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RESEARCH BULLETIN 101

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LINCOLN, NEBRASKA
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SUMMARY

A study was made of the vitamin A content of soybean silage, and of A.I.V., molasses, and common corn silage. The silages were fed to groups of cows and the vitamin A content of their milk determined. The vitamin A determinations were made by feeding the silage or the milk to groups of rats whose body stores of this vitamin had been depleted by being fed a vitamin-A-deficient ration. Approximately 780 rats were used in these experiments. There were no apparent ill effects of feeding as much as 3.2 grams of the A.I.V. silage per rat per day for eight weeks. This was 20 to 30 per cent of the food consumed. The A.I.V. silage contained only slightly more vitamin A than did the molasses silage. The ordinary corn silage contained less vitamin A than either the A.I.V. or molasses silage. The soybean silage was inferior to any of the other silages as a source of vitamin A.

Milk produced by cows receiving these silages as the only source of roughage ranked in the same order of vitamin A potency as did the silages, namely A.I.V. silage, molasses silage, and common silage. On the other hand when a good grade of alfalfa hay served as the only source of roughage, the milk produced contained more vitamin A than did the milk produced by cows receiving A.I.V. silage as the only roughage.

In another experiment one group of cows was fed both molasses silage and alfalfa hay, while a second group received A.I.V. silage and alfalfa hay. The hay was fed *ad libitum* and the group which received the molasses silage consumed the most hay. In this instance the milk produced by the group receiving the molasses silage contained more vitamin A than did the milk produced by the A.I.V. silage group, which was probably due to the greater consumption of alfalfa hay.

The Vitamin A Content of Soybean Silage and of A.I.V., Molasses, and Common Corn Silages, and the Effect of Feeding these Materials upon the Vitamin A Content of Milk

I. L. HATHAWAY, H. P. DAVIS, AND J. C. BRAUER

During the past few years a number of attempts have been made to improve methods of forage-crop preservation. Several factors have been responsible for these efforts. The first is the problem, common in many regions, of unfavorable weather conditions at the time of harvesting. The second is the desire on the part of progressive dairymen to increase the food value of milk by the use of feeds of high nutritive value. Lastly, the extreme shortage of feeds, which was caused by the drouth of the past few years, has in some instances made it desirable to preserve any available forage and also to conserve as much of its nutritive value as possible. The purpose of this investigation was to determine the relative vitamin A content of soybean silage and of A.I.V., molasses, and common corn silage and to study the effect of feeding these silages upon the vitamin A content of milk.

REVIEW OF LITERATURE

Various substances have been added to different crops during the process of ensiling in order to insure their preservation. One of the most widely used methods is that developed by Virtanen (8-14) which is known as the A.I.V. method. In order to prevent the destruction of carbohydrates which occurs when lactic acid is formed in silage, mixtures of hydrochloric and sulphuric acids were used. The mineral acids may be replaced by lactic acid, sodium sulphate, or other compounds (sulphur trioxytetra-chloride, sulfuril oxychloride, thionyl chloride, and chlorosulphonic acid). Sufficient acid is sprinkled on the fodder to produce a pH of 3.6 to 4.0. According to Virtanen, fodder with a pH of less than 3.0 is not suitable for consumption and if the pH is above 4.0 there is a loss in feeding value. It has been said that this method checks plant-cell respiration and bacterial activity, inhibits proteolysis, preserves soluble carbohydrates, carotene, and vitamin C, and prevents losses due to unfavorable weather conditions at the time of harvesting.

Peterson and associates (4) used the A.I.V. method to preserve two lots of alfalfa and one lot of soybeans. There was no apparent loss of carotene in the silage and no unusual changes were noted in milk production. There was a definite increase in the carotene and vitamin A content of the butterfat when the A. I. V. silage was fed. However, the carotene and vitamin A values were not as high as those previously found for pasture feeding.

Hayden and associates (1) preserved a mixture of alfalfa and clover with mineral acids. The silage had a pH of 4.31 instead of 3.6 to 4.0 as recommended by Virtanen. One group of cows received the acid silage as the sole roughage while another group was fed dry chopped hay. Two other groups received corn silage at the rate of two pounds per hundredweight and were reversed between acid silage and long

alfalfa hay as the remaining roughage in periods of six weeks. The acid silage was fed *ad libitum* and the hay feeding regulated to supply the check group with about the same amount of dry matter. The vitamin A and carotene values per unit of fat produced were almost identical for the different groups at the same stage of the experiment.

Krauss and Washburn (3) reported that the milk of cows fed A.I.V. alfalfa silage produced slightly better responses when fed to rats than the milk produced by cows fed alfalfa hay.

Watson and Ferguson (15) measured the value of artificially dried grass, grass silage made with added molasses, and A.I.V. grass fodder on the yield and quantity of milk produced when these materials were fed to Shorthorn cows. Artificially dried grass, molasses silage, and A.I.V. fodder raised the carotene content of milk to a level similar to that of the average of the grazing season for the pasture-fed cows. However, the A.I.V. fodder was not as efficient as were the other two materials.

Peterson, Bird, and Beeson (5) studied the vitamin A content of A.I.V. alfalfa silage and green alfalfa. From the results obtained, they suggested that the carotene in green alfalfa was more effectively utilized than that in A. I. V. silage, since green alfalfa, when fed to rats on the same dry-weight basis as A.I.V. silage, increased the vitamin A potency of the butterfat over the A.I.V. values.

Petersen, Fitch, and Allen (6) found that the carotene content of the butterfat produced by cows declined when alfalfa hay was fed and increased when A.I.V. alfalfa silage was fed. This decline in carotene content was due to the necessary reduction in amounts of hay to approximate the dry matter in the silage.

Hegsted and Bohstedt (2) found that the milk produced by cows fed molasses alfalfa silage and A.I.V. alfalfa silage contained approximately the same carotene and vitamin A values.

PREPARATION OF SILAGES¹

Permission to prepare A.I.V. silage was obtained from the Chapman Dairy Farms, of Kansas City, Missouri, who control the licensing of patents of the A.I.V. silage in the United States.

During the second week of August, 1934, a hollow-tile upright silo was filled with 109 tons of corn silage preserved by the A.I.V. method. This fodder was short and immature and contained little or no grain. It was finely cut and sufficient water was added to allow the corn to pack properly. The chopped fodder was elevated into the silo by means of a blower and the acid solution was pumped into the silo by means of an electric pump. The acid solution was sprayed on the corn as it fell from the blower in order to get a light but even covering of the acid. Acidity tests were run with the corn fodder to determine the quantity of the mixture of hydrochloric and sulphuric acids, with a molecular ratio of 4:1, that should be added. These determinations showed that approximately 12 gallons of the acid mixture should be added per ton of fodder

¹ The silage was prepared under the supervision of Dr. P. A. Downs.

in order to give a pH of 3.5 in the fodder after treatment. The acid solution was pumped from 200-gallon galvanized iron water tanks, coated with asphalt paint. In filling this silo with 109 tons of corn fodder, 17 carboys of hydrochloric and $2\frac{1}{2}$ carboys of sulphuric acid were used. The cost amounted to 50.3 cents for the hydrochloric acid and 13 cents for the sulphuric, or 63.3 cents per ton for acid. When the silo had 103 tons of corn silage in it, the silage was sprinkled with a solution of 4 ounces of oil of mustard to 3 gallons of water. It was then covered with tar paper and on top of the tar paper were placed 6 tons of firmly packed silage. The top two feet of silage beneath the paper were given twice as much acid as was applied to the remainder. The silage showed little or no evidence of heating during settling. The silo was not opened until about the middle of the winter and there was practically no spoilage beneath the paper. The soluble acidity was determined daily for sixty days. As the silage came from the silo, it varied from 0.96 cubic centimeter of one-tenth normal sodium hydroxide to 3.82 cubic centimeters per gram, or an average of 2.86. In this work the method outlined by the Association of Official Agricultural Chemists was followed and phenolphthalein was used as an indicator.

During the third week in August, 1934, a trench silo holding 210 tons was filled with corn fodder of the same quality, but instead of having acid added, cane molasses was added at the rate of 50 pounds of molasses to a ton of corn fodder. The molasses was mixed with water, at the rate of five pounds of water to one pound of molasses, and the molasses-water was added by means of a hose which extended into the blower. This silage went through the various heating stages that ordinary silage goes through, but produced a very palatable and good quality of silage. The acidity varied from 1.71 cubic centimeters of one-tenth-normal sodium hydroxide to 2.21 cubic centimeters per gram of silage, or an average of 1.97.

During the fall of the year 1935, the filling of the silos was begun September 27 and was completed by October 3. A trench silo was filled with molasses silage, the hollow tile upright silo was filled with ordinary silage, and a second upright silo with the A.I.V. silage. The methods of preserving the silages were the same as were used during 1934.

The soybean silage was made during August, 1935, and was placed in the bottom of a stucco silo.

COLONY MANAGEMENT

The breeding colony consisted of pied male and female rats. The room had east light, tile floor, steam heat, and ventilation by means of fans. The temperature was governed by a thermostatic control during the winter, and summer heat was reduced by a water cooling system.

The breeding stock was reared on the Steenbock ration composed of the following (in percentages):

1. Yellow corn, 76.
2. Linseed oil meal, 16.
3. Crude casein, 5.
4. Ground alfalfa, 2.0.
5. Sodium chloride, 0.5.
6. Calcium carbonate, 0.5.

The material for the ration was finely ground and then 5 per cent by weight of butter was added. In addition to this ration the animals received milk daily and lettuce several times each week.

VITAMIN A DETERMINATIONS

Since vitamin A is a growth-promoting vitamin, the vitamin A determinations were made by comparing the growth produced when groups of rats whose body stores of vitamin A had been depleted by being fed a vitamin-A-free ration, were fed the silages or milks as a source of this vitamin. Pied rats reared in our laboratory were used in all the experiments. Male and female rats 22 to 25 days old and weighing between 35 and 45 grams were fed a vitamin-A-free basal ration until depleted. This ration was composed as follows:

Basal Ration I

	<i>P.ct.</i>
Casein ²	18
Starch ³	53
Yeast ⁴	15
Hydrogenated vegetable oil ⁵	10
Salt mixture ⁶	4

The antirachitic factor was supplied by irradiation of the starch and the yeast with a Cooper-Hewitt quartz mercury-vapor lamp ⁷ run at 2.4 amperes, 250 volts. One hundred grams of starch or yeast was spread uniformly over an area of 60 centimeters square in metal trays and then irradiated for 40 minutes at a distance of 60 centimeters. All experimental animals received distilled water to which iodine was added once a week in the manner suggested by Sure (7). The rats were weighed once each week except near the end of the depletion period, at which time they were weighed daily. The litters were allotted so that as far as possible related animals were distributed throughout all the groups. Each rat was placed in an individual cage at weaning, and was housed alone until the experiment was completed. A negative control which received only the basal ration, and a positive control, which received the basal ration supplemented with reference cod-liver oil ⁸ daily, were selected from each litter except when several litters were started on the vitamin A supplements at approximately the same time. The feeding of the silages and milks was begun when ophthalmia was in its incipient stage or when there was a cessation of growth. As a rule the pre-experimental or depletion period

² A special grade of purified casein (No. 453) was purchased from the Casein Company of America, New York City. It was extracted continuously for five days with 95 per cent ethyl alcohol.

³ Powdered starch was obtained from the Corn Products Refining Company, New York City.

⁴ Compressed yeast was obtained from the Fleischmann Yeast Company, New York City. It was dried with a current of air at room temperature, finely ground, and fed without further treatment.

⁵ The hydrogenated vegetable oil was "Primex," a purely vegetable hydrostearalcine, manufactured by Proctor and Gamble Co.

⁶ The salt mixture was McCollum's Salt Mixture (No. 185).

⁷ Uviarc Poultry Treater, Type R. I. special 100.

⁸ Reference cod-liver oil containing 3,000 U. S. P. units per gram was used as the source of vitamin A.

lasted for about five weeks, while the feeding trial was carried on eight weeks.

The silage samples which were used for the vitamin-A determinations were finely ground in a food chopper and stored in an electrical refrigerator at 4°C. At this time the pH of the A.I.V. silage samples was determined by the quinhydrone electrode and was found to be between pH 3.5 and 4.0. Once each week a rat's weekly allowance of silage was weighed into a bottle and this quantity was fed during the following week. The milk was bottled in half-pint milk bottles and stored at 0°C. until fed.

RESULTS

A preliminary experiment was first conducted in order to learn whether or not the rats would consume the silage in the moist condition and also to determine what quantities of the silage must be fed. In this preliminary trial the vitamin A content of the A.I.V. silage was compared to that of the molasses silage. The various silages were fed as shown in Table 3 from 0.2 to 3.2 gram levels. In this preliminary trial no apparent ill effects resulted from feeding as much as 3.2 grams of the A.I.V. silage per rat per day. The rats ate the silages readily, and it was evident that smaller levels should be fed.

In Experiment I (Table 4) the vitamin A content of the A.I.V. silage was compared to that of molasses silage. The groups of rats receiving 0.025 gram and 0.05 gram of the A.I.V. silage made an average gain per

TABLE 1.—*A comparison of the groups of cows used in the experiments.*

Experiment	Cow	Breed	Ration	Times milked daily	Age	Months fresh	Lactation periods	Bred	Milk produced	Av. fat
No.	No.			No.	Yrs., mos.	No.	No.	Date	Lbs.	P.ct.
II Milk stored March, 1935	503A	Jer.	A.I.V.	3	6-7	10.6	4	8-28-34	18.1	4.1
	265A	Hol.	silage,	3	5-8	10.1	3	1-7-35	45.6	
	59	Ayr.	hay, and	3	5-5	3.5	4	Not bred	44.7	
	160	Guer.	grain	3	5-4	1.0	2	1-14-35	15.4	
IV Milk stored July, 1935	516A	Jer.	Molasses	3	4-9	12.0	3	7-14-34	11.5	5.3
	268	Hol.	silage,	3	13-5	1.6	8	Not bred	50.7	
	74	Ayr.	hay, and	3	4-3	3.3	2	Not bred	38.3	
	156	Guer.	grain	3	5-9	4.6	3	1-22-35	12.5	
VI Milk stored Dec., 1935	283A	Hol.	A.I.V.	4	4-10	2.0	3	Not bred	55.7	3.9
	47	Ayr.	silage	3	6-11	6.7	5	3-14-35	15.3	
	516A	Jer.	and grain	3	5-2	3.0	3	Not bred	18.8	
	272A	Hol.	Hay	4	5-6	4.0	3	Not bred	26.2	
V Milk stored Dec., 1935	74	Ayr.	and	3	3-7	6.4	2	4-20-35	12.9	4.4
	515A	Jer.	grain	3	5-2	2.0	3	Not bred	21.1	
	534A	Jer.	A.I.V.	3	2-10	5.5	2	7-9-35	14.1	5.8
	526A	Jer.	silage	3	3-7	5.7	3	7-2-35	16.0	
	80	Ayr.	and grain	3	2-10	6.0	1	10-4-35	22.6	
	545A	Jer.	Molasses	3	2-11	7.0	1	7-16-35	17.2	5.2
	523A	Jer.	silage	3	5-4	6.0	3	11-8-35	13.2	
	78	Ayr.	and grain	3	3-3	5.7	1	10-15-35	21.8	
511A	Jer.	Ordinary	3	6-0	7.0	4	8-23-35	15.9	5.2	
516A	Jer.	silage	3	5-7	8.0	3	4-13-35	10.7		
81	Ayr.	and grain	3	7-0	8.0	3	4-19-35	24.4		

TABLE 2.—Average daily feed consumption per cow.

Group	Grain	Alfalfa hay	Silage		
			A.I.V.	Molasses	Ordinary
	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>	<i>Lbs.</i>
Experiment II					
Molasses silage.....	10.5	14.5	...	26.5	...
A.I.V. silage.....	12.5	12.2	29.7
Experiment IV					
A.I.V. silage.....	13.8	...	43.3
Hay	13.8	18.8
Experiment VI					
A.I.V. silage.....	7.0	...	52.7
Molasses silage.....	7.0	56.8	...
Ordinary silage.....	7.0	54.8

TABLE 3.—Summary of the average gains in weight of rats fed various daily quantities of A.I.V. silage or molasses silage as the source of vitamin A.

Source of vitamin A	Rats in a group	Av. when feeding source of vitamin A began	Av. at end of experiment	Average gain
<i>Grams</i>	<i>No.</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
Preliminary trial begun Oct. 20, 1934				
0.2A ¹	4	106	234	128
0.4A	4	102	222	120
0.8A	3	99	206	107
1.6A	3	103	216	113
3.2A	4	96	218	122
0.2M ²	4	103	216	113
0.4M	3	96	169	73
0.8M	4	92	195	103
1.6M	4	111	232	121
3.2M	4	98	229	131
Positive control ³	11	125	232	107
Negative control ⁴	8	130	87 ⁵	Died

¹ A = silage to which acids were added (A.I.V. silage).

² M = silage to which molasses was added.

³ Positive controls received 0.1 cc. of sweet-cream butter as the source of vitamin A.

⁴ Negative controls received only the vitamin-A-free diet.

⁵ Average weight at death.

rat of 17 grams and 26 grams, respectively. The groups fed 0.05 gram, 0.10 gram, and 0.15 gram of molasses silage gained 22 grams, 31 grams, and 42 grams respectively. Although the difference in vitamin-A content of these two kinds of silage was not marked, there seemed to be a slight trend in favor of the A.I.V. silage.

In Experiments II and III (Table 5) the vitamin A contents of the milks produced by cows receiving either A.I.V. silage or molasses silage

TABLE 4.—*Summary of the average gains in weight of rats fed various daily quantities of A.I.V. silage or molasses silage as the source of vitamin A.*

Source of vitamin A	Rats in a group	Av. when feeding source of vitamin A began	Av. at end of experiment	Average gain
<i>Grams</i>	<i>No.</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
Experiment I, begun June 14, 1935				
0.025A ¹	9	116	133	17
0.05A	9	111	137	26
0.05M ²	7	112	134	22
0.10M	12	113	144	31
0.15M	17	111	153	42
Positive control ³ . .	12	114	163	49
Negative control ⁴ . .	11	105	77 ⁵	Died

¹ A = silage to which acids were added (A.I.V. silage).² M = silage to which molasses was added.³ Positive controls received 2.25 units of reference cod-liver oil daily as source of vitamin A.⁴ Negative controls received only the vitamin-A-free diet.⁵ Average weight at death.TABLE 5.—*Summary of the average gains in weight of rats fed various daily quantities of milk as the source of vitamin A. This milk was produced by cows receiving alfalfa hay in addition to either A.I.V. silage or molasses silage as the source of roughage.*

Source of vitamin A	Rats in a group	Av. when feeding source of vitamin A began	Av. at end of experiment	Average gain
<i>cc.</i>	<i>No.</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
Experiment II, begun March 2, 1935				
0.50AM ¹	4	123	128	5
0.75AM	12	120	149	29
1.00AM	11	120	162	42
0.50MM ²	7	122	142	20
0.75MM	12	120	162	42
1.00MM	15	118	178	60
Positive control ³ . .	17	126	176	50
Negative control ⁴ . .	10	145	96 ⁵	Died
Experiment III, begun April 4, 1935				
0.75AM ¹	7	106	148	42
1.00AM	9	105	155	50
1.50AM	11	107	179	72
0.75MM ²	9	102	155	53
1.00MM	8	103	168	65
1.50MM	11	106	192	86
Positive control ³ . .	9	112	167	55
Negative control ⁴ . .	5	89	77 ⁵	Died

¹ AM = milk produced by cows fed A.I.V. silage.² MM = milk produced by cows fed molasses silage.³ Positive controls received 2.25 units of reference cod-liver oil daily as source of vitamin A.⁴ Negative controls received only the vitamin-A-free diet.⁵ Average weight at death.

in addition to grain mixture composed of corn (yellow) 50 per cent, bran (wheat) 32 per cent, cottonseed meal 15 per cent, salt 1 per cent, steamed bone meal 1 per cent, lime flour 1 per cent, and alfalfa hay were determined. The cows which produced the milk were from the Experiment Station herd and were selected so far as possible (Table 1) for uniformity of breed, age, stage of lactation, number of lactations, and stages of gestation. This silage was from the same lots that were used in Experiment I. The food consumption of the cows which produced this milk is shown in Table 2. No attempt was made to control the food consumption. It will be noted that the cows on molasses silage ate less grain and silage but more alfalfa hay than did the cows on the A.I.V. silage. In Experiment II the groups of rats receiving 0.50, 0.75, and 1.0 cubic centimeter of A.I.V. silage milk made an average gain per rat of 5, 20, and 42 grams respectively, while the groups fed corresponding levels of molasses-silage milk made an average gain of 20, 42, and 60 grams respectively. In Experiment III the groups of rats receiving 0.75, 1.0 and 1.5 cubic centimeters of A.I.V.-silage milk made an average gain per rat of 42, 50, and 72 grams respectively, while the groups fed corresponding levels of molasses-silage milk made an average gain of 53, 65, and 86 grams respectively. In both experiments the molasses-silage milk was superior to the A.I.V.-silage milk in vitamin A potency, although the results of Experiment I indicate no marked difference in the vitamin A content of these two lots of silage. The difference in vitamin A content of these milks was probably due to the fact that the cows in the molasses-silage group ate more alfalfa hay than did the cows in the A.I.V.-silage group.

In Experiment IV (Table 6) the vitamin A contents of the milks produced by cows receiving either A.I.V. silage or alfalfa hay as the only source of roughage were determined. As shown in Table 2 each

TABLE 6.—*Summary of the average gains in weight of rats fed various daily quantities of milk as the source of vitamin A. This milk was produced by cows receiving either A.I.V. silage or alfalfa hay as the only roughage.*

Source of vitamin A	Rats in a group	Av. when feeding source of vitamin A began	Av. at end of experiment	Average gain
<i>cc.</i>	<i>No.</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
Experiment IV, begun July 17, 1935.				
0.75SM ¹	7	117	136	19
1.00SM	8	121	143	22
0.75HM ²	11	120	146	26
1.00HM	11	123	156	33
Positive control ³ . .	9	115	174	56
Negative control ⁴ . .	6	128	114 ⁵	Died

¹ SM = milk produced by cows fed A.I.V. silage as the only roughage.

² HM = milk produced by cows fed alfalfa hay as the only roughage.

³ Positive controls received 2.25 units of reference cod-liver oil daily as source of vitamin A.

⁴ Negative controls received only the vitamin-A-free diet.

⁵ Average weight at death.

group of cows was allowed 13.8 pounds of grain per head per day. The silage and hay were fed *ad libitum*. The silage group consumed 43.3 pounds of A.I.V. silage while the hay group ate 18.8 pounds of alfalfa hay per head per day. The groups of rats receiving 0.75 and 1.0 cubic centimeter of A.I.V.-silage milk made an average gain of 19 grams and 22 grams respectively, while the groups fed corresponding levels of alfalfa hay milk made an average gain of 26 and 33 grams respectively. In other words the hay milk was superior to the A.I.V. milk in vitamin A potency.

In Experiment V (Table 7) the vitamin A contents of A.I.V., molasses, and ordinary silages were determined. These lots of silage were those that were preserved during 1935. The groups of rats fed 0.10 gram and 0.15

TABLE 7.—*Summary of the average gains in weight of rats fed various daily quantities of A.I.V., molasses, or ordinary silage as the source of vitamin A.*

Source of vitamin A	Rats in a group	Av. when feeding source of vitamin A began	Rats surviving experiment	Rats that gained weight during experiment	Av. at the end of the experiment	Average gain
Grams	No.	Grams	No.	No.	Grams	Grams
Experiment V, begun October 27, 1935						
0.10A ¹	15	118	10	10	153	35
0.15A	14	120	13	13	178	58
0.10M ²	15	108	11	11	141	33
0.15M	14	115	13	13	162	47
0.10N ³	15	112	2	None
0.15N	14	114	2	1	107	..
Positive control ⁴	12	119	12	12	164	45
Negative control ⁵	15	106	83 ⁶	Died

¹ A = silage to which acids were added (A.I.V. silage).

² M = silage to which molasses was added.

³ N = silage prepared in the usual way.

⁴ Positive controls received 2.25 units of reference cod-liver oil daily as source of vitamin A.

⁵ Negative controls received only the vitamin-A-free diet.

⁶ Average weight at death.

gram of A.I.V. silage made an average gain per rat of 35 grams and 58 grams respectively, while the groups fed corresponding levels of molasses silage made an average gain per rat of 33 grams and 47 grams respectively. On the other hand the groups fed corresponding levels of ordinary silage failed to make any growth. It was evident from this experiment that, as in Experiment I, though there was no marked difference in the vitamin A potency of the A.I.V. and molasses silage, there was a slight trend in favor of the A. I. V. silage. The ordinary silage on the other hand contained less vitamin A than did either the A.I.V. or the molasses silage.

In Experiment VI (Table 8) the vitamin A content of the milk produced by cows receiving either A.I.V., molasses, or ordinary silage as the only roughage was determined. These silages were from the same lots

TABLE 8.—*Summary of the average gains in weight of rats fed various daily quantities of milk as the source of vitamin A. This milk was produced by cows receiving A.I.V., molasses, or ordinary silage as the only source of roughage.*

Source of vitamin A	Rats in a group	Av. when feeding source of vitamin A began	Rats surviving experiment	Rats that gained weight during experiment	Av. at the end of the experiment	Average gain
cc.	No.	Grams	No.	No.	Grams	Grams
Experiment VI, begun December 24, 1935						
1.0AM ¹	13	99	11	11	155	56
1.5AM	12	97	12	12	178	81
1.0MM ² . . .	13	95	12	12	145	50
1.5MM	12	91	12	12	167	76
1.0NM ³ . . .	13	95	9	9	126	31
1.5NM	12	92	11	11	151	59
Positive control ⁴ . . .	13	88	13	13	137	49
Negative control ⁵ . . .	11	81	63 ⁶	Died

¹ AM = milk produced by cows fed A.I.V. silage.

² MM = milk produced by cows fed molasses silage.

³ NM = milk produced by cows fed ordinary silage.

⁴ Positive controls received 2.25 units of reference cod-liver oil daily as source of vitamin A.

⁵ Negative controls received only the vitamin-A-free diet.

⁶ Average weight at death.

as were used in Experiment V. The food consumption of these groups of cows is shown in Table 2. The groups of rats receiving 1.0 and 1.5 cubic centimeters of A.I.V. milk made an average gain per rat of 56 and 81 grams respectively, while the groups fed corresponding levels of molasses-silage milk gained 50 grams and 76 grams respectively. The groups fed the corresponding levels of ordinary silage milk gained 31 and 59 grams. The A.I.V.-silage milk seemed slightly more potent in vitamin A than was the molasses-silage milk, but the ordinary-silage milk was inferior to both the A.I.V. and molasses milks.

In Experiment VII (Table 9) the vitamin A contents of A.I.V., molasses, ordinary, and soybean silages were compared. These silages were preserved during 1935. The groups of rats fed 0.10 and 0.15 gram of A.I.V. silage made an average gain per rat of 67 grams and 86 grams respectively, while the groups fed corresponding levels of molasses silage gained 67 grams and 89 grams respectively. The groups of rats fed 0.15 gram, 0.30 gram, and 0.35 gram of ordinary silage made an average gain per rat of 62 grams, 95 grams and 99 grams respectively. The groups of rats fed 0.10 gram, 0.20 gram and 0.30 gram of the soybean silage made an average gain per rat of 52 grams, 84 grams and 90 grams respectively. Again there was no appreciable difference between the vitamin A content of the A.I.V. and molasses silage, and the ordinary silage was inferior to those silages as a source of this vitamin. The soybean silage contained less vitamin A than any of the other silages.

TABLE 9.—*Summary of the average gains in weight of rats fed various daily quantities of A.I.V., molasses, ordinary, or soybean silage as the source of vitamin A.*

Source of vitamin A	Rats in a group	Av. when feeding source of vitamin A began	Av. at end of experiment	Average gain
Grams	No.	Grams	Grams	Grams
Experiment VII, begun July 13, 1936				
0.10A ¹	6	118	185	67
0.15A	12	113	199	86
0.10M ²	8	110	177	67
0.15M	11	118	207	89
0.15N ³	8	105	167	62
0.30N	12	124	219	95
0.35N	10	107	206	99
0.10S ⁴	6	112	164	52
0.20S	9	112	196	84
0.30S	12	117	207	90
Positive control ⁵	8	112	147	35
Negative control ⁶ ..	7	118	108 ⁷	Died

¹ A = silage to which acids were added (A.I.V.).

² M = silage to which molasses was added.

³ N = silage prepared in the usual way.

⁴ S = soybean silage.

⁵ Positive controls received 2.25 units of reference cod-liver oil daily as source of vitamin A.

⁶ Negative controls received only the vitamin-A-free diet.

⁷ Average weight at death.

CONCLUSIONS

From these data it was evident that under the conditions of these experiments the milks produced by cows receiving A.I.V., molasses, or ordinary silage as the only roughage ranked in the same order of vitamin A potency as did the silages, namely A.I.V.-silage milk, molasses-silage milk, and ordinary-silage milk. On the other hand when a good grade of alfalfa hay served as the only source of roughage the vitamin A content of the milk produced was greater than that produced by cows receiving the A.I.V. silage as the only roughage. When both alfalfa hay and A.I.V. silage were fed as the roughage and when the amount of alfalfa hay consumed by the cows in this group was less than that consumed by the comparable group of cows on alfalfa hay and molasses silage, then the vitamin A content of the milk produced was greater in the milk of the group which consumed the greatest amount of alfalfa hay. The soybean silage contained less vitamin A than any of the other silages.

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