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EFFECT OF PHOSPHORUS DEFICIENCY ON METABOLISM OF CAROTENE AND VITAMIN A BY BEEF COWS

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THE metabolism of carotene and vitamin A in domestic animals has been shown to be influenced by several compounds. Reference to investigations of the importance of these compounds has been made in a previous publication (Gallup *et al.*, 1951) dealing with protein supplements and urea.

The possibility that phosphorus affects the utilization of carotene was suggested by an observed inverse relationship between the inorganic phosphorus and carotene content of the blood plasma of Hereford cows maintained on adequate- and low-phosphorus rations (Ross and Gallup, 1949). This inverse relationship was also apparent in Hereford steers fed adequate- and low-phosphorus rations and known amounts of carotene (Thomas, Gallup and Ross, 1951). Although inefficient conversion of carotene to vitamin A was indicated in the results with steers, supporting evidence was not obtained in similar studies conducted with lambs (Thomas, 1951); vitamin A levels in the blood and liver of lambs fed nine times their minimum requirement of carotene were unaffected by phosphorus deficiency. Further work, as reported in the present paper, was therefore initiated with beef cows. The development of a biopsy technique (Van Arsdell, Whitehair and MacVicar, 1951) has made it possible in this latter work to secure liver samples for carotene and vitamin A determinations at regular intervals during the experimental period.

Procedure

Nine bred grade Hereford cows averaging 11 years of age were used in this study. They were grazed during the summer and fall of 1950 on native grass supplemented with a mineral mixture consisting of three parts bonemeal and one part salt. On November 16, 1950, the cows were placed in dry-lot and fed a low-phosphorus ration composed of 1.5 lb. corn gluten meal with prairie hay, free choice. The

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ration provided an average daily intake of about 0.6 gm. of phosphorus per 100 lb. liveweight and 113 mg. of carotene. Blood samples were taken by venous puncture at regular intervals, and at the end of 71 days, which was estimated to be about 4 to 8 weeks before parturition, liver samples were obtained by biopsy technique (VanArsdell *et al.*, 1951). The blood plasma was analyzed for carotene and vitamin A (Kimball, 1939) and inorganic phosphorus (Fiske and Subbarow, 1925). Liver samples were analyzed for carotene and vitamin A (Gallup and Hoefer, 1946). The results of these analyses were used as a guide in dividing the cows into two comparable lots. From this time until the end of the experiment, four cows making up Lot 1 were continued on the phosphorus-deficient ration, and five cows as Lot 2 were fed the same ration supplemented with sufficient bonemeal to provide an intake of approximately 3 gm. of phosphorus per 100 lb. liveweight. Calcium carbonate was used to equalize calcium intake. The carotene content of the ration was adjusted during the experiment at the desired level by substitution of beet pulp and cottonseed hulls for part of the prairie hay. Carotene intakes were kept relatively high during the first part of the experiment and low during the last part.

At the time of calving, the cows were placed in individual pens so that carotene and phosphorus intakes could be individually controlled. Blood, liver and colostrum samples were obtained from each cow at parturition. Thereafter blood samples were taken at weekly intervals for 4 weeks, and at the 6th, 8th, 12th, 16th, and 20th week of lactation; milk samples were taken at the same time up to and including the 8th week; liver samples were taken at the 4th, 8th, 16th, and 20th week of lactation. Milk samples were kept frozen until analyzed for carotene and vitamin A (Boyer *et al.*, 1944). Liver samples were wrapped in cellophane and frozen until analysis could be made the following day.

Although it was originally planned to discontinue the experiment at the 8th week of lactation, two cows in each lot calved at an earlier date than the others, making it possible to extend the observations with these four cows through the 12th week. The results were of sufficient interest that observations were continued beyond the 12th week to 20 weeks. From the 12th to the 20th week, the ration for these four cows was made extremely low in carotene by complete replacement of prairie hay with beet pulp.

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Results and Discussion

The pertinent data from these studies are summarized in table 1 showing the carotene and vitamin A content of the blood plasma, liver and milk of the cows in relation to phosphorus intake and phosphorus levels in the blood.

The cows in the high-phosphorus lot required no assistance at the time of calving, and all calves appeared to be strong. In the low-phosphorus lot one cow required assistance at calving, and another lost her calf at calving time. The latter cow was given a Holstein calf to suckle. All calves in the low-phosphorus lot were weak at birth.

At the time the cows were divided into two lots and phosphorus supplementation started for Lot 2, the average plasma-inorganic phosphorus level of both lots was 2.8 mg. per 100 ml. of plasma. At parturition, the average was 4.0 mg. for the low-phosphorus lot and 7.9 mg. for the high-phosphorus lot. Eight weeks after parturition, these values were 2.6 and 5.6 mg., and at 20 weeks, 2.8 and 4.2 mg., respectively. It is evident, therefore, that plasma phosphorus in cows of Lot 1 was being kept at a low level by the low-phosphorus intake.

Plasma Carotene and Vitamin A

Initially, the cows of Lot 1 and Lot 2 had similar plasma carotene values of 194 and 190 mcg. per 100 ml., respectively. Differences in these values became greater as the experiment progressed to 6 weeks after calving. At parturition, plasma carotene was 180 mcg. per 100 ml. in the cows of Lot 1 and 165 in those of Lot 2. During the first 4 weeks after calving, the average values were 158 for Lot 1 and 138 for Lot 2; this difference approached statistical significance. During the next four-week period, when carotene intake was greatly reduced for both lots, consistent but small differences in plasma carotene between lots were still apparent. Although carotene values at each bleeding date from the time of phosphorus supplementation until 8 weeks after parturition were higher for the cows of Lot 1 than for those of Lot 2, these differences were never greater than 31 mcg. In view of the wide range of carotene values associated with normal performance of beef cows (Long *et al.*, 1952) differences of this magnitude, despite their reality, are not usually regarded as having physiological importance. Carotene values for the two cows in each lot which received 40 mg. of carotene per day during the 8 to 12 week period after calving, and only 11 mg. thereafter, decreased

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TABLE 1. CAROTENE AND VITAMIN A CONTENT OF PLASMA, LIVER AND MILK OF BEEF COWS ON LOW- AND ADEQUATE-PHOSPHORUS RATIONS [1]

Lot No. and Phosphorus Level		Number of Cows	Phosphorus Intake,[2] Daily gm./100 lb., Live Weight	Carotene Intake [2] Daily mg.	Plasma Inorganic Phosphorus mg./100 ml.	Plasma		Liver		Milk	
						Carotene mcg./100 ml.	Vitamin A mcg./100 ml.	Carotene mcg./gm.	Vitamin A mcg./gm.	Carotene mcg./100 ml.	Vitamin A mcg./100 ml.
Initial (at time of allotment for phosphorus supplementation)											
1	Low	4	0.62	113	2.8	194	8.9	11.9	273.1	.....	.....
2	High	5	0.62	113	2.8	190	9.9	10.1	197.4	.....	.....
Parturition											
1	Low	4	0.60	91	4.0	180	9.2	9.7	260.8	126.7	195.1
2	High	5	3.25	85	7.9	165	9.3	8.5	161.4	169.1(89.8)[3]	276.0
First 4 weeks postpartum											
1	Low	4	0.71	116	3.0	158	7.5	9.4	276.3	4.9**	6.3
2	High	5	3.38	116	6.6	138	7.4	7.5	155.3	3.4	7.4
4–8 weeks postpartum											
1	Low	4	0.86	36	2.6	129	12.7	6.3	173.4	3.4	3.0
2	High	5	3.25	38	5.6	126	12.9	6.8	132.7	1.4	5.4
8–12 weeks postpartum											
1	Low	2	1.00	40	3.8	49	16.0	3.9	77.5	.....	.....
2	High	2	3.77	40	4.4	82	22.4	2.3	21.9	.....	.....
12–20 weeks postpartum											
1	Low	2	0.73	11	2.8	34	25.3	3.1	71.7	.....	.....
2	High	2	3.72	11	4.2	27	21.4	1.6	22.7	.....	.....

** Significant at 1% level over Lot 2.

[1] Average of individual values. Samples of blood and milk were collected at parturition, each week for 4 weeks post-partum and at the 6th and 8th week of the 4–8 weeks period. Blood was collected at the 12th week of the 8–12 weeks period and at the 16th and 20th week of the 12–20 weeks period. Liver samples were taken at the end of each period.

[2] Phosphorus and carotene intake values are for 71 days preceding allotment, 56 days preceding parturition and for the number of weeks indicated in each subsequent period.

[3] Average with one erratic value of 486 mcg. omitted.

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markedly in a manner that was unrelated to phosphorus intake or plasma phosphorus levels.

The average initial vitamin A content of the plasma of cows of Lots 1 and 2 was 8.9 and 9.9 mcg. per 100 ml., respectively. At the time of parturition, the values for both lots were slightly over 9 mcg., but during the 4 weeks thereafter they dropped to about 7.5 mcg. Despite a lowered carotene intake in the next four-week period, plasma vitamin A increased to over 12 mcg. Further, the vitamin A content of the plasma of the two cows in each lot that were continued on experiment increased markedly and reached maximum average values of 25.2 mcg. for Lot 1 and 31.2 for Lot 2 at 16 weeks; at 20 weeks these values were 25.3 and 21.4, respectively. During the latter period, the cows received only 11 mg. of carotene per day. The only indication that these increases resulted from mobilization of tissue reserves during the last stages of vitamin depletion is contained in the results of the liver analysis and in the observation of typical deficiency symptoms (night blindness and muscular incoordination) in one of the cows of Lot 2 after the 16th week. With respect to phosporus intake and plasma vitamin A levels, the results reveal no definite relationship or trend.

Liver Carotene and Vitamin A

The initial amount of carotene in the liver was about 18 percent higher in the cows of Lot 1 than in those of Lot 2. Liver carotene values decreased in both lots at parturition and during the 4 weeks thereafter. These decreases, which amounted to 2.5 and 2.6 mcg. per gram, were only slightly less for the low-phosphorus lot than for the high-phosphorus lot, on a percentage basis. An abnormally high value of 10.2 mcg. was obtained with one cow in the high-phosphorus lot at the end of the 4 to 8 week period after calving. At 16 weeks the two cows that were continued on experiment in each lot had lost 66 percent of the carotene present in the liver previous to parturition; at the end of 20 weeks about 75 percent had disappeared. These changes were unrelated to phosphorus intake.

Of particular interest are the liver vitamin A values which in the low-phosphorus lot stayed above 260 mcg. per gm. through parturition and for 4 weeks thereafter, but decreased in the high-phosphorus lot from 197.4 to 155.3 mcg. during the same period of relatively high carotene intake. These unexpected results lend no support to the idea that phosphorus deficiency may adversely affect the conversion of carotene to vitamin A and its storage in the liver. They may indicate,

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however, that phosphorus deficiency slows down the mobilization of vitamin A from liver stores and its transfer to milk during early lactation. At 8 weeks after calving, the cows in both lots had lost about an equal amount, 34 percent, of their initial liver stores of vitamin A. The low carotene intake during this period and the subsequent periods resulted in a further loss, and by the 20th week the two cows in Lots 1 and 2 had lost 69 percent and 83 percent of their initial liver vitamin A, respectively.

Milk Carotene and Vitamin A

Colostrum from one of the cows of Lot 2 contained over four times the amount of carotene found in any of the other samples. With this one value of 486 mcg. omitted from the average, there was a definite trend for both the milk and colostrum from the cows on the low-phosphorus ration to be higher in carotene, but lower in vitamin A, than that from the cows fed adequate phosphorus. Differences in carotene of milk between the low- and high-phosphorus lots were highly significant (Snedecor, 1946) 4 weeks after calving and closely approached significance during the 4 to 8 week period. Differences in vitamin A of milk approached significance only during the 4 to 8 week period. Further work with dairy cattle seems warranted.

Summary

Nine bred Hereford cows, after a preliminary period of low-phosphorus intake, were used to study the effect of phosphorus deficiency on carotene metabolism. For an average period of 8 weeks before calving and 8 weeks or longer thereafter, four of the cows were continued on the low-phosphorus ration and five were given a phosphorus supplement. All of the cows received equal but limited amounts of carotene supplied by prairie hay. Determinations were made of the phosphorus content of the plasma and the carotene and vitamin A content of the plasma, liver, and milk of the cows at regular intervals.

Average plasma-carotene levels were generally higher in the phosphorus-deficient cows than in those fed adequate phosphorus; these differences approached statistical significance (5% level). Plasma-vitamin A levels appeared to be unaffected by phosphorus deficiency. At 8 weeks after calving both groups of cows had lost similar amounts of carotene and vitamin A from the liver. Milk from the phosphorus-deficient cows contained more carotene but less vitamin A than that from the cows fed adequate phosphorus.

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