr. The authors believe there is no evidence to support the opinions that pasteurization of milk causes the bacteria to disintegrate during the cooling period. Heating may cause a denaturation of bacterial proteins and also affect the buffer system of the milk, thus influencing the adsorption of the dye by the bacteria. H. H. Weiser

433. Om koliprovet. (For bacteria calculations.) A. LEESMENT. Mejeritekniska Medd., no. 2: 17–19, 26–27. Apr.–May, 1948.

A new method for counting bacteria in milk has been tried. Measuring the milk was done with a calibrated platinum loop which contained 0.033 ml. milk. Experiments showed that the platinum loop was just as useful in estimating the bacteria when streaking on media in petri dishes or in Bergman flasks as when estimates were done with roll flasks. Estimation of colon organisms with the roll culture method proved highly successful. The results were less variable than with the streak method. The saving of media was a

noteworthy factor since about 3 ggr. (?) less was needed for a culture by the roll method than by the petri dish method. The roll method therefore would seem to be suitable for laboratories with large numbers of milk samples to be examined. Some special equipment is necessary.

G. H. Wilster

434. Ein Universal-Agarnährboden für das molkerei-bakteriologische Laboratorium. (A universal agar medium for the bacteriological dairy laboratory.) English summary. A. TODOROFF AND K. ASSENOWA. Die Milchwissenschaft, **3,** 9: 263–266. Sept., 1948.

Whey agar, nutrient agar and wort agar were tried alone or in various combinations with single cultures of *Streptococcus lactis, Streptococcus cremoris, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus helveticus, Escherichia coli, Aerobacter aerogenes, Bacillus subtilis,* a lactose-fermenting yeast, a pellicle-forming yeast and *Penicillium camemberti candidum.* All lactic bacteria tried preferred the combined whey and nutrient agar to the prescribed nutrient agar plus 1% lactose. All test organisms showed good growth and development on a universal medium containing two parts of whey agar, two parts of nutrient agar and one part of wort agar at pH 6.5. This universal medium is recommended to replace the three separate media and the lactose medium formerly used in dairy laboratories in Germany. I. Peters

435. High colony counts in pasteurized milk caused by bacteria from efficiently sterilized plant. C. S. MORRIS AND M. EDWARDS, Natl. Agr. Advisory Serv., Sarcross, Devon. Proc. Soc. Applied Bact., **1947,** 1: 21–23. 1947.

Samples of pasteurized milk from one dairy consistently were found to have colony counts of about 10 million/ml. after storage at 18° C. for 24 hr., although counts before storage were less than 10,000/ml. prior to the incubation. Swabbings from equipment were made, inoculated into pasteurized milk and incubated 24 hr. at 18° C. Pipe lines, the filler bowl, filler valve and bottles harbored the organisms which were capable of the rapid growth at 18° C. These bacteria were present only in small numbers on the equipment. The bacteria were gram-negative and did not ferment sugars or effect any change in litmus milk. They also were not detected by the resazurin test, the test originally recommended for use in conjunction with the storage quality test. M. L. Speck

436. The incidence of thermoduric organisms in farm milk supplies with some observations on the dominant types. S. B. THOMAS, Natl. Agr. Advisory Serv., Crosswood, AND E. J. EVANS AND L. B. JONES, Natl. Milk Testing Serv., Brynawel, Aberystwyth. Proc. Soc. Applied Bact., **1947,** 1: 15–18. 1947.

Milk samples, collected from cans on arrival at the milk plant, were stored at atmospheric shade temperature until 22–28 hr. old and then 10 ml. volumes were pasteurized at 63.5 ± 0.1° C. for 30 min. Plates were poured using yeastrel milk agar and were incubated at 30 ± 0.5° C. for 4 d. Thermoduric counts were found to be the highest during Dec.–Feb. and June–Aug. Careless cleaning of equipment was found to be the cause of the winter peak, while higher initial thermoduric counts of summer milk were considered the cause of the peak at that season. Hypochlorite and boiling water sterilization of utensils were found to be very effective in keeping the thermoduric counts low; steam was moderately effective; warm water washing was very ineffective. The types of thermoduric bacteria isolated in their order of occurrence were: microbacteria (approx. 70%), followed by micrococci (and sarcina), spore-formers, gram-negative rods, streptococci, actinomycetes and yeasts. The method of utensil sterilization had no effect on the order of the occurrence and but slight effect on the % of these types of bacteria present in the milk. M. L. Speck

437. The seasonal incidence of thermoduric organisms in farm milk supplies. S. B. THOMAS, P. M. HOBSON AND P. M. FRANKLIN, Natl. Agr. Advisory Service, Trawscoed, Aberystwyth. Dairy Ind., **14,** 1: 31–37, 44. Jan., 1949.

Monthly thermoduric bacterial counts were obtained on milk from 210 farms over a period of 1 yr. The thermoduric bacterial content, both at 30 and at 37° C., of farm milk supplies of poor as well as of fair hygienic quality generally was highest in summer and lowest in winter. This is to be expected, since the incidence of high thermoduric counts increases with an increase in raw milk colony counts.

Milk supplies handled in thoroughly sterilized utensils, so that the heat resistant bacteria rarely exceeded 10,000 per ml., had a higher incidence of thermoduric organisms in winter than in summer. Probably farms with facilities for steam sterilization apply it more carefully and more consistently during warm weather, thus reducing the thermoduric colony counts during summer.

G. H. Watrous, Jr.

438. A modified procedure for determining the thermoduric bacterial content of milk. D. A. MCKENZIE AND J. LAMBERT, Provincial Laboratory, Leeds 6. Proc. Soc. Applied Bact., **1947,** 1: 19–20. 1947.

The method is based on that of Myers and

Pence. Samples of milk are pasteurized and then 0.01 ml. of the sample, as obtained by a calibrated loop, is inoculated into yeastrel agar contained in a 2-oz. flat medical bottle. Then, as is customary in the oval tube technic, the bottles are laid on a flat side and the agar allowed to solidify. Incubation is for 3 d. at 30° C. Thermoduric counts made by this method were lower than those made by the plating method. Therefore, it was proposed that the maximum count allowable by the plate method be reduced by 50% in the modified technic. It was suggested that a keeping quality test be performed simultaneously with the thermoduric count, as the latter alone cannot be used to determine poor production methods.

M. L. Speck

439. Preliminary observations on various temperature characteristics of some facultative psychrophilic bacteria. C. V. CHANDRA SEKHAR AND N. WALKER, Univ. College Wales, Aberystwyth. Proc. Soc. Applied Bact., **1947,** 1: 24–27. 1947.

Some of the cultures which grew during the refrigeration of milk grew in broth and on agar at 3–5° C. but the optimum temperature was about 24° C. Similar results were obtained with the fermentation of carbohydrates and certain other biochemical tests. CO_2 production was greater at 24 than at 37° C. by two cultures, while a third produced more CO_2 at 37° C. Lactate dehydrogenase activity of six cultures showed the temperature optimum to be at 44° C. M. L. Speck

440. Die Milchsäurestreptokokken und Degenerations-Erscheinungen im Säurewecker. (The lactic acid streptococci and their degeneration in starters.) English summary. A. HADZI-ANTIC. Die Milchwissenschaft, **3,** 9: 260–263. Sept., 1948.

Sixty-five per cent of the strains isolated from a commercial starter were constant in their morphological characteristics, whereas the other 35% were not. Degenerated *Streptococcus cremoris* strains, forming pairs or short chains, were regenerated in size and chain length by from five to seven transfers in litmus milk containing from 1 to 100 mg. potassium meta bisulfite per 100 ml. of medium. A similar treatment with constant *Streptococcus lactis* strains did not result in chain formation. Potassium meta bisulfite and ascorbic acid were used for the regeneration of *S. lactis.*

The step-wise degeneration of starter cultures begins with decrease in aroma, followed by decrease in volatile acids and slow lactic acid production. Mixed cultures are more resistant toward degeneration than are single-strain cultures. The most ideal growth conditions for both the constant and variable strains exist under facultative anaerobic conditions, such as are in the lower level of the starter. For best results inoculum should be taken from the bottom of the mother culture. I. Peters

441. Coliforms, their significance and control in ice cream making. G. W. SHADWICK, Beatrice Foods Co., Chicago, Ill. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., **2:** 69–73. Oct., 1948.

The presence of coliform organisms in ice cream does not mean necessarily that the product is unsafe from the public health standpoint or that fecal material is present. However, it may be an indication of careless and unsanitary conditions of manufacture. A list of steps that should be taken in the manufacturing plant in order to eliminate contamination with coliform and other organisms was presented. H. B. Naylor

442. The testing of frozen eggs for pathogens. S. E. HARTSELL, Purdue Univ., Lafayette, Ind. J. Milk and Food Technol., **12,** 2: 107–108. Mar.–Apr., 1949.

A comparative study was made on frozen eggs to evaluate the usefulness of different differential culture media in determining the longevity of certain pathogens in the product. *Staphylococcus aureus, S. typhosa, S. oranienburg, S. aertrycke* and *Escherichia coli* were able to survive in frozen eggs up to 10 mo. at −17.8° C. (O° F.). The culture media used were glucose tryptone agar, yeast water and veal infusion agar in addition to selective media—desoxycholate agar, MacConkey agar and staphylococcus medium no. 110. The selective media tended to inhibit all of the test organisms, except *S. aureus,* while the nutritive media increased the growth of the pathogens. The author suggested a more satisfactory plating culture medium should be devised for testing the presence of pathogens in frozen eggs. H. H. Weiser

443. Framställning av myölksyra ur vassle. (The preparation of lactic acid from whey.) G. NILSSON, Mjölkcentralens Centrallaboratorium. Svenska Mejeritidningen, **40,** 20: 207–210. May, 1948.

It is possible from the results obtained in the experiments to state that under non-aseptic conditions for a period of 48 hr. at 43° C. the milk sugar in whey was changed to lactic acid with a mixed culture of a lactobacillus and a mycoderma, accomplished in such a manner that the fermentation took place with a pH which did not differ particularly from the optimum value (5.6–5.8). The production of Ca lactate in creameries is described. G. H. Wilster

444. Method of enhancing the yield of yeast in a whey medium. A. M. HANSON, N. E. RODGERS AND R. E. MEAD. (Assigned to Western Condensing Co.) U. S. Patent 2,465,870. 1 claim. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, **620,** 5: 1516. 1949.

After adjusting the pH to between 1.5 and 3.5 the wehy is heat sterilized, after which the pH is adjusted to between 5 and 8 and then inoculated and cultured. R. Whitaker

Also see abs. no. 424, 425, 481.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

445. Verfahren zur Bestimmung des Vedorbenheitsgrades von Fetten. (Procedure for the determination of degree of fat deterioration.) English summary. H. SCHMALFUSS Die Milchwissenschaft, **3,** 8: 225–233. Aug., 1948.

Six approved methods for the detection and determination of spoilage in fat are described and discussed. The methods are those for measuring fat acidity of colored and colorless fats, peroxides, free aldehydes, epihydrinaldehydes and ketones.

I. Peters

446. Methode ter bepaling van de hoeveelheid gekristalliseerd vet in room. (Method for determining the quantity of crystallized fat in cream.) English and German summaries. W. ADRIANI AND A. F. TAMSMA, Laboratorium der Cooperatieve Fabriek Van Melkproducten, Bedum, Holland. Verslag. Landbouwk. Onderzoek., **51,** 6G: 79–90. 1945.

The dilatometric method for cream yielded lower values for the amount of crystallized fat than it did for fat from the same cream. As the last value agreed very well with the results of the manufacturing process of butter, the dilatometric results for cream were unreliable. Sources of error are discussed. A new method based on thermic principle was elaborated, giving reliable results for the quantity of crystallized fat in cream. The latent heat necessary to change partly crystallized cream fat into liquid fat was determined, being a good indication for the percentage of crystallized fat. The determination was arranged in a simple way by using as calorimeter a thermos bottle, closed with a rubber stopper and a thermometer accurate to 0.01° C. The cream is mixed in this bottle with hot water and the temperature effect found by extrapolating the curves of temperature decrease before and after the cream was poured into the bottle. Constants necessary for calculating the results can be found easily with the same kind of experiments. This method is suitable for use in factory laboratories. The accuracy was about 1% with small thermos bottles, but can be increased by using large size bottles. It was found that nearly the same amount of fat crystallized in cream and separated butterfat with winter cream at 18 to 25° C. and with summer cream at 26 to 30° C. both during a day.

The authors arrive at the following conclusions: The dilatometric method is excellent in examining butterfat, but should be wholly abandoned for cream as giving erroneous results. To ascertain the quantity of crystallized fat in cream the new method should be used, which opens a wide perspective, because now for the first time it is possible to control the manufacturing process.

A. F. Tamsma

447. Milk treatment with oxidation-inhibiting gases. M. E. DUNKLEY. U. S. Patent 2,463,363. 2 claims. Mar. 1, 1949. Official Gaz. U. S. Pat. Office, **620,** 1: 285. 1949.

Oxidation of such dairy products as butter, evaporated milk, whole milk powder, etc., is prevented by a system of handling all processing of the milk from the milking machine under an atmosphere containing 8.2% carbon dioxide, 0.1 to 0.2% acetylene, 0.1 to 0.2% oxygen, 3.8% carbon monoxide, 2.2% methane and 85.5% nitrogen. This gas is produced by burning fuel gas.

R. Whitaker

448. Fosfataseenzymets Varmedestruktion. (Destruction of the milk phosphatase by heating.) R. HANSEN. Nord. Mejeri Tid., **14,** 8: 3–10. 1948.

A new technic in establishing curves of destruction of the milk phosphatase at different temperatures was developed by the State Experiment Station, Denmark. Glass ampules, 100 mm. high, 19–20 mm. in diameter and 0.5 mm. thick were utilized. An injection syringe was used to fill the ampules with enzymconcentrate. Three tables and two graphs are given with temperatures and the number of seconds necessary for the destruction of milk phosphatase.

The following is a summarization of the data:

No. of sec. to destroy phosphatase

Pasteurization temp.	% destruction of enzyme		
	99.6	96.0	90.0
(°C.)	(sec.)	(sec.)	(sec.)
60	3,540	1,740	1,086
63	810	400	256
66	199	100	64.5
68	75.0	38.4	24.6
71	20.5	10.3	6.6
74	5.9	2.95	1.90
77	1.65	0.85	0.55
80	0.44	0.23	0.15

G. H. Wilster

449. Visual observation and silhouette projection apparatus. G. D. AMERDING. (Assigned to Mojonnier Bros. Co.) U. S. Patent 2,461,623. 7 claims. Feb. 15, 1949. Official Gaz. U. S. Pat. Office, **619,** 3: 721. 1949.

The image of a Babcock fat test bottle is projected on a diaphragm by means of a beam of parallel light rays in such a manner that the reading of the fat column is facilitated. A heater is provided for maintaining the device at an elevated temperature. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

450. Interessant planløsning av kombinert meieri. (Ineresting plan for a multiple-product dairy plant.) ANONYMOUS. Meieriposten, **37,** 14: 245–246. Apr., 1948.

To arrange a creamery so it has a practical layout for carrying out all of its processes for all products constitutes a problem which this article attempts to solve. It suggests a star-shaped floor plan. The center of the star plan would contain the platform and separator room. An illustration shows the various star wings housing the equipment for buttermaking, cheesemaking, market milk and one star wing left vacant for future development. Two large storage rooms could be built across the end of one star wing where cheese could be cured. Room is allowed for the boiler in this creamery plan which lends itself to an expanding plant. The star plan allows for plenty of light and ventilation. It has the advantage of having all rooms join the center room which houses the separator and milk handling equipment. Expansion can be accomplished by adding on to the star wings without disrupting the general plant activities. It is as economical to build the star plant building as to build a single building of more than one story, containing the necessary halls and stairways. The plan was developed by A. P. Andersen and I. Nielsen. G. H. Wilster

451. Do's and dont's on cold-room insulation. ANONYMOUS. Operating Engineer, **2,** 1: 36–37. Jan., 1949.

Insulation is used to reduce the heat flow from the warmer to the cooler area to a reasonable amount. After thickness of insulation for a given heat flow is decided upon, building design should be studied to ascertain that the full thickness can be applied everywhere.

Foundation concrete should be poured short to allow sub-floor to overlap wall to make room for full thickness of insulation at sub-floor and building wall joint. Door sills must be made properly or door may become cause of greatest refrigeration loss. Roof joists placed too closely to the building walls may prevent proper thickness of insulation. Roof flashing also is very important here. Cover building walls with asphalt paint or paper to prevent leaks of moisture-laden air into insulation.

Steps in repairing damaged corkboard are: (a) On masonry walls, smooth surface with cement plaster. (b) When dry, cover with asphalt primer paint. (c) Apply first layer of corkboard with hot asphalt. (d) With additional layers, fasten each board to first layer with hardwood pulp.

Protect insulation with an airproof, moistureproof and odorless cover. Portland-cement plaster and asphalt plastic may be used. Illustrations present correct design for critical points in cold-room construction. H. L. Mitten, Jr.

452. How to choose economical type of roof construction. H. J. SCHARRES, Graham, Anderson, Probst and White, Architects, Chicago. Heating, Piping Air Conditioning, **21,** 3: 93–95. Mar., 1949.

Because each project presents different problems, rules for achieving economy cannot be set to apply to all types of roof construction. Heating and air conditioning designs must be correlated with the rest of the structure. Usually this takes much calculation to produce results which are understood readily by the architect and the client. To make the problem easier tables were prepared for eight of the most-used types of roof decks with seven kinds of insulation. The tables show the construction, heat transfer coefficient for the kind of insulation and thickness selected, thermal conductivity and thermal conductance. H. L. Mitten, Jr.

453. Temperature control system for pasteurizers. J. I. HALL. (Assigned to Ex-Cell-O Corp.) U. S. Patent 2,465,532. 1 claim. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, **620,** 5: 1429. 1949.

This device correctively controls the magnitude of the current applied to milk when it is pasteurized by resistance heating, in spite of variations in flow, composition and initial temperature of the milk. The direct current corresponding to the temperature of the milk downstream from the electrodes is amplified and employed to vary the current between the electrodes.

R. Whitaker

454. Safe application and operation of ammonia equipment in ice cream plants. V. C. PATTERSON, V. C. Patterson & Associates, York, Pa. Rept.

Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., **2:** 74–78. Oct., 1948; and Ice Cream Trade J., **45,** 3: 50, 52, 96, 97. Mar., 1949.

Safety in operation of direct expansion refrigeration requires a knowledge of the properties of ammonia. Ammonia in amounts of 13.1 to 26.8 by volume in air becomes explosive and gas masks are not effective when the concentration reaches 3 to 4%. In the application of safety first practices in ammonia plant designs an operator's attention should be given to the following: (a) Locate condenser, high pressure receiver and charging connections outside building or on roof. (b) Locate compressor room on ground floor, separate from boiler room, with ample exits and good ventilation. (c) Design so all ammonia charge can be transferred to low pressure side while plant is under full operation without liquid slopping over into any compressor. (d) Equip all evaporators with relief valves which open to atmosphere, not into the suction line. (e) A fireman's emergency station located outside the building, equipped with a stop switch for all machinery in compressor room, a transfer valve to dump high pressure ammonia into a low pressure surge drum to relieve all high pressure lines and a hand-operated valve to relieve ammonia pressure into a low temperature surge drum over into a water mixing tank. Definite instructions should be not to dump ammonia unless pressure is either 100 lb. or over, or 200 lb. or over. Other precautions include an emergency fire hose, protected ammonia lines, storage of minimum amounts of ammonia on high side, high pressure cut-outs on all high stage compressors and all lines properly hung, braced and protected against physical damage. A colored floor diagram of the cycle should be framed, covered with glass and hung in compressor room. Ammonia masks and CO_2 fire extinguishers should be located at convenient places and operators trained to handle an emergency. When automatic operation is used, the best plan is to have two small compressors with one operating all the time and one thermostatically controlled coming on and off as required.

W. H. Martin

455. Basic principles of piping (a review of fundamentals). H. VETTER, Consulting Engineer, Los Angeles. Heating, Piping Air Conditioning, **21,** 3: 79–82. Mar., 1949.

The difference between steam and refrigeration plants is in the boiling temperatures and application. In refrigeration plants the evaporator coil corresponds to the boiler and the compressor is in the same position as the engine. The piping between an evaporator and engine should be pitched toward the evaporator so that condensate formed in the line will drain back to the evaporator. When globe valves are placed in horizontal lines (valve stem vertical) the engine side of the line should be tapped and connected to a drip trap to prevent the collection of condensate in the line.

Pipe line size should be determined by desired pressure drop and not by velocity; however, high velocities should be avoided. Equations, graphs and tables are presented which give pressure drops and velocities for steam pipes. An ideal leader arrangement is one in which the pressure drop to each engine (or compressor) is the same. Difference in pressure drop is more important in refrigeration compressors than in steam engines, for compressors do not have a governor to compensate for the difference. A bottleneck in the piping will reduce the capacity of the system. Locate stop valves in the outlets of all pressure vessels so that, in the event of leaks, the pressure can be shut off to allow repairs without shutting down the evaporator.

H. L. Mitten, Jr.

456. Flexible couplings. J. J. O'CONNOR, Power, McGraw-Hill Publishing Co., New York, N. Y. Power, **93,** 4: 87–102. Apr., 1949.

The coupling transmits all power between driving and driven units. Couplings should be used to protect against misalignment which occurs under operating conditions. They should not be used to connect two shafts originally out of line.

There are many types of couplings and their applications overlap. This article attempts to explain couplings so that the plant engineer may select, install and operate flexible couplings successfully.

If a machine and its driving unit are in line they usually are connected by a coupling. Since perfect alignment is practically impossible, flexible couplings are necessary. Misalignment of machines connected by rigid couplings causes excessive bearing wear and power consumption.

Use of yielding material or a mechanical design which allows movement between rigid elements are the two general means of obtaining flexibility in couplings. Some couplings provide flexibility in four directions, tangential, angular, radial and axial. Others are limited to two or three directions. The number of directions of flexibility is dependent upon the application.

Proper selection of a coupling requires it to be large enough to fit the shafts, capable of carrying the load and capable of operating under the prevailing plant conditions. Couplings usually are rated in horsepower/100 r.p.m.

One of the most common causes for trouble is the selection of couplings which are too small for the application. Centrifugal pumps and fans cause little wear. A plunger pump subjects the coupling to considerable shock.

H. L. Mitten, Jr.

457. Fuels and firing. Part I. P. SWAIN, J. MCCABE, AND B. SKROTZKI, McGraw-Hill Pub. Co., New York, N. Y. Operating Engineer, **2,** 2: 19–34. Feb., 1949.

Combustion is high-speed oxidation in which the gas generated from the fuels burns, rather than the solid or liquid fuel itself. Yellow flames are caused by the glow of a concentration of individual carbon molecules just before they are burned. If the gaseous mixture containing hydrogen and carbon is cooled, the hydrogen may burn and the carbon may deposit out as soot. When a yellow flame in a boiler is cooled by such things as boiler tubes, unburned carbon is deposited or swept up the stack as smoke. Soot and smoke can be prevented by allowing complete combustion before the flame reaches the tubes. When combustion is complete the hydrogen always ends up as H_2O and the carbon as CO_2. When the carbon is not burned entirely it may appear in CO, which is capable of further burning.

Coke and charcoal are mostly solid carbon. Bituminous coal contains, in addition, distillate hydrocarbon. Natural gas is almost all gaseous hydrocarbon. Carbureted water gas is largely CO and hydrogen. Fuel oil vaporizes into gaseous hydrocarbon before burning. If cracked, it may produce some solid carbon and hydrogen. Coal decomposes into gaseous hydrocarbon, CO and hydrogen before burning.

Suspension firing of oil and coal requires that each be broken into as many particles as possible. To prevent smoke, combustion steps must be complete during the travel of the fuel from the burner to the furnace outlet. Good combustion depends upon temperature, time and turbulence. In fuel bed firing, volatile matter distills off and coke left in the grate burns to mixture of CO_2 and CO. The gases from the fuel bed are mixed with the air which flows through the grate. Secondary air may be admitted above the grate to furnish oxygen for complete combustion. From 40 to 60% of coal's heat is liberated above the fuel bed. In furnaces, the less excess air used, the larger must be the combustion space to insure complete combustion. Modern furnaces designed to keep size and investment low and efficiency high tend to use high speed air jets for more turbulence.

Natural gas, manufactured gas, blast-furnace gas, commercial fuel oils and coal are discussed. In designing a new plant a fuel survey should be made to avoid a plant which will prevent a free choice of fuel. Facts needed are (a) fuels available, (b) how available fuels compare now, (c) how they will compare in the future. Don't skimp on equipment. Plan for flexibility in fuel selection.

Combustion chemistry is discussed and a short-cut for combustion calculation is presented.

H. L. Mitten, Jr.

458. How to size your blowoff tanks H. B. NICKELSPORN, Radiant Engineering Co. Operating Engineer, **2,** 1: 24–25. Jan., 1949.

Blowoff tanks prevent direct discharge of steam-water blow-down mixture into sewer lines which are not built to take blow-down temperatures. Direct discharge into sewers also might cause steam to back up into other equipment which empties into the sewer line. Blowoff tank provides space for blow-down to cool and lose pressure. Flash steam is vented to the atmosphere.

American Society of Mechanical Engineers Code requires blowoff valves to be no larger than 2.5 in., no smaller than 1 in. Valves such as globe valves having pockets in which sediment might collect cannot be used. All valves and fittings must have American Standards Association rating 25% higher than boiler safety-valve setting. When pressure exceeds 100 psi. each blow-down must have two valves in series. One table shows per cent of flash steam in blow-down at various temperatures and another gives vent and discharge sizes.

Blowoff tank size is dependent upon the weight and volume of blow-down mixture at a single period. A tank volume equal to 1/5 the water-holding capacity of the boiler or water volume of one guage on water column may be used. Boiler pressure does not affect tank size. Allow for a cold water seal to remain in the tank between blow-downs.

Tank design must be approved by local authorities. As a guide use A.S.M.E. Code for unfired vessels. Tank should be designed to withstand half the boiler pressure. Tanks may be vertical or horizontal. Vent line must be twice inlet size. Making vent larger than necessary causes less water to be carried out by flash steam. No valves or other restrictions should be in the vent line. Line must rise 7 ft. above places where people may walk. Discharge lines must be twice the area of the blowoff pipe and must have an elbow inside the tank at least 6 in. from bottom. The discharge line should have a siphon breaker at the highest point.

H. L. Mitten, Jr.

459. Refrigerating apparatus including hydraulic lift. S. G. PRICE. U. S. Patent 2,463,307. 4 claims. Mar. 1, 1949. Official Gaz. U. S. Pat. Office, **620,** 1: 270. 1949.

To facilitate insertion and removal of cans of milk or other perishable food products in a tank of cooling liquid, a platform is raised from or lowered to the floor of the tank by hydraulic means. R. Whitaker

Also see abs. no. 418, 419, 420, 427, 466, 467.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

460. Controlling fat losses. L. C. THOMSEN, Univ. of Wisconsin, Madison. Milk Dealer, **38,** 6: 52, 126–134. Mar., 1949.

Data are presented showing the causes of fat losses in dairy plants. Hidden losses result from the use of wrong conversion factors in converting from volume to weight or vice versa, from incorrect test samples, incorrect testing procedures, or when one testing procedure is to be reconciled with another. Apparent losses include spillage, spoilage, incorrect standardization for composition, and larceny. In the control of losses it is suggested that all employees should be made waste and loss conscious by showing the cash value of losses and then offering bonuses for keeping them within pre-established limits. C. J. Babcock

Also see abs. no. 475.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

461. Measurement of sperm activity before artificial insemination. LORD ROTHSCHILD. Nature, **163,** 4140: 358. 1949.

This method is based on the observation that semen, when active, exhibits well-defined changes in electrical impedance. A conventional alternating current bridge, energized by a 5 kc. oscillator, is used for measuring the electrical changes. The detector is an oscilloscope with an amplifier. The unknown arm of the bridge consists of a pair of platinized platinum electrodes which dip into the semen at 37° C. The voltage across it is about 50 m.v. The frequency is greatest when first collected, decreasing with temperature and reading zero when the sperm are dead or feebly motile. Phosphate buffer added to the semen maintains the initial frequency. The impedance is increased greatly by concentration of the semen, i.e., centrifugation. R. Whitaker

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

462. Cutting milk production costs with pen barns. CLARON BURNETT, Assoc. Ed. Milk Dealer, **38,** 6: 42–43, 112–116. Mar., 1949.

Comparisons of pen type stabling of cows with the conventional stanchion barn at the University of Wisconsin experimental farm indicate more milk can be produced with less labor and lower feed costs when the newer type barns and milking parlors are used. Studies at the Huntley, Montana, experiment station show cows produce as much as 19% more milk and 18% more butterfat when housed in pen barns than when kept in a stanchion barn. Studies of the two types of barns at Wisconsin have shown: (a) Over-all labor requirements have run about 10% less for pen barn operations than for the stanchion barn. (b) Cows housed in pen barns have better appetites and consume more roughage with the result that cost/lb. of 4% fat-corrected milk was only 96.11% of that produced by cows in the stanchion barn. (c) There is little difference in the quality of the milk as shown by bacterial counts between the individual barns. (d) Cows have remained healthier and there has been less trouble with mastitis in pen barns. Other advantages of pen barns include economy of construction, ample fresh air and sunshine for cows and better-preserved manure for application to the soil. Disadvantages include larger bedding requirements and the necessity of dehorning the cows. C. J. Babcock

463. Insecticide studies with dairy cattle. L. A. MOORE, R. H. CARTER AND F. W. POOS, U. S. Dept. of Agr., Washington. J. Milk and Food Technol., **12,** 2: 103–104. Mar.-Apr., 1949.

The authors indicate the danger of using DDT to control insects on crops fed to cows and the milk containing appreciable quantities of the insecticide. However, if the amounts of DDT are kept to a minimum for the control of the insects, no large amount of this compound will appear in the milk.

No doubt, other insecticides will be developed that will be just as effective as DDT in controlling insects and will not be absorbed and secreted in the milk as readily. H. H. Weiser

464. Teat cup. A. C. WEIBY. (Assigned to Solar Corp.) U. S. Patent 2,462,583. 3 claims. Feb. 22, 1949. Official Gaz. U. S. Pat. Office, **619,** 4: 1096. 1949.

This milking machine teat cup consists of a rigid metal outer shell, within which and re-

leasably secured to it is a flexible tube molded to receive the teat. Intermittent suction contracts the tube, withdraws the milk and discharges it to the milker through a tube attached to the lower end of the outer shell. R. Whitaker

465. Alarm device for milking machines. A. G. PERKINS. U. S. Patent 2,461,439. 8 claims. Feb. 8, 1949. Official Gaz. U. S. Pat. Office, **619,** 2: 567. 1949.

A receptacle is mounted inside the milking machine pail directly under the ports which deliver the milk from the cow. This receptacle is lowered as it fills with milk and stays down as long as there is a full flow of milk. When the flow decreases at the completion of the milking the receptacle rises, thereby sounding an alarm and finally breaking the vacuum if the attendant does not remove the milker from the cow when the alarm is sounding. The rise and fall of the receptacle is governed by a drain which discharges the milk from the receptacle at a slower rate than the normal milk flow but faster than the reduced flow toward the end of the milking. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

466. Short time high temperature pasteurization of ice cream mixes. C. M. MINTHORN, Chester Dairy Supply Co., Chester, Pa. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., **2:** 47–50. Oct., 1948.

Mix prepared from concentrated products must be preheated to 125° F. before it enters the first section of the Ste-Vac heater. Condensed mix can be pumped directly from the vacuum pan through this heater where the temperature is raised to 157° F. At this point, the mix is homogenized. Friction raises the temperature to 162° F. and the mix is pumped through the second section of the heater. It enters the holding tube at 175° F and is held 23 sec. before it is cooled. Bacterial counts in mix pasteurized by this method compare favorably with counts in mix pasteurized by the batch method. H. B. Naylor

467. The use of the vacreator for high temperature pasteurization of ice cream mixes. G. H. WILSTER, Oregon State College, Corvallis. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., **2:** 24–40. Oct., 1948.

The use of the vacreator for pasteurizing cream and ice cream mix and the application of the vacreator for condensing milk and ice cream mix was discussed. Mixes pasteurized by the vacreator process had lower bacterial counts, lower viscosities, better flavor and better whipping properties than control mixes pasteurized by the vat method. The finished ice cream had a better body and fresher flavor than ice cream from the vat-pasteurized mixes. H. B. Naylor

468. Some factors influencing shrinkage in ice cream. J. J. SHEURING, Univ. of Georgia, Athens. Ice Cream Rev., **32,** 8: 44, 129–133. Mar. 1949.

Factors studied which were found to increase the amount of vacuum-induced shrinkage of batch-frozen ice cream were: (a) Increasing the fat content of the mix, particularly within the range of 12 to 15% fat. (b) Use of sweet cream as compared with similar mixes prepared with butter or frozen cream. (c) Neutralization of high acid mixes with either sodium bicarbonate or magnesium carbonate increased the amount of shrinkage over that observed with low acid mixes to which no neutralizing agent had been added. (d) Reconstituting a dehydrated mix and freezing immediately resulted in more shrinkage than when reconstituted mixes were aged overnight prior to freezing. Storage of dehydrated mix for a period of 40 d. had no significant effect on shrinkage when reconstituted, provided the reconstituted mix was aged overnight before freezing. (e) Freezing of unaged mixes was found to increase the amount of shrinkage; however, no advantage was observed in aging mixes longer than 48 hr.

Any factor which will tend to produce large air cells and large ice crystals will reduce shrinkage, whereas small air cells and small ice crystals will increase shrinkage in ice cream. W. J. Caulfield

469. Forsøg med Emulgatoren "Creminovo" i Flødeis. (Experiments on the emulsifying agent "Creminovo" for ice cream purposes.) English summary. O. S. HANSEN, Ladelund. Nord. Mejeri Tid., **14,** 4: 8–10. 1948.

Experiments in ice cream making have been undertaken with a new synthetic emulsifying agent "Creminovo" manufactured by Emulsion, Ltd., Juelsminde. Creminovo is a fine, very light, yellowish powder resembling egg yolk and, like the latter, containing about 55% fat. It is easily soluble in water at 60–70° C. but possible clots cannot be stirred easily into the ice cream compound any more than clots of milk powder and egg yolk, for which reason Creminovo preferably should be mixed with milk powder, sugar and gelatin before being stirred into the ice cream compound. By such procedure it is dissolved easily at a temperature of 65–70° C. within 10

to 20 min. Creminovo increased the viscosity and whipping ability of the compound and produced an ice cream that was less watery and "cold" and had a more velvety and fat consistency. The effect was in all respects equal to that of the same quantities of egg yolk and an emulsifying agent. G. H. Wilster

470. The use of emulsifiers in ice cream P. H. TRACY, Univ. of Illinois, Urbana. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., **2:** 53–67. Oct., 1948.

See abs. no. 376, p. A78.

471. Controlling viscosity in chocolate ice cream mixes. C. D. DAHLE, W. R. DAVEY AND W. D. SWOPE, Pennsylvania State College, State College. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., **2:** 5–14. Oct., 1948.

The results of studies dealing with the effect of processing methods, type of stabilizer and type of cocoa on the viscosity of chocolate mixes were presented.

When gelatin was used as the stabilizer, mixes flavored with domestic cocoa had much higher viscosities than mixes flavored with Dutch cocoa. The viscosity of mixes containing domestic cocoa could be controlled satisfactorily by reducing the homogenizing pressure or by standardizing the acidity with sodium bicarbonate. Raising the homogenizing temperature increased the viscosity somewhat with both types of cocoa.

Dariloid as the stabilizer usually caused excessive viscosity when the mixes contained Dutch process cocoa. This condition was not corrected by standardization of mix acidity with sodium bicarbonate, or by the addition of stabilizing salts. The viscosity of mixes containing domestic cocoa was not excessive, but increased when soda or a stabilizing salt was added.

Cellulose gum gave results similar to those obtained with Dariloid. Mixes containing Dutch process cocoa had extremely high viscosities when this stabilizer was used. A satisfactory mix could be prepared using domestic cocoa. Chocolate mixes require less stabilizer than plain mixes, due to higher total solids content. H. B. Naylor

472. Galliker packs pints in bowls in a premium promotion. ANONYMOUS. Ice Cream Trade J., 45, 3: 42, 85, 86. Mar., 1949.

Premium promotion as a means of selling ice cream has been used successfully by the Galliker Dairy Co., Johnstown, Pa. The premium consists of a kitchen bowl. The color of the bowl and flavor of ice cream are changed monthly. The wholesale price to dealers for this item is $1.76 per gallon, the retail price to consumers is 37 cents per pint. The dairy pays 6 cents for the bowl and 22 cents for the ice cream less carton cost. The bowls are packed in a 5.5 × 5.5 × 2.625 in. knock-down style carton, which eliminates wrapping at the store. W. H. Martin

473. Current sales trends. ANONYMOUS. Ice Cream Trade J., **45,** 3: 46, 88. Mar., 1949.

Jan., 1949, ice cream sales were about 4% below Jan., 1948, for the country as a whole. Areas hit by heavy snow showed a loss of about 14% and the west coast was down 33%. Some areas in the east had slight losses and some areas showed a gain in Jan. sales. W. H. Martin

474. Supermarkets. M. M. ZIMMERMAN, Editor and Publisher, Super Market Merchandising. Ice Cream Trade J., **45,** 3: 74, 104–106. Mar., 1949.

Supermarkets have increased from 1,200 in 1936 to 12,000 in 1948, doing nearly 30% of the grocery store volume, with an average store volume of $643,000 per yr. Stores attract from 25,000 to 50,000 customers per wk. The average gross mark-up is 17 cents on the dollar and the average net, 1 to 2 cents on the dollar. Eighteen hundred to 3,000 items, each in its own package, are handled. Markets with two to four frozen food cases for dairy foods are common. Under such conditions the style, design and type of package is of great importance in merchandising ice cream. W. H. Martin

Also see abs no. 441, 454.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

475. Three day delivery. ANONYMOUS. Milk Dealer, **38,** 6: 76–78. Mar., 1949.

Indianapolis dealers operating under the 3-day delivery plan report as follows: On wholesale sales, three had an increase up to as high as 9.9%, one had a decrease and one made no comment. On retail sales, five indicated increases up to as high as 7.2%, three reported no change and two had decreases. On gasoline the average was a saving of 6.1%, with a low of 2 and a high of 12.6%. On manpower, seven saved no men and three did save men. Average increase in bottles in service under the 3-day system (nine dealers) was 41%, and average increase in bottle cases in service was 36%. Nine dealers advised no hardship was inflicted in the plant under the new system and eight reported it caused no overtime. Six said it required no additional cooler space while two said it did. C. J. Babcock.

476. Milk strainer. E. ZIKA. U. S. Patent 2,465,623. 1 claim. Mar. 29, 1949. Official Gaz. U. S. Pat. Office, **620,** 5: 1453. 1949.

This strainer, designed for placement on a milk can, has a throat which has a flat perforated plate for supporting the filter medium. The outside of the throat is tapered and fits snugly into the neck of the can. Vacuum is applied to the can through a tube built in the strainer which hastens the rate of straining. R. Whitaker

477. Tote box. C. W. PRAEGER, H. BLUM AND H. JOCHIMSEN. (Assigned to Sturdibilt Milk Box Corp.) U. S. Patent 2,464,343. Mar. 15, 1949. Official Gaz. U. S. Pat. Office, **620,** 3: 840. 1949.

A box for holding milk bottles and paper cartons of milk for convenient handling in milk plants and on delivery vehicles is described. The upper edges of the ends of the box are indented and bent outward to facilitate handling.
R. Whitaker

478. Apparatus for collecting liquid sediments. N. FERRAEZ. U. S. Patent 2,463,481. 4 claims. Mar. 1, 1949. Official Gaz. U. S. Pat. Office, **620,** 1: 313. 1949.

This device is designed to draw quickly and easily a sample of milk from the bottom of a can and pass it through a sediment pad. The apparatus is operated by vacuum which is applied by actuating a foot-operated valve. R. Whitaker

479. Tillsats av bikarbonat till homogeniserad grädde. (The addition of sodium bicarbonate to homogenized cream.) ANONYMOUS. Mejeritekniska Medd., No. 5–6: 83. Dec., 1948.

Homogenization stabilizes the dispersion properties of fat so that the formation of a cream plug is hindered. At the same time the stability of the protein is reduced and coagulation is favored. As coffee contains a substance that favors coagulation, the homogenized cream sometimes is curdled when added to coffee. To prevent this, about 30 g. $NaHC0_3$ can be added per 100 l. cream. Citrate or Na_2HPO_4 also can be used for this purpose. G. H. Wilster

Also see abs. no. 432, 435, 436, 437, 438, 482.

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

480. Forsatte undersøkelser over innholdet av A-vitamin og karotin i norsk smør og mjølk. (Further investigations on the content of vitamin A and carotene in Norwegian milk and butter.) English summary. HARALD HVIDSTEN, LIS GILBE HANSTEEN AND GERD BROCH. Meieriposten, **37,** 11: 186–192. Mar., 1948.

As a supplement to earlier investigations, some determinations of carotene and vitamin A in 33 samples of butter from cow's milk, five from goat's milk and two from sheep's milk have been carried out. The carotene content of some of the feeding stuffs used for the production of the butter samples also has been determined. The determinations of carotene and vitamin A have been effected by photometric methods. The samples of butter from cow's milk stem partly from pasture (cultivated pasture and highland pasture) and partly from barn feeding, in which the carotene content of the daily ration ranges from 33 to 1,360 mg.

The carotene content of butter samples varied from 1.2 to 7.6 γ/g. of butterfat (2–12.7 I.U. vitamin A), the vitamin A content from 14 to 32 I.U. and total computed content of vitamin A (vitamin A effect) from 16 to 42 I.U./g. of butterfat. The following correlation was found between mg. of carotene in the daily ration (x) and: (a) total I.U. of vitamin A effect per g. of butterfat (Y): $Y = 20.7 + 0.0129$ x; $r = 0.69$; (b) total I.U. of vitamin A effect in milk per day (Y): $Y = 10200 + 8.6$ x; $r = 0.78$; (c) the vitamin A effect in daily milk yield in % of the daily carotene content in feed (Y): $\log Y = 2.2074 + 0.7204 \log x$.

In butter from goat's milk, 40 to 66 I.U of vitamin A were found per g. of butterfat. The carotene content was small. In butter from milk of sheep, 30 I.U. of vitamin A were found per g. of butterfat.

Determinations of carotene and vitamin A directly in milk have given results which were in good agreement with the determinations in butter. G. H. Wilster

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

481. The sterilization of milk bottles at farms and dairies. S. B. THOMAS, Natl. Agr. Advisory Serv., Crosswood, D. GRIFFITHS, T. LEWIS, K. J. MORGAN, AND E. P. DAVIES, Natl. Milk Testing Serv., Brynawel, Aberystwyth. Proc. Soc. Applied Bact., **1947,** 1: 6–12. 1947.

A large number of bottles examined after hand washing, washing by rotary spray machines or straight-through machines revealed that a large number contained over 600 bacteria per bottle, a number which was "unsatisfactory" according to prescribed standards. Causes for the high counts usually were the result of using incorrect

alkalinity of insufficient time in and temperature of the detergent. Dairies using steam sterilization showed lower counts on bottles than those using chlorine. M. L. Speck

482. Seasonal variation in the sterility of washed milk churns. G. ELLIS JONES, Natl. Agr. Advisory Serv., Crosswood, Aberystwyth. Proc. Soc. Applied Bact., **1947,** 1: 13–14. 1947.

During a 4-year study, a peak in the number of bacteriologically unsatisfactory milk cans (rinse count $>$ 250,00 when dry or $>$ 50,000 when wet) occurred during June–Sept. A minor peak also was observed in Feb., this peak becoming less marked with improved sanitation. The following were considered causes for the summer peak: (a) higher bacterial content of the milk residues left in the cans during the warmer weather; (b) less careful washing during the period of maximum milk intake at the dairies; (c) shortage of milk cans during the rush season which led to the use of old, dented and rusty cans; (d) the use in some instances of old and inefficient can washers which are brought to use in the rush season. The winter peak was thought to be caused by the higher thermoduric bacterial content of milk as reported by some to be present in winter. M. L. Speck

483. We have made progress. RAY TARDIFF, Breyer Ice Cream Co., Philadelphia, Pa. Ice Cream Trade J., **45,** 3: 89–92. Mar., 1949.

The dairy industry committee has made much progress in plans to standardize all equipment used for the production, transportation and processing of milk so that it is easy to clean, operate and maintain. Standards for equipment are developed and submitted to the Dairy Industry Committee for approval and then to the Committee on Sanitary Procedures of the International Association of Milk and Food Sanitarians and to the Milk and Food Unit of the U. S. Publ. Health Service. Then a joint meeting of these three groups is held to arrive at standards.

Standards have been developed for centrifugal and positive rotary pumps, sanitary fittings, storage tanks, weigh cans and receiving tanks for raw milk. Tentative standards are being considered for milk transportation tanks, can washers, homogenizers and high pressure pumps, gauges for milk storage tanks, dairy ware, milk pails, strainers, milking machines, electric motors, plant heat exchangers, surface type heat exchangers and tubular type heat exchangers. Work also has been started on cabinets and soda fountains.

W. H. Martin

484. Studies of quaternaries as bactericides. G. A. WEBER, U. S. Pub. Health Service, Cincinnati, O. J. Milk and Food Technol., **12,** 2: 107. Mar.–Apr., 1949.

More information on the quaternary compounds as bactericides is urged by the author. Since these compounds are surface-active, they are inactivated readily by anionic agents such as soaps and synthetic detergents. The amphoteric nature of protein foods enhances their absorption by forming a film over the surfaces, thus protecting the bacteria beneath the film. Anionic agents can break up this film readily.

In order to evaluate the germicidal efficiency of quaternary compounds, more satisfactory activators are needed as compared to those used on chlorine. The author suggests additional studies, such as the efficiency of quaternaries against pathogenic bacteria and viruses, a chemical measure for effective quarternary residual, more information on interfering substances in natural waters, the longevity of the germicides in the rinse vat and suitable concentration levels for adequate disinfection. H. H. Weiser

485. Studies on high temperature dishwashing. W. L. MALLMAN AND D. KAHLER, Dept. of Bact. and Pub. Health, Michigan State College, East Lansing. J. Milk and Food Technol., **12,** 2: 105. Mar.–Apr., 1949.

The destruction of *Micrococcus caseolyticus* used as the test organism was made on four leading makes of single-tank conveyor curtain rinse dishwashing machines. The authors suggest that anything less than 99.5% kill of the organism studied failed to meet this requirement. A minimum standard of performance is (a) temperature, 170° F., (b) exposure, 10 sec., (c) 1.5 gal. of water per 20 in. tray or 9 gal. per min.

Single-tank machines should maintain a wash temperature of 160° F. and rinse at 170° F. for maximum efficiency. In other types of machines equipped with rinses, the temperature should be set at 170° F. for 10 sec. or held at a range between 140 and 160° F. H. H. Weiser

486. Insect and rodent control. E. M. SEARLS, Sealtest, Inc., New York, N. Y. Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 44th Ann. Conv., **2:** 87–94. Oct., 1948.

See abs. no. 414, p. A85.
Also see abs. no. 435, 437, 478.

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