CAROTENE CONTENT OF THE BLOOD PLASMA OF DAIRY CATTLE IN RELATION TO VITAMIN A DEFICIENCY*

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INTRODUCTION

A deficiency of vitamin A in rations of dairy cattle leads to a general breakdown in health, poor growth, and unsuccessful reproduction. Early symptoms of a deficiency involve vision; the eyes "water" and lesions of the cornea frequently develop. Other manifestations of injury are respiratory disorders, bloating, general weakness, excitability and occasional spasms. A permanent type of blindness may develop from injury to the optic nerve. In mature cows, a border line deficiency leads to reproductive failure. Pregnant cows often produce weak and dead calves. The minimum amount of vitamin A required to prevent these losses is being investigated in a group of grade Jerseys at the Oklahoma Agricultural Experiment Station (Kuhlman and Gallup, 1941)**. All of the symptoms noted above have been observed at one time or another in this group of animals.

Sources of vitamin A. Vitamin A may be supplied in the ration in any one of several forms; as carotene and/or cryptoxanthin, natural yellow pigments present in roughage and vegetable products, and as vitamin A or one of its esters. Under the usual conditions of feeding, however, carotene is the principal, frequently the only, source of vitamin A in dairy rations. True vitamin A, being found in animal products, is seldom present. Cryptoxanthin, which is present in yellow corn, contributes only a small amount toward the total vitamin intake. Carotene, however, is abundant in green pasture grasses, alfalfa and cured green hay. Although three forms of carotene exist, only beta-carotene, the one which predominates, need be considered here.

Plasma Carotene. Animals cannot synthesize carotene but must rely upon feed sources. After ingestion, much of the carotene is absorbed, some is probably destroyed, and some passes through the digestive tract unchanged. The absorbed carotene is converted into vitamin A by the liver. The extent of this conversion depends largely upon the animal species. In cows the process is incomplete, and only a part of the absorbed carotene is changed to vitamin A; a small amount is stored as such in the tissues and some is secreted along with vitamin A in the milk. Cows show individual as well as breed differences of a quantitative nature in their metabolism of carotene.

The level of carotene in the blood plasma of cows, although modified by factors just mentioned, is indicative of carotene intake and should, therefore, be of importance in studying vitamin A requirements. This paper reports, in part, the progress that has been made in determining the blood plasma carotene of cows receiving carotene in amounts above and below the minimum amount required for successful reproduction.

EXPERIMENTAL

The experimental cows were grade Jerseys which received different levels of prairie hay as the only source of carotene in the ration. The levels

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** For general discussions of the subject, consult the papers of Guilbert and associates, J. Nutri. 8, 25 (1934); 10, 409 (1935); 19, 91 (1940); Moore, L. A., J. Nutri. 9, 533 (1934); 17, 443 (1939); J. Dairy Sci. 22, 863 (1939); Converse and Meigs, Proc. Amer. Soc. Animal Prod., 32nd meet., 67 (1940).

at which the hay was fed represented approximately a full, a fifty percent and a twenty-five percent normal hay allowance. This manner of feeding simplified the feeding schedule, but made no allowance for seasonal changes in the carotene content of the hay, consequently, carotene intakes varied considerably whenever different shipments of hay were fed. The other constituents of the ration, which were devoid of vitamin A and carotene, were molasses beet pulp, cottonseed meal, and minerals. A carotene concentrate was used in the ration of a few animals that received no hay.

The carotene content of the hays fed was determined on composite samples at monthly intervals (Peterson, Hughes, and Freeman 1937). The carotene content of the blood plasma of individual animals was determined at monthly, or more frequent, intervals during complete gestation and lactation periods. The method employed was similar to that described by Moore (1939). Blood was drawn from the jugular vein three or four hours after the morning feeding and collected in 50 ml. flasks containing lithium citrate. The cells were thrown down by centrifuging in 50 ml. tubes for 30 minutes. Ten ml. of the clear plasma were added to 10 ml. of 95 percent alcohol in a 40 ml. heavy-wall centrifuge tube. The necks of these tubes are constricted to take a No. 0 stopper. After precipitation of the proteins and shaking, the tubes were chilled in ice water and 10 ml. of petroleum ether, having a boiling range of 80° to 90° C., were added to extract the carotene. All of the carotene was taken up by the petroleum ether by shaking thoroughly and allowing the tubes to stand for about 30 minutes. Ten ml. of cold water were added and the tubes centrifuged for about 10 minutes. In this manner, a clear upper layer of carotene in petroleum ether was obtained. A portion of the upper layer was removed with a 2 ml. Ostwald-Van Slyke pipette with stop cock, and its depth of color compared in a semi-micro colorimeter with that of 0.02 percent potassium dichromate. The color of the dichromate solution was taken as equivalent to that of a 0.0001 percent solution of carotene in petroleum ether. Colorimetric determinations were frequently checked by spectrophotometric determinations.

RESULTS

The carotene content of prairie hay, harvested in 1938 and fed from October of the same year until June of 1939, decreased during this period from 30 to 9 p. p. m. Likewise, it decreased from 31 to 12 p. p. m. in hay harvested in 1939 and fed from August 1939 until February 1940. Another shipment of hay, harvested in 1939 and fed from February until September of 1940, showed a decrease from 24 to 18 p. p. m. An unusually good grade of hay, harvested in 1940 and fed during the past five months, has decreased in carotene from 52 to 40 p. p. m. These figures serve to emphasize, not only the yearly variation in the carotene of prairie hay, but also the loss that occurs during winter and spring storage.

Data on the blood plasma carotene of eleven cows, which received different levels of prairie hay during complete lactation periods, are now available and are summarized in Table I. Carotene intakes were calculated from monthly feed intake records. The variations in carotene intake during the course of lactation were due largely to seasonal changes in the carotene content of the hay. Despite these variations, the results fall into three fairly well-defined groups. Cows receiving a 25 percent level of hay maintained an average plasma-carotene content of 80 to 167 micrograms per 100 ml. For those receiving the 50 percent level, the average plasma carotene ranged from 175 to 201, and, for those receiving a full hay ration, from 250 to 479 micrograms per 100 ml. Individual variations in some instances were large, as shown by a comparison of the average plasma carotene of No. 604-2 with that of No. 338-3, and No. 1338-1 with that of No. 3313-1. Carotene intakes preceding and during lactation do not account for these differences. It is evident from these and other data that some cows during lactation

naturally maintain a higher level of carotene in the plasma than do others. It may be noted that No. 436, despite a lower average carotene intake during the first lactation period, maintained a higher average plasma carotene during the first period than during the second. This is an unusual case for which no explanation is offered. Experiments in progress indicate a close relationship between plasma carotene and carotene intake of the same individual during successive lactations.

Detailed data, not presented here, on carotene intake, plasma carotene, and stage of lactation, have failed to reveal any consistent change in plasma carotene with advance in lactation. These changes, if they occur, may be brought out in data now being secured with cows on a constant carotene intake. It was found, however, that plasma carotene usually dropped immediately after the cows calved. Figures for 19 calvings are available. The plasma carotene determinations were made during the month preceding calving and again within several days after calving. In 13 instances, plasma carotene decreased, in one instance there was no change, and in 5 instances it increased. In those cases in which there was an increase in plasma carotene, the blood sample had been taken two weeks or more after calving; in other cases the sample was usually taken within the first week. Further work is necessary to determine the true significance of these changes.

Results on reproduction in cows as related to plasma carotene and carotene intake are summarized for 26 gestations (16 cows) in Table II. Carotene intake figures are given in relation to body weight during the last 90 days of gestation. The average daily carotene intake was practically the same during the entire gestation period as that shown for the last 90 days.

Inspection of the figures in Table II shows that when the avorage plasma carotene was below 150 micrograms per 100 ml. the cows gave birth to weak calves. Of the eleven calves produced, five died; one of these was dead at birth, one died on the day it was born, and three died of pneumonia. Four of the cows in this group were in poor condition after calving. When plasma carotene was maintained above 150 micrograms by an increased carotene intake, only two of the fifteen calves died and one cow showed poor condition.

These results emphasize the value of plasma carotene determinations in revealing border line deficiencies of vitamin A due to low carotene intake. Apparently a border line deficiency is reached in pregnant Jersey cows when the carotene intake is insufficient to maintain an average plasma carotene content of about 150 micrograms per 100 ml. This is a somewhat higher value than that found to be associated with early symptoms of vitamin A deficiency in Jersey calves (Gallup and Kuhlman 1941).

SUMMARY

The carotene content of the blood plasma of Jersey cows receiving prairie hay at three levels of intake throughout complete gestation and lactation periods was determined at monthly, or more frequent, intervals. The carotene of the hay furnished the only source of vitamin A in the ration in amounts above and below that required for successful reproduction. The levels at which the hay was fed represented approximately a full, a 50 percent, and a 25 percent normal hay allowance. Despite yearly and seasonal variation in the carotene content of the hay, carotene intake over extended periods was roughly in proportion to hay allowance.

Plasma carotene values usually dropped immediately or soon after the cows calved. There was no consistent change in these values with advance in lactation. The range of average carotene content of the plasma throughout lactation was from 250 to 479 micrograms per 100 ml. for 4 animals receiving a full hay allowance, 179 to 201 micrograms for 2 animals

(3 lactations) receiving a 50 percent allowance, and from 80 to 165 micrograms for 5 animals receiving a 25 percent allowance.

Cows whose average plasma carotene during the last 90 days of gestation was less than 150 micrograms per 100 ml. of plasma produced 11 calves. Five of the calves from this group died, and 4 of the cows were in poor condition after calving. Cows whose carotene intake was sufficient to maintain the plasma carotene above this level produced 15 calves. Only two of the calves from this latter group died and one cow showed poor condition. Apparently, a plasma carotene value of less than 150 micrograms per 100 ml. in pregnant Jersey cows indicates a border line deficiency of vitamin A.

TABLE I

Blood plasma carotene of Jersey cows in relation to carotene intake during complete lactation periods

Cow and lactation no.	Hay allowance in percent of normal	Daily carotene intake in mg.		Plasma carotene in micrograms per 100 ml.	
		Range	Ave.	Range	Ave.
424-2	25	16-58	33	50-115	85
604-2	"	16-58	37	80-280	167
613-1	"	16-58	38	75-195	122
338-3	"	21-48	41	40-105	80
431-2	"	21-56	41	102-210	136
436-1	50	28-114	67	125-300	201
331-3	"	32-116	71	88-270	188
436-2	"	67-177	110	125-236	179
3313-1	100	85-156	126	340-600	479
1338-1	"	50-210	128	118-370	250
713-1	"	74-198	150	235-390	320
13-9	"	101-235	163	200-330	257

TABLE II

Reproduction in Jersey cows as related to carotene intake and the carotene content of the blood plasma

Gestations	Range of daily carotene intake during last 90 days of gestation*	Range of plasma carotene during last 90 days of gestation	Cows showing poor condition after calving	Data on calves		
				Born	Weak at birth	Died
Number	Micrograms. per lb. body weight	Micrograms. per 100 ml. of plasma	Number	Number	Number	Number
7	23-75	62-100	2	7	3	4
4	39-66	132-140	2	4	2	1
6	42-129	150-210	1	6	0	0
9	68-244	223-441	0	9	1	2

* These values have been taken from data presented elsewhere (Kuhlman and Gallup, 1941) in which it was shown that the minimum daily carotene intake necessary for normal calving of Jerseys was between 40 and 45 micrograms per pound body weight. This represents a daily intake of from 40 to 45 milligrams for a 1000-pound animal.

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