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THE VITAMIN D CONTENT OF ROUGHAGES

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MANY investigators have demonstrated that farm animals have a fundamental need for vitamin D. Roughages are the only natural feeds which supply significant amounts of this vitamin for livestock. Hence, quantitative information about the vitamin D content of roughages is of obvious importance. Despite the importance of roughages in livestock feeding only a comparatively few values for vitamin D content have been published. Johnson and Palmer (1941), Wallis (1944), Bechdel *et al.* (1936), Moore *et al.* (1948), and Newlander (1948) have provided some information but this pertains mostly to alfalfa.

Keener (1954) found that the vitamin D activity of fresh green plants was variable but of a low order. Ultraviolet irradiation of artificially dried samples showed that first cutting samples were potentially much poorer sources of vitamin D than subsequent cuttings made at similar stages of maturity. Weits (1954) has shown that roughages contain an antivitamin D factor.

The study reported here was designed to extend our knowledge about the vitamin D content of roughages available for livestock feeding by (1) obtaining samples from widely different regions of the United States, (2) by including a wide variety of roughages upon which no published information is available, and (3) by including a substantial number of samples of sun-cured hay. A rather wide-spread feeling exists that if hay is sun-cured it will be high in vitamin D, but published experimental evidence on this point is extremely meager.

Procedure

Ten agricultural colleges and experiment stations, widely scattered geographically, supplied samples of the types of roughages most prevalent or of particular interest in their respective localities. The samples covered a wide range with respect to quality; some were of the best, some fair, and others of poor quality. Final selection and preparation of the samples were left to the discretion of the cooperating colleges and experiment sta-

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tions. In all, 65 samples of roughages were studied, including 40 of sun-cured hay, 6 of mow-cured hay, 7 of winter range grass, 2 of fodder, 4 of silage, and 6 samples of dehydrated roughages. A representative sample of each roughage was obtained. A 250–350 gm. portion of each was finely ground and sent for vitamin D assay to the Laboratory of Industrial Hygiene, an independent commercial and research laboratory. For the hay samples, another portion (unground) was sent to the U.S.D.A. for government classification and grade.

TABLE 1. THE VITAMIN D CONTENT OF SUN-CURED HAY

Forage sample [a]	U.S. grade	U.S.P. units of Vitamin D per lb.[b] (air-dry basis)	Forage sample [a]	U.S. grade	U.S.P. units of Vitamin D per lb.[b] (air-dry basis)
Alfalfa			Prarie (cont'd)		
K-2	1	400	SD-5018	Sample	550
W-1 (2) [b]	1	280	SD-5019	Ungraded	510
SD-5020 (2)	1	200	Lespedeza		
SD-5021	2	690	NC-2	2	1020
			Timothy and alfalfa		
T-El Paso	2	460	NY-702 (4) [b]	1	690
W-2	2	1250	NY-777	1	910
W-4	2	830	NY-714 (3)	3	470
K-1	3	510	NY-726 (3)	Sample	900
T-Brazos	3	900	Timothy and clover		
C-SB6 (2)	Ungraded	250	NY-729	1	1110
C-4 (2)	Ungraded	80	NY-795	1	1160
C-3 (2)	Ungraded	220	NY-773	3	970
Clover			Oat and vetch		
NY-783	1	810	C-1	Ungraded	230
NY-812	2	1160	C-2	Ungraded	70
NY-799	2	1060	Brome-quack-alfalfa		
Timothy			M-4 [c]	1	790
NY-802	1	1180	Alfalfa-heavy grass		
NY-709	3	1200	Mixed hay		
NY-801	3	930	M-1	2	730
NY-764	Sample	1140	M-3	Sample	1280
Soybean			Brome grass and alfalfa		
NC-3 [c]	1	260	M-2	2	1140
NC-4 [c]	1	700	Mixed hay		
NC-5	1	260	(grass, clover, alfalfa)		
I-1 [c]	Ungraded	160	W-3	2	1210
I-2 [c]	Ungraded	330	Mixed grass (redtop,		
Peanut			clover, timothy)		
NC-1	2	1420	W-5	3	1440
Prairie			Grass hay (Brome,		
T-Blackland	2	650	blue grass, alfalfa)		
SD-5017	3	580	M-5 [c]	Sample	570

[a] Letters indicate state from which sample was obtained: C-California; F-Florida; I-Illinois; K-Kansas; M-Michigan; NY-New York; NC-North Carolina; SD-South Dakota; T-Texas; W-Washington.

[b] Multipliers and divisors for estimating the upper and lower probable limits of potency within which values would be expected to fall two out of three times in subsequent assays.

Number of assays on sample	Multiplier or divisor
1	1.315
2	1.213
3	1.172
4	1.148

Potencies were determined by single assays except as noted by the numbers in parentheses following sample designation.

All vitamin D assays were made under the direction of Miss Grace McGuire at the Laboratory of Industrial Hygiene, New York City.

[c] Mow-cured.

Estimations of the vitamin D content of each hay or roughage were made according to the Method of Assay for vitamin D essentially as prescribed in the U. S. Pharmacopoeia, Revision XIII. Statistical procedures and computations followed were those proposed by C. I. Bliss (1952). Estimates of the error to be attached to the values for potency reported in tables 1 and 2 were based upon the results of those assays conducted more than once on the same sample; i.e., the "repeatability" of the assays. (See footnote (b), table 1, for the multipliers and divisors for estimating repeatability).

Results and Discussion

The vitamin D potencies of the 40 samples of sun-cured hay and the 6 samples of mow-cured hay are shown in table 1. Samples of alfalfa, clover, timothy, soybean, peanut, prairie, and lespedeza hay are included as

TABLE 2. THE VITAMIN D CONTENT OF MISCELLANEOUS ROUGHAGES

Kind	Sample[a]	U.S.P. units of vitamin D per lb.[b]
Winter range (air-dry basis)		
Native grass	SD–5005	290
Bermuda grass		
(just after first frost–Jan.)	F–12	890
(same weathered on range–Mar.)	F–10A	290
(new growth-simultaneous with weathered sample–Mar.)	F–10B	270
Pangola grass		
(just after first frost–Jan.)	F–27(2)[b]	390
(same weathered on range–Mar.)	F–14A	350
(new growth-simultaneous with weathered sample–Mar.)	F–14B	470
Fodder (air-dry basis)		
Atlas sorgo	K–3	250
Sorghum	SD–5039	200
Silage (wet basis-as fed)		
Corn	SD–5022	80
Corn and sorghum	SD–5023	70
Hegari	T–hegari	110
Johnson grass and cane	T	90
Dehydrated roughages		
Alfalfa	K–4	280
17% alfalfa meal	C–SB3	80
17% alfalfa meal	C–SB4	110
20% alfalfa meal	C–SB5	150
Cereal grass	W–SB1	210
Cereal grass	W–SB2	170

[a] See footnote (a) table 1.
[b] See footnote (b) table 1.

well as several different kinds of mixed hay listed according to government classification and grade. The results obtained for samples of winter range grasses, fodder, silage, and artificially dehydrated roughages are shown in table 2.

For sun-cured hay, the lowest vitamin D value found was 70 U.S.P. units of vitamin D per pound in a sample of oat and vetch hay, while the highest value found was 1440 U.S.P. units in a sample of mixed grass (redtop, clover, timothy) hay. Within the limits of one kind of hay, alfalfa for instance, the range for sun-cured samples was from 80 to 1250 units per pound—a 16-fold variation. The data in table 1 show the variations found for the other kinds of sun-cured hay covered by the survey.

Samples of sun-cured hay from regions where curing conditions are generally good and sunshine abundant present some surprising and unexpected information, as for instance the values of 80, 220, and 250, respectively, for 3 samples of sun-cured alfalfa from California (see alfalfa samples, C-SB6, C-4, and C-3, table 1). The results are even more striking when compared with the vitamin D content found for dehydrated alfalfa. As shown in table 2, dehydrated alfalfa ranged from 80 to 280 units per pound which is essentially the same as for the California sun-cured alfalfa. Thus, some artificially dehydrated alfalfa was found to contain more vitamin D than some of the sun-cured samples.

These results provide substantial evidence that sun-cured hay, regardless of kind or government grade, varies over a wide range in vitamin D content. This observation is of significance because it is the vitamin D content of a specific batch of hay or other roughage, with which the livestock raiser or research man is concerned in livestock feeding. In view of the wide variations which occur, the use of an average value for the vitamin D content of a roughage may be decidedly misleading.

The mow-cured hay samples assayed from 160 to 790 units per pound which is within the range of 70 to 1440 covered by the sun-cured samples. Thus the simple knowledge that a sample of hay is mow-cured or sun-cured really indicates little or nothing with regard to its vitamin D content.

The samples of winter range grass included in this study varied from 270 to 890 units when calculated on the air-dry basis as shown in table 2. It is interesting to note that old-crop weathered Bermuda grass taken from a Florida range the last of March had a potency of 290 units while the fresh new growth taken at the same time contained 270 units on a comparable air-dry basis. Similar figures for Pangola grass were 350 and 470 units respectively. The close similarity in vitamin D content between the old weathered grass and the fresh new grass is contrary to usual assumptions.

Sorghum fodder ran fairly low, varying from 200 to 250 units per pound; silages on the wet basis as fed, 70 to 110 units; and dehydrated roughages 80 to 280 units.

The results of this study are in general agreement with the earlier observations of Wallis (1944) who stated, ". . . there are influences other than the amount of sunshine received which greatly affect the vitamin D content of the resulting hay". They also extend the work of Newlander (1948), Moore *et al.* (1948) and Keener (1954) with reference to the variability of the vitamin D content of roughages.

Calculations from Newlander's data show an average of 216 units of vitamin D per pound in a 1946 crop of field-cured mixed timothy, grass, alfalfa and clover hay. The 1947 crop from the same field which received approximately the same curing and handling contained 862 units—approximately 4 times as much.

Moore *et al.* (1948) harvested alfalfa from the same field for silage, barn-dried hay and field-cured hay. When converted to units per pound of air-dried forage, the results for the 1946 crop show 395 units of vitamin D per pound as wilted for silage. When ready for barn-drying it had received about twice as much solar radiation but contained only 263 units per pound. As field-cured hay it had received approximately six times as much solar radiation as the wilted silage. It then contained 400 units per pound—almost the same as for the wilted silage.

To study possible effects of stage of maturity on vitamin D content of forage plants, Keener (1954) cut and artificially dried samples of ladino clover, red clover, and timothy at three stages of maturity. The average vitamin D content for the three samples (one of each forage) cut June 25 was 20 units per pound; August 2, 302 units; and September 15, 650 units. In samples of alfalfa harvested from the same field and dried inside on a barn drier, Thomas *et al.* (1951) found 96 units per pound when harvested at the bud stage, 145 units at half-bloom or hay stage, and 291 units at seed stage. Differences from year to year and from field to field appear to be much greater than differences due to stage of maturity within the limits compatible with the making of a good-quality nutritious hay. As Keener (1954) points out, "The higher vitamin D content of more mature forage is of considerable scientific interest but of little apparent practical importance because of the lowered feeding value in other respects".

Some of the variation in the vitamin D content of roughages may be related to the amount of dry, dead, and brown material in the forage as cut. Thomas *et al.* (1951) reported 2,700 I.U. of vitamin D per pound for dead leaves on the alfalfa plant, 295 units for half dead or yellowed leaves, and less than 33 units for entirely green leaves. However, Newlander (1948) reported much smaller differences. His values were 0.84 units per gram (380 units per pound) for hand picked green leaves and 1.10 units per gram (500 units per pound) for brown leaves.

Keener (1954) also reported differences in the vitamin D content of first, second, and third cuttings of ladino clover, red clover, and timothy made at comparable stages of maturity and dried artificially. First cutting samples averaged 20 units per pound; second cuttings, 151 units; and third cuttings, 216 units.

The vitamin D values reported in this study and in other scientific literature could be influenced in varying degrees by the antivitamin D factor which has been found in roughages, Weits (1954). The evidence indicates that this factor is carotene. It measurably decreases healing responses of rachitic rats to known amounts of vitamin D.

Vitamin D values reported for artificially dried grass and for most hay samples after chromatography to remove the antivitamin D factor are higher than those made before chromatography. However, vitamin D values for roughages as made in this and other studies by methods which reflect the net vitamin D activity for the animal (true potency minus antivitamin D effects) are undoubtedly preferable and serve a more useful purpose in most situations where such information is employed.

It seems evident from the results of the present study and from the work of other investigators that the amount of sunshine received does not give a reliable indication of the vitamin D content of roughages. The fact that a hay is sun-cured and of good quality gives no assurance that it will rank high as a roughage source of vitamin D.

Summary

The vitamin D content has been determined in 65 roughage samples including sun-cured hay, mow-cured hay, winter range grass, fodder, silage and artificially dehydrated roughages. The results show that wide and unpredictable variations occur in the vitamin D content of roughages.

Sun-cured hay varied from 70 to 1440 units per pound; mow-cured hay, 160 to 790; winter range grass, 270 to 890; fodder 200 to 250; silage (wet basis) 70 to 110; and artificially dehydrated roughages 80 to 280 units per pound.

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