THE INFLUENCE OF THE RATION OF THE COW

ON

VITAMIN C CONTENT OF MILK

By

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TABLE OF CONTENTS

INTRODUCTION

Since the report by Butcher, Pearson and Biester. '19,[1] '20,[2] . and Butcher, et al. 20.[3], "that guinea pigs fed oats and 'spring' milk developed scurvy later, often gained more weight and lived longer than those getting milk from cows on 'winter' rations." there have been many investigations to learn more of the causes of these differences. These workers concluded from this work, that the diet of the cow was an important factor in controlling scurvy, weight and longevity of experimental animals.

Hess, Unger and Supplee, '20,[4] reported that, "if the ration of the cow was low in vitamin C, the milk possessed no antiscorbutic properties and that the milk from cows on pasture contained a fair amount of vitamin C."

Olsen and Copeland, '24,[5] confirmed the report that the vitamin C content of the milk was dependent upon the feed of the cow and that silage contained an appreciable amount of the vitamin C. Ellis, Steenbock and Hart, '21,[6] found that silage contained little if any vitamin C.

For many years many investigations were made to ascertain the nutritional differences in "spring" and "winter" milk. The vitamin content of A, B, and D in milk was studied and the greater growth quality of the "spring" milk was not attributed to any of these three vitamins.

The difference in growth is well established; however, it is not entirely clear whether this difference is due to vitamin C or to another factor. If it can be definitely established that the vitamin C content of milk is not influenced by a change in the ration of the cow, a viewpoint which is supported by much evidence at the present time, it would seem that the super-

ior growth quality of "spring" milk must be due to another factor.

PURPOSE

The purpose of this investigation was to study the influence of the ration of the cow upon the vitamin C content of milk. The determinations were made by the biological assay method and by the chemical titration method. The cows were given four kinds of rations. Similar determinations have been made by other workers. A brief resume of their technique and conclusions follows:

Hughes, Fitch, Cave and Riddell, '26,[7] reported that the presence of vitamin C in cows milk was independent of the ration of the cow. After feeding a dry ration consisting of beet pulp, pearl hominy, tankage and bone ash, for three years to cows which were kept on a dry lot, the milk was found to be rich in vitamin C. The milk when compared with milk from cows getting carrots, fresh green alfalfa and pasture grass was found to be equally high in vitamin C. A biological assay was made by using guinea pigs which were fed a basal ration of 89 parts of ground oats, 10 parts of dried alfalfa and 1 part of cod liver oil. Thirty (30) c.c. of milk were fed each day. The animals getting the two types, "spring" milk and "winter" milk, showed no significant differences. These authors concluded that the vitamin C content of the milk was independent of the ration of the cow and that dairy cows had, to some extent, the ability to synthesize vitamin C. There were no reports given of the lactation periods, the pregnancy periods or the ages of the cows.

Swartz, Murphy and Hamm, '30,[8] reported that the vitamin C potency of milk from ensilage fed cows was almost as high as that of milk from cows on pasture. These workers also reported that 50 c.c. of raw milk from cows on

pasture were insufficient to protect guinea pigs from milk scurvy, quoting from Hess that 80 to 100 c.c. of raw milk were required to protect guinea pigs from scurvy. Swartz reported, however, no severe scurvy cases nor deaths from scurvy on the 50 c.c. amount.

Bessey and King, '33,[9] established a method for the titration of vitamin C in animal and plant tissues. This offered a chemical means of ascertaining the vitamin C content of foods to parallel the biological assay method which had been the only method employed up to this time. Dann and Cowgill, '34,[10] established the scurvy protective dose of 0.6 to .7 mg of ascorbic acid per 100 grams of body weight per day of the guinea pigs.

Kohler, Elvehjem and Hart, '34,[11] reported a difference in growth in "spring" and "winter" milk. Rats were used as the experimental animals. The juice of grass when added to "winter" milk stimulated growth to such an extent that it approached "spring" milk in growth properties. Artificially dried alfalfa had been found to produce "winter" milk which was more nutritious than the ordinary type, but not as good as produced on green grass or A. I. V. silage.

Hogan and Johnson, '36,[12] stated that when the basal diet of 60 per cent of ground oats, 33 per cent skim milk powder, 4 per cent wheat germ oil, 1 per cent cod liver oil, 1 per cent sodium chloride and 1 per cent calcium carbonate was fed to guinea pigs, muscular atrophy resulted; if 10 per cent of alfalfa meal was added, the diet was adequate. Juice from barley plants also rendered the diet adequate. Juice of fresh grass also stimulated growth, 3 c.c. from filtered centrifuged lawn clippings stimulated growth of 4 grams daily. Adding B_1 and B_2 in pure form was no aid, neither

vitamins A, C. or D of any value as shown by adding carotene, cod liver oil, or orange juice. When the diet was irradiated there was no increased growth.

Kohler, Elvehjem and Hart, '36[13] stated that, "It is evident that in clear fresh grass juice there are important water soluble substances which contribute to the nutritive value of "spring" and "winter" milk and are directly stimulating to growth when added to "winter" milk."

Riddell et al.[14] reported on this problem in 1936. After repeating the work done in 1926, additional determinations of the vitamin C content of milk were made by both the biological assay and chemical titration methods. Cows were fed silage, dry feed, hay and pasture. Guinea pigs were fed the Sherman, La Mer, Campbell diet. The guinea pigs which were fed milk from cows on pasture made the most substantial growth yet the vitamin C content of the milk, from the cows getting the four kinds of feed, did not vary widely. It was concluded that the ration of the cow did not influence the vitamin C content of the milk, but the difference was due to another factor in pasture milk.

Butcher, '36.[15] reviewed the vitamin C investigations. He gave some additional findings from his own work, viz., that the ascorbic acid content of milk, e.i., the vitamin C content, was influenced by the stage of lactation of the cow. He also stated that the breeds of the cows showed consistent differences.

From this brief summary of the investigations, the problems of the causal factor or factors of the increased growth resulting from "spring" milk is not clearly understood after 20 years' work. By co-operating with a feeding experiment of the Dairy Department of the Virginia Experiment Station it became possible to undertake a short time investigation of this controversial problem.

The feeding experiment consisted of feeding four different feeds for a three months' period, namely, clover silage, clover hay, corn silage, and pasture to Holstein cows. The usual grain feeding was given to all the cows except the one on pasture.

Daily samples of milk were obtained for a period of 74 days and the vitamin C content determined by the biological assay method and at intervals by chemical titrations. The purpose of the investigation was to ascertain any differences in the milk from the four cows since they were fed four types of rations, (e.i., to see whether the type of ration made any difference in the vitamin C content in milk from cows of the same breed. This was the study originally planned. When it was apparent that the data did not warrant definite conclusions it was decided to continue the study for an additional 60 day period, this time using the same ration for all of the cows, namely, pasture grass. The milk was titrated frequently during this period.

BIOLOGICAL ASSAY

A. Experimental Method

Four "types" of milk were assayed each day for 74 days. "Types" of milk refer to the kinds of ration the cows were fed, (e.i., "clover hay milk" was milk from a cow getting clover hay, "clover silage milk" from a cow getting clover silage, "corn silage milk" from a cow getting corn silage, and "pasture grass milk" from a cow on pasture.

The Sherman, La Mer, Campbell vitamin C technique was used and the procedure was as follows: Forty young guinea pigs averaging 225 grams in weight were purchased from a commercial firm. Each was weighed, numbered and placed in a separate wire cage. After a preliminary feeding period of a few days during which the pigs were offered the basal ration, cabbage and milk ad libitum, a selection was made of the guinea pigs which had attained the weight of 300 grams. Thirty-four animals were selected. These were divided into two groups, group A the test group, and group B the control group.

A kilogram of vitamin C free basal diet was prepared consisting of 29.5 per cent rolled oats, 29.5 per cent wheat bran, 30 per cent skim milk powder heated to 110° C for 4 hours, 8 per cent filtered butter fat, 2 per cent of cod liver oil and 1 per cent of sodium chloride. The assay was carried out as follows: forty milliliters of raw milk were placed in a feeding cup in the cage of each guinea pig within 30 minutes after the time of milking. The basal diet was offered ad libitum. Water was kept in the cups in the cages throughout the assay period.

Since there were four types of milk to assay, each type was given to a group of 5 guinea pigs in the test group, and to 5 guinea pigs in the con-

trol group. From the review of literature, it was apparent that the determination by assay of vitamin C in milk was one of detecting small differences. A control group was set up to check the variation by adding ten units of vitamin C daily. The amount was small enough to detect differences at the threshold level.

The control group B, therefore, received in addition to the four types of milk ten International Units of pure crystaline vitamin C (0.5)* mg. of l-ascorbic acid in solution) daily in addition to the milk.

Weighings were made twice each week and clinical observations recorded. The usual laboratory hygiene was carried out.

B. Experimental Results

The data recorded during the assay consisted of weights of the guinea pigs taken twice each week, clinical observations and autopsy findings. Indications of hemorrhages such as tenderness in the joints, "face ache" position denoting hemorrhages in the buccal regions and muscular atrophy were recorded during the clinical observations. Any one of these conditions was recorded as an evidence of vitamin C deficiency. The degree of the deficiency was determined by the multiplicity of such conditions and the degree of hemorrhage. At the autopsy, hemorrhages and enlargements of joints were recorded as indicators of a vitamin C deficiency.

The records were tabulated and are presented in Table I.

*Ten International units of 0.05 l. ascorbic acid = 1 Sherman Unit 0.5 mg ascorbic acid.

Table I. Scurvy Scores and Weight Records of Two Groups of Guinea Pigs; Group A. fed 40 Milliliters of Raw Milk Daily from Cows on Four Types of Rations and Group B. fed Ten International Units of Vitamin C (0.5 mg. of l. ascorbic acid) in Addition to 40 ml. of Raw Milk.

Type of Milk Fed	No. of Pig	Scurvy Score	Day on- set of scurvy (das.)	Weights During Weeks of Experiment										
				0	1	2	3	4	5	6	7	8	9	10
				gms	gms	gms	gms	gms	gms	gms	gms	gms	gms	gms
Clover Hay A	67	0	74	280	318	336	360	400	410	426	454	504	500	480
	78	0	74	280	308	348	384	362	380	392	404	440	460	430
	13	2	28	268	285	280	264	290	286	316	340	380	394	400
	37	7	38	298	316	336	352	380	384	396	400	380		
	36	2	35	296	280	292	326	360	400	420	380			
		2.2	49	284	301	320	337	358	372	390	396	426	450	433
	61	0	74	286	290	300	312	350	372	392	422	448	460	486
	39	0	74	324	328	332	360	384	414	436	430	360	384	412
B	43	2	24	280	270	280	328	344	328	350	360	400	460	450
	31	0	74	284	316	344	354	390	424	450	454	500	500	
	52	0	74	310	300	328	284	254	240	240	310	304		
		0.4	64	296	300	316	327	344	355	373	395	402	450	450
Corn Silage A	45	10	28	286	296	312	320	356	362	374	354	372	396	384
	38	2	28	282	272	290	332	372	396	394	420	440	496	484
	65	5	31	320	320	276	298	320	392	266	260	265	260	250
	2	14	47	280	324	344	370	390	428	444	440	460	410	
	84	8	31	316	330	368	372	368	420	420	424			
		7.2	35	296	309	318	338	361	379	379	379	384	415	372
	10	9	45	286	312	300	344	358	358	374	404	400	390	396
	77	2	28	298	304	296	316	324	336	366	380	350	360	350
B	3	2	42	294	296	290	298	316	318	338	380	370	410	
	29	0	14	280	306	356	359	380	400	420	454	476		
	53	2	31	300	336	333	354	380	376	400	390	390		
		3.0	44	291	310	315	337	351	351	379	401	401	404	378
Clover Silage A	85	2	35	286	298	312	300	340	364	410	384	390	420	450
	12	12	56	290	324	376	408	424	428	422	424	412	420	430
	73	12	36	280	256	280	262	242	270	308	364	376	354	
	47	13	38	304	354	356	374	390	398	388	370	320		
	4	13	14	296	332	350	336	306	320	290	256	220		
		10.4	37.5	291	318	334	336	340	356	363	359	343	408	440
	68	9	59	284	270	244	293	320	319	350	356	384	358	312
	57	4	45	316	338	384	390	390	390	414	430	450	491	490
B	30	13	36	280	310	324	304	338	352	320	320	330	310	
	15	2	35	312	328	326	340	350	340	370	420			
	64	8	31	308	290	322	330	370	376	416	440	380		
		7.1	41.2	300	307	320	331	353	355	384	393	386	386	401

Table I. (Continued)

Type of Milk Fed	No. of Pig	Scurvy Score	Day on set of scurvy (das.)	Weights During Weeks of Experiment 0 gms	1 gms	2 gms	3 gms	4 gms	5 gms	6 gms	7 gms	8 gms	9 gms	10 gms
Pasture	6	12	25	290	284	330	374	340	330	324	320			
A	25	14	10	300	322	350	360	362	370	360				
		13.0	17.5	295	303	340	367	351	350	332				
B	63	11	32	270	322	390	370	378	320	320	340			
	33	10	21	270	274	324	340	376	360	320	336			
		10.5	26.5	270	298	357	355	327	340	320	338			

There was a marked difference in the guinea pigs' conditions. The pigs getting the "pasture grass milk" showed more evidence of severe scurvy than all of the others; those getting "clover hay milk" showed very little evidence of scurvy; the pigs getting the "clover silage milk" and the pigs getting the "corn silage milk" had a mild type of scurvy.

The King method" was used in scoring scurvy, which is an evaluation of the observations during the life of the test animal and at the time of the autopsy. Each item observed is scored from 0 to 3 according to the severity, a total score of 0 to 3 being considered no evidence of scurvy; a score of 3 to 5 is considered indicative of mild scurvy; 5 to 10 is considered moderate scurvy; 10 to 15 severe scurvy, and an animal with a score of 15 to 20 usually indicates a degree of scurvy which ends in death.

The King Score:

I. Hemorrhages

1.	muscles	0 - 3
2.	joints	0 - 3
3.	rib muscles	0 - 3
4.	intestines	0 - 3

*Personal correspondence from King, C. G., Professor of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania, 1939.

II. Brittle bones and bone hemorrhages

5.	ribs (beading at intercostal junction)	0 - 3
6.	femur	0 - 3
7.	teeth	0 - 3
8.	jaws	0 - 3
Total score		0 -24

The guinea pigs of group A on the "clover hay milk" had a score of 2.2, on the "corn silage milk" 7.1, on "clover silage milk" 10.4, and those on "pasture grass milk" 13.0.

The guinea pigs of group B, those getting ten additional units of vitamin C each day, plus the four "types" of milk had the following scores: "clover hay milk" 0.4, "corn silage milk" 3.0, "clover silage milk" 7.1, and "pasture grass milk" 10.5.

It was evident that the unit of vitamin C had the effect of decreasing the degree of scurvy in all instances. The unit of vitamin C being an arbitrary unit, the amount of l-ascorbic acid that will afford complete protection from scurvy to a standard guinea pig (300 grams), gave complete protection up to the twenty-sixth day and to 362 grams of weight.

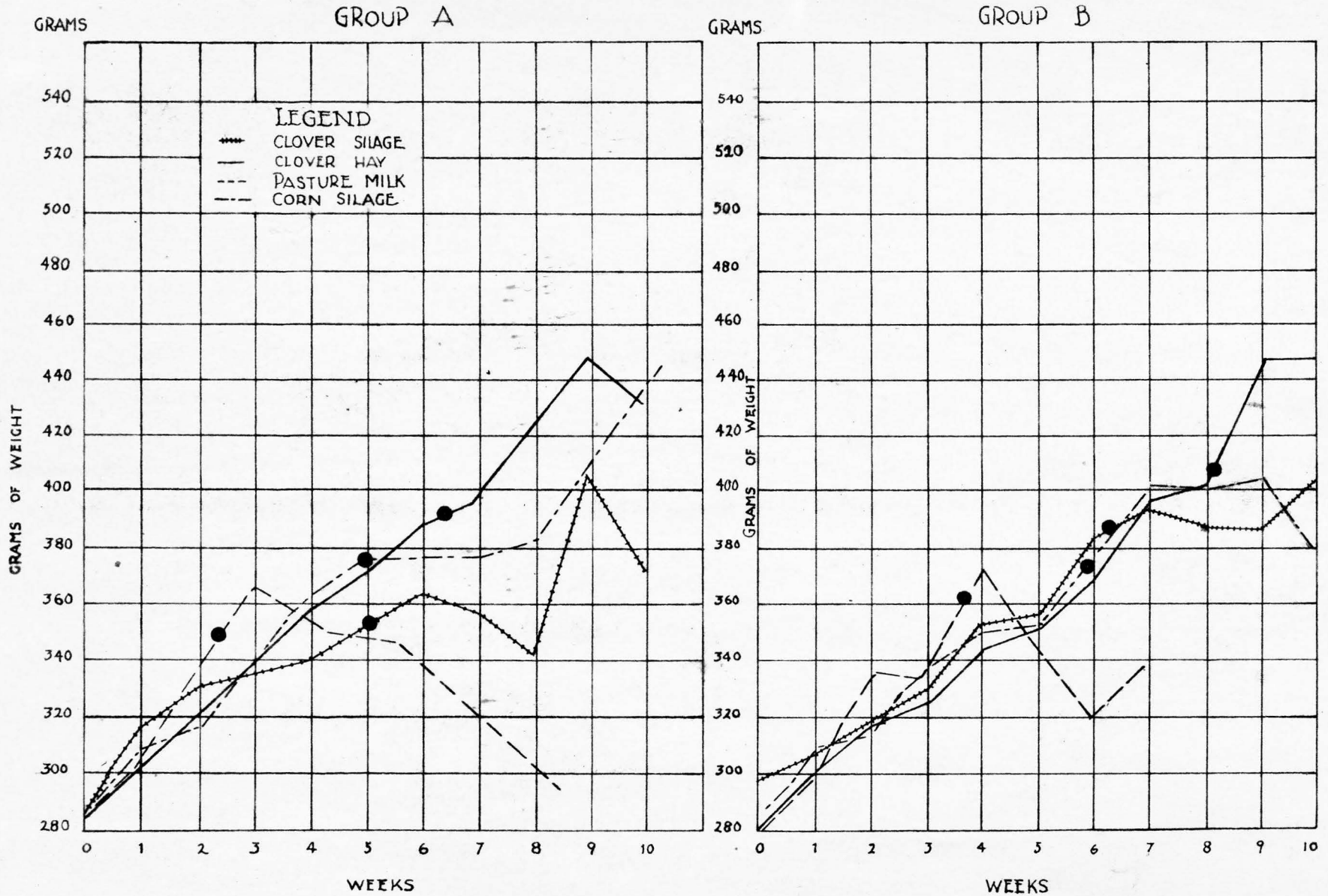
The guinea pigs of the test groups developed scurvy sooner than those in the control groups and developed scurvy when they weighed from 10 to 20 grams less than those in the control groups. Earlier workers[10] established the quantitative protection of 0.6 mg. of ascorbic acid of 100 grams of body weight increase. These guinea pigs received 0.5 mg. of ascorbic acid for 60 grams of body weight increase.

The differences appeared after the twenty-sixth day and continued to the end of the experiment or the seventy-fourth day.

The weight record of each group as well as the particular time the first symptom of scurvy was noted are shown graphically in Figure I.

FIGURE I.

WEIGHT CURVES OF GROUPS A AND B OF GUINEA PIGS WITH FIRST DAY OF OBSERVED SCURVY SYMPTOMS INDICATED



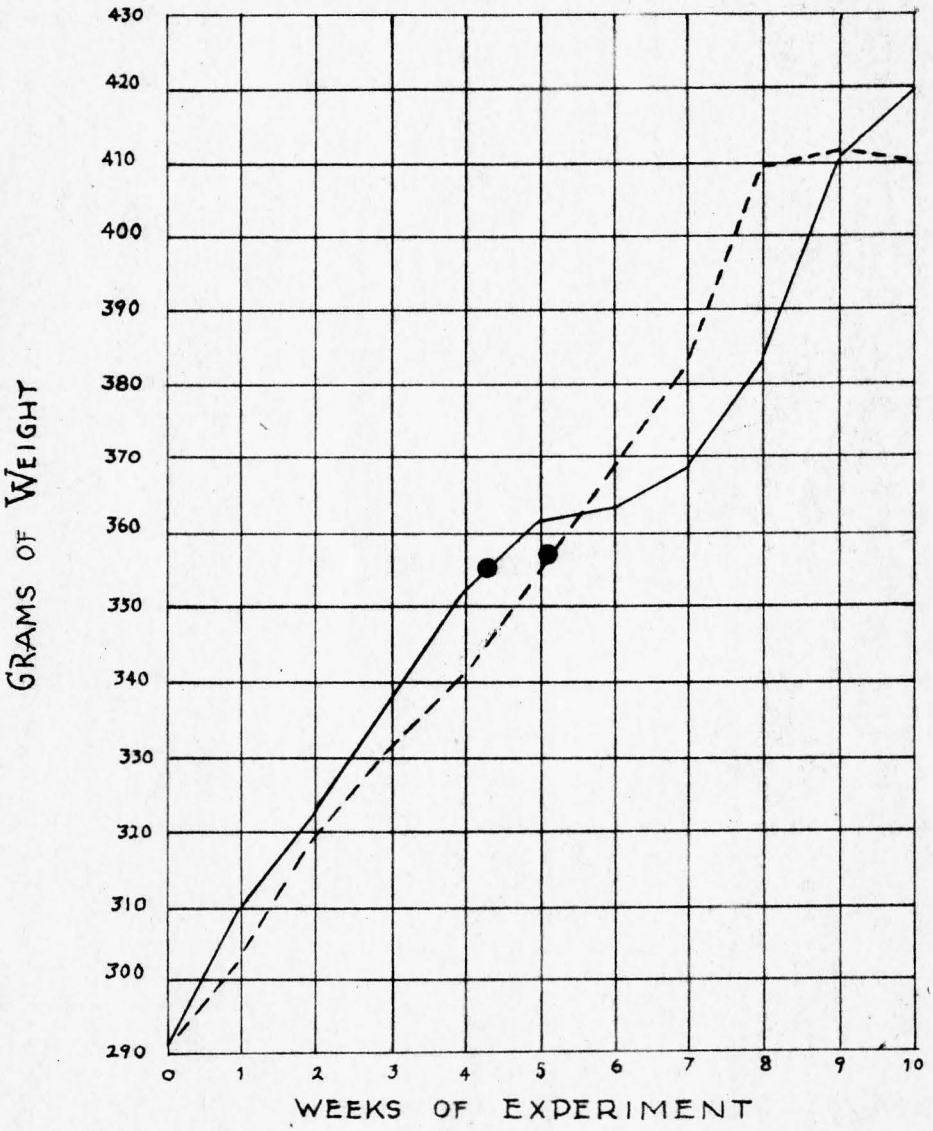
12

Weight increase is a controversial measure of the presence of vitamin C. It is evident that there is a weight increase when the diet is adequate which is greater than when the diet is inadequate. From these recorded data the weight increase continued for a period of seven to ten days after the first observed clinical evidence of scurvy appeared. The decrease in weight appeared as the severity of scurvy increased. Therefore, the weight records were not always in accord with the scurvy scores which were summations of both the clinical observations and autopsy findings.

A comparison of the weight records of the guinea pigs of both the test and control groups is given in Figure II for each type of milk fed. The groups receiving the additional units of vitamin C daily weighed very little more than those not receiving vitamin C; up to the fifth week they weighed less than those receiving only milk. It appears that the unit of vitamin C did not influence weight except where the diet was inadequate, and the unit of vitamin C did, however, prevent the abnormal condition or scurvy. These data, when regrouped from Table I, and organized in the two groups, test and control, irrespective of the types of milk fed, are presented graphically in Figure II.

FIGURE II.

WEIGHT CURVES OF GROUPS A AND B IRRESPECTIVE OF THE TYPES OF MILK FED AND AVERAGE FIRST DAY OF OBSERVED SCURVY SYMPTOMS INDICATED



LEGEND

— GROUP A, 40 ML. OF MILK DAILY

--- GROUP B, 40 ML. OF MILK DAILY PLUS 1 UNIT OF VITAMIN C

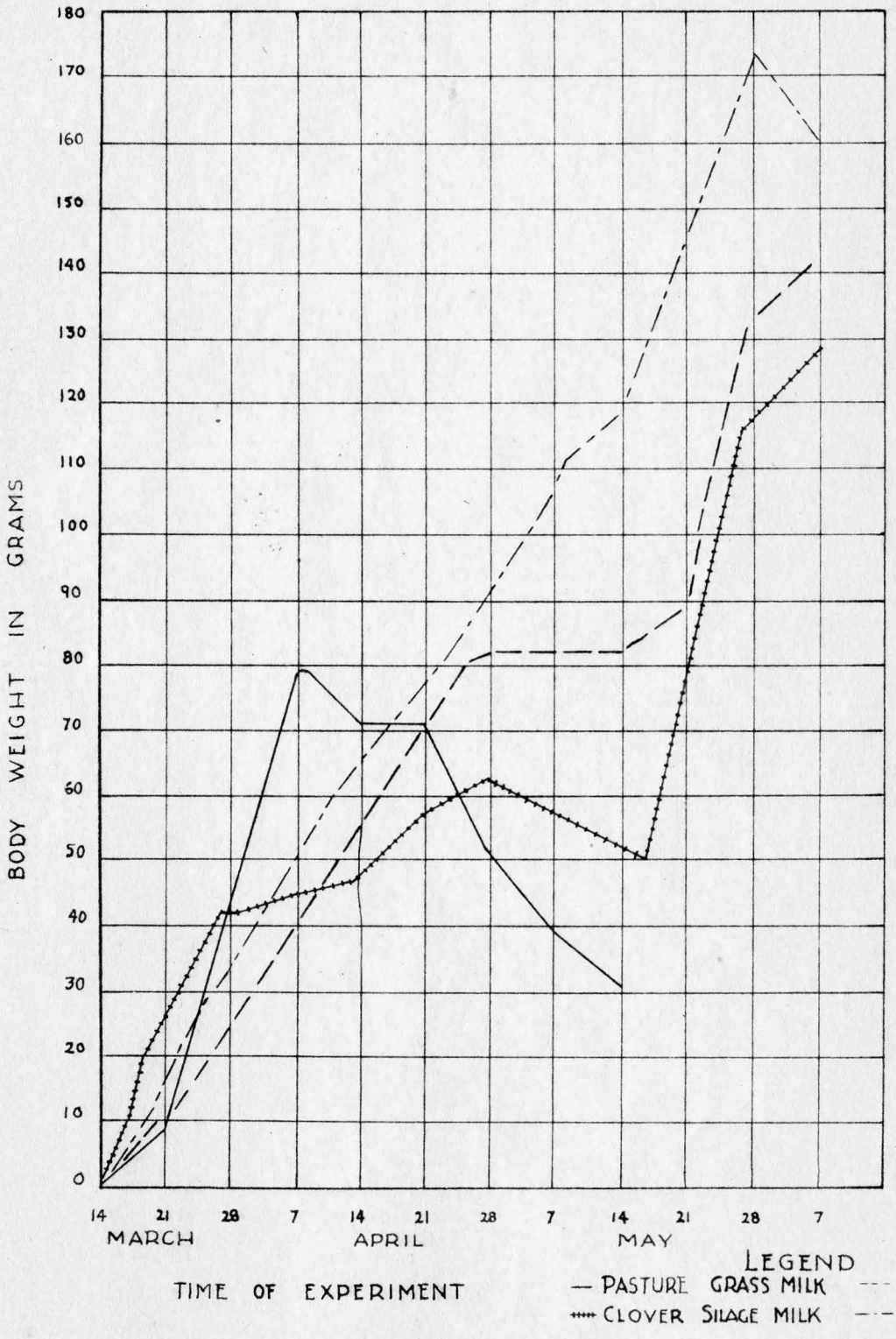
There were differences in the rapidity of growth of the four types of milk fed as shown in Figure I. The pigs getting the "pasture grass milk" gained much more rapidly than all of the other pigs before the onset of scurvy. A summary from these data presented in Table I is as follows:

Type of Milk Fed	Av. Initial Weight of Group	Av. Max. Weight of Group	Days to Attain Max. Wgt.	Av. Gain per Day until Max. Weight Attained
Test Group A	gms.	gms.	da.	gms.
Clover Hay	301	450	70	2.1
Clover Silage	318	440	57	2.1
Corn Silage	309	415	45	2.3
Pasture Grass	295	367	20	3.6
Control Group B				
Clover Hay	300	450	71	2.1
Clover Silage	307	401	48	1.9
Corn Silage	310	404	56	1.7
Pasture Grass	270	377	28	3.9

This material is shown graphically in Figure III.

FIGURE III.

WEIGHT INCREASES AND DECREASES AT INTERVALS OF ONE WEEK OF GROUPS OF GUINEA PIGS FED 4 "TYPES" OF MILK : CLOVER HAY MILK, CLOVER SILAGE MILK, CORN SILAGE MILK AND PAS-TURE GRASS MILK



Summary of biological assay data:

Type of Milk	Scurvy Score	Gain per Day until Maximum Weight
		gms.
Clover Hay	2.2	2.1
Corn Silage	7.2	2.3
Clover Silage	10.4	2.1
Pasture Grass	13.0	3.6

"Clover hay milk" had the highest amount of vitamin C; "pasture grass milk" had the lowest amount of vitamin C; "clover silage milk" had less than "clover hay" and "corn silage milk," but more than "pasture grass milk." The guinea pigs receiving "pasture grass milk" made the most rapid gains up to the onset of scurvy. This was not in accord with the earlier report by Dutcher et al. It appeared from these data that the differences of vitamin C content were either due to differences in ration of the cow or to some condition of the cow as Dutcher suggested. Records of the period of lactation and pregnancies of each animal will be given later in the report.

To confirm the biological assay data chemical titrations were made. The method used and the results obtained follow.

CHEMICAL TITRATIONS

A. Experimental Method

Freshly drawn morning milk was taken to the laboratory in glass containers and held at a constant temperature of 36° - 40° F for a period not exceeding two hours before the tests were made. The determinations were made in the following manner: A solution of the dye, 2 - 6 dichlorophenol-indophenol was prepared just previous to being used by dissolving 0.1 gm. of the dye in successive portions of warm water, diluting to 200 ml., then cooling and filtering. A small quantity of phosphate buffer p.h. 6.8 was added and the solution was stored in a dark bottle. A 5 ml. aliquot of freshly prepared lemon juice was titrated with a 0.01 normal iodine solution until a permanent blueing of the starch indicator resulted. Ten ml. of an 8 per cent acetic acid solution was added to a second 5 ml. aliquot of the lemon juice and the dye solution was run in from a burette until the end point was reached. Since 1 ml. of 0.01 normal iodine solution reacts with 0.88 mg. of ascorbic acid, the ascorbic acid equivalent of the dye was easily calculated.

Ten ml. of milk were titrated with 5 ml. of 10 per cent tri-chloracetic acid in order to precipitate the proteins. The supernatant liquid was removed from the precipitated materials by filtration. The precipitated residue was then washed with 8% of acetic acid, and titrated with a standardised solution of 2 - 6 dichlorophenolindolphenol.

B. Experimental Results

The ascorbic acid or vitamin C content of the four types of milk is recorded in tabular form, Table II. As has been previously stated, after the four cows were fed different rations for 74 days after which all of the cows were put out on pasture grass for 60 days. These data are also included as well as data from three additional cows on pasture during the same period.

Table II. Milligrams of Ascorbic Acid in 1 Liter of Raw Milk from Holstein Cows.

Type of Milk	Name of Cow	5/17	5/20	5/23	5/26	5/28	5/30	6/5	6/8	6/13	7/12	7/22
		mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg
Clover Hay	Rosni Bess Belle	19.0	18.0	16.0	18.0	16.0	16.0	16.0	20.0	20.0	14.0	14.0
Clover Silage	Fanny	23.0	26.0	24.0	22.0	24.0	22.0	16.0	16.0	-	-	-
Corn Silage	Burke Vieman Daisy	20.0	22.0	20.0	20.0	22.0	20.0	16.0	20.0	20.0	16.0	14.0
Pasture Grass	Homestead Rose DeKol	-	14.0	15.0	12.0	12.0	12.0	9.0	16.0	16.0	14.0	-
Pasture Grass	Burke Belle*								16.0	16.0	12.0	12.0
Pasture Grass	Rosni Daisy Belle*								18.0	18.0	8.0	8.0
Pasture Grass	Vaeman Daisy*								13.0	13.0	-	-

*On pasture with the cows from which the milk had been assayed.

Three samples of milk from each cow were titrated within two hours after the time of milking. The determination of the ascorbic acid was usually in agreement for the three samples. In case of any disagreement, if two readings were the same, these determinations were recorded. It was noted that the milk from cows having an ascorbic acid reading below 10 mg. per liter was slow in coagulating. The low readings preceded the cessation of lactation. All of the cows were out on pasture after June 1.

The ascorbic acid content of the four types of milk tested during the later part of May showed a difference in the amounts of ascorbic acid pres-

ent, "spring milk" or "pasture grass milk" having the smallest amount of vitamin C, "clover silage milk" the highest amount, "corn silage milk" the next highest and "clover hay milk" the next to the lowest.

The amounts of vitamin C as determined by the titration method was not in entire agreement with the determinations by biological assay. The "pasture grass milk" was the lowest by both methods and the "corn silage milk" the next to the highest. The chemical titrations were run only during the last fifteen days of the biological assay period.

After observing the amount of ascorbic acid in the four types of milk it appeared that the milk had a decreasing amount of vitamin C even during the fifteen days when titrations were made, as well as that different milk had different amounts.

All of the cows were put out on pasture June 1 and titrations were run periodically as well as on milk from three other Holstein cows. There was a slight rise in the vitamin C content just after the cows were put on pasture but a generally decreasing trend even though all were on the same ration, grass, for 60 days. This seemed to indicate some influence other than ration, which was affecting the ascorbic acid content.

The ascorbic acid content of the milk did not show a fairly constant value for each cow, as had been reported. This is graphically shown in Figure IV and Figure V.

FIGURE IV.

AVERAGE CONTENT OF ASCORBIC ACID IN LITER OF MILK AND TIME OF READINGS

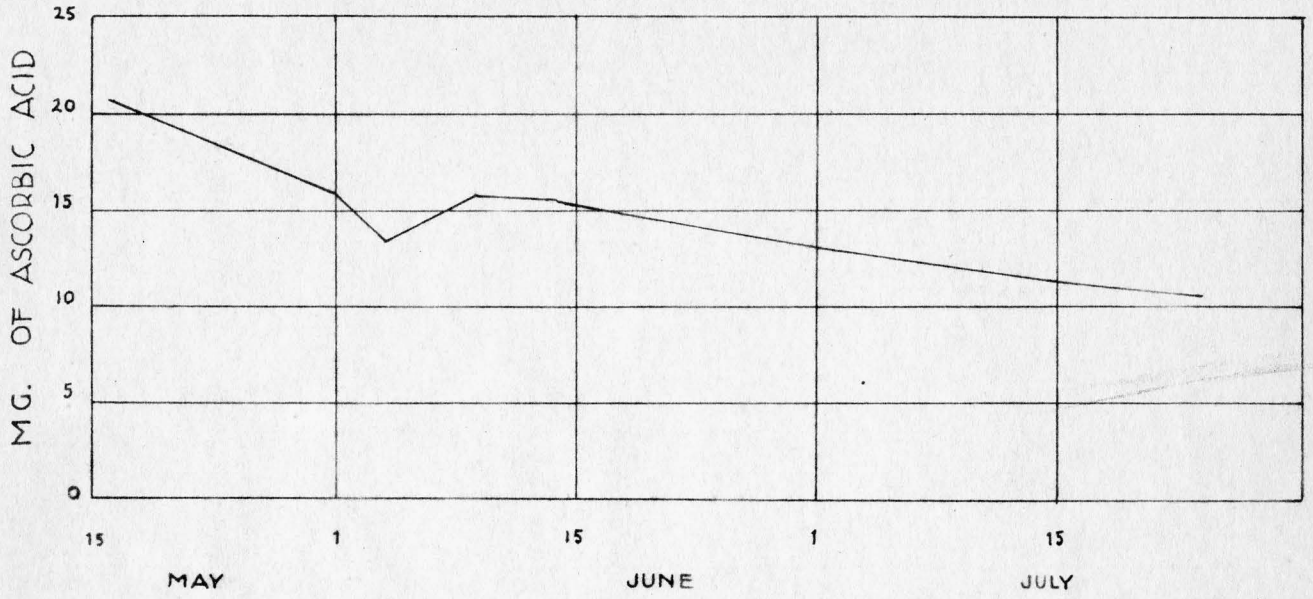
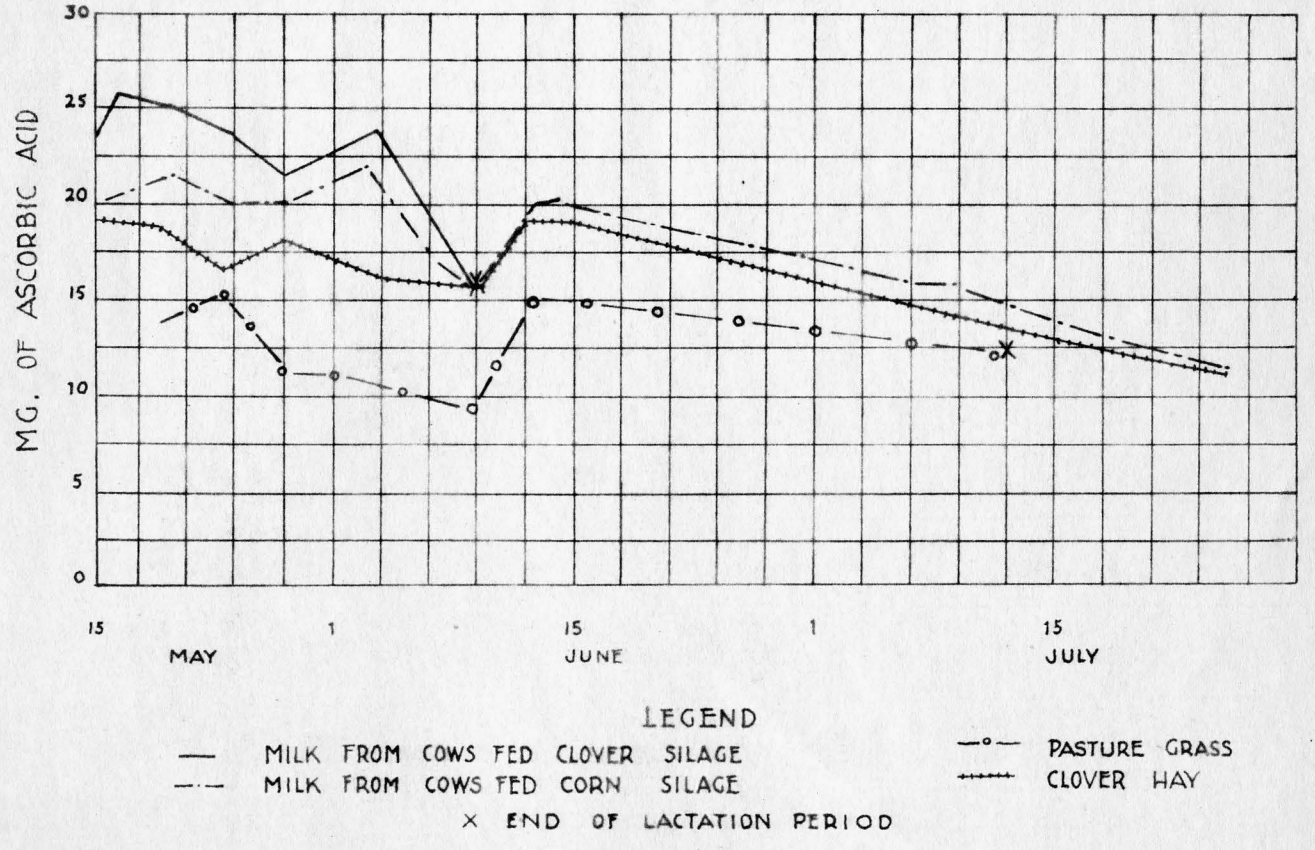


FIGURE V. (3 SAMPLES DAILY)

AVERAGE CONTENT OF ASCORBIC ACID IN LITER OF FOUR TYPES OF MILK AND TIME OF READINGS



Breed:

Since all of the cows were of the same breed, the difference in vitamin C content could not be due to this factor.

Milk Flow:

The total amount of milk given and the total milligrams of ascorbic acid contained therein were next investigated to determine whether the ascorbic acid increased or decreased as the milk flow increased or decreased. These data are presented in Table III.

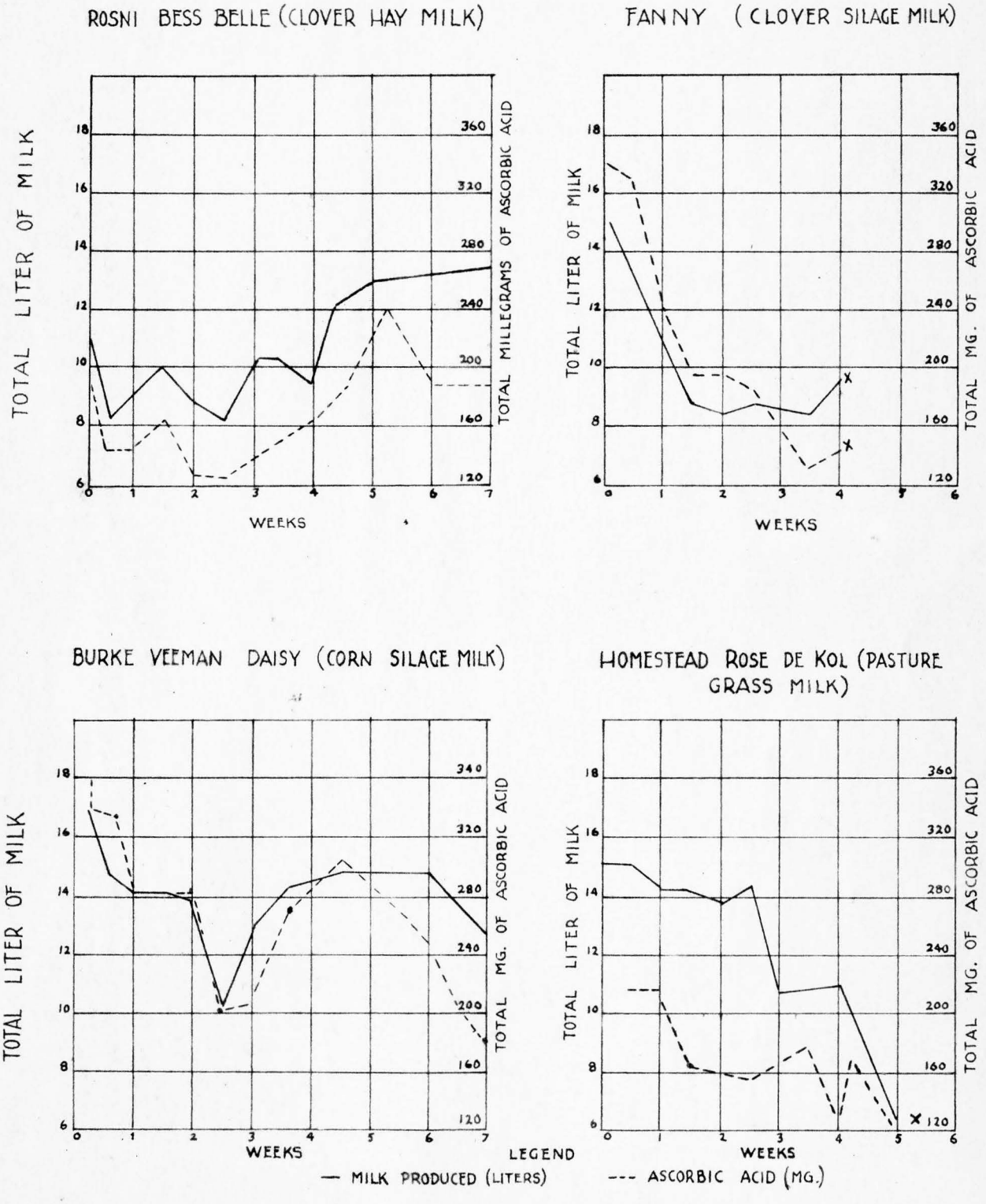
Table III. Total Liters of Milk and Total Milligrams of Ascorbic Acid Produced Daily During Ninty Day Period by Four Holstein Cows on Four Kinds of Rations.

Name of Cow	Production	Dates of Sampling									
		5/17	5/20	5/23	5/26	5/28	5/30	6/5	6/8	6/13	7/12	7/22
Rosni Bess Belle (clover hay)	Liters of Milk	11.3	8.9	9.1	9.9	8.5	8.1	10.2	9.5	12.0	13.1	13.8
	Mg. of ascorbic acid	209	144	144	162	128	128	160	180	240	182	182
Fanny (clover silage)	Liters of Milk	15.0	13.5	10.5	9.7	8.5	8.8	8.3	9.7	-	-	-
	Mg. of ascorbic acid	345	338	240	198	192	176	128	144	-	-	-
Burke Veeman Daisy (corn silage	Liters of Milk	17.1	15.0	14.7	14.2	13.2	10.1	13.1	14.7	15.9	15.8	13.2
	Mg. of ascorbic acid	340	330	280	280	286	200	208	280	300	240	182
Homestead Rose DeKol (pasture grass)	Liters of Milk	13.4	15.6	14.8	14.5	13.9	14.6	11.7	11.8	11.5	4.8	-
	Mg. of ascorbic acid	-	210	210	168	156	168	100	176	176	49.0	-

In general, the production of ascorbic acid increased as the flow of milk increased and decreased as the flow of milk decreased. The records of the individual cows are shown in Figure VI.

FIGURE VI.

TOTAL MILK PRODUCTION AND TOTAL ASCORBIC ACID CONTENT OF MILK PER DAY, GIVEN BY THE FOUR COWS, ON DIFFERENT RATIONS FOR FOUR WEEKS, THEN FED SAME RATION FOR FOUR WEEKS.



Age and Stage of Lactation

Since the ascorbic acid content apparently increases or decreases with the milk flow, and varies within the same breed, it seemed that the production of vitamin C might be correlated with some other condition of the cow. The age of the cow, date of calving, date due to calve, months of lactation period during titration were next studied. These findings are presented in tabular form in Table IV.

Table IV. Milligrams of Ascorbic Acid in 1 Liter of Milk with Dates of Lactation and Pregnancy for Seven Holstein Cows.

Name of Cow and Type of Feed Used	Age	Date of Calving	Due to Calve	Months of Lactation Period which Titrations* were made	Months of Period of Pregnancy	Mg. of Ascorbic Acid in 1 Liter of Milk during 3 months, May, June, July
	yrs.			mo.		mg.
Rosni Bess Belle (Clover Hay)	5	12-3-37	10-28-38	5. 6. 7	4,5,6	17.18.14
Fanny (Clover Silage)	9	10-3-37	0	7. 8. x	0	23.16. x
Burke Veeman Daisy (Corn Silage)	3	12-27-37	12-19-38	5. 6. 7	2,3,4	20.19.15
Homestead Rose DeKol (Pasture)	8	8-5-37	9-19-38	9. 10. 11	5,6,7	13.13.12
Burke Belle (Pasture)	9	10-18-37	2-3-39	7. 8. 9	0,1,2	- .16.13
Veeman Daisy (Pasture)	8	8-29-37	9-8-38	9. 10. 11	5,6,7	- .13.0
Rosni Daisy Belle (Pasture)	4	9-3-37	9-28-38	8. 9. 10	5,6,7	- .18.8

* Titrations were run during May, June and July.

Table V. Assay Results and Date of Pregnancy and Lactation of Four Cows

Name of Cow	Feed during Assay	Months of Lactation Period which Assay was made	Months of Pregnancy during which Assay was made	Scurvy Score of Guinea Pigs of Assay*
Rosni Boss Belle	Clover Hay	3, 4, 5	2, 3, 4	2, 2
Fanny	Clover Silage	5, 6, 7	0	10, 4
Burke Veeman Daisy	Corn Silage	3, 4, 5	0, 1, 2	7, 2
Homestead Rose DeKol	Pasture Grass	7, 8, 9	3, 4, 5	13, 0

*Assays were carried on during March, April and May

It seemed from these data that the milk from the cows in an earlier stage of the lactation period had a lower ascorbic acid content; that the ascorbic acid in the milk was less in the milk from pregnant cows than in the milk from the cow which was not pregnant; that the ascorbic acid decreased as months of pregnancy increased and that age did not influence the amount of ascorbic acid present in the milk.

DISCUSSION

There were many variables, starting with feed, losses in collection of milk samples, interferring reducing substances which should not be titrated, biological variables with the experimental animals et cetera which may have influenced the assay and titration results.

The disagreement between the determinations of the vitamin C content of the milks by the assay method and the titration method may have been due to the behavior of the cow, Fanny, the one which was fed clover silage. She was an unsatisfactory experimental animal because at frequent intervals during the assay period she went off feed. The weight record of the guinea pigs which were fed her milk showed a rapid increase in weight at the beginning of the assay while she was eating constantly. The fluctuations in weight during the remainder of the assay occurred while she ate irregularly. It is possible that there may be some influence on the scurvy reading which was due to this condition. Too, this cow was the only animal of the group which was not pregnant. This fact also may have influenced vitamin C values. Some workers[16] report a lesser amount of vitamin C in the blood of pregnant women than in non-pregnant women.

The titrations which were run only during the last 15 days of the biological assay period do not give a simultaneous reading with the assay. The plan of the investigation called for titrations to be made at some period to check the assays. There was no evidence in literature to indicate that the vitamin C content of the milk of the individual cow changed over a period of time. When it appeared that there was a decrease in the vitamin C content of the milk of each of the cows, the problem seemed to be quite a

different one. It is regretted that the titrations were not carried out during the entire assay period. It is evident that a more comprehensive investigation could have been made if the titrations had been run throughout all the time that the milk was assayed.

From this investigation which was limited both in time and in comparative data, it seems that the stage of lactation of the cow was a primary factor in the vitamin C content of milk, e.i., the earlier in the period, the more ascorbic acid. However, the number of cows studied was not adequate to give data for all of the stages of lactation. Bechtel[18], reported on this phase of the problem after this investigation was undertaken.

The increased rate of weight gain of the animals fed "spring milk" was the noticeable difference. These guinea pigs gained much more rapidly than any of the other groups. Weight increase is not in itself a measure apparently of the vitamin C content of milk as has been pointed out in the preceding data. That there was a rate of gain independent of the vitamin C content seems to be apparent. To establish this fact more studies obviously are needed.

This was in accord with the report by Butcher et al[1]. The vitamin C content of the milk when tested by the assay method and the chemical titration method was, however, lower than all other types of milk. This was not in accord with the earlier observations. Riddell et al was not in agreement with the earlier work stating "that the guinea pigs grew as well and did not develop scurvy sooner when they were fed winter milk than when the pigs were fed spring milk. The difference in the technique however was in the test ration. Riddell feeding alfalfa in addition to oats. This one

food apparently influenced the weight record of the guinea pigs fed these two ways and confused the influence of the vitamin C and another factor.

This rate of gain apparently is caused by some factor carried in the ration of the cow, and must be greater in amount in "spring milk". Elvehjem has named this factor the "grass juice factor." In a recent publication[17] a report was given that molasses contained this "grass juice factor." The clover silage feed may, therefore, contain this factor because of the presence of molasses and no doubt accounts for the greater growth of the guinea pigs getting the clover silage milk. The vitamin C content of silage has been a controversial one. This confusion may be the result of the presence of the "grass juice factor" as well as of vitamin C. Until some biological evidence is established for the lack of the "grass juice factor" confused interpretations may continue to be made. It was observed in this investigation that the animals which grew most rapidly, namely, the "spring milk" group, also developed scurvy earliest. This has been noted by other workers.

CONCLUSIONS

From the results of this investigation, it is concluded:

1. That the ascorbic acid content of milk decreases as the stage of lactation increases.
2. That the ascorbic acid of the milk of individual cows varies within the breed.
3. That the age of the cow does not influence the vitamin C content of the milk.
4. That the rate of gain of the assay animals over a period of 7½ days was independent of the ascorbic acid content.
5. That the vitamin C content seems to be independent of the ration of the cow.
6. That the "spring milk" carried some factor which influenced a greater gain of weight than other "winter" milk rations.

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