B-VITAMIN LEVELS IN THE BLOOD OF YOUNG DAIRY CALVES
FED A MILK REPLACEMENT DIET WITH AND
WITHOUT AUREOMYCIN

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The mechanism by which antibiotics exert a growth-stimulating effect in
chicks, pigs, and calves has not been definitely established. Jukes and Williams
(9) have reviewed the various hypotheses proposed to account for the mode of
action of antibiotics, and the basis for most of the proposals is the assumption
that the antibiotics exert an influence on the intestinal microflora.

Since it has been established (10) that certain B-complex vitamins are syn-
thesized in the intestinal tract of young calves on a limited whole milk regime,
it seems that an antibiotic possibly may alter the intestinal microflora in a
manner such that greater or less than normal quantities of B-vitamins may be
present in the intestinal tract. The availability to the calf of intestinally synthe-
sized vitamins has not been adequately determined, but it is generally assumed
that part of these vitamins may be absorbed. Whether blood levels of the B-vita-
mins in calves are correlated with absorption and/or state of metabolism of
these vitamins has not been ascertained.

The objectives of the present study were to determine the blood levels of
thiamine, riboflavin, niacin, pantothenic acid, and vitamin B₁₂ activity in Hol-
stein calves at 4 days of age and to ascertain the influence of orally fed aureo-
mycin on the blood levels of these B-vitamins in Holstein calves on a whole milk
replacement feeding regime for a period of 12 weeks.

EXPERIMENTAL PROCEDURE

Two groups of Holstein calves were selected for this study. Calves of one
group (ten males and ten females) from the Iowa State College dairy herd were
allowed to remain with their respective dams for 3 days following birth. To
characterize the early postnatal blood B-vitamin levels, samples of venous blood
(potassium oxalate anticoagulant) were drawn on the fourth day and analyzed
as described below for thiamine, riboflavin, pantothenic acid, niacin, and
vitamin B₁₂.

A second group of eight Holstein calves (four males and four females) at 4
days of age were placed on a milk replacement feeding program. The milk
replacement, containing primarily dried whey product reconstituted with water
(14% dried whey product, 86% water), was fed for the first 7 weeks at the
following daily rates per 100 lb. body weight: 3.0, 5.4, 10, 8, 8, 6, and 4 lb.

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respectively. Whole milk averaging 3% fat was fed at the daily rates of 5.0 lb. and 3.6 lb. per 100 lb. body weight during the first and second weeks on experiment. No milk or milk replacement was fed subsequent to 7 weeks. A calf starter containing 40% ground corn, 20% crushed oats, 28% soybean oil meal, 10% wheat bran, 1% steamed bone meal, and 1% iodized salt was fed ad libitum until a maximum of 4 lb. was consumed daily per calf. Medium quality mixed hay, largely alfalfa and brome grass, was fed free choice. Four calves, two males and two females, served as controls while the other four animals in this group were fed the same ration plus aureomycin. The antibiotic was fed to each animal at the daily rate of 40 mg. of aureomycin via the liquid portion of the diet during the first 7 weeks and at the daily rate of 80 mg. in the concentrate mixture from 8 to 12 weeks on experiment. With a few exceptions, venous blood samples were drawn at the start of the experiment and at 1, 2, 4, and 8 weeks thereafter. In addition, several samples were taken at 12 weeks.

After enzymatic hydrolysis of the blood with a combination of clarase and papain, riboflavin, pantothenic acid, and niacin were determined microbiologically with \textit{Lactobacillus casei} by a modification of the method of Clegg, Kodicek, and Mistry (5). The thiochrome method (2) was employed for measuring blood thiamine values. Extraction of vitamin B$_{12}$ activity from the blood involved heating the sample to which cyanide had been added, mixing with water in a Waring blendor, and filtering. The vitamin B$_{12}$ activity was estimated with \textit{Lactobacillus leichmannii} by a procedure based upon the method of the United States Pharmacopeia (23).

### TABLE 1

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine ($\mu$/ml)</td>
<td>0.074 ± 0.011$^a$</td>
<td>0.069 ± 0.010$^b$</td>
</tr>
<tr>
<td>Riboflavin ($\gamma$/ml)</td>
<td>0.21 ± 0.03</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>Pantothenic acid ($\gamma$/ml)</td>
<td>1.91 ± 0.33</td>
<td>2.04 ± 0.44</td>
</tr>
<tr>
<td>Niacin ($\gamma$/ml)</td>
<td>9.96 ± 3.4</td>
<td>12.1 ± 2.9</td>
</tr>
<tr>
<td>Vitamin B$_{12}$ activity ($mc\gamma$/ml)</td>
<td>0.90 ± 0.20</td>
<td>0.94 ± 0.16$^a$</td>
</tr>
</tbody>
</table>

$^a$Mean value ± standard error.  
$^b$Values for 8 calves only.

### RESULTS

The blood B-vitamin levels in 4-day-old Holstein calves are summarized in Table 1. No statistically significant differences between male and female animals were found in the vitamins studied. The variations in values for each vitamin, however, were great.

The average blood B-vitamin values for calves fed the basal diet with and without aureomycin are presented in Figures 1 and 2. Only the calves for which all the vitamin values within an age period were determined are included

$^a$As Aurofasc D supplied by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.
Fig. 1. Changes in the blood pantothenic acid, riboflavin, and thiamine values in control and aureomycin-supplemented dairy calves.

in these graphs. The values at 12 weeks represent only two calves, whereas the other values are averages for three. Missing plot estimations were employed to supply single values for the aureomycin-supplemented group at 2 weeks (thiamine), 4 weeks (vitamin B$_{12}$), and 12 weeks (vitamin B$_{12}$). There were no statistically significant differences between the aureomycin-supplemented group and the control group at any age. Also, there were no apparent differences between male and female animals.

Since the differences between experimental groups were not significant, all of the available blood B-vitamin data for the eight calves at various ages were combined and summarized in Table 2. The average vitamin levels appear in certain cases to change with age. Since the values at various ages include a variable number of determinations, differences between various ages were tested by comparing values for calves from which samples were obtained at both age-
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Fig. 2. Changes in the blood niacin values and vitamin B₁₂ activity in control and aureomycin-supplemented dairy calves.

periods in question. By this procedure it was found that during the first week on experiment the decreases in pantothenic acid and thiamine levels were significant at the $P = 0.05$ level, and the niacin and vitamin $B₁₂$ activity changes approached significance ($P = 0.1$). The decline in riboflavin values during the same period was not significant, but from 4 to 8 weeks the drop in blood riboflavin values was significant at the $P = 0.05$ level. Although other trends in the blood B-vitamins at several intervals were observed, none was significant at the $P = 0.05$ level.

The aureomycin-supplemented animals grew at an accelerated rate. This observation is in accordance with previous reports ($11, 14$).

DISCUSSION

The variability of the blood B-vitamin values, except for vitamin $B₁₂$, was considerably greater in calves at 4 days of age than that observed at later age periods. These data suggest possible differences in the state of nutrition of the calves due, at least in part, to the levels of the B-vitamins in the colostrum of the dam. Another factor which may be involved is the total quantity of nutrients consumed during the colostral period. Calves on experiment subsequent to
### TABLE 2

B-vitamin levels for Holstein calves at various ages

<table>
<thead>
<tr>
<th>Weeks on expt.</th>
<th>No. calves</th>
<th>Thiamine (μg/ml)</th>
<th>Riboflavin (μg/ml)</th>
<th>Pantothenic acid (mg/ml)</th>
<th>Niacin (mg/ml)</th>
<th>Vitamin B$_{12}$ activity (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6</td>
<td>0.062 ± 0.008$^*$</td>
<td>0.28 ± 0.05</td>
<td>1.61 ± 0.38</td>
<td>15.1 ± 5.5</td>
<td>1.11 ± 0.18</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>0.057 ± 0.007</td>
<td>0.22 ± 0.03</td>
<td>0.80 ± 0.16</td>
<td>12.0 ± 1.0</td>
<td>0.73 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>0.062 ± 0.007(6)$^b$</td>
<td>0.21 ± 0.02</td>
<td>0.69 ± 0.12</td>
<td>8.6 ± 1.1</td>
<td>0.80 ± 0.18(7)</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.079 ± 0.006(5)</td>
<td>0.23 ± 0.02</td>
<td>0.49 ± 0.06</td>
<td>7.3 ± 0.8</td>
<td>0.62 ± 0.14(5)</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>0.081 ± 0.005</td>
<td>0.15 ± 0.02</td>
<td>0.72 ± 0.10</td>
<td>6.1 ± 0.2</td>
<td>1.00 ± 0.32</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>0.086 ± 0.007</td>
<td>0.17 ± 0.03</td>
<td>0.60 ± 0.11</td>
<td>7.1 ± 0.6</td>
<td>1.33 ± 0.25(3)</td>
</tr>
</tbody>
</table>

$^*$ Mean values ± standard error.

$^b$ Numbers in parentheses denote number of calves when different from column 2.
4 days of age received approximately equal quantities of feed, and the variability in blood B-vitamin values decreased.

The data presented in this report indicate that the feeding of aureomycin had no marked effect on the blood levels of thiamine, riboflavin, pantothenic acid, niacin, and vitamin B₁₂. These results fail to explain an earlier observation by Murley (13) wherein an apparent riboflavin deficiency occurred in one young calf and a thiamine deficiency in another, both of which were receiving aureomycin in the diet. It now seems probable that factors other than antibiotic supplementation per se were responsible for the apparent B-vitamin deficiencies observed by Murley.

Evidence that indicates gastro-intestinal synthesis of B-vitamins in ruminants is available. Kesler and Knodt (10) found that on a dry matter basis the concentrations of thiamine, riboflavin, niacin, and pteroylglutamic acid were higher in various regions in the digestive tract of the young dairy calves than in the feed consumed. Further evidence of intestinal synthesis of some of the B-vitamins has been reported by Pearson et al. (16) in studies with sheep. It is of interest to note that Chance et al. (3), in a study of the effect of aureomycin on the rumen synthesis of some of the B-vitamins, found that the antibiotic appeared to have no marked effect on the rumen synthesis of riboflavin, pantothenic acid, or nicotinic acid.

The effect of antibiotics on the intestinal synthesis of some of the B-vitamins by various species has received attention during the past few years. Several reports (4, 8, 17) have shown that vitamin B₁₂ is synthesized in the intestines of rats and that aureomycin feeding results in an increase in the intestinal level of this vitamin. The oral administration of streptomycin to humans apparently has no marked effect on the urinary excretion of folic acid, thiamine, riboflavin, and pyridoxine compounds (19). This finding suggests that streptomycin did not increase intestinal synthesis of the vitamins studied. Sauberlich (20) has shown, however, that the addition of penicillin to a diet caused a marked stimulation in the growth of rats fed diets free of or low in thiamine, pyridoxine, and pantothenic acid. The inclusion of penicillin or aureomycin in the complete diet had no effect upon the growth of the animals. The recent report by Guggenheim et al. (7) indicates that aureomycin, streptomycin, and terramycin added to diets low in pantothenic acid cause a significant increase in the fecal excretion of this vitamin in the rat, and also that the antibiotics caused increased urinary excretion of thiamine and pantothenic acid at all levels of vitamin intake. It seems apparent, therefore, that one might expect little adverse effect, and quite probably a beneficial effect, of aureomycin-supplementation on the intestinal synthesis of several of the B-complex vitamins in most species.

The blood B-vitamin levels reported herein are within the range of the values reported for bovine blood levels of riboflavin (6, 21), niacin (15, 22), thiamine (6, 24), and vitamin B₁₂ activity (1, 18). The authors are not aware of reports of blood pantothenic acid values in young dairy calves. Moreover, virtually no data are available to show the trends in blood levels of the B-vitamins during the early life of the young calf. A recent report by Moinuddin et al. (12) indicates
that the levels of both riboflavin and niacin in lambs' blood decrease gradually from birth to 6 weeks, whereas the blood levels of vitamin B$_{12}$ tend to increase slightly from birth to 8 weeks. Similar trends were observed in calves in the present study.

The observed trends in the blood B-vitamin values cannot be explained solely by gradual change in the feeding regime over the 12-week experimental period. However, the significant drop in the riboflavin level during the 4-to-8-week period may be due in part to the discontinuation of whey product (high in riboflavin) feeding and the subsequent consumption of hay and grain (low in riboflavin) during this period. The onset of rumination may be an important factor in the observed changes in blood levels of vitamins.

Since the urinary excretions of the B-vitamins under consideration in this report were not measured, one cannot state that the B-vitamin absorption from the intestinal tract was not influenced by the oral administration of aureomycin. However, since the blood B-vitamin levels were essentially the same in each group of calves, it is apparent that the antibiotic had no demonstrable adverse effect. Additional studies, which should include urinary excretions of the B-vitamins, are needed to clarify the over-all problem of B-vitamin metabolism in calves receiving antibiotics.

SUMMARY

The blood levels of thiamine, riboflavin, pantothenic acid, niacin, and vitamin B$_{12}$ activity were determined for ten male and ten female Holstein calves at 4 days of age. No significant differences between sexes were observed.

Eight Holstein calves at 4 days of age were placed on a milk replacement diet and were assigned to two comparable groups, one of which received aureomycin orally (40 mg. daily per calf for 7 weeks, 80 mg. daily per calf from 8 to 12 weeks) while the other served as a control. Venous blood samples drawn at the beginning of the experiment and at 1, 2, 4, 8, and 12 weeks thereafter were analyzed for thiamine, riboflavin, niacin, pantothenic acid, and vitamin B$_{12}$. No significant differences in the blood levels of these vitamins were found between the two groups of animals. Moreover, no apparent differences were observed between male and female calves. Certain trends in the B-vitamin blood levels with age are evident.

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REFERENCES


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(23) U. S. Pharmacopeia, 14th Rev., Suppl. 3: 15. 1951.