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THE SPECTROPHOTOMETRIC DETERMINATION OF THE COLOR OF MILK

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In the commercial manufacture of evaporated milk, considerable effort is given to the production of milk of uniform quality throughout the year. While ideas on quality vary, flavor, color and viscosity generally are regarded as the chief factors in quality rating.

In order to have a record of quality ratings, some method of measurement must be used which can be related to an accepted standard. Viscosity can be determined easily, but color and flavor have been difficult to rate, since no convenient or wholly satisfactory standards have been available. In most laboratories color and flavor remain a matter of the personal judgment of the inspector. However, color can be referred to known standards. The purpose of this paper is to report on the spectrophotometer as a means of evaluating the color of evaporated milk and related products.

METHODS AND APPARATUS

Some years ago Webb and Holm (4) and more recently Bell and Webb (1) measured the color produced in the processing of evaporated milk by means of the Munsell system of disc colorimetry. This system is relatively convenient, inexpensive and fairly accurate in its specifications of color. However, its lack of high sensitivity excludes it from the measurement of the minute changes in color which accompany variations in the heat processing of milk, especially those changes occurring at the lower temperatures, *e.g.*, at 220° F.

In recent years several spectrophotometers of relatively low cost have been introduced, and among these the Beckman provides a reflectance attachment for measuring the color of opaque solids. This attachment is so designed that it easily can be adapted for the measurement of opaque liquids such as milk.

Since no containers for liquids were included in the equipment, it was necessary to construct them in the laboratory. The containers were constructed from tin plate, were circular in shape, 0.5 inch in depth and 1.125

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inches in diameter. The reference standard ordinarily used is a magnesia block, but some difficulty was experienced in obtaining a block of uniformly high reflectance. Hence, for the data herein reported, the standard used was one of the sample cups filled with reagent grade magnesium carbonate. For simplicity, this standard was considered as having a reflectance of 100 per cent. A weighed amount of milk was used in order to insure a constant depth of milk in the cup. Since the surface of the reference magnesia standard and the surface of the liquid should be at the same level for accurate comparison, the surface of the standard was lowered to the level of the milk by the insertion of a metal plate with a 1-inch diameter circular opening and proper thickness between the top of the cup and the retaining plate. Measurements then were made as usual over the wave front of the instrument.

EXPERIMENTAL

The experimental part of this work consisted in comparing the reflectance of milk samples before and after processing with the reflectance of the

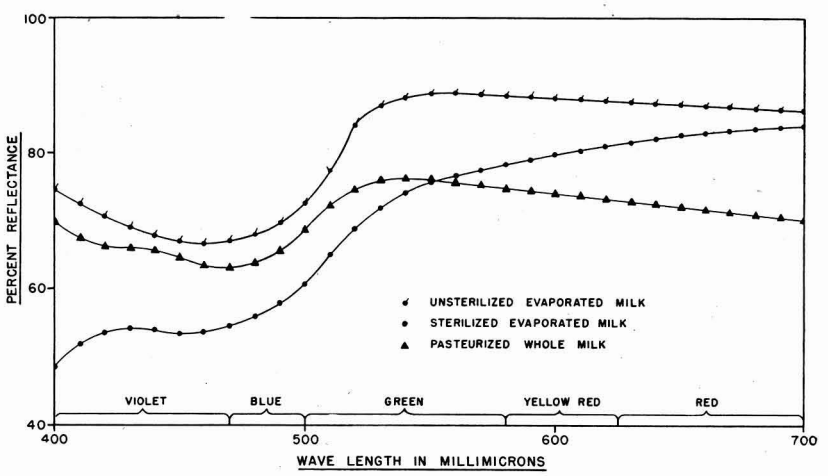

FIG. 1. Variations in reflectance values of pasteurized whole milk, sterilized milk and unsterilized milk between 400–700 mμ.

magnesia standard. These samples consisted of pasteurized milk, unsterilized evaporated milk, sterilized evaporated milk, and six lots of evaporated milk which had received varying preheater and sterilization treatments.

A comparison of the color of pasteurized milk with unsterilized evaporated milk and sterilized evaporated milk is shown by the curves on figure 1. The color difference between the three curves is indicated by their relative positions. The brightness of each color is indicated by the average level of the curve, *e.g.*, the color of unsterilized evaporated milk is the brightest.

with the pasteurized milk and the sterilized milk averaging about the same. An accurate statement regarding hue shift is possible only on the basis of colorimetric data to be calculated from these curves. The saturation of each color is indicated by the relative slope of the curves; *e.g.,* the pasteurized milk curve is the flattest, therefore the least saturated, while the other curves are steeper and therefore more saturated.

It should be noted that the spectrophotometer provides a means for the analysis of the spectral composition of a color sample, while the visual impression is the effect produced on the observer by the combined effect of the spectral composition of the sample, the spectral composition of the illuminant under which the sample is viewed, and the observer's own visual mechanism (which is more receptive to wave lengths in the middle portion of the visible spectrum than those on either end).

With regard to the data plotted on figure 1, it is interesting to note that concentration of milk produces an increase in reflectance in the green, yellow, and red wave lengths but little change in the blue and violet. There

TABLE 1

Conversion of curve data into I.C.I. and Munsell notation

Curve	I.C.I. color notation			Munsell color notation		
	x	y	Y	Hue	Value	Chroma
Pasteurized milk	0.3330	0.3470	0.738	7.3Y	8.8	1.7
Unster. evap. milk	0.3315	0.3435	0.853	5.8Y	9.3	1.55
Ster. evap. milk	0.3435	0.3500	0.738	1.7Y	8.8	2.3

is a possibility that the decrease in the blue-violet region is produced in the forewarming and evaporation processes.

Sterilization of milk produces a marked decrease in reflectance at all wave lengths, especially marked in the violet region. This inequality in reflectance loss is the primary reason for the brown appearance of sterilized evaporated milk, since the result is a relative increase in red and yellow and not an actual increase in these colors.

The conversion of the spectrophotometric data into the I.C.I. (2) and Munsell notation (3) is given in table 1. The Munsell values derived are in good agreement with those obtained by Bell and Webb (1) on evaporated milk. Therefore, it appears that no serious error is introduced by the fluorescence of riboflavin or other compounds.

In the routine grading of freshly sterilized evaporated milk, it is desirable to know the relative color of milk in terms of a simple index number. While this index number cannot represent accurately the true color, it can indicate the direction of shift in hue, brightness and chroma and thus afford to the inspector a quick estimate of the change in color. Since the

heat treatment of milk produces a loss of reflectance, especially marked in the green region, a wave length of 520 mμ appears particularly suitable for routine work on standard evaporated milk, since small visual changes give large instrumental readings. Furthermore, no special light bulb is needed, since the ordinary light source is relatively strong at this wave length.

The data plotted on figure 2 provide a comparison of the spectral composition of sterilized and unsterilized milk over a greater range than that provided by the data in figure 1. In this second experiment it was desirable to measure the degree of darkening, from a visual point of view, produced in the high temperature pretreatment of evaporated milk and in the subse-

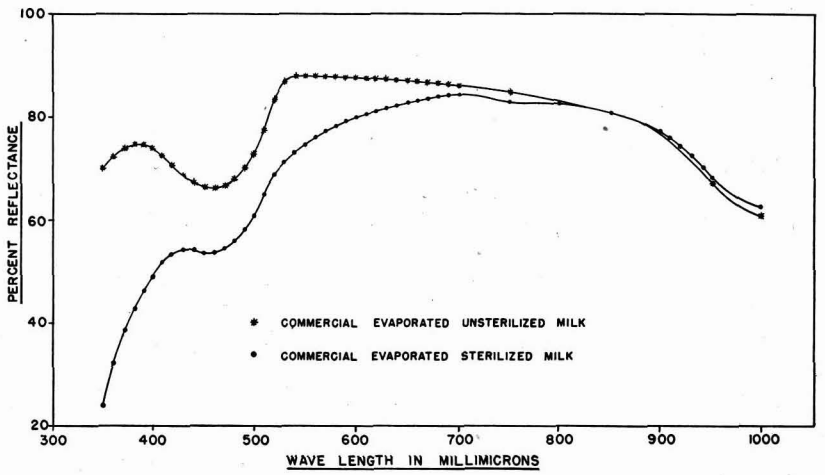

FIG. 2. Variations in reflectance values of sterilized and unsterilized milk between 350–1000 mμ.

quent sterilization. Since these data are from pilot plant research on high temperature-short time sterilization, a note of explanation is offered.

The data are presented because they represent extreme time-temperature variations in treatment, interesting for the purpose of illustrating the effect on color values. A brief outline of the data follows: The treatment raised the temperature of the milk to the desired temperature in a few seconds. The pretreatment time (table 2) refers to the time the evaporated milk was held at the given temperature in a jacketed holder. The corresponding reflectance value was that taken after rapid cooling of the milk.

The cooled evaporated milk was filled into cans of 14.5-ounce capacity. The filled cans then were closed and treated in an experimental continuous sterilizer as follows: The cans were conveyed through a pre-heating chamber, the temperature of which increased at a uniform rate from 225° F. at the portal of entrance to 235° F. at the portal of exit of the cans. The cans of

TABLE 2.

The effect of pretreatment and sterilization on the reflectance value of milk
(Sterilization time was 4.7 min.)

Group	Lot no.	Pretreatment temp.	Pretreatment time	% Reflectance 520 mμ	Sterilizer temp.	% Reflectance 520 mμ	Viscosity M.U.[a]
		(°F.)	(min.)		(°F.)		
I	A	215	0	78.8	261	67.7	92
	B	"	8	78.3	260	68.2	50
	C	"	16	77.5	255	68.2	70
	D	"	24	75.9	254	66.2	85
	E	"	32	74.4	255	67.7	32
II	A	220	0	80.2	262	68.7	47
	B	"	5	80.1	256	66.5	48
	C	"	10	78.4	259	67.7	66
	D	"	15	77.4	255	64.5	80
	E	"	20	73.8	252	65.4	66
III	A	230	0	80.2	261	69.6	41
	B	"	4	79.3	259	66.2	25
	C	"	8	77.6	259	65.4	70
	D	"	12	75.7	254	62.2	125
	E	"	16	77.5	252	62.2	106
IV	A	240	0	76.9	260	68.0	39
	B	"	3	78.0	259	64.8	20
	C	"	6	73.9	259	61.6	68
	D	"	9	73.5	254	58.6	105
	E	"	12	71.2	252	62.2	52
V	A	250	0	77.6	262	69.0	19
	B	"	2	75.7	259	58.4	50
	C	"	3.5	75.4	257	59.8	50
	D	"	5	71.8	254	57.2	15
	E	"	6	71.2	262	60.7	37
VI	A	260	0	75.6	260	67.3	22
	B	"	0.5	74.4	262	58.4	18
	C	"	1.0	75.0	262	59.2	20
	D	"	1.5	66.0	254	51.3	12
	E	"	2.0	67.7	.....	57.3	15

[a] M.U. = (Centipoise + 10)/1.9 (approximate).

milk then were conveyed through a second chamber, where they were subjected to the indicated temperature for 4.7 minutes. After this sterilization treatment the samples were cooled in the usual manner and tested for reflectance loss and viscosity. Viscosity values are in terms of Mojonnier units. There was no "burn-on" or other abnormality which would affect viscosity or color values.

Some discrepancies may be noted in the data. These could well be due to variations in the color of the original milk used and to some unavoidable departures from the temperatures given. In the case of the sterilized product, variations in the rate of cooling affected the color. In any case deviations from the expected color are not large when considered from the standpoint of visual perception, except for some notable exceptions in Groups V and VI. In these latter groups considerable unexplained variation was found in the sterilizing and color characteristics of the various lots of milk.

SUMMARY

1. The Beckman spectrophotometer provides basic data for spectral composition of energy reflected from a sample and when it is combined with standard colorimetric data (as the I.C.I. Standard Observer and one of the I.C.I. Standard Illuminants) it provides a good means of estimating the color.

2. A convenient index for routine estimations of the darkening in color of evaporated milk can be determined by noting changes in reflectance of light of 520 mμ wave length.

The author is indebted to Mr. Paul C. Wilbur, A. E. Pech and Dr. C. R. Stumbo for their valuable suggestions and criticism.

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THE COLOR OF EVAPORATED MILK WITH RESPECT TO TIME AND TEMPERATURE OF PROCESSING

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It is well known in the evaporated milk industry that the color of evaporated milk can be improved by using a high-temperature-short-time sterilization process. Recently, Tarassuk (3) showed that color in evaporated milk could be reduced a significant amount by reducing the oxygen content of the milk before sterilization. However, there is almost no information available, except that given by Bell and Webb (1), on the rates of color formation at the various sterilization temperatures.

Information on the rate of color development always has been desirable, but until recently no entirely satisfactory method has been available. In this paper the technique of color measurement used by Nelson (2) is applied to the investigation of the rates of color formation during the processing of evaporated milk.

METHOD AND APPARATUS

The apparatus consisted of a thermostatically controlled oil bath, a preliminary heating oil bath maintained at 175° F., 75 mm. × 10 mm. test tubes, a wire tray for holding the tubes, a cold water bath for cooling the tubes quickly after heating and a Beckman spectrophotometer for reflectance measurements.

Because of the small milk sample used, a small container made from plastic was used instead of the larger container used by Nelson (2). Tests were made to insure comparableness of the two containers.

One and one-half milliliters of commercial unsterilized evaporated milk of 26 per cent total solids content was inserted carefully into the small tubes with the aid of a hypodermic needle. The tubes were sealed over a small pointed flame, the hot tip being drawn into a loop so that it could be suspended on a wire and placed in the wire basket.

The desired number of tubes filled with the evaporated milk was placed in the basket and held in the preliminary oil bath for 3 minutes before immersion in the constant temperature process bath. After immersion in the process bath, tubes were withdrawn at stated intervals, cooled in the water bath, dried, numbered and later analyzed.

EXPERIMENTAL

The data obtained in this work are represented graphically in figures 1 to 5.

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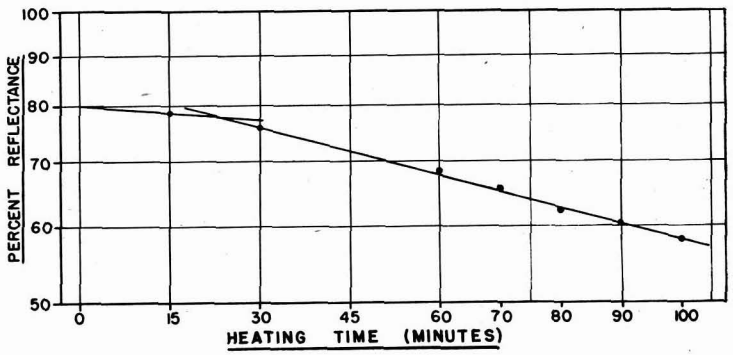

FIG. 1. The relationship between time of heating at 220° F. and reflectance at 520 mμ wave length.

The preliminary heating of the tubes to 175° F. is not essential but it is convenient, since the time then necessary to arrive within a degree of the desired temperature in the process oil bath is reduced to approximately 3 minutes, as determined by thermocouple measurements and the well known logarithmic nature of the heat penetration curve. Consequently, zero time in this experiment is 3 minutes after immersion in the process oil bath.

The data are plotted on semi-logarithmic paper, since it was found that a straight line was obtained if the logarithm of the reflectance was plotted against time.

Some reflectance loss is noted at zero time at 250° F. (fig. 4). However, all the data are plotted without correction, since the lag in the reflectance loss at the lower temperatures, or the rate of loss, is so low that a measurable reflectance loss cannot be found for several minutes. In any case, the error in zero time does not affect the slope of the curves, although it

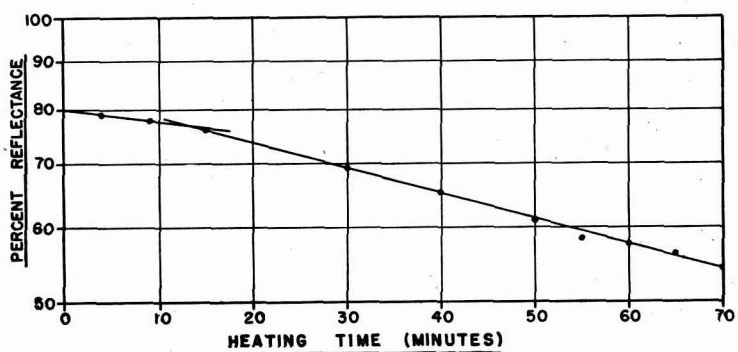

FIG. 2. The relationship between time of heating at 230° F. and reflectance at 520 mμ wave length.

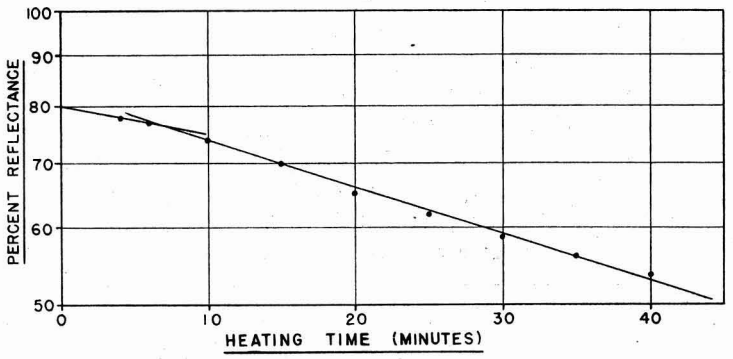

FIG. 3. The relationship between time of heating at 240° F. and reflectance at 520 mμ wave length.

does affect, to a minor degree, the values of the 250° F. curve. However, if it is desired to correct for this loss it seems not unreasonable to assume that projection of the curve until it crosses the 80 per cent line will give the time—about 30 seconds in this case—which should be added to the time plotted. (The reflectance of the original milk was 79.8 per cent at 520 mμ wave length.)

Plotting the data on semi-logarithmic paper was found advantageous. The data for the 250° F. curve are represented by a single straight line, while the data for the other curves are represented most conveniently by two straight lines. While the data for the short curves are inconclusive in determining the character of the curves, they are represented as straight lines for convenience and also to indicate the change in slope of the longer curves. In any event, there is a lag in the darkening in color of evaporated milk during processing, a situation also noted by Townley and Gould (4), who found that a visible color change occurred at the time of marked de-

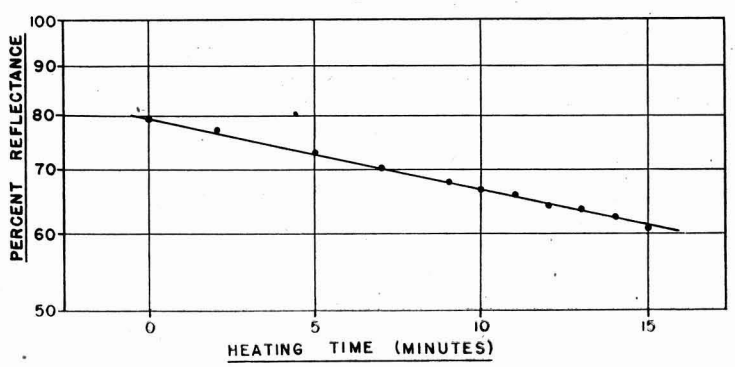

FIG. 4. The relationship between time of heating at 250° F. and reflectance at 520 mμ wave length.

crease in labile sulfur liberation. Whether this point of decrease marks a decided increase in the oxidation-reduction potential has not been determined, but in view of the effect of oxygen on color, it may be significant.

Curves 1 and 2 on figure 5 were derived from the slopes of the curves marked 220°, 230°, 240° and 250° F. The numerals on Curve 1 indicate the time in minutes at these particular points for which this curve is valid. After this time period, values should be selected from Curve 2.

In connection with this experiment, it should be noted that the ratio of volume of air to milk is greater than in commercial canning. Therefore,

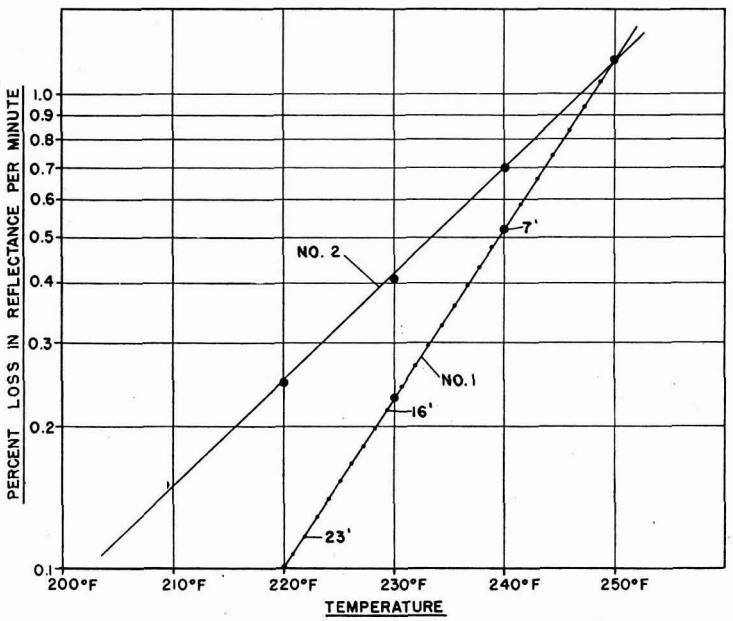

FIG. 5. The relationship between temperature and reflectance loss per minute.

it is quite possible that Curve 1 represents the condition of minimum lag period likely to be encountered in commercial practice. On the other hand, Curve 2 normally will represent the conditions prevailing after the lag phase of sterilization is concluded.

The data obtained in this experiment find application in the color evaluation of sterilization processes. While it is difficult to arrive at absolute values because of the variables introduced by the pretreatment a milk receives, the relative color values of processes can be determined with reasonable accuracy. Given the heat penetration curve of a process, a new curve can be constructed by substituting rate of reflectance loss values for temperature and integrating graphically the curve produced. For example, it will be found that the color produced in a commercial cooker

process of 15 minutes at 243° F. is greater than a comparable process of 6 minutes at 254° F. Not only is the high temperature process short, but the reflectance loss values are relatively low, since a large part of the process occurs in the lag phase of the curve.

SUMMARY AND CONCLUSIONS

1. The loss in reflectance at the temperatures studied decreased logarithmically with time after a lag period.

2. A lag period in reflectance loss was noted at temperatures below 250° F. The character of this curve is not known with certainty.

3. The data obtained are applicable to the color evaluation of sterilization processes.

The author is indebted to Mr. Paul C. Wilbur, A. E. Pech and Dr. C. R. Stumbo for their valuable suggestions and criticism.

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EFFECT OF RAW SOYBEANS AND OF SOYBEAN OIL ON PLASMA CAROTENE AND ON VITAMIN A AS MEASURED BY ACTIVATED GLYCEROL DICHLOROHYDRIN[1]

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Interference with vitamin A metabolism in dairy cows fed soybeans and soybean products has been reported previously. Hauge *et al.* (3, 4) were among the first to demonstrate a factor in soybeans that suppressed the transfer of the vitamin A potency of the ration to the milk fat. These workers found that the factor could be removed from soybean oil by adsorption on activated charcoal. Although their data showed no lowering of carotene, they stated that carotene as well as the vitamin A values may be lowered by additions of large amounts of either soybeans or soybean oil to the ration. Cannon *et al.* (1) observed a bleaching effect on the milk fat of cows fed raw soybeans. Their observations were based on color comparisons rather than chemical determinations of vitamin A and carotene. Shaw *et al.* (6) recently reported the occurrence of a vitamin A deficiency in dairy calves from dams fed raw soybeans.

The effects of raw soybeans and soybean oil on the blood plasma carotene and vitamin A concentrations of lactating cows fed alfalfa hay, silage, concentrates and a carotene supplement are reported herein.

EXPERIMENTAL

Procedure. In this feeding trial either 9 lb. of raw soybeans or 1.7 lb. of expeller-process soybean oil, an amount calculated to be equivalent to the oil supplied by the raw beans, were incorporated into the rations of dairy cows to test their effect on the concentrations of blood plasma carotene and vitamin A. This quantity of raw soybeans, based on previous experiments (1), was selected as the probable maximum amount that could be fed daily over a prolonged experimental period.

Six Holstein cows were divided into two comparable groups. One of three experimental rations was assigned at random to each cow of Group I; these rations were duplicated for Group II. The daily feeding schedule indicating the concentrates fed is presented in table 1.

In addition to the concentrates, all cows were fed a poor quality alfalfa hay throughout the trial. Corn silage was provided for the first 6 weeks of the experimental period, at the end of which time the supply was exhausted and alfalfa hay became the only roughage. Before the trial started it was found that the cows selected had plasma carotene levels that were

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less than 300 γ per 100 ml. Since it was desired to conduct the studies with cows having more plasma carotene than this, so as to allow sufficient latitude for a possible depression, each cow also received daily both before and during the trial 0.5 lb. of a carotene preparation[2] containing 250,000 USP units of vitamin A per pound. This 0.5 lb. of carotene preparation was mixed with 2 lb. of the basal concentrate mixture and fed each cow

TABLE 1

Daily concentrate feeding schedule of the three cows in each of the two groups during each period

Cow no.	Group no.	Basal concentrate mixture[a] (*lb.*)	Materials tested (*lb.*)
		Basal period (2 weeks)	
1947	I	14.0	.....
2470	I	13.0	.....
2379	I	15.5	.....
2210	II	14.0	.....
2392	II	15.0	.....
2472	II	14.0	.....
		Experimental period (9 weeks)	
1947	I	5.0	9.0 Raw soybeans
2470	I	11.3	1.7 Soybean oil
2397	I	15.5	Control
2210	II	5.0	9.0 Raw soybeans
2392	II	13.3	1.7 Soybean oil
2472	II	14.0	Control
		Cross-over period (4 weeks)	
1947	I	14.0	Control
2470	I	11.3	1.7 Soybean oil
2397	I	6.5	9.0 Raw soybeans
2210	II	14.0	Control
2392	II	13.3	1.7 Soybean oil
2472	II	5.0	9.0 Raw soybeans

[a] The basal concentrate mixture consisted of 250 lb. of ground yellow corn, 250 lb. crushed oats, 200 lb. linseed oil meal, 100 lb. wheat bran, 9 lb. common salt, and 16 lb. bone meal.

between the morning and evening feeding periods apart from the soybean products in order to avoid possible *in vitro* destruction of the carotene (2).

The trial was initiated with a 2-week basal period during which the plasma of each cow was characterized for its carotene and vitamin A content. An experimental period of 9 weeks followed the basal period. At the end of the 9-week experimental period the rations of the cows fed the control diet and those fed the raw soybeans were switched (table 1). This cross-over period was continued for 4 weeks. All other experimental

[2] "Super Carex", a carrot oil preparation taken up in a dry carrier, was obtained from Nutritional Research Associates, Inc., South Whitley, Indiana.

conditions were maintained with these two groups. The cows receiving the soybean oil, however, were continued on their starting experimental ration throughout the trial.

Venous blood samples were collected weekly from each cow and were analyzed immediately for vitamin A and carotene contents. Sufficient blood was drawn to supply duplicate 9-ml. plasma samples. Kimble's (5) procedure was used for extracting the vitamin A and carotenoids from the plasma. Five-milliliter portions of these extracts were used for determining the carotenoids. The per cent transmission readings obtained with a Coleman Universal Spectrophotometer set at 440 mμ were converted into carotene values by means of a standard curve.[3] A new reagent, activated glycerol dichlorohydrin (G.D.H.), was used to determine the vitamin A of the blood plasmas. G.D.H. was selected in view of the potential advantages of this colorimetric reagent (7).

For the determination of vitamin A, 12-ml. portions of the plasma extracts were placed into 50-ml. centrifuge tubes. These tubes were heated in a water bath, which at no time exceeded 65° C., to evaporate the solvent. Immediately following the removal of the solvent, the tubes were cooled to room temperature and the residue in each tube dissolved in 1.5 ml. of chloroform. One milliliter of each chloroform solution was transferred to a Coleman cuvette and 4 ml. of G.D.H. added. The contents of the cuvette were mixed by inversion and the color allowed to develop for 4 minutes in the dark at room temperature, after which the readings were made with the spectrophotometer. The transmission readings were converted into vitamin A values by means of the standard curve and then corrected for carotene interference.

Validity of the reagent used for the determination of vitamin A. Sobel and Werbin (8) previously have shown G.D.H. to be satisfactory for the determination of the vitamin A of fish oils. Since information on the applicability of G.D.H. for the determination of vitamin A of bovine blood plasmas was unavailable, it was necessary to ascertain its validity for the type of study reported herein.

A series of recovery studies was made on pooled samples of bovine plasmas containing from 400 to 550 γ carotene per 100 ml. Natural vitamin A ester was added at four different levels and 95.9 to 100.0 per cent recovery was obtained using G.D.H. as the colorimetric reagent. These results indicate that vitamin A could be determined satisfactorily with G.D.H.

[3] A Coleman Universal Spectrophotometer, Model 11, was used for all analyses. It previously was standardized at 440 mμ with crystalline B carotene for estimating the carotenoids, and at 550 mμ with a natural vitamin A ester, PC 3 capsule, obtained from Distillation Products, Inc., for estimating the vitamin A. A carotene interference curve was plotted from data obtained by the addition of G.D.H. to crystalline B carotene in chloroform.

TABLE 2

The effect of raw soybeans and soybean oil on plasma carotene and vitamin A

Weeks	Group I						Group II					
	Control		Raw soybeans		Soybean oil		Control		Raw soybeans		Soybean oil	
	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A	Carotene	Vit. A
	(γ/100 ml.)						(γ/100 ml.)					
Basal period												
1	.....	.....	343	30.0	394	37.4	367	31.2	425	30.3	446	37.6
2	307	43.2	338	37.9	422	36.3	388	42.0	442	34.8	458	39.0
Experimental period												
1	365	47.3	324	45.5	422	34.9	430	45.0	420	40.9	418	47.0
2	408	45.6	317	36.8	420	53.0	437	32.3	367	39.8	449	35.1
3	550	33.4	398	43.7	518	30.0	499	37.2	386	45.8	480	30.4
4	528	31.0	346	26.0	382	30.1	422	30.5	314	34.4	473	31.0
5	504	39.2	350	40.0	396	33.2	422	33.0	259	42.6	514	32.5
6	480	40.0	420	41.6	458	41.8	420	33.7	317	34.8	593	41.0
7	662	34.6	420	24.4	509	26.5	535	30.9	341	33.6	533	29.2
8	662	47.0	425	36.0	550	45.4	535	45.3	427	45.0	480	39.3
9	571	37.0	437	40.3	566	41.2	564	45.4	403	39.5	490	40.6
Cross-over period[a]												
1	427	38.1	502	26.3	599	24.7	482	27.3	461	34.7	456	22.1
2	398	25.0	542	25.8	518	21.1	427	32.1	470	26.0	446	26.5
3	432	27.8	553	39.4	542	36.6	432	40.2	490	44.7	384	31.0
4	418	31.2	533	35.5	499	28.3	391	33.9	494	34.0	434	44.1

[a] The cows fed soybean oil were not switched but continued on the same ration in this period.

RESULTS AND DISCUSSION

The effects of feeding raw soybeans or soybean oil were measured by the changes that occurred in the concentrations of blood plasma carotene and vitamin A found in the cows fed these products. These changes also were compared with those that occurred in the blood plasma carotene and vitamin A of the cows fed the control ration.

In table 2 are listed the amounts of carotene and vitamin A that were found in the blood plasma of each of the cows at weekly intervals. In order to get a clearer picture of the changes that occurred in the concentration of carotene in the blood plasma of the cows under the three feeding

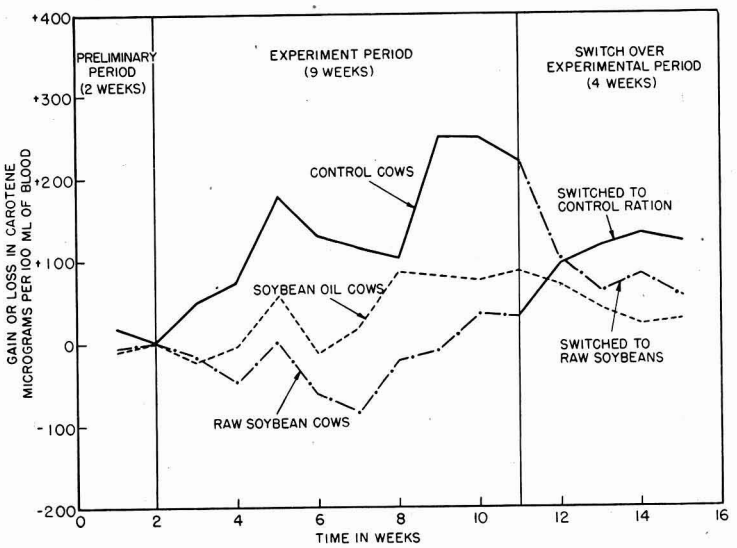

FIG. 1. Variations from the final determinations in a preliminary period, of average blood plasma carotene content for control cows, cows fed raw soybeans and cows fed soybean oil.

schedules, the data were plotted out as shown in figure 1. The zero point on these curves is the average concentration of carotene in the blood plasma at the end of the preliminary period for cows fed each feed. The changes in carotene concentration are plotted from that point.

It is apparent from the curves that the feeding of raw soybeans to cows caused their plasma carotene to decline somewhat in concentration and to remain considerably under that of cows fed the control ration. During the first 4 weeks the differences between these two lots of cows increased rapidly, then more slowly up to 7–9 weeks, when differences in concentration between the control cows and those fed raw soybeans seemed to level off at about 250 γ carotene per 100 ml. of blood plasma.

That this difference in carotene concentration was effected by feed and not by cow differences is strongly supported by what happened when the feed of these cows was switched. Those cows formerly being fed raw soybeans and then fed the control ration showed increasing concentrations of carotene from the time the rations were changed. In opposition, those cows which were changed from the control ration to raw soybeans showed a constantly decreasing carotene concentration in their blood plasma until it was considerably under that of the other group with which its ration was switched. Although the switch-over period lasted only 4 weeks, yet in this time the differences in plasma carotene concentration reached almost one-half the magnitude that existed in a similar period before the switch-over was made.

The changes in blood plasma carotene concentration of the cows receiving soybean oil were intermediate, lying between those of the control cows and those fed raw soybeans. Apparently the oil depressed the plasma carotene concentration but not to the extent of the raw beans. The effects of feeding the oil are not as clear as with feeding raw soybeans, since no switch-over of rations was made with these cows. It is not known whether their position in relation to the control cows would have been reversed had these rations been switched. Presumably such a result would have occurred.

As was noted in outlining the feeding procedure, changes in the kind of roughages that were fed occurred during the progress of the trial. At the end of the sixth week corn silage was eliminated from the ration and alfalfa hay fed in greater amounts. Also, during the fifth week (third week of the first experimental period) all the cows were inadvertently permitted access to fresh grass for approximately 2 hours.

These changes in feed no doubt affected the carotene intake of the cows. Since all cows were fed alike and supposedly increased their carotene intakes together, these changes should have caused no serious influence on the differences in carotene concentrations between groups. Increased or decreased intakes of carotene would cause fluctuations in the plasma carotene, but each group would be affected in the same way.

The differences that occurred in the blood plasma vitamin A concentrations among the cows fed the control, raw soybean and the soybean oil rations (table 2) were small and showed no particular trends. If destruction of vitamin A was being caused by either the raw soybean or soybean oil, the physiological processes of the cows quickly replenished the supply in the blood from carotene or from liver storage. Perhaps if the carotene intake of the cows were low enough, the feeding of raw soybeans and maybe soybean oil would cause a decline in the vitamin A concentration in their blood. Hauge *et al.* (3) have specified a factor that adversely affects the vitamin A concentration in milk fat. This factor might be operative on blood plasma vitamin A.

SUMMARY

Feeding raw soybeans in the amount of 9 lb. daily to lactating cows caused marked differences in their blood plasma concentration of carotene from that of cows fed a control ration containing no soybean products. During the first 4 weeks the differences increased rapidly, but by 7–9 weeks they seemed to level off at about 250 γ carotene per 100 ml. of blood plasma. The reversal in blood plasma concentrations of carotene that took place after a switch-over of rations was made between the cows receiving the control and the raw soybean rations indicates that the causative factor was the feed rather than the individuality of the cows.

The feeding of expeller process soybean oil to lactating cows caused differences in their blood plasma concentrations that were intermediate with the concentrations found in the blood plasma of cows fed a control ration containing no soybeans or soybean products and cows fed raw soybeans. The oil apparently depressed the carotene concentrations but not to the extent of the raw soybeans.

The differences that occurred in the blood plasma vitamin A concentrations among the cows fed the control, raw soybean and soybean oil rations were small and showed no particular trends.

Activated glycerol dichlorohydrin, a new reagent for the determination of vitamin A, proved to be satisfactory for this determination in bovine blood.

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SIMPLE VERSUS COMPLEX CONCENTRATE MIXTURES FOR YOUNG BREEDING BULLS. I. GROWTH, BLOOD COMPOSITION, AND COST[1]

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In a study of the relative value of a simple and a complex concentrate mixture for young breeding bulls, the composition of the whole blood and of the plasma was investigated on the chance that the feeds might reflect different physiological effects upon these tissues. Quantitative studies of various blood constituents have proved invaluable in experimental, diagnostic and clinical work, despite the variability in "normals" observed in different individuals, sexes, species, physiological functions, regions, seasons and climates. Most studies of bovine blood composition have concerned the female rather than the male.

Table 1 summarizes the levels of several constituents of whole blood and plasma of bulls as found in previous studies.

Since the literature involving the relationships of whole blood and plasma constituents of cattle to diet, age, physiological functions and pathological conditions is too extensive to be considered here, recognition has been given only to those data which reflect the blood picture of healthy bulls and which are pertinent to the present study.

The purpose of this study was to ascertain the effects of a simple and a complex concentrate mixture upon growth as determined by wither height and heart girth measurements and upon the concentration of some of the constituents of blood. The cost of maintaining breeding bulls on these feeding regimes was examined.

EXPERIMENTAL PROCEDURE

Sixteen Holstein bulls of similar blood lines were obtained at birth and reared under the same management and feeding regime until the commencement of the experiment. From these animals the 12 bulls used in this investigation were selected at 18 months of age on the basis of uniformity of age, size, and blood and semen pictures. The 12 bulls composed two groups of six each. Group I received a simple concentrate mixture of

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TABLE 1

Summary of previous studies on levels of several constituents of whole blood and plasma in bulls

Investigator	No. of animals	Age	Whole blood			Blood plasma			
			Hb	R.B.C.C.[a]	R.B.C.V.[b]	Ca	Inorg. P	Total proteins	Ascorbic acid
			(%)	(*mill./mm.*^{3})	(%)	(*mg.%*)	(*mg.%*)	(%)	(*mg.%*)
Abderhalden (1)	1	24 mo.	10.64	.....	33.43	7.93	2.73	6.97	.....
Kusner (12)			11.74	8.24	.....	.....	.....	.....	.....
Knoop *et al.* (11)	6	2 days–37 wk.	10.52	9.78	.....	.....	.....	.....	.....
Anderson *et al.* (2)	5	1–5 mo.	11.81	.....	.....	.....	.....	.....	.....
	5	birth to 10 mo.	.....	.....	.....	12.62	3.34	.....	.....
McCay (15)	6	Mature	12.80	.....	.....	.....	.....	.....	.....
Brook and Hughes (5)	24	.....	11.92	.....	.....	.....	.....	.....	.....
Dimock and Thompson (7)	3	.....	10.66	5.81	.....	.....	.....	.....	.....
Lamarre (13)	4	.....	.....	6.50–10.90	.....	.....	.....	.....	.....
Payne *et al.* (18)	45	12 mo.	.....	.....	.....	10.46	7.30	.....	.....
	27	Over 12 mo.	.....	.....	.....	13.03	4.76	.....	.....
Schwob (24)	.....	.....	.....	.....	.....	.....	.....	7.28	.....
Bortree *et al.* (3)	10	.....	.....	.....	.....	.....	.....	.....	0.19–0.39
Hamersma (9)	1	26 mo.	12.79	.....	.....	11.40	7.2	.....	.....
	1	27 mo.	12.18	.....	.....	10.60	7.5	.....	.....
Phillips *et al.* (19)	22	.....	.....	.....	.....	.....	.....	.....	0.27

[a] Red blood cell count.
[b] Red blood cell volume.

which corn and corn gluten meal constituted a large portion, while Group II was fed a complex concentrate mixture (table 2). Both groups received the same average grade timothy-clover hay. The average composition of the concentrate mixtures and hay used during the feeding trial is shown in table 3.

The bulls were fed 1 lb. of hay per 100 lb. body weight daily with concentrate feed in sufficient quantity to provide an average daily digestible nutrient intake of approximately 1.02 and 1.18 lb. per 100 lb. body weight before and after an average age of 760 days, respectively. Feed intake was adjusted at average intervals of 43 days following heart girth and

TABLE 2

Composition of concentrate mixtures

Ingredients	Group I	Group II
	(%)	(%)
Ground yellow corn	54.0	10.0
Beet pulp	25.0	.....
Corn gluten meal	10.0	.....
Cane molasses	10.0	10.0
Linseed meal	.....	12.0
Soybean meal	.....	17.0
Crushed oats	.....	25.0
Wheat bran	.....	10.0
Dehydrated alfalfa	.....	10.0
Limestone	.....	2.0
Iodized salt	1.0	1.0
Bone meal	.....	0.7
Brewers yeast	.....	1.95
Mineral salt mixture[a]	.....	0.1
Fish liver oil[b]	.....	0.2
Irradiated yeast[c]	.....	0.05
	100.00	100.00

[a] Mineral salt mixture consisted of: $MnSO_4.4H_2O$, 50%; $FeSO_4.7H_2O$, 44.5%; $CuSO_4.5H_2O$, 5.0%; and $Co(NO_3)_2.6H_2O$, 0.5%.

[b] Fish liver oil containing 15,000 I.U. or more of vitamin A per g.

[c] Irradiated yeast containing 9,000 USP units of vitamin D per g.

wither height measurements. The average daily digestible nutrient intake immediately following body measurement was 1.08 and 1.22 lb. per 100 lb. body weight before and after an average age of 760 days, respectively, and regressed to about 0.96 and 1.11 lb., respectively, before the next adjustment as calculated from Morrison's tables (17). Body weight was calculated from the heart girth measurement according to the equation suggested by Branton and Salisbury (4).

An attempt was made to obtain about the same amount of semen from the bulls of each group; however, more was being taken from Group II during the latter part of the experiment than from Group I. The average daily semen volume and accumulative semen volume taken from the two groups are represented by the graphs and curves, respectively, shown in

TABLE 3

Average chemical composition of feeds (per cent of dry matter)

Group	Protein	Fat	Fiber	Ash	N.F.E.	P	Ca	Mn
				Concentrate mixtures				
I	12.00	2.72	8.70	4.73	64.47	0.30	0.33	0.0044
II	21.76	4.67	10.08	9.03	49.03	0.66	0.97	0.0233
				Hay				
	6.44	2.24	38.73	4.85	44.02	0.15	0.30	0.0053

figure 1, heart girth measurements also being included. The difference in the accumulative semen volume does not indicate that the bulls of Group II were capable of producing greater volumes of semen but merely that more semen was taken from these animals than from the bulls of Group I.

No data were obtained on the semen of one bull in Group II, since this animal manifested a fear which precluded obtaining semen from him in the usual manner. One bull was eliminated from Group I about mid-trial because of tuberculosis reaction. The animals were 18 months old at the beginning and 33 months old at the termination of the experiment. Twenty days was the greatest difference between the ages of any of the bulls.

The data on the phase of the study dealing with the blood constituents have been grouped into 3-month age periods for convenience of study. The

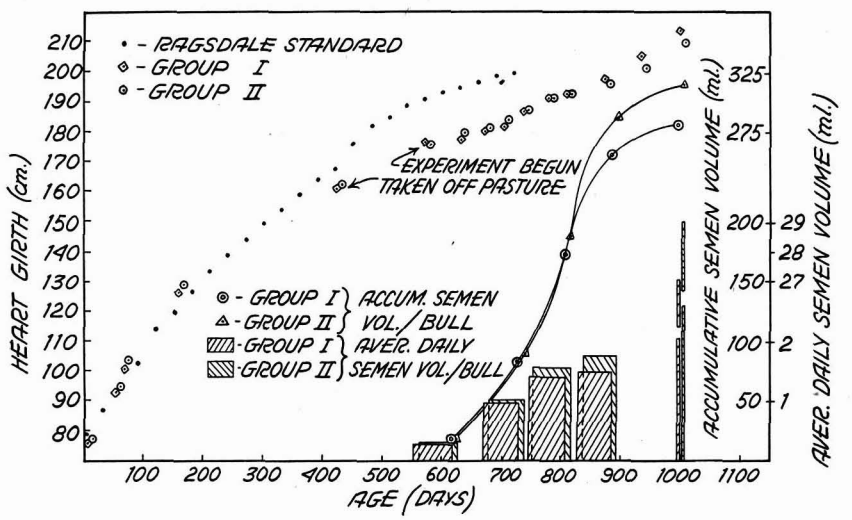

FIG. 1. Heart girth measurements of bulls, the Ragsdale standard (20) for Holstein bulls, and the average daily and accumulative volumes of semen produced per bull.

chemical methods used to determine the levels of certain blood and plasma constituents are as follows: hemoglobin, Sanford *et al.* (23); glutathione, Woodward and Fry (26); calcium, Clark and Collip (6); inorganic phosphorus, Fiske and Subbarow (8); phosphatase, method of King and Armstrong (10) as modified by Wiese *et al.* (25); plasma proteins, albumin and globulins, Looney and Walsh (14); and ascorbic acid, modification of method of Mindlin and Butler (16). The red blood cell count and volume (hematocrit) were determined on the same blood samples according to the standard procedures.

RESULTS

Growth. Good growth of bulls was effected by both feeding regimes once the retarded growth which was incurred on the late fall pasture

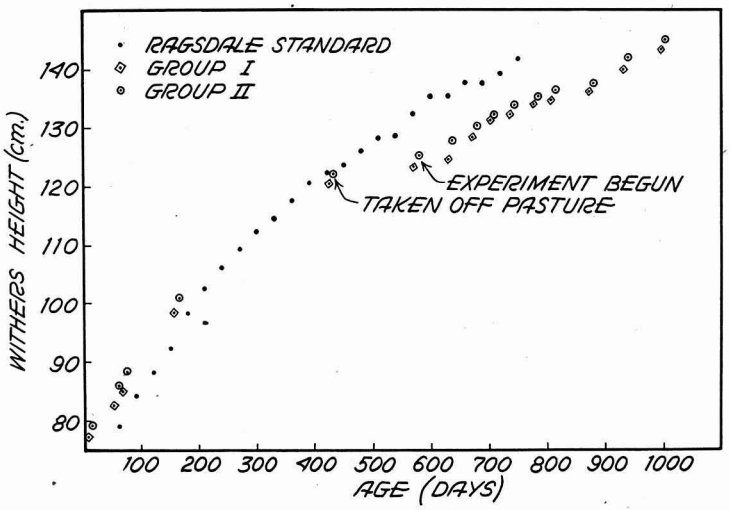

Fig. 2. Withers height measurements and the Ragsdale standard (20) for Holstein bulls.

prior to the commencement of the feeding trials was overcome (figs. 1 and 2). Semen production did not appear to affect growth (fig. 1). A study of the body measurements of the bulls of both groups would indicate a slightly more rapid growth of these animals during the actual feeding trial than is considered standard for Holstein bulls of the same age (20). It should be pointed out, however, that the standard proposed by Ragsdale (20) is based upon the measurements of only two bulls subsequent to 540 days of age. Group I animals gained body weight at an average rate of 0.18 lb. per day faster than Group II bulls. The rate of increase in height at the withers was similar for both groups (Group I, 1.57 and Group II, 1.54 mm. per day), as shown in figure 2. A similar general appearance and degree of fleshiness was observed in the animals of both groups.

TABLE 4

Blood constituents at various ages

Constituent	Group[a]	Age (mo.)									
		18–21		21–24		24–27		27–30		30–33	
		No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content
Hb (gm.%)	I	12	12.07 ± 0.31	18	12.70 ± 0.29	23	13.50 ± 0.25	10	14.16 ± 0.31	15	14.49 ± 0.21
	II	12	12.32 ± 0.26	18	13.15 ± 0.16	24	13.40 ± 0.13	12	14.09 ± 0.13	18	15.16 ± 0.16
R.B.C.C.[b] (mill./mm.^{3})	I	12	9.88 ± 0.38	18	9.56 ± 0.35	23	8.88 ± 0.25	10	8.85 ± 0.47	15	8.83 ± 0.31
	II	12	9.98 ± 0.36	18	9.62 ± 0.24	24	8.62 ± 0.18	12	8.55 ± 0.25	18	9.36 ± 0.22
R.B.C.V.[c] (%)	I	12	35.98 ± 1.60	18	33.59 ± 0.88	23	36.67 ± 0.78	10	37.74 ± 1.03	15	40.12 ± 0.77
	II	12	35.76 ± 1.17	18	34.45 ± 0.52	24	35.58 ± 0.49	12	37.54 ± 0.86	18	40.26 ± 0.78
Mean corpuscular Hb (yy)	I	12	12.22	18	13.28	23	15.20	10	16.00	15	16.41
	II	12	12.34	18	13.67	24	15.55	12	16.48	18	16.20
Mean corpuscular volume (μ^3)	I	12	36.42	18	35.14	23	41.30	10	42.64	15	45.44
	II	12	35.83	18	35.81	24	41.28	12	43.91	18	43.01
Reduced glutathione (mg.%)	I	12	28.66 ± 0.66	17	30.17 ± 1.20	23	34.79 ± 1.05	10	35.51 ± 2.12	15	41.12 ± 1.47
	II	12	27.47 ± 1.38	18	31.22 ± 1.35	24	35.06 ± 1.02	12	34.40 ± 1.45	18	41.86 ± 1.21
Oxidized glutathione (mg.%)	I	12	4.94 ± 0 53	17	8.84 ± 0.47	.....	.....	.....	.....	10	5.60 ± 0.83
	II	12	6.54 ± 0.57	18	7.99 ± 0.49	.....	.....	.....	.....	12	6.95 ± 0.43
Total glutathione (mg.%)	I	12	33.60 ± 0.71	17	39.01 ± 1.18	.....	.....	.....	.....	10	45.27 ± 2.13
	II	12	34.01 ± 1.45	18	39.21 ± 1.24	.....	.....	.....	.....	12	48.64 ± 1.64

[a] Group I received simple concentrate mixture and mixed hay.
Group II received complex concentrate mixture and mixed hay.

[b] Red blood cell count.

[c] Red blood cell volume.

TABLE 5

Plasma constituents at various ages (mean and standard error)

Constituent	Group[1]	Age (mo.)									
		18-21		21-24		24-27		27-30		30-33	
		No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content	No. cases	Content
Ca (mg.%)	I	12	8.79 ± 0.22	18	10.49 ± 0.15	23	10.60 ± 0.10	10	11.02 ± 0.16	10	11.65 ± 0.38
	II	12	9.37 ± 0.22	18	10.09 ± 0.11	24	10.37 ± 0.09	12	10.61 ± 0.09	12	11.52 ± 0.74
Inorg. P (mg.%)	I	12	8.26 ± 0.18	18	7.76 ± 0.26	23	7.61 ± 0.21	10	7.13 ± 0.14	15	7.16 ± 0.24
	II	12	7.56 ± 0.24	18	7.78 ± 0.13	24	7.23 ± 0.26	12	6.86 ± 0.26	18	7.34 ± 0.18
Phosphatase, acid (units/100 ml.)	I							5	2.32 ± 0.18	10	1.43 ± 0.29
	II							6	1.72 ± 0.20	12	1.36 ± 0.19
Phosphatase, alkaline (units/100 ml.)	I					5	8.06 ± 0.39	10	6.09 ± 0.59	10	11.18 ± 0.56
	II					6	9.53 ± 1.81	12	7.61 ± 1.67	12	12.15 ± 0.88
Total proteins (g.%)	I	6	7.55 ± 0.06	18	6.81 ± 0.10	23	7.35 ± 0.07	10	6.80 ± 0.13	10	6.90 ± 0.10
	II	6	7.68 ± 0.17	18	6.99 ± 0.07	24	7.21 ± 0.08	12	6.90 ± 0.10	12	6.90 ± 0.07
Albumin (g.%)	I					5	2.91 ± 0.13	5	5.21 ± 0.08	9	4.05 ± 0.14
	II					6	4.14 ± 0.36	6	5.18 ± 0.13	12	4.02 ± 0.16
Globulins (g.%)	I					6	4.76 ± 0.25	5	1.82 ± 0.12	14	2.65 ± 0.14
	II					6	3.31 ± 0.35	6	1.93 ± 0.14	18	2.71 ± 0.11
Ascorbic acid (mg.%)	I	12	0.20 ± 0.01	18	0.28 ± 0.03	23	0.32 ± 0.02	10	0.37 ± 0.02	15	0.27 ± 0.02
	II	12	0.26 ± 0.02	18	0.27 ± 0.02	24	0.29 ± 0.01	12	0.34 ± 0.03	18	0.24 ± 0.01

[1] Group I received simple concentrate mixture and mixed hay.
Group II received complex concentrate mixture and mixed hay.

Blood composition. Remarkably similar values were found for various constituents and characteristics of the blood and plasma of the animals of both groups during the same age period. These data are summarized by groups in tables 4 and 5. Since no appreciable group differences were observed, the average data from both groups would seem to be normal for bulls of similar age. Although diet did not appear to influence the composition of the blood of these animals, various trends were observed which appeared to be associated with aging.

The concentration of hemoglobin, the red blood cell volume, and the mean corpuscular hemoglobin and volume gradually increased, whereas the number of erythrocytes decreased very little as age progressed from 18 to 33 months. Other constituents tending to increase with age were plasma calcium and reduced and total glutathione. The plasma level of inorganic phosphorus tended to decrease, whereas no definite relationship between the plasma concentration of ascorbic acid and age was observed.

The variations in alkaline plasma phosphatase were attributed to the rate of semen collection as reported previously (22). Likewise, the fluctuation in the levels of albumin and globulin may have been related to the production of semen.

Data on total and oxidized glutathione are not given for the periods 24 to 27 months and 27 to 30 months because estimations of this compound could not be obtained. Inability to measure this compound was concomitant with an increased rate of semen collection and appeared to be caused by a factor(s) existing in blood plasma under these conditions which prevented the reduction of oxidized glutathione by metallic zinc (21).

Cost. A comparable gain in body weight cost approximately 50 per cent more in Group II than in Group I. The average cost of maintaining a bull on the Group II regime was $52.75 more per year than that of a bull receiving the other diet.

DISCUSSION

No important differences were found in the growth, general health, and blood constituents of two groups of breeding bulls receiving markedly different concentrate feeds. On the basis of these criteria, it would seem that costly, complex concentrate mixtures are not necessary for animals of similar age and producing semen at similar rates. The final evaluation of complex feeds, however, necessarily lies in their effects upon the production of fertile semen. A subsequent report in this series will consider the production of semen by the bulls used in this study.

Although this investigation revealed no group differences, various trends appeared to be associated with aging. In view of the lack of dietary influence, the concentration of some blood constituents investigated in this study would appear to be standard for bulls of similar age and breed when maintained under climatic conditions similar to those of northern New Jersey (tables 4 and 5).

The results of this study would seem to indicate the importance of the rumen in the nutrition of the bull. Regardless of the supposed limitations of the simple concentrate feed received by Group I animals, these bulls were able to maintain, at levels similar to those of bulls receiving a more complex diet, not only growth but also blood constituents believed to be indicative of physical well being. It should be pointed out that these relationships may not necessarily hold for bulls of greater age or for the same bulls over a longer period of time, as these data were obtained from bulls during the interval of 18 to 33 months of age. The importance of the poor to average grade hay fed to both groups may be underestimated in these considerations. Since hay and concentrates were fed in a manner believed to be consistent with good feeding practice, and since the same hay was fed to both groups, the main considerations involved comparisons of the effects of the two concentrate mixtures. Additional data dealing with the merits of these diets are presented in the subsequent paper on semen production of young bulls.

Although the cost of maintaining a sire by the ordinary breeder is of no great significance if satisfactory performance is being obtained, large bull studs such as those used in some artificial breeding units would effect a considerable saving by using simple concentrate mixtures similar to the one employed in this study rather than complex, high protein mixtures.

SUMMARY

1. Comparable rates of growth and concentrations of several blood constituents were found in bulls receiving a simple and a complex concentrate mixture.

2. Since the levels of certain blood and plasma constituents were similar for the two groups, these figures are presented as standards for healthy Holstein bulls of similar age and producing semen at similar rates.

3. The hematocrit, mean corpuscular hemoglobin and volume, the level of hemoglobin, reduced and total glutathione, and plasma calcium tended to increase with aging, whereas the plasma concentration of inorganic phosphorus decreased.

4. The maintenance of bulls on the complex concentrate feed cost approximately 50 per cent more than that of bulls receiving the simple concentrate mixture.

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SIMPLE VERSUS COMPLEX CONCENTRATE MIXTURES FOR YOUNG BREEDING BULLS. II. SEMEN PRODUCTION[1]

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The comparative merits of simple and complex concentrate mixtures for semen production by bulls have not been ascertained previously. Various investigations, however, have demonstrated the efficacy and the lack of effect of certain feeds and specific nutritional factors upon the quantity and quality of semen ejaculated by bulls. Jones *et al.* (12) showed that rations which are satisfactory for normal growth to 3 years of age are adequate for normal reproductive performance. Recent Cornell investigations (6, 26) in which total digestible nutrient levels of 100, 120, and 140 per cent of the Morrison dry cow maintenance requirements (21) were fed to breeding bulls demonstrated that neither the quantity and quality of semen produced nor the fertility of the bulls was related to the digestible nutrient intake within the limits studied. Concentrate mixtures containing 12, 16, and 20 per cent total protein did not affect significantly the fertility of bulls (6, 26). However, bulls receiving the 20 per cent protein concentrate produced significantly greater concentrations of spermatozoa and lower ejaculate volume and motility and less total spermatozoa per ejaculate than did bulls receiving the other concentrate mixtures. For bulls in active service, these workers (6, 26) suggested feeding at the rate of 1 lb. of hay and 0.4 to 0.5 lb. concentrate mixture containing 12 per cent protein per 100 lb. body weight daily.

Jones *et al.* (11) found that bulls fed alfalfa hay supplemented with 1 lb. each of skim milk powder and oats groats daily grew faster, matured earlier, were in better condition, and produced good quality semen earlier than bulls receiving a basal ration of hay supplemented with salt, phosphorus and iodine. These differences were attributed to the greater energy intake rather than to the quality or quantity of protein ingested.

Since it was not possible in this experiment to use the semen from the unregistered bulls for breeding purposes, a number of measures of semen quantity and quality were employed as criteria of the relative merits of the two concentrate mixtures fed. Numerous reports support the use of the following tests of semen quality and quantity as an evaluation of rela-

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tive fertility: spermatozoa concentration (15, 16, 23, 29, 32, 34); initial motility (9, 15, 16, 20, 28, 30, 31); livability or maintenance of motility (1, 10, 17, 20, 30, 31, 34); pH (2, 3, 4, 8, 9, 10); change in pH upon incubation (1, 4, 16); number of morphologically abnormal spermatozoa (1, 10, 15, 19, 32, 36); semen and spermatozoa volume (1, 3, 7, 16, 32); semen level of ascorbic acid (8, 13, 14, 22); and reducing capacity (5, 27, 28, 33).

It was the purpose of this study to evaluate the relative merits of a simple and a complex concentrate mixture for the production of semen by young bulls as determined by various tests for semen quantity and quality.

EXPERIMENTAL PROCEDURE

A study of the semen obtained from the two groups of bulls (one receiving a simple concentrate mixture and the other a complex mixture) was made simultaneously with the investigation of the blood composition, growth, and cost of maintenance of these animals reported in the first paper of this series (25). For details on the composition of feeds, rate of feeding, age and growth of the bulls, and the cost of the concentrate feeds, the reader is referred to the previous paper (p. 429). Of the 12 animals composing the two groups (reported in the previous paper), five in each group yielded semen during the entire experiment.

Semen quantity and quality were studied during four periods ranging from 56 to 65 days in length, separated by rest periods of 14 to 51 days, as shown in table 1. These collection periods loosely represent the four seasons.

The quantity of semen produced was expressed as the number of ejaculates obtained, the average ejaculate volume, and the average daily semen volume per bull. The quantity of spermatozoa was determined by direct counts using a cytometer and by centrifugation of semen in hematocrit tubes. These data were expressed as the concentration of spermatozoa, the proportion of the whole semen volume consisting of spermatozoa, and the average size of a spermatozoan in terms of volume (cubic micra).

Since a constant temperature stage incubator was found to be necessary but was not obtainable during the first period, no attempt was made to procure data on motility and allied characteristics for this period. During subsequent periods, the initial motility, the motility at intervals following the initial estimation, and the livability of spermatozoa were determined by means of a constant temperature stage incubator adjusted to a temperature of 100° F. The data on motility are expressed in terms of arbitrary units derived from separate estimations of the number of living spermatozoa and the spermatozoa engaged in progressive motility. The motility units used here would be approximately equivalent to motility data expressed in terms of per cent ÷ 5. Livability of spermatozoa was calculated as the percentage of the initial motility persisting at 100 hours subsequent to ejaculation.

TABLE 1

Semen characteristics and constituents

	Period no.		I	II	III	IV	Summary[b]
	Period date		3/5–5/9/46	6/29–8/28/46	9/12–11/14/46	12/5/46–1/30/47	3/5/46–5/22/47
	Av. beginning and terminal ages of bulls	I[a]	556–621	672–732	747–810	831–887	556–998
		II[a]	564–629	680–740	755–818	839–895	564–1006
Volume	Av. ejaculate volume (ml.)	I	2.95(40)[c]	3.52(110)	3.70(144)	4.13(101)	3.82(423)
		II	3.18(33)	3.79(91)	4.15(120)	4.44(111)	4.19(383)
	Av. daily semen volume/bull (ml.)	I	0.30	1.07	1.41	1.49	.....
		II	0.32	1.15	1.58	1.76	.....
Spermatozoa	Av. spermatozoa conc. (mill./mm.^{3})	I	0.735(25)	0.891(41)	1.026(52)	0.823(47)	0.869(193)
		II	0.775(23)	0.962(34)	1.020(45)	1.087(47)	0.950(177)
	Av. % spermatozoa[d]	I	.....	6.62(31)	7.44(51)	9.24(42)	7.84(124)
		II	.....	6.54(24)	7.48(45)	9.56(42)	8.06(111)
	Av. spermatozoan volume (μ^3)	I	.....	84.03(30)	78.22(51)	105.26(42)	88.87(123)
		II	.....	72.86(24)	77.63(45)	90.63(42)	81.52(111)
Motility	Initial motility[e]	I	.....	13.31(36)	13.17(52)	11.05(47)	11.98(163)
		II	.....	12.76(29)	13.29(45)	12.72(47)	12.61(149)
	Motility at 100 hours[f]	I	.....	2.55(34)	5.65(52)	7.54(47)	5.53(133)
		II	.....	2.49(27)	4.51(45)	7.77(46)	5.32(118)
	Livability at 100 hours[g]	I	.....	17.23(34)	35.46(52)	58.49(47)	38.94(133)
		II	.....	19.68(27)	31.82(45)	52.93(46)	37.27(118)
Reducing substances	Total reducing substances (mg.%)	I	30.32(19)	37.30(41)	36.48(47)	26.47(21)	34.18(128)
		II	33.55(17)	37.94(34)	41.15(41)	34.79(25)	37.75(117)
	Reducing substances in oxidized state (mg.%)	I	15.89(13)	14.07(41)	13.55(38)	5.84(21)	12.58(113)
		II	18.40(10)	13.55(33)	15.13(33)	6.83(23)	13.01(99)
	Potential reducing capacity (mg.%)	I	43.21(15)	51.37(41)	50.04(38)	32.32(21)	46.39(115)
		II	53.59(12)	50.73(33)	56.05(33)	39.26(23)	50.19(101)
	Ascorbic acid (mg.%)	I	7.01(23)	8.22(41)	8.28(47)	5.17(19)	7.58(130)
		II	8.59(22)	7.96(34)	9.32(40)	7.08(23)	8.36(119)
pH	Initial pH	I	6.86(16)	6.55(34)	6.68(50)	6.70(25)	6.67(138)
		II	6.77(14)	6.57(28)	6.59(45)	6.61(25)	6.62(123)
	Post incubation pH	I	6.56(16)	6.13(34)	6.28(50)	6.45(25)	6.32(138)
		II	6.56(14)	6.19(28)	6.33(45)	6.41(25)	6.35(123)

[a] Roman numerals represent group number.

[b] Summary includes data obtained during entire feeding experiment. (Therefore, data on semen obtained in a special study made of semen phosphatases subsequent to Period IV are included.)

[c] Figures in parentheses indicate number of samples studied.

[d] Represents proportion of total semen volume consisting of spermatozoa.

[e] Estimated at 100° F. Motility value $\times 5$ is approximately equivalent to per cent motility.

[f] Motility rating at 100 hours subsequent to ejaculation (rating $\times 5$ is approximately equivalent to the motility expressed as per cent).

[g] Livability is expressed as the per cent of original motility persisting at 100 hours subsequent to ejaculation.

The reducing substances in semen were measured by a procedure similar to that outlined by Woodward and Fry (37) for the estimation of glutathione in whole blood (24).

Ascorbic acid was determined according to the method outlined by Mindlin and Butler (18).

The pH of semen was measured immediately after ejaculation and after incubation at 37° C. for 1 hour, using a Beckman pH meter equipped with a glass electrode. The decrease in pH effected by incubation was calculated from these estimations.

TABLE 2

Percentages of various types of morphologically abnormal spermatozoa

Abnormality	Group	Period				Av. over 426 days
		I	II	III	IV	
		(%)	(%)	(%)	(%)	(%)
Head						
Pyriform	I	5.92	4.70	4.16	4.99	4.76
	II	4.49	2.31	2.05	1.94	2.48
Tapering	I	2.60	1.04	1.04	0.99	1.28
	II	2.51	0.94	0.75	0.58	1.04
Others	I	0.52	0.79	0.84	1.16	0.84
	II	0.34	0.45	0.76	0.63	0.59
Midpiece						
Filiform	I	0.31	0.46	0.44	0.22	0.37
	II	0.49	0.48	0.32	0.23	0.36
Beaded	I	0.54	1.15	1.59	1.46	1.28
	II	1.93	1.15	1.82	1.25	1.55
Others	I	1.17	2.07	5.03	8.41	4.44
	II	0.57	1.60	4.25	9.64	4.34
Tail						
Coiled	I	0.96	0.46	0.83	2.86	1.21
	II	1.26	0.94	0.63	1.60	1.03
Beaded	I	0.32	0.12	0.35	0.34	0.29
	II	0.45	0.12	0.47	0.44	0.38
Others	I	0.33	0.18	0.59	0.70	0.47
	II	0.56	0.09	0.72	0.62	0.53
Total abnormalities	I	12.67(22)[a]	10.97(33)	14.87(52)	21.13(30)	14.94(137)
	II	12.60(20)	8.08(27)	11.77(46)	16.93(29)	12.30(122)

[a] Figures in parentheses indicate number of samples studied.

Semen smears were prepared for the estimation of morphologically abnormal spermatozoa. Priority was given to the abnormalities in the order listed in table 2 (*i.e.*, a spermatozoan showing both head and tail abnormalities was registered as possessing an abnormal head) in order that the influence of an abnormal spermatozoan would be reflected but once.

RESULTS

The average data for several characteristics and constituents of the semen produced by both groups of bulls at intervals during the 442-day experiment are presented in tables 1 and 2.

The Group II bulls produced slightly larger ejaculates containing a greater total number of spermatozoa than did Group I bulls. No appreciable differences were found in the concentration of spermatozoa or in the proportion of semen constituted by spermatozoa ejaculated by the two groups. The difference in the average spermatozoan volume is largely the reflection of one animal in Group I.

No appreciable differences were found in the initial motility, motility at 100 hours after ejaculation, and livability of spermatozoa of the groups. The improved livability observed in both groups during the colder months may have been a seasonal effect upon this characteristic.

Similar levels of reducing substances, reducing substances in oxidized form, and ascorbic acid were found in the semen obtained from both groups. A marked decrease was observed in the level of reducing substances in oxidized state (which was reflected in the potential reducing capacity) during Period IV.

The initial pH of semen was similar for both groups; however, semen from Group I underwent a greater decrease in pH during incubation than did that of Group II.

The data in table 2 summarize the proportions of the various types of abnormal spermatozoa found in the semen from each group of bulls. Generally, the abnormalities occurred at about the same rate in both groups, with Group I spermatozoa manifesting a greater proportion of the heads of the pyriform type. It will be noted that a greater quantity of morphologically abnormal spermatozoa appeared during Periods III and IV than previously. This may have been effected by the increased rate of semen ejaculation. The differences found between the groups in total abnormalities were attributed largely to one bull in Group I, whose semen contained a characteristically high number of abnormal spermatozoa.

DISCUSSION

In the evaluation of the comparative merits of simple and complex concentrate feeds for breeding bulls, the final conclusion must be based upon the over-all effects of these mixtures upon the character and/or the fertility of the semen produced. Although it has been recognized that no single test presently exists which allows an adequate prediction of the relative fertility of a semen specimen, a combination of tests involving various semen properties and characteristics is believed to contribute valuable information relative to forecasting the impregnating capacity.

In general, the quality of semen produced by the two groups of bulls receiving markedly different concentrate feeds was essentially the same, as determined by various tests. The concentration of spermatozoa in the semen was similar for both groups; however, bulls receiving the complex concentrate mixture yielded ejaculates of larger volume and greater num-

bers of spermatozoa than those ejaculated by bulls consuming the simple mixture. These differences were not regarded as of great importance, since neither group of animals produced semen which was subnormal in these respects. The spermatozoa produced by bulls receiving the two diets possessed similar average degrees of motility and livability, with a slightly higher degree of livability in the semen of bulls receiving the simple mixture during Periods III and IV and in that of bulls receiving the complex feed during Period II.

Various investigators (1, 4, 16) have pointed out the usefulness of the measure of pH change during incubation as an index of semen quality, since this test affords a gross picture of the metabolic activity of spermatozoa, probably involving the effects of spermatozoa numbers, activity and chemical changes. Other studies (1, 10, 17, 20, 30, 31, 35) have demonstrated conclusively the reliability of livability or longevity estimates as forecasters of relative fertility of semen. Because of the strong evidence offered in their support, these two measures were accorded higher recognition as criteria of semen quality than the others used in this study. From this standpoint semen of similar character was produced by the bulls on both feeding programs.

The reducing properties of semen were examined previously and found to be related to the general metabolism of spermatozoa (27, 28, 33) and to states of fertility (5, 27, 28). In view of the results of these investigations, a method was devised for the analysis of semen in which reducing materials probably not measured in previous studies could be accounted for and measured as absolute quantities. No great differences were observed in the quantities of total reducing substances, reducing substances in oxidized form, potential reducing capacity, and ascorbic acid content of the semen of the two groups of bulls.

The small differences observed between the groups relative to the percentage of abnormal spermatozoa were not regarded as important, since both groups appeared to be within a safe range as determined in a very critical examination, and since these differences are explicable on the basis of the consistently high percentage of abnormal spermatozoa shown by one bull in Group I.

Apparently when sufficient energy is provided for growing, breeding bulls, a simple mixture of concentrate ingredients is equivalent to a high protein, complex mixture from the standpoint of the quality of semen produced. Satisfactory growth was found to accompany the production of good semen when the daily digestible nutrient intake was approximately 1.18 lb. per 100 lb. body weight. It is not known whether or not the same results would be found in older bulls or in the same bulls over an extended period of time.

SUMMARY

A study was made of the relative merits of a simple and a complex concentrate mixture when fed with a poor to average grade mixed hay for the production of semen by young bulls during a 442-day experimental period.

Various analyses of 423 ejaculates yielded by the bulls receiving the simple concentrate feed and of 383 ejaculates produced by the bulls consuming the complex concentrate mixture would indicate that good quality semen of similar character resulted from the ingestion of both diets when provided at an average rate of 1.18 lb. digestible nutrients per 100 lb. body weight daily.

Although bulls consuming the complex mixture yielded slightly larger ejaculates containing more spermatozoa per ejaculate and fewer abnormal spermatozoa than those of bulls fed the simple mixture, the decrease in pH upon incubation of semen ejaculated by the latter group was greater than that of the semen produced by the bulls fed the complex concentrate feed.

Regardless of the diet fed, the concentration of spermatozoa in semen, the initial motility, the degree of livability, the size of spermatozoa, the quantity of total reducing substances, reducing substances in oxidized state, potential reducing capacity, ascorbic acid, and the initial pH of semen were similar.

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A STUDY OF THE USE OF THE ANTIOXIDANT NORDIHYDROGUAIARETIC ACID IN DAIRY PRODUCTS. I. ITS ANTIOXYGENIC PROPERTIES IN MILK

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The oxidized flavor is one of the most prevalent off-flavors which develop in market milk. This off-flavor may appear even though the raw milk is of the highest quality and the processing methods are carefully supervised in approved equipment.

REVIEW OF LITERATURE

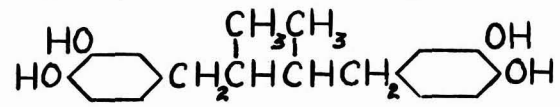
The oxidized flavor in dairy products has been studied extensively, as indicated by the voluminous amount of literature reviewed by Brown and Thurston (4), who cited 412 references.

The compounds or substances proposed as antioxidants for fats are numerous. Matill (9) studied a large number of compounds and found that the active groups of the phenolic compounds were two hydroxyl groups in either the ortho or para configuration. When these groups were in the meta position, the compound did not possess antioxidant properties.

Nordihydroguaiaretic acid (NDGA), one of the compounds which has been used as an antioxidant, was first synthesized in 1918 from hydroguaiaretic acid (12). During cooperative investigations by the United States Department of Agriculture and the University of Minnesota, NDGA was found to occur in a common desert plant, the creosote bush (*Larrea divaricata*), which grows in the southwestern United States (20). Pure NDGA is prepared by crystallization from a crude extract of the plant material.

White, crystalline NDGA is practically odorless but has a slight astringent flavor. It is only slightly soluble in water but is 50 per cent soluble in ethyl alcohol, 20 per cent soluble in propylene glycol, about 15 per cent soluble in glycerol and from 0.3 to 3 per cent soluble in fats and oils (17).

The following chemical formula has been assigned to NDGA (20):



On the basis of Matill's study (9), this phenolic compound would be expected to have antioxygenic properties since the hydroxyl groups are in the ortho position.

Extensive toxicity experiments (5) conducted for over two years indicate that NDGA is entirely harmless (1, 2) in amounts far in excess of

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that required to prevent the oxidation of fat over extended periods of storage.

NDGA has been used successfully in retarding the development of rancidity[1] in lard (6, 7, 8), in bacon (16), and in salt-cured fish (15); in retarding the oxidation of esters of fatty acids (18); in stabilizing carotene in vegetable oil solutions (3, 11); and in retarding oxidative changes in vegetable oils (10) and frozen cream (19).

EXPERIMENTAL METHODS

The milk used in this study was produced by the University dairy herd. In cases where it was important to have milk with little or no metal contamination, the milk was taken directly from the stainless steel milking machines. In other cases, the milk was taken from aluminum milk cans after it arrived at the University Creamery.

Milk samples were scored or criticized for flavor by three or more judges and the consensus taken as the score or criticism. The judges were not aware of the history or treatment of the samples.

Vitamin C determinations were made using the rapid method of Sharp *et al.* (13).

RESULTS

The concentration of NDGA needed for antioxidant protection—the effect of method of adding. Because concentrations of 0.005 per cent were being used successfully in the treatment of fats and oils (1, 6, 7, 8, 10, 11, 15, 16, 18, 19), concentrations of 0.0075 per cent or less, expressed on the basis of the fat content of the milk, were used in this study. The NDGA was added to 4 per cent milk before it was pasteurized at 143° F. for 30 minutes. The development of the oxidized flavor was induced by adding 0.3 p.p.m. copper. The results of a representative trial are found in table 1. The trials were conducted during the period of April through August. A concentration as low as 0.00125 per cent NGDA added either in glycerol solution or in water suspension inhibited the development of the oxidized flavor during 5 days of storage at 40° F. in milk containing 0.3 p.p.m. added copper.

The effect of NDGA on the disappearance of vitamin C in pasteurized milk. The disappearance of vitamin C is reported by Sharp *et al.* (14) to be related to the development of the oxidized flavor in milk. For this reason a series of experiments was conducted to determine whether or not NDGA would retard the loss of vitamin C in milk under normal conditions of storage. Milk was taken directly from the milking machine and samples were prepared containing 0.00125 per cent and 0.0075 per cent NDGA

[1] In other branches of the food industry, the term rancidity usually is used synonymously with the term oxidation. In the dairy industry, oxidation is used to denote oxidative changes in fat, whereas rancidity characterizes hydrolytic changes.

added both in glycerine solution and in water suspension. The milk was pasteurized and cooled. Vitamin C determinations and flavor scores were made every 24 hours. The results of a representative trial are presented in table 2. The trials were conducted during the period of April through August.

The data show that NDGA retarded the destruction of Vitamin C in pasteurized milk stored at 40° F. without added copper. At the end of 96 hours of storage, all of the vitamin C had disappeared in the control samples of the milk which did not contain added copper. At the end of

TABLE 1

The concentration of NDGA needed for antioxidant protection (storage at 40° F.)

Sample no.	Treatment	Flavor comments				
		24 hr.	48 hr.	72 hr.	96 hr.	120 hr.
1	Control	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
2	Control + 0.3 p.p.m. cu.	Sl. cooked Sl. feed	Oxidized 1[a]	Oxidized 2	Oxidized 3	Oxidized 3
3	Control + 0.3 p.p.m. cu. + 0.00125% NDGA in water	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
4	Control + 0.3 p.p.m. cu. + 0.00125% NDGA in glycerol	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
5	Control + 0.3 p.p.m. cu. + 0.0075% NDGA in water	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed
6	Control + 0.3 p.p.m. cu. + 0.0075% NDGA in glycerol	Sl. cooked Sl. feed	Sl. feed	Sl. feed	Sl. feed	Sl. feed

[a] The intensity of the oxidized flavor was given values from 1 to 5, as the level of the defect increased.

the same storage period, the loss of vitamin C in the samples which contained NDGA ranged from 54.9 per cent at the lower concentration (0.00125 per cent) to 41.9 per cent at the higher concentration (0.0075 per cent).

Even though the vitamin C disappeared in 24 to 48 hours in the milk which contained 0.3 p.p.m. added copper, the oxidized flavor did not develop during 5 days of storage in the samples which contained NDGA.

The antioxidant retarded the destruction of vitamin C during pasteurization. In the milk which contained no added copper, 18.7 per cent of the vitamin C was destroyed in the control samples during pasteurization. The loss of vitamin C in the similar samples which contained NDGA ranged from 6.2 per cent at the higher concentration (0.0075 per cent) to 12.5 per cent at the lower concentration (0.00125 per cent).

TABLE 2

The effect of NDGA on the disappearance of vitamin C in pasteurized milk stored at 40° F.

Sample no.	Treatment	Per cent loss of vitamin C						Flavor criticisms					
		0 hr.[a]	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	0 hr.	24 hr.	48 hr.	72 hr.	96 hr.	120 hr.
1	Control	18.7[b]	21.9	25.0	66.8	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
2	0.00125% NDGA	12.5	12.5	15.6	51.6	52.4	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
3	0.00125% NDGA in glycerine	12.5	12.5	12.5	45.6	54.9	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
4	0.0075% NDGA in water	6.2	6.2	15.6	47.8	41.9	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
5	0.0075% NDGA in glycerine	9.4	15.6	15.6	40.7	41.9	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
	0.3 p.p.m. copper added												
6	Control	56.3	84.4	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Oxidized 1[c]	Oxidized 2	Oxidized 2	Oxidized 4
7	0.00125% NDGA in water	56.3	87.5	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
8	0.00125% NDGA in glycerine	53.2	87.5	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
9	0.0075% NDGA in water	49.9	81.2	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed
10	0.0075% NDGA in glycerine	41.8	75.1	100	100	100	100	Sl. feed Sl. cooked	Sl. feed Sl. cooked	Sl. feed	Sl. feed	Sl. feed	Sl. feed

[a] Immediately after pasteurization and cooling to 40° F.
[b] Vitamin C content of raw milk, 14.8 mg./l.
[c] The numbers 1 to 5 indicate increasing levels of oxidized flavor defect.

In the milk which contained 0.3 p.p.m. added copper, 56.3 per cent of the vitamin C was destroyed in the control sample during pasteurization. The loss of vitamin C in the similar samples which contained NDGA ranged from 41.8 per cent at the higher concentration (0.0075 per cent) to 53.2 per cent at the lower concentration (0.00125 per cent).

There was no significant difference between the protective effect exerted by the antioxidant which was added in solution and that which was added in water suspension.

CONCLUSIONS

1. Concentrations of 0.00125 to 0.0075 per cent nordihydroguaiaretic acid will prevent the development of the oxidized flavor during 5 days of storage at 40° F. in whole milk containing 0.3 p.p.m. added copper.

2. In the absence of added copper, the addition of 0.00125 to 0.0075 per cent nordihydroguaiaretic acid will retard the destruction of vitamin C in whole milk stored at 40° F.

3. In the absence of added copper, concentrations of 0.00125 to 0.0075 per cent nordihydroguaiaretic acid will retard the destruction of vitamin C during pasteurization at 143° F. for 30 minutes.

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VITAMIN D CONTENT OF ROUGHAGES[1]

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Very little vitamin D generally is considered to exist in the green growing plant. Ergosterol or some other provitamin is present and may be changed over to vitamin D when the plant is cut and exposed to the radiant energy of the sun, as in the sun curing of roughages. From this viewpoint it could be assumed that flue or barn-cured hay would have less vitamin D than sun-cured hay and that little or no vitamin D would be present in roughages cured in the dark or artificially dried with no sun exposure. However, this is not the case according to results obtained at this Station. Assays made on sun-cured and barn-cured hays show practically equal amounts of vitamin D in the two hays. Furthermore, appreciable amounts of vitamin D are present in hays dried without exposure to the sun, as in a dehydrating machine or natural drying in a dark place.

A few trials at other stations have been reported showing that the nutritive and antirachitic values of barn-cured hay compare favorably with that of sun-cured hay for dairy animals, as based on such criteria as rate of growth, physical condition and analyses of certain bones from slaughtered animals. However, no vitamin D contents of the hays were given.

Wylie *et al.* (8), in a trial with yearling heifers, compared the feeding value of barn-cured hay with that of sun-cured hay. The feeding periods extended through three successive winters, each trial being 150 days in length. Each animal received daily 2 lb. of grain, 10 lb. of corn silage and hay *ad libitum*. The heifers in both groups made normal growth with no marked difference in favor of either.

Moore and Thomas (4) report the results of a feeding trial with dairy calves comparing the antirachitic values of field-cured alfalfa hay, barn-dried alfalfa hay and wilted alfalfa silage. They used three groups of dairy calves, six in each group. The calves were first depleted of their body stores of vitamin D and then fed on the above roughages for a period of 6 months. From the results obtained, it was concluded that further fundamental work is necessary. The indications to date are that barn-dried hay and wilted silage will provide sufficient vitamin D for normal functions in growing calves when fed at the usual levels of roughage feeding, *i.e.*, at the rate of 2 to 3 lb. of hay, or the equivalent, per 100 lb. of body weight.

EXPERIMENTAL

In the fall of 1946 the author started a trial with 16 dairy calves (3 days of age) to compare mainly the antirachitic value of barn-cured hay

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[1] Published with the approval of the Director of the Vermont Agricultural Experiment Station.

with that of sun-cured hay up to first calving. A number of vitamin D assays were made upon the hays, bloods of living animals, and livers of slaughtered animals. At the end of 12 months the animals on barn-cured

TABLE 1

Vitamin D in sun-cured, barn-cured and artificially dried hays

	Av. dry matter	Date sampled	Date assayed	USP units per g. hay		
				First sampling	Second sampling	Third sampling
	(%)					
1946 crop cut 6/27 and 7/1						
Sun-cured—used in trial	89.85					
1st sampling		6/30 and 7/4/46	8/29/46	0.61		
2nd "		1/6/47	1/24/47		0.34	
3rd "		6/19/47	6/25/47			0.32
" "		"	"			0.61
" "		"	12/2/47			0.55
" "		"	12/16/47			0.43
" " av.						0.48
Barn-cured—used in trial	89.61					
1st sampling		7/26/46	8/29/46	0.51		
2nd "		1/6/47	1/24/47		0.33	
3rd "		6/19/47	6/25/47			0.26
" "		"	"			0.18
" " av.						0.22
Artificially dried (Ardrier)	90.50					
1st sampling		6/27 and 7/1/46	12/16/46	1.14		
" "		"	5/22/47	1.40		
2nd "		3/17/47	3/27/47		0.42	
1947 crop cut 7/14, 7/16 and 7/24						
Sun-cured—used in trial	92.19					
1st sampling		7/16 and 7/25/47	8/13/47	2.00		
2nd "		1/14/48	1/27/48		1.80	
Barn-cured—used in trial	90.50					
1st sampling		8/7/47	8/13/47	2.33		
2nd "		1/14/48	1/27/48		2.00	
Artificially dried (Ardrier)	91.95					
1st sampling		7/14 and 7/16/47	8/28/47	0.59		
2nd "		1/14/48	1/27/48		0.75	

hay were fully equal to those on sun-cured hay in rate of growth, activity and physical appearance. This trial is still in progress and the detailed procedure and results will be reported at a later date. The present paper

deals with the vitamin D content of the various lots of hay handled in different ways. Vitamin D determinations were made according to the rat assay method essentially as set forth in the U. S. Pharmacopoeia XII (5). The vitamin content was calculated by establishing an equation for a curve of response essentially as outlined by Coward (3). Tables 1 and 2 give the USP units of vitamin D per gram of material in the various roughages. The three hays of the 1946 crop were from the same cuttings (table 1). Two lots of hay were harvested and in each case representative samples of the three hays were obtained. Lot I was cut 9 a.m. on June 27; the hay for barn curing was hauled in at 2 p.m. June 28, and the sun-cured hay at 4 p.m. June 29. The weather was sunny except for a shower of short duration on the first day. The material for artificial

TABLE 2

Vitamin D in hays cured in the dark (except no. 7)

Plot	Sample	Date cut		Dry matter	USP units
		(1947)		*(%)*	*(per g.)*
A	1	7/3	Plants cut at 5 a.m.	90.92	0.75
	2	7/3	Plants cut at 5 p.m.	91.80	0.54
B	3	8/7	Top of plants, green	91.78	0.84
	4	8/7	Bottom of plants, mostly brown	91.73	1.00
C	5	8/18	Green leaves, hand picked	91.41	0.84
	6	8/18	Brown leaves, hand picked	91.33	1.10
	7	8/18	Hay, whole plant, sun-cured	91.27	2.30

drying was obtained by following the mower and taking a handful of grass every few feet. This was placed in burlap bags so as not to allow any sun exposure. As soon as the mowing was completed, these bags of grass were taken to the barn, chopped and put through the hay drier (Ardrier). The drying involved but a few minutes. The dried material then was spread out on the floor of a dark barn loft for cooling and to complete the drying. Normally a roughage would be wilted until the moisture content was down to around 65 per cent before putting it through the drier. A few days later the material was mixed and sampled. The sample of sun-cured hay was taken from each load as it was hauled in and the barn-dried sample was taken after it had cured, which required approximately 2 weeks.

Lot II was handled in the same manner. It was cut at 4 p.m. July 1; the hay for barn curing was hauled in at 4 p.m. July 2 and the sun-cured hay at 4 p.m. July 3. A small shower occurred during the first night after cutting; otherwise the weather was sunny. The composition of a mixture of these two lots would average approximately 63 per cent timothy and grass, 27 per cent alfalfa, 7 per cent clover and 3 per cent weeds.

Composite samples were made of the two lots each of sun-cured, barn-cured and artificially dried hay. They then were milled and appropriate amounts sent to the assaying laboratory.

The 1947 crop was handled similarly. Lot I was cut at 9 a.m. July 14; the hay for barn curing was hauled in at 11 a.m. July 15 and the sun-cured hay at 2 p.m. July 16. The weather was sunny for the most part and no rain occurred. Lot II was cut at 9 a.m. July 16; the hay for barn curing was hauled in at 10 a.m. July 17. The sun-cured hay was thoroughly soaked with a shower and so it was decided to discard this hay and make another cutting from the same field to obtain sun-cured hay without rain. Another supply for barn curing and artificial drying was obtained at the same time and these samples were mixed with those from Lots I and II. This cutting was made at 9 a.m. July 24; the hay for barn curing was hauled in at 10 a.m. July 25, and since it was a fast-drying day, the sun-cured hay was ready to be hauled in at 4 p.m. on the same day.

Table 1 shows the vitamin D contents of the sun-cured and barn-cured hays used in the calf-feeding trial. Assays for the artificially dried hay harvested from the same lots are included for comparison. Three samples were taken from the 1946 crop of sun-cured and barn-cured hays—one at harvest time or soon afterward, one in January and the last one the following June. The same number will be taken from the 1947 crop. In the 1946 crop there is very little difference in the vitamin D content of the sun-cured and barn-cured hays in the first two samples taken, the units per g. being 0.61 and 0.34 for the sun-cured hay and 0.51 and 0.33 for the barn-cured hay, respectively. The June sample, however, being reassayed several times, showed greater differences, the sun-cured hay averaging 0.48 and the barn-cured hay 0.22 unit. These results suggest that there may be a tendency for some loss of vitamin D in storage.

The hay which was dried artificially in 1946 had a decided advantage in the amount of vitamin D. The first assay showed 1.14 units per g. and a re-assay of the same sample showed 1.40 units. Another sample from the reserve supply which was stored unmilled from July, 1946, to March, 1947, contained 0.42 unit. These relatively high figures for artificially dried hay were surprising. However, Wallis (7) reported 812 units of vitamin D per pound of artificially dried hay. His sample was cut after dark and dried artificially in a dehydrating machine, thus eliminating any exposure to sunshine. Bechdel *et al.* (1) reported 150 and 300 units of vitamin D per lb. of dehydrated alfalfa hay for two successive seasons. The alfalfa was cut after sundown and dried in an artificial drier to prevent exposure to sunlight after mowing. It was planned to use this hay in a rachitogenic diet for dairy calves, but the amounts of vitamin D were found to be too large. On the other hand, Bechdel and Landsburg (2) found a measurable difference in the antirachitic potency of

dehydrated and sun-cured alfalfa hay when fed to calves as supplements to a basal rachitic diet. Two and one-half pounds of dehydrated alfalfa did not prevent the development of a mild rachitic condition over a 6-month feeding period whereas an equal amount of sun-cured alfalfa served as a complete preventive.

The first two samples of the 1947 crop show higher vitamin D contents in both the sun-cured and barn-cured hays over the previous year. The barn-cured hay was a little higher than the sun-cured hay, the former containing 2.33 and 2.00 and the latter containing 2.00 and 1.80 units. However, the artificially dried hay contained only 0.59 and 0.75 unit per g. Other samples of these hays will be assayed, but to date the barn-cured hay for both years is practically equal to the sun-cured hay in vitamin D content.

The results of the assays on these hays indicate that vitamin D may be present in the growing plant to a larger extent than heretofore has been considered to be the case. In order to throw some light upon this point, several samples of hay were cut from the same field and cured in a dark barn loft, the plants thus receiving no exposure to the sun after being cut. Samples 1 and 2 were cut at 5 a.m. and 5 p.m., respectively, to note any effect the sun might have upon the standing plant (table 2). Samples 3 and 4 were taken to show any differences in the vitamin D content of the top and bottom parts of the plants. The upper part was clipped from the lower without taking any special pains to separate the green stems and leaves from the brown. The lower part, nevertheless, consisted mainly of browned leaves and stems. Samples 5 and 6 were hand picked to obtain only green leaves and brown leaves, respectively, no stems being included. A sample of the entire plants from the same area was sun-cured for comparison. It required around 7 to 10 days for the samples to cure in the dark barn loft. They then were milled and sampled for assaying.

All the samples cured in the dark contained appreciable amounts of vitamin D. They contained lower amounts of the vitamin than did the sun-cured sample, but were higher for the most part than the sun-cured hays obtained in 1946 (table 1). Comparing samples 1 and 2, more vitamin D was present in the morning-cut sample than in the evening-cut hay, even though the standing plants of the latter received 12 hours of sunshine, the respective amounts being 0.75 and 0.54 unit per g. Samples 3 and 4 show a little less vitamin D in the upper than in the lower part of the plants, the respective amounts being 0.84 and 1.00 unit. Thus the brown part appears to contain more of the vitamin than the green part. This also is shown in a comparison of samples 5 and 6, the amounts of vitamin D in the green and brown leaves being, respectively, 0.84 and 1.10 units. The entire plant, as represented by the sun-cured sample 7, contained 2.30 units, which is more than double the amount in either sample 5 or 6 taken at the same time and cured in the dark. This shows the effect of sunshine

in increasing the vitamin D content. The amount in all probability would be higher still if only the leafy portion was considered. Wallis (6) found that the leaves of a good quality green colored alfalfa hay were about six times as potent in vitamin D as the stems. The International Units were 10.45 and 1.72 per g., respectively. Since these hays cured in the dark contained, for the most part, more vitamin D than the sun-cured hays (1946 crop) fed during the first year of the calf feeding trial, one might conclude that they also would provide sufficient amounts of vitamin D to prevent rickets.

CONCLUSIONS

More work needs to be done on the vitamin D content of roughages. The limited results of this study suggest that plants cured in the dark contain appreciable amounts of vitamin D. The brown leaves on growing plants appear to have a somewhat higher vitamin D content than the green leaves. Sunshine plays an important part in the formation of additional vitamin D during the curing process of roughages.

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THE EFFECT OF SOYA-PHOSPHATIDES ON THE ABSORPTION AND UTILIZATION OF VITAMIN A IN DAIRY ANIMALS[1]

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It has been shown by various research workers (2, 6, 8, 35) that the liver of the newborn calf contains either little or no vitamin A. Analyses of blood of newborn calves also revealed that the level of vitamin A is exceptionally low (19, 20, 27, 32, 35).

Colostrum, which has been found to be very rich in this vitamin (6, 16, 24, 29, 35), is the first natural material which the calves consume to overcome this deficiency. There is a wide variation in colostral vitamin A among cows of the same breed (29) as well as in different seasons. Attempts have been made to increase the vitamin A potency of colostrum by feeding extra vitamin A during the latter part of the gestation period. Stewart and McCallum (30) failed to find an increase of this vitamin in colostrum following the feeding of carrots or cod-liver oil. Contrary to this finding, Spielman *et al.* (28) were able to demonstrate an increased amount of vitamin A in colostrum and in the blood and livers of calves following the feeding of large doses of the vitamin during the later stages of the gestation period. Wise *et al.* (35) also found higher levels of vitamin A in both the blood and the liver of the newborn calves when the dams were fed supplementary vitamin A during the gestation period. When large doses of vitamin A or carotene were fed during the later stages of gestation, the decrease in the blood plasma vitamin A and carotene of cows at parturition was not prevented (5, 17, 33, 34), although a higher level was maintained than was observed in the controls.

Numerous reports have been published regarding the importance of vitamin A in calfhood nutrition. Attempts were made to raise calves on skim milk supplemented with vitamin A concentrate (18, 19), usually with disappointing results. Wisconsin workers (19) reported that vitamin A, together with ascorbic acid and nicotinic acid, would increase the survival rate of calves on skim milk. Later work (12, 21) showed no beneficial effect of nicotinic acid feeding, especially in conjunction with large doses of vitamin A supplement.

The question is raised as to whether or not other sources of vitamin A or carotene along with skim milk can be utilized in the same way as the

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colostral vitamin A or carotene. Spielman *et al.* (26) have shown that the carotene of commercial concentrates is poorly utilized by the very young calf. They have reported further that the vitamin A and carotene in colostrum are used more effectively than are vitamin A and carotene added to skim milk or similarly fortified reconstituted skim milk. Knowledge of the exact mechanism of absorption and utilization of vitamin A in the animal system still is inadequate.

Considerable work has been done during recent years regarding the synergism of vitamin E and vitamin A (10, 13) as well as of lecithin and vitamin A. It has been reported that in the case of rats (25), chicks (22), and man (1), the utilization of vitamin A and carotene can be influenced by soybean phosphatides.

The purpose of the present investigation was twofold: (a) To determine the extent to which the vitamin A level of the blood and colostrum of cows and the vitamin A reserve of the newborn calves may be affected by feeding large doses of vitamin A along with soybean phosphatides during the later stages of gestation, and (b) to throw some light on the mechanism of absorption and utilization of vitamin A by calves on a skim milk ration from the time of birth.

EXPERIMENTAL PROCEDURE

This experiment was undertaken during the late winter and early spring months of 1947 while all the animals were on a winter herd ration. Twenty-six pregnant dairy cows of the Jersey and Holstein breeds previously maintained under similar conditions of feeding and management were divided into three dietary groups approximately 30 days prior to parturition. Group I, consisting of ten cows, was again subdivided into two groups of five each. Group I–A received only the usual herd ration[3] and Group I–B the same ration plus 10 g. of soybean lecithin[4] daily. Group II, consisting of eight cows, was given one million I.U. (International Units) of vitamin A[5] daily, in addition to the herd ration. Group III, consisting of eight animals, received the herd ration plus one million I.U. of vitamin A and 10 g. of lecithin daily.

These rations were continued to the seventh day following the day of parturition. The vitamin A was given orally in gelatin capsules at approximately the same hour each day. The lecithin was mixed carefully with the vitamin A concentrate (fish-liver oil) and special gelatin cap-

[3] The herd ration consists of ground corn 400, ground oats 300, wheat bran 100, soybean oil meal 100, salt iodized 9, and the silage and hay *ad libitum*.

[4] Soybean lecithin supplied by the American Lecithin Company, Long Island City, New York, contained 70 per cent soya phosphatides (lecithin, cephalin and lipositol) and 30 per cent soybean oil.

[5] Vitamin A capsules were supplied by the Gelatin Products Company, Detroit, Michigan. One gram of this capsule had 25,000 I.U. vitamin A.

sules each containing 3.33 g. of lecithin and 333,333 I.U. of vitamin A were prepared each week and stored in the refrigerator. Three of these capsules were administered daily to the cows in Group III.

Blood plasma vitamin A and carotene were determined each week before parturition, at parturition, and at 1, 3, 7, 14, and 21 days after parturition. Vitamin A, carotene and lecithin analyses were made on the colostrum and milk samples successively at parturition, and at 1, 3, 7, 14, and 21 days after parturition. Two cows in each of Groups II and III were fed in the same way for approximately 60 days before parturition in order to determine the possible effect of feeding vitamin A for a longer period on the changes of vitamin A and carotene levels in the blood plasma of the cows and their calves and in the liver of the calves as well as in the colostrum and milk.

Blood plasma vitamin A and carotene were determined in the newborn calves before they had access to colostrum or other feed. A few calves from each of the representative groups were slaughtered after birth to determine the liver storage of vitamin A and carotene. All of the calves which were not slaughtered at birth from the above experiment (15 in all) were divided into three groups. There was no predetermined basis for the allotment of the calves to the various groups except that the largest number of calves from cows fed vitamin A and lecithin were allotted to group B, which was assumed to be the group receiving the most rigorous treatment. Preliminary data had shown higher liver storage in the calves from these cows. The calves in Group A were fed colostrum at the rate of 10 lb. per 100 lb. of bodyweight for 7 days after birth. A composite mixture of colostrum was made and stored previously to standardize the feeding in every case. The vitamin A, carotene and lecithin contents of this mixture of colostrum were determined, and the total vitamin A and lecithin consumed by each calf were calculated. The calves in Group B were fed skim milk at the same rate for 7 days after birth. They were not permitted to receive any colostrum. The same quantity of vitamin A consumed daily by the calves in the colostrum-fed group (Group A) was added to the skim milk every day. The calculated quantity of vitamin A oil (25,000–37,500 I.U.) was homogenized with the skim milk before each feeding. Group C was fed skim milk plus the same quantity of vitamin A and the same quantity of lecithin (3–4.5 g.) as was consumed daily by the calves in Group A. This feeding schedule was continued for 7 days following birth. The appropriate quantities of lecithin and vitamin A oil were homogenized with a small amount of skim milk and then mixed with the skim milk to be fed at each feeding. The surviving calves of all the groups were fed whole milk after the seventh day.

Vitamin A and carotene of blood plasma were determined successively at birth, and at 1, 3, 7, 14, and 21 days after birth. After 21 days, some

of the calves from each of the representative groups were slaughtered to determine the total liver storage of vitamin A and of carotene.

Vitamin A and carotene of blood plasma were determined according to the method of Kimble (15). The vitamin A of the blood samples having carotene concentrations exceeding 300 was determined by the method of Boyer *et al.* (3). The vitamin A and carotene of the colostrum and milk samples were determined by the method of Boyer *et al.* (4), with slight modifications. Instead of cold saponification, a hot saponification procedure was adopted. Five milliliters of colostrum or 25 ml. of milk plus 10 g. of caustic potash plus 50 ml. of methyl alcohol were refluxed in a boiling water bath for 10 minutes in a low actinic flask with a ground glass fitted reflux assembly. After cooling, the mixture was transferred to a separatory funnel, rinsing the flask with 55 ml. of water. The mixture was extracted successively with 50-ml. and with 25-ml. quantities of diethyl ether. The remainder of the procedure was the same as that of Boyer *et al.* (4). Liver vitamin A and carotene were determined by using the extraction procedure of Guilbert and Hart (8), with slight modification. Lecithin was determined according to the procedure adopted by Horrall (14). All of the colorimetric measurements were made in an Evelyn Photoelectric Colorimeter, using the appropriate filters.

RESULTS AND DISCUSSION

Effect of prepartal vitamin A and lecithin feeding on the vitamin A and carotene levels in the blood plasma of cows. The individual animal data are too voluminous to report; therefore the data are summarized in table 1. It will be noted that the feeding of lecithin or of vitamin A, or a combination of lecithin and vitamin A, did not prevent a decrease in blood plasma vitamin A and carotene at the time of parturition and beginning lactation. However, higher vitamin A levels were maintained when vitamin A was fed with or without additional lecithin (34). When both vitamin A and lecithin were fed, the highest blood vitamin A level was maintained; the level following parturition was higher (statistically significant) than that found 4 weeks prior to parturition. These data are considered as presumptive evidence that lecithin facilitates the absorption of vitamin A. There was considerable individual variation both in the time when the maximum decrease was noted after parturition and in the magnitude of the decrease. If samples had been obtained at more frequent intervals, perhaps these differences between groups would have been more clear-cut. Feeding of lecithin apparently has a tendency to delay the time of maximum postpartum decrease in blood plasma vitamin A. Although the vitamin A and lecithin feeding was discontinued on the seventh day postpartum, a carry-over effect was still apparent on the twenty-first day, the greatest carry-over effect being observed when both vitamin A and

TABLE 1

The effect of feeding vitamin A and vitamin A plus lecithin on the average concentration of vitamin A and carotene in the blood plasma of cows for the period 4 weeks before parturition to 3 weeks following parturition

Groups	No. of cows	Days prepartum				At parturition	Days postpartum				
		28	21	14	7		1	3	7	14	21
Vitamin A in blood plasma (γ/100 ml.)											
I–A (No lecithin)	5	20.5	19.1	17.8	18.0	13.7	9.9	13.8	11.5	17.1	18.8
I–B (With lecithin)	5	17.9	18.8	11.9	10.1	12.4	12.4	6.7	12.1	13.7	12.4
II (Vitamin A)	8	19.5	29.6	25.8	26.1	20.4	18.4	21.8	26.0	23.1	22.1
III (A and lecithin)	8	18.9	38.9	38.9	31.2	29.9	25.4	27.5	24.7	26.8	28.7
Carotene in blood plasma (γ/100 ml.)											
I–A (No lecithin)	5	285	335	320	273	212	157	212	215	242	371
I–B (With lecithin)	5	303	368	340	347	318	273	303	331	297	303
II (Vitamin A)	8	422	351	294	222	181	160	141	145	170	263
III (A and lecithin)	8	390	322	240	216	159	145	128	93	135	172

lecithin were fed. These data provide additional evidence that high levels of vitamin A in the blood plasma of cows can be maintained by high levels of vitamin A feeding (34) and that this can be done more effectively by feeding a combination of vitamin A and lecithin.

TABLE 2

The effects of feeding vitamin A and vitamin A plus lecithin on the changes in the vitamin A content of cows' colostrum and milk up to 21 days postpartum

Cow no.	Vitamin A of colostrum and milk (γ/100 ml.)					
	At parturition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I–A (control group without lecithin)						
851H	496	67	35	19	17	18
873J	145	79	21	16	15	11
820J	141	52	33	11	14	15
866J	134	69	68	22	13	14
620H	218	113	29	18	.....	.....
Mean	226	76	37	17	15	15
Group I–B (control group with lecithin)						
798H	143	46	.....	18	22	25
616H	132	60	58	22	19	17
801H	98	40	21	.....	...	
763J	216	105	43	27	17	18
800J	134	92	22	25	26	16
Mean	145	69	36	23	21	19
Group II (vitamin A)						
755H	229	167	123	31	25	32
692J	420	131	179	71	23	25
812J	163	172	141	97	14	
711J	304	.....	169	201	74	27
649J	216	222	32	144	28	27
752H	705	302	161	191	.....	.....
Mean	339	197	134	122	33	28
Group III (vitamin A plus lecithin)						
852H	552	113	175	70	31	24
806H	598	156	195	77	31	39
743H	377	158	112	148	146	22
675J	847	210	125	148	25	40
760J	1119	506	165	162	29	32
Mean	698	229	154	121	52	32

The effect of feeding lecithin alone is not statistically significant although the decrease in blood carotene following parturition appears to be about 10 per cent less when lecithin is fed.

The effects of feeding vitamin A and vitamin A plus lecithin on blood plasma carotene at the time of parturition are difficult to interpret be-

cause of the depressing effect (7) of vitamin A feeding on blood plasma carotene. This depressing effect was strikingly shown in the case of three cows fed vitamin A and lecithin for 8 weeks prepartum. The average carotene content of the blood of these cows was 329, 53 and 73 γ per 100 ml. at 8 weeks prepartum and at 7 and 21 days postpartum, respectively.

TABLE 3

The effects of feeding vitamin A and vitamin A plus lecithin on the changes in the carotene content of cows' colostrum and milk up to 21 days postpartum

Cow no.	Carotene of colostrum and milk (γ/100 ml.)					
	At parturition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I–A (control group without lecithin)						
851H	195	42	18	9	14	17
873J	200	157	17	13	20	19
820J	191	74	36	18	22	28
866J	179	105	52	22	22	9
620H	133	71	18	7	.....	.....
Mean	180	90	28	14	19	18
Group I–B (control group with lecithin)						
798H	223	53	.....	11	10	14
616H	200	89	46	16	35	8
801H	105	34	17	.....	.....	.....
763J	218	216	53	16	14	13
800J	172	58	10	8	12	9
Mean	183	90	31	13	18	11
Group II (vitamin A)						
755H	140	108	48	12	8	8
692J	137	36	20	8	8	15
812J	113	62	18	10	7	.....
711J	152	.....	34	27	8	9
649J	103	69	10	18	7	28
752J	115	47	10	6	.....	.....
Mean	126	64	23	14	8	15
Group III (vitamin A plus lecithin)						
852H	171	24	15	6	6	6
806H	195	27	25	9	6	12
743H	168	30	16	6	17	7
675J	243	71	26	6	7	15
760J	200	42	16	6	6	6
Mean	195	39	20	7	8	9

Effect of prepartal vitamin A and lecithin feeding on the vitamin A, carotene and lecithin content of colostrum. The data in table 2 show that when vitamin A was fed, the vitamin A in the colostrum was significantly higher than that produced by the groups receiving no vitamin A. When

both vitamin A and lecithin were fed, the vitamin A in the first milking of colostrum was approximately double that of the colostrum from cows receiving vitamin A alone. This difference became less as the milk approached normal and had vanished by the seventh day postpartum. The effects of vitamin A feeding on the potency of the milk still were evident 2 weeks following the end of the vitamin feeding.

The data on the effect of feeding lecithin and vitamin A on the carotene content of the colostrum and early milk are presented in table 3. The feeding of vitamin A depresses the level of carotene in colostrum and milk, a result which has been reported previously (7). It appears also that the feeding of lecithin with the vitamin A enhances this suppressing effect. Additional data are needed to confirm this point.

As previously noted, three animals were fed vitamin A and lecithin for a period of 8 weeks before freshening. The limited data obtained from these animals indicated that higher levels of vitamin A were maintained in the milk after the third day following parturition and the milk carotene was further depressed. These limited data need further confirmation.

The carotene content of colostrum and milk follows the same trend as in the blood. The apparent antagonistic effect of supplemental vitamin A on blood and milk carotene is not explainable in the light of present knowledge; however, its occurrence seems to be beyond doubt.

The total output of vitamin A in International Units per milking is presented in table 4. Although the level of carotene has been depressed in the vitamin A supplemented groups, the total output of vitamin A is higher in these groups. When lecithin was fed with vitamin A, the total output of vitamin A was highest, especially at the first milking. This difference is so great that there is little doubt of its significance. Following the discontinuation of vitamin A supplementation on the seventh day, a sudden marked drop in vitamin A occurred. In the case of those animals receiving both vitamin A and lecithin, the drop was more gradual. Results in the control groups are interesting. Although the vitamin A concentration in the milk of the control group I–A (without lecithin) was higher than that of the control group I–B (with lecithin, see table 2), the total output of vitamin A is higher in the lecithin-fed group. This is due to the greater milk yield. Whether this increased milk-yield is due to lecithin feeding is to be determined by further experiments with a larger number of cows.

The effects of lecithin feeding on the lecithin content of colostrum and milk are shown in table 5. The lecithin content of colostrum and milk seems to be maintained at a higher level when both vitamin A and lecithin are fed. The carry-over effect is still apparent in the milk on the twenty-first day, 2 weeks after the supplemental feeding was discontinued. When

lecithin is fed without vitamin A or vitamin A fed without lecithin, the amount of lecithin in the colostrum and milk is little different from that of the control group which received no supplement. It appears from these data that there may be a reciprocal relationship in the absorption and/or metabolism of these two compounds.

TABLE 4

The effects of feeding vitamin A and vitamin A plus lecithin on the changes in total output of vitamin A per milking up to 21 days postpartum

Cow no.	I.U.[a] of vitamin A per milking					
	At parturition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I–A (control group without lecithin)						
851H	136,140	13,557	13,648	6,717	7,541	8,323
873J	16,565	11,831	3,696	3,769	4,482	3,601
820J	17,998	15,731	8,759	4,839	5,723	6,937
866J	9,456	8,361	8,480	5,133	4,732	3,932
620H	50,592	23,273	6,445	4,147	.....	.....
Mean	46,150	14,550	8,206	4,921	5,620	5,698
Group I–B (control group with lecithin)						
798H	55,600	34,610	.....	9,899	13,075	17,668
616H	51,950	37,710	25,264	11,195	13,078	8,400
801H	87,780	19,854	9,351	.....	.....	.....
763J	72,350	5,304	19,440	8,629	6,353	7,770
800J	20,512	30,058	5,994	8,146	8,781	3,964
Mean	57,638	25,507	15,012	9,467	10,321	8,950
Group II (vitamin A fed)						
755H	58,883	90,360	47,782	16,685	12,693	16,993
692J	112,493	41,939	60,538	25,285	7,645	12,844
812J	45,740	37,675	45,764	32,017	6,355	.....
711J	36,652	.....	52,145	67,821	21,279	8,831
649J	34,285	24,123	6,137	41,377	8,436	13,443
752H	280,100	125,412	60,156	72,996	.....	.....
Mean	94,692	63,902	45,420	42,697	11,282	13,027
Group III (vitamin A plus lecithin)						
852H	102,850	22,056	55,220	19,315	9,649	6,955
806H	455,910	51,539	112,227	33,145	14,169	23,099
743H	295,940	66,164	35,697	59,653	76,830	10,979
675J	146,240	55,198	30,774	42,357	9,092	15,751
760J	141,713	56,997	45,183	51,164	9,417	9,253
Mean	228,530	50,391	55,820	41,127	23,830	13,207

[a] One microgram of vitamin A = 4 I.U. Vitamin A. One microgram of carotene = 1.66 I.U. vitamin A.

Effect of supplementing the maternal diet with vitamin A and lecithin on the blood plasma vitamin A and carotene of the newborn calf. The results of this phase of the study are presented in table 6. The plasma vita-

min A of the calves from cows receiving vitamin A was significantly higher than that of those from the control cows. These results are in agreement with those of Wise *et al.* (35) and Spielman *et al.* (27). The

TABLE 5

The effects of feeding vitamin A and vitamin A plus lecithin on the changes in the lecithin content of cows' colostrum and milk

Cow no.	Percentage of lecithin in colostrum and milk					
	At parturition	24 hr.	3 d.	7 d.	14 d.	21 d.
Group I–A (control group without lecithin)						
851H	0.062	0.051	0.049	0.028	0.029	0.024
873J	0.076	.....	0.044	0.039	0.038	0.038
820J	0.058	0.059	0.043	0.038	0.021	0.020
866J	0.068	.....	0.049	0.039	0.029	0.036
620H	0.060	0.080	0.048	0.038	0.034	.....
Mean	0.065	0.063	0.047	0.036	0.030	0.029
Group I–B (control group with lecithin)						
798H	0.076	0.056	.....	.....	0.042	0.026
616H	0.076	.....	0.056	.....	0.021	0.025
801H	0.051	0.043	0.023	.....	0.042	0.026
763J	0.073	.....	0.074	.....	.....	.....
800J	0.055	0.053	0.055	0.048	0.026	0.029
Mean	0.066	0.051	0.052	.....	0.033	0.026
Group II (vitamin A)						
755H	0.068	0.063	0.055	0.046	0.031	0.025
692J	0.064	0.046	0.052	0.047	0.024	0.023
812J	0.056	0.046	0.044	0.039	0.036	.....
711J	0.088	.....	0.074	0.055	0.036	0.040
649J	0.064	0.035	0.026	0.060	0.036	0.038
752J	0.084	0.067	0.059	0.052	.....	.....
Mean	0.071	0.051	0.052	0.049	0.033	0.032
Group III (vitamin A plus lecithin)						
852H	0.094	0.062	0.057	0.058	0.044	0.036
806H	0.094	0.060	0.050	0.051	0.046	0.051
743H	0.069	0.049	0.040	0.040	0.046	0.030
675J	0.093	0.100	0.180	0.081	0.059	0.055
760J	0.093	0.102	0.075	0.056	0.052	0.051
Mean	0.089	0.075	0.060	0.057	0.049	0.045

slightly higher mean value of the blood plasma vitamin A of calves from cows receiving both vitamin A and lecithin is insignificant.

It appears from the data on blood plasma carotene that a combination of lecithin and vitamin A in the maternal diet exerts a suppressing action on the level of carotene in the blood of the newborn calf. These data, however, are within a range where experimental error of determination is

apt to be rather high, and the difference may not be as significant as the statistic indicates.

The effect of supplementing the maternal diet with vitamin A and lecithin on the liver storage of vitamin A and carotene in the newborn calf. Seven of the calves were sacrificed at birth to determine total liver storage

TABLE 6

The effect of increasing the vitamin A and lecithin content of the maternal diet on the blood plasma and liver storage of vitamin A and carotene in the newborn calf

Dam no.	Calf no.	Blood plasma data		Liver storage data		
		Vitamin A	Carotene	Liver wt.	Vitamin A	Total A
		(γ/100 ml.)	(γ/100 ml.)	(g.)	(γ/g.)	(γ)
Calves from Group I (control cows)						
866J	819J	2.4	4.2	.....	.....	.....
873J	820J	3.5	6.3	.....	.....	.....
851H	825H	6.5	11.4	.....	.....	.....
800J[a]	944J	7.7	4.2	.....	.....	.....
820J	821J	5.4	9.2	434	0.59	249
620H	948H	8.9	0.0	1000	0.25	252
801H[a]	828H	8.4	5.6	793	0.08	67
	Mean	6.1	5.8	.....	0.31	190
Calves from Group II (cows fed vitamin A)						
755H	905H	7.2	11.4	.....	.....	.....
812J	946J	9.8	8.5	.....	.....	.....
711J	823J	8.6	8.4	.....	.....	.....
853H	830H	9.3	0.0	.....	.....	.....
752H	829H	10.7	0.0	.....	.....	.....
692J	822J	11.5	4.9	435	12.0	5241
649J	827J	6.2	7.0	500	13.1	6580
	Mean	9.0	5.7	.....	12.5	5910
Calves from Group III (cows fed vitamin A plus lecithin)						
855J	832J	9.2	0.0	.....	.....	.....
760J	947J	6.6	2.8	.....	.....	.....
743H	950H	10.7	0.0	.....	.....	.....
852H	949H	10.2	0.0	.....	.....	.....
857H	951H	13.6	0.0	.....	.....	.....
675J	824J	8.2	5.7	438	22.0	9625
806H	826H	15.1	0.0	945	14.6	13820
	Mean	10.5	1.2	.....	18.3	11722

[a] These cows were in Group I–B; lecithin was fed.

of vitamin A and carotene. Three of these calves were from cows in Group I, two from cows in Group II, and two from cows in Group III. As previously reported by other workers (27, 35), a statistically significant greater liver storage was found in newborn calves from dams receiving massive vitamin A supplements. The cows receiving both vitamin A and

lecithin gave birth to calves with almost double the liver vitamin A storage of those from cows receiving vitamin A alone. As can be noted from table 6, this difference was due in part to higher concentration and in part to greater liver weight. Although the numbers are limited, the magnitude of the difference is so great that there is little doubt of the significance, particularly in the light of the other data presented in this paper. Fur-

TABLE 7

The effects of feeding colostrum, skim milk plus vitamin A and skim milk plus vitamin A and lecithin on the plasma vitamin A levels of calves

Calf no.	Dam no.	Dam group	Vitamin A fed daily (*I.U.*)	Vitamin A in plasma (γ/100 ml.)					
				At birth	24 hr.	3 d.	7 d.	14 d.	21 d.
Group A (colostrum-fed calves)									
945H	755H	II	37,500	7.2	4.6	27.9	16.9	9.4	9.3
819J	866J	I–A	25,000	2.4	8.2	24.0	9.9	13.6	8.3
820J	873J	I–A	25,000	3.5	11.9	25.4	23.3	13.5	11.1
944J	800J	I–B	25,000	7.7	13.5	18.2	19.9	14.6	14.1
832J	855J	III	25,000	9.2	11.9	20.2	20.7	18.2	15.9
			Mean	6.2	10.1	23.5	18.2	13.8	11.7
Group B (skim milk plus vitamin A)									
825H	851H	I–A	37,500	6.5	5.3	12.0	Fell sick and died on the 7th day		
946J	812J	II	25,000	9.8	6.6	.....	"	"	"
947J[a]	760J	III	25,000	6.6	6.6	8.7	13.9	11.3	6.6
950H[a]	743H	III	37,500	10.7	10.4	12.2	10.1	9.3	22.0
831J[b]	854J	III	25,000	5.9	9.3	13.2	13.3	died on the 11th day	
			Mean	7.9	7.6	11.4	12.4	.....	.....
Group C (skim plus vitamin A plus lecithin)									
823J	711J	II	25,000	8.6	15.7	15.7	18.7	16.8	13.9
949H	852H	III	37,500	10.2	10.7	30.3	35.0	20.8	16.0
951H	857H	III	37,500	13.6	10.7	19.2	27.8	17.2	10.5
830H	853H	II	37,500	9.3	18.3	14.3	28.1	12.2	15.3
829H	752H	II	37,500	10.7	13.6	16.8	22.8	12.7	12.2
			Mean	10.5	13.8	19.3	26.3	15.9	13.6

[a] Given 2% cocoanut oil per feeding.
[b] Given lecithin from the 6th day.

ther evidence of the effect of vitamin A feeding on liver storage is shown in table 9. Calves 832J, 831J, and 830H were from dams that received the vitamin A supplement for 8 weeks prior to parturition. When these calves were sacrificed at 21 days of age (calf 831J died at 11 days of age), the liver storage was significantly higher than that of other comparable calves with similar histories and treatment.

Effect of the diet on the blood plasma vitamin A and carotene levels and the liver vitamin A storage of young calves. As previously indicated, the calves from the cows in this experiment were removed from the dams at birth (before nursing) and given special dietary treatments for the first 7 days. These dietary treatments have been described earlier in this paper.

The data obtained are presented in tables 7 and 8. The feeding of colos-

TABLE 8

The effects of feeding colostrum, skim milk plus vitamin A and skim milk plus vitmain A and lecithin on the plasma carotene levels of calves

Calf no.	Dam no.	Dam group	Vitamin A fed daily (*I.U.*)	Carotene in plasma (γ/100 ml.)					
				At birth	24 hr.	3 d.	7 d.	14 d.	21 d.
Group A (colostrum-fed calves)									
945H	755H	II	37,500	11.4	5.7	24.7	38.8	20.6	23.2
819J	866J	I–A	25,000	4.2	6.4	4.9	62.1	49.4	37.2
820J	873J	I–A	25,000	6.3	2.8	26.3	58.7	29.0	21.8
944J	800J	I–B	25,000	4.2	21.7	48.1	22.4	51.0	35.6
832J	855J	III	25,000	0.0	5.7	29.4	35.6	57.0	32.4
			Mean	5.2	8.4	26.6	43.5	41.3	30.0
Group B (skim milk plus vitamin A)									
825H	851H	I–A	37,500	11.4	7.8	13.6	died on the 7th day		
946J	812J	II	25,000	8.5	5.6	.....	" " " " "		
947J[a]	760J	III	25,000	2.8	5.6	7.7	0.0	12.8	5.9
950H[a]	743H	III	37,500	0.0	0.0	3.5	4.9	17.2	21.8
831J[b]	854J	III	25,000	0.0	0.0	0.0	3.5	died on the 11th day	
			Mean	4.5	4.0	6.2	2.8	.....	.....
Group C (skim plus vitamin A plus lecithin)									
823J	711J	II	25,000	8.4	15.3	15.3	2.5	15.3	27.9
949H	852H	III	37,500	0.0	0.0	0.0	4.9	32.4	30.2
951H	857H	III	37,500	0.0	0.0	0.0	4.9	9.6	26.3
830H	853H	II	37,500	0.0	2.6	0.0	2.8	26.3	14.3
829H	752H	II	37,500	0.0	0.0	0.0	3.5	12.8	20.9
			Mean	1.7	3.5	3.1	3.7	19.3	24.0

[a] Given 2% cocoanut oil per feeding.
[b] Given lecithin from the 6th day.

trum resulted in a marked increase in the amount of vitamin A in the blood, as has been noted by others (19, 20, 32). There was an increase in blood plasma carotene following the feeding of colostrum (table 8), but the carotene remained low in both the groups fed vitamin A, as was expected. The carotene increase in the latter groups on the fourteenth and twenty-first days resulted from whole milk feeding following 7 days of age. There was no evidence of scours in the colostrum-fed group. The calves receiving skim milk plus the vitamin A supplement did very poorly.

Serious scours developed on the third day. Three of the calves died, two on the seventh day and one on the eleventh day. The calf that died on the eleventh day was from a cow that received both vitamin A and lecithin, and at the time of death this calf had an appreciable liver storage of vitamin A (see calf 831J, table 9). Perhaps the liver storage of vitamin A permitted this calf to endure the rigors of the diet longer than those which died on the seventh day. The other two calves, 947J and 950H, were given 2 per cent cocoanut oil and were able to survive. These calves also scoured from the third to the tenth day, and vitamin A absorption, as indicated by the low blood level, was poor.

TABLE 9

The effects of feeding colostrum, skim milk plus vitamin A and skim milk plus vitamin A plus lecithin on the storage of vitamin A in the liver of calves at 21 days of age

Calf no.	Dam no.	Dam group	Wt. of the liver (*g.*)	Vitamin A (γ/*g.*)	Total vitamin A (γ)
Calves from Group A (colostrum-fed)					
819J	866J	I–A	519.6	7.2	3,724.0
820J	873J	I–A	526.8	8.7	4.630.0
832J[a]	855J	III	608.0	53.0	32,244.0
Calves from Group B (skim milk plus vitamin A)					
825H[b]	851H	I–A	988.0	0.15	149.0
946J[b]	812J	II	466.5	11.8	5,524.0
831J[c]	854J	III	453.8	56.3	25,570.0
Calves from Group C (skim milk plus vitamin A plus lecithin)					
823J	711J	II	549.0	35.4	19,470.0
830H[a]	853H	II	1124.0	69.0	77,556.0
829H	752H	II	953.0	36.6	34,900.0

[a] The dams got vitamin A or vitamin A plus lecithin for 8 weeks prepartum.
[b] Died on the 7th day.
[c] Died on the 11th day.

The low level of blood vitamin A in the group receiving skim milk plus vitamin A indicates poor vitamin A absorption on this type of diet. When lecithin was added along with the vitamin A, the blood levels were comparable to those of the colostrum-fed group. These results again indicate that soya lecithin enhances the absorption of vitamin A. There were a few mild cases of scours among the calves in this group, but, in general, they did quite well and were comparable to the colostrum-fed calves in rate of growth and general appearance.

It has been suggested that colostral vitamin A may be superior in the nutrition of newborn calves to the vitamin A of fish-liver oil or other concentrated sources (9). The data presented herein provide evidence that the higher concentration of lecithin present in colostrum may be partially responsible for the better absorption and utilization of colostral vitamin A.

An attempt was made to start calves on a skim milk ration plus cocoanut oil (two calves) and a skim milk ration plus lecithin (two calves). These attempts failed and all the calves died on the fourth day following birth.

The results of this phase of the investigation show that unless adequate quantities of vitamin A or its precursor are present in the ration and unless favorable circumstances for vitamin A absorption are provided, the animal will quickly succumb to vitamin A deficiency, even when there is considerable liver storage.

Limited data on the liver storage of vitamin A determined on the twenty-first day are presented in table 9. Here again it will be noted that the calves fed the vitamin A plus lecithin had appreciably higher liver storages. While the data cannot provide conclusive proof that vitamin A storage is greater when vitamin A and lecithin are fed, rat data (31) have proved this point conclusively.

Previous workers (12) have shown that the feeding of vitamin A in capsules resulted in increased liver storage. The results of the present investigation provide evidence that the storage will be increased still further if lecithin is fed along with vitamin A.

SUMMARY

Twenty-six healthy pregnant dairy cows of the Jersey and the Holstein breeds were divided into three dietary groups approximately 30 days prior to parturition. Each group received the basic herd ration. Group I, consisting of ten cows, was again subdivided into two groups; Group I–A received no supplement and Group I–B the herd ration plus 10 g. of soya-lecithin daily. Each of the eight cows in Group II was given one million I.U. of vitamin A (fish-liver oil) daily. Each of the eight cows in Group III was fed one million I.U. of vitamin A and 10 g. of lecithin daily. The supplements were continued up to the seventh day following parturition. Assays of blood vitamin A and carotene and of milk vitamin A, carotene and lecithin were made at intervals up to 21 days postpartum. Blood plasma vitamin A and carotene were determined in all calves, and representative animals were sacrificed at birth to determine vitamin A liver storage.

At parturition the plasma vitamin A level in the control cows fell almost to half of the 4 weeks prepartum level. The level in the cows fed vitamin A supplements remained fairly high, especially for the cows fed lecithin plus vitamin A, indicating that lecithin enhanced the absorption of vitamin A. There was no significant effect of feeding lecithin without vitamin A, although the decrease in blood carotene following parturition appears to be about 10 per cent less when lecithin is fed.

Blood plasma carotene was depressed in both vitamin A supplemented

groups. However, when vitamin A was fed for a longer period, the carotene level was depressed still further in the cows fed lecithin along with vitamin A. These limited data indicate that lecithin enhanced the action of vitamin A in depressing the carotene level.

The vitamin A in the colostrum of cows fed vitamin A was greater than that of the control group; when both lecithin and vitamin A were fed, the colostral vitamin A at the first milking was approximately double that of the vitamin A supplemented cows. This shows that lecithin, when added to vitamin A, increased the transmission of colostral vitamin A. The transmission of vitamin A and carotene in milk closely followed the trend found in the blood plasma.

The lecithin content of milk was highest when lecithin was fed to the cows along with vitamin A, and a higher level was maintained in the normal milk. Feeding lecithin without vitamin A had no effect on the transfer of lecithin to milk. When both vitamin A and lecithin were fed, the lecithin of the colostrum and milk was increased.

The blood plasma vitamin A level in the newborn calf was highest and the plasma carotene level was the lowest in the calves from dams fed both lecithin and vitamin A.

The total liver storage vitamin A in the newborn calves from the control group was low (190 γ); it was 5,910 γ in the vitamin A supplemented group and 11,722 γ in the vitamin A plus lecithin supplemented group. Thus, the addition of lecithin to the vitamin A supplement remarkedly increased the liver storage.

Three groups of five calves each were fed from birth to 7 days of age as follows: Group A, colostrum; Group B, skim milk plus the same daily quantity of total vitamin A consumed by the calves in the colostrum group (25,000–37,500 I.U. of vitamin A); and Group C, skim milk plus the same quantity of vitamin A and the same quantity of lecithin (3–4.5 g.) available in the colostrum given to Group A.

Every calf in Group B developed serious scours from the third day. Two of them died on the seventh day and a third one on the eleventh day. Their blood plasma vitamin A level was much below that of the colostrum-fed calves. All the calves in Groups A and C grew quite well with slight evidence of digestive disturbance. Blood plasma levels in Group A and C were almost identical, showing the ability of lecithin to increase absorption and utilization of vitamin A.

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CAROTENE AND VITAMIN A IN THE COLOSTRUM OF COWS OF TYPICAL INDIAN BREEDS

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Since the discovery of vitamin A as an anti-infective factor, many workers (3, 8, 14, 15, 18, 21, 24, 27) have studied its importance in the nutrition of dairy calves. It is now recognized that vitamin A is indispensable and must be supplied in adequate amounts during the earlier part of life, since the calf is born with practically no reserve of vitamin A (2, 4, 10, 17). Krauss *et al.* (17) reported a decrease in the incidence of pneumonia in calves which received 15,000 I.U. of vitamin A concentrate daily. Gullickson and Fitch (11), in an experiment involving 72 calves, reported less trouble from digestive disturbances in young calves that were fed cod-liver oil than in calves not given the vitamin A supplement. Phillips *et al.* (24) observed that the administration of shark-liver oil with a high vitamin A potency and certain members of the B-complex eliminated diarrhea and lowered the mortality resulting from pneumonia. Nelson *et al.* (23) recommended the feeding of fish-liver oil as a vitamin A supplement when there was difficulty in raising calves.

Under natural feeding conditions vitamin A supplementation is not usually practiced; however, the value of colostrum as a source of vitamin A for newborn calves has been the subject of investigation by some workers. Stewart and McCallum (30) made an extensive study of the correlation between the incidence of white scours in calves and the vitamin A content of the colostrum. In 83 calves which received colostrum containing more than 250 blue units of vitamin A, only 10.8 per cent developed white scours or allied infections; whereas, in 28 calves which received colostrum containing less than 250 blue units of vitamin A, 25 per cent developed white scours or allied infections. Moore and Berry (22) also have pointed out the significance of adequate colostrum feeding in building up the vitamin A reserve in the calf. Apart from these observations, several papers report that cow colostrum contains more vitamin A than the milk (7, 9, 12, 16, 20). Dann (5) and Kramer *et al.* (16) have shown that cows' colostra are ten to one-hundred times richer in vitamin A activity than the normal milk. On the first day of life a calf is supposed to receive a supply of vitamin A greater than the later milk can give in 20 to 50 days. Henry *et al.* (13) have noted that the colostrum of first-

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calf heifers is richer in vitamin A than that of cows, although Stewart and McCallum (29) did not note such a difference. However, they did report: (a) The length of the dry period between successive calvings affected the colostral vitamin A. (b) The vitamin A content was independent of season in contrast to that of carotene. The vitamin A content of the colostrum collected from 100 cows varied from 35 to 1,181 I.U. per 100 ml. By the third or fourth day the amount of vitamin A was from one-tenth to one-twentieth of that present immediately after parturition.

The variations in the carotene and vitamin A content of colostral fat from various breeds of cattle have been studied by Gillam *et al.* (9) and Semb *et al.* (26). These investigators have shown that the concentration of carotene and vitamin A in colostral fat is from five to fifteen times that of the fat prepared from normal milk and that these constituents decrease very rapidly during the first week postpartum. Stewart and McCallum (31) were unable to raise the vitamin A content of colostrum of cows on winter feed by feeding 3 lb. of carrots or one-seventh pint of cod-liver oil per day. Spielman *et al.* (28) have studied the relationship of the prepartum diet to the carotene and vitamin A content of bovine colostrum. Colostrum from cows receiving a low carotene ration for 60 days before parturition contained significantly less vitamin A per gram of butterfat than did colostrum from cows receiving a comparatively rich carotene ration. The effect of feed was more pronounced on the carotene content of the butterfat of the colostrum than on the vitamin A content, although vitamin A supplementation for 60 days before calving increased the colostral vitamin A to a considerable extent.

As no comparable data are available for any of the milking breeds of cows in India, it seemed desirable to initiate a study along this line. The results obtained from such a study are presented in this paper.

EXPERIMENTAL PROCEDURE

The colostrum and milk samples were collected from 15 cows in the Institute dairy herd for a period of 8 days postpartum. Nine cows and one first-calf heifer of the Hariana breed and five first-calf heifers of the Sahiwal breed were used. The animals were fed 3.5 lb. of a dairy mixture, 1 oz. of iodized salt and 1 oz. of bonemeal per head daily. The nature of the roughage fed to the cows depended on the season of the year and has been discussed in a previous paper (25).

The colostrum and milk samples were collected each day for 8 days and stored in a refrigerator for subsequent analysis. The percentage composition with respect to fat, solids-not-fat, protein and ash was determined according to the methods outlined in the A.O.A.C. (1). The extraction procedure of Dann (5) was followed for the determination of carotene and

vitamin A. Vitamin A was measured spectrographically in an alcoholic solution of the unsaponifiable matter, and the proper correction for the absorption due to carotene was made. For the conversion of corrected density readings to micrograms of vitamin A, the factor, $E\ {}^{1\%}_{1cm.}\ 328\ m\mu = 1800$, was used. Carotene was estimated colorimetrically in a petroleum ether solution.

Table 1 gives the data pertaining to the history and breed of the animals used in this experiment.

TABLE 1

Data pertaining to the history of the cows

Animal no.	No. of lactation	Length of dry period	Calving date
	Hariana breed		
		(days)	
1	10	166	8–22–42
2	3	180	10–17–42
3	3	76	10–25–42
4	10	144	11–14–42
5	2	146	11–15–42
6	2	149	11–18–42
7	9	287	12–28–42
8	2	148	1–20–43
9	2	37	5–16–43
10	1	.....	5–17–43
	Sahiwal breed		
11	1	.....	10–26–42
12	1	.....	1– 3–43
13	1	.....	1– 7–43
14	1	.....	1–17–43
15	1	.....	5–17–43

RESULTS

Composition of colostral milk. The data on the average daily milk yield and the percentage composition of the colostral milk with respect to fat, solids-not-fat, protein and ash are presented in table 2. Individual variations are quite apparent. The comparatively lower yields of colostrum and the higher percentages of the above constituents were found mostly in the samples obtained from the Sahiwal heifers, which were considered at one time as being sterile. All of the colostrum samples were characterized by a high percentage of solids-not-fat, protein and ash. The fat content of the colostral milk from individual cows varied widely from day to day but the percentage of fat in the first two days' samples was lower than in the later milk. The change from colostrum to milk was a gradual one, a fact which has been established by others (6) but, on the whole, the colostral samples tended to approach normal milk after the fourth day. The protein content was much higher than that usually obtained for the

TABLE 2

Average daily yield and the percentage composition of colostrum

Days after parturition	Yield		Fat		S–N–F		Protein		Ash	
	Range	Av.[a]	Range	Av.[a]	Range	Av.[b]	Range	Av.[b]	Range	Av.[b]
	(*lb.*)	(*lb.*)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1	3–13	6.4	1.6– 8.3	4.37	12.94–24.85	18.37	8.40–18.26	13.16	0.865–1.253	1.028
2	2–14	8.3	0.9– 7.4	4.23	11.16–16.50	14.07	8.55–11.57	9.00	0.734–0.965	0.877
3	4–18	11.2	1.1– 8.1	5.25	9.33–11.90	10.35	5.46–8.26	6.30	0.747–1.043	0.858
4	2–18	11.3	4.3–10.0	5.72	8.43–11.11	9.63	4.01–7.40	5.30	0.702–0.855	0.821
5	2–18	9.5	4.4– 7.4	5.59	8.40–10.21	9.15	4.40–5.10	4.81	0.765–0.920	0.824
6	2–17	8.8	2.1– 8.4	5.17	8.55–10.32	9.18	3.78–5.04	4.46	0.725–0.835	0.790
7	4–19	10.3	4.6– 7.2	5.47	7.39–9.32	8.85	3.90–4.90	4.35	0.736–0.842	0.794
8	4–18	12.0	4.3– 7.8	5.70	9.10–9.37	9.23[c]	4.62–4.73	4.66[c]	0.792–0.846	0.812[c]

[a] Average for 15 animals.
[b] Average for 9 animals.
[c] Average for 4 Sahiwal heifers.

Institute herd milk. Further progress in lactation, however, might cause more diminution in the percentage of protein.

Carotene and vitamin A in colostral milk. The data showing the variations in the carotene and vitamin A content of the colostral milk are presented in table 3. The first day's colostrum contained more than four times

TABLE 3

Variations in the carotene and vitamin A content of colostral milk during the first 8 days of lactation

Days postpartum	Carotene		Vitamin A	
	Range	Av.	Range	Av.
	(γ/*100 ml.*)		(γ/*100 ml.*)	
1	33.6–153.9	85.5	63.2–571.8	313.4
2	7.7–160.7	65.1	43.2–500.9	218.6
3	12.1–130.1	49.0	58.3–358.8	204.2
4	17.2–109.0	39.9[a]	63.1–438.0	157.5[a]
5	13.6– 70.3	31.1[b]	58.6–361.4	118.4[b]
6	12.1– 42.0	23.0[b]	49.0–107.8	79.5[b]
7	10.4– 32.3	20.1[a]	51.5–140.3	77.1[a]
8	10.8– 43.7	19.7[c]	47.3–111.6	70.9[c]

[a] Average for 14 animals.
[b] Average for 11 animals.
[c] Average for 12 animals.

as much carotene and vitamin A as the eighth day's sample, which might be considered equivalent in potency to a sample of milk obtained under pasture conditions. This variation was not so pronounced, however, as that reported by some English workers (5, 13, 29). As compared to the other constituents of colostrum and milk, the drop in carotene and vitamin A obviously is more marked. It might be mentioned also that the first day's colostrum did not always contain more carotene and vitamin A than the samples obtained within 4 days postpartum, although the average values showed consistent decreases. The lower carotene values were found for the animals which received very little carotene in the ration before calving. Some of these animals also secreted correspondingly lower amounts of vitamin A. The wide individual variations that are apparent in this investigation also have been observed by other workers. The average sample of colostrum obtained from the cows on the day of parturition was found to contain 85.5 γ of carotene and 313.4 γ of vitamin A per 100 ml. as compared to 107 γ of carotene and 374 γ of vitamin A for the Cornell University dairy herd on a standard dry-cow ration (28). The vitamin A-carotene ratio remained practically constant during the 8-day experimental period, indicating the flushing of these constituents from the mammary gland after their accumulation during the dry period. The concentration of both carotene and vitamin A decreased to a greater extent during the first 4 days of lactation rather than in the next 4 days. From the standpoint of vitamin A feeding, the samples for the first 4 days assume particular importance. According to Lewis and Wilson (19), the daily intake of vitamin A for a calf should be 11,000 I.U. per 100 lb. of liveweight. On this basis, the ingestion of 3 lb. of an average sample of colostrum during the first 4 days of lactation probably would be adequate for ensuring an appreciable storage of vitamin A in the liver and a satisfactory level of carotene and vitamin A in the blood of the calf. None of the calves born from the above cows showed any signs of vitamin A deficiency. The calves received colostrum *ad libitum;* consequently, the vitamin A supply was satisfactory even though the carotene and vitamin A contents of colostrum were low in a few cases.

Carotene and vitamin A in colostral fat. In order to obtain more detailed information on the carotene and vitamin A contents of the colostral fat, values for each individual cow were determined. The results are presented in tables 4 and 5. Table 4 gives the data on the carotene content of the colostral fat. A marked drop on the second day of lactation occurred in all but two cows (nos. 13 and 15), and a further sharp drop occurred on the third day in all of the cows except no. 4. Thereafter the decline was slow and the carotene level became almost constant by the seventh day. The average first day's colostrum contained seven times as much carotene as the average eighth day's sample. No appreciable dif-

TABLE 4

Variations in the carotene content of colostral fat during the first 8 days of lactation

Animal no.	Days after parturition							
	1	2	3	4	5	6	7	8
	(γ *carotene/g. colostral fat*)							
1[a]	67.5	40.7	25.5	14.6	9.3	6.7	5.6	5.6
2	29.3	19.6	6.4	5.5	3.9	3.3	3.0	.....
3[b]	5.7	5.1	4.5	4.6	3.6	3.2	2.3	2.5
4	24.8	8.0	18.6	5.7	.....	.....	6.1	.....
5	28.5	9.8	4.9	.....	.....	.....	2.7	2.5
6	31.8	22.5	8.0	4.7	.....	.....	2.3	2.1
7	27.9	16.8	8.4	5.2	4.5	4.0	4.1	3.9
8	18.1	10.2	9.8	5.8	5.3	5.3	4.7	3.7
9[b]	5.4	3.6	2.7	3.5	5.4	5.7	3.3	3.4
10[b]	20.9	16.2	3.9	3.4	2.6	3.8	3.0	2.9
11	20.2	14.5	8.3	6.8	3.1	3.1	2.0	.....
12	25.7	15.8	10.6	10.9	7.3	8.8	4.7	2.7
13	14.7	17.1	8.7	7.2	4.1	2.4	3.3	3.9
14	23.1	14.4	8.0	8.1	9.5	5.0	5.2	5.6
15[b]	8.2	18.0	12.2	8.1	.....	.....	.....	2.4
Av.	23.5	15.5	9.4	6.7	5.3	4.7	3.7	3.4

[a] Received comparatively large quantity of green fodders before calving because of the monsoon months.

[b] Received very little carotene in the ration before calving because of the drought.

ference was noted between the Hariana and the Sahiwal breeds in regard to their ability to secrete carotene in butterfat. Owing to the small number of animals, the effect of feed on the carotene content of the butterfat could not be studied thoroughly. However, an examination of the data in

TABLE 5

Variations in the vitamin A content of colostral fat during the first 8 days of lactation

Animal no.	Days after parturition							
	1	2	3	4	5	6	7	8
	(γ *vitamin A/g. colostral fat*)							
1	147.7	77.9	52.0	39.0	28.0	22.0	17.5	16.2
2	72.6	60.0	20.9	17.1	14.3	14.2	12.8	.....
3	41.0	45.4	52.1	15.8	15.3	15.0	11.7	11.7
4	69.4	43.1	50.0	24.5	.....	.....	22.7	.....
5	89.1	38.6	23.6	.....	.....	.....	23.0	18.0
6	106.0	75.9	28.1	11.9	.....	.....	11.1	10.1
7	82.9	47.3	29.2	21.1	15.5	11.0	11.0	11.2
8	49.8	25.2	39.7	25.4	18.3	15.1	13.2	12.8
9	30.2	17.4	18.2	17.9	14.2	12.3	13.2	12.7
10	24.3	33.2	29.0	45.0	13.4	9.8	8.9	8.6
11	76.7	60.4	48.6	27.9	24.0	19.2	13.9	.....
12	94.8	52.0	44.3	43.8	49.5	46.4	15.2	12.3
13	116.7	127.0	56.8	36.3	20.6	13.7	13.0	13.1
14	117.2	78.1	50.5	12.9	13.7	12.6	11.2	12.0
15	80.0	64.5	53.0	25.2	.....	.....	.....	11.0
Av.	79.9	56.4	39.7	26.0	20.6	17.4	14.2	12.5

table 4 reveals that there was no consistent relationship between the carotene intake and the carotene content of the colostral fat from all the animals except for a few. Animals 3, 9, 10 and 15 secreted very small amounts of carotene, whereas animal no. 1 secreted a comparatively large amount in the colostral fat. The first four animals were on a carotene-poor ration due to the drought period, whereas the fifth one received large quantities of green feed due to the periodic monsoon. These results tend to show that, in spite of a number of variables, the carotene content of colostral fat also is affected, like butterfat, by the type of feed.

The data in table 5 show the decrease in the vitamin A content of the colostral fat during the first 8 days. The average first day's colostrum contained more than six times as much vitamin A as the average eighth day's sample. These results compare favorably with those of Semb *et al.* (26), who observed that this ratio varied from five to fifteen. Although a few of the animals secreted less vitamin A in the colostrum on the first day than on the second, this was not generally the case in subsequent samples. The change in the vitamin A content as a result of the dry ration was not so apparent as it was in the case of carotene. This might be explained on the basis of the relative ease with which carotene is mobilized as compared to vitamin A. Although there was no difference between the average carotene content of the colostral fat from first-calf heifers and cows, the vitamin A content tended to be higher in the case of the former. It is difficult to say definitely, under the present experimental conditions, whether or not first-calf heifers secrete more vitamin A in colostral fat than do cows, an observation also made by Dann (5) and Henry *et al.* (13).

Although the numerical values reported in this investigation are not the same as those found by other investigators, there is some parallelism in the findings, especially when due consideration is given to such differences as diet, breed, and environment.

SUMMARY

Colostrum samples from Hariana and Sahiwal cows have been analyzed for the percentage composition of carotene, vitamin A, fat, solids-not-fat, protein and ash.

1. The colostrum contains more solids-not-fat, protein, and ash than does the normal milk.

2. Colostrum contains more than four times as much carotene and vitamin A as milk.

3. Colostral fat was found to be six to seven times richer in carotene and vitamin A than the fat of normal milk, but both of these constituents decreased markedly during the first week postpartum. The decrease thereafter was relatively slow and carotene appeared to be affected more than vitamin A by the type of ration fed to the animals.

4. The carotene content of colostral fat of first-calf Sahiwal heifers was comparable to that secreted by the Hariana cows, but the heifers secreted more vitamin A in the colostral fat than did the cows.

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COMPARATIVE ANTIRACHITIC VALUE OF FIELD-CURED HAY, BARN-DRIED HAY, AND WILTED GRASS SILAGE FOR GROWING DAIRY CALVES

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According to present opinion, hay crops contain little or no vitamin D before they are cut. It is only after the crop is cut and while it is exposed to the rays of the sun during the curing process that activation of certain plant sterols takes place to form vitamin D.

With the newer methods of conserving hay crops, such as curing the hay in the barn or making wilted silage, the time of exposure to the rays of the sun is less than when the crop is cured in the field. Consequently, when barn-cured hay or wilted silage is the sole source of vitamin D for calves, it might be questionable whether they would obtain enough of the vitamin to meet their requirements. A review of the literature does not supply a direct answer to this question.

Some information on the vitamin D content of forage plants subjected to various curing processes, as determined by rat bioassays, is available. Steenbock *et al.* (9) reported that clover leaves cured without exposure to direct sunlight showed no vitamin D activity when fed to rats at the 1- and 5-per cent levels. On the other hand, leaves from the same field that were cured in the sun and fed on the same basis showed definite vitamin D activity. In later experiments Hart *et al.* (4) found that alfalfa hay cured in Colorado with limited exposure to the sun contained some vitamin D but less than hay cured with full exposure to the sun. Russell (7) reported some vitamin D activity in alfalfa leaves cured out of sunlight but considerably less than in leaves cured in the sun or leaves cured in the sun and irradiated. Smith and Briggs (8) reported very little vitamin D activity of alfalfa leaves cured in the dark. Leaves cured for 15 hours in sunlight had considerable activity but not so much as leaves exposed for 57 hours. However, Hodgson and Knott (5) found that an artificially dehydrated pasture mixture of English ryegrass, Italian ryegrass, and white clover from irrigated land had as much calcifying activity as the same material sun cured. Wallis (10) reported a considerable increase in the vitamin D activity of alfalfa hay after sun curing. However, considerable variation was found between crops in this respect, and it was concluded that "there are other influences than the amount of sunshine received which greatly affect the vitamin D content of the resulting hay." One

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sample of hay cut after dark and dried artificially contained 812 I.U. per pound. This value is equal to or greater than some of the values reported for sun-cured hay. Bechdel *et al.* (1) found that artificially dried alfalfa contained considerably less vitamin D than sun-cured hay. These same workers report values of 150 to 300 USP units of vitamin D per pound in two different lots of night-harvested dehydrated alfalfa hay. If such a hay were fed to calves to the extent of their roughage requirements, it should furnish the minimum requirements.

Some experimental data on the antirachitic value of hay for calves have been reported in the literature. Huffman *et al.* (6) found that 2 lb. per day of sun-cured timothy prevented rickets up to 1 year of age and 3 lb. per day cured rickets in a 9-month-old calf. Two pounds of sun-cured alfalfa hay per day prevented rickets in one calf up to 195 days of age. In using the curative method, these workers were unable to obtain a sufficient intake of timothy hay cured in the dark to determine its antirachitic effect. In studying the data of these workers, one comes to the conclusion that about 0.7 lb. of sun-cured hay per 100 lb. of body weight is about a minimum for the prevention of rickets in growing calves.

The Pennsylvania Agricultural Experiment Station (2) found that a mild rachitic condition developed in a 6-month feeding period with 1 lb. of sun-cured alfalfa hay per day added to the basal diet, whereas on the same basis artificially cured alfalfa permitted a severe rachitic condition to develop. Two and one-half pounds of good sun-cured alfalfa hay per hay prevented the development of a rachitic condition during a 6-month period, whereas the same quantity of artificially cured hay permitted the development of a mild rachitic condition.

The review of literature gives little information for making practical recommendations on the question of whether vitamin D supplements should be used when barn-cured hay or wilted silage is fed as the sole source of vitamin D for calves. Results reported in the literature on rat bioassays with hays show that hay cured without exposure to the sun contains considerably less vitamin D than sun-cured hay. The data with calves likewise give the same indication. In the experiments thus far conducted with calves, limited quantities of hay were fed to bring out differences between sun curing and artificial curing. This raises the question of whether the artificially dried hay would not have furnished sufficient vitamin D for calves had it been fed according to body weight or the appetite of the calf (2 to 2.5 lb. per 100 lb). For this reason the data on artificially cured hay cannot be used in making practical recommendations for vitamin D supplementation either for artificially cured hay, barn-cured hay, or wilted silage.

The present study was undertaken to determine whether barn-cured hay or wilted silage will supply sufficient vitamin D to growing calves when it

is fed at adequate levels, *i.e.*, at levels which ordinarily would be fed under practical feeding conditions.

EXPERIMENTAL PROCEDURE

In the summer of 1945 a second cutting of alfalfa was harvested simultaneously as field-cured hay, barn-cured hay, and wilted silage. Good weather conditions prevailed during the time the crop was being harvested by the three methods, so there was a maximum exposure to the sun during daylight hours. The wilted silage was exposed for 4 to 6 hours between sunrise and sunset, the barn-cured hay for 12 to 16 hours, and the field-cured hay for 30 to 40 hours, although there was considerable variation in this respect.

Holstein and Jersey male calves were reared to 90 days of age on a ration of skim milk, grain, alfalfa hay and cod-liver oil. In addition, it was necessary to use two crossbred calves, one of which was placed in the Holstein group and one in the Jersey group. Flaxseed jelly, corn meal, or grain were added to the skim milk, beginning when the calves were about 10 days of age, in order to increase the energy intake. Skim milk was discontinued at 30 days of age for the Holsteins and at 45 days for the Jersey calves. Three calves on the experiment (503, 701, 703) received whole milk to 60 days of age, along with alfalfa hay and grain.

At 90 days of age the calves were placed on the basal ration made up as follows: Corn meal, 60 parts; wheat bran, 30 parts; soybean meal, 20 parts; linseed meal, 10 parts; iodized salt, 1 part; calcium carbonate, 2 parts. In addition, 1 lb. of beet pulp per 100 lb. of body weight, 100 g. of dehydrated alfalfa leaf meal, and 4 lb. of skim milk were fed daily. The calves were kept on this ration for a period of 50 days or until they were 140 days of age in order to deplete their vitamin D stores. Calcium, phosphorus and phosphatase values of the blood were used to measure depletion. Following the depletion period the calves were fed, in addition to the basal grain ration, the particular experimental forage they were to receive for a period of 180 days. In some instances it was necessary to place the calves on their respective forages before the end of the 50-day depletion period because of blood values which indicated the incipient stage of rickets. The calves were kept in a darkened barn out of direct sunlight. They were turned to a dry lot for exercise at night.

Groups of six calves each were fed the alfalfa forage cured by the three different methods. Within each group three different levels of forage were fed with two calves on each level (table 1). The Jersey and Holstein calves were distributed equally between and within groups.

The wilted silage was fed on a hay-equivalent basis, taking into consideration the moisture content. The calves received, in addition to the basal grain ration and the specified forage, 4 lb. of skim milk daily. Total

digestible nutrients were fed according to the Morrison standard by adjusting the grain intake after allowing for the T.D.N. in the skim milk and forage. Adjustments of forage and grain were made each 2 weeks.

Two positive control calves were continued on the depletion ration but received 10,200 USP units of vitamin D daily in the form of irradiated yeast after the 50-day depletion period. One negative control animal was used which received the depletion ration but no vitamin D.

After the calves received the forage for 180 days they were slaughtered and the eighth and ninth ribs were saved for ash analysis. Ash determinations were made on the distal 10 per cent of the two ribs after they were subjected to hot alcohol extraction. The calcium, inorganic phosphorus and phosphatase contents of the blood were determined each week, except toward the end of the experiment, when the determinations were made each 2 weeks.

Rat bioassays for vitamin D were made on the forage put up by the

TABLE 1

Rate of forage feeding per 100 lb. of body weight

No. of calves	Field-cured hay (Group 1)	Barn-cured hay (Group 2)	Wilted silage (hay equivalent) (Group 3)
	(*lb.*)	(*lb.*)	(*lb.*)
2	0.5	0.7	0.7
2	1.0	1.2	1.2
2	1.5	1.7	1.7

three procedures in order to obtain comparative values. The usual line test procedure was used by including 10 per cent of the forage in the basal rachitogenic diet.

In 1946 another crop of wilted alfalfa silage was fed to two calves, beginning as soon after birth as the calves would consume the silage. They received a limited quantity of whole milk to 60 days of age, but after this time their sole source of vitamin D was from the wilted alfalfa silage. The silage was fed on a hay-equivalent basis of 1.5 lb. per 100 lb. of body weight. The two calves were slaughtered at 8 and 9 months of age.

RESULTS AND DISCUSSION

The effect of feeding alfalfa cured by the three different methods on rate of growth is shown in table 2. These data show that the best rate of gain was made by the calves on the wilted silage, their average daily gain being 1.71 lb. per day for the 180-day period. The next best gain was by the calves on barn-cured hay, which averaged 1.65 lb. a day, whereas the field-cured hay produced a daily gain of 1.48 lb. Since the feed intake was well-controlled, these results indicate that good gains can be obtained with

TABLE 2

Gain in weight on field-cured and barn-cured hay and on wilted silage (180-day feeding period)

Field-cured hay			Barn-cured hay			Wilted silage		
Calf no.	Rate[a]	Total gain	Calf no.	Rate[a]	Total gain	Calf no.	Rate[a]	Total gain
	(*lb.*)	(*lb.*)		(*lb.*)	(*lb.*)		(*lb.*)	(*lb.*)
705–H[b]	0.5	330	503–H	0.7	270	703–H	0.7	350
2380–J	0.5	218	2384–J	0.7	288	2379–J	0.7	259
250–H	1.0	300	2557–H	1.2	344	120–H	1.2	330
504–J	1.0	279	2385–J	1.2	266	505–J	1.2	263
330–X	1.5	279	2558–H	1.7	301	701–H	1.7	342
2383–J	1.5	248	332–X	1.7	314	506–J	1.7	282
Total av. gain		267	.....	.....	297	.....	.....	307
Av. daily gain		1.48	.....	.....	1.65	.....	.....	1.71

[a] Rate = hay or hay equivalent daily per 100 lb. of body weight.
[b] H = Holstein; J = Jersey; X = Crossbred.

wilted silage. There was very little feed refusal by the two calves that were fed at the highest level (an equivalent of 1.7 lb. of hay per day per 100 lb. of body weight). One Holstein calf, weighing 650 lb., consumed as much as 30 lb. of wilted silage per day, which was the sole roughage. The calves on the wilted silage were very sleek in appearance and appeared to do well throughout the experiment.

The results of the ash analyses of the distal 10 per cent of the eighth and ninth ribs are shown in table 3. The results of the analyses of the two ribs were averaged. These results show that all three forages possessed definite antirachitic properties for calves. There does not appear to be any dif-

TABLE 3

The ash values of rib ends

Field-cured hay			Barn-cured hay			Wilted silage		
Calf no.	Rate[a]	Ash	Calf no.	Rate[a]	Ash	Calf no.	Rate[a]	Ash
	(*lb.*)	(%)		(*lb.*)	(%)		(*lb.*)	(%)
705–H	0.5	53.1 N[b]	503–H	0.7	50.9 Sl.st	703–H	0.7	53.0 N
2380–J	0.5	55.8 Sl.st	2384–J	0.7	56.6 N	2379–J	0.7	55.0 St
250–H	1.0	56.5 Sl.st	2557–H	1.2	61.1 Sl.st	120–H	1.2	54.9 St
504–J	1.0	56.7 N	2385–J	1.2	56.2 Sl.st	505–J	1.2	59.0 N
330–X	1.5	56.0 N	2558–H	1.7	59.4 Sl.st	701–H	1.7	57.1 Sl.st
2383–J	1.5	61.2 N	332–X	1.7	58.8 N	506–J	1.7	62.0 N
Negative 2570–H		39.0 St						
Positive			2571–H		50.8 Sl.st			
Positive			508–J		51.6 N			

[a] Rate = hay or hay equivalent per day per 100 lb. of body weight.
[b] N = normal; Sl.st = slightly stiff; St = stiff.

ference in this respect between the three lots of calves that were fed the three different kinds of forage. In all three groups, the calves that were fed at the lowest roughage level showed the lowest ash values. The ash value for the negative control was only 39 per cent and it was necessary to remove this calf from the experiment after 160 days because of the extreme rachitic condition. The ash values for the two positive control animals were not so high as for the calves that were fed the various levels of forage, even

TABLE 4

The effect of feeding 1.5 lb. field-cured hay per 100 lb. body weight on blood calcium, phosphorous and phosphatase
(Data on one calf)

Age	Calcium	Phosphorus	Phosphatase
(days)	*(mg./100 ml.)*	*(mg./100 ml.)*	*(units/100 ml.)*
	Basal ration		
95	8.1	8.1	14.3
102	7.0	6.2	20.6
109	6.7	6.4	18.2
116	7.1	4.8	17.2
	Hay added		
122	8.0	5.8	18.2
130	7.8	4.5	17.9
136	8.1	5.8	9.4
147	9.0	6.8	18.8
161	11.3	8.4	12.4
175	11.3	9.3	10.7
189	11.4	9.3	9.9
196	10.3	8.0	9.2
218	10.6	6.4	10.4
231	9.3	6.9	9.9
245	10.5	7.6	9.2
259	10.0	5.8	5.8
274	10.1	7.4	6.6
287	10.4	8.6	5.0
302	10.0	8.7	5.7
316	9.6	8.1	3.9

though the controls were fed 10,200 USP units of vitamin D per day. The calcium intake of these two calves was not so high as for the calves receiving forage, since no extra calcium was fed, yet the intake at the end of the experimental period was as much as 18 g., or well above a 10-g.-minimum. Therefore, forages may contain factors other than vitamin D which aid in calcification.

The calves were examined periodically for evidence of stiffness (table 3). While there was less stiffness or indication of clinical rickets in the group that received field-cured hay, between groups the differences probably are not significant. There did not appear to be a direct correlation between stiffness and the bone ash values. It also was noted that the calves that

showed stiffness did not show abnormal blood values for calcium, inorganic phosphorus and phosphatase at the end of the experiment. During the depletion period, however, abnormal blood values for calcium, phosphorus and phosphatase usually preceded the clinical signs of rickets.

All the detailed data of blood analyses for each calf cannot be presented. However, the data for one crossbred and two Holstein calves that were fed the largest intake on each kind of forage are shown in tables 4, 5

TABLE 5

The effect of feeding 1.7 lb. barn-dried hay per 100 lb. body weight on blood calcium, phosphorus and phosphatase
(Data on one calf)

Age	Calcium	Phosphorus	Phosphatase
(days)	*(mg./100 ml.)*	*(mg./100 ml.)*	*(units/100 ml.)*
	Basal ration		
91	10.9	8.8	8.4
108	11.3	8.0	10.2
115	10.5	6.8	13.9
122	8.5	7.4	14.6
128	7.6	7.7	.....
136	6.4	8.6	19.4
	Hay added		
142	6.3	8.2	16.2
153	8.4	7.4	14.2
167	11.6	8.8	8.4
181	10.8	9.6	7.2
195	11.2	8.9	7.1
210	10.3	8.3	7.3
224	9.6	7.0	6.6
237	9.3	7.8	6.6
251	10.3	6.8	8.0
265	9.8	6.8	9.2
280	9.7	6.4	5.6
293	9.8	8.6	5.0
308	9.5	6.2	7.0
322	9.8	7.0	7.0

and 6. A study of the detailed data does not reveal any marked differences between the three groups of calves. The addition of forage in the three different forms caused the blood values to return to normal following the depletion period.

The quantity of solar radiation received by the forage, as shown in table 7, was calculated from hourly figures covering the period the forage was in the swath and windrow. The values are in terms of gram-calories per square centimeter of horizontal surface. The vitamin D content of the forage as determined by rat bioassays also is shown in the table in terms of International Units of vitamin D per g. of air-dried forage. There does not appear to be any close correlation between the amount of solar radia-

tion and the vitamin D content of the forage, although the vitamin D content in the field-cured hay was somewhat higher.

Using the figure 0.47 I.U. per g. for the barn-dried hay, when the calves were fed at the rate of 0.7 lb. of barn-dried hay per 100 lb. of body weight the intake of vitamin D would be 150 I.U. per 100 lb. of body weight. On the same basis, when the calves were fed at the rate of 1.2 lb. of barn-dried hay per 100 lb. of body weight, the vitamin D intake would be 256 I.U., and when they were fed at the rate of 1.7 lb. the intake would be 363 I.U. per

TABLE 6

The effect of feeding 1.7 lb. hay equivalent of silage per 100 lb. body weight on blood calcium, phosphorus and phosphatase
(Data on one calf)

Age	Calcium	Phosphorus	Phosphatase
(days)	*(mg./100 ml.)*	*(mg./100 ml.)*	*(units/100 ml.)*
	Basal ration		
101	11.8	9.4	.....
107	10.5	9.5	.....
115	11.6	9.1	12.5
122	10.5	8.1	16.4
129	10.6	.....	17.9
136	9.4	8.2	15.0
	Silage added		
142	10.8	7.5	16.5
150	10.6	6.8	10.6
156	11.9	7.3	8.7
167	12.1	7.9	6.9
181	.....	8.1	6.3
195	10.8	8.7	8.1
209	12.3	7.4	8.6
225	10.0	7.8	7.2
238	10.9	6.6	6.2
251	9.7	6.3	6.9
265	10.5	6.7	6.1
279	9.7	6.8	7.6
294	9.7	6.9	5.9
307	10.0	7.1	3.7
322	9.7	7.0	7.8

100 lb. of body weight. If 300 I.U. per 100 lb. of body weight is taken as the minimum requirement, it would be met by the feeding of 1.5 to 1.7 lb. of barn-dried hay per 100 lb. of body weight. On this basis, one would not expect marked rickets to develop in the calves fed wilted silage and barn-cured hay in this experiment even though the vitamin D intake was near the minimum allowance or slightly below. The observations on stiffness and bone ash values confirm this opinion.

The two calves that were fed from birth the wilted silage that was made in 1946 showed no evidence of rickets at any time during the experiment.

The calcium, phosphorus and phosphatase values of the blood remained within the normal range. When these two calves were slaughtered at 8 and 9 months of age, respectively, they were in excellent condition and had sleek hair coats. These two calves did not consume as much dry matter from wilted silage up to 90 days of age as they would be expected to consume from hay. However, after 90 days of age they easily consumed the 1.5 lb. (hay equivalent) of wilted silage offered per 100 lb. of body weight.

The vitamin D content of the three forages put up simultaneously in 1946 as determined by rat bioassays is shown in table 7. The figure 0.87 for the wilted silage was checked and found to be correct. Thus, one would not expect the two calves that were fed the wilted silage containing 0.87 I.U. per g. to develop rickets. The high vitamin D content of this lot

TABLE 7

Exposure of forage to solar radiation and its vitamin D content

	Radiation exposure[a]			Vitamin D content
	In swath	In windrow	Total	
	(*g.-cal./cm.*²)	(*g.-cal./cm.*²)	(*g.-cal./cm.*²)	(*I.U./g.*)
		1945 crop		
Wilted silage	104	117	221	0.56
Barn-dried hay	364	158	522	0.47
Field-cured hay	573	777	1350	0.97
		1946 crop		
Wilted silage	134	109	243	0.87
Barn-dried hay	323	218	541	0.58
Field-cured hay	515	958	1473	0.88

[a] Calculated from data furnished by Dr. W. F. Shenton of American University of Washington, D. C.

of wilted silage probably was due to the presence of a larger quantity of foreign material in the crop than was present in the field-cured hay and barn-cured hay. It was necessary in 1946 to use one field which was somewhat weedy in order to have sufficient silage for the planned experiments.

Therefore, the results indicate that barn-cured hay and wilted silage conserved under the conditions of this experiment will contain sufficient vitamin D to prevent rickets in dairy calves when these forages are consumed at adequate levels. The rat bioassay data show that these forages contained less vitamin D than the field-cured hay. The difference, however, was not sufficiently great to precipitate rickets when the forages were fed at the rate of 1.5 to 1.7 lb. of hay equivalent per 100 lb. of body weight.

A greater difference in the vitamin D content of these forages might have been expected in view of the accepted concept of the mechanism of formation of vitamin D in forage crops. However, while the field curing of hay promotes an increase in its vitamin D content, there may be con-

siderable vitamin D present in the crop as it stands in the field. The amount of vitamin D probably is governed by the quantity or area of dead plant tissue, such as dead stems or leaves or partially injured leaves. The amount of dead tissue might vary, depending on such factors as climatic conditions, stage of maturity, disease, and insect injury. Thus, in the case of leaf hopper injury, minute injured areas exist on the surface of the leaf, where activation of the sterols might take place. Probably an absolutely green plant without injury of any sort would contain no vitamin D. However, under practical conditions in the eastern section of the country it is doubtful whether such a condition ever exists.

The suggestion that the vitamin D content of hay crops might be affected by the quantity of dead material in the crop at time of cutting is found in the paper of Bechtel *et al.* (3). For instance, these investigators found that, in the corn plant at the silage-making stage, the silks, tassels, and dried leaves were excellent sources of vitamin D, whereas the green part of the plant was devoid of vitamin D. The effect of some of these factors on the vitamin D content of tissues of forage plants, now is under investigation.

While it seems probable that wilted silage and barn-cured hay contain sufficient vitamin D so that no supplementary feeding of vitamin D is needed for calves kept out of direct sunlight, further fundamental studies on the factors affecting the vitamin D content of plant tissue as it stands in the field at the hay stage need to be carried out. It would seem quite probable that a higher vitamin D intake might be possible where a good quality of barn-cured hay or wilted silage is fed than where a poor quality of field-cured hay is fed, because of the greater palatability of the former.

Rickets has been reported in dairy calves under practical farm conditions where grain was fed in excess so that very little sun-cured hay was consumed. Vitamin A and calcium deficiency also probably would be present where such a feeding practice is being used. It would seem more logical to advocate proper management practices to correct such conditions rather than the addition of supplements to the grain mixtures fed. Limiting the grain fed so that hay consumption could be increased and exposure of the calves to sun would be of benefit. Calves exposed to direct sunlight during the winter months and receiving no other source of vitamin D do not develop rickets at Beltsville.

Vitamin D sometimes is added to commercial grain mixtures for mature dairy cattle. While the cost is low, it is the opinion of the authors that there are not sufficient concrete scientific data at the present time to warrant such a practice. Later developments may justify such supplementation, but scientific fiction is not sufficient justification for such a practice.

SUMMARY AND CONCLUSIONS

1. Wilted silage fed as the sole roughage to growing dairy calves produced gains as good or better than those observed in calves fed barn-cured

and field-cured hay. The calves that were fed the wilted silage were sleek and excellent in appearance.

2. Wilted silage, made in two different years, contained sufficient vitamin D to prevent rickets in growing calves that were kept out of sunlight when the silage was fed at the rate of 1.2 to 1.7 lb. per 100 lb. of body weight on the hay-equivalent basis (3 to 4 lb. per 100 lb. body weight on the silage basis).

3. Barn-cured hay made one year contained sufficient vitamin D to prevent rickets in calves that were kept out of sunlight when it was fed at the rate of 1.2 to 1.7 lb. per 100 lb. of body weight.

4. Rat bioassays of the forages fed for vitamin D, which confirmed the results of the calf-feeding experiment, showed that they contained sufficient vitamin D to prevent rickets in growing calves.

5. While further fundamental data must be collected on the factors affecting the vitamin D content of forages harvested with a minimum exposure to the sun, it seems quite likely that barn-cured hay and wilted silage, at least as conserved under Beltsville conditions, contain sufficient vitamin D for growing calves to prevent rickets if fed at the usual levels of roughage feeding.

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THE EFFECT OF STREPTOMYCIN UPON THE LIVABILITY AND BACTERIAL CONTENT OF BOVINE SEMEN[1]

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Various antibacterial agents have been used in efforts to overcome the problems associated with the presence of bacteria in bull semen used for artificial breeding (1, 4, 5, 6). Earlier investigations at this Experiment Station (1) upon the use of penicillin in diluted bull semen showed that certain organisms were resistant to penicillin at levels as high as 2,000 units per ml. Since streptomycin inhibits the growth of a number of organisms which are insusceptible or only slightly susceptible to penicillin, it seemed desirable to study its effect on bacteria commonly found in bull semen.

Gunsalus *et al.* (2, 3) have reported that bulls harboring *Pseudomonas aeruginosa* in their reproductive tracts were apt to have low breeding efficiencies and be poor risks for use in artificial breeding. Since Waksman and Reilly (7) have found streptomycin to be bactericidal for *Pseudomonas aeruginosa,* its addition to semen might restore normal breeding efficiency to bulls of lowered fertility known to disseminate this organism in their semen.

EXPERIMENTAL

Effect of streptomycin upon the livability of spermatozoa. In a preliminary study to determine the relative resistance of stored bull spermatozoa to streptomycin, this antibiotic was added to four ejaculates diluted 1:24 with yolk-citrate diluter at levels of 100, 500, 1,000, 1,500, 2,000, 2,500, 5,000 and 10,000 units or γ per ml. of diluted semen. When compared to untreated controls, no marked differences in spermatozoan livability were noted during the 20-day storage period in the levels ranging from 100 to 1,500 γ. However, concentrations of 2,500, 5,000 and 10,000 γ per ml. of diluted semen greatly reduced motility during storage. On the basis of these results, streptomycin was added to ten samples of bull semen at the rate of 100, 250, 500, 750, 1,000, 1,250, 1,500 and 2,000 γ per ml. of diluted semen with appropriate controls. Each of the ten ejaculates was diluted 1:24 with yolk-citrate diluter composed of one part of fresh egg yolk and one part of citrate buffer prepared by dissolving 3.6 g.

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of sodium citrate dihydrate in 100 ml. of water distilled over glass. The streptomycin powder was dissolved in sterile sodium citrate solution and mixed with egg yolk to provide a diluter with a 1:1 ratio of yolk to buffer.

The diluted samples were stored at 4.5° C. and the percentages of motile spermatozoa were determined every 2 days for 20 days. In order to avoid bias on the part of the observer making the motility estimations, randomized numbers were placed on the test tubes of diluted semen containing the various levels of streptomycin. Bacterial counts and streptomycin assays were made on these samples after 0, 8 and 16 days of storage.

The ten ejaculates had a mean concentration of 1,054,000 spermatozoa per cubic millimeter, a mean initial motility of 69 per cent active spermatozoa, and a mean methylene blue reduction time of 9.4 minutes.

The mean motility data for the ten ejaculates are shown in table 1.

TABLE 1

The effect of streptomycin upon the livability of bovine spermatozoa
(Mean of 10 determinations)

Streptomycin units per ml. of diluted semen	Per cent motile spermatozoa					
	Before storage	After storage at 4.5° C. for				
		4 days	8 days	12 days	16 days	20 days
Control	69	59	44	33	16	10
100	69	57	47	33	19	8
250	69	58	47	34	17	8
500	69	61	47	34	19	5
750	69	59	46	31	18	5
1000	69	59	45	32	15	9
1250	69	59	45	25	14	3
1500	69	60	42	27	13	6
2000	69	60	43	25	14	4

Using the observations made at each 2-day interval, analysis of variance showed no significant differences in spermatozoan livability between levels of streptomycin of 0, 100, 250, 500, 750 and 1,000 γ per ml. However, the three highest levels (1,250, 1,500 and 2,000 γ per ml. of diluted semen) brought about a highly significant decrease in livability as compared to untreated diluted semen.

The relationship between spermatozoan livability and concentration of streptomycin was studied further by means of regression. While both highly significant linear and curvilinear regressions were calculated, a test for significance of departure from linearity showed that a straight line was more applicable to the livability data (fig. 1.). Compared to untreated control samples, the mean percentage of motile spermatozoa during storage for 20 days decreased by 0.5 per cent for each addition of 250 γ of streptomycin.

Effect of streptomycin upon the bacterial content of diluted semen. Bacterial plate counts were determined on nine samples of diluted semen

after 0, 8 and 16 days of storage using veal infusion agar containing 4 per cent sterile defibrinated ox blood. The samples were plated in dilutions of 1:10, 1:100, 1:1,000 and 1:10,000 and incubated for 48 hours at 37° C. Desoxycholate agar plates incubated at 37° C. were used for determining the number of bacteria belonging to the coliform group. The same procedure was followed in obtaining bacterial counts on portions of undiluted semen and plain yolk-citrate diluter stored for 0, 8 and 16 days.

The results of the bacterial plate counts are shown in figures 2, 3 and 4. Logarithmic rather than arithmetic means have been used to express the mean number of bacteria in the nine semen samples. Since 1:10 was the lowest serial dilution employed and at least 25 colonies were required at

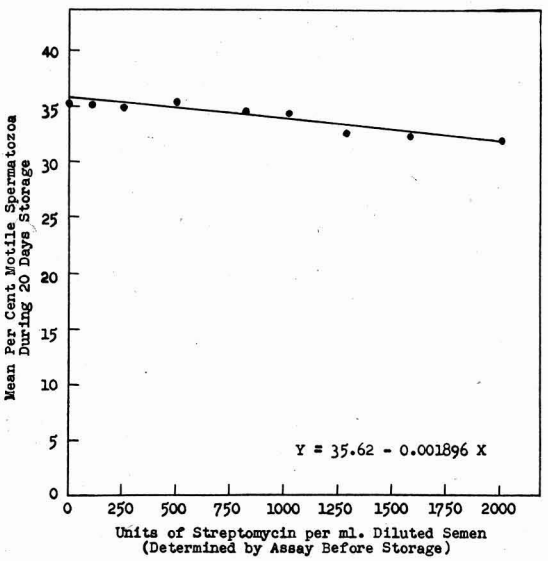

FIG. 1. Relationship of per cent motile spermatozoa during 20 days of storage to level of streptomycin, as shown by regression.

this dilution before a count was considered significant, any counts below log number 2.40 only indicate that the material was not sterile.

As shown in figure 2, levels of streptomycin above 100 γ per ml. were most effective in inhibiting growth of bacteria in freshly diluted semen. Complete inhibition was obtained at all levels of streptomycin in seven of the nine samples. Freshly diluted semen without streptomycin contained an average of 5,000 bacteria per ml., as compared to an average of only 120 bacteria per ml. for the portions of diluted semen containing added streptomycin.

Figure 3 shows that all levels of streptomycin retarded bacterial growth in diluted semen stored for 8 days at 4.5° C. The tubes of untreated

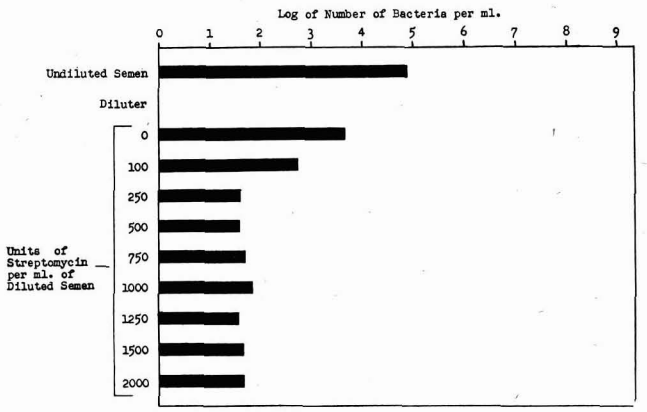

FIG. 2. The effect of streptomycin upon bacterial growth in freshly diluted semen.

semen averaged 82,000 bacteria per ml. while those containing streptomycin averaged 2,000 bacteria per ml. of diluted semen. The high average counts at the 500 and 1,500 γ levels were due to one sample of semen. Possible explanations are contamination during bacteriological analysis or contamination of the individual test tubes of diluted semen with streptomycin-resistant organisms when the tubes were opened for routine motility observations during storage.

Culture plate counts made after 16 days of storage, as shown in figure 4, were rather erratic. In four of the nine diluted samples, minute, pinpoint colonies were present which made counting rather difficult. However, in the remaining five samples streptomycin showed fairly good inhibition of bacterial growth as compared with the controls. The average bac-

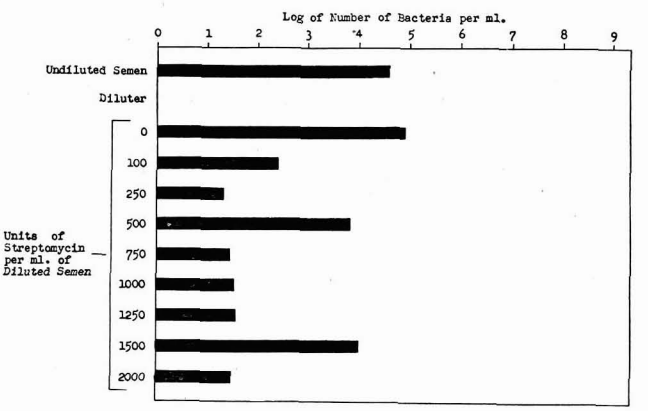

FIG. 3. The effect of streptomycin upon bacterial growth in diluted semen stored for 8 days at 4.5° C.

terial count for diluted semen without streptomycin was 131,000,000 per ml., while the average for diluted semen containing the various levels of the antibiotic was 137,000 per ml. Thus, the antibacterial activity of streptomycin was greatest in freshly diluted semen and semen stored for 8 days.

Very few typical bacteria of the coliform group were present on the desoxycholate agar plates. Only one sample had countable plates and these were present only in the undiluted semen and the tube of diluted semen which did not receive streptomycin. The number of bacteria of the coliform group increased in these two tubes during storage. While there were only a few organisms of this type per ml. in the fresh undiluted semen, counts of 250 and 50,000 per ml. were obtained after storage for 8

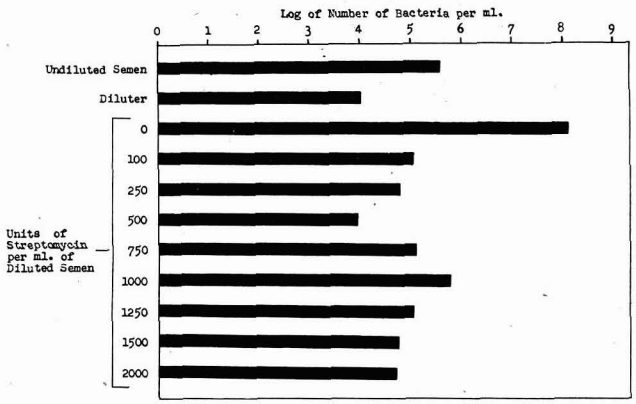

FIG. 4. The effect of streptomycin upon bacterial growth in diluted semen stored for 16 days at 4.5° C.

and 16 days, respectively. The number of coliform bacteria in the untreated diluted semen increased from a few colonies before storage to 5,000 per ml. after 8 days of storage and 1,000,000 per ml. after 16 days of storage.

Total plate counts on the ten samples of fresh, undiluted semen used in the final livability study ranged from 2,000 to 350,000 bacteria per ml., with a mean of 73,000 bacteria per ml. The samples were collected with an artificial vagina and only two of the ten ejaculates had counts exceeding 100,000 bacteria per ml., while seven of the remaining eight had counts of 30,000 or less per ml.

Stability of streptomycin in diluted semen. The stability of streptomycin in diluted semen stored at 4.5° C. was determined by assays at 0, 8 and 16 days with the standard cylinder plate method, using *Bacillus subtilis* as the test organism. The results of the assays made on ten diluted semen samples are presented in table 2. There was no appreciable decrease in the amount of streptomycin over the 16-day storage period.

Studies are now in progress to test the effect of streptomycin upon the fertility of diluted semen used for artificial breeding. Its use in combination with penicillin also is being studied and will be reported as soon as the work is completed.

TABLE 2

The stability of streptomycin in diluted semen stored at 4.5° C.
(Mean of 10 determinations)

Theoretical units of streptomycin[a]	Units of streptomycin by assay (per ml. of diluted semen)		
	Before storage	After storage for	
		8 days	16 days
Control	0	0	0
100	96	96	95
250	246	252	247
500	501	520	502
750	814	774	756
1000	1014	1094	988
1250	1282	1250	1267
1500	1586	1511	1529
2000	2012	2065	2014
Diluter alone	0	0	0

[a] No. of units expected, based on the total units in the ampules according to the producer.

SUMMARY

1. The additions of 100, 250, 500, 750 and 1,000 γ of streptomycin per ml. of diluted semen did not significantly affect the livability of bull spermatozoa during a 20-day storage period. Levels of 1,250, 1,500 and 2,000 γ per ml. of diluted semen brought about a significant decrease in spermatozoan livability during a storage period of 20 days.

2. A significant linear relationship was found between spermatozoan livability and concentration of streptomycin. The mean percentage of motile spermatozoa during storage for 20 days decreased by 0.5 per cent for each addition of 250 γ of streptomycin.

3. Streptomycin inhibited bacterial growth in diluted semen as compared with untreated controls. Levels above 100 γ per ml. were especially effective; the greatest antibacterial activity was obtained in freshly diluted semen and diluted semen stored for 8 days. The initial plate counts for ten ejaculates studied ranged from 2,000 to 350,000 bacteria per ml. of fresh, undiluted semen, with a mean of 73,000 bacteria per ml.

4. There was no significant loss in streptomycin activity in diluted semen stored for 8 and 16 days at 4.5° C.

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EFFECT OF ULTRAVIOLET IRRADIATION ON BACTERIOPHAGE ACTIVE AGAINST *STREPTOCOCCUS LACTIS*[1]

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Various methods have been advocated for decreasing the incidence of slow acid production due to bacteriophage action during the manufacture of cheese. The control measures have included protection of the mother culture, bulk culture and cheese milk from bacteriophage, and also methods for the destruction of bacteriophage within the cheese plant. Chlorination and irradiation with ultraviolet light commonly have been employed for the destruction of bacteriophage. Since bacteriophage particles frequently are found in the air of the cheese plant, mists containing active chlorine have been used for their destruction. Chlorine mists have the disadvantage of corroding equipment and fixtures within the plant. Use of ultraviolet irradiation for the destruction of bacteriophage would possess various advantages over the use of chlorine compounds, provided it was as effective.

HISTORICAL

Appelmans (2) and Zoeller (10) found that *Shigella* bacteriophage was killed by a short exposure to ultraviolet rays. Mizuno (7) noted that the depth of solution containing bacteriophage, type of suspending liquid, and concentration of bacteriophage influenced the time required for destruction of *Shigella* bacteriophage by ultraviolet rays.

Gates (3) exposed a culture of *Staphylococcus aureus* and its homologous bacteriophage to ultraviolet light and noted a direct relation between the energy required to kill the organism and that needed for inactivation of the bacteriophage; bacteriophage required expenditure of more energy for its destruction.

Sutton (8) exposed various quantities of a bacteria-free filtrate containing bacteriophage active against *Streptococcus cremoris* to ultraviolet rays. When 2-, 4-, and 9-ml. quantities of the filtrate were placed in petri dishes at a distance of 3 inches from a Westinghouse Sterilamp, the bacteriophage was destroyed completely in 6 minutes.

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Luria and Delbrück (5) stated that a suspension of bacterial virus, after inactivation by ultraviolet rays, may have the ability to interfere with growth of a second virus acting on the same host, *Escherichia coli* in this case. A virus inactivated by ultraviolet irradiation inhibited the growth of senstive organisms but did not lyse bacterial cells.

Anderson (1) conducted experiments to determine whether cells of *E. coli* which had been inactivated by ultraviolet irradiation could be used as hosts for the propagation of bacterial virus. Bacterial cells which were not able to form colonies were able to support growth of virus. Irradiation appeared to reduce the ability of the host to adsorb virus, liberate a virus-inhibiting substance from the host, reduce burst size of the host, inactivate the virus when adsorbed on the host, and kill bacteria.

Whitehead and Hunter (9) stated that bacteriophage active against lactic streptococci could be destroyed by ultraviolet light if the necessary exposure was given. The practical value of ultraviolet light for the destruction of bacteriophage within a cheese plant was considered questionable because of constant reinfection.

Latarjet and Wahl (4) noted that bacteriophage preparations and homologous strains of *E. coli* irradiated separately were not destroyed in the same length of time. Bacteriophage was two to six times more sensitive to ultraviolet irradiation than the homologous strain of *E. coli*. However, when a mixture of bacteriophage and cells was irradiated, the bacteriophage was more resistant.

Luria and Latarjet (6) state that *E. coli* loses its ability to liberate bacteriophage after irradiation, due to inactivation of the intracellular bacteriophage. When bacteria were irradiated between the time of infection with bacteriophage and lysis of the organisms, a rapid increase in resistance was noted. The increase in resistance was thought to be caused by an accumulation of ultraviolet-absorbing materials around the bacteriophage particle. Analysis of the survival curves for bacteriophage indicated that more than one bacteriophage particle grew in one host cell.

METHODS

Source of ultraviolet light. The sources of ultraviolet light were commercial low-pressure mercury-vapor lamps, releasing 85 per cent of their radiations in the range of 2537 Å. One bulb had an output of 279 microwatts and the other bulb an output of 364 microwatts at the surface of the light source.

Determination of effective radiant energy. Measurement of the effective radiant energy of the ultraviolet lamps was made with a Luckiesh-Taylor germicidal light filter in combination with a standard General Electric light meter.

Preparation of bacteria-free filtrates. The bacteria-free filtrates were prepared by adding 1 ml. of a sensitive culture of *Streptococcus lactis* to 100 ml. of sterile skim milk. Bottles containing the sensitive culture were placed in an incubator at 30° C. for 3 hours; then 1 ml. of bacteriophage active against the *S. lactis* culture was added. The bottles containing bacteriophage and a sensitive strain of *S. lactis* were incubated at 30° C. for 48 hours. After incubation, the bottle contents were coagulated with sterile 10 per cent lactic acid, filtered through coarse filter paper, and the resulting filtrate passed through a Selas microporous filter of 03 porosity.

Determination of bacteriophage titer of bacteria-free filtrates. The serial dilution method was used to determine the concentration of bacteriophage in a bacteria-free filtrate. The bacteriophage titer was recorded as the smallest amount of bacteria-free filtrate, in milliliters, which would cause a significant retarding effect on the production of acid, reduction of litmus, or coagulation of the milk by a sensitive culture of *S. lactis.*

Irradiation of bacteria-free filtrates containing bacteriophage. A pure culture of *S. lactis* (Hl–1) and its homologous bacteriophage (Hl–7) were used throughout this series of experiments. The ultraviolet lamps used in the studies were permitted to burn for a period before use in order to stabilize the radiant energy output.

Irradiation experiments were carried out by placing the desired quantity of bacteria-free filtrate containing bacteriophage in a petri dish, distributing the filtrate evenly over the bottom surface and irradiating with the cover removed from the petri dish. Petri dishes having a flat bottom surface were selected for use; they had average inside diameters of 90 mm.

After a bacteria-free filtrate containing bacteriophage was irradiated for a given time, a portion of the filtrate was added to tubes of litmus milk which had been inoculated just previously with a sensitive culture. A significant retarding effect on the production of acid, reduction of litmus, or coagulation of the milk, as compared with the control cultures, denoted the presence of active bacteriophage.

RESULTS

Irradiation of 1-ml. quantities of bacteria-free filtrates containing bacteriophage. One-milliliter quantities of bacteria-free filtrates, having bacteriophage titers of 10^{-3}, 10^{-6}, and 10^{-11}, were irradiated with two commercial low-pressure, mercury-vapor ultraviolet bulbs. With each bulb, 1-ml. portions of the bacteria-free filtrates were irradiated at distances of 3, 6, 9, 12, 18 and 24 inches from the source of light. The times required for destruction of bacteriophage, using the two bulbs individually and bacteria-free filtrates containing various concentrations of bacteriophage, are presented in table 1.

The data show that bacteriophage in a bacteria-free filtrate having a titer of 10^{-3} was destroyed by irradiation in a shorter time than bacteriophage in a filtrate having a titer of 10^{-6}, when the irradiation distance was the same and comparisons were made with the same ultraviolet bulb. Also, a bacteria-free filtrate having a bacteriophage titer of 10^{-6} was destroyed by irradiation in a shorter time than bacteriophage in a filtrate having a titer of 10^{-11} when the irradiation distance was the same and comparisons were made with the same ultraviolet bulb.

The time necessary for destruction of bacteriophage increased as the distance between the ultraviolet bulb and the bacteria-free filtrates containing bacteriophage was increased. This relationship was noted with filtrates having different bacteriophage concentrations and with both ultraviolet lamps.

TABLE 1

Inactivation times during irradiation of 1-ml. quantities of bacteria-free filtrates containing various concentrations of bacteriophage

Bacteriophage titer of bacteria-free filtrate	Lamp output (microwatts)	Minutes required for destruction of bacteriophage when irradiated at the following distances from the lamp:					
		3 in.	6 in.	9 in.	12 in.	18 in.	24 in.
10^{-3}	279	7.5	7.5	10	20	45	90
10^{-3}	364	5.0	7.5	7.5	10	25	35
10^{-6}	279	15.0	30.0	45	75	150	210
10^{-6}	364	7.5	15.0	30	45	75	120
10^{-11}	279	120.0	150.0	270	360	420	900
10^{-11}	364	90.0	120.0	210	300	330	420

The output of radiant energy by an ultraviolet bulb influenced the time required to destroy bacteriophage in a bacteria-free filtrate. Bacteriophage in a bacteria-free filtrate was destroyed in a shorter time by an ultraviolet bulb having an output of 364 microwatts than it was by a bulb having an output of 279 microwatts, when comparisons were made at the same irradiation distance and using filtrates of the same bacteriophage titer.

Irradiation of 2.5 mm. depths of bacteria-free filtrates containing bacteriophage. Quantities of bacteria-free filtrates sufficient to form a layer 2.5 mm. deep in petri dishes and having bacteriophage titers of 10^{-3}, 10^{-7} and 10^{-10} were irradiated with the two ultraviolet bulbs described previously. The irradiation distances were the same as those used for the irradiation of 1-ml. quantities of filtrates. The times required for destruction of bacteriophage, using the two bulbs individually and bacteria-free filtrates containing various concentrations of bacteriophage, are presented in table 2.

The data show that the time necessary for destruction of bacteriophage in bacteria-free filtrates by ultraviolet light was dependent upon the titer of the filtrate. Bacteriophage in a filtrate having a titer of 10^{-3} was destroyed in a shorter time than bacteriophage in filtrates having titers of 10^{-7} or 10^{-10}, when comparisons were made at the same irradiation distance and with the same ultraviolet bulb. Under the same conditions, bacteriophage in a filtrate having a titer of 10^{-7} was destroyed in a shorter time than bacteriophage in a filtrate having a titer of 10^{-10}.

As in the previous experiment, the time necessary for destruction of bacteriophage by ultraviolet light was increased by increasing the irradiation distance. Also, the output of radiant energy by the ultraviolet bulb influenced the time required to destroy bacteriophage in bacteria-free filtrates; the bulb having the greater energy output destroyed bacteriophage in a shorter time under the same conditions.

TABLE 2

Inactivation times during irradiation of 2.5-mm. films of bacteria-free filtrates containing various concentrations of bacteriophage

Bacteriophage titer of bacteria-free filtrate	Lamp output (microwatts)	Minutes required for destruction of bacteriophage when irradiated at the following distances from the lamp:					
		3 in.	6 in.	9 in.	12 in.	18 in.	24 in.
10^{-3}	279	21	26	39	60	70	80
10^{-3}	364	16	20	35	45	55	70
10^{-7}	279	40	60	75	90	90	115
10^{-7}	364	35	45	60	60	75	105
10^{-10}	279	120	135	180	210	225	255
10^{-10}	364	60	75	90	135	180	225

Comparison of irradiation times necessary to destroy bacteriophage in thin and thick films. The data presented in table 1 show that there were only slight differences in the time required for destruction of bacteriophage in a bacteria-free filtrate having a titer of 10^{-3} by two ultraviolet lamps having different energy outputs at irradiation distances of 3, 6 and 9 inches. With irradiation distances of 12 inches or more, there were greater variations in destruction time between the two lamps. The same general relationship was noted with this filtrate (titer 10^{-3}) in table 2.

A comparison of the results obtained with bacteria-free filtrates having bacteriophage titers of 10^{-3} shows that at irradiation distances of 3, 6, 9, 12 and 18 inches, bacteriophage in 1-ml. quantities (thin film) was destroyed in a shorter time than bacteriophage in films 2.5 mm. thick. Similar results were obtained with both lamps. At an irradiation distance of 24 inches, the filtrate having a bacteriophage titer of 10^{-3} was destroyed in less time in a 2.5 mm. depth with one lamp (279 microwatts output) than

it was in a thinner film (1 ml.). With the other lamp (364 microwatts output), bacteriophage was destroyed in a shorter time in a thin film than in a thicker film.

Bacteriophage in a bacteria-free filtrate having a titer of 10^{-6} (thin film) was destroyed in a shorter time than bacteriophage in a bacteria-free filtrate having a titer of 10^{-7} (2.5-mm. film) at irradiation distances of 3, 6, 9 and 12 inches but not at 18 and 24 inches.

Bacteriophage in a bacteria-free filtrate having a titer of 10^{-10} and irradiated in a 2.5-mm. film was destroyed in a shorter time at all irradiation distances than bacteriophage in a preparation having a titer of 10^{-11} and exposed in the thin film formed by 1 ml. of the material.

DISCUSSION

In the trials in which 1-ml. quantities of bacteria-free filtrates were irradiated, the thin films dried after a time, frequently before bacteriophage was destroyed completely. The time required for the moisture to evaporate from the filtrates varied, but on some occasions they appeared dry after 15 minutes. As the filtrate containing bacteriophage dried, the time necessary for destruction of bacteriophage increased appreciably.

The influence of drying on the destruction of bacteriophage by ultraviolet light can be demonstrated by a comparison of data obtained with the filtrate having a titer of 10^{-11} in table 1 (1-ml. film) and the filtrate having a titer of 10^{-10} in table 2 (2.5-mm. film). The bacteriophage present in the thicker film was destroyed in a shorter time at all irradiation distances than was the bacteriophage in the thin film, which had an opportunity to dry before the end of the irradiation period.

The comparatively long time required to destroy bacteriophage active against *S. lactis* by ultraviolet light at short distances from the lamp indicates that this method of destruction may be of limited value in commercial cheese plants. Since bacteriophage particles are present in the air of cheese plants experiencing difficulty with slow acid production due to bacteriophage, the bacteriophage particles might not be in contact with the rays of an ultraviolet lamp for any appreciable length of time. Certain areas of a cheese plant would be difficult to irradiate, such as the area beneath various pieces of equipment.

SUMMARY AND CONCLUSIONS

Bacteriophage active against *Streptococcus lactis* was destroyed by irradiation with ultraviolet light in a shorter time by a bulb having an output of 364 microwatts than by a bulb having an output of 279 microwatts.

Bacteria-free filtrates having bacteriophage titers of 10^{-3} were destroyed in a shorter time than filtrates having higher bacteriophage titers.

Increasing the distance between the ultraviolet bulb and the bacteria-

free filtrate resulted in an increase in the time required to destroy bacteriophage in the filtrate.

Bacteriophage in a thin film of bacteria-free filtrate was destroyed in a shorter time than bacteriophage in a thicker film, provided that the thin film did not dry before the bacteriophage was destroyed. Drying appeared to make bacteriophage significantly more resistant to destruction by ultraviolet light.

The long time necessary to destroy bacteriophage by ultraviolet light at relatively short distances from the lamp and the increased resistance of dry bacteriophage to ultraviolet light appear to make this procedure of doubtful value for the destruction of bacteriophage in commercial plants experiencing difficulty with bacteriophage.

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