EFFECT OF THE STAGE OF MATURITY AND METHOD OF CURING UPON THE VITAMIN B AND VITAMIN G CONTENT OF ALFALFA, CLOVER, AND TIMOTHY HAYS[1]

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INTRODUCTION

In the past hays have been produced largely with one object in mind—that of obtaining the utmost in quantity or total yield. More recently attention has been directed to the factors that affect the nutritive properties of hays, with the object of producing a product with the maximum feeding value.

Many investigators have pointed out the importance of hays as a source of vitamins for growing and producing animals. These investigations have in the main dealt with vitamins A and D. Practically no information is available regarding the vitamin G content of hays and the factors that affect it. This report presents the results of a study of the vitamin B (B_1) and G (B_2) content of alfalfa, clover, and timothy hays as they are influenced by the stage of maturity of the plant when cut and by natural climatic factors. It also includes the results of growth experiments with chicks, designed to show the value of the chick method of assay for the vitamin G complex as compared with the rat method.

REVIEW OF LITERATURE

In reviewing the literature, one finds data showing that certain practices or procedures affect the vitamin content of hays. Steenbock, Hart, and their associates (*10*)[2] found that clover hay which had been exposed to sunlight and rain was less valuable as a source of vitamin A and that it had better calcifying properties than hay which had been cured quickly and still retained a good green color. Russell (*9*) confirmed these findings, by showing that alfalfa hay dried artificially was about seven times as potent in vitamin A as hay cured in the usual way. He also noted an increase in the vitamin D content when hay was cured in the sun. Unpublished data obtained at the Ohio Station confirm the foregoing results. Bethke and Kick (*1*) observed a loss of vitamin A in alfalfa hay cured in the sun and exposed to rain and dew. Hauge and Aitkenhead (*7*) also observed a loss of vitamin A and explained that the destruction was due to enzymes. Hathaway, Davis, and Graves (*6*) found that artificially cured alfalfa was twice as potent in vitamin A as field-cured alfalfa. Douglass, Tobiska, and Vail (*5*) reported that alfalfa cut at the early bloom stage contained more vitamin A and vitamin G than hay cut at a later stage. They also reported that exposure to rain and sunshine lowered the vitamin A, vitamin B, and possibly the vitamin G

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[2] Reference is made by number (italic) to Literature Cited, p. 258.

content. Hunt and Krauss (*8*), in a study of pasture grasses, found that the vitamin G content decreased as the plants matured.

SOURCE OF HAYS

The alfalfa and clover hays used in this investigation were either grown on the station farm at Wooster or were obtained from the Department of Farm Crops of Ohio State University at Columbus. The timothy samples were grown on the station farm or on plots of the Timothy Breeding Station of the United States Department of Agriculture, at North Ridgeville, Ohio. Unless otherwise stated, the samples were cured indoors in subdued light.

RAT EXPERIMENTS

METHODS

In the first series of experiments for evaluating the vitamin content of the hays, rats were used as the experimental animals. These animals were born to mothers maintained on a modified Steenbock stock ration (*2*). When 24 days of age, or when weighing from 45 to 60 g, the young were placed in screen-bottom cages and were given a diet free from vitamin B and vitamin G. This diet consisted of casein (extracted if found to contain vitamin B or G), 18 percent; cornstarch, 64; hydrogenated fat (Crisco), 10; agar, 2; cod-liver oil, 2; and salt mixture (185), 4 percent.

If the rats were to be used for the determination of vitamin G each was given daily 400 mg of yeast (Northwestern), autoclaved for 4 hours at 15 pounds pressure, in addition to the basal diet. When the animals had ceased gaining in weight, which required from 14 to 21 days, they were placed in individual wire cages and fed daily the hay supplement with 400 mg of autoclaved yeast. The supplements were moistened with distilled water to prevent scattering.

If the rats were to be used for the determination of vitamin G, the basal diet was supplemented with 1 cc of an extract of rice polishings per rat daily, to furnish vitamin B. When the weights of the animals became stationary, or when they had lost slightly in weight, they were transferred to individual wire cages and the supplement (hay), moistened with 1 cc of the vitamin B preparation, was fed daily. The depletion period required on an average about 24 days.

The vitamin B extract was prepared as follows: Four kilograms of rice polishings were shaken up with 8 l of 0.1-percent hydrochloric acid and allowed to stand overnight. The clear supernatant liquid was then siphoned off. This procedure was repeated 5 or 6 times. The combined extracts were evaporated to 1,200 to 1,400 cc and then made up to 80 percent alcohol by weight. The precipitate was filtered off and washed with alcohol of the same strength. The combined alcoholic filtrates were partially concentrated under vacuum and the last traces of alcohol removed before an electric fan. The residual liquid was then diluted so that 1 cc was equivalent to 1 g of rice polishings.

The hays were ground to a fine flour, in a ball mill, for feeding purposes. The experimental feeding period in all cases was of 6 weeks' duration. The results are expressed in terms of rat units, as outlined by Bourquin and Sherman (*3*) and Chase and Sherman (*4*).

TABLE 1.—*Vitamin B and vitamin G value of hays*

VITAMIN B

Sample and no.[1]	Date cut	Rats	Level fed daily	Total gain or loss (−) in weight	Weekly gain or loss (−) in weight	Vitamin units per gram (approximate)[2]	Source and description of hay
		No.	*Milligrams*	*Grams*	*Grams*		
Alfalfa (1)	May 31, 1932.	8	350	11.7	1.9	2.2	University farm; first cutting.
		8	450	22.3	3.7		
	June 10, 1932.	8	400	15.3	2.5	2.0	
		8	500	18.1	3.0		
	June 21, 1932.	8	500	8.1	1.5	1.6	
		8	600	18.3	3.0		
	July 28, 1932.	8	400	15.8	2.6	2.0	Second cutting; injured by leaf hoppers.
		8	600	22.3	3.7		
	Sept. 10, 1932.	8	450	13.3	2.2	2.0	Third cutting.
		8	500	18.2	3.0		
Alfalfa (2)	June 8, 1932.	8	400	19.0	3.1	2.5	Station farm.
		8	500	26.0	4.3		
Timothy (3)	June 12, 1931.	8	600	7.2	1.2	.7–0.8	U. S. Timothy Breeding Station.
	June 24, 1931.	8	700	5.1	.85	[3].6	
	July 13, 1931.	8	600	−2.0	−.33	[3].5	
	June 18, 1932.	8	700	11.1	1.8	[3]1.2	
		8	900	21.8	3.6		
Timothy (3), 15150	do	8	600	12.5	2.0	1.4	U. S. Timothy Breeding Station; late maturing.
		8	800	23.8	3.9		
Timothy (3), 12368	July 1, 1932.	8	600	6.5	1.1	[3].5	
Timothy (3)	do	8	1,000	11.5	1.9	[3].6	Regular cutting.
Clover (1)	June 10, 1932.	8	500	13.1	2.2	1.6	University farm.
		8	600	22.3	3.7		
	June 21, 1932.	8	500	13.1	2.2	1.6–1.8	
		8	600	27.2	4.5		
Alfalfa from alfalfa and timothy mixture (2).	June 8, 1932.	8	400	6.6	1.1	1.6	Station farm, grown as a mixed hay.
		8	600	22.2	3.7		
Timothy from alfalfa and timothy mixture (2).	do	8	600	11.4	1.9	1.2	
		8	800	24.5	4.1		
Alfalfa and timothy mixture (50:50) (2).	do	8	650	16.7	2.8	1.4–1.5	
		8	700	21.3	3.5		
400 mg autoclaved yeast (controls).		[4] 18					

VITAMIN G

Sample and no.[1]	Date cut	Rats	Level fed daily	Total gain or loss (−) in weight	Weekly gain or loss (−) in weight	Vitamin units per gram (approximate)[2]	Source and description of hay
Alfalfa (1)	May 31, 1932	8	100	25.1	4.2	10–12	University farm; first cutting.
		8	150	40.8	6.8		
	June 10, 1932	8	100	24.9	4.1	10.0	
	June 21, 1932	8	150	27.4	4.5	6.6	
		8	200	34.8	5.8		
	July 28, 1932	8	150	24.0	4.0	6.6	Second cutting; injured by leaf hoppers.
	Sept. 10, 1932	8	75	22.6	3.8	13.0	Third cutting.
Alfalfa (2)	June 8, 1932	8	100	15.9	2.6	6.6	Station farm; first cutting.
		8	150	16.7	2.8		
	July 21, 1932	8	100	18.5	3.0	10.0	Second cutting, sun-cured; no rain.
		8	200	20.7	3.4	5.0	Second cutting, sun-cured; 0.68-inch rain.
Timothy (3)	June 12, 1931	8	100	15.0	2.5	6.6	Heads emerging.
		8	150	24.0	4.0		
	June 24, 1931	8	150	14.2	2.3	3–4	Full bloom.
		8	300	25.4	4.2		
	July 13, 1931	8	250	13.0	2.2	2.5	Ripe, brown color.
		8	400	25.0	4.1		
	June 18, 1932	8	150	9.7	1.6	3.3	Early bloom.
		8	300	19.7	3.3		
	July 1, 1932	8	300	17.4	2.9	2–3	Late bloom.
		8	450	24.7	4.0		

[1] The same number indicates that the hays were grown in the same or in adjacent fields.
[2] A unit of vitamin B or G is the weight of the supplement which, when fed to rats under standard conditions will produce an increase in weight of 3 to 4 g per week.
[3] Estimated, animal could not consume sufficient hay to produce the required increase in weight.
[4] All rats died in 3 weeks.

TABLE 1.—*Vitamin B and vitamin G value of hays*—Continued

VITAMIN G—Continued

Sample and no.	Date cut	Rats	Level fed daily	Total gain or loss (−) in weight	Weekly gain or loss (−) in weight	Vitamin units per gram (approximate)	Source and description of hay
		No.	*Milligrams*	*Grams*	*Grams*		
Timothy (3), 15150	June 18, 1932	8	150	7.5	1.2	2–3	U. S. Timothy Breeding Station, late-maturing variety.
		8	300	16.0	2.6		
Timothy (3), 12363	July 1, 1932	8	150	9.4	1.5	3.3	Do.
		8	300	18.0	3.0		
Timothy (3), 12321	June 20, 1933	8	100	16.0	2.6	8.0	U. S. Timothy Breeding Station, late-maturing variety, sun-cured.
		8	125	19.9	3.3		
Clover (1)	June 10, 1932	8	100	24.9	4.1	10.0	University farm.
		8	150	28.7	4.8		
	June 21, 1932	8	100	27.7	4.6	10.0	
		8	200	37.8	6.3		
	June 17, 1932	8	100	17.1	2.9	8	Cured indoors.
		8	100	17.4	2.9	8	96-hour exposure, no rain.
Alfalfa from alfalfa and timothy mixture (2).	June 8, 1932	8	100	16.0	2.7	6.6	Station farm, grown as mixed hay.
		8	150	21.3	3.5		
Timothy from alfalfa and timothy mixture (2).	do	8	100	15.5	2.6	5.0	
		8	200	23.3	3.9		
Alfalfa and timothy mixture (50:50) (2).	do	8	100	16.4	2.7	6.6	
		8	200	29.0	4.8		
Controls		16		−2.0			

RESULTS

The results of the biological analyses for vitamin B, presented in table 1, show that hays are comparatively low in this factor. Alfalfa (bud stage, May 31) appeared to have a higher vitamin B content than either clover or timothy cut June 10 and 12, respectively, at approximately the same stage of maturity. The data also suggest that the vitamin B content of the plant decreases as the plant matures.

The results of the vitamin G assays (table 1) show that hays may serve as a good source of this vitamin. In general, the vitamin G content was highest in the young plant, and decreased as the plant matured. The relation of the maturity of the plant to its vitamin G content is particularly evident in the case of alfalfa and timothy; clover, however, showed no such relation. Apparently, in the case of the clover the time elapsing between the two cuttings was not long enough to bring out any differences that may have existed between the more and the less mature plants. It is of interest to note that clover and timothy may have as high a vitamin G content as alfalfa when the plants are harvested at corresponding stages of maturity. The comparative vitamin B and G content of alfalfa and timothy cut at the same time is readily seen in the case of the samples grown as mixed hay.

Leaf-hopper injury to alfalfa apparently reduces its vitamin B content. This reduction no doubt is brought about primarily by the loss of leaves. Exposure of alfalfa and clover to the weather (day and night) for 96 hours, more than one-half of which was sunshine, did not seem to affect the vitamin G content of the hay. Rain (0.68 inch), on the contrary, lowered the vitamin G content of alfalfa by 50 percent (see alfalfa (2), second cutting, table 1).

In table 2 an attempt is made to show the relationship between the protein and the vitamin B and G content of hays. It will be observed that, in general, a high protein content is correlated with a high vitamin G content. It is well known that as the plant matures the proportion of leaves to the entire plant decreases and consequently the protein and the vitamin B and G decrease. Accordingly, any procedure or process that would reduce the leaf content of the hay, such as insect injury, excessive handling, or late cutting, would lower the protein and vitamin content and also the quality of the hay.

TABLE 2.—*Protein, crude fiber, and vitamins B and G in hays*

Sample and no.[1]	Date cut	Crude fiber	Protein	Vitamin B units per gram[2]	Vitamin G units per gram[2]	Description of hay
		Percent	*Percent*			
Alfalfa (2)	May 14, 1931	17.42	22.32	2.5	13.0	60 percent leaves.
	June 10, 1931	30.02	13.83	1.8	6.6	40 percent leaves.
Alfalfa (1)	May 31, 1932	25.27	20.14	2.2	10–12	Early bud.
	June 10, 1932	32.41	17.60	2.0	10.0	Early bloom.
	June 21, 1932	34.76	15.59	1.6	6.6	Late bloom.
	July 28, 1932	32.51	12.91	2.0	6.6	Second cutting, hopper injury.
	Sept. 10, 1932	23.49	19.92	2.0	13.0	Third cutting.
Clover (1)	June 10, 1932	23.30	15.67	1.6	10.0	Early bloom.
	June 21, 1932	28.73	12.26	1.8	10.0	Late bloom.
Clover (2)	June 17, 1933	--------	12.13	----------	8	Sun-cured.
Timothy (3)	June 12, 1931	23.79	8.50	.7–.8	6.6	Heads emerging.
	June 24, 1931	31.05	7.25	.6	3–4	Full bloom.
	July 13, 1931	--------	5.00	.5	2.5	Brown (ripe) color.
	June 18, 1932	25.65	6.65	1.2	3.3	Early bloom.
	July 1, 1932	26.97	5.21	.6	2–3	Late bloom.
Timothy from alfalfa and timothy mixture.	June 8, 1932	23.80	8.66	1.2	5.0	Grown as mixed hay.
Alfalfa from alfalfa and timothy mixture.	-----do-------	25.95	15.76	1.6	6.6	Do.
Mixed alfalfa and timothy (about 50:50).	-----do-------	24.23	10.20	1.4	6.6	Do.

[1] See footnote 1 to table 1. [2] Approximately.

The correlation of protein and vitamin G content with greenness is only relative and varies with the amount of bleaching due to light, extent of insect injury (leaf hopper), and whether or not the plant is diseased. A similar greenness in alfalfa, clover, and timothy does not necessarily indicate the same vitamin G content. In general, however, the vitamin G values follow in the same order as the protein, leafiness, and greenness of the particular plant.

CHICK EXPERIMENTS

In order to obtain further data on the comparative value of vitamin G in different alfalfa and alfalfa-leaf meals, day-old chicks were used as the experimental animals. These experiments gave a direct comparison of the value of the chick and rat methods of testing for vitamin G.

White Leghorn chicks from the same parent stock were used. They were confined in pens equipped with hardware cloth floors, and fed a ration known to be deficient in vitamin G. Each lot contained 25 chicks. The basal ration was composed of yellow corn, 58 percent; ground wheat, 20; wheat bran, 5; casein (Argentine), 12; steamed bone meal, 3; salt, 1; and cod-liver oil, 1 percent. The meals were incorporated in this ration in the amounts indicated in table 3. The casein and corn were so adjusted that the protein content was approximately the same in all lots. The meals used represented composite

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samples of three or more lots, with the exception of the Ohio leaf meals, which involved only one sample. The Ohio leaf-meal fed to lots 10 and 11 (table 3) was prepared from sun-cured hay that had received 0.68 inch of rainfall while in the swath. Liver meal is considered to be a very rich source of vitamin G and for that reason one lot of chicks was fed 3 percent of this as a positive control.

TABLE 3.—*Effect of alfalfa, alfalfa-leaf, and liver meals on growth and leg paralysis of chicks*

Lot no.	Supplement	Protein in meal	Meal consumed per chick in 8 weeks	Rat units of vitamin G per gram of meal	Total rat units of vitamin G consumed per chick in 8 weeks	Average weight per chick in 8 weeks	Leg paralysis	
							Total [1]	Recovered [2]
		Percent	*Gram*			*Gram*	*Percent*	*Percent*
1	None					168.8±4.8	67.0	0.0
2	5 percent alfalfa meal [3]	16.0	38.7	8.0	309.6	278.8±14.0	68.0	8.0
3	10 percent alfalfa meal [3]	16.0	118.5	8.0	948.0	466.8±13.3	29.0	17.0
4	5 percent alfalfa-leaf meal [3]	20.25	49.9	13.0	648.7	384.6±15.2	48.0	12.0
5	10 percent alfalfa-leaf meal.[3]	20.25	130.6	13.0	1,697.8	517.3±11.8	.0	
6	5 percent alfalfa meal [4]	14.25	35.6	8.0	284.8	251.9±11.3	87.0	35.0
7	10 percent alfalfa meal [4]	14.25	119.7	8.0	957.6	454.9±15.0	28.0	16.0
8	5 percent alfalfa-leaf meal [4]	20.56	55.2	13.0	717.6	438.3±14.4	48.0	48.0
9	10 percent alfalfa-leaf meal [4]	20.56	132.4	13.0	1,721.2	528.3±9.9	.0	
10	5 percent alfalfa-leaf meal [3] (0.68 inches rain).	20.31	32.8	5.0	164.0	240.6±11.9	83.0	33.0
11	10 percent alfalfa-leaf meal [3] (0.68 inches rain).	20.31	107.1	5.0	535.5	397.9±13.6	36.0	20.0
12	3 percent dried pork liver					602.9±12.3	.0	

[1] The figures in this column indicate the percentage of chicks that developed leg paralysis during the experiment.
[2] The figures in this column indicate the percentage of chicks that recovered before the end of the experiment.
[3] Meal from hays grown in Ohio.
[4] Meal from hays grown in Colorado.

It is evident from the data presented in table 3 that the growth of the chicks and the incidence of leg paralysis are directly correlated with the vitamin G content of the meals as determined with rats. Leaf meals having a higher protein and vitamin G content than straight meals caused greater growth and less incidence of leg paralysis than the regular meals of lower protein and vitamin G content. The poor growth and comparatively large percentage of leg paralysis in the case of lots 10 and 11, which received 5 and 10 percent respectively of the leaf meal subjected to rain, suggest that a large part of the vitamin G was lost, and corroborate the results obtained with rats. The data also show that alfalfa meals and leaf meals of approximately the same vitamin G potency produce comparable responses in chicks, whether the meals are produced in Ohio or in Colorado.

A second experiment was conducted partly to check the results of the first trial and partly to compare meals prepared from clover and timothy with those of alfalfa. The same basal ration and procedure used in the first test were employed. The meals were incorporated in the basal ration in amounts shown in table 4. The casein and corn were so adjusted that the total protein content of the rations in all lots was comparable. The clover and timothy meals were prepared from single samples of hay, while the alfalfa meals represented composite samples of three or more lots. Twenty chicks were started in each lot.

The results presented in table 4 show the ineffectiveness of 10 percent of the straight hay meals in preventing leg paralysis-substantiating the results obtained in the first experiment. Although the results show some variation between different meals, the data in general reveal a close correlation between the response in growth and incidence of leg paralysis and the vitamin G content of the meals as determined on rats. Clover meal gave results comparable to alfalfa meals of the same vitamin G potency but a higher protein content. The results obtained with the timothy meals also compare favorably with those of alfalfa when the vitamin G content of the meals is considered. As in the previous experiment, there was no difference in the results with the Ohio and Colorado meals when compared on the same vitamin G basis.

TABLE 4.—*Effect of alfalfa, clover, timothy, and liver meals on growth and leg paralysis of chicks*

Lot no.	Supplement	Protein in meal	Meal consumed per chick in 8 weeks	Rat units of vitamin G per gram of meal	Total rat units of vitamin G consumed per chick in 8 weeks	Average weight per chick in 8 weeks	Leg paralysis: Total [1]	Leg paralysis: Recovered [2]
		Percent	*Gram*			*Gram*	*Percent*	*Percent*
1	None					176.0±5.3	95.0	15.0
2	5 percent clover [3]	11.54	38.0	8.0	304.0	243.0±12.3	85.0	35.0
3	10 percent clover [3]	11.54	125.7	8.0	1,005.6	453.5±20.2	15.5	0.0
4	5 percent timothy [4]	7.63	38.9	5.0	194.5	261.8±9.0	65.0	5.0
5	10 percent timothy [4]	7.63	100.2	5.0	501.0	345.5±11.8	70.0	45.0
6	10 percent timothy [5]	5.62	92.3	3.0	276.9	314.0±10.6	70.0	25.0
7	5 percent alfalfa meal [6]	12.25	48.2	8.0	385.6	306.0±11.1	80.0	35.0
8	10 percent alfalfa meal [6]	12.25	110.8	8.0	886.4	389.8±13.2	40.0	5.0
9	10 percent alfalfa meal [7]	14.25	101.1	6.6	667.2	368.7±11.9	45.0	35.0
10	5 percent alfalfa meal [7]	15.56	49.6	8.0	396.8	325.9±11.1	55.0	25.0
11	10 percent alfalfa meal [7]	15.56	125.7	8.0	1,005.6	430.0±20.2	20.0	10.0
12	3 percent dried pork liver					612.6±9.1	5.0	5.0

[1] The figures in this column indicate the percentage of chicks that developed leg paralysis during the experiment.
[2] The figures in this column indicate the percentage of chicks that recovered before the end of the experiment.
[3] See table 1. Clover cut June 17; 96 hours exposure.
[4] Timothy cut June 9 (heads emerging). Cured in sun.
[5] Cut in late-bloom stage.
[6] Meal from hays grown in Colorado.
[7] Meal from hays grown in Ohio.

The data in tables 3 and 4 show a close correlation between the total consumption of vitamin G and the weight of the chick and incidence of leg paralysis. The figures in the column showing the total consumption do not include the vitamin G content of the basal ration.

The data obtained by the method used in this investigation appear to show that 10 percent of an alfalfa-leaf meal containing 13 rat units of vitamin G per gram is necessary to induce good growth and prevent the occurrence of leg paralysis.

SUMMARY AND CONCLUSIONS

Alfalfa, clover, and timothy hays cut at different times and cured under different conditions were tested for vitamin B and G with rats as the experimental animals. The results show that these hays contain significantly more vitamin G than vitamin B. The vitamin B and vitamin G content of the hays decreased as the plant matured and, in general, were correlated with the leafiness, greenness, and protein content of the plant.

The exposure of alfalfa to the weather (day and night) for 96 hours—over half of which was sunshine—without rain, did not affect the vitamin G content. Rain (0.68 inch), on the contrary, removed as much as 50 percent of this vitamin.

Timothy and clover cut early may have as high a vitamin G content as alfalfa cut later, and with a much greener color.

The method of testing for vitamin G by growth and incidence of leg paralysis in chicks compared favorably with the rat-assay method.

Ten percent of an alfalfa leaf meal containing 13 rat units of vitamin G per gram was required to induce good growth in chicks and prevent the occurrence of leg paralysis.

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