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The Influence of Feed and Sunshine on the Vitamin D Content of Cow's Milk

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THE INFLUENCE OF FEED AND SUNSHINE ON THE VITAMIN D CONTENT OF COWS' MILK

by

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INTRODUCTION

For the past several years there has been a growing tendency among dairymen, particularly those located near the large cities, to keep their cows in the barn much of the time. Under such a system of management the cows receive but little direct sunshine. In some localities it is also possible to find many cows which are fed the year around on dry feeds receiving very little, if any, green feed, either as soiling crops or as pasture. From many standpoints such practices are, of course, necessary and desirable, but from the standpoint of the nutritional value of the milk produced there are those who question the desirability of such practices.

On July 1, 1927, an experiment was begun at South Dakota State College to determine the effect of direct sunlight upon the growth and development of dairy calves. Eight grade Holstein heifer calves, all less than two weeks old, were secured for this experiment. Four of these calves were reared in a shed which opened into a large outside lot on the south. They were allowed to run in this outside lot at will and consequently received an ample supply of direct sunshine. The other four heifers were reared in the west half of a shed which had two large windows in the east end. These heifers had no other source of sunshine and were not allowed outside.

The eight heifers in this experiment were bred and were kept under the conditions just given until some of them had completed their first lactation period. Breeding difficulties of an undetermined

nature were encountered and only one of the sunshine heifers and two of the no sunshine heifers became pregnant and freshened. A heifer calf from one of these no sunshine heifers was kept and was reared under the same no sunshine conditions which the previous no sunshine heifers had been subjected to. This second heifer (whose herd number is 104591) gave birth to a normal calf April 6, 1932, and is now near the end of her first lactation period. Her milk was used in the rat feeding trials to be discussed later.

The three feeding trials reported in this paper were conducted to compare the nutritional value of the milk from these no sunshine heifers with that of milk from cows which had been allowed access to sunshine. Particular emphasis was placed upon the vitamin D content of the milk in all trials. Trials were also conducted to determine, if possible, the immediate effect of direct sunlight and pasture on the vitamin D content of milk produced by cows handled in a normal way.

REVIEW OF LITERATURE

Investigators are not in agreement on the value of milk as a source of vitamin D. This is probably due to the fact that the amount of vitamin D in milk seems to vary in different localities, and in different seasons. It is quite generally conceded that milk contains some vitamin D, but the amount present is not to be depended upon to prevent rickets.

Golding and Zilva (9) report that stall fed cows on a good practical ration, including silage, gave milk with a fair amount of vitamin D whether or not cod liver oil was given in such moderate

quantities as 2 ounces daily. More cod liver oil, 6 or 8 ounces, increased the antiricketic potency of the butter decidedly, but decreased the percentage of fat in the cows' milk. A poor winter ration, without silage or hays, poorer than would ordinarily be fed, resulted in a butter practically free from vitamin D.

Outhouse, Macy, and Brekke (10) conducted a carefully controlled experiment with white rats in which care was taken to maintain a Ca:P ratio of 5 in all rations. They obtained a marked healing of ricketic lesions by feeding 50 cc. of certified cows' milk daily for 7 days. This milk had a butterfat test of 4 percent. It was obtained from a herd of 450 cows fed a concentrate mixture of grain, bone meal, and bran in addition to silage and alfalfa. These cows were kept in light, well ventilated stalls but had no direct exposure to sunshine and had little access to green pasture. The antiricketic properties of 5 or 10 cc. of milk were so slight that no beneficial effects were obtained when these amounts were fed. The degree of curing obtained with 50 cc., however, was about the same as when 5 to 6 drops of cod liver oil were fed daily.

Steenbock and associates (2) found that after rickets had been produced in rats by their ration No. 2965 it took 12 cc. of non-irradiated milk to produce a favorable response while 1 cc. of directly irradiated milk produced a favorable response. They also found that 12cc. of normal goat's milk was the minimum amount that would produce healing. After irradiation with ultra violet light 5 cc. of the goat's milk produced healing and 2 cc. produced healing after the animal had been irradiated.

Honeywell, Dutcher and Dahle (7) corroborated the results of Steenbock and associates using the same ration in a preventative type of

experiment. Luce (1), however, demonstrated that 2 to 5 cc. of milk from cows kept on pasture were potent in preventing rickets when fed to rats on the McCollum ration No. 3143, whereas 15 to 20 cc. of milk were ineffective if the cow was kept in the dark on either dry or green feed. Hess and Weinstock (8) state that when from 20 to 25 cc. of cow's milk were added to a standard ricketic ration the development of rickets was prevented.

Chick and Roscoe (4) conducted some rat feeding experiments as a continuation of those conducted by Luce (1). Chick and Roscoe, however, carried on their work after methods had been developed for determining the difference between the effects of vitamins A and D, while Luce did her work before these methods had been developed. These investigators found that the growth of rats was limited by a lack of vitamin D when milk produced by a cow kept in a darkened stall was depended upon to furnish this vitamin. This was true whether the cow was on a ration of dry feed or one of fresh green grass although their results showed a slight improvement when the latter ration was fed. When the cow was turned out on pasture a definite increase in the vitamin D content of the milk was obtained. Previous to this, however, the cow had been turned out in the open without a change in diet. This latter treatment resulted in a two to three fold increase in the vitamin D content of the milk. They also found that when 12 cc. or more of milk was fed daily to a rat that enough phosphorus was added to the ration to alter results even though the milk contained no vitamin D. To iron out any discrepancies which this factor might have introduced quantitative vitamin D determinations were also made on the butter produced during the various

feeding periods mentioned above. Results of these trials checked with those made with the whole milk but in cases where differences were found they were somewhat larger when whole milk was fed than when butter was fed. From their results Chick and Roscoe concluded that sunshine was more important in increasing the vitamin D content of milk than feed, but they obtained their greatest vitamin D potency from a combination of sunshine and pasture.

McCollum and associates (6) found that rats would be protected from rickets if 5 percent of butterfat was added to an otherwise ricketic diet. When 1 or 2 percent was included no protection was secured. They also found that different samples of butterfat varied considerably in anti-ricketic potency.

Krauss (11) found that it required 8 grams of butterfat from cows on a high protein ration and 4 grams from cows on a low protein ration to produce definite healing in ricketic rats. Krauss says, "These results indicate that cows' milk is a relatively poor source of vitamin D since about 25 cc. of milk are required to produce normal bone formation in rats on a ricketic diet". No explanation as to why a high and low protein ration would have such an effect on the milk, is offered.

It has been shown by Chick and co-workers (2) that calcium and phosphorus determinations, based on the ash content of the bones, are of no significance in vitamin D and mineral metabolism experiments since these values will remain approximately constant irrespective of diet, sex, or age of the animal.

EXPERIMENT

Comparisons of the vitamin D content of milk from cows exposed to sunlight and those not exposed to sunlight were made both with young growing pigs and with white rats.

I.-MILK AS A SOURCE OF VITAMIN D FOR GROWING PIGS

Procedure

In the feeding trial made with pigs the following procedure of feeding and weighing was followed.

The basal ration consisted of white corn 75 parts, linseed oil meal 15 parts, and wheat middlings 10 parts. This ration was fed in self feeders. All feed was weighed when it was put into the feeders and at the end of each two week period any feed which was left was removed from the feeder and weighed.

All pigs had access to a mineral mixture which was fed in a self feeder. Any mineral which had not been eaten was weighed back at the end of each two weeks the same as was done with the basal ration. The mineral mixture used was made up of 28 pounds of steamed bone meal, 50 pounds of ground limestone and 20 pounds of salt.

The pigs were weighed on three consecutive days both at the beginning and at the end of the trial. The averages of these 3 weights were taken as the initial and final weights respectively. The total weight of each lot was obtained every week and every two weeks each pig was weighed individually. All pigs were kept inside where they had no access to direct sunshine.

Whole milk was fed in metal troughs soon after milking in the morning and again in the evening. The milk was weighed and the same

amount was fed to both lots receiving milk. The pigs in each lot drank together so there was no absolute check on the amount of milk consumed by each pig. The milk which will be described as coming from "no sunshine cows" was produced by the two no sunshine heifers which freshened as described in the introduction. Most of the milk which will be described as coming from "sunshine cows" was produced by the one heifer which, as was pointed out in the introduction, was the only one of the sunshine group to freshen. When she did not produce enough milk the shortage was made up by taking milk from some of the other Holstein cows in the herd. This other milk was essentially the same because the method used in rearing the experimental sunshine heifers was that which is commonly practiced. In addition to this qualification the "sunshine cows" from which milk was taken were kept outside, in the sun, during the entire duration of the feeding trials with the pigs.

One lot of pigs received cod liver oil. The cod liver oil was mixed with the basal ration at the rate of 1 pint to 100 pounds of feed.

Care was exercised in this trial to have all lots of pigs as nearly equal in weight, breed, sex, vitality, and other observable factors as possible.

The trial was begun January 24, 1930, and continued to June 14, 1930. The pigs in Lot I and II received 4 pounds of whole milk per head daily until March 22. After this date they were fed 8 pounds per head daily until the end of the trial. Lot I received milk from the sunshine cows and Lot II received milk from the no sunshine cows. Lot III received the basal ration plus cod liver oil.

Results

Table I gives detailed information concerning the gains made and the feed consumed by the pigs in this trial.

Table I - Results Of Feeding Trial With Pigs

Basal ration plus	Lot 1 Sunshine Milk	Lot 2 No sunshine Milk	Lot 3 Cod Liver Oil
Number of pigs	5	7	5
Number of days fed	142	142	142
Average initial weight per pig-lbs.	63.6	65.8	60.0
Average final weight per pig-lbs.	218.8	184.7	218.0
Total gain per pig-lbs.	155.2	118.9	158.0
Average daily gain per pig-lbs.	1.09	.84	1.11
Total milk consumed per pig-lbs.	905.	905.	—
Average milk consumed per pig daily-lbs.	6.57	6.57	—
Total basal ration consumed per pig-lbs.	487.2	387.4	528.5
Total mineral consumed per pig-lbs.	4.8	2.9	5.5
Basal ration consumed per 100 lbs. gain-lbs.	313.9 ~~309.8~~	325.8 ~~325.5~~	334.4
Mineral consumed per 100 lbs. gain-lbs.	3.09 ~~3.05~~	2.44 ~~2.4~~	3.48 ~~3.11~~
Milk consumed per 100 lbs. gain-lbs.	583.1	761.1	—
T.D.N. consumed per 100 lbs. gain-lbs.	342.2	325.4	367.3

T.D.N. - Total Digestible Nutrients.

Table I shows that the pigs in Lot I made an average daily gain of .25 of a pound per head more than Lot 2. Lots 1 and 3 made practically the same daily gain per pig there being a small difference in favor of Lot 3. Lot 1 required the smallest amount of basal ration per 100 pounds of gain, and Lot 3 required the most with Lot 2 ranking in between.

The difference here, however, is not large and when the requirements for 100 lbs. of gain are computed on the basis of total digestible nutrients, so as to make possible a direct comparison between Lots 1 and 2, receiving milk, and Lot 3, receiving no milk, it will be noted that Lot 3 required about 75 pounds less total digestible nutrients for 100 pounds of gain than Lot 1 while Lot 2 required 41 pounds more than Lot 1. These results may be taken as an indication that both Lot and Lot 2 did not receive a sufficient amount of vitamin D. Cod liver oil, fed in the amount used in this trial, should have furnished Lot 3 an ample amount of this vitamin. The information presented in Table I indicates a higher nutritive value in the sunshine milk than in the no sunshine milk. Under the conditions of this trial it is believed that this difference is due to a larger amount of vitamin D in the sunshine milk than in the no sunshine milk.

During the progress of this feeding trial a number of notes were taken which are of considerable significance in interpreting the results. These notes, with minor changes for the sake of coherence or brevity, will be given here just as they were taken.

March 29, 1950 - LOT I. PB-TL-Tor. (a Poland barrow with ears notched to read, tip left - tip outer right) showing very decided symptoms of rickets, crampy on hind legs, does not stand up but a few minutes at a time and then is constantly shifting weight from one leg to the other.

DS-OTL-IOR. (Duroc sow, outer tip left, inner outer right) - Symptoms of rickets becoming quite evident.

The other pigs in Lot I do not show hesitation in movement or gait when taken to the scales to be weighed as do the two pigs above.

LOT 2. PB-TL-IR. Showing more evidence of rickets than last week.

PS-IL-NoR. (a Poland sow) - Getting crampy on hind legs.

PB-2OL-OR. Weak on front pasterns and getting crampy on hind legs.

The other pigs in this lot are all showing symptoms of rickets. The Duroc sow is getting weak and crampy in hind legs. None of the pigs in this lot show weakness so much as the two pigs in Lot 1.

LOT 3. The pigs in LOT 3 do not show symptoms of rickets.

April 10, 1950. LOT 1. The pigs are showing symptoms of rickets. The Poland barrow continues in bad condition without any indication of improvement.

LOT 2. PS-IL-NoR. Showing a very pronounced case of rickets. Moves with extreme difficulty. Can hardly get to feeder to eat. The joints of both front and hind legs are swollen. This condition seems to be accompanied with extreme soreness.

All the other pigs in this lot also are showing evidence of rickets as indicated by swollen joints, a knuckling over of the pasterns, and crampiness.

LOT 3. All pigs continue active and show no evidence of rickets.

April 12, 1950. LOT 1. All pigs in this lot seem to be showing more evidence of rickets than they did 4 or 5 days ago. PB-T1-TOR will not stand on his feet to eat or drink as much as he should.

LOT 2. PS-IL-NoR. Is in very bad condition. Cannot stand to eat or drink. Had to be helped to scales for weighing. An extreme case of rickets. All pigs in LOT 2 are foot sore and lying around.

April 15, 1930. LOT 2. PS-IL-NoR. Weighed and taken off experiment today. She will be kept in a room used for straw storage until there is opportunity to get a good picture of her.

April 22, 1930. Pictures taken of all hogs. The pig PS-IL-NoR from Lot 2 was put in shed where she can get plenty of sunlight.

The development of rickets in the pigs in Lot 1 seems to have been checked since the increase in the amount of milk fed. (The increase was made March 22). The development of rickets in the pigs in Lot 2 also seems to have been checked. No evidence of rickets in Lot 3.

May 1, 1930. PS-IL-NoR. showing improvement even though there has been some cloudy weather.

May 15, 1930. LOT 1. The increase of milk has checked the development of rickets but has not brought about recovery.

LOT 2. These pigs are gradually developing rickets. DS-IL-TR and CB-2OL-TOR (a Chester White barrow) move with difficulty and both will soon be down.

The difference in the way the pigs in the different Lots feel is very noticeable when they are driven to the scales for weighing. The pigs in Lot 3 run and play, those in Lot 1 come out of the pen slowly and walk as if they were sore, and those in Lot 2 object to being driven and walk to the scale with difficulty.

May 19, 1930. The Poland sow PS-IL-NoR from Lot 2 can get around without help and is making a rapid recovery.

Discussion

A study of the notes just given support the conclusions indicated in the discussion of Table 1; namely, that the cod liver oil supplied an ample amount of vitamin D for the pigs in Lot 3 and that both Lots 1

and 2 suffered from a vitamin deficiency. Lot 1, however, received more of this vitamin from the sunshine milk than Lot 2 received from the no sunshine milk. Had the pigs in Lot 3 received a protein supplement equal to the milk received by Lot 1 and Lot 2 it seems reasonable to predict that their rate of gain and their economy of gain would have excelled that of the other lots by a wider margin than was evident in this trial.

II. MILK AS A SOURCE OF VITAMIN D FOR WHITE RATS

Trial 1.

The Effect Of Sunlight And Pasture On The Vitamin D Content Of Milk

Procedure

Sixty-two white rats were received from E. G. Steinhilber, Oshkosh, Wisconsin, on May 2. These rats were all born on April 1 and weighed from 45 to 65 grams each. They all appeared healthy and thrifty.

The rats were divided into 5 groups as follows:

Group 1 - 15 rats; 4 males, 9 females

Group 2 - 15 rats; 9 males, 4 females

Group 3 - 12 rats; 8 males, 4 females

Group 4 - 12 rats; 8 males, 4 females

Group 5 - 12 rats; 8 males, 4 females

The framework of the cages in which the rats were housed was wood. The wood was covered with wire on the inside of the cages and coarse mesh wire bottoms kept the rats clean and prevented them from eating their own droppings. Each cage was about two feet square. They were placed in a corner of a room where they were amply protected from any direct sunlight. Four rats were kept in each cage with the exception of one cage of females in Group 1 and one cage of males in Group 2 in each of which there were five rats. The males and females were kept

separate but the conditions under which the rats were obtained made it impossible to secure an even distribution of litter mates. The groups were made to be as nearly equal in weight as possible at the beginning of the experiment. It was felt that division on this basis together with the comparatively large number of rats used would eliminate most, if not all, of the discrepancies which an uneven division of litter mates might introduce.

Each cage of rats was weighed daily. No attempt was made to keep individual weight records of rats in the same cage.

Steenbock's ricketic ration No. 2965 consisting of yellow corn 76 parts, wheat gluten 20 parts, calcium carbonate 3 parts, and sodium chloride 1 part was fed ad libitum.

Two cages of rats in Group 1 received only the basal ration while one cage of females in this group received the basal ration plus 8 percent of lactose. All rats in Group 2 received the basal ration plus 1 percent of Squibbs cod liver oil with a stipulated potency of 2800 units of vitamin D per 100 grams of oil. In addition one cage of males in this group received 8 percent lactose in their ration. Since some investigators have found lactose to have a favorable influence upon calcium assimilation, and since the other three groups of rats would receive lactose in the milk fed, it was deemed advisable to add lactose to the ration of one cage of rats in each of the first two groups to determine whether or not it would have any bearing upon the results obtained.

Group 3 was fed the basal ration plus milk from cow No. 104591. Group 4 received the basal ration plus milk from cow No. 4A and group 5 the basal ration plus milk from cow No. 365. Milk was fed in all cases at the rate of 12 cc. of milk per rat daily. It was fed twice each day

soon after milking. Record was kept of the milk not consumed.

The following information concerning the cows used in this trial should be given.

No. 104591 is from a well marked grade Holstein cow, which had been reared in a darkened stall, and sired by a purebred Jersey bull. Her butterfat test for May was 5.3 per cent and for June 4.4 per cent. She has spent her entire life to date in a darkened stall. Her ration during this time has consisted of silage, hay, and a grain mixture. During this experiment she was fed corn silage, alfalfa hay, and a grain mixture of 4 parts corn, 4 parts oats, 1 part bran and 1 part cottonseed meal.

No. 4A is from a purebred Holstein cow, and a purebred Jersey bull. Her butterfat test during May was 4.0 per cent and during June 4.5 percent. She was reared under normal herd conditions. During this trial she was on rye and sweet clover pasture. She was on rye pasture from April 26 until May 15. After this latter date she was on sweet clover pasture. She received no other roughage and was fed a grain mixture of 4 parts corn, 4 parts oats, and 1 part cottonseed meal.

No. 565 is a purebred Holstein. Her butterfat test for May was 4.2 per cent and for June 4.1 per cent. She was reared under normal herd conditions. During this trial she was fed a ration of alfalfa hay, corn silage, and a grain mixture made up of 6 parts corn, 3 parts oats, 4 parts bran, 3 parts linseed oil meal, 2 parts cottonseed meal, and 1 part beet pulp. She had not been on pasture since the preceeding summer and received no pasture during this trial. Beginning with April 26 she was let out in a dry lot every day where she was exposed to the direct rays of the sun. She was handled in this way until the termination of

the trial.

Distilled water was used for the rats to drink. As previously mentioned, the rats were received on May 2. They were all fed the basal ration plus all the pasteurized milk they wanted until the morning of May 5 when they were put on their experimental rations. This was done to give all rats an equal chance to recover from any ill effects of being shipped before being put upon different rations.

At the termination of the trial rats from each group were killed. Their blood was analyzed for calcium and phosphorus. The femur and tibia were freed of flesh, were allowed to dry, and were extracted with ether for 20 hours. Ash, calcium, and phosphorus determinations were made upon them. The method of Kramer and Howland (3) was used for the calcium and phosphorus determinations. The ash percentages are all expressed on the dry fat free basis. X-ray pictures were taken of the skeletons of representative rats from each group. Rats were killed on June 21 and on June 28 for these purposes.

Results

GROWTH. In comparing the growth made by the different groups only gains made to June 21 inclusive will be considered. All rats were continued on the experimental rations for one week longer than this when a second group was killed for blood samples and X-ray pictures. The irregularities introduced when a number of rats were taken away on June 21, however, made it very difficult to include any gains made during the next week and obtain an accurate average for the whole period. In as much as the rats were kept on experiment during this last week only to make it possible to check the blood analyses and X-ray pictures taken

June 21, and in as much as the rate of gain did not change, it is not considered erroneous for the purposes of comparison to omit these latter weights.

In interpreting the following table it will be necessary to keep in mind that cow No. 104591 has been reared and kept inside, that cow No. 565 was allowed sunshine but had had no pasture since the preceding summer, and that cow No. 4A received both sunshine and pasture. The following table shows the average gain per rat made during the entire period, May 4 to June 21.

Group	Ration	Gain (grams)
1	Basal	52.6
2	Basal and cod liver oil	54.7
3	Basal and milk from cow No. 104591	158.1
4	Basal and milk from cow No. 4A	162.3
5	Basal and milk from cow No. 565	155.2

The rats receiving milk gained considerably more than those not receiving milk. This, of course, should be expected. The milk fed groups show that there is an improvement in the nutritive qualities of the milk when the cow received sunshine, and that it is further improved when the cow got both sunshine and pasture. Since vitamin D is the only known pronounced deficiency in the basal ration fed it seems logical to assume that the milk produced under the different conditions contained varying amounts of this vitamin which accounted for the results obtained.

BLOOD ANALYSIS. Blood samples were taken on June 21 for calcium and phosphorus determinations. The samples taken from rats in groups 4 and 5 became clotted and consequently these results could not be relied upon. For this reason results of this first analysis have

been excluded from the data. Samples were again taken on June 28 and June 29. The results obtained follow.

Group No.	Ration	Ca Mg per 100 cc. June 28	Ca Mg per 100 cc. June 29	P Mg per 100 cc. June 28	P Mg per 100 cc. June 29
1	Basal	11.2	7.8	2.64	2.85
2	Basal and cod liver oil	14.2	7.6	4.5	6.8
3	Basal and milk from cow No. 104591	10.0	10.0	2.7	4.9
4	Basal and milk from cow No. 4A	13.4	7.2	5.15	7.0
5	Basal and milk from cow No. 565	15.2	11.0	2.64	5.5

It will be noted that the blood of all groups is low in phosphorus content which is characteristic of rats fed on a high calcium, low phosphorus ration. Little information was obtained from these results which will aid in comparing one group with another.

BONE ANALYSES. The following table shows the results of the bone analyses. All percentages are determined on the dry, fat free bone.

Group No.	Ration	Ash%	Ca%	P%
1	Basal	57.5	10.7	6.4
2	Basal and Cod Liver Oil	55.8	18.27	9.25
3	Basal and Milk from cow No. 104591	56.5	18.75	9.7
4	Basal and Milk from cow No. 4A	58.7	19.57	10.6
5	Basal and Milk from cow No. 565	57.9	19.62	10.2

A study of these results substantiate the statements made when discussing growth. The highest bone ash content was obtained by feeding milk from the cow on pasture, the next highest by feeding milk from the cow kept in the sun but not allowed pasture, and the lowest with milk from the no-sunshine cow. The fact that the bones of all milk fed groups have a higher ash, calcium, and phosphorus content than the cod liver oil group can probably be accounted for by the more favorable mineral balance

Obtained in the ration when milk was added. It was also observed that the rats in the cod liver oil group ate sparingly of their ration during the early part of the trial. This may have had some bearing on the results.

X-RAY STUDIES. X-ray pictures of the entire skeleton of representative rats of each group were taken on June 21 and June 28. Both groups of pictures lead to the same conclusions. The bones of Group 4 appear to be denser, the joints cleaner, and the ends of the bones more clearly defined than in any of the other groups. It is impossible to denote much difference between Groups 3 and 5. In some of the joints, however, there seems to be indications that slightly better calcification may have taken place in Group 5 than in Group 3. Differences noted between these two groups, however, are too slight to justify definite statements.

The pictures of Group 2 reveal bone development about equal to that of Groups 3 and 5. All pictures of this group, however, reveal crooked spinal columns which it is difficult to account for. It is probable that the small amounts of feed eaten in the early part of the trial is responsible for this condition. The pictures of Group 1 all show definite and severe ricketic symptoms.

Discussion

It was stated in the procedure that one pen of rats in each of the Groups 1 and 2 was fed lactose in addition to the rest of their ration. Results obtained with lactose did not differ from results obtained without lactose in each of these groups. These data were therefore omitted as of no significance in making the comparisons desired in this trial.

The following table will briefly sum up the results of this trial by showing the ranking of each group in the different phases of the

results. The group showing the most favorable results in each case is listed at the top of the column with the others coming in their proper order.

Gain per rat		Ash in Bones		Bone development by x-ray
Group	Gain Gms.	Group	% Ash	
4	162.3	4	58.7	4
5	155.2	5	57.9	5) differences slight
3	138.1	3	56.5	3) and indistinct
2	54.7	2	54.7	2
1	52.6	1	37.5	1

The rats in Group 1 began to show ricketic symptoms after 3 weeks and all had pronounced rickets after 4 weeks on the basal ration.

The rats of Group 2 were perhaps the most active of any of the groups and showed no ricketic symptoms but were thin in appearance and their growth was unsatisfactory. Reference has been made to their crooked spines.

Two days after the experiment was completed two of the rats remaining in Group 3 died. Before death they entered a state of mild convulsions, became unable to get to their feet, and shortly before death their muscles became rigid resulting in a state of tetanus. The cause of this has not been determined.

The amount of milk not consumed was small and was practically the same in all milk fed groups. Consequently these data were omitted as of no importance in interpreting the results.

From the results which have been presented it may be concluded that the milk from cow No. 4A (receiving both pasture and sunshine) contained more Vitamin D than any of the other milk fed. Indications are that the milk from cow No. 365 (receiving sunshine and no pasture) was next

best in this respect. The poorest results with the milk fed groups were obtained with milk from cow No. 104591 (kept in a darkened stall) although the difference between this milk and that from 365 was very slight.

Trial 2

A Comparison Of The Vitamin D Content Of Whole Milk And Skim Milk

Introduction

The results of Trial 1 indicate a difference in the nutritive value of milk produced by cows handled in the different ways as outlined in the presentation of that trial. The fact that the only known pronounced deficiency in the basal ration was vitamin D indicates that the milk from the different cows varied in the amount of vitamin D present. It must be borne in mind, however, that when milk is added to a ration many things besides vitamin D are added. In addition to the fat there is the protein, mineral, and lactose of the milk, all of which are active nutrients and any of which might have had an influence on the results obtained. It will be recalled, however, that no different results were obtained when lactose was added to the ration of Groups 1 and 2 in Trial 1.

Skim milk contains everything found in whole milk except the butterfat. The butterfat contains both vitamins A and D, but since the basal ration used in these trials contains an ample supply of vitamin A, this latter vitamin should not be of any importance in determining the results. A comparison, therefore, between the results obtained with feeding whole milk and skim milk should establish whether or not the results obtained in Trial 1 were due to variations in the amount of vitamin D or of some other nutrient in the milk.

Procedure

To make such a comparison nineteen white rats, 35 to 45 days old, which had been grown on a ration of cracked corn (yellow), whole wheat, skim milk, and bread were divided into 3 groups as follows:

Group 1 - 6 rats - received the Steenbock ration No. 2965. (same basal ration used in trial)

Group 2 - 6 rats - received the basal ration plus skim milk.

Group 3 - 7 rats - received the basal ration plus whole milk.

The skim milk was caught from the separator in the College Creamery as the milk from the general herd was separated. The whole milk came from the milk bottled in the College Creamery but was taken before pasteurization.

Ten cubic centimeters of milk per rat was fed once a day. The rats were kept in individual cages so that a record could be kept of any milk refused.

Daily weights of all rats were kept.

Distilled water was supplied in glass dishes.

At the end of 5½ weeks two rats were killed from each of the milk fed groups and ash determinations were made on the femur and tibia by the following method. After the bones had been freed of flesh as much as possible they were allowed to dry until the remaining fleshy material could be removed and until they could be well pulverized in a mortar. After pulverization they were extracted with ether in a Soxklet's tube for 20 hours and dried to constant weight at 100° C. They were then ashed to constant weight in an electric furnace at red heat. The percent ash was determined on the dry, fat free basis.

At the end of 7½ weeks two rats from each of the three groups

were killed and ash determinations were made on the femur and tibia as before.

Results

At the end of 4 weeks the rats on the basal ration (Group 1) all showed definite ricketic symptoms. The other two lots showed no such symptoms and remained in a healthy, thrifty condition to the end of the trial.

BONE ANALYSIS. The following tabulation shows the results of the bone analysis made. All percentages are expressed upon a dry fat free basis.

	Basal % Ash	Whole milk % Ash	Skim milk % Ash
After 5½ weeks	No analysis	51.9	45.9
After 7½ weeks	33.3	58.9	49.2

GAIN IN WEIGHT. The following tabulation shows the average gain in grams made by the three groups.

	Basal grams	Whole Milk grams	Skim Milk grams
Average initial weight per rat	67.5	69.0	70.3
Average final weight per rat	106.0	121.7	128.0
Average gain per rat	38.5	52.7	57.7

Discussion

The rats on the basal ration were decidedly inferior to both the other lots in the amount of gain made, in the amount of ash developed in the bones, and in general appearance and thriftiness. The whole milk contained enough vitamin D to cause a distinctly superior deposition of ash in the bones when compared with skim milk. The addition of skim milk to the basal ration, in the amount used in this

experiment, apparently changed the calcium phosphorus ratio and improved the quality of the protein sufficiently to prevent the development of active rickets and to promote more favorable growth than was possible on the basal ration. It is also possible that there may have been enough vitamin D present in the small amount of butterfat contained in the skim milk to have had a little influence on the results. Although the skim milk fed rats showed more gain than the whole milk fed rats the difference is hardly large enough to be considered significant.

Trial 5

A Comparison Of The Vitamin D Content Of Skim Milk, Of Whole Milk From A Cow Reared In A Normal Way, And Of Whole Milk From A Cow Reared Without Sunshine

Procedure

Fifteen white rats were carried on the Steenbock ration No. 2965 for 5 weeks. After this time they were started on this milk feeding trial. At the end of the 5 week period very slight symptoms of rickets were beginning to develop. These rats were divided into 5 groups of 5 rats each and were fed as follows:

Group 1 - Steenbock ration 2965 plus skim milk.

Group 2 - Steenbock ration 2965 plus milk from Cow 8A.

Group 3 - Steenbock ration 2965 plus milk from cow 104591.

The cow 104591 is the same cow used to furnish milk for one of the groups of rats in the first trial. (The reader is referred to the discussion of that trial for her history and description). The cow 8A is from a purebred Holstein cow and a purebred Jersey bull. She was reared by South Dakota State College under usual herd conditions. The milk from cow No. 104591 had an average butterfat test of 5.5 percent during the

trial while the milk from cow 8A averaged 3.8 percent fat.

The rats were fed and handled in exactly the same way as in the second trial.

It was planned to kill one rat from each group and make an ash determination on the femur and tibia at the end of each week until all rats were gone. This schedule was followed with the exception of the second week which was unavoidably left out. Consequently the milk feeding period lasted six weeks instead of five as planned.

Results

BONE ANALYSIS. The percent of bone ash is expressed on the dry fat free basis as in the previous two trials and the determinations were made by the same procedure. The following tabulation shows the results obtained.

	Skim-milk % Ash	Milk from Cow 8A % Ash	Milk from Cow No. 104591 % Ash
First week	32.8	40.4	35.5
Third week	31.5	41.2	35.7
Fourth week	32.5	35.8	37.9
Fifth week	36.0	35.5	34.1
Sixth week	34.8	31.8	30.2

GAIN IN WEIGHT. With the plan used in this trial it is impossible to give an average daily gain per rat for the three groups. The following tabulation will show the gain made by each rat from the time milk feeding was started until the rat was killed.

	Skim-milk Grams	Milk from Cow 8A Grams	Milk from Cow No. 104591 Grams
First week	1	5	9
Third week	17	30	29
Fourth week	8	0	21
Fifth week	35	38	22
Sixth week	0	12	45

The rat reported at the end of the sixth week in the skimmilk group died 4 days before the end of the week. It weighed exactly the same as when milk feeding started, but ten days before death it showed a 20 gram gain. The rat killed at the end of the fourth week in the group receiving milk from Cow 8A showed no gain at any time during the milk feeding period. The writer believes it would have died before another week had passed. This rat and the one that died in the skim-milk group showed severe symptoms of rickets. The rat reported at the end of the sixth week in the group receiving milk from Cow 104591 was the most active and the most difficult to handle of any of the rats during the entire feeding period.

Discussion

It is quite evident from the results of this trial that none of the milk used contained enough vitamin D to bring about a recovery from the ricketic conditions which had developed in the rats before milk feeding was started. In all cases the percentage of ash contained in the bones is but little, if any, higher than that obtained in the two previous trials with the rats on the basal ration only. The

results of the bone analyses indicate that the milk from cow 8A may have been slightly more anti-ricketic than the milk from cow 104591. The differences, however, are too small and irregular to justify definite conclusions.

It will be noted that growth was unsatisfactory in all cases.

CONCLUSIONS

From the feeding trials with pigs and with white rats which have been discussed the following conclusions are justified.

1. Whole milk contains vitamin D, but the amount is not constant for all milk.

2. Exposure of the cows to direct sunshine increases to some extent the amount of vitamin D in the milk produced.

3. A combination of pasture and direct sunshine will result in the production of milk of a higher vitamin D content than direct sunshine alone.

4. The rearing of dairy cows under conditions depriving them of exposure to direct sunshine apparently does not cause them to produce milk lower in vitamin D than milk produced by cows reared in a normal way but kept inside during their lactating period. The third trial with rats provides evidence for this statement although further trials of the preventative rather than of the curative type should be conducted to definitely establish this point.

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