THE COMPARISON OF THE VITAMIN A CONTENT OF TOBACCO CURED AND ORDINARY CURED ALFALFA HAY

by

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B. A., Nebraska Wesleyan University, 1925

A THESIS

submitted in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

KANSAS STATE AGRICULTURAL COLLEGE

1928

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INTRODUCTION

The importance of the vitamins in farm feeds is being recognized in animal nutrition. Vitamin A has received special attention. Lack of this vitamin according to Hughes, Aubel, and Lienhart (1928) causes severe nervous disorders in hogs, susceptibility to sore eyes and respiratory disorders in poultry. Deficiency in Vitamin A may result in failure to reproduce, or cause weak offspring due to inability to endow young with normal amounts of the vitamin.

The only good sources of Vitamin A are yellow corn and leafy hay. Kansas has always grown more white than yellow corn because it produces better yields. According to Steenbock and Coward (1927) white corn does not contain Vitamin A. The small grains grown in western Kansas are also poor sources of Vitamin A. It was thought that yellow milo might contain A similar to yellow corn, but such was not the case.[1] This is not an exception to the rule that yellow grains contain Vitamin A, because the milo kernel is white, the color all being concentrated in the outer covering.

The only dependable source of Vitamin A in farm feeds then is the leafy hay. It has long been known that alfalfa contained a good supply of this vitamin. Steenbock et als

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1. Unpublished data, Kansas Agricultural Experiment station.

(1925) have shown that different treatment of the hay varied the vitamin content. It is very important that the curing process should retain as much of the vitamin content as possible. The dairy industry is especially interested in the effects of curing on the vitamin content of the hay. Vitamin A cannot only be stored in the animal body, but also may be transmitted in the milk. Milk as an important item in the human diet must contain an adequate supply of Vitamin A. Macy, Outhouse, Graham, and Long (1927a) state that a diet low in Vitamin A is closely associated with a child's susceptibility to infection and certain eye disorders. Chick and Roscoe (1926) found that the Vitamin A content of the milk is dependent on the diet of the cow, being a maximum when the cow is fed upon fresh green feed. From this it can be seen that the Vitamin A content of dairy feeds is very important.

The process of tobacco curing alfalfa hay is receiving considerable attention at the present time. According to Kritter (1920), dairy cows do better on that kind of hay, showing an increase from one-half to one-third in milk production. Swanson, Call, and Salmon (1919) stated that this hay is alfalfa partially wilted, and stacked while it still contains a large per cent. of moisture. The weight of the hay excludes the air and fermentation takes place. This hay

contains from thirty-eight to fifty per cent. of moisture in contrast to fresh green hay which contains seventy per cent. moisture. The tobacco cured hay comes out of storage a rich brown color, soft, pliable and palatable with all the leaves attached. So far there is no information available concerning the effect of this method of curing on the vitamin content of the alfalfa. The purpose of this experiment was to secure such information.

ACKNOWLEDGMENT

At this time I wish to express my thanks and appreciation to Doctor J. S. Hughes, Professor of Physiological Chemistry, for his valuable suggestions and assistance in carrying out this experiment. I also wish to thank other members of the Chemistry Department for help received from them.

METHODS

There are two general methods to determine the Vitamin A content of a material, known as the colormetric method and the animal feeding method. The failure to obtain uniform results and the time required to complete a biological assay has led several investigators to devise chemical tests which they believe give an indication of the Vitamin A con-

tent of the material.

Colormetric Methods

Rosenheim and Drummond (1925) put forth the following test for Vitamin A in cod liver oil: "One to two drops of cod liver oil are dissolved in five cubic centimeters of anhydrous fat solvent. One drop of sulphuric acid is added and the mixture gently shaken. A deep violet color is the positive test. The color fades or is replaced by red or brown shades." They also got a blue color with arsenic chloride, dimethyl sulphate, trichloroacetic acid and acetyl chloride, (the last two only in the presence of zinc chloride). In a later communication Drummond, Coward, and Handy (1925) state that the quantitative comparison of color tests with animal feeding tests has proven a harder task than they expected.

Carr and Price (1926) describe the following colormetric test for Vitamin A: "Two cubic centimeters of a solution of fat in chloroform is added to two cubic centimeters of a thirty per cent. solution of antimony chloride. A blue color results which varies in depth with the amount of reacting substance present. The color fades rapidly. The time of disappearance depending on the depth of the original color. Rosenheim and Drummond (1925) used arsenic chloride and found it from two to three times as sensitive as the

antimony chloride. However this test has not been proven satisfactory, as Carr and Price failed to establish a quantitative relationship between the color test and the biological assay.

Pearon (1925) found cod liver oil reacted with phosphorus pentoxide to give a blue color, he also states that pyrogallol, phloroglucinal, a napthol and substituted phenols give definite color reactions with cod liver oil in the presence of trichloroacetic acid. Rosenheim and Webster (1926) state that Pearon's pyrogallol reaction accompanies the saponifiable fraction of the oil. The unsaponifiable fraction is the one which contains the Vitamin A and is the fraction used by Rosenheim and Drummond (1925) for their arsenic chloride reaction. Up to the present time efforts to establish a quantitative relationship between the biological assay and the colorometric method have not been successful. The colorometric method yields qualitative information but not accurate quantitative information

Animal Feeding Methods

The biological assay is the best way known at the present time to determine accurate and quantitative information regarding the amount of Vitamin A present in the material to be tested. A number of investigators have formulated a Vi-

tamin A technique. The method put forth by Sherman and Munsell (1925) with modification suggested by Steenbock and Coward (1927) for the Vitamin D factor was used in the biological assay. This may be accomplished by either the protective method or the curative method.

The protective method is described by Hume and Smith (1928). Healthy, growing, young albino rats of known family and nutritional history are fed ad libitum a ration containing a definite amount of test material. The variations in growth rate gives the relative amounts of vitamin in the material. The basal ration is adequate for all nutritional needs of the rats, but devoid of Vitamin A. It consists of dextrin seventy-three per cent.; casein (alcohol extracted) eighteen per cent.; dried yeast five per cent.; and salt mixture (40) four per cent. Salt mixture (40) is described by Steenbock and Nelson (1923). The salt mixture contains:

Na cl.	23.3 parts
$K_2 HPO_4$	69.6 parts
$Ca_2H_2(PO_4)_2\ 4H_2O$	68.8 parts
$Na_2\ H\ PO_4\ 12H_2O$	35.8 parts
$MgSO_4\ 7H_2O$	24.6 parts
Ca Lactate	15.4 parts
Ferric Citrate	5.98 parts
K I	.16 parts

The dextrin furnishes the carbohydrates necessary for energy production. The casein the protein for body building and repair. This casein was rendered free of Vitamin A by the method of Sherman and Munsell (1925) by extraction with ninety-eight per cent. alcohol. The yeast furnished Vitamin B. This vitamin according to Sherman and Axtmayer (1927) furnishes two factors, an antineuritic factor, and a growth promoting factor. Macy et al. (1927b) stated that the rat can neither store or synthize much Vitamin B hence the need of a good supply in the basal ration. The yeast was not placed in the basal ration until after irradiation as Hogan and Hunter (1928) found irradiation destroyed the vitamin. The four per cent. salt mixture furnished the proper mineral balance. The Vitamin D necessary for proper utilization of calcium and phosphorus was supplied by irradiation of the ration by a quartz mercury vapor lamp. The rat does not require the antiscorbutic factor Vitamin C. The test material was always added to the basal ration after irradiation, as Titus et al. (1926) found that the action of the ultraviolet light destroyed the Vitamin A.

The curative method was carried out as described by Supplee and Dow (1927), and Dutcher and Honeywell (1927). The rats, similar to those used for the preventative method are fed the same basal ration, the only difference being that

no test material was included as a source of Vitamin A. When the vitamin store in the rat's body was depleted they began to show typical signs of avitaminosis A, (decrease in weight, loss of hair, respiratory trouble or sore eyes). The time required to deplete the store of Vitamin A according to Sherman and Stormes (1925) depends on the age and the Vitamin A content of its food previous to the time of the experiment. At the onset of avitaminosis A they were given a predetermined graduated allowance of the material to be tested as a source of Vitamin A.

Rose and Smith (1928) in the discussion of their results on evaluating Vitamin A by the growth of rats recommend that the best method consists in giving a range of doses of the substance to be tested from the start of the experiment. Macy et al. (1927c) mentions that a larger dose of Vitamin A is required after a depletion period than when the vitamin is given from the start. Sherman and Hessler (1927) state that the amount of food which produces a gain in weight of three grams a week contains one unit of Vitamin A. The protective method gives a quantitative comparison of test materials, but does not furnish any definite conclusion as to the amount of test material consumed in a given time. The curative method furnished a means of determining the level at which the test material must be fed to furnish adequate growth. The concentration of this vitamin is judged to be inversely pro-

portional to the amount of material which is fed, if the test animal and all conditions of the test are quantitatively standardized and controlled.

Special Methods

The alfalfa used in this experiment was obtained from the Dairy Department of the Kansas State Agricultural College. The samples of green and tobacco cured hay were prepared by stripping the leaves from the stems of a large quantity of dry hay and grinding them to a fine powder. The feed for the animals on the protective diet was prepared by mixing twenty grams of alfalfa with nine hundred and eighty grams of basal ration. In this manner the two types of hay were fed as two per cent. of the total feed given. As a positive check against the alfalfa another ration was prepared containing five per cent. cod liver oil and ninety-five per cent. basal ration. A control ration consisted of basal ration and no addition to supply the Vitamin A. The alfalfa was fed to the curative group in three levels; one-half, one-fourth, and one-eighth grams per day. As the alfalfa was in the form of a very fine powder, it was necessary to furnish the curative dose in some form which could not be easily lost, and which could be easily administered; this was accomplished by feeding the alfalfa in the form of pellets.

Fifty grams of alfalfa and fifty grams of basal ration were weighed into the same container and thoroughly mixed. The amount of this alfalfa mixture that would supply a certain level for a definite number of days was weighed out and made into pellets with glycerine as a binding agent. For example twenty grams of alfalfa mixture would make enough pellets to supply one animal for twenty days on a one-half gram level, or enough for one animal for forty days on a one-fourth gram level. The same animal received all the pellets from a single weighing so that it was not necessary to have the pellets absolutely uniform in size. If an animal refused to eat the pellets, the basal ration would be removed for a time; however if it still refused to eat them after a reasonable time, the feed was returned so that loss in weight was due to vitamin deficiency not lack of food.

EXPERIMENTAL

The comparison of the Vitamin A content of the tobacco cured and green cured alfalfa was determined entirely by the growth of albino rats. These rats were from five to six weeks old and were of known nutritional history. Each rat was weighed, ear marked, and given a number at the beginning of the experiment. Every animal was weighed on the same day and approximately the same hour once a week. The sexes were segregated during most of the experiment. The animals were kept in steel wire cages fourteen inches square and ten inches deep. The cages had raised wire floors to prevent excess to excreta. Each cage stood in a shallow galvanized tray over a pad of absorbent paper. A similar tray served as a lid to the cage. Glazed earthen ware dishes were used for water, and pint fruit jars fitted with galvanized wire cones served as feeders. The cages, feeders, trays, and water dishes were sterilized once a week and the animals were always kept in a sanitary condition. Water and food were supplied constantly.

The numbering system used through this experiment designates the number of the animals in a certain lot, as rat number 109 is the number ten rat in the ninth lot; rat 51 is the fifth rat in the first lot. The rats on the protec-

tive experiment were divided into lots according to rations, while those on the curative were divided in lots according to litters. In order to minimize individual differences the rats in a single lot were well distributed through out the various levels. As far as possible each animal placed on the green alfalfa had a litter mate of similar characteristics placed on the tobacco cured hay on the same level.

Experiment I

In this experiment the object was to compare the rate of growth in animals receiving various protective rations. Table I describes the rations and number of animals on each.

Table I

Distribution of Animals on Protective Rations

	Kind of Ration	No. of animals
I	Basal Ration - 2% tobacco cured alfalfa	9
II	Basal Ration - 2% green cured alfalfa	9
III	Basal Ration - 5% Cod Liver Oil	5
IV	Basal Ration	8

Two animals on ration I and one on ration IV died of typical Vitamin A deficiency before the end of the experiment. One animal on ration II died from an unknown cause, another animal on ration II failed to grow from the first of the experiment and was not considered in any calculations. This left

seven animals on rations I, II, and IV, and five on ration III which completed the ten weeks experiment. The results of this experiment are given in Tables II and III, inclusive. Figure 1 gives the growth curves for the various rations. These were computed from the average of the weekly gains of each animal on a certain specified ration.

The animals grew normally on the green alfalfa, but those on the tobacco cured on the same level did not do any better than those on the basal ration. The graph shows clearly the superiority of the green hay over the tobacco cured when fed at a level of two percent as a source of Vitamin A. The total average gain in weight for a period of ten weeks of the animals on green alfalfa was 87 grams, and of those one the tobacco cured was 74 grams. Table II summarized the results on the protective experiment.

Table II

Comparison of Protective Rations

Type of Ration	No. of animals		Average gain		Ration Average
	♂	♀	♂	♀	
Basal Ration - 2% tobacco cured alfalfa	3	4	83	66	74
Basal Ration - 2% green alfalfa	3	4	96	79	87
Basal Ration - 5% cod liver oil	1	4	80	86	87
Basal Ration	2	5	104	68	77

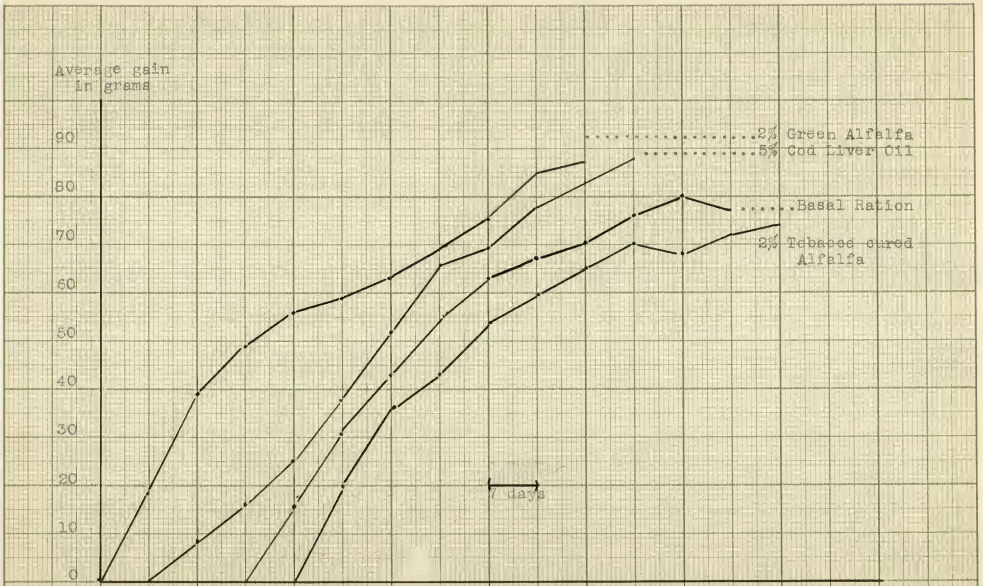

Figure 1
The Comparison of Various Protective Rations

Table III

Individual Results for Protective Experiment

Basal Ration - 2% tobacco cured alfalfa					
Number	Sex ♂	Sex ♀	Weight in Grams: Original	Weight in Grams: Final	Gain in Weight
11		♀	70 g.	108 g.	38 g.
21	♂		80 g.	160 g.	80 g.
41		♀	68 g.	142 g.	74 g.
15	♂		66 g.	147 g.	81 g.
25		♀	64 g.	139 g.	75 g.
35	♂		56 g.	146 g.	90 g.
45		♀	59 g.	138 g.	79 g.
Average Gain in 10 weeks					74 g.

Basal Ration - 2% green cured alfalfa					
Number	Sex ♂	Sex ♀	Weight in Grams: Original	Weight in Grams: Final	Gain in Weight
12	♂		110 g.	157 g.	47 g.
32		♀	76 g.	138 g.	62 g.
42	♂		72 g.	200 g.	128 g.
16	♂		57 g.	171 g.	114 g.
26		♀	55 g.	142 g.	87 g.
36		♀	50 g.	140 g.	90 g.
46		♀	48 g.	128 g.	80 g.
Average Gain in 10 weeks					87 g.

Table III (continued)

Basal Ration + 5% cod liver oil					
Number	Sex ♂	Sex ♀	Weight in grams Original	Weight in grams Final	Gain in Weight
13	♂		112 g.	192 g.	80 g.
23		♀	70 g.	169 g.	99 g.
33		♀	63 g.	153 g.	90 g.
43		♀	58 g.	145 g.	87 g.
53		♀	62 g.	152 g.	90 g.
Average Gain in 10 Weeks					87 g.

Basal Ration					
Number	Sex ♂	Sex ♀	Weight in grams Original	Weight in grams Final	Gain in Weight
24		♀	42 g.	139 g.	47 g.
34	♂		64 g.	150 g.	86 g.
44		♀	70 g.	145 g.	75 g.
17	♂		65 g.	178 g.	113 g.
27		♀	61 g.	145 g.	84 g.
37		♀	70 g.	136 g.	65 g.
47		♀	56 g.	127 g.	71 g.
Average Gain in 10 Weeks					78 g.

Experiment 2

This experiment was to determine the amount of green and tobacco cured alfalfa required to furnish an adequate supply of Vitamin A. Animals similar to those in Experiment 1, were kept in wire cages on Ration IV until they began to show definite signs of avitaminosis A, then they were placed in individual cages, and a definite level of alfalfa given to restore normal health and growth. The following table gives the distribution on the various levels of the two kinds of alfalfa.

Table IV

Distribution of Animals on Curative Rations

Grams per day	Green cured Alfalfa		Tobacco cured alfalfa	
	♂	♀	♂	♀
.5 grams	5	5	6	5
.25 "	5	5	4	7
.125 "	4	3	5	3

The animals on the curative diet were weighed every other day and the weights recorded. The individual records for the forty day period are given in Tables V, VI, VII, inclusive. A record was made of the number of pillets eaten by each animal and from that number and the average gain in weight, the minimum level for each kind of alfalfa was cal-

culated.

Table V

Individual Results of Curative Diet at ½ Gram Level

Number	Sex	Pellets Eaten	Survival in days	Grams of Alfalfa Eaten	Total Gain
Green alfalfa .5 g. per day					
11	♂	40	40	20 g.	-3 g.
187	♀	40	40	20 g.	+10 g.
157	♂	40	40	20 g.	0 g.
68	♂	40	40	20 g.	+13 g.
27	♂	40	40	20 g.	+39 g.
47	♂	40	40	20 g.	+39 g.
611	♀	40	40	20 g.	+35 g.
49	♀	40	40	20 g.	+25 g.
610	♀	40	40	20 g.	+18 g.
69	♀	40	40	20 g.	+12 g.
Average		40	40	20 g.	15.9 g.

Number	Sex	Pellets Eaten	Survival in days	Grams of Alfalfa Eaten	Total Gain
Tobacco cured alfalfa .5 g. per day					
41	♂	40	40	20	-13g.
117	♀	27	28	13½	- 7g.
137	♂	39	40	19½	-23g.
28	♂	3	18	1½	-34g.
17	♀	40	40	20	- 4g.
37	♂	40	40	20	- 3g.
210	♂	40	40	20	-28g.
411	♀	40	40	20	+17g.
59	♀	39	40	18½	+ 5g.
1110	♀	40	40	20	-11g.
1310	♀	35	35	16½	-46g.
Average		34.63	36.6	17⅓	-1.94

Table VI

Individual Results of Curative Diet at ½ Gram Level

Number	Sex	Pellets Eaten	Survived in days	Grams of Alfalfa Eaten	Total Gain
Green Alfalfa .25 g. per day					
167	♂	13	15	3½	-14 g.
97	♀	40	40	10	+70 g.
57	♀	40	40	10	- 2 g.
38	♂	40	40	10	-26 g.
810	♀	40	40	10	+30 g.
211	♀	40	40	10	+ 6 g.
99	♂	40	40	10	+52 g.
109	♂	40	40	10	+ 8 g.
110	♂	40	40	10	+32 g.
710	♀	40	40	10	+ 2 g.
Average		37.3	37.5	9 1/3	11.85
Tobacco cured alfalfa .25 g. per day					
107	♀	33	33	8¼	-21 g.
67	♀	15	19	2¾	-23 g.
177	♀	23	23	5¾	-40 g.
58	♂	40	40	10	-30 g.
1111	♀	40	40	10	+ 7 g.
44	♂	40	40	10	+10 g.
811	♂	40	40	10	+22 g.
89	♂	33	34	8¼	+18 g.
79	♂	37	40	10	+ 8 g.
410	♂	40	40	10	-27 g.
310	♂	40	40	10	-13 g.
Average		34.6	35.3	8 2/3	-3.15

Table VII

Individual Results of Curative Diet at 1/8 Gram Level

Green alfalfa .125 g. per day					
Number	Sex	Pellets Eaten	Survival in days	Grams of Alfalfa Eaten	Total Gain
24	♀	18	18	2¼ g.	-34 g.
127	♀	40	40	5 g.	- 3 g.
34	♀	24	24	3 g.	-27 g.
18	♀	40	40	5 g.	+31 g.
910	♀	40	40	5 g.	+93 g.
111	♀	40	40	5 g.	0 g.
311	♀	40	40	5 g.	+59 g.
Average		37.5	37.5	4.32 g.	19.6 g.

Tobacco cured alfalfa .125 g. per day					
Number	Sex	Pellets Eaten	Survival in days	Grams of Alfalfa Eaten	Total Gain
87	♀	14	14	1¾ g.	-39 g.
147	♀	15	15	1 7/8 g.	-39 g.
21	♀	40	40	5 g.	-12 g.
48	♀	24	24	3 g.	-15 g.
510	♀	40	40	5 g.	+ 8 g.
1010	♀	33	34	4 1/8 g.	-33 g.
511	♀	40	40	5 g.	-20 g.
711	♀	40	40	5 g.	+18 g.
Average		30.7	30.7	3.8 g.	-5.92g.

The gain during each five day period of all rats on a particular level was averaged and the results are given in figures 2, 3, and 4. The average gain in weight was calculated from the averages for each five day interval.

The results of this experiment indicate that five grams of green alfalfa are sufficient to produce normal growth during a forty day test period. Twenty grams of this sample of tobacco cured alfalfa does not appear to have enough Vitamin A to cause normal growth during a similar period. Table VIII gives a summary of results on Experiment 2. Figures 2, 3, and 4 show plainly the effect of each kind of hay at various levels on the growth of animals.

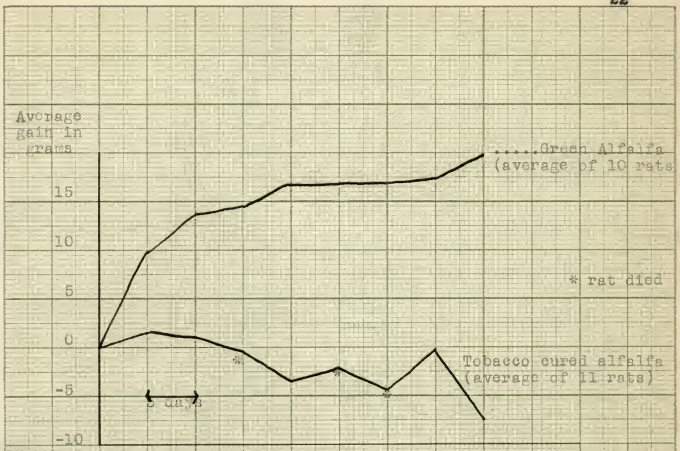

Figure 2
Effect of Curative Diet at $\frac{1}{2}$ Gram level

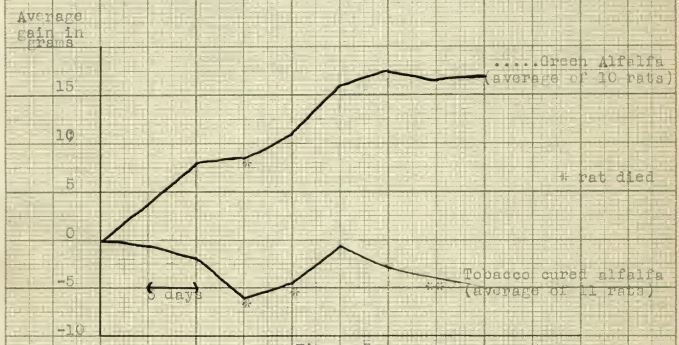

Figure 3
Effect of Curative Diet at $\frac{1}{4}$ Gram level

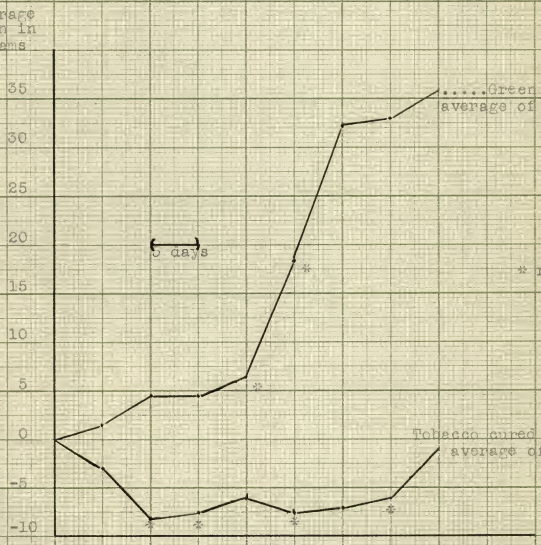

Figure 4

Effect of Curative Diet at 1/8 Gram Level

Table VIII

Quantitative Measurements of Vitamin A Values

Material Tested	Amount Fed Daily	Number of Animals	Average Gain in Experimental Period	Remarks
Green Alfalfa	.5 g.	10	15.9	All animals survived test period.
Tobacco Cured Alfalfa	.5 g.	11	-1.94	Seven animals survived test period.
Green Alfalfa	.25 g.	10	11.85	Nine animals survived test period.
Tobacco Cured Alfalfa	.25 g.	11	-3.15	Seven animals survived test period.
Green Alfalfa	.125 g.	7	19.6	Five animals survived test period.
Tobacco Cured Alfalfa	.125 g.	8	-5.92	Four animals survived test period.

DISCUSSION

The condition of the animal when the pellets were first administered had a great deal to do with gain or loss in weight. Certain individuals were showing bad signs of avitaminosis A when put on the curative diet, in some cases these animals never did show any increase in weight, but with one exception the animals receiving Vitamin A from the green alfalfa showed increased activity and improved eyes. For example, rat number 187 had a very bad case of sore eyes and respiratory trouble when put on .25 g. of green alfalfa. At the end of the test period the eyes were cured and the respiratory trouble was gone, but there was no gain in weight. Number 97 showed a definite increase in weight, but still had the respiratory trouble at the end of the experiment. The curative method does not seem entirely satisfactory as the effects of avitaminosis A are so lasting and severe that a larger dose is required to restore normal growth, than is required to sustain it on the protective method.

There were only three animals which failed to survive the test period on green alfalfa. Number 167 was too deficient in Vitamin A when placed on green alfalfa to ever recover. Rats number 24 and number 34 were negative controls

for experiment number 1. They were placed on one-eighth of a gram of green alfalfa, but they had evidently gone too long because they never lived to finish the experiment. Rat number 11 was formerly on Ration 1, Experiment 1, upon showing signs of avitaminosis A, it was placed on .5 grams of green alfalfa per day. Number 41 from the same lot was placed on .5 grams of tobacco cured alfalfa. Neither of these animals gained in weight, but the rat on the tobacco cured alfalfa lost ten grams more in forty days than the rat on the green alfalfa.

The green alfalfa seemed to furnish enough Vitamin A when fed at one-eighth of a gram a day to rats not badly deficient in Vitamin A. This is evidently the minimum dose to procure normal growth as the higher levels of green alfalfa did not produce as much growth as the one-eighth of a gram.

Most of the animals receiving tobacco cured hay decreased in weight. In a very few cases was any increase in weight noticed. If the animal was at the very initial stage of avitaminosis A the tobacco cured hay seemed to furnish enough Vitamin A to continue the growth for the forty day test period. The tobacco cured hay however when fed in a protective ration failed to sustain the growth during the entire experiment. Several individuals on rations 1 and 2 of the protective experiment were held to these rations for

twenty weeks. The tendency was for the animals on the two per cent. tobacco cured ration to develop severe signs of avitaminosis A. The tobacco cured hay was increased to five per cent. of the ration with no better result. The males and females on the two per cent. tobacco cured alfalfa ration were kept together for eight weeks. There was no reproduction, giving another indication that the ration was deficient in Vitamin A.

The loss in weight of animals on the tobacco cured curative diet was inversely proportional to the size of the dose. This would seem to indicate that the tobacco cured hay contained some Vitamin A. It appears, however, that the levels at which the tobacco cured hay was fed were too low. The minimum dose of tobacco cured alfalfa is evidently more than one-half of a gram per day. In order to secure definite information in regard to average loss of Vitamin A by the process of tobacco curing it would be necessary to study more samples and to feed hay at different levels. The results of this experiment indicate clearly that tobacco cured hay is inferior to ordinary alfalfa as a source of Vitamin A.

SUMMARY

The results indicate that tobacco cured alfalfa is not an adequate source of Vitamin A, when fed as two per cent. of the ration.

The green alfalfa furnished enough Vitamin A, when fed at the one-eighth of a gram level.

The tobacco cured alfalfa furnished an inadequate supply of Vitamin A, when fed at the one-half of a gram level.

A larger amount of green alfalfa is required to produce normal growth in animals which have had their supply of Vitamin A depleted, than in those which have received a definite amount from the first of the experiment.

The tobacco cured hay caused a decrease in weight inversely proportional to the amount fed. This would indicate that this sample of tobacco cured alfalfa contained a small amount of Vitamin A.

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