

FURTHER STUDIES ON
VITAMINS IN ALFALFA HAY

C. E. VAIL, J. W. TOBISKA, and EARL DOUGLASS



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Further Studies on VITAMINS IN ALFALFA HAY

C. E. VAIL, J. W. TOBISKA, and EARL DOUGLASS

DURING the seasons of 1930 and 1931 the Chemistry Section of the Colorado Experiment Station made a study of the vitamin values (A, B, C, and G) of alfalfa hays, most of the samples being Colorado hays. The results of this work were reported in Technical Bulletin 4 of this station.

The importance of the method of curing upon the vitamin values of alfalfa having been forcefully indicated, it was decided to carry our investigations further. This bulletin is developed from efforts to determine:

1. How much of vitamins A, B, and G may be lost from hay stacked in the field?
2. Since sunlight destroys vitamin A, how much of this loss can be accounted for by the action of ultraviolet light?
3. What is the effect of other methods of drying, such as rolling or crushing the green hay, or dehydrating with hot air?
4. What is the best practical way to store alfalfa meal?
5. Is there a difference in the vitamin content of different varieties?

The results of our investigation as presented here are treated under the following principal topics:

- Rapid artificial drying.
- Drying in ultraviolet light.
- Green crushing and drying.
- Manner and length of storage.
 - (a) In the stack.
 - (b) In the bale.
 - (c) Laboratory conditions.
- Comparison of four varieties.

THE SAMPLES

The samples were collected during the growing season of 1932 and were mostly from the plots on the college east farm. Several samples were obtained from private farms in the vicinity of Fort Collins and Longmont.

Although the spring of 1932 was somewhat cold and dry, the experiment station farm had an abundance of irrigation water. The

first crop of alfalfa was rank and coarse, making the heaviest first cutting in several years. The alfalfa web-worm was present on all first-cutting samples except those from Longmont, but the hay was not badly damaged.

The second crop was cut August 9, in mid-bloom. Grasshoppers had damaged the Grimm and Turkestan plots but not the large field of common alfalfa. The second cutting was in better than average condition, although woody and cut a little late.

The third cutting was harvested September 24 but dried very slowly. It was stacked October 3. This crop was much better than usual, due to the long growing season. We baled enough of this cutting to make a pile 8 bales wide and 2 bales high under cover of a barn, so that an inside bale could be sampled at a later date.

In Colorado alfalfa is stacked in the field, with no cover of any kind. The first cutting is stacked during the latter part of June, the second cutting during the middle of August, and the third cutting in middle or late September. Our stacked hays were all sampled on January 6 and 7, 1933. By using a hay knife, a cut 18 inches wide was made from the top to the bottom of the stack, and for the first cutting, 1 foot deep. The next sample of first cutting consisted of the next 9 inches in the same cut. For the third sample a hole was cut 5 feet deep and the sample taken at the bottom of this hole.

As practically no rain had fallen since the third cutting had been stacked, the hay was in excellent condition, and only the outside of the stack was even discolored. The surface sample was taken with a hay knife about 9 inches deep, the second sample was the next 12 inches under this, and the third sample $4\frac{1}{2}$ to $5\frac{1}{2}$ feet deep in the stack.

An inside bale from the pile of baled hay was also sampled at the time the stacks were opened.

When the sample of first-cutting hay dried in diffused light was taken, another sample was dried indoors in the same way except for treatment with a sun-lamp for about 20 hours on three successive days while drying. The lamp was from 2 to $2\frac{1}{2}$ feet distant from the hay, which was turned every 2 hours. However, since the hay was spread upon a table and the position of the lamp had to be changed, the direct rays of the lamp played upon the hay only about 5 hours.

Another sample was dried quickly with hot air, in imitation of a mechanical dryer.

Samples of second- and third-cutting hays were passed through tinner's rolls set tight enough to crack the stems but not to wring out any sap. This hay was then dried in sunshine out-of-doors. It was surprising how quickly the crushed hay dried. Placed in imitation of a swath on a canvas at 9 a. m., by 1 p. m. it could have been

raked and by 3 o'clock stacked—a matter of 6 hours instead of 3 days.

As alfalfa meal is an important feed in Colorado, the best method for its preservation becomes important. Two samples of the same hay were stored, one in a cloth bag such as is usually used, and one in a paper bag, and the vitamin-A content determined after 10 months' storage.

Samples of hay, field-cured by use of a side-delivery rake, were compared with the same hay cured with a dump rake, but no significant differences in vitamin content were found. We obtained

TABLE 1—*Alfalfa hay samples, season of 1932*

Sample no.	Cutting	Variety	Treatment	Location	Date
3243	First	Common	Diffused light	Exp. farm	6/27
3244	First	Common	Mercury lamp	Exp. farm	6/27
3245	First	Common	Oven-dried (45° C.)	Exp. farm	6/27
3248	First	Turkestan	Diffused light	Exp. farm	6/21
3249	First	Grimm	Diffused light	Exp. farm	6/22
3250	First	Cossack	Diffused light	Fort Collins	6/22
3251	First	Grimm	Diffused light	Longmont	6/23
3252	First	Turkestan	Diffused light	Longmont	6/23
3253	Second	Common	Oven-dried (45° C.)	Exp. farm	8/9
3254	Second	Common	Diffused light	Exp. farm	8/9
3255	Second	Common	Mercury lamp	Exp. farm	8/9
3256	Second	Common	Crushed and sun-dried	Exp. farm	8/11
3259	Second	Turkestan	Diffused light	Exp. farm	8/9
3260	Second	Grimm	Diffused light	Exp. farm	8/9
3261	Second	Cossack	Diffused light	S. W. Fort Collins	8/6
3262	Third	Common	Oven-dried (45° C.)	Exp. farm	9/26
3263	Third	Common	Diffused light	Exp. farm	9/26
3264	Third	Common	Mercury lamp	Exp. farm	9/26
3265	Third	Common	Crushed and sun-dried	Exp. farm	9/26
3268	Third	Cossack	Diffused light	Exp. farm	9/26
3269	Third	Grimm	Diffused light	Exp. farm	9/26
3270	Third	Turkestan	Diffused lamp	Exp. farm	9/26
3271	First	Common	6 mos. storage in stack, surface foot	Exp. farm	1/6-33
3272	First	Common	6 mos. storage in stack, 12"-21" in stack	Exp. farm	1/6-33
3273	First	Common	6 mos. 5'-6' stack	Exp. farm	1/6-33
3274	First	Common	6 mos. 6'-7' stack	Exp. farm	1/7-33
3275	Third	Common	3 mos. storage in stack, surface 9"	Exp. farm	1/7-33
3276	Third	Common	3 mos. storage in stack, 9"-21" in stack	Exp. farm	1/7-33
3277	Third	Common	3 mos. 5'-5.5' stack	Exp. farm	1/7-33
3278	Third	Common	Baled at stacking time (sheltered)	Exp. farm	1/7-33
3245-A	First	Common	Meal, stored in cloth bag	Exp. farm	6/27
3245-B	First	Common	Meal, stored in paper bag	Exp. farm	6/27

evidence, however, confirming the data given in Technical Bulletin 4, page 66, relative to the loss of vitamins by curing in sunlight.

Table 1 lists the samples reported on in this bulletin, with notes on location, cutting, variety, and treatment.

FODDER ANALYSES

Table 2 shows the difference in composition of alfalfa hay cured in diffused light compared with the samples which were crushed between rolls before drying in the sunshine. It was not supposed any chemical differences would be found, but there is a decided increase in the ether-soluble portion as well as in the carbohydrates, and a decided decrease in crude fiber. Honcamp (Land. Vers.-Stat. 86:215-276) found that hay desiccated in a vacuum apparatus was as digestible as fresh grass, while that which was dried naturally was less digestible, and concluded that certain substances soluble in ether are either converted into indigestible substances or are entirely decomposed when dried naturally in the sunshine. Likewise the carbohydrates are easily decomposed in the natural-drying process.

An abstract in the Experiment Station Record from an article in the Mark Lane Express (1915): "In ordinary hay making there is considerable loss from oxidation. This may be avoided by a rapid drying. The changes in digestibility seem to be due rather to changes of a physical nature than to chemical decomposition."

The rolled sample of alfalfa proved to be exceptionally high in vitamin A, exceeding by a wide margin the same hay cured in diffused light and confirming the comparatively slight increase in content indicated by the fodder analysis.

Table 3 groups the alfalfas by varieties and allows comparison with the samples of common alfalfa cured in diffused light given in table 2. There is a much greater difference in the composition of the three cuttings of the same variety than between varieties.

Samples of alfalfa stacked in the open field are given in table 4 and may be compared with the same hay cured indoors. The first-cutting samples 3271, 3272, and 3273, which had been stacked since the latter part of June and were sampled January 6—6½ months later—show decided changes when compared with 3243, which is the same hay cured in diffused light. The crude fiber is much higher in the stacked samples, while the carbohydrates, protein, and ether extract are lower. The third cutting, which had been stacked only 3 months under ideal weather conditions, showed almost no change except a lowering of the ether extract. The baled alfalfa lost only a small amount of its ether extract in 3 months' storage.

TABLE 2—*Fodder analyses of common alfalfa, Colorado Experiment Station Farm, season of 1932*

Sample no.	Cutting	How cured	Moisture at 100° C.	Ash	Crude fiber	Nitrogen-free extract	Crude protein	Ether extract
3243	First	Diffused light	8.64	10.01	34.31	31.17	14.82	1.05
3254	Second	Diffused light	8.52	9.50	33.05	28.60	19.12	1.21
3256	Second	(Rolled) sunshine	8.38	10.20	29.20	31.50	19.03	1.69
3263	Third	Diffused light	8.15	10.97	24.09	32.95	22.15	1.68
3265	Third	(Rolled) sunshine	8.02	11.32	22.58	34.73	21.44	1.91

TABLE 3—*Fodder analyses of three varieties of alfalfa, season of 1932 (all cured in diffused light)*

Sample no.	Cutting	Variety	Moisture at 100° C.	Ash	Crude fiber	Nitrogen-free extract	Crude protein	Ether extract
3248	First	Turkestan	8.04	9.95	34.35	30.85	15.53	1.29
3249	First	Grimm	8.30	9.44	33.63	31.09	16.36	1.19
3250	First	Cossack	8.70	10.73	31.52	32.61	15.43	1.01
3259	Second	Turkestan	8.29	10.89	34.68	26.84	18.10	1.21
3260	Second	Grimm	8.45	9.68	32.70	29.47	18.44	1.27
3261	Second	Cossack	8.69	10.39	27.93	30.66	20.89	1.44
3270	Third	Turkestan	7.95	10.98	25.43	32.40	21.44	1.80
3269	Third	Grimm	8.10	11.43	26.14	32.42	20.18	1.73
3268	Third	Cossack	8.34	10.77	21.16	34.56	23.19	1.98

TABLE 4—*Fodder analyses of stacked alfalfa hay, season of 1932*

Sample no.	Cutting	Source	Moisture at 100° C.	Ash	Crude fiber	Nitrogen-free extract	Crude protein	Ether extract
3271	First	Outside 12" deep	6.57	9.11	44.10	28.72	10.86	0.64
3272	First	From 13" to 22"	7.23	10.66	41.42	27.54	12.41	0.74
3273	First	From 5' to 6' deep	7.16	9.45	37.82	30.57	14.11	0.89
3274	First	From 6½' to 7' deep	7.56	10.81	38.82	27.57	14.44	0.80
3275	Third	Outside 9" deep	7.35	10.54	25.57	35.28	20.13	1.13
3276	Third	From 10" to 22" deep	7.23	10.79	28.08	32.75	19.96	1.19
3277	Third	From 4½' to 5½' deep	6.85	10.55	25.99	35.56	19.85	1.30
3278	Third	Baled	7.70	10.52	25.71	35.25	19.41	1.41
3243	First	Cured in diffused light	8.64	10.01	34.31	31.17	14.82	1.05
3263	Third	Cured in diffused light	8.15	10.97	24.09	32.95	22.15	1.68

TABLE 5—*Fodder analyses of alfalfa hays as made in 1931 and again in 1933*

Sample no.	Year analyzed	Stage of growth	Cutting	Moisture at 100° C.	Ash	Crude fiber	Nitrogen-free extract	Crude protein	Ether extract
3192	1931	Early bud	First	5.35	10.24	26.00	36.40	20.33	1.87
3192	1933	Early bud	First	7.59	11.23	25.93	32.55	21.44	1.26
3193	1931	Early bloom	First	5.35	9.52	29.18	35.74	18.22	1.99
3193	1933	Early bloom	First	7.36	10.32	29.87	31.38	18.92	1.25
3209	1931	Late bloom	Second	6.17	7.23	40.36	29.88	14.82	1.54
3209	1933	Late bloom	Second	7.78	8.63	40.31	26.92	15.31	1.05
3220	1931	Early bloom	Third	5.46	8.43	32.84	34.21	17.45	1.61
3220	1933	Early bloom	Third	7.73	10.45	40.01	22.60	18.20	1.01

Since storage makes such a difference, four samples of alfalfa hay which had been analyzed in 1931 and stored in fairly tight tin cans were again analyzed in 1933 (tab. 5). There is no significant difference in the protein content, but in every case the ether extract and carbohydrates are lower and the crude fiber and ash are higher after the lapse of 2 years between analyses.

TECHNIQUE OF VITAMIN DETERMINATIONS

A description of the technique used for the vitamin determinations is given in our previous bulletin (Tech. Bul. 4, pp. 5-14). We used the same basal diets, a similar series of controls, and the same method of calculating the results. The depletion and routine handling of the experimental rats was essentially the same as heretofore. Our results reported here should therefore be quite comparable with our previous work.

In Technical Bulletin 4 it was shown that alfalfa cured in diffused light retained most of its vitamin values. Accordingly, it should be noted that in this bulletin all values found are compared with those of hays dried in ordinary diffused light. As will be shown, in some cases the samples cured in diffused light were lower in vitamin content than samples cured otherwise.

VITAMIN-B CONTENT OF ALFALFA HAYS, CROP OF 1932

In the vitamin-B studies the animals were all depleted before being put on test, and the tests were carried over an 8-weeks period.

The fate of our control animals was as set forth in table 6.

TABLE 6—*Fate of control animals in vitamin-B studies*

	Depleted weight	Final weight	Days of life	No. of animals	Av. weekly food	Fate
Negative	-----*73.0	43.2	19	5	---	All died
Positive	----- 72.3	156.7	56	8	70.8	All lived

*(All weights in tables expressed in grams unless otherwise specified.)

ARTIFICIAL DRYING

Both our previous studies and those by other investigators have shown that a number of artificial methods of curing alfalfa tend to produce hays of higher vitamin content. Table 7, while not exhaustive, presents comparative data on three artificial methods of drying.

TABLE 7—*Vitamin content of alfalfa hays, crop of 1932; effect of artificial drying (five animals per sample, 8-weeks' period)*

Sample no.	Cutting	Variety	Depleted weight	Final weight	Units B per g. hay	Av. weekly food	Treatment
3243	First	Common	61.2	95.6	2.1	38.0	Check (diffused light)
3244	First	Common	63.2	101.2	2.3	39.4	Sun-lamp (ultra-violet)
3245	First	Common	65.0	81.0	1.0	34.4	Oven-dried
3254	Second	Common	62.8	91.4	1.8	33.0	Check (diffused light)
3255	Second	Common	76.4	94.6	1.1	36.5	Sun-lamp (ultra-violet)
3253	Second	Common	59.2	114.6	3.4	40.8	Oven-dried
3263	Third	Common	72.6	119.0	2.9	47.8	Check
3264	Third	Common	76.0	107.0	1.9	44.0	Sun-lamp (ultra-violet)
3262	Third	Common	66.0	108.6	2.6	43.1	Oven-dried

The table seems to indicate that the use of a mercury lamp does not tend to increase the vitamin-B content of the hay; however, the rather rapid drying in a warm-air oven, as well as the slow drying out of contact with sunlight, again indicate their value as regards vitamin-B content in the resulting hay.

EFFECT OF CRUSHING AND FIELD DRYING

In California and other states the farmers have resorted to the expedient of crushing the green alfalfa as it is cut and then allowing it to sun-dry. We treated samples of our second and third cuttings of common alfalfa in this manner, and table 8 represents the effect of such treatment upon the vitamin-B content.

TABLE 8—*Effect of roller-crushing and drying (five animals per sample, 3-weeks' period)*

Sample no.	Cutting	Variety	Depleted weight	Final weight	Units B per g. hay	Av. weekly food	Treatment
3254	Second	Common	62.8	91.4	1.8	33.0	Check (diffused light)
3256	Second	Common	69.2	107.2	2.3	40.3	Crushed and air-dried
3263	Third	Common	72.6	119.0	2.9	47.8	Check (diffused light)
3265	Third	Common	70.0	81.0	1.6	31.9	Crushed and air-dried

A more thorough study should be made because of the contradictory results for B vitamin obtained in the two cuttings. The meager results here presented may be taken to indicate, at least, that vitamin B is not destroyed by the crushing treatment.

TIME AND MANNER OF STORAGE

Owing to the recent developments in vitamin studies, and the nutritional value attached to their presence, it became our natural curiosity to know the fate of these accessory food values in alfalfa after periods of storage in stacks out under the skies.

TABLE 9—*Effect of manner of storage (five animals per sample; average life, 53 days)*

Sample no.	Cutting	Variety	Depleted weight	Final weight	Units B per g. hay	Av. weekly food	Treatment
3271	First	Common	78.0	74.2	—(1.2)	30.6	Stacked hay (6mos.) 1st ft. depth of stack
3272	First	Common	77.2	85.4	+0.5	35.9	12"-21" depth
3273	First	Common	71.8	102.0	+1.8	39.0	5'-6' depth
3274	First	Common	84.0	58.6	—(1.57)	22.0	6'-7' depth (moldy) stacked hay (3 mos.)
3275	Third	Common	83.2	100.2	1.4	45.7	1st 9" depth
3276	Third	Common	68.4	87.2	1.1	41.3	9"-21" depth
3277	Third	Common	72.0	75.4	.2	34.0	4.5'-5.5' depth
3278	Third	Common	72.0	80.6	.5	42.6	Baled at stacking time
3245A	First	Common	72.5	107.0	2.1	42.6	Ground and stored in cloth
3245B	First	Common	68.0	92.0	1.5	39.1	Ground and stored in paper

Table 9 is a statement of our findings in that respect.

As far as concerns vitamin B, the greatest losses are those brought about by surface weathering of the stack, and also perhaps those deep within the stack when the heat generated in curing serves to destroy the B factor. Small samples of ground hay stored in a cabinet preserved this factor fairly well.

COMPARISON OF VITAMIN-B CONTENT OF FOUR VARIETIES OF ALFALFA

Common alfalfa is the variety grown on the largest acreage in this section. The Agronomy Department of Colorado State College has been experimenting with other varieties for several years; our samples of these varieties came largely, though not entirely, from their plots, through the courtesy of Dr. D. W. Robertson.

TABLE 10—*Comparison of B content of four varieties commonly grown, dried in diffused light (five animals, 8-weeks' period)*

Sample no.	Cutting	Variety	Depleted weight	Final weight	Units B per g. hay	Av. weekly food
3243	First	Common	61.2	95.6	2.1	38.0
3250	First	Cossack	58.4	76.4	1.1	33.1
3248 & 52*	First	Turkestan	62.6	83.5	1.3	34.6
3249 & 51*	First	Grimm	62.7	65.5	0.2	28.4
3254	Second	Common	62.8	91.4	1.8	33.0
3261	Second	Cossack	67.0	106.6	2.4	39.0
3259	Second	Turkestan	69.2	95.4	1.6	38.7
3260	Second	Grimm	67.4	94.6	1.7	36.0
3263	Third	Common	72.6	119.0	2.9	47.8
3268	Third	Cossack	74.0	98.6	1.5	41.8
3270	Third	Turkestan	72.8	101.8	1.8	43.2
3269	Third	Grimm	75.0	116.4	2.5	44.0

*Ten animals.

Our table 10 sets forth the comparative results obtained with these four varieties carried through the three crops of the growing season of 1932.

SUMMARY AND CONCLUSIONS

(1) The B-vitamin factor is present in Colorado alfalfa hays in amounts so small (1 to 3 units per gram) that a supplement of this accessory would appear advisable where stock subsists largely on this type of roughage.

(2) The B factor, being heat-labile, can deteriorate with storage and other harsh treatment to the point of total destruction.

(3) Insofar as relates to the data in table 10, we are inclined to make the deduction that one may expect more variation in B-vitamin content in one and the same variety, dependent upon the manner of curing, than from any differences inherent in the particular variety.

(4) In striking an average for the vitamin-B content of each of the above varieties for all three cuttings, we did find a small difference in favor of the common variety. The other three varieties were about on a par.

VITAMIN-G CONTENT OF ALFALFA HAYS, CROP OF 1932

Owing to pressure of time and because of the relative stability of the vitamin-G factor in Colorado alfalfa hays, these studies were not made to cover all our samples, as had been done with the A and B factors.

Previous work (Tech. Bul. 4) had given the general information that Colorado alfalfas, when properly cured, may carry from three to more than five units per gram of this factor. Since this factor is heat-stable to a high degree, one would not expect great variations in hays which were cured in the regular manner.

The fate of a small number of control animals, interspersed along with the positive measurements, was as shown in table 11.

TABLE 11—*Fate of control animals in vitamin-G studies*

	Depleted weight	Final weight	Days of life	No. of animals	Av. weekly food
Negative -----	56.8	48.4	55.8	5	34.7
Positive -----	---	157.8	56.0	12	78.7

As was true in previous work, our negative controls generally lived through an 8-weeks period.

In table 12 are set forth all the comparisons made for vitamin-G content.

TABLE 12—*Vitamin-G content of alfalfa hays, crop of 1932 (five animals per sample, 8-weeks' period)*

Sample no.	Cutting	Variety	Depleted weight	Final weight	Units G per g. hay	Av. weekly food	Treatment
EFFECT OF METHOD OF CURING							
3243	First	Common	48.8	78.6	3.5	39.1	Diffused light
3244	First	Common	47.8	76.2	3.3	37.3	Ultraviolet lamp
3245	First	Common	52.8	80.6	3.3	40.1	Oven-dried
EFFECT OF ROLLER CRUSHING AND AIR-DRYING							
3256	Second	Common	51.0	93.0	5.0	44.4	Crushed and air-dried
EFFECT OF STORAGE							
							Stacked hays
3271	First	Common	49.8	69.6	2.3	39.0	1st foot depth (6 mos.)
3274	First	Common	52.2	77.4	3.0	39.8	6'-7' depth
COMPARISON OF G CONTENT OF FOUR VARIETIES OF ALFALFA							
3243	First	Common	48.8	78.6	3.5	39.1	Diffused light
3250	First	Cossack	51.0	83.8	3.8	42.0	Diffused light
3248	First	Turkestan	51.8	81.0	3.4	40.4	Diffused light
3249	First	Grimm	59.2	95.2	4.3	57.0	Diffused light

SUMMARY AND CONCLUSIONS

(1) Fresh Colorado alfalfa hay carries from three to five units per gram of the G factor.

(2) From a perusal of the table it is apparent that methods of artificial drying do not affect the G factor notably.

(3) There is not a very significant difference in the G content as between varieties of alfalfa.

(4) It would appear that top-weathering in a stack does cause some loss of the G factor, and apparently also, the one green crushed sample indicates a material gain in vitamin G from that procedure. The latter test should be checked by further experiment.

VITAMIN A IN THE ALFALFA HAY SAMPLES OF 1932

Table 13 gives a summary of the data obtained for vitamin A on 41 hays and 2 sets of controls, based in general on results from feeding the animals at the lowest adequate levels. For convenience the table is divided into five sections.

TABLE 13—Vitamin-A content of alfalfa hays, crop of 1932, Fort Collins area (10 animals per sample, usually; 8-weeks' period)

Sample no.	Cutting	Variety	Depleted weight	Final weight	Units A per g. hay	Av. weekly food	Treatment	
Section I EFFECT OF ARTIFICIAL DRYING								
3243	First	Common	88.7	122.8	56	72	Check (diffused light)	
3244	First	Common	105.7	138.6	28	66	Sun-lamp	
3245	First	Common	92.9	141.7	80	71	Oven-dried	
3254	Second	Common	92.3	127.7	60	70	Check (diffused light)	
3255	Second	Common	100.9	128.2	48	70	Sun-lamp	
3253	Second	Common	105.6	145.2	107	75	Oven-dried	
3263	Third	Common	104.9	144.7	64	75	Check (diffused light)	
3264	Third	Common	101.5	153.8	44	81	Sun-lamp	
3262	Third	Common	111.1	156.0	127	82	Oven-dried	
Section II EFFECT OF ROLLER CRUSHING AND DRYING								
3254	Second	Common	92.3	127.7	60.0	70	Check (diffused light)	
3256	Second	Common	111.4	139.3	87.0	73	Crushed and air-dried	
3263	Third	Common	104.9	144.7	64.0	75	Check (diffused light)	
3265	Third	Common	103.1	145.0	120.0	75	Crushed and air-dried	
Section III EFFECT OF MANNER OF STORAGE								
3271	First	Common	108.7	138.5	6.5	72	Stacked hay (6 mos.)	
3272	First	Common	108.7	154.7	9.5	84	12"-21" depth	
3273	First	Common	107.4	145.7	7.5	78	5'-6' depth	
3274	First	Common	109.8	137.8	6.0	76	6'-7' depth (Moldy) Stacked hay (3 mos.)	
3275	Third	Common	102.6	155.3	22.0	78	1st 9" depth	
3276	Third	Common	101.9	137.5	30.0	71	9"-21" depth	
3277	Third	Common	103.7	147.1	36.0	70	4.5'-5.5' depth	
3278	Third	Common	103.3	129.1	44.0	69	Baled at stacking time.	
3245-A	First	Common	106.8	163.9	48.0	78	Ground and stored in cloth	
3245-B	First	Common	108.4	144.9	60.0	78	Ground and stored in paper	
3220	Third	Common	108.8	142.0	64.0	66	Original sample	
3220	Third	Common	101.5	111.0	20.0	62	*Stored 3 yrs.	
3230	Third	Common	105.6	150.8	76.0	70	Original sample	
3230	Third	Common	105.0	133.4	52.0	71	*Stored 2 yrs.	
3243	First	Common	88.7	122.8	56.0	72	Original sample	
3243	First	Common	106.0	131.2	40.0	69	*Stored 14 mos.	
Section IV COMPARISON OF VITAMIN-A CONTENT OF FOUR VARIETIES OF ALFALFA (DRIED IN DIFFUSED LIGHT)								
3243	First	Common	88.7	122.8	56	72		
3250	First	Cossack	91.2	125.1	60	68		
3248 & 52	First	Turkestan	96.6	132.6	60	67		
3249 & 51	First	Grimm	99.0	146.7	60	76		
3254	Second	Common	92.3	127.7	60	70		
3261	Second	Cossack	97.8	128.5	48	69		
3259	Second	Turkestan	104.9	131.6	48	69		
3260	Second	Grimm	103.1	127.2	56	65		
3263	Third	Common	104.9	144.7	64	75		
3268	Third	Cossack	104.2	158.6	86	81		
3270	Third	Turkestan	110.9	171.0	100	85		
3269	Third	Grimm	105.2	161.0	96	80		
Section V								
					Days life	No. ans.	Av. weekly food	Fate
Negative controls			108.4	81.1	23	14	42	All died
Positive controls			88.6	179.0	56	9	82	All lived

*In air-tight tins.

CURING PROCESSES

In section I of the table the data show that rapid oven-drying tends to conserve the vitamin A to a marked degree. The inference is that natural, slow drying in diffused light, although superior to field curing, nevertheless allows a distinct loss of vitamin A. The difference in the A content amounts to as much as 98 percent in the case of the third-cutting samples 3262 and 3263. Our data agree with the results of a number of other experimenters with artificial dryers.

The attempt to reproduce the effect of direct sunlight by curing some hay samples indoors under a sun-lamp gave us data found also in section I of the table. As compared with the checks, the sun-lamp samples show losses in vitamin A ranging from 20 to 50 percent.

The results of roller crushing and subsequent sun drying are seen in section II of the table. The vitamin-A contents of the crushed samples were 45 and 87 percent higher than of the respective check samples. Although the drying of the crushed samples was completed in direct sunlight, they compare favorably with the oven-dried samples.

CHANGES WHILE IN STORAGE

In section III we have data on the samples of hay that were stored in various ways. In the first-cutting stacks the loss of vitamin A ranges from 27 to 64 percent, while in the third-cutting stack the loss is from 21 to 52 percent. Comparisons were made with first-cutting samples 3246 and 3247 and with second-cutting sample 3266. The baled sample was also compared with sample 3266 and was found to be only about 4 percent lower in vitamin A.

The character of alfalfa meal is such that aeration and oxidation of the vitamins may proceed rather effectively. Samples 3245-A and -B gave results indicative of the effect of differing storage conditions on ground alfalfa. The hay used was the oven-dried first cutting, 3245. This ground hay lost 40 percent of its vitamin A after 9 months' storage in the cloth sack, and 25 percent in the paper sack.

The progressive loss of vitamin A from chopped alfalfa hay is shown in the last group in section III. The original hays were stored in air-tight tin containers, and probably represent optimum laboratory-storage conditions, since they were kept in the dark, at moderate temperatures, and without free aeration. There was 28 percent loss in the sample stored 14 months, 31 percent after 2 years, and 68 percent after 3 years.

COMPARISON OF VARIETIES

Section IV exhibits the data obtained in the study of four varieties. These data may be further condensed into tabular statements of averages, thus:

Varieties	Units A per Gram
Common	60
Turkestan	68
Grimm	64
Cossack	65
Cuttings	
6 hays, 2d cutting.....	59
4 hays, 2d cutting.....	55
4 hays, 3d cutting.....	87

Except in the third cutting, the differences between varieties are not especially significant.

CONCLUSIONS REGARDING VITAMIN A

- Loss or inactivation of vitamin A in alfalfa hay results from:
 - Usual practice of curing and stacking.
 - Exposure to ultraviolet light in presence of moisture.
 - Storage of alfalfa meal in cloth sacks.
- Conservation of vitamin A in alfalfa hay results from:
 - Curing indoors.
 - Curing by rapid artificial drying.
 - Curing by crushing and rapid drying.
 - Storage in the bale.
 - Storage of alfalfa meal in paper sacks.
- Common alfalfa seems to carry less vitamin A in the third crop than Turkestan, Grimm, or Cossack. The differences among these varieties are much less in the other cuttings.
- Vitamin A in alfalfa hay tends to become more inactive the longer it is stored.

BIBLIOGRAPHY

This reference list on vitamins in alfalfa is intended to supplement the one published on pp. 67 and 68, Technical Bulletin 4, Colorado Experiment Station.

BECHDEL, S. I., and LANDSBURG, K. G.

1932. Pa. Agr. Exp. Sta. Bul. 279, p. 9.

BECHDEL, S. I., LANDSBURG, K. G., and HILL, O. J.

1933. Pa. Agr. Exp. Sta. Tech. Bul. 291.

BEESON, W. M.

1934. Rec. Proc. 27th Ann. Meeting, Am. Soc. Animal Prodn., p. 54.

FRAPS, G. S., and TREICHLER, R.

1933. Ind. Eng. Chem., 25:465.

1933. Texas Agr. Exp. Sta. Bul. 477.

GUILBERT, H. R.

1935. J. Nutrition, 10:45.

HARTMAN, A. M.

1931. J. Biol. Chem., 92:VII.

HATHAWAY, I. L., and DAVIS, H. P.

1934. Nebr. Agr. Exp. Sta. Res. Bul. 73, p. 3.

HAUGE, S. M.

1934. J. Assoc. Official Agr. Chem., 17:304.

1935. J. Biol. Chem., 108:331.

HEYWANG, B. W.

1933. Poultry Sci., 12:167.

HILTON, J. H., HAUGE, S. M., and WILBUR, J. W.

1935. J. Dairy Sci., 18:795.

HUFFMAN, C. F., and DUNCAN, C. W.

1935. J. Dairy Sci., 18:511.

HUNT, C. H.

1933. Fertilizer, Feeding Stuffs and Farm Supplies J., 18:275.

HUNT, C. H., BETHKE, R. M., WILDER, O. H. M., and BELL, D. S.

1933. Ohio Agr. Exp. Sta. Bul. 516, p. 89.

HUNT, C. H., and PERKINS, A. E.

1935. Ohio Agr. Exp. Sta. Bul. 548, p. 74.

HUNT, C. H., RECORD, P. R., and BETHKE, R. M.

1935. J. Agr. Research, 51:251.

HUNT, C. H., RECORD, P. R., WILDER, W., and BETHKE, R. M.

1933. Ohio Agr. Exp. Sta. Bimo. Bul. 18:104.

JACOBSON, C. O.

1935. Ark. Agr. Exp. Sta. Bul. 318.

JONES, T. N., and PALMER, L. O.

1934. Agr. Eng., 15:198.

KEMPSTER, H. L., and FUNK, E. M.

1934. Mo. Agr. Exp. Sta. Bul. 340, p. 67.

KISHLAR, L. M.

1933. Agr. Eng., 14:129.

LEVY, L. F., and FOX, F. W.

1935. Biochem. J., 29:884.

McCLURE, H. B.

1913. U. S. D. A. Bur. of Plant Ind. Circ. 116, p. 27.

MEIGS, E. B., and CONVERSE, H. T.

1933. Rec. Proc. 26th Ann. Meeting, Am. Soc. Animal Prodn.,
p. 58.

MILLER, M. W., and BEARSE, G. E.

1934. Wash. Sta. Bul. 292.

MYBURGH, S. J.

1935. Onderstepoort J. Vet. Sci., 5:475.

RICHARDSON, H. E.

1935. Agr. Eng., 16:469.

RUSSELL, W. C., TAYLOR, M. W., CHICHESTER, D. F., and
WILSON, L. T.

1935. N. J. Agr. Exp. Sta. Bul. 592, p. 3.

RYGH, O.

1934. Biochem. Z., 270:227.

SCHEUNERT, O., and SCHIEBLICH, M.

1934. Biedermann's Zentr. B. Tierernahr., 6:112, 133.

SHERWOOD, R. M., and FRAPS, G. S.

1934. Texas Agr. Exp. Sta. Bul. 468.

- SHINN, L. A., KANE, E. A., WISEMAN, H. G., and CARY, C. A.
1934. Rec. Proc. 27th Ann. Meeting, Am. Soc. Animal Prodn.,
p. 190.
- SMITH, M. C., and BRIGGS, I. A.
1933. J. Agr. Res., 46:229, 235.
- STEENBOCK, H., HART, E. B., ELVEHJEM, C. A., and
KLETZIAN, S. W. F.
1925. J. Biol. Chem., 66:425.
- TAYLOR, M. W.
1934. N. J. Agr., 16, no. 6, p. 3.
- WOODMAN, H. E., and EDEN, A.
1935. J. Agr. Sci., 25:50.
- WOODS, E., ATKESON, F. W., SHAW, A. O., SLATER, I. W., and
JOHNSON, R. F.
1935. J. Dairy Sci., 18:573.
- ZINK, F. J.
1933. Agr. Eng., 14:71.
-
1932. Calif. Sta. Rept., p. 21.
1933. N. J. Agr., 15:7.