THE VITAMIN A AND RIBOFLAVIN VALUES OF BUTTER AND MILK

by

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B. S., Marymount College, 1935

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1937

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INTRODUCTION

A previous study reported from this laboratory showed interesting variation in the vitamin A value of milk and butter produced at different stages of lactation. Similarly, the vitamin G content of milk of another group of cows was determined. As feeding conditions are known to influence milk production and vitamin content, this study was undertaken to add information concerning the vitamin A value of butters and riboflavin content of milks produced in late summer and fall, by cows in the Kansas State College herd.

REVIEW OF LITERATURE

Vitamin A

The observation of Morton and Heilbron (21) that vitamin A exhibits a specific absorption band at 328 mμ, the intensity of which is proportional to the concentration, has led to further developments of physical-chemical methods. Baumann and Steenbock (2), in 1933, reported a method for the determination of the vitamin A content of butter based on absorption phenomenon. As a precursor, β-caro-

tene, is converted into vitamin A in the animal body (20), the determination of both the vitamin per se and its precursor is necessary to give the total vitamin A value. Several methods for determining carotene in butter and butter concentrates as well as total vitamin A value have been developed.

Sherman (31) has recently emphasized the significance of the term "vitamin A value", as opposed to "vitamin A content". The vitamin A value of a food is the combined nutritional value of vitamin A itself and/or any precursor or precursors. Harris (14) is now using "vitamin-A activity" with the same connotation as "vitamin A value". It now seems that certain foods of animal origin contain vitamin A, while foods which are plant products may contain carotene or other precursors which account for their vitamin A value. Of the precursors now described, β-carotene is usually determined quantitatively for vitamin A assays. Leuschen (18) has compared basic biological assay for the vitamin A value of butter with certain physical-chemical methods and reported good agreement between biological estimations and those values obtained by adding vitamin A content determined spectrographically to the β-carotene content determined spectrophotometrically. These results

indicate that the vitamin A value of butter can be satisfactorily determined by physical-chemical methods.

Much of the vitamin A biological work done in this country followed the Sherman-Munsell procedure (29). Results were subsequently expressed in terms of the "Sherman unit", later called A. C. S. or U. S. D. A. units (31) which are defined as the amount of food necessary to give a growth of 3 grams per week in the test animal during the 4- to 8-week test period. The "International unit" (I. U.) expresses vitamin A values in terms of standard β-carotene, distributed by the Health Organization of the League of Nations. It is convenient to record quantities of carotene and vitamin A in gamma (γ) = 0.000001 gm. The factor 1.66 is used in converting gamma of β-carotene into I. U. of vitamin A because 1 gram of carotene is capable of being transformed into 1.067 grams of vitamin A. Vitamin A may be expressed in I. U. by multiplying the quantity in gamma by 1.56. The 1934 U. S. Pharmacopoeia (U. S. P.) unit = the International unit. The Sherman units may be converted into I. U. units by multiplying by 1.4. Final vitamin A values of this study will be expressed in the now more widely used I. U.

Riboflavin or Vitamin G

Between the years 1919 and 1928, investigators grad-

ually contributed evidence that at least two substances, the heat labile antineuritic vitamin and something more heat-stable were involved in the functions which until then had been attributed to vitamin B. The extensive work done during this period has been authoritatively reviewed by Sherman and Smith (30). By this time the American workers had adopted the term vitamin G (B_2) for the heat-stable, growth-promoting factor in water soluble B extracts.

In the attempts to isolate and concentrate the G (B_2) factor, further complications presented themselves. The numerous theories and findings of this factor presented during the last ten years of vitamin research have been thoroughly reviewed by Munsell (22). The literature at this time indicated that the term "vitamin G" might include flavin and also a still unidentified factor B_6 or vitamin H. Vitamin G values given in literature probably are flavin values.

Flavins have been isolated, purified and synthesized from liver, hepatoflavin; from egg, ovoflavin; and from milk, lactoflavin. The chemistry of the flavin molecule and the present formula have been brought to light and their conclusions have been confirmed by laboratory synthesis. Developments in the recognition of lactoflavin as

an entity of vitamin G (B_2) are attributed to Ansbacher, et al. (1). There now exists sufficient evidence (4) that the biological method of Bourquin and Sherman for vitamin G, measured the flavin factor (3). Recently Sherman (31) has ably reviewed these later developments in the vitamin G (flavin) values.

Rapid changes in terminology of vitamin G necessitates a review of recent usage. Jansen (15) suggested in 1935 the discontinuance of the use of letters in the terminology of vitamins, and that vitamin G (B_2) be designated as flavin. Many investigators in recent years have proposed the use of the term "flavin" instead of vitamin G. Sherman has used the term "vitamin G"; some workers in continental Europe have used the term "vitamin B_2", and English workers have used the term "flavin", while others use the term "lactoflavin". A recent article in the United States Health Bulletin (26) has this foot-note regarding lactoflavin, "since Karrer and co-workers and Kuhn and co-workers have shown that lactoflavin is 6, 7 dimethyl 9 d-riboflavin we consider the term 'riboflavin' preferable to lactoflavin". In April 1937, the Committee on Vitamin Standards of the American Society of Biological Chemists (24) decided to omit the prefix "lacto" because flavins

obtained from numerous sources are identical in structure and possess the properties of vitamin G (B_2). However, since the term flavins represents a class of compounds, some of which have no vitamin activity, the Committee on Nomenclature decided to use the prefix "ribo" to indicate that the compound formerly designated as vitamin G (B_2) is a ribose derivative of isoalloxazine. Since the Council of Pharmacy and Chemistry (24), upon the recommendations of the Committee on Nomenclature, has accepted the term "riboflavin", this term will also be used in this study instead of vitamin G or lactoflavin.

Results of vitamin G (riboflavin) assay have been expressed in different ways. Most vitamin G values in the literature of this country are in the terms of the Bourquin-Sherman unit. György (13) uses the term "a rat day dose" to denote the daily amount of substance which will promote a gain of 3 grams per week during the 6- to 8-week experimental period. After lactoflavin, now called riboflavin, became available in crystalline form, it was expressed in terms of units of weight, 0.000001 gram = 1 gamma = 1 microgram. Day and Darby (8) have suggested that approximately 4 gamma (4 micrograms) of lactoflavin are equivalent to 1 Bourquin-Sherman unit of vitamin G.

The gamma has been frequently used, but at present the term "microgram" is more prevalent in the literature and is the one used in this study.

PROCEDURE

Samples of raw milk and butter (table 1) for these experiments were secured from the Department of Dairy Husbandry, this college. Figures for per cent of fat and total solids in milk and fat in butter were supplied by the Department of Dairy Husbandry. Twenty-four hour collections of milk were made, and in most cases a composite sample was retained for riboflavin determinations and butter was churned from the remainder for vitamin A assay. Milk #1 and butter #1 came from composite 24-hour milk collections from a group of 7 Holsteins that had been kept on an experimental ration without supplements of green feed or pasture since May, 1934. The experimental ration consisted of prairie hay and grain mixture made up of equal parts of white corn, bran, and cottonseed meal. Fortunately these samples could be secured as examples of milk and butter produced by cows kept on a rigidly restricted diet for a long period of time. In contrast, the next two lots

of samples, #2 to #4 and #5 to #7, were secured respectively from 7 Holstein and 5 Jersey cows on the regular dairy herd schedule. Samples were collected early in September, at the end of the dry summer of 1936, when no pasture had been available for several months to supplement the regular herd ration of alfalfa hay, grade No. 2; Atlas Sorgo silage; and grain mixture consisting of 400 parts yellow corn, 200 parts bran, 200 parts oats, 100 parts cottonseed meal, and 50 parts linseed oil. Later, samples were collected from the same cows in October 10 days after the fall rains began and early in December when cows had had access for one month to good pasture of oats, wheat, and rye, to supplement the regular herd ration.

Samples of butters, #8 and #9, churned on a commercial scale at the college dairy from cream purchased from neighboring farms, were secured in late August and in October for comparison. Milk #10 was secured from the college herd in the winter when the cows had been receiving the unsupplemented dairy herd ration for some weeks.

All samples of milk and butter were held below 0° C. until time for use. Grayson (12) found that no deterioration of vitamin G (riboflavin) takes place in a frozen sample.

For the spectrographic determination of vitamin A, duplicate samples of 15 gm. butter were prepared by following the method given by Baumann and Steenbock (2). The butter was saponified 30 minutes with 125 ml. of 12 per cent alcoholic potash from which aldehydes and ketones had been recently removed, 125 ml. of water were added, and the mixture was packed in ice. When cooled to 4° C. 150 ml. of ether purified according to Semb et al. (27) was added followed by the addition of 500 ml. of cold distilled water. After shaking the mixture, the ether layer was drawn off and the aqueous alcoholic fraction was extracted several times with 50 ml. portions of ether. The combined ether solutions were washed with distilled water repeatedly, dried over Na_2SO_4 and freed from the greater portion of ether with nitrogen under reduced pressure. The residue was dissolved in about 15 ml. of hot methyl alcohol and the impurities crystallized out by cooling several hours to -21° C. The cold solution was filtered, washed with cold methyl alcohol and made up to 25 ml. (1 ml. equivalent to 0.6 gm. of butter). This solution was divided into approximately two equal portions and sealed in ampules in an atmosphere of nitrogen. These ampules were kept dark until vitamin A could be determined in the

Physics Laboratories of Iowa State College by measuring the intensity of the absorption band at 328 mμ in a quartz spectrograph.

A Bausch and Lomb quartz spectrograph was used for making the spectrograms. The light beams from a Ni-Fe arc, served as a light source. Two small cylindrical cells with quartz ends and 1.5 ml. capacity containing solution and solvent were placed side by side in the path of the light beam entering the spectrograph. The solution to be tested was diluted to such a concentration with the methyl alcohol that the intensity of the light passing through it could be matched by that getting through the solvent. A biprism placed immediately in front of the slit on the spectrograph served to produce the two spectra, one due to light coming through the solvent, and the other due to the light coming through the solution. The spectrograms were photographed on Wratten and Wainwright panchromatic plates 4" by 10" and developed according to the Eastman Kodak Co. directions for these plates.

The intensity of the absorption was determined visually by comparing the spectra on the plates and obtaining from the recorded rotating sector settings the values for the densities at 328 mμ for the strips having the match

points. Concentration of vitamin A was calculated from the Beer-Lambert law $D = E.c.l.$; where $D = \log I_o/I$ (I_o is intensity of light getting through solvent and I is intensity of light getting through solution), E = extinction coefficient, c = concentration and l = length of the cell. For the instrument used, l = 1 cm. The Castle et al. (7) value for $E^{1\%}_{1\ cm.}$ for vitamin A = 1600 was used in calculating the per cent concentrations.

The solutions prepared for spectrographic reading were further treated according to Gillam and Heilbron (11) in order to separate the carotene from all xanthophyl and major portion of vitamin A by adding Skellysolve, a light petroleum ether. To 9 ml. of the methyl alcohol solution 1.2 ml. of distilled water were added and shaken sufficiently to insure thorough mixing. Skellysolve was added in 4 to 8 ml. portions, the mixture shaken and with the rising of the Skellysolve, the methyl alcohol layer was withdrawn by means of a burette. The methyl alcohol portion was washed several times with small amounts of Skellysolve. The combined Skellysolve portions were washed three times with distilled water and dried over Na_2SO_4 for approximately 24 hours. The solution was filtered and made up to 10 ml. and read directly in a visual spectro-

photometer at 455, 470 and 480 mμ. In making the computations, the extinction coefficients for Skellysolve by Peterson et al. (23) were used.

The Bourquin-Sherman (6) biological method was used for vitamin G (now called riboflavin) determinations in milk. This method of assay has been validated by Booher, Blodgett and Page (4), and more recently by Bisbey and Sherman (3) as a reliable one for testing, quantitatively, the flavin factor in foods.

Albino rats of the Wistar Institute strain were used. The stock animals were fed the diet suggested by Sherman and Crocker (28) ad libitum. Young, normally growing rats were taken for depletion of vitamin G reserves when they had reached weights of 38 to 40 grams, following the suggestions of Roscoe (25) and also of Lassen (17). The basal diet planned to contain all known nutritional factors except vitamin G was prepared according to Bourquin and Sherman (6) as follows:

	per cent
Purified casein . . .	18
Cornstarch	68
Butterfat	8
Cod liver oil . . .	2
Osborne and Mendel salt	4
	100

The whole wheat extract, used as a source of vitamin B, was prepared according to the method outlined by Bourquin

(5) and dried over the cornstarch. The amounts used were such as to introduce the alcoholic extract of 50 grams of wheat into each 100 grams of diet. The vitamin G was extracted from the casein according to the method described by Ellis (9). The Osborne and Mendel salt was made by the short-cut method suggested by Wesson (35). The butterfat was melted and filtered at 45 to 55° C.

During the early part of the depletion period, the rats were confined in groups of five in cages with rasied screen floors to prevent access to excreta. The body weight of each animal was recorded at regular intervals for the first week and then every day until the end of the depletion period. Toward the end of this depletion period, each animal was put into a separate cage with a raised bottom. Evidence of depletion consisted of such decline in weight that when started on the assay period the rat was less or equal in weight to his weight 2 days before. Under conditions in this laboratory the weight usually became stationary in from 11 to 16 days.

In the test period which followed the depletion period the animals were divided into experimental groups, comparable in size, weight, sex, and litter. During the 8-week experimental period, each animal continued to receive the

basal vitamin G-free diet and water ad libitum. The samples of milk to be tested were measured with a pipette and fed on the basis of 3 grams per day for 6 days a week. During the test period the animals were weighed each week and records kept of the amount of food consumed. The animals used as negative controls received only the basal diet. The standard fed for reference to the positive controls was riboflavin (lactoflavin, PX grade), a highly potent product, secured from the Biological and Chemical Laboratories of the Borden Company Research Division. Five mg. of this dry crystalline material was accurately weighed and made up to 500 ml. with distilled water. The solution, containing 10 micrograms per ml., was fed at two different levels; 0.25 ml. and 0.50 ml. daily or 2.5 micrograms and 5 micrograms daily. A Luer Tuberculin syringe, graduated in 0.01 ml., was used in feeding the material directly to the animals.

Riboflavin exhibits a marked greenish-yellow fluorescence when subjected to the blue-violet rays of a carbon arc. Because these fluorescent characteristics have been correlated with the growth promoting properties, the fluorescent properties of riboflavin were used by Supplee (32) and associates (33) as a basis for the quantitative esti-

mation of this dietary factor. The method of Supplee et al. (32) has been modified for rapid determinations of the vitamin G (riboflavin) content of milk (36), and so used in this present work. Following this method of Whitnah, Kunerth, and Kramer (36), 15 ml. of 10 per cent trichloroacetic acid were added to 10 ml. of milk, which stood for 30 to 60 minutes, and were then centrifuged for 5 minutes at about 200 r. c. f. A 10 ml. portion of the resulting serum was neutralized, with methyl orange as an indicator, and diluted until the sample could be matched in the light of an Eveready Flouray lamp with standard flavin solutions (Labco PX grade) containing 0.12 to 0.06 micrograms of flavin (riboflavin) per ml. The riboflavin content was calculated on the basis of the dilutions made. Values were most easily and accurately read when the diluted portions contained less than 0.12 micrograms per ml.

Table 1. Data concerning milk and butter samples.

Source of sample	Milk				Butter	
	Date	Sample #	Fat %	Total solids %	Sample #	Butter fat %
7 Holstein cows on experimental ration	9- 9-36	1	4.0	11.21	1	85.3
7 Holstein cows on herd ration	9- 3-36	2	3.5	12.07	2	82.7
Little pasture	10-19-36				3	85.0
Good pasture	12- 3-36	4	3.1	11.72		
5 Jersey cows on herd ration	9- 3-36	5	4.5	13.89	5	89.0
Little pasture	10-19-36				6	85.0
Good pasture	12- 3-36	7	5.6	15.30	7	85.5
Commercial Dairy sales counter	8-29-36				8	80.0
	10-19-36				9	80.0
College dairy herd	winter 1936-37	10	4.0	12.75		

Table 2. Vitamin A and carotene determinations on butter obtained by chemical and physical methods.

Butter sample and determination		Vitamin A content 328 mμ: 1% "pure" vitamin A E 1 cm. = 1600: 1% butter E 1 cm.	Vitamin A content 328 mμ: Vitamin per gm. butter: Av. %	Vitamin A content 328 mμ: Vitamin per gm. butter: Av. I. U.	β-carotene content 480 mμ: 1% β-carotene E 1 cm. = 2120: 1% butter E 1 cm.	β-carotene content 470 mμ: 1% β-carotene E 1 cm. = 2000: 1% butter E 1 cm.	β-carotene content 455 mμ: 1% β-carotene E 1 cm. = 2270: 1% butter E 1 cm.	β-carotene %	β-carotene I. U.	Vitamin A value (Vitamin A content plus β-carotene)
Holstein, #1	A	.01033			.000079	.000083	.000092			
	B	.01033	.000646	10.08	.000075	.000077	.000083	.000081	1.34	11.42
Holstein, #2	A	.01600			.000205	.000208	.000232			
	B	.01770	.001053	16.43	.000201	.000199	.000223	.000211	3.50	19.93
Holstein, #3	A	.01466			.000328	.000310	.000355			
	B	.01300	.000864	13.48	.000310	.000310	.000343	.000326	5.31	18.79
Jersey, #5	A	.01660			.000742	.000759	.000807			
	B	.01700	.001049	16.36	.000699	.000694	.000742	.000740	12.28	28.64
Jersey, #6	A	.01033			.000559	.000583	.000624			
	B	.01033	.000646	10.08	.000524	.000528	.000595	.000568	9.43	19.51
Jersey, #7	B	.09500	.005937	92.62	.000734	.000722	.000775	.000744	12.35	104.97
Commercial, #8	A	.01660			.000236	.000241	.000253			
	B	.01470	.000978	15.26	.000249	.000245	.000253	.000269	4.46	19.72
Commercial, #9	A	.01233			.000406	.000407	.000444	.000419	6.95	
	B	.01233	.000771	12.03	-	-	-			18.98

Table 3. Summary of data from rat feeding experiments.

Milk sample	Daily portion fed	No. of rats	Av. depletion period	Av. weekly weights during test period in gm.									Av. for 8-weeks	
				1	2	3	4	5	6	7	8	9	Gain in weight	Diet consumed
	gm.		days										gm.	gm.
Holstein, #1	3	10	13	46	55	64	71	77	81	85	87	92	46	345
Holstein, #2	3	10	13	49	55	58	62	67	72	79	84	89	40	388
Holstein, #4	3	10	12	47	57	65	71	77	86	90	93	97	50	343
Jersey, #5	3	10	13	48	55	61	64	70	75	82	87	91	43	372
Jersey, #7	3	10	13	47	54	61	68	77	85	92	99	103	56	359
Herd, #10	3	13	16	45	53	60	66	73	77	80	83	85	40	318
Negative controls	0	19	14	46	46	45	45	45	46	45	46	46	0	250
	micro-grams													
Lactoflavin	2.5	10	13	46	50	52	55	57	60	62	63	63	17	235
Lactoflavin	5.0	9	14	49	53	60	65	67	72	75	80	82	33	343

Table 4. Comparison of results of riboflavin determinations by two methods.

Milk sample	Biological					Fluorimetric		
	Daily portion fed	No. of rats	Av. gain for 8-weeks	Estimated riboflavin per portion	Estimated riboflavin per gm. milk	Riboflavin per gm. milk Fluoray lamp	Differences from biological value per gm.	
	gm.		gm.	micrograms	micrograms	micrograms	micrograms	%
Holstein, #1	3	10	46	7.0	2.3	2.20	-0.10	- 5
Holstein, #2	3	10	40	6.0	2.0	1.76	-0.24	-12
Holstein, #4	3	10	50	7.5	2.5	1.88	-0.62	-25
Jersey, #5	3	10	43	6.5	2.2	2.30	+0.10	+ 4
Jersey, #7	3	10	56	8.5	2.8	2.55	-0.25	- 9
Herdmilk, #10	3	13	40	6.0	2.0	-	-	-
	micro-grams							
Lactoflavin	2.5	10	17					
Lactoflavin	5.0	9	33					

Table 5. The vitamin A value of butter and the riboflavin content of milk.

Sample	Vitamin A value, Physical-chemical methods						Riboflavin in milk			
	In butter			In butterfat	Fat yield per cow per day	Av. value of daily yield	Biological assay per gm.	Fluoray lamp per gm.	Av. milk yield per cow per day	Av. riboflavin yield per cow per day
	β-carotene per gm.	Vitamin A per gm.	Total Vitamin A value per gm.	Total Vitamin A value per gm.						
	I. U.	I. U.	I. U.	I. U.	gm.	1,000 I. U.	micrograms	micrograms	gm.	1,000 micrograms
Holstein, #1	1.34	10.08	11.39	13.35	290	4	2.3	2.20	7,258	17
Holstein, #2	3.50	16.43	19.93	24.10	556	13	2.0	1.76	15,876	32
Holstein, #3	5.31	13.48	18.79	21.11	419	9	-	-	12,701	-
Holstein, #4	-	-	-	-	380	-	2.5	1.88	12,246	31
Jersey, #5	12.28	16.36	28.64	32.18	449	14	2.2	2.30	9,979	22
Jersey, #6	9.43	10.08	19.51	22.95	445	10	-	-	9.076	-
Jersey, #7	12.35	92.62	104.97	122.77	330	41	2.8	2.55	5,897	17
Commercial, #8	4.46	15.26	19.72	24.65	-	-	-	-	-	-
Commercial, #9	6.95	12.03	18.98	23.73	-	-	-	-	-	-
College herd, #10	-	-	-	-	-	-	2.0	-	-	-

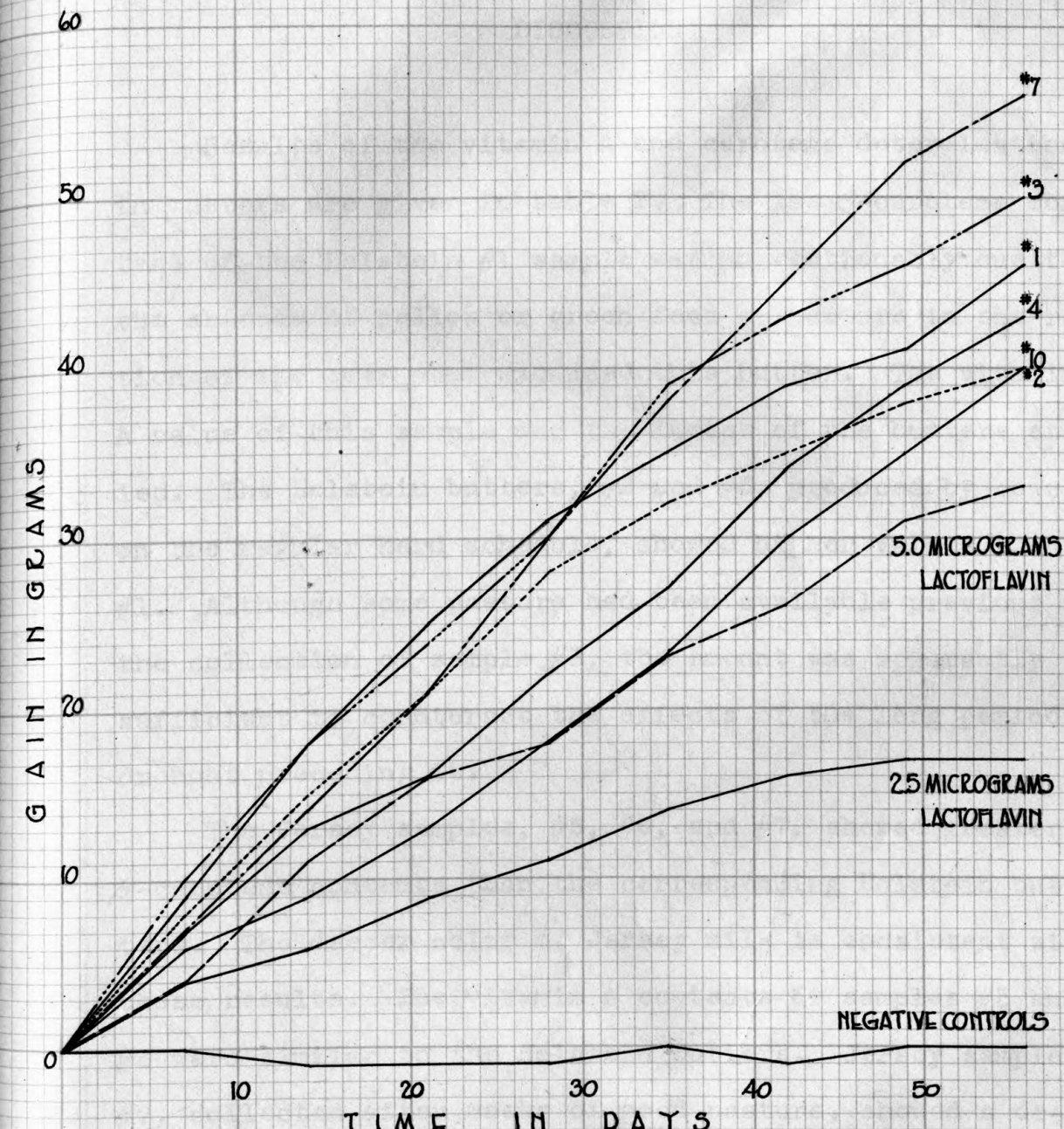

FIG. 1. AVERAGE GAIN CURVES FOR EXPERIMENTAL RATS.

DISCUSSION

Results of the vitamin A and carotene determinations in butters are given in table 2. The low β-carotene content of the Holstein #1 sample was unquestionably due to the absence of yellow or green feed or pasture in the ration of the cows that produced this butter. The vitamin A value of this sample was the lowest of the butters studied. The Holstein butters, #2 and #3, produced by cows on the regular herd schedule, showed higher values than #1. Although some pasture had been available previous to the collection of sample #3, the amount was apparently insufficient to counteract the effects of the long period of drought preceding it.

The Jersey samples, #5, #6, and #7, showed greater β-carotene contents than the corresponding Holstein butters. The deeper color of Jersey milk is consistent with these results. The vitamin A contents of samples #5 and #6 were similar to the Holstein butters. Jersey sample #7, collected after weeks of good pasture, showed a decidedly higher vitamin A content. The plate for determination A of this butter was available but due to the high

concentration of the solution a good reading was impossible. Determination B, as suggested by the first plate, gave a high reading for vitamin A. The total vitamin A value for this butter was twice as high as Sherman's (31) value compiled for ordinary butter.

The commercial fall butters, #8 and #9, churned from cream obtained from neighboring farms, were similar to the Holstein (#2 and #3) and Jersey (#5 and #6) butters prepared at the same seasons. All these samples showed total vitamin A values that were approximately one-half the value given recently by Sherman (31) for average butter.

Table 3 gives a record of the rats used for biological assay of riboflavin. The columns indicated give the average weekly weights of the groups of animals during the 8-week test period. Ten or more animals were used in every group except one. Although the experiments extended over a period of 10 months, the groups were quite uniform in average length of depletion period and in initial weight. The average length of the depletion periods ranged from 12 to 16 days, and the average initial weights from 45 to 49 grams. The animals in every test group showed good weekly gains as indicated in figure 1. Only one curve, that of the lower lactoflavin level, flattened during the last 2 weeks. However, the animals fed at this lower level

of standard lactoflavin (riboflavin) showed one-half the gain made by the group fed at the 5.0 microgram level. These gains are consistent with the growth response curve shown by Supplee (16). The negative controls were satisfactory in that they maintained approximately the same average weight throughout the experiment. The average weight of vitamin G-free diet consumed per rat during the 8-week test period was recorded. These records were kept carefully and showed the general trend of food consumption with growth; however, the groups that consumed the larger amounts of food did not always show the greatest gains.

Estimated riboflavin contents of milks based on biological findings are compared in table 4 with the readings obtained from the lamp. Estimates of riboflavin value of daily portions of milk were made on the assumption that the growth response is in direct proportion to the riboflavin content. Supplee (16) has suggested that the growth response is not in direct proportion when feedings much larger than 5 micrograms are given daily. Some daily portions fed contained distinctly more than 5 micrograms, but not so much as to prevent reliable estimates, as shown by the good checks with the Eveready Fluoray lamp. Gains of

33 and 17 grams by the animals receiving daily 5.0 and 2.5 micrograms, respectively, of pure riboflavin indicated an approximate gain of 6.5 grams per microgram of standard riboflavin. Using this gain as a basis, the riboflavin was estimated per portion of milk fed. These values are also expressed per gram of milk. This table also presents riboflavin values obtained from the readings made with the Eveready Fluoray lamp. The estimates made from biological assay were in accord with the lamp readings. In no case were the lamp readings excessively high; rather they tended to be lower than the estimates derived from biological data. The maximum difference in comparing the two methods was 25 per cent, with an average difference of ±10 per cent.

Biological assays of samples #2 and #5, collected at the end of the dry summer, indicated the presence of at least 2 micrograms of riboflavin per gram of milk. Later samples, collected after good pasture had been available for one month, gave results about one-fourth higher. It is interesting to note that the milk produced by the Holsteins on the restricted ration was at least as rich as samples #2 and #5 produced by cows on the regular schedule, and the winter milk #10 from the herd. Comparable Hol-

stein and Jersey milk samples were similar in riboflavin content. These riboflavin values for milk are consistent with findings from other laboratories. Euler and Adler (10) have estimated that milk contains 2 to 3 micrograms of riboflavin per ml. MacLeod et al. (19) reported that milk from the Walker-Gordon dairy contained 0.3 Bourquin unit per gram, equivalent to 1.2 micrograms, for Day and Darby (8) suggested that 1 Bourquin unit equals 4 micrograms of riboflavin. Subsequently, Todhunter (34) found 0.74 Bourquin unit (2.96 micrograms) per gram of pasteurized milk.

A summary of the vitamin A values of butters and the riboflavin contents of the milk samples is presented in table 5. In terms of total production, the Holsteins that furnished sample #1 showed lowest total daily yield of vitamin A value. The total vitamin A value of the daily yield of Jerseys, when samples #5 and #6 were collected, were comparable to those of the Holsteins, when samples #2 and #3 were collected. However, when Jersey sample #7 was collected, the total vitamin A value of the daily yield was 10 times as high as when Holstein #1 butter was secured, and 3 to 4 times as high as the other yields. In terms of yields of riboflavin per cow, the experimental

Holsteins produced the lowest total daily amount, giving only half as much per day as did the other cows of the same breed. The other Holsteins on the regular dairy schedule produced a greater average daily yield of riboflavin at both seasons studied than did the Jerseys, because of higher milk production. Some of the cows in this group were approaching the end of their lactation periods, which may account for decreasing milk yields.

The vitamin A values per gram of daily yield of fat showed results comparable to those expressed in terms of total production. Samples collected from cows of different breed or on different rations tended to vary much less in riboflavin content of the milk than in the vitamin A value of the butter.

SUMMARY AND CONCLUSIONS

Samples of butter and milk for vitamin A and riboflavin determinations were produced from the Department of Dairy Husbandry, this college. Samples were collected early in September of 1936, when good pasture had not been available on account of the dry summer, again in October, and early in December, when the cows had had access to the

good fall pasture for 1 month. Composite samples were also secured from 7 Holsteins that had been kept on an experimental ration without supplement of green feed or pasture since May 1934. For each sample, complete 24-hour collections of milk were made. In most cases, a composite sample of milk was retained for riboflavin determination and butter was churned for the vitamin A work. Records available show total daily yield of milk per cow, total solid content of milk composite and fat content of milk and butter samples. Commercial samples of butter produced locally in August and October were purchased for comparison.

Vitamin A content of the butter was estimated by measuring the absorption spectra at 328 mμ with a quartz spectrograph (Courtesy of Physics Department, Iowa State College). The amount of β-carotene, a precursor of vitamin A, was measured spectrophotometrically.

The biological assay for riboflavin was made according to the procedure of Bourquin and Sherman. Pure lactoflavin (riboflavin) was procured and fed as a standard for reference to the positive controls at two levels; 2.5 and 5.0 micrograms daily.

A new fluorimetric method for rapid determination of riboflavin was used on the same samples of milk which were

tested biologically.

Results of this study were computed and expressed as International Units of vitamin A value per gram of butterfat and as micrograms of riboflavin per gram of milk.

The September samples collected at the end of the dry summer showed low vitamin A values, about one-half the average value for butter in the Sherman tables. The commercial sample was similar to that produced by the Holsteins.

Although the October samples of butter were collected after the fall rains had begun, the amount of green pasture was not yet sufficient to influence the vitamin A value of the butter obtained from nearby farms or from the college dairy where approved scientific practices are followed. In fact, each sample of butter was lower in vitamin A value than at the close of the summer.

At the end of the fall season the Jersey butter, the only sample available, showed marked improvement following good fall pasture supplement. The vitamin A value rose to about twice the average value shown in the Sherman compilations.

The low vitamin A value of the experimental Holstein butter showed the effect of the prolonged feeding of a

restricted ration. The total vitamin A value was about one-fifth that given by Sherman for ordinary butter.

Biological assays of the September samples of milk indicated the presence of at least 2 micrograms of riboflavin per gram of milk. Three months later, after good pasture had been available, the samples gave results about one-fourth higher.

The experimental Holsteins on the restricted ration yielded milk at least as rich in riboflavin as the September milks.

The riboflavin determination of the milks by the new fluorimetric method gave results comparable to biological assay findings and valuable as checks of the range of these findings. The maximum difference between the biological findings and the fluoray lamp readings was -25 per cent with an average of ±10 per cent.

Samples collected from cows of different breed or on different rations tended to vary much less in riboflavin content of the milk than in the vitamin A value of the butter.

ACKNOWLEDGMENT

The writer wishes to acknowledge her sincere appreciation and gratitude to Dr. Martha M. Kramer, Professor of Foods and Nutrition, and Bernice L. Kunerth, Instructor of Foods and Nutrition, for their kind assistance and helpful suggestions in this study; to Dr. W. H. Riddell, Department of Dairy Husbandry, for the collection of the samples; to Dr. J. W. Woodrow, Head of the Department of Physics and to Dr. Wm. Kunerth, both of Iowa State College, for their assistance with the quartz spectrograph; and especially to Mother Mary Rose and to the Community of the Sisters of St. Joseph of Concordia, Kansas, through whom the privilege of graduate study has been made possible.

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