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SUMMARY

A comparative study of the vitamin A content of field-cured and artificially cured alfalfa was made by comparing the growth produced by 124 rats receiving alfalfa hay as their source of vitamin A. From the results of this study, in which the experiments were duplicated, it is concluded that under the conditions of these experiments the artificially cured alfalfa was twice as potent in vitamin A as was the field-cured alfalfa.

The comparative study of the vitamin E content of these hays was made by comparing the number of litters produced by groups of female rats which received graded quantities of either field-cured or artificially cured alfalfa as their source of vitamin E. It is concluded that the artificial drying of the alfalfa tended to preserve its vitamin E content to a greater degree than did field curing.

The Vitamin A and the Vitamin E Content of Field-Cured and Artificially Cured Alfalfa Hay

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The high nutritive value of alfalfa has been one of the factors responsible for its extensive use in the rations of domestic farm animals. It was pointed out by Maynard (22), in a review of the literature in 1929, and later confirmed by still others (32), that in general the vitamin content of milk is dependent upon the vitamin content of the ration of the cow. Bethke, Kennard, and Sassaman (2) reported a similar relationship between the vitamin A and vitamin D content of the ration and the occurrence of these vitamins in egg yolks. As milk and eggs are possibly the most important, year-round sources of vitamins in the human diet and as these foods are of the utmost importance in infant and child feeding, any agricultural practice which may affect the nutritive value of the rations of the animals which produce these foods is, therefore, of interest. With the development of artificial-heat drying of hay arose the question of the effect of the curing process upon the nutritive value of the hay produced. It is well known that some vitamins are heat-stable, while others are heat-labile, and also that artificial drying uniformly results in the production of hay having a choice green color. A number of investigators have noted an association of greenness in plant tissues with vitamin A content (4, 5, 6, 8, 10, 15, 16, 18, 24, 26, 35). Hague and Aitkenhead (14), however, reported that while bright green color is associated with quality in field-cured alfalfa, green color is not necessarily an index of vitamin A preservation since a sample of hay was turned from a bright green to an olive-drab color by autoclaving, without apparent destruction of the vitamin. In view of these facts it was thought advisable to make a study of the comparative effect of artificial and field curing upon the vitamin content of alfalfa hay.

REVIEW OF LITERATURE

The results obtained by a number of investigators indicate that the nutritive value of hay may be influenced by the cur-

[1] This is a report of a co-operative experiment between the Bureau of Dairy Industry, United States Department of Agriculture, and the Dairy Husbandry Department of the Nebraska Agricultural Experiment Station.

ing process. Dutcher (9) noted that practical feeders have observed that bright green alfalfa, properly dried and cured, is superior to the light-colored crops, bleached out by unsatisfactory climatic conditions or careless agricultural practice. Steenbock (31) and co-workers in studies designed to test the stability of vitamin A reported that alfalfa hay, cured under a skylight in an attic and allowed to remain there for many months until completely bleached, no longer possessed any growth-promoting properties for rats. Bethke and Kick (3) exposed alfalfa hay to the sun, rain, and dew for several days and reported a marked loss in its vitamin A content. Russell (27) studied the effect of artificial drying upon the vitamin A content of leaves from alfalfa plants, and from the results obtained concluded that the green leaves of the artificially cured plants contained seven times as much vitamin A as the brownish-green leaves from the field-cured plants. Woodman, Bee, and Griffith (36) made two inquiries into the influence of artificial drying on the digestibility of young leafy pasturage. It was shown that the digestibility of young grass is not reduced when it is dried at the temperature of steam or by direct heat in a kiln. Bechdel (1) fed mechanically dried, mixed timothy and clover hay to grade Holstein heifers and stated that "the explanation would appear to be that the high heat employed in the presence of air in this particular type of drier, which is no longer on the market, destroys vitamin A or a similar dietary factor necessary for growth and well-being of young cattle." The influence of the method of curing Kudzu hay upon its vitamin A content was investigated by Sewell and Cottier (28). Samples were field-cured, cock-cured, or shade-cured. These writers stated that although the samples were fed at levels too low to constitute a Sherman unit, the results indicated that in vitamin A potency shade-cured, cock-cured, and sun-cured hays rank in the order named. Kiesselbach and Anderson (17) reported briefly the results of experiments by Mussehl in which two lots of chicks were fed artificially cured and field-cured alfalfa hay, respectively. They reported that differences in growth due to feeding the alfalfa cured under these two extreme conditions were not significant. The studies of Hague and Aitkenhead (14), unlike those of Russell, were made with the alfalfa stems and leaves ground together. The results showed that the artificial drying tended to preserve the vitamin A content while the field-curing process caused a market diminution in the vitamin A content. They also presented evidence which indicated that enzymes are the important factor in the destruction of vitamin A during the field-curing process.

EXPERIMENTAL PROCEDURE

The samples of alfalfa used in these experiments were obtained from Brook-Hill Farm, Genesee Depot, Wisconsin.[2] The hay was cut on July 15 at 9 a. m. The day was clear and dry. At 3 p. m. when the plants were wilted, but not dry, they were raked and at 5 p. m. put up in small cocks. On July 22 the hay was hauled in and stored. On August 4 it was chopped, blown into bags, and stored in a dry warehouse. All possible precautions were taken to keep the loss of leaves at a minimum. This hay was undoubtedly superior in all respects and had much less loss of leaves than ordinary field-cured hay. The sample which was artificially dried was obtained from the same field and cutting. It was hauled to the drier as soon after cutting as possible. The drying was done in an Arnold drier and required less than 15 minutes. In other words, the field-cured hay was cured in cocks in the field for eight days before being stored, while the artificially dried hay was dried and stored within an hour after cutting. It was not possible to determine either the maximum temperature to which the hay was subjected in the drying machine or the temperatures in the different parts of the drier, since it was constantly revolving while in operation. The color of the artificially dried sample was a dark green, while that of the field-cured sample was an olive green. The results of the fodder analyses are shown in Table 1.

TABLE 1.—*The comparative composition of the field-cured and artificially cured alfalfa* [1]

Method of drying	Moisture	Ash	Nitrogen	Crude protein	Fat	Nitrogen free extract	Crude fiber
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Field	10.0	6.9	2.65	16.6	2.2	38.2	26.1
Artificial	9.5	7.2	2.79	17.4	2.8	37.3	25.8

[1] These samples were analyzed by Mr. C. B. Parker, U. S. D. A., Bureau of Dairy Industry.

VITAMIN A STUDIES

The management of the rat colony and the procedure for the determination of vitamin A was essentially the same as that outlined by Davis and Hathaway (7) except where otherwise indicated. The breeding stock was maintained on the Steenbock Stock Ration, composed as follows:

[2] Brook-Hill Farm was owned by Mr. Howard Green. The writers are indebted to Mr. Green and his men for their kind assistance in obtaining these samples.

Stock Ration I

Yellow corn	76.0%	Ground alfalfa	2.0%
Linseed oil meal	16.0%	Sodium chloride	0.5%
Crude casein	5.0%	Calcium carbonate	0.5%

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

Pied male and female rats, 24 to 28 days old and weighing between 35 and 45 grams, were fed a vitamin A free basal ration composed of casein,[3] 18 per cent; corn starch,[4] 78 per cent; and salt mixture (McCollum No. 185) (23), 4 per cent. One gram of dried yeast [5] daily was fed separately as the source of vitamin B and vitamin G. The antirachitic factor was supplied by irradiation of the corn starch and the yeast with a Cooper-Hewitt quartz mercury vapor lamp [6] run at 2.4 amperes, 250 volts. One hundred grams of starch or yeast was spread uniformly over an area 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 each week, according to the method of Sure (33). The rats were weighed three times per week and a record of the amount of food consumed was kept. The litters were allotted so that, as far as possible, related animals were in each group of the experiment. A negative control, receiving only the basal ration, and a positive control, which received the basal ration supplemented with 0.1 cubic centimeter of cod-liver oil [7] daily, were selected from each litter except when several litters were started on the alfalfa supplements at approximately the same time. The feeding of the alfalfa was begun when ophthalmia was in its incipient stage or when there was a cessation of growth. At this point various percentages of the finely ground alfalfa were incorporated into the basal ration by replacing a portion of the corn starch with an equal quantity of alfalfa. The plan adopted was similar to the method for the quantitative determination of vitamin A as recommended by Sherman and Burtis (29). A unit was to be the minimum quantity of alfalfa required to cure induced symptoms of vitamin A starvation in young rats and to cause an average gain in

[3] 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.

[4] Powdered starch was obtained from the Corn Products Refining Company, New York City.

[5] Compressed yeast was furnished through the courtesy of the Fleischmann Yeast Company. It was dried in a current of air at room temperature, finely ground, and fed without further treatment.

[6] Uviarc Poultry Treater, Type R. I. Special 100.

[7] Standardized cod-liver oil from Mead Johnson & Company, Evansville, Ind., was used.

weight of three grams per week. The vitamin A potencies could then be determined by a comparison of the unit of each type of alfalfa. The field-cured hay was fed at slightly lower levels than the artificially cured hay, since the results of other investigators as well as the difference in color of the two kinds of hay suggested a difference in vitamin A content. The pre-experimental or depletion period generally lasted for about five weeks, while the feeding trial was carried on eight weeks.

The results of two experiments are summarized in Table 1 and are statistically examined in Table 2. In the first experiment each kind of alfalfa was fed in three different amounts. Male and female rats were used in Experiment I, while only male rats were used in Experiment II. The field-cured alfalfa was fed at 1, 3, and 5 per cent levels, while the artificially dried sample was fed at 0.5, 2, and 4 per cent levels. The groups fed 3 per cent and 5 per cent of field-cured hay made about equal gains. The groups fed 2 per cent and 4 per cent of artificially cured alfalfa also made similar gains. It was therefore evident that these groups received so much alfalfa that an amount of vitamin A in excess of unit requirements was supplied and hence no differences in growth became apparent at these levels. One per cent of field-cured alfalfa produced an average gain of 63.4 ± 3.14 grams, while only one-half that amount of artificially cured hay or 0.5 per cent gave an average gain of 76.0 ± 4.64 grams. When this difference of 12.6 grams was divided by its probable error, a value of 2.25, or approximate odds of 6.26 to 1 were obtained. Since only differences of at least 3.2 times the probable error, or odds of 30 to 1, are considered significant, it is evident that no difference between the vitmin A content of one per cent of field-cured and 0.5 per cent of artificially cured alfalfa was established. In other words, the results indicate that under the conditions of these experiments the artificially cured alfalfa was twice as potent in vitamin A as was the field-cured. As it was shown by Sherman and Burtis (29) that this method for the quantitative determination of vitamin A is most delicate when the rats make an average gain of 3 grams per week and since even the smallest gains made by our rats were much in excess of this amount, a second experiment was conducted in which smaller amounts of the alfalfa were fed. In this trial 0.5 per cent of field-cured alfalfa was compared with 0.25 per cent of artificially cured alfalfa. The group fed 0.5 per cent of field-cured hay made an average gain of 23.9 ± 1.1 grams, while the group fed the 0.25 per cent of artificially cured alfalfa made an average gain of 21.6 ± 1.78 grams. This difference of 2.3 grams divided by its probable error equals 1.08, or approximately odds of 1 to 1. It is evident

TABLE 2.—*Summary of the average gains in weight of rats fed various percentages of alfalfa hay, field-cured, or artificially cured, as the sources of vitamin A*

Quantity of alfalfa fed		Rats in group	Av. when feeding of alfalfa began	Av. at end of experiment	Average gain
Field-cured	Artificially cured				
P. ct.	*P. ct.*	*No.*	*Grams*	*Grams*	*Grams*
EXPERIMENT NO. I					
1.0	....	11	93.8	157.2	63.4
3.0	....	11	95.4	187.6	92.2
5.0	....	11	89.2	180.1	90.9
....	0.5	11	93.2	169.2	76.0
....	2.0	11	93.2	178.1	84.9
....	4.0	11	89.3	176.7	87.4
Positive control [1]		8	94.5	176.1	81.6
Negative control [1]		6	99.1	80.8 [2]	Died
EXPERIMENT NO. II					
0.5	....	19	106.6	130.5	23.9
....	0.25	14	104.3	125.9	21.6
Positive control		6	110.3	213.5	103.2
Negative control		4	99.5	76.7 [2]	Died

[1] Positive controls received 0.1 c.c. of cod-liver oil daily as the source of vitamin A, while the negative controls received only the vitamin A free diet.
[2] Average weight at death.

that the difference was not statistically significant and that the conclusion drawn from Experiment I was confirmed.

VITAMIN E STUDIES

It has been shown by Evans and Bishop (11), Sure (34), Mattill, Carman, and Clayton (20) and confirmed by many investigators that rats are unable to reproduce if maintained on a diet deficient in vitamin E. We have been unable to find any comparative studies of the vitamin E content of field-cured and artificially cured alfalfa. The individual variation of rats in response to vitamin E has made comparative studies of this vitamin rather unsatisfactory. Such variation has been noted by Evans and Burr (12), Mattill and Clayton (21), and Simmonds, Becker, and McCollum (30), as well as by many others. From the nature of assay results the methods for the study of vitamin E are essentially qualitative rather than quantitative. Munch (25) stated that the quantitative accuracy of the method appeared to be about 50 per cent. In other words, the effect of doses differing by 50 per cent may be determined. Despite the limitation of the method it was thought advisable to attempt this study.

TABLE 3.—*Average gains in weight of rats produced by alfalfa hay, field-cured or artificially cured, as the source of vitamin A—statistical examination of results* [1]

Quantity of alfalfa fed		Rats in group	Mean gain per animal and P. E. of the mean		Difference of means and P. E. of difference	Difference / P. E. of D.	Approximate odds
Field-cured	Artificially cured		Field-cured	Artificially cured			
P. ct.	*P. ct.*	*Number*	*Grams*	*Grams*	*Grams*		
EXPERIMENT NO. I							
1.0	0.5	11	63.4 ± 3.14	76.0 ± 4.64	12.6 ± 5.60	2.25	6.26-1
3.0	2.0	11	92.2 ± 3.21	84.9 ± 4.44	7.3 ± 5.47	1.33	1.63-1
5.0	4.0	11	90.9 ± 3.65	87.4 ± 4.20	3.5 ± 5.56	0.63	less than 1-1
EXPERIMENT NO. II							
0.5	0.25	19-14 [2]	23.9 ± 1.1	21.6 ± 1.78	2.3 ± 2.09	1.08	1-1

[1] The following formulae were used in these calculations:

(1) $M = \frac{\Sigma x}{n}$

M=arithmetical average. Σx=sum of the variates. n=total variates.

(2) S. D. $= \sqrt{\frac{\Sigma (Fd^2)}{n}}$

S. D. = Standard deviation.
F = frequency.
d = deviation of a class from the mean.
n = number of individuals.

(3) P. E. of the mean $= \frac{.6745 \text{ S. D.}}{\sqrt{n}}$

P. E. = probable error.

(4) P. E. of a difference $= \sqrt{a^2 + b^2}$

a = P. E. of first mean.
b = P. E. of second mean.

[2] There were 19 rats in the group receiving field-cured alfalfa and 14 rats in the group receiving artificially cured alfalfa.

The comparative study of the vitamin E content of the field-cured and the artificially cured alfalfa was made by comparing the number of litters produced by groups of female rats which received graded quantities of either field-cured or artificially cured alfalfa as their source of vitamin E. Female pied rats, 24 to 28 days old, were placed on a vitamin E deficient ration essentially the same as that employed by Evans and Burr (13). It was composed of casein,[8] 32 per cent; corn starch, 30 per cent; lard,[9] 22 per cent; yeast, 10 per cent; cod-liver oil, 2 per cent; and mineral mixture (McCollum No. 185) (23), 4 per cent. This ration has been fed to 200 females and in no instance has initial fertility been encountered. Evans and Burr (12) obtained four cases of initial fertility in 200 cases. The females were maintained on this ration until they were 90 to 120 days old and then mated for a period of 15 days to males previously shown to be fertile by matings with stock females. Each female was weighed three times per week, during this period of mating, and until 21 days after separation from the males. Only those females which showed resorption of a fetus as evidenced by their weight curve were used for the vitamin E tests. The weight curve of the pregnant female on a normal ration generally shows a steady rise until parturition, at which time there is a precipitous fall in the curve. On the other hand the weight curve of the pregnant female on a vitamin E deficient ration shows a steady rise until about the fourteenth day. From this time on there is a gradual diminution in weight and the period of gestation passes without a litter being cast. During this selection 35 females were discarded, as it was not clearly evident whether or not they had resorbed a fetus.

Beginning with the twenty-second day after the males were removed from the females, graded quantities of the field-cured or artificially cured alfalfa were incorporated into the vitamin E deficient ration. This was accomplished by replacing a portion of the corn starch with an equal quantity of the finely ground alfalfa. After the females had been on the supplemented rations for four days they were again mated. During this second gestation period the females were weighed daily and watched closely to detect parturition. As our cages were not equipped with any special device, such as the one described by Long and Evans (19), for determining the number of young dropped, our counts may not be accurate since some pups are at times devoured by the mother shortly after birth. In three cases it was not possible to determine whether a litter

[8] The casein, starch, yeast, and cod liver oil were the same brands and were prepared in the same manner as described under Vitamin A studies.

[9] The lard, labeled Star Lard, was purchased locally from Armour & Company.

had been dropped or a resorption had taken place. These cases, therefore, were not considered in the tabulations.

In order to obtain some idea of the smallest quantity of alfalfa which would permit successful casting of litters, a preliminary trial was first run with 24 females. The results are shown in Table 4.

TABLE 4.—*Summary of the number of litters dropped by rats fed artificially cured alfalfa as the source of vitamin E—preliminary trial*

Sources of Vitamin E	Females in group	Litters dropped	Resorptions	Young dropped	Young weaned
	No.	*No.*	*No.*	*No.*	*No.*
Alfalfa *(per cent)*					
5	6	1	5	1	0
10	6	2	4	15	2
15	6	6	0	34	27
Ground wheat *(per cent)* 15	4	3	1	14	6
Vitamin E free ration	2	0	2	0	0

From the results of this preliminary trial it seemed that from 10 to 15 per cent of artificially cured alfalfa was required to furnish sufficient vitamin E to allow successful casting of litters. Therefore, in comparing the vitamin E content of the field-cured with the artificially cured alfalfa (Table 5) the former was fed at levels of 10, 15, 20, and 25 per cent, while the latter was fed at levels of 10, 15, and 20 per cent. The 25 per cent field-cured group was added so that in case this type of alfalfa should have a lower vitamin E content, as was found with the vitamin A, the minimum quantity required might still be determined. Negative controls received only the vitamin E free ration, while positive controls were fed the vitamin E free ration supplemented with 15 per cent of ground whole wheat. Since the supply of alfalfa unfortunately became exhausted, it was necessary to terminate the experiment after the females had cast a litter or the period of gestation had passed.

From the results shown in Table 5 it is evident that 15 per cent of artificially cured alfalfa furnished sufficient vitamin E to permit the successful casting of litters, since each of the females in the 15 and 20 per cent groups produced a litter. Identical results were obtained with 15 per cent of the artificially cured alfalfa in the preliminary trial. On the other hand only five, or 50 per cent, of the females in the group fed 15 per cent of the field-cured alfalfa produced a litter.

TABLE 5.—*Summary of the number of litters dropped by rats fed various percentages of field-cured and artificially cured alfalfa as the sources of vitamin E*

Source of vitamin E	Females in group	Litters dropped	Resorptions	Young dropped
	No.	*No.*	*No.*	*No.*
Artificially cured *(per cent)*				
10	12	6	6	29
15	12	12	0	107
20	12	12	0	125
Field-cured *(per cent)*				
10	10	3	7	16
15	10	5	5	22
20	12	11	1	87
25	11	9	2	82
Ground wheat *(per cent)*				
15	4	3	1	33
Vitamin E free ration	7	0	7	0

At least 20 per cent of the field-cured hay was required to approximate perfect reproduction of all females in the group. Furthermore, 15 per cent of the field-cured hay resulted in no better reproduction than 10 per cent of the artificially cured hay. It seems evident, therefore, that the artificial drying of alfalfa tended to preserve its vitamin E content to a greater degree than did the field curing.

Since as little as 3 per cent of field-cured alfalfa was sufficient to supply enough vitamin A for good growth and since 20 per cent produced good reproduction, it seems that a shortage of these vitamins would never be responsible for failures in growth or reproduction in livestock having a good grade of field-cured alfalfa included liberally in their ration, even though the minimum requirements are smaller with artificially cured than with field-cured alfalfa. This statement is made on the assumption that farm animals respond similarly to rats. It remains for future investigation to establish whether there is a relationship between artificial and field-curing practices and the vitamin content of dairy and poultry products.

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