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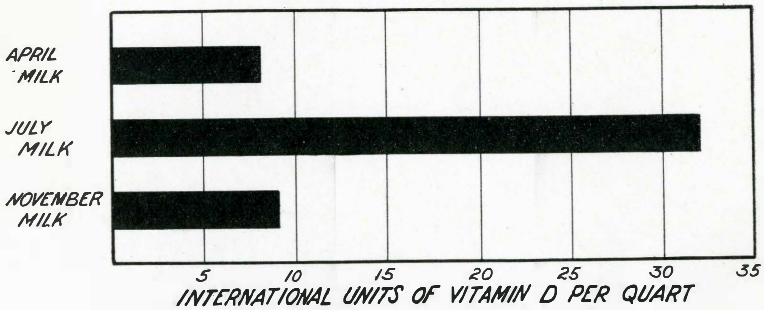

Chart 1.—The seasonal variation in the vitamin D content of milk

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One would scarcely believe that a small amount of material no larger than the head of a pin scattered evenly through 1,000 quarts of milk would make any difference in the food value of that milk. However, it has been found in recent years that there are just such small traces of materials in some of our food articles that are very important in maintaining health and vigor.

One of these materials is a substance known as vitamin D. This vitamin helps the body use the calcium and phosphorus in food for the building of strong, sturdy bones and sound teeth. Without it, children, babies, and the young of many farm animals are likely to develop rickets. In this disease the cartilage continues to develop but sufficient minerals are not deposited in the growing bones to make them rigid enough to support the weight of the body and the pressure resulting from the movement and contraction of the muscles. As a result the bones tend to become bent and twisted, and enlargements appear at the joints.

There are only a very limited number of natural foods that contain any appreciable amount of vitamin D. In fact, milk and eggs are about the only ones on the list. The well-known antirachitic effect of sunshine acting on the skin also adds to the possible sources of this factor during the seasons of the year when sunshine is available. Milk enjoys one advantage not common to other sources of vitamin D in that it furnishes liberal amounts of the bone-building minerals along with some of the vitamin which is necessary for converting these minerals into strong bones and sound teeth. Add to this the fact that milk forms a large part of the diet of children and babies during the time when they are most likely to suffer from a deficiency of vitamin D and minerals and you have several reasons why studies have been made of some of the factors which influence the amount of vitamin D in milk.

Further information as to the effect of the season of the year and the feeds given the cow on the vitamin D content of the milk produced has been secured since the publication of South Dakota Bulletin No. 296, "Vitamin D in Milk." Some of these results will be presented in this bulletin.

Experimental Methods

Managing the Herd and Collecting the Fat Samples.—Six grade Holstein cows were used in this experiment to study the seasonal effect on the vitamin D content of milk. They were housed and fed as any good dairy herd under South Dakota conditions during the season of 1935. In April, shortly before the pasture season opened, a sample of butterfat was obtained from the mixed milk of this group of cows. The cream from this milk was churned, the butter was melted, and the pure butter oil was filtered off and placed in a glass jar, which was sealed and kept in a refrigerator until the vitamin D content was determined. In a similar way, a sample of butterfat was secured in July while the cows were on pasture under normal summer conditions, and a third sample was obtained in November to represent early winter conditions.

These same cows were used during the winter and spring of 1935-36 to study the effect of vitamin D in the feed on the vitamin D content of the milk. In this work alfalfa hay was compared with prairie hay as to its vitamin D content and its influence on the antirachitic properties of the milk produced. These two roughages were selected because they are important feed crops in South Dakota and also because roughages constitute the major source of vitamin D in the ordinary rations of dairy cattle. Most grains and concentrates have not been found to carry measurable amounts of vitamin D. Sufficient alfalfa and prairie hay were obtained during the preceding summer to complete the entire experiment. Care was taken to get hay of good average quality. Each lot of hay was exposed to about the same amount of sunshine during the curing process and was otherwise handled in a comparable manner. The vitamin D content of each of the lots of hay was determined at the beginning and again at the close of the experiment.

All the cows had freshened in the summer or fall and about the middle of December they were placed in the experimental barn and kept out of direct sunlight until the experiment was completed, so their only source of vitamin D during this period was that in the feed or possibly some stored up in their body. All the cows were placed on a ration deficient in vitamin D for two months in order to use up any excess vitamin D reserves stored in their bodies and thus make them as comparable as possible in this respect. For this purpose beet pulp was used as the only roughage material as most common roughages carry more or less vitamin D. Tests on the beet pulp showed that it contained no measurable amount of vitamin D. A grain mixture composed of ground yellow corn, ground oats, corn gluten meal, salt, and a little bone meal was used to balance the ration. Vitamin A was supplied in a special concentrated form to avoid a deficiency of this important food factor. In suitable weather, the cows were turned out into a dry-lot after dark for exercise.

On February 15, after two months on the vitamin D deficient ration, the cows were divided into three groups of two animals each. Each cow in group No. 1 was given 20 pounds of alfalfa hay in place of the beet pulp, and the grain mixture was changed to one composed of equal parts of ground corn and oats. Each animal in group No. 2 was given 20 pounds of prairie hay daily in place of the beet pulp in the previous ration. The animals in group No. 3 were continued on the vitamin D deficient beet pulp ration as a control group.

Immediately previous to February 15, a sample of butterfat was obtained from the mixed milk of all six cows to be used in determining the amount of vitamin D in the milk at the time the alfalfa and prairie hay feeding was started. Additional samples were saved separately from each group of cows at successive thirty-day intervals for a period of three months. The vitamin D content of these samples was also determined to learn what effect the feeding of different kinds of hay had had on the vitamin D content of the milk.

Determining the Vitamin D Content of the Hay and Fat Samples.— Unfortunately there is no chemical method available at present which is sensitive and accurate enough to make possible a direct chemical determination of vitamin D. The amount of vitamin D in any material is determined by the use of animals, hence the procedure is called a bio-assay. The laboratory animal most commanly used for this purpose is the white rat. It is necessary to maintain a colony of old rats under special condi-

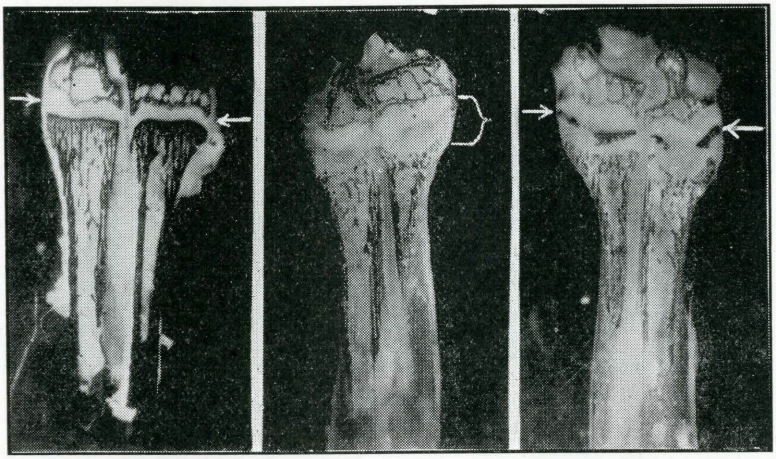
tions for producing the young animals to be used in the determination, or assay, of vitamin D. Young rats are taken from this colony at weaning time when they are three or four weeks old and are placed on a diet deficient in vitamin D. Under these conditions they usually develop rickets in about three weeks and they are then ready to be used for making a vitamin D determination, or assay.

Fig. 1.—A rachitic rat ready for use in a vitamin D assay

Fig. 1 shows the picture of a rachitic rat ready for use in a vitamin D assay. Such an animal walks with a shambling gait and has considerable swelling and tenderness in the wrist joints at the end of the long bones (radius and ulna) of the front legs. A picture of the longitudinal section through these bones from a rachitic rat is shown in Fig. 2 (b). A longitudinal section of the corresponding bones from a normal rat of the same age is shown in Fig. 2 (a) for comparison. The dark areas indicate mineral deposits in the bone while the clear areas are largely cartilage. The development of unmineralized cartilage has caused the enlargement of the end of the bones from the rachitic rat. The cartilage is indicated by the wide clear area which occupies most of the enlarged end of the bone shown in Fig. 2 (b). The normal bone shown in Fig. 2 (a) has a more dense mineral deposit and contains only a small amount of cartilage which may be seen as a narrow, white line running crosswise between the dark areas indicating mineral deposits in the head and shaft of the bones. When vitamin D is fed to a rachtic rat such as the one shown in Fig. 1 healing of the rachtic condition will be started. Among other things calcium and phosphorus will be deposited in the large area of cartilage at the end of the long bones and if just the right amount of vitamin D is fed a line of mineral deposit such as that indicated by the dark line across the wide band of cartilage in Fig. 2 (c) will be laid down in this region.

In making a vitamin D assay, it is necessary to find out how much of the material being tested contains enough vitamin D to start the healing process as indicated by fresh mineral deposits in the cartilage that formed during the development of rickets. Preliminary trials are made by taking a few rahitic rats such as is shown in Fig. 1 and feeding them individually on different amounts of the test material in an effort to find

out the approximate amount that will give a narrow line of mineral deposit such as is illustrated in Fig. 2 (c). The rats are given the prescribed amount of test material daily for eight days as a supplement to their regular rachitogenic ration. They are then fed the rickets-producing diet only for two more days to make a total of 10 days for the test period. At the end of the 10-day period they are killed with ether and the



a b c

Fig. 2.—Longitudinal sections through the ends of the long bones (radius and ulna) of the front legts of a young rat; (a) normal animal, showing a narrow band of cartilage between the head and shaft of the bone; (b) rachitic animal, showing a wide band of cartilage; and (c) a rachitic animal which has received enough vitamin D to start a line of mineral deposition in the wide band of cartilage.

long bones are removed from the front legs and placed in a 10 per cent formaldehyde solution for a couple of days. The bones are then washed in running water, split longitudinally, placed in a silver nitrate solution for 5 minutes and then exposed to sunlight. In a few minutes the areas having a mineral deposit will turn dark and may be seen under a magnifying glass as shown in Fig. 2.

When the preliminary tests have shown the approximate amount of the test material that will give a narrow line of healing such as that shown in Fig. 2 (c), we are ready for the final determination. For this purpose four groups of rats are usually assembled in such a way that each group receives the same number of rats from any one litter. If there were eight rats in a litter each of the four groups would be assigned two of the rats. Assignments are made to the groups until each group has at least ten rats in it. Each rat in the first group is fed the amount of test material found in the preliminary trials to give a narrow line of healing. Each animal in the second group recives slightly more of the test material, and each animal in the third group receives slightly less of the test material than was given to the rats in the first group. The fourth group is given a definite amount of International Standard Reference Oil of known vitamin D potency.

The amount of the Vitamin D Standard fed is selected so that it will give a degree of healing like that shown in Fig. 2 (c). All the rats are carried through the testing procedure as previously outlined for the rats on the preliminary tests. The amount of healing for each rat is assigned a numerical value on the basis that a narrow continuous line of mineral deposit as shown in Fig. 2 (c) would be called a 1+ healing. Values range from 0 for no healing to 4+ for complete healing. The average healing for the 10 rats in each group is calculated. It is hoped that the average healing induced by the vitamin D in one of the three levels at which the unknown material was fed will be equal to the average in the group of ten rats that received a known amount of vitamin D in the International Standard Reference Oil. If such is the case, then the amount of the test material that contains one International Unit of vitamin D can be readily calculated.

Vitamin D potencies are usually expressed in International Units so work in one laboratory can be compared with work in other laboratories not only in this country but also in foreign countries. One Intenational Unit is defined as the vitamin D activity of a small amount (1 milligram) of the International Standard Reference Oil. Under average conditions it usually takes about three or four International Units of vitamin D to produce a line of healing such as that shown in Fig. 2 (c). If it was found in a determination that 5 grams of a certain sample of butterfat produced the same amount of healing as 4 International Units of vitamin D then by simple division (5÷4) it is found that 1.25 grams of butterfat will contain 1 International Unit of vitamin D.

Results

Seasonal Variations.—Using the above methods, the vitamin D potency of the butterfat samples collected from the mixed milk of the six cows in the different seasons of the year was determined. Knowing the amount of vitamin D in the fat and the percentage of fat in the milk it is possible to calculate the number of International Units of vitamin D per quart for the various samples of milk. The results of these calculations are shown graphically in Chart 1. (See front cover.)

For the sample collected in April at the close of the winter feeding period it was found that 1 gram of the butterfat contained only 0.23 International Units of vitamin D. According to the Babcock test the milk contained 3.6 per cent of butterfat which would make about 35 grams, or 1.25 ounces of butterfat per quart. Multiplying the 35 grams by 0.23, the potency per gram, we find that each quart of April milk contained 8 International Units of vitamin D.

Similar tests run on the butterfat sample collected in July while the cows were handled under summer pasture conditions showed that one gram of this fat contained 0.83 International Units of vitamin D. The July milk contained 3.9 per cent of butterfat so there were 38 grams of fat making a potency of 32 International Units of vitamin D per quart for this sample of summer milk.

The November fat showed a potency of 0.28 International Units per gram. The milk tested 3.3 per cent which would make 32 grams of fat

and therefore 9.0 International Units of vitamin D per quart for this sample of early winter milk.

The Influence of the Feeds on the Vitamin D Potency of the Milk.— The results of that part of the experiment dealing with the effects of the vitamin D in alfalfa and prairie hay on the antirachitic value of the milk are summarized in Table 1. Using the methods for measuring vitamin D which have already been explained, it was found that the alfalfa hay used in this experiment contained 500 International Units in one pound and the prairie hay contained just half as much, or 250 International Units of vitamin D per pound. The beet pulp contained no measurable amount of vitamin D as indicated by the fact that the rats fed this material showed no signs of healing at the end of the assay, or test period.

TABLE 1.—The Vitamin D Content of Milk as Affected by the Vitamin D in the roughage consumed

Experimental Group	Vitamin D fed per cow daily	Vitamin D per quart of milk			
		At start of experiment	After experimental feeding for		
			1 month	2 months	3 months
	I.U.*	I.U.*	I.U.*	I.U.*	I.U.*
Alfalfa hay group	10,000	5.4	6.3	10.0	13.0
Prairie hay group	5,000	5.4	5.6	6.4	8.4
Beet pulp (control) group		5.4	Too weak to measure		

*I.U.—International Unit of Vitamin D.

According to the plans which have been previously explained, the cows were placed on a vitamin D deficient ration for two months to equalize them as far as possible with respect to possible body reserves of vitamin D. They were then divided into three groups. Each animal in group No. 1 was given 20 pounds of alfalfa hay daily and each cow in group No. 2 was given 20 pounds of prairie hay daily. The cows in group No. 3 were continued on the beet pulp ration to serve as a control group. Twenty pounds of alfalfa hay having 500 International Units of vitamin D per pound supplied 10,000 units of vitamin D daily per cow, as shown in Table 1. Similarily, the cows receiving 20 pounds of prairie hay having 250 International Units per pound were supplied with 5,000 units of vitamin D daily.

The mixed milk of all the cows just before they were divided into separate groups for feeding the different kinds of hay had a vitamin D potency of 5.4 International Units per quart as shown in Table 1. The vitamin D potency of the milk from the group receiving alfalfa hay which supplied 10,000 International Units of vitamin D daily increased from 5.4 units at the beginning of the trial to 6.3 units per quart at the end of one month of alfalfa hay feeding, 10.0 units per quart at the end of two months, and 13.0 units per quart at the end of three months. The vitamin D potency of the milk from the group receiving the prairie hay which supplied 5,000 International Units daily showed a slight increase over the three months' period. The beginning value was 5.4 units per quart which increased to 5.6, 6.4, and 8.4 International Units per quart at the end of one, two, and three months of hay feeding, respectively. The potency of the milk from the beet pulp control group was too weak to be measured. This information is summarized in Table 1.

Discussion

The information obtained in this experiment shows quite clearly that summer milk contains more of the rickets-preventing vitamin than winter milk. Similar observatioins have been reported from other experiment stations. Whether the increase in vitamin D content results from the antirachitic effect of sunshine on the cow or from the foodstuffs commonly consumed during the summer season can not be determined from the results of this investigation. However, it has not been possible to show that green pasture furnishes any more vitamin D than other kinds of feed so it is quite likely that the effect of the summer sunshine on the cow is responsible for a large part of the increased vitamin D content of the summer milk.

An interesting question arises as to how effective the vitamin D content of normally produced milk may be in meeting requirements for this factor. As vitamin D is largely concerned with the proper mineralization of the developing bone, the requirement for this vitamin is more pronounced for infants than for older children and adults. In this connection the American Medical Association states that milk with 135 International Units of vitamin D per quart will usually prevent clinical rickets when fed to infants in the customary quantities. They further state that when milk contains as much as 400 units per quart the amount of vitamin D is greater than that usually required for the prevention of rickets in normal infants so that there is a margin of safety when customary amounts of milk are taken. On this basis it is evident that even summer milk containing 32 units per quart is not potent enough to be used as the only source of vitamin D for infants. There are three methods in use now to produce milk on a commercial basis which contains increased amounts of vitamin D to meet these special conditions. Just what the vitamin D requirements may be for older children and adults is not definitely known but there is a growing feeling that a more liberal intake of vitamin D, calcium, and phosphorus than is commonly practiced would prove beneficial in improving the health of children and adults. An increased intake of vitamin D and minerals would have special significance in promoting the development of sound teeth in children and in meeting the added requirements of expectant and nursing mothers. Milk furnishes one of the few food sources available for obtaining these health-giving materials more abundantly.

It is interesting to observe that the bulk of our surplus butter that goes into cold storage is produced during the summer pasture season. As the vitamin D is associated with the butterfat portion of milk this butter is relatively high in its antirachitic value. It is a fortunate coincidence that most of this butter comes on to the market again during the winter months when vitamin D deficiencies are more likely to occur. From the standpoint of its vitamin D content, butter which goes into storage during the summer may have more value than has been recognized here-to-fore.

This experiment also furnishes some interesting information as to the vitamin D content of roughages and their effect on the antirachitic value of the milk produced when they are fed to dairy cows. The alfalfa hay obtained for this investigation had about twice the vitamin D content of the prairie hay. Whether alfalfa hay will generally be found to contain more vitamin D than prairie hay can not be decided until more tests have

been made. It would give alfalfa another important advantage as a hay crop if it proved to be generally true. It has been known for some time that calves need a certain amount of vitamin D and recent work at this experiment station has shown that milking cows also must have some vitamin D or serious consequences may be expected. The vitamin D content of roughages is an important item in meeting these requirements, especially during the winter months when sunshine is not very effective.

The alfalfa and prairie hay supplied enough vitamin D to meet the needs of the cows and to provide some for the milk which showed an increase in potency over the three months' period. The larger vitamin D content of the alfalfa hay was responsible for a considerable increase in the potency of the milk produced, while the smaller vitamin D content of the prairie hay allowed for only a slight increase in the potency of the milk above what it was at the beginning of the trial. Too much confidence should probably not be placed in the absolute values reported for the vitamin D content of the milk, especially for the samples having low values as, for instance, those from the cows receiving prairie hay. Amounts of vitamin D as small as these are difficult to measure with absolute accuracy. However, the general relationship indicated between the amount of vitamin D in the feed and the amount in the milk is undoubtedly significant. When more vitamin D is fed there is a tendency for more to appear in the milk. Thus, the milk from the cows getting 10,000 units of vitamin D daily in alfalfa hay showed more potency each month of the experiment than the milk from the cows getting 5,000 units daily from the prairie hay. And the milk from cows getting 5,000 units daily in the prairie hay contained more vitamin D than the milk from the cows getting beet pulp which supplied no measurable amount of vitamin D. As the body reserves of vitamin D decreased in the beet pulp control group the potency of the milk declined until it was too weak to measure.

Only a small amount of the vitamin D fed the cows in the alfalfa and prairie hay was recovered in the milk. Calculations based on the fat percentage of the milk and the amount of milk given indicate that about 1 or 2 per cent of the vitamin D in the feed was recovered in the milk. It seems quite likely that the amount of vitamin D recovered from any given amount in the feed will be influenced by the quantity of milk being produced and by the fat percentage of the milk, but just what the relationship may be can not be determined from the results of this experiment.

It is evident from the results of this investigation that the amount of vitamin D in milk is a variable quantity. It is influenced by the amount of vitamin D in the food eaten by the cow and probably by the effects of summer sunshine on the cow, as summer milk is ordinarily higher in vitamin D content than winter milk. As milk is one of the few food sources of vitamin D it is all the more important that every opportunity be taken to make it as potent as possible in this factor. Some of the methods of doing this have been discussed in this bulletin but the value of other possible procedures can be determined only ofter further investigation.

Summary

Six grade Holstein cows were used to study the effect of the season of the year and of the feeds consumed by dairy cows on the vitamin D content of the milk produced. A marked seasonal effect was found. Summer milk contained 32 International Units of vitamin D per quart which was about four times the amount of vitamin D contained in the winter milk produced under the conditions of this experiment.

The vitamin D in the feed eaten by the cow also had an important influence on the amount in the milk produced. When the vitamin D intake was increased the milk became proportionately richer in this important food factor. The alfalfa hay used in this experiment contained 500 International Units of vitamin D, and the prairie hay 250 International Units of vitamin D per pound. The hay was fed at the rate of 20 pounds per day so the cows getting alfalfa hay received 10,000 International Units of vitamin D daily, and the cows getting prairie hay received 5,000 units. This difference in intake was reflected in the higher vitamin D content of the milk produced by the cows receiving alfalfa hay. However, only a small proportion (between 1 and 2 per cent in this case) of the vitamin D in the feed consumed was recovered in the milk.

Because of the importance of milk as one of the few food sources of vitamin D, advantage should be taken of all factors which will contribute towards increasing the antirachitic value of milk. Some of these factors have been indicated in this bulletin while others will require further study.