RATE OF ABSORPTION OF CAROTENE AND OF VITAMIN A FROM THE ALIMENTARY TRACT OF DAIRY CALVES. 
I. EFFECT OF METHOD OF ADMINISTRATION

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The importance of vitamin A activity in the nutrition of dairy calves has continued to focus attention on the quantitative dietary needs for carotene and vitamin A. The establishment of optimal allowances of these nutrients is contingent upon a knowledge of factors affecting utilization. Among the multitude of variables that may be related to efficiency of absorption and utilization are the vehicle of the vitamins, the dispersion of the vitamin concentrate and the methods of administration. Of these, only the last will be considered herein.

Lemley et al. (5) observed that when vitamin A in an oil medium was injected either subcutaneously or intramuscularly the effectiveness was 35 per cent and 2 per cent, respectively, as great as when taken per os. Water-solubilized carotene given intramuscularly, however, was utilized efficiently by rats (11). Aqueous dispersions of vitamin A also were utilized effectively when injected intramuscularly into children (4).

Niedermeier et al. (8) found that injections of an aqueous dispersion of vitamin A into the small intestine of the goat effected higher blood plasma levels of this vitamin than did similar injections into either the abomasum or the large intestine. Moreover, when vitamin A was injected into the small intestine of sheep (1), the rate of absorption was more rapid than when placed into the rumen or administered orally. There was little absorption of either carotene or vitamin A from the cecum and the colon.

Since feeding vitamin A and carotene concentrates to calves at different stages of development involves managemental problems as well as nutritional consequences, the objective of this investigation was to compare effects of various methods of administering (nipple feeder, stomach tube and gelatin capsule) supplements on the rates of absorption.

GENERAL EXPERIMENTAL PROCEDURES

Experimental subjects, feeding and management. Dairy calves representing four different breeds, Brown Swiss, Guernsey, Holstein and Jersey, were used in carotene and vitamin A absorption tests. During the first 3 days following birth, each calf received colostrum from its dam. Subsequently, either fresh whole milk or reconstituted milk was fed twice daily at the rate of 10 lb. per day per 100 lb. body weight of calf. The routine method of feeding was from a nip-

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ple pail. At various stages of growth of several of the calves, a concentrate mixture and hay were incorporated in the diet. All the experimental subjects were confined to individual pens bedded with wood shavings. Whenever calves were restricted to milk diets, the individuals were muzzled to minimize consumption of foreign material.

Carotene and vitamin A supplements. In most of the trials the source of carotene was "Carex"\(^3\), a carrot oil that contained 5,000 I. U. of carotene per gram, but during the terminal stages of the investigation, carotene in cottonseed oil\(^4\), 50,000 I. U. per gram, was administered. The source of vitamin A was fish liver oil concentrates. The potency of the product used during the early trials was 25,000 I. U. per gram\(^5\), whereas that given in the later studies was 30,000 I. U.\(^6\).

The quantity of supplement given in absorption tests was 1,000 I. U. per lb. of body weight. The measured amount for each subject was administered either dispersed (by homogenization) in milk or enclosed in gelatin capsules. The milk-dispersed supplement was given either orally from a nipple feeder or intra-ruminally through a stomach tube. Milk fed from a nipple normally traverses the esophageal groove and enters the abomasum directly (12). In the stomach-tube method of administration the supplements dispersed in milk were passed through a horse catheter into the rumino- retal cular cavity. The volume of fluid used for the dispersion medium was approximately the same for either system, nipple or tube. It is possible, however, that in some instances the volume administered by tube might have exceeded the capacity of the rumen and reticulum, thus resulting in an overflow into the abomasum. A balling gun was used to administer the capsules, special care being taken to avoid their rupture before swallowing. Capsules thus administered would be expected to pass into the rumen.

Blood collection and analytical procedures. The criteria of the rates of absorption of carotene and of vitamin A were the levels of these substances in samples of plasma from venous blood collected at the time of feeding and at 2, 4, 8, 12 and 24 hr. thereafter. Blood plasma carotenoids and vitamin A were determined by procedures described by Squibb et al. (10).

TRIALS AND RESULTS

Trial I—Nipple feeder vs. stomach tube. At intervals of approximately 1 wk., the milk normally given at the morning feeding was replaced with reconstituted separated milk in which either a carotene or a vitamin A concentrate had been dispersed. The nipple and the stomach-tube methods of administration were alternated from period to period for each calf. In these comparisons eight animals received carotene supplements and six, vitamin A.

The mean pre-absorption carotenoid and vitamin A values in blood plasma are shown in table 1 (trial I). Each initial value was considered as the base

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\(^3\) Obtained from Nutrition Research Associates, South Whitley, Ind.

\(^4\) Obtained from General Biochemicals, Inc., Chagrin Falls, O.

\(^5\) Obtained from White Laboratories, Inc., Newark, N. J.

level (zero) from which subsequent changes were determined. Mean responses to the respective methods of administering the supplements are depicted in figures 1 and 2.

Although the magnitude of the increases resulting from each method of administration was variable in the different calves and in the same calf at various periods, the nipple system uniformly resulted in more rapid rises than did the stomach-tube procedure. The differences in the levels of carotenoids were more pronounced than those of vitamin A. The maximum values for vitamin A, however, were attained more quickly than those for carotenoids.

**TABLE 1**

*Mean pre-supplementation concentrations of carotenoids and vitamin A in the blood plasma of calves*

<table>
<thead>
<tr>
<th>Trial</th>
<th>Supplement</th>
<th>Method of administration</th>
<th>Mean level in blood plasma ((\gamma/100 \text{ ml.}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carotene in oil</td>
<td>Nipple feeder</td>
<td>Carotenoids: 15.3, Vitamin A: 11.7</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>Stomach tube</td>
<td>Carotenoids: 18.9, Vitamin A: 11.6</td>
</tr>
<tr>
<td></td>
<td>Vitamin A conc.</td>
<td>Nipple feeder</td>
<td>Carotenoids: 19.6, Vitamin A: 13.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stomach tube</td>
<td>Carotenoids: 16.5, Vitamin A: 12.5</td>
</tr>
<tr>
<td>II-a</td>
<td>Carotene in oil</td>
<td>Nipple feeder</td>
<td>Carotenoids: 19.8, Vitamin A: 7.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Capsule</td>
<td>Carotenoids: 17.0, Vitamin A: 6.5</td>
</tr>
<tr>
<td></td>
<td>Vitamin A conc.</td>
<td>Nipple feeder</td>
<td>Carotenoids: 14.2, Vitamin A: 9.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Capsule</td>
<td>Carotenoids: 13.6, Vitamin A: 8.2</td>
</tr>
<tr>
<td>II-b</td>
<td>Carotene in oil</td>
<td>Nipple feeder</td>
<td>Carotenoids: 57.3, Vitamin A: 16.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Capsule</td>
<td>Carotenoids: 60.0, Vitamin A: 17.8</td>
</tr>
<tr>
<td></td>
<td>Vitamin A conc.</td>
<td>Nipple feeder</td>
<td>Carotenoids: 35.0, Vitamin A: 15.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Capsule</td>
<td>Carotenoids: 42.4, Vitamin A: 15.7</td>
</tr>
</tbody>
</table>

Small but relatively uniform increases in concentrations of vitamin A in the blood plasma occurred during at least the first 12 hr. following the administration of carotene (fig. 1). Even though the carotenoid level was higher at 24 hr. than at 12, the vitamin A concentration was lower. The true relationship between the values of these constituents in plasma is obscure. Following vitamin A absorption (fig. 2), the carotenoid values in the plasma decreased, the degree and rate of depression being somewhat greater from the nipple administration than from the stomach tube.

In the absence of any well-established law relating concentrations of vitamin A and of carotenoids in the blood plasma to time after feeding massive doses of these substances, it was decided, for the purpose of statistical analysis, to obtain the average (or linear) rate of increase of concentration over the period
Fig. 1. Mean changes in levels of carotenoids and vitamin A in blood plasma of eight calves that received massive doses of a carotene concentrate homogenized in milk and administered by nipple feeder and by stomach tube.

Fig. 2. Mean changes in levels of carotenoids and vitamin A in blood plasma of six calves that received massive doses of a vitamin A oil concentrate homogenized in milk and administered by nipple feeder and by stomach tube.
0 to 12 hr. The curvature in this relationship was examined by evaluating the quadratic component orthogonal to the linear component. If the increases at 2, 4, 8 and 12 hr. are denoted by $I_2$, $I_4$, $I_8$ and $I_{12}$, the linear rate of uptake $L$ and the orthogonal quadratic component $Q$ are apart from constant numerical divisors, thus

\[
L = -8I_2 - 3I_4 + 7I_8 + 17I_{12}
\]
\[
Q = -20I_2 - 109I_4 - 113I_8 + 115I_{12}
\]

The $L$ and $Q$ values were appraised by a simple analysis of variance. The variations in these values were separated into those due to differences between calves, those due to differences between treatments and those due to treatment by calf interactions. The significance of treatment effects on either $L$ or $Q$ was determined by comparing the average effect with a variance which measures the failure of the effect to be the same for all calves. Thus, the test of significance was made by comparing the mean square for treatment with the mean square for calf-treatment interactions.

The analysis of the $L$ values for the first set of data, table 2, revealed a dif-

TABLE 2

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves</td>
<td>7</td>
<td>609,260</td>
<td>87,037</td>
</tr>
<tr>
<td>Treatments</td>
<td>1</td>
<td>682,235</td>
<td>682,235</td>
</tr>
<tr>
<td>(Nipple pail versus stomach tube)</td>
<td>7</td>
<td>503,927</td>
<td>71,990</td>
</tr>
</tbody>
</table>

\[
F = \frac{682,235}{71,990} = 9.28^a
\]

\[^a\text{Significance } P_{0.05} = 5.59 \]
\[P_{0.01} = 12.25\]

ference significant at the 5 per cent level in the linear rates of increase of blood plasma carotenoid levels following administration of carotene by the two methods. A like analysis of the corresponding $Q$ values also showed a difference significant at the 5 per cent level of probability.

Although the remaining data were analyzed in a manner similar to those illustrated in table 2, only summary statements are presented.

In this first trial the data on vitamin A uptake during the initial 12 hr. following administration of this vitamin showed a difference in curvatures that approached significance at the 5 per cent level, whereas the differences in the linear components were non-significant. This was due, in part, to the marked downward trend in the nipple-fed group after the eighth hour.

Trial II. Nipple feeder vs. capsule. The experimental subjects were 60-day old calves that had been used in a previous study (7) in which all subjects were restricted to a fortified filled-milk diet. Since the calves had not consumed solid feed, it was assumed that the rumen was underdeveloped. To gain
information on the effects of diet and/or rumen development, two series of absorption trials were conducted: the first, while the animals were on a milk diet and, the second, after 2 mo. on a conventional milk, concentrate and hay regime.

a. Whole milk diet. The diet of the calves was changed from the filled milk to whole milk. Subsequently, the animals were divided into two units: group A consisted of eight Holsteins and two Guernseys and group B, seven Holsteins and one Guernsey. Each group was divided further into two subgroups (table 3). At approximately weekly intervals, carotene and vitamin A supplements were given at the rate previously indicated. The methods of administration were as outlined in table 3.

The initial values of carotenoids and of vitamin A in blood plasma are shown in table 1 (trial II-a) and the responses to supplementation in figures 3 and 4. The rate of carotene (oil concentrate homogenized in milk) absorption (fig. 3) following ingestion from the nipple feeder was similar to that

<table>
<thead>
<tr>
<th>Supplements</th>
<th>Group</th>
<th>Sub-group</th>
<th>No. of calves</th>
<th>Method of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotene in oil</td>
<td>A</td>
<td>1</td>
<td>5</td>
<td>Nipple feeder</td>
</tr>
<tr>
<td>(carotene)</td>
<td></td>
<td>2</td>
<td></td>
<td>Capsule</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish liver oil</td>
<td>B</td>
<td>1</td>
<td>4</td>
<td>Nipple feeder</td>
</tr>
<tr>
<td>(vitamin A)</td>
<td></td>
<td>2</td>
<td></td>
<td>Capsule</td>
</tr>
</tbody>
</table>

*Administered at rate of 1000 I. U./lb. body wt.*

in trial I (fig. 1) but more rapid than when the oil was given in a capsule (fig. 3). The differences in linear trends resulting from the two methods of administration were significant at the 1 per cent level, but the differences in curvatures were non-significant statistically.

The rate of absorption of vitamin A (fig. 4) was somewhat greater when the fish liver oil concentrate was fed from a nipple than when given in a capsule, but in the former the maximum level was attained at approximately 12 hr. after ingestion, whereas in the latter the maximum occurred later. During the initial 12 hr., the difference in the linear trends of vitamin A in blood plasma of calves receiving the supplement by the two methods was statistically significant at the 5 per cent level, but the difference in curvatures of rates of uptake was not significant.

When the nipple system of feeding was employed, the corresponding responses in trials I and II-a to carotenoid intake (figs. 1 and 3) and to vitamin A (figs. 2 and 4) were strikingly similar. A comparison of the stomach-tube method (figs. 1 and 2) with the capsule procedure (figs. 3 and 4) indicates that the rate of absorption of the supplements was more rapid when the former of the two methods was used. Moreover, the extent of carotenoid suppression in the blood plasma following vitamin A supplementation was somewhat greater in trial I than in trial II-a.
Fig. 3. Mean changes in levels of carotenoids and vitamin A in blood plasma of ten 2-mo.-old calves that received a basal diet of whole milk and a supplement of massive doses of carotene administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

Fig. 4. Mean changes in levels of carotenoids and vitamin A in blood plasma of eight 2-mo.-old calves that received a basal diet of whole milk and a supplement of massive doses of vitamin A administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.
b. Buttermilk (reconstituted), concentrate mixture and alfalfa hay diet. After this diet was fed to the same calves employed in trial II-a (less one calf in carotene group) for a period of 2 mo., the plan of administering carotene and vitamin A, table 3, was repeated. Since the dry separated milk available was more readily reconstituted and, thus, was a more desirable dispersion medium for the supplement than the dry buttermilk commonly fed, the former was substituted for the latter when the vitamin substances were administered. Other components of the diet, concentrate mixture and hay, were unchanged on the day of the tests.

As a result of hay consumption, the base levels of carotenoids and of vitamin A in the blood plasma of the calves were higher in trial II-b than in II-a (table 1). The post-supplementation changes from these bases are shown in figures 5 and 6.

![Graph](image-url)

Fig. 5. Mean changes in levels of carotenoids and vitamin A in blood plasma of nine 4-mo.-old calves that received a basal diet of reconstituted buttermilk, alfalfa hay and a concentrate mixture and a supplement of massive doses of carotene administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

The values of plasma carotenoids following carotene administration were slightly greater when the supplement was fed from a nipple than when given by a capsule (fig. 5). During the first 12 hr., the difference in linear trends approached significance at the 5 per cent level, but the difference in curvatures was non-significant. The striking features of the responses in this trial, in comparison with those in trial II-a (fig. 3), were the delayed increases and the subsequent low magnitude. Although, as in preceding trials, the accompanying increases of plasma vitamin A were slight, the higher level of vitamin A corresponded to the higher values for carotenoids.

In contrast to the exceptionally slow rise in carotenoid concentrations in the blood plasma (fig. 5), the increase of vitamin A was rapid (fig. 6). The rate of uptake of this vitamin and the level reached were even greater in this trial than in the preceding (fig. 4). In accord with observations in other trials, vita-
min A was absorbed more rapidly when the nipple procedure of administration was employed than when the capsule method was used (fig. 6). The difference in linear trends, however, during the period from 0 to 12 hr. was not significant, largely due to the precipitous drop in the "nipple" curve after the eighth hour. On the other hand, the difference of curvatures was significant at the 1 per cent level.

A further comparison of responses during the liquid (trial II-a) and the solid (trial II-b) dietary regimes indicates that when vitamin A concentrates were given by capsule, the concentration of this vitamin in the blood plasma was greater in the former trial (fig. 4) at 24 hr. after administration than at 12 hr., whereas in the latter (fig. 6) the converse was true. This difference in time suggests a more rapid passage of the supplement in the animals having the greater ruminal activity. In trial II-b the depression of carotenoids following vitamin A administration was greater than in trial II-a.

DISCUSSION

Although the concentration of any nutrient in the blood at a given time involves many metabolic processes, the results reported herein seem to indicate a relationship between the methods of administering carotene and vitamin A and the rate at which these substances are absorbed from the alimentary tract of dairy calves. There are several possible explanations for the difference ob-
served when vitamin substances were administered by stomach tube and by
nipple. Milk ingested by this latter procedure is mixed with relatively large
quantities of oral and esophageal secretions (14). This exposure of the dis-
persed supplements might have evoked physical and chemical alterations that
enhanced subsequent absorption. Moreover, the slower rate of uptake of vita-
min supplements following stomach-tube administration may be ascribed to
their gradual passage from the rumino-recticular cavity and thence into the
other stomach compartments and the small intestine. Inasmuch as it has been
demonstrated (2) that vitamin A in oil is absorbed in the bovine largely through
the lymph of the small intestine, the rapidity with which this absorptive area
was contacted by the vitamin substances used in the present experiment might
have affected the rate of transmission to the blood. This delay in the fore part
of the digestive tract conceivably also could have resulted in an increased loss
of potency of the supplements.

Since the carotene and the vitamin A administered in gelatin capsules pre-
sumably passed into the rumino-recticular cavity, the retarded rate of absorption
probably resulted, in part, from factors similar to those affecting uptake of
vitamin substances dispersed in milk and administered by stomach tube. As
the rate of uptake in the latter instance was somewhat more rapid, it would
seem that absorption might have been enhanced by dispersion of the vitamin sup-
plements. Frazer and associates (3) found that the average particle size of
ingested triglyceride fats in the intestine of the rat is less than 0.5 μ and that
paraffin, which normally does not pass through the intestinal wall, is absorbed
when similarly dispersed. Since it has been shown (9) that fats and vitamin A
are absorbed in a like manner, it seems possible that vitamin A uptake, like fat
absorption, may be influenced by dispersion. The need for further experimen-
tation, however, is indicated since Lundbaek and Maaløe (6) were unable to con-
firm the paraffin absorption observations.

The marked reduction in rate of uptake of carotene following the transition
from a diet of whole milk to one composed of reconstituted buttermilk, hay and
concentrates is difficult to interpret. Possibly the relatively high initial blood
plasma carotenoid values of calves in trial II-b (solid diet) might have masked
the effects of supplemental carotene. It would seem, however, that this ap-
parent reduced rate of absorption might have been due, in part, to changes in
the amount and the type of oil in the carotene supplement and to the quantity
of fat in the milk in which the concentrate was dispersed. It is possible that
the reduced absorption might have resulted not from any single factor but
rather from the combined effect of several of the foregoing.

The relationship between blood plasma values for vitamin A and those for
carotenoids following the administration of massive doses of carotene is obscure.
Maximum vitamin A levels, subsequent to carotene administration, usually were
reached earlier than the corresponding carotenoid maxima. Since the changes
in vitamin A values were small, additional experimentation is necessary before
this relationship can be clarified.

The maximum blood plasma levels of the vitamin substances fed were at-
tained earlier after vitamin A administration than after carotene feeding, thus suggesting a possible difference in the metabolism of these materials. Since the rates of administration of these substances were similar on the I.U. basis, the quantity of carotene, in micrograms, was greater, thus possibly affecting the time required for maximum levels to be attained.

The 24-hr. experimental period employed in this investigation was too brief to characterize the entire absorption curves. Limited data (13), however, indicate that the blood plasma carotenoid and vitamin A levels following administration of carotene by stomach tube and by capsule increase over a longer period of time and decline more gradually than those resulting from nipple pail feeding. Studies of this nature, even though conducted over an extended interval, may not indicate the efficiency of utilization of vitamin supplements fed by the various methods. Whether a relationship exists between rate of increase in the blood and total absorption remains to be determined by further experimentation.

**SUMMARY**

Carotene and vitamin A given at the rate of 1000 I.U. per lb. of body weight of calf were administered, respectively, by nipple feeder, stomach tube and gelatin capsule.

Comparisons of initial blood plasma carotenoid and vitamin A levels with those 2, 4, 8, 12 and 24 hr. after feeding the vitamin substances were employed as criteria of the rates of absorption.

Carotene and vitamin A dispersed in milk by homogenization and fed by nipple were absorbed more rapidly than similar preparations administered by stomach tube. The rates of absorption of carotene and of vitamin A from concentrates administered by gelatin capsules were somewhat less rapid than those resulting from the foregoing procedures.

The rate of absorption of vitamin A by calves restricted to whole milk was less rapid than the rate of uptake by the same calves after having received a diet of reconstituted buttermilk, hay and grain concentrates for approximately 8 wk. Conversely, the rate of absorption of carotene was more rapid under the former dietary regime than under the latter.

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**REFERENCES**


