THE VITAMIN D CONTENT OF SOME SOUTH DAKOTA ROUGHAGES

G. C. Wallis and Henry Lardy
South Dakota State College

An understanding of the units used for expressing the amount or quantity of vitamin D and some idea of the method used in determining the vitamin D potency of feeds will no doubt add to the value of this paper. Unfortunately we have at present no chemical method which is accurate enough to make possible a quantitative determination of vitamin D, so it is necessary to use a bio-assay procedure. The animal most commonly used for the bio-assay is the white or hooded rat. A brood colony is maintained on a diet which is complete yet carries only a minimum amount of vitamin D. Young rats are taken from this brood colony when they reach a weight between forty-five and sixty grams which usually occurs by the time they are four weeks of age. The young rats are then fed a diet deficient in vitamin D until a rachitic condition has been established which usually takes an additional twenty-one to twenty-five days. At this time they walk with a hesitant, shambling gait and have considerable swelling and tenderness in the carpal (wrist) and other joints. A longitudinal section through the radius and ulna will show that an enlarged band of unmineralized cartilage (metaphysis) has developed at the distal end of the bones.

After the rats have reached the rachitic stage they are ready to be used in a vitamin D determination, or assay. In making such an assay it is necessary to find out how much of the material being tested contains sufficient vitamin D to start the healing process as indicated by a narrow band of fresh mineral deposit in the wide metaphysis that developed at the end of the radius and ulna during the rachitogenic period. (These conditions were illustrated by lantern slides). Preliminary tests are made with a few rachitic rats by feeding them individually on different amounts of the test material. The test material is fed for eight days as a

supplement to their regular rachitogenic ration. They are then fed the rickets-producing diet for two more days to make a total of ten days for the test period. At the end of the ten-day period the rats are killed with ether and the long bones from the fore legs are removed and placed in 10 per cent formaldehyde for a few days. The bones are then washed in running water, split longitudinally, placed in a 2 per cent silver nitrate solution for five minutes and then exposed to sunlight in a shallow dish of distilled water. The areas having a mineral deposit will turn dark. The amount of healing initiated by the vitamin D administered is evaluated with the aid of a binocular giving a 12-15 X magnification. A narrow line of fresh mineral deposit extending across the wide rachitic metaphysis is usually evaluated as a 1+ healing (illustrated by lantern slides). Values vary from zero for no healing to 4+ for complete healing.

When the preliminary tests have shown the approximate amount of the test material that will give a narrow line (1+) healing, we are ready for the final determination. For this purpose four groups of rats are assembled so that each group receives the same number of rats from any one litter. At least ten rats are placed in each group. One group is fed the amount of test material found in the preliminary trials to give about a 1+ healing. Each rat in the second group is fed slightly more of the test material and each rat in the third group is fed slightly less than the rats in the first group. Each rat in the fourth group is fed the International Standard Reference Oil of known vitamin D potency in such an amount as to give a 1+ healing. This usually takes three or four International Units of vitamin D. All the rats are carried through the procedure as outlined for the preliminary trials. The vitamin D potency is determined by comparing the average healing produced by the test material with the healing produced by the known amount of vitamin D in the International Standard Reference Oil. For example, if two grams of alfalfa produced an average healing in ten rats equivalent to that produced by four International Units of vitamin D in ten litter-mate rats, then

by simple division (4÷2) we find that one gram of alfalfa contains two International Units of vitamin D.

It has been known for some time that farm animals such as calves, pige, and poultry are subject to rickets. Recent work at our station has shown that mature, heavy-milking dairy cows also have a specific vitamin D requirement which must be met if serious consequences are to be avoided. A study of the vitamin D content of roughages is of importance because for cattle, at least, they constitute the chief source of this factor during the winter months when the amount received from the action of sunlight is very low. While we have no specific project at present for investigating the vitamin D content of feeds, considerable data have been accumulated in connection with related experiments at our station. Some of these results are shown in Table 1.

Table I

The Vitamin D Contents of some South Dakota Roughages

Material	Vitamin D per pound I.U.*
1. Alfalfa hay	500
2. Alfalfa hay	1588
3. Alfalfa hay (calculated)	2760
(a) Stems (50.6% of wt. of hay)	780
(b) Leaves (49.4% of wt. of hay)	4740
4. Prairie hay	250
5. Beet pulp	000
6. Beet pulp	000

* I.U.=International Units.

Several interesting observations are indicated by the data in the table. There is quite a wide variation in the potency of the different samples of alfalfa hay. Undoubtedly the length of time they were exposed to the action of sunlight during the curing process accounts in part for the variability noted. There is a tendency for the potency to increase with increasing exposure to sunshine during the curing process. In fact, alfalfa hay cut after dark and cured in the absence of sunlight usually shows no vitamin D at all. Alfalfa sample No. 3 was exposed to about fifteen

days of bright sunny weather before it was hauled to the barn and it shows a comparatively high vitamin D content. This figure was obtained by calculation from the potencies determined for the stems and leaves separately. A sample of this hay was collected and carefully divided into stems and leaves. The stems represented 50.6 per cent of the weight of the original hay sample and the leaves 49.4 per cent. Upon assaying these separately it was found that the leaves were six times as potent in vitamin D as the stems. If this relationship proves to be generally true then alfalfa leaf meal has added significance for the winter rations of farm animals which are subject to vitamin D deficiency such as calves, pigs, and poultry.

The prairie hay shown as No. 4 had a curing history quite comparable to the alfalfa hay No. 1 but still it was only about one half as potent in vitamin D. More tests will be necessary of course to establish a significant difference between these two kinds of hay but it would be still another important advantage for alfalfa hay should it prove to be more potent in vitamin D than other common types of hay. It was impossible to demonstrate any vitamin D in the two samples of dried beet pulp that have been assayed.

Some idea of the significance of the amount of vitamin D found in these hay samples is indicated by the fact that the minimum vitamin D requirement of calves has been reported by one station to be three hundred to four hundred International Units daily per one hundred pounds of live weight from birth to six months of age. On this basis a three hundred pound calf would require nine hundred to twelve hundred International Units of vitamin D daily. Two pounds of alfalfa hay No. 1 would supply the indicated amount of vitamin D whereas it would take four or five pounds of the prairie hay to meet the requirements of such an animal. Sufficient vitamin D would be furnished by less than a pound of alfalfa hay No. 2 and by less than half a pound of alfalfa hay No. 3. Experimental work at different stations has shown that amounts of hay comparable to those indicated above have been effective in preventing the development of rickets in calves.