VITAMIN G(FLAVIN) CONTENT OF MILK OF INDIVIDUAL COWS WITH SPECIAL REFERENCE TO DIFFERENCES DUE TO THE INDIVIDUAL, THE BREED, AND THE STAGE OF LACTATION

by

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INTRODUCTION

Cow's milk is recognized as a valuable source of vitamin G in the diet. Sherman expresses its value by saying "Milk is doubtless the most important source of vitamin G for the American and European peoples" (27). Likewise colostrum, the first milk secreted by a mammal after parturition, is considered an important food for the young of the species. Since little is known of the amounts of vitamin G to be expected in fresh milk from various breeds of cows at different stages of lactation, it seemed desirable to undertake an experiment which would add to the available information. This study deals with the vitamin G (flavin) content of milk with special reference to the individual cow, the breed of cow, and the stage of lactation.

REVIEW OF LITERATURE

The multiple nature of vitamin $B(B_1)$ has engaged the attention of scientists for many years. Early work led to the differentiation of vitamin $G(B_2)$ from the vitamin B complex. A review of this field, as well as of the investigation of vitamin $G(B_2)$ up to 1931, is given by Sherman and

Smith (29). In 1935, Elvehjem (11) summarized the status of the vitamin B complex, Harris (20) reviewed new developments in vitamin work, including vitamin $G(B_2)$, and, more recently, Munsell (23) reviewed the literature on vitamin G.

In 1929, a committee representing the American Society of Biological Chemists (1) suggested the retention of the letter "B" to indicate the anti-neuritic factor while the letter "G" would be used to represent the pellagra-preventive factor first described by Goldberger, Wheeler, Lillie, and Rogers (13). English workers have used the term "vitamin B_2" instead of "vitamin G". Since 1929, work indicates that vitamin G, which was thought to be a single factor, is in reality complex in nature. As a consequence the nomenclature has become complicated. The majority of investigators have used the term "$G(B_2)$" to represent the growth-promoting component, while "B_6", "Y", and the "P-P factor" are terms which have been applied to the dermatitis component.

Gyorgy (16), in 1934, reported investigation showing two distinct factors of vitamin $G(B_2)$, one flavin or the growth-promoting factor and the other probably vitamin B_6 or the anti-pellagra factor. Elvehjem and Koehn (12) shortly afterwards published work showing similar findings. Gyorgy (17) again reported further differentiation and stated definitely that there are two vitamin $G(B_2)$ components. Lactoflavin, according to Gyorgy, has nothing to do with the cure or prevention of dermatitis, but rats given a diet deficient in the supplementary factor, B_6, develop dermatitis in 100 per cent of the cases. Chick, Copping, and Edgar (8), also concerned with the differentiation of the components of vitamin $G(B_2)$, reported two distinct factors, flavin which was thought to have a slight curative effect on generalized dermatitis and which supported growth, and vitamin B_6 which did not induce growth and which alone seemed to have little curative effect on dermatitis. The two factors, when fed simultaneously, were said to promote normal growth and to prevent dermatitis. About the same time, Harris (19) published data which indicated that flavin did not prevent or cure rat pellagra. He therefore concluded that vitamin $G(B_2)$ must consist of at least two factors. Further, Birch, Gyorgy and Harris (3) late in 1935 reported two components of vitamin $G(B_2)$ in addition to flavin and vitamin B_6; namely, the human pellagra-preventive factor and the extrinsic factor of pernicious anemia. Vitamin B_6 was thought to affect only the rat type of pellagra or dermatitis.

This extensive investigation of the components of vitamin $G(B_2)$ has yielded gratifying new knowledge but has aroused concern for the validity of methods of biological assay. Fortunately, Booher, Blodgett, and Page (5) and, more recently, Bisby and Sherman (4) have confirmed the accuracy of the Bourquin (6) method for measuring vitamin G or flavin values.

There is little information in the literature regarding the vitamin G(B_2) content of raw milk. Gyorgy (18) tested some natural foods to determine their relative value as sources of the components of vitamin B_2 and found that cow's milk contained about the same amounts of lactoflavin and vitamin B_6 and that, during the summer months, 10 ml. was a rat-day dose of each. Donelson and Macy (9) in 1934 fed human milk to rats to determine the influence of yeast in the maternal diet on the vitamin B and G potency of the milk secreted. They found that the milk contained 0.2 Bourquin unit of vitamin G per ml. prior to yeast consumption by the mother and 0.3 unit per ml. afterwards. Investigation which nearest resembles the present study was that of MacLeod, Brodie, and Macloon (22) in 1932. They fed rats a composite of milk obtained from a herd of Walker-Gordon dairy cows, and concluded that each gram of milk contained 0.3 Bourquin unit of vitamin G and that approximately 3 ml. would be the daily feeding necessary to induce a 25-gram gain in rats during an 8-week experimental period.

METHOD OF PROCEDURE

The biological method of Bourquin (6) was used for the determinations. This method, developed to measure vitamin $G(B_2)$ values, has been shown by Booher, Blodgett, and Page (5) and by Bisby and Sherman (4) to be accurate for the assay of vitamin G or flavin and is so used in the present investigation.

Albino rats from Wistar Institute stock were used for this study. The stock animals were fed a diet suggested by Sherman and Crocker (28). Its constitutents were:

Dried whole milk	1 part
Ground whole wheat	2 parts
Sodium chloride	2 per cent of weight of wheat

This diet was supplied ad libitum, and distilled water was available at all times. The young rats used in the preliminary experiments and in series I were kept on the stock diet until 4 weeks of age, according to the Bourquin and Sherman (7) method. Those animals used in series II were fed the stock diet until they had reached a weight of 38 to 40 grams, since a number of investigators thought the lesser weight more desirable. Roscoe (26) in 1933 reported the use of rats weighing from 30 to 40 grams. Supplee, Ansbacher, and Bender (30) in 1935 stated that they used rats from 21 to 26 days old and preferably weighing from 40 to 45 grams. Prunty and Roscoe (25) used rats taken immediately after weaning with a weight range from 35 to 50 grams. Lassen (21) reported that he preferred 3-week-old animals to 4-week-old ones as they were more uniform in size and caused less variation in the subsequent response.

Upon reaching the proper weight or age, the rats were placed on the vitamin G-free diet prepared according to Bourquin and Sherman (7) until their body store of vitamin G was depleted. During the first part of this period they were kept in groups of four in cages with raised screen floors. They were weighed at regular intervals for about 1 week, then were placed in individual cages and were weighed daily. When their weights remained stationary for 3 days or when there was a decrease in weight, the animals were considered ready for experiment.

The Bourquin and Sherman (7) diet was made up as follows:

G-free casein	18 per cent
Osborne and Mendel salt	4 per cent
Cod-liver oil	1 per cent
Butterfat (filtered)	9 per cent
Cornstarch and alcoholic extract of whole wheat	68 per cent

The vitamin G-free casein was extracted according to the method described by Ellis (10). It was found necessary to add 1/3 ml. of N/10 ammonium hydroxide to each liter of 60 per cent alcohol to make the pH of the casein mixture approach the isoelectric point and thus prevent inconvenient swelling of the casein. The Osborne and Mendel salt was made by the short-cut method suggested by Wesson (33). The butterfat was melted and filtered at 45° to 55° C. The alcoholic extract was prepared by the method proposed by Bourquin and Sherman (7). The extract was added as part of the 68 per cent cornstarch in amounts such that the extract from 50 grams of wheat was introduced for each 100 grams of the diet.

When the rats were depleted and ready to place on experiment, they were divided into groups of 10 or more. The groups were made comparable in size, weight, sex, and litter so that each would be an average of the entire series of animals. During the 8-week experimental period, each animal continued to receive the basal vitamin G-free diet and distilled water ad libitum. The sample to be tested was fed on the basis of 6 feedings a week for 8 weeks. The amounts fed were either weighed to the nearest milligram on an analytical balance or were measured with a pipette after equivalent measurements and weights had been determined.

It was found necessary to weigh some of the colostrum since it was too thick to be determined accurately by volume. Records were kept of the weekly weight and food consumption as well as of the physical condition of each animal.

Since various authors (15, 19, 27) have reported the harmful influence of coprophagy upon vitamin B and G experiments, an attempt was made to prevent it throughout the entire investigation. All animals were kept in cages with raised screen floors, a precaution thought sufficient by most authors. A few rats from each group were kept in harnesses similar to those described by Page (24) and made after the pattern supplied by Bourquin. These prevented the consumption of feces upon excretion. Accurate record of the number of fecal particles found in cages of animals in harness was checked against that of animals not harnessed. Food consumption was also considered in making the comparison. Doubtful animals were harnessed and observed and later were eliminated if there was any question of their having practiced coprophagy.

Since there were no data available as to the comparative vitamin G or flavin potency of milk at the different stages of lactation, a group of preliminary experiments was run in order to estimate the amount of colostrum and milk suitable for daily feedings. The animals used were fed graded amounts of the material to be assayed. The amounts which seemed to result in approximate unit gains as described by MacLeod, Brodie, and Macloon (22) were taken as a basis for feeding during the investigation. They were 1 gram of first-day colostrum, 2 grams of fifth-day colostrum and 3 grams of milk after 1 month of lactation. This latter figure agrees with the amount used by the above investigators (22).

The colostrum and milk used in this investigation were obtained from the cows of the dairy herd, Department of Dairy Husbandry, Kansas State College of Agriculture and Applied Science. The cows received the regular ration which included a good quality of alfalfa hay, Atlas sorgo or corn silage, and a grain mixture of corn, bran, and cotton seed meal. All of the cows used in this experiment freshened in the fall or winter and did not have access to pasture during the collection of samples.

Twenty-four hour composite samples of the colostrum and milk were saved by the Department of Dairy Husbandry through the courtesy of Dr. W. H. Riddell. These were immediately bottled and frozen and were then stored at 0° F. until ready for use. This method of keeping samples was considered desirable as Grayson (14) has shown that there is no deterioration of vitamin G during storage in a frozen condition.

The total yield of milk, the per cent of fat, and the per cent of total solids were determined for each composite by the cooperating department. Samples for series I were collected from cows on the first or freshening day and at the end of the first month of lactation. Collections for series II were made at the same intervals, with an additional composite saved on the fifth day of lactation, which was thought to be a good sample to test for determining the rate of decline in the vitamin G potency of milk which had shown up in the same laboratory (2) indicated that milk at the fifth day level is approaching normality in potency for that particular vitamin. It was thought this might carry over into vitamin G.

Ten or more rats were used to test each sample of colostrum or milk, but the records of a few of these were later eliminated as they either were improperly depleted or apparently had resorted to coprophagy. The negative control group included at least 1 rat from each litter used in the experiment. When they were depleted they were continued on the basal vitamin G-free diet and distilled water with no additions. It was thought advisable to have also a group of positive controls receiving composite portions of raw milk from the dairy herd. These were used as a check on growth gains.

Table 1. Records of cows used in experiment.

Cow	Date	Fat per cent	Total solids per cent	Total yield milk pounds	Total yield fat pounds	Total yield solids pounds
Elmbar Crystal	3- 4-35	5.1	22.76	8.0	0.41	1.821
Born 1-13-32	3-28-35	4.2	13.10	34.6	1.45	4.533
2nd calf						
Ayrshire						
S. L. Maggie	5- 8-35	3.4	22.66	11.0	0.37	2.493
Born 3-21-29	6- 1-35	4.8	12.77	59.2	2.84	7.560
5th calf						
Ayrshire						
Dean Trixie	10- 4-35	2.85	13.504	7.0*	0.1995	0.945
Born 1-8-32	11- 4-35	3.768	11.930	46.2	1.741	5.512
2nd calf						
Holstein						
Emily Emperor	10-27-35	5.40	26.171	4.4*	0.238	1.152
Born 8-12-19	10-31-35	5.332	14.486	47.0	2.506	6.808
4th calf	11-27-35	4.433	12.870	63.5	2.815	8.172
Holstein						
F. B. Maid	10- 7-35	1.50	17.919	6.0*	0.09	1.075
Born 8-14-31	11- 7-35	6.375	15.588	30.1	1.919	4.692
3rd calf						
Jersey						
F. B. Titan	10-30-35	4.20	17.319	6.5	0.273	1.126
Born 9-15-32	11-4 -35	6.398	15.872	23.9	1.529	3.793
2nd calf	11-30-35	6.706	16.551	28.8	1.932	4.767
Jersey						

* Freshened in afternoon and yield includes only evening milking.

Table 2. Summary of data from rats of preliminary series.

Portion fed grams	No. of rats	Initial Wt. grams	Depletion days	Weight after depletion grams	Weekly gains--grams 1	2	3	4	5	6	7	8	Total gain grams	Food consumption grams
First-day colostrum														
0.5	7	53	15	68	5	2	-1	1	1	2	2	-4	8	159
1.0	2	67	15	65	5	13	5	2	4	-2	5	6	38	298
2.0	2	72	17	81	11	19	14	4	1	2	9	16	76	394
Thirtieth-day milk														
1.0	7	53	15	67	1	2	1	-1	-1	3	-2	-1	2	174
2.0	7	54	15	68	6	3	4	3	3	1	-3	1	18	186
3.0	2	55	16	67	11	2	3	8	2	2	0	2	30	194
Negative controls														
0.0	7	49	16	65	-1	1	-1	-2	-2	0	-4	-1	-10	153

Table 3. Summary of data from rats of series I.

Samples fed	No. of rats	Initial Wt. grams	Depletion days	Weight after depletion grams	Weekly gains--grams 1	2	3	4	5	6	7	8	Total gain grams	Food consumption grams
Holstein (Trixie)														
1st day 1 gram	9	59	19	68	3	6	9	8	0	3	5	2	36	346
30th day 3 grams	10	56	18	63	3	10	6	4	3	5	4	4	39	338
Jersey (Maid)														
1st day 1 gram	10	57	19	62	5	5	5	8	0	7	5	1	36	326
30th day 3 grams	10	51	20	62	7	6	10	5	4	9	5	2	48	326
Controls														
Herd milk: 3 grams	3	48	27	69	6	13	-1	10	7	3	-2	19	55	362
Negative	7	56	17	66	-3	1	0	3	-2	7	0	0	6	290

Table 4. Summary of data from rats of series II.

Samples fed	No. of rats	Initial Wt. grams	Depletion days	Weight after depletion grams	1	2	3	4	5	6	7	8	Total gain grams	Food consumption grams
					Weekly gains--grams									
Holstein (Emily)														
1st day 1 gram	11	39	21	47	6	10	6	6	6	5	4	7	50	309
5th day 2 grams	11	39	19	50	6	4	5	7	7	12	8	6	55	335
30th day 3 grams	10	38	19	46	11	3	7	6	7	6	6	7	53	299
Jersey (Titan)														
1st day 1 gram	11	38	22	48	5	6	5	1	5	2	3	7	34	282
5th day 2 grams	11	39	22	46	7	4	4	7	7	7	9	4	49	289
30th day 3 grams	11	39	22	48	9	7	6	8	7	3	3	9	52	294
Controls														
Herd milk: 3 grams	14	39	18	45	8	6	5	5	6	4	6	6	46	281
Negative	8	38	18	47	0	0	1	0	1	2	0	2	6	276

Table 5. Summary of data from rat feeding experiment.

Cows	Amt. fed grams	Date produced	No. rats fed	Gains due to feeding grams	Gains per gram of feeding grams	Estimated Bourquin units per gram	Yield of milk per day grams	Yield per day Bourquin units of vitamin G 1000 units
Dean								
Trixie	1	10- 4-35	9	30	30	1.2	3,175	3.8
(Holstein)	3	11- 4-35	10	33	11	0.4	20,956	8.4
Emily	1	10-27-35	11	44	44	1.8	1,996	3.6
Emperor	2	10-31-35	11	49	25	1.0	21,319	21.3
(Holstein)	3	11-27-35	10	47	16	0.6	28,803	17.3
F. B. Maid	1	10- 7-35	10	30	30	1.2	2,722	3.3
(Jersey)	3	11- 7-35	10	42	14	0.6	13,653	8.2
F. B.	1	10-30-35	11	28	28	1.1	2,948	3.2
Titan	2	11-4 -35	11	43	22	0.9	10,841	9.8
(Jersey)	3	11-30-35	11	46	15	0.6	13,063	7.8
Herd milk	3		17	48	16	0.6		

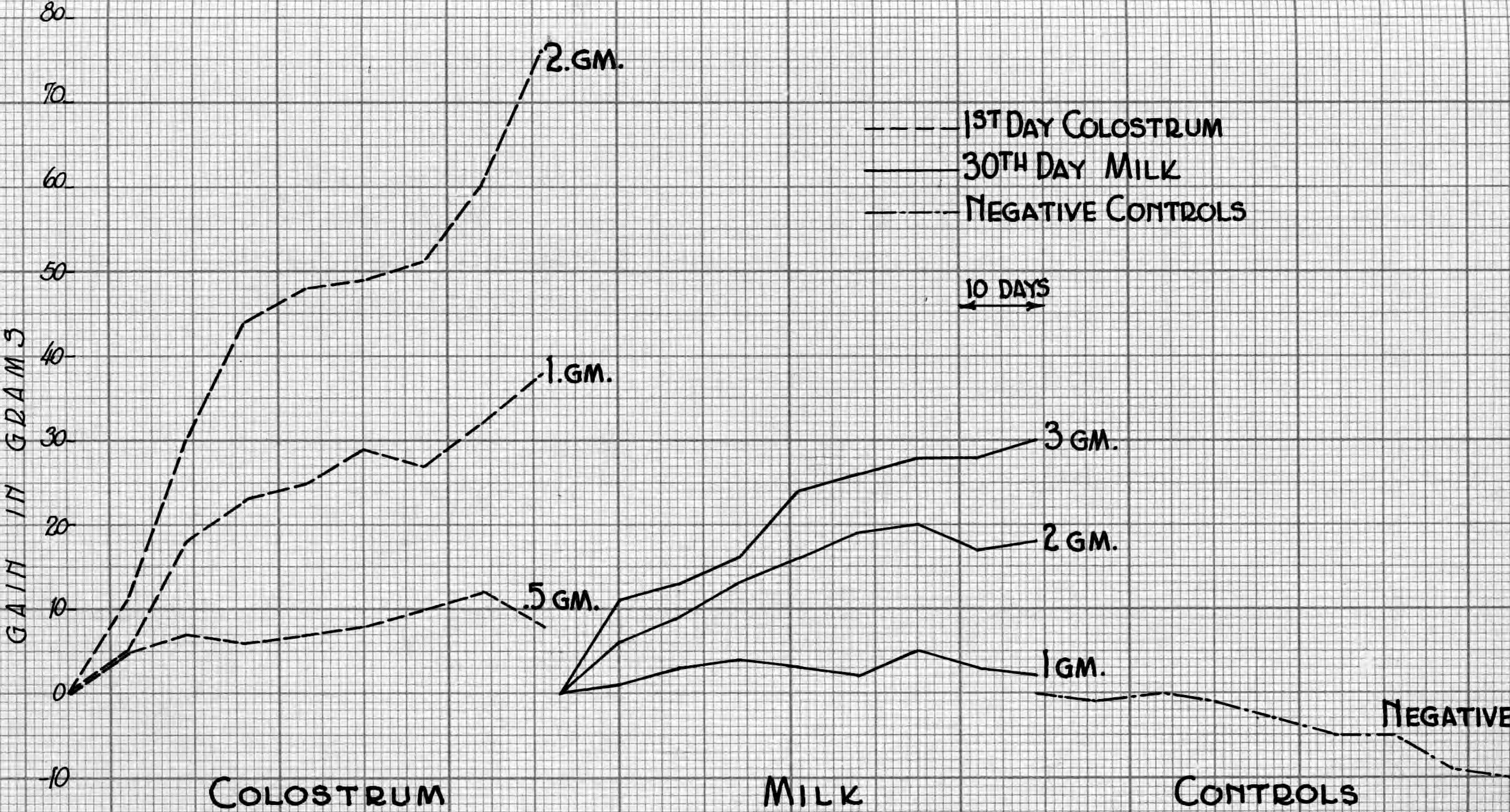

FIG. 1. AVERAGE GAIN CURVES FOR RATS OF PRELIMINARY SERIES.

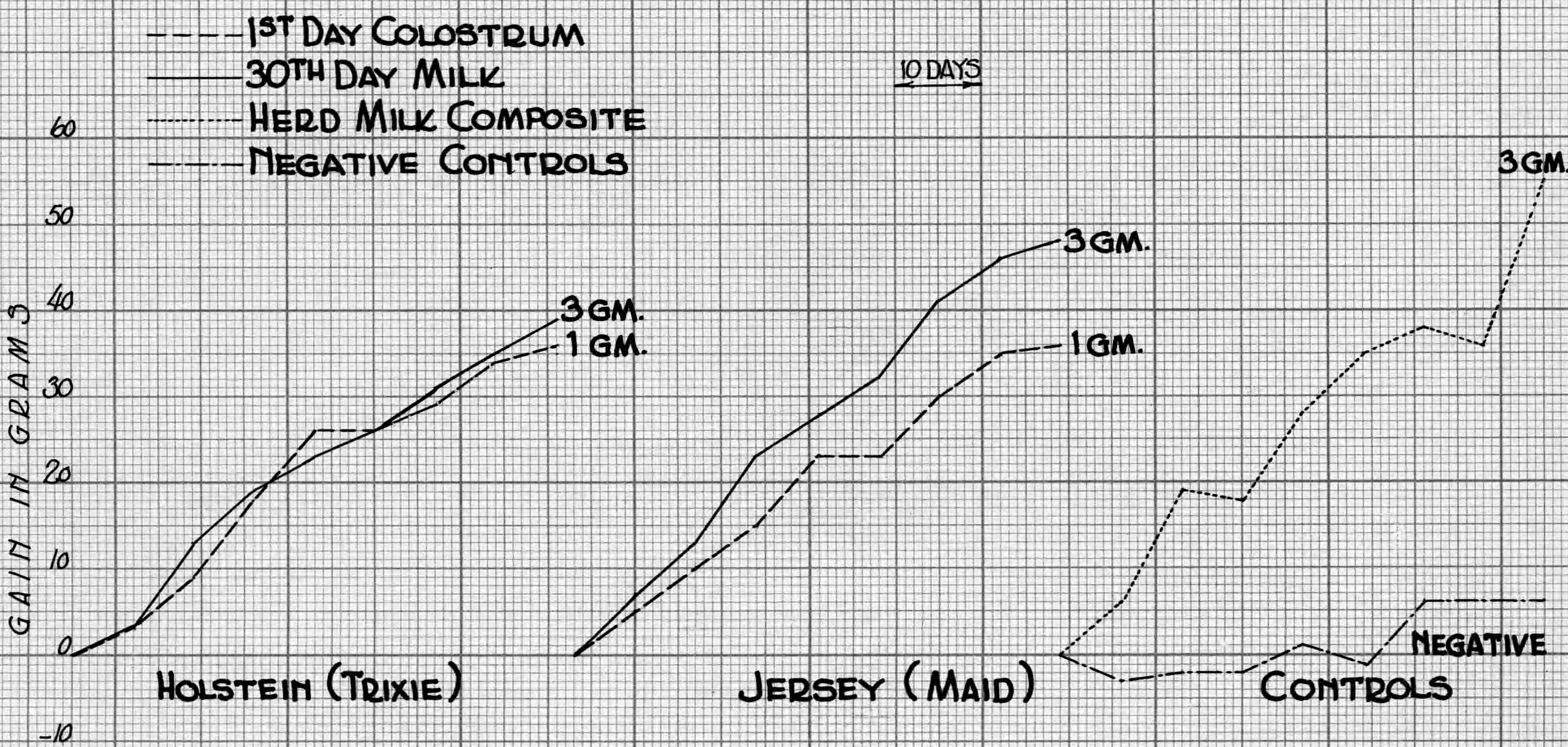

FIG. 2. AVERAGE GAIN CURVES FOR RATS OF SERIES I.

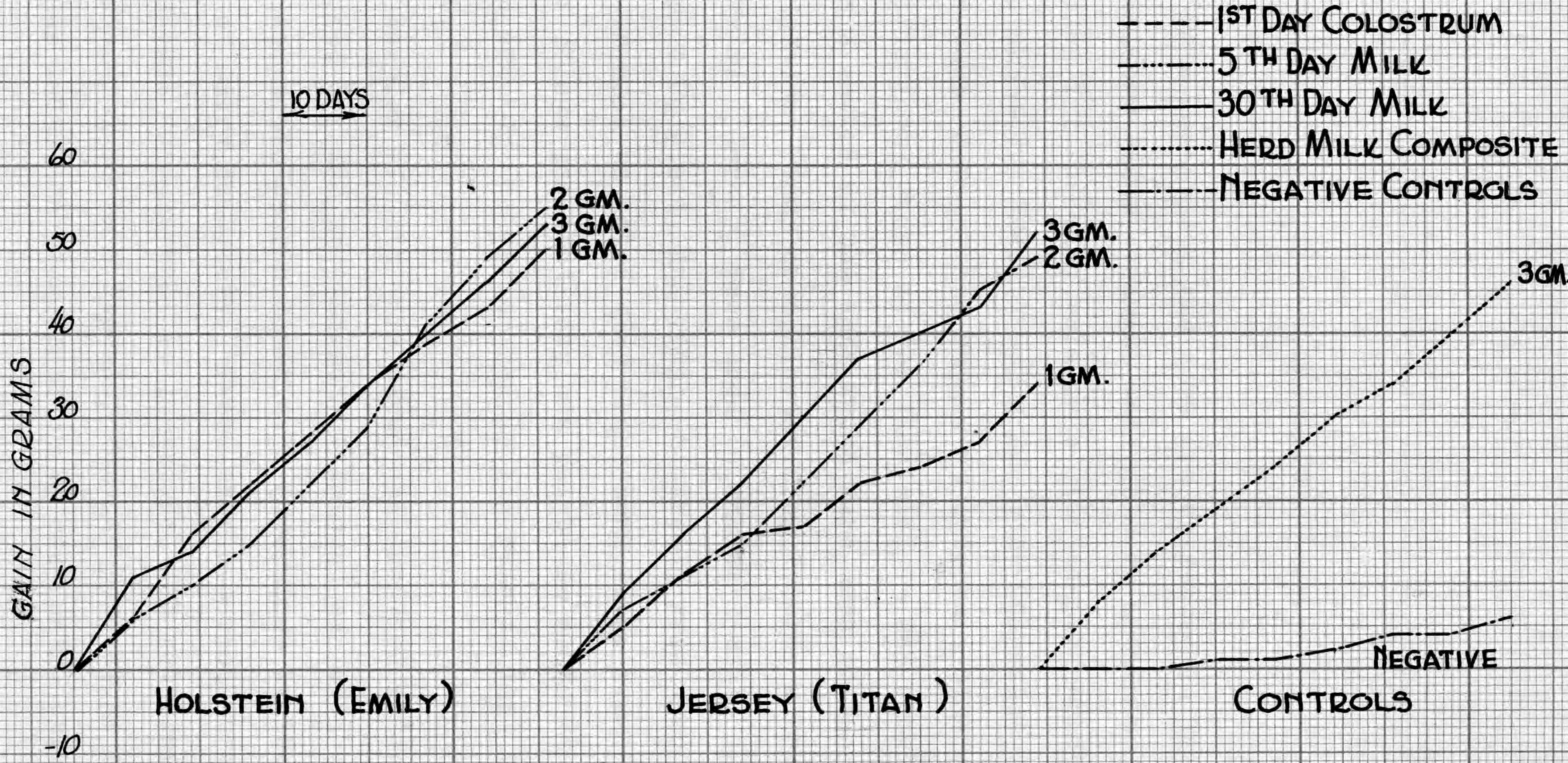

FIG. 3. AVERAGE GAIN CURVES FOR RATS OF SERIES II.

DISCUSSION

Data concerning the cows are presented in Table 1. The preliminary tests, thought desirable to determine suitable portions for feeding, were made with samples which happened to be on hand. These samples had been collected from 2 Ayrshire cows, Crystal and Maggie at intervals similar to the specifications drawn up for this study. The same table also gives a record of the cows which were used in the later investigation. Samples collected from Trixie, a Holstein, and Maid, a Jersey, were used in series I while those from Emily, a Holstein, and Titan, a Jersey, were used in series II. It will be noted that these cows range from 3 to 6 years in age and that they are paired so as to make the average age for one series nearly the same as that of the other.

Samples of milk were saved and examined as indicated in Table 1. The percentages of fat and of total solids for each composite sample saved were determined by the Department of Dairy Husbandry. These show a marked variance in individual cows as well as in breeds. As was expected, there seemed to be a tendency for the percentage of fat to rise as lactation progressed up to one month, while the reverse was true for the percentage of total solids, which decreased as the lactation period advanced.

The total yield of milk in pounds was obtained by combining the weights of the morning and evening milkings on the days the samples were saved. There is some question as to the accuracy of the yields of colostrum, as complete milking at this time is difficult; hence, the figures given may not represent the entire production. When cows freshened in the afternoon, only the colostrum obtained in the evening was saved, while a day's composite was made when freshening occurred in the morning. Table 1 indicates the procedure followed for each cow.

The calculated 24-hour yield of fat and total solids presents further data. The total yield of fat and of total solids increased as the stage of lactation progressed, findings consistent with accepted averages. The larger amounts were due both to increased percentage and increased yield of milk in figuring fat, but only to increased yield in computing total solids. Because of heavier daily production, the total yield of total solids was usually larger for the Holstein cows than for the Jersey cows.

Ten or more rats were started on each level of feeding in series I and II, with the exception of the positive controls in the first series as shown in Table 2. Data from the first rats showed a larger gain in weight than expected from other reports (22); therefore, after the series was started a few of the remaining available rats were fed herd composite raw milk to parallel the animals which were on experimental feedings. Data from a few rats were eliminated as it was apparent that they had resorted to coprophagy, had refused to eat their daily portions of milk, or had been over-depleted.

The original records of the rats of the preliminary series have been studied and used in compiling the data given in Table 2 and Figure 1. These show the gains which resulted when first day colostrum and milk at the end of the first month of lactation were fed in three different amounts. The number of days required to deplete the body store of vitamin G was about the same for the rats on each level of feeding. The average weights of the rats after depletion are also approximately the same, with the exception of the group which later received 2 grams of first-day colostrum as a daily feeding. When the preliminary experiments were finished, it was apparent that 1 gram of colostrum and 3 grams of milk produced gains which were nearly equal; therefore, these amounts were accepted as feeding portions to use throughout the experiment. Most of the curves of the rats on the lower levels of feeding flatten toward the end, probably because of intense heat, since the preliminary work was finished in July and August, 1935.

Table 3 and Figure 2 summarize the data of rats of series I, in which first-day colostrum and milk at the end of the first month of lactation from a Jersey cow, Maid, and a Holstein cow, Trixie, were fed. The average length of time necessary to deplete the body stores of vitamin G and the average depleted weights were about the same for the groups of rats in this series. It will be noted that the curves of the rats fed colostrum show the same total gain for each breed of cow. The Jersey milk on the thirtieth day of lactation produced a slightly higher gain than the Holstein milk at the same stage of lactation while the composite milk produced an even higher gain. This would tend to indicate a similar potency of vitamin G in colostrum produced by different breeds with a variation in the normal milk. Gains resulting from feeding 1 gram of colostrum and 3 grams of milk are approximately the same for the Holstein cow, while the gain produced by 3 grams of Jersey milk is slightly higher than that from 1 gram of colostrum.

Table 4 and Figure 3 present the compilation of data accumulated from the rats used in series II. Again, there is found a similarity in the period required for body depletion of vitamin G and in the average depleted weights of animals in each group. The rats in this series were put on the vitamin G-free diet when they reached a weight of 38 to 40 grams, so that their depleted weights are from 15 to 20 grams less than those of animals used in either of the preceding series. It is significant that a different relative vitamin G potency is shown in this series from that shown in series I. The first-day colostrum collected from Emily, a Holstein, appears to have more vitamin G content than that collected from Titan, a Jersey, while the colostrum used in series I apparently was of the same potency. Milk at the end of the first month of lactation is of practically the same potency from cows of the two breeds, but the Jersey milk appeared to have a greater vitamin G content in series I. Fifth-day milk from the two breeds is of similar vitamin G potency. The rats used as positive controls show approximately the same total gain on the 3-gram feedings of herd composite milk as rats fed 3 grams of thirtieth-day milk from each individual cow of the two breeds. One of the most interesting features of this series of the investigation is shown in the nearly equal gains of rats fed on varying amounts of milk produced at different stages of lactation. One gram of first-day colostrum, 2 grams of fifth-day milk and 3 grams of milk at the end of the first month of lactation gave approximately the same total gains in weight.

It will be noticed that the negative controls in series I and II each gained an average of 6 grams. While this is not a large gain, it is more than is reported in other vitamin work (4, 6). A slight gain rather than a loss may be due to the fact that the animals were obtaining a small amount of vitamin G since new fluorometric estimates show (31, 32) that it is seldom possible to remove all of the vitamin G occurring in casein. If this is true, the basal diet should properly be called vitamin G-deficient rather than vitamin G-free. In estimating the vitamin G content according to the growth of the animals, the gain of the negative controls was subtracted from the gain of the animals receiving each supplementary feeding.

Table 5 gives data concerning the total 24-hour yields of vitamin G for each of the 4 cows used in the investigation at the various stages of lactation. Yields of the vitamin are estimated in Bourquin units and are expressed as thousands of units. It will be noted that the total production of vitamin G varied from 3.2 thousands to more than 21 thousands of units. The yields of vitamin G for the first day of lactation are similar, ranging from 3.2 thousands to 3.8 thousands with a mean of 3.5. Data from only 2 cows are available concerning the fifth day of lactation and these show wide variation. However, it is interesting to note that each cow produced on the fifth day somewhat more vitamin G than she later produced on the thirtieth day of lactation, but it is evident that the fourth cow, Emily, was a higher producer at this stage as well as on the fifth day. The same cow, a Holstein, excelled in other ways. At the end of the first month of lactation, when she was yielding more than twice as much vitamin G (17.3 thousand) per day as the mean of the other cows (8.1 thousand), she produced 81 per cent more milk, 51 per cent more butterfat, and 43 per cent more total solids than the mean production of the other cows.

Table 5 presents the number of Bourquin units of vitamin G per gram of milk produced by each cow at the different stages of lactation studied. It will be noticed that milk produced at the end of the first month of lactation was similar in vitamin G content to the herd milk fed to the positive control rats. The thirtieth day milk from one cow gave a result somewhat lower than that from the other cows. The vitamin G content of milk from the herd or from the individual cows at the end of one month of lactation may be estimated at 0.6 Bourquin unit per gram or 585 units per quart of 975 grams. The samples of fifth-day milk tested were approximately 50 per cent richer in vitamin G than the milk later obtained from the same cows or the composite herd milk. Of the materials studied, first-day colostrum was richest in vitamin G. It was estimated to contain 1.1 to 1.8 Bourquin units per gram, which is two to three times the number of units found in a gram of milk produced by the same cows after 30 days of lactation.

SUMMARY AND CONCLUSIONS

Twenty-four hour composite samples of colostrum and milk from 2 Jersey and 2 Holstein cows on the first, fifth, and thirtieth days of lactation were obtained through the cooperation of the Department of Dairy Husbandry. These were stored at 0° F. until needed for testing. The percentage of fat, the percentage of total solids, and the total yield of milk for the day were recorded for each sample according to the determinations made by the cooperating department.

Albino rats were depleted of their body store of vitamin G by feeding a vitamin G-free diet. Upon depletion, the animals were divided into groups of ten or more, comparable in weight, sex, and litter. One of the groups served as negative controls, receiving only vitamin G-free food throughout the experiment. The group used as positive controls received feedings from a composite sample of raw milk obtained from the college dairy herd. The other groups were fed the material to be tested, following the method of Bourquin, which consists of feeding the animals weighed portions of milk 6 days a week for 8 weeks. Each rat was weighed weekly. Composite growth curves were plotted from the records of the rats on each sample of milk fed. Computations were made to show the vitamin G content per gram of sample as expressed in Bourquin units. The total yield of vitamin G per day for each cow was also calculated and expressed in the same terms.

In the preliminary series, and in series I, 1 gram of colostrum and 3 grams of milk produced similar gains, indicating that colostrum may contain three times as much vitamin G as later milk. In the other series, 1 gram of first-day colostrum, 2 grams of fifth-day milk and 3 grams of milk at the end of the first month of lactation gave approximately the same total gains in weight for the groups of rats fed. For the small number of cows studied individually, the Jersies and the Holsteins seemed to produce samples of similar vitamin G content.

Comparisons may be made on the basis of the vitamin G produced per cow per day. On the first day of lactation, the cows all yielded somewhat more than 3 thousand units. The largest total daily outputs were on the fifth day for the cows from which such samples were saved, but these totals approached the yields for the thirtieth day. On the thirtieth day of lactation 3 cows produced about 8 thousand units each, but the other cow, a high milk producer, yielded twice this amount of vitamin G.

Milk produced at the end of the first month of lactation was similar in vitamin G content to the herd milk which was fed to the positive control rats, although the thirtieth day milk from one cow gave a result 25 per cent lower than the others. The vitamin G content of milk from the herd or from the individual cows studied at the end of one month of lactation may be estimated as about 0.6 Bourquin unit per gram or 585 units per quart of 975 grams. The samples of fifth day milk tested were approximately 50 per cent richer in vitamin G than the later milk from the same cows or the regular herd milk. First-day colostrum was richest in vitamin G and was estimated to contain 1.1 to 1.8 Bourquin units per gram. The samples of colostrum studied were found to contain two to three times as much vitamin G, per unit of weight, as the milk produced by the same cows at the end of the first month of lactation.

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