Lactational Trends in Vitamin B₁₂ and Restricted-Roughage Rations

C. K. WALKER¹ and J. M. ELLIOT
Department of Animal Science, Cornell University
Ithaca, New York 14850

Abstract
Sixteen Holstein cows were assigned to one of two dietary treatments, control or restricted-roughage, for the first 34 weeks of lactation. Vitamin B₁₂ in rumen fluid, blood serum, liver, milk and urine was monitored at intervals to determine general trends and to test the hypothesis that roughage restriction would alter the B₁₂ status of animals on this treatment. The per cent of total vitamin B₁₂ activity in rumen fluid represented by the vitamin itself was reduced by roughage restriction. This treatment resulted in higher serum, lower liver and milk and higher urine vitamin B₁₂ activity. Lactational trends are described. Interrelationships among variables suggested the possibility that higher serum B₁₂ of cows on restricted roughage might be due in part to vitamin B₁₂ analogues.

Introduction
The microorganisms in the rumen synthesize large quantities of vitamin B₁₂ provided the diet contains at least minimal amounts of cobalt (23, 25, 26). In addition to the vitamin itself, analogues, including Factors A and B, pseudovitamin B₁₂, and others (9, 13, 14) are also synthesized, some in even larger amounts. Relatively little attention has been given to dietary factors other than cobalt affecting ruminal synthesis of B₁₂ and its analogues although a few observations suggesting such effects have been made (6, 9, 11, 18).

Vitamin B₁₂ activity of liver, milk and blood appears generally to consist almost entirely of the vitamin itself (7, 13, 15, 20) suggesting that the analogues are either poorly absorbed or rapidly and preferentially excreted. Although lactational trends in the B₁₂ content of milk are well documented (16, 17, 19), only limited data of this type are available for serum or liver.

A preliminary study of vitamin B₁₂ synthesis in the sheep (11) suggested that a greater amount of the vitamin and a smaller proportion of analogues were produced on a dried grass diet than on an all-concentrate diet. These observations together with the suggestion of an effect of roughage restriction on liver vitamin B₁₂ (32) prompted the present study which was designed to test the hypothesis that roughage restriction in the dairy cow would be reflected in an altered vitamin B₁₂ status.

Experimental Procedure
Sixteen Holstein cows were grouped according to age and within age groups randomly assigned to one of two treatments: a) Control: long hay ad libitum, plus a pelleted concentrate at 1 kg per 4 to 4.5 kg of milk; or b) Restricted roughage: long hay as 20 to 25% of dry matter intake plus pelleted concentrate fed essentially to appetite for 5 to 6 months of lactation but subsequently restricted slightly to prevent cows on this treatment from gaining excessive weight. Hay and concentrates were fed twice daily in individual mangers. Cows were gradually adapted to their diets, beginning 3 to 4 weeks prepartum. They were maintained on the experiment through 34 weeks of lactation. Feed intake and milk production were recorded daily. During the entire experiment, cows were housed indoors except for daily exercise 1 to 2 hr. Iodized salt was available free choice during exercise. Bedding consisted of sawdust or shavings.

The average chemical composition of the feeds is in Table 1. Fiber fractions, including lignin, were determined by methods of Van Soest (29) and Van Soest and Wine (30). At predetermined intervals during the experiment, eight samples of milk (morning milking), urine, and blood serum, and three samples of liver and rumen fluid were collected from each cow for vitamin B₁₂ assay. Mercuric chloride (2 ml saturated solution per 100 ml sample) was added to the rumen fluid samples after collection by stomach tube. They were
TABLE 1. Average chemical composition of feeds.

<table>
<thead>
<tr>
<th></th>
<th>Concentratea</th>
<th>Hay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>18.2</td>
<td>15.2</td>
</tr>
<tr>
<td>Neutral detergent fiber</td>
<td>11.3</td>
<td>50.1</td>
</tr>
<tr>
<td>Acid detergent fiber</td>
<td>3.4</td>
<td>35.0</td>
</tr>
<tr>
<td>Lignin</td>
<td>.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Ash</td>
<td>5.9</td>
<td>6.7</td>
</tr>
<tr>
<td>Cobaltb</td>
<td>3.0 ppm</td>
<td>.2 ppm</td>
</tr>
</tbody>
</table>

a Ingredients (%): Ground corn (72.5), soybean meal (18.0), molasses (6.0), iodized salt (1.0), monocalcium and dicalcium phosphate (1.25), calcium carbonate (.75), trace-mineral mixture (.50). Vitamin A was added to supply 2,204 IU/kg.

b Cobalt analyses were obtained courtesy of V. A. Lazar, U. S. Plant, Soil and Nutrition Laboratory, Ithaca, New York.

strained through cheese cloth. Liver samples were obtained by the liver biopsy technic described by Butler and Elliot (2) and were homogenized in distilled water before storage. Milk samples were diluted tenfold with distilled water for storage. All samples were stored frozen until analyzed.

Serum, milk, liver homogenates, urine and rumen fluid extracts (11) were assayed for vitamin B12 by the radioisotope dilution method of Lau et al. (21) as modified by Corse and Elliot (5). This method is sensitive to at least two of the common analogues (Factor A and pseudo B12) although it measures them with a lower efficiency than the vitamin itself (31). Liver protein determinations were by the procedure of Lowry et al. (22). Vitamin B12 in the rumen fluid extracts was also estimated by the microbiological (Ochromonas) assay of Ford (12) which, in contrast to the isotope assay, is reasonably specific for vitamin B12.

Data were initially analyzed by an analysis of variance of unweighted means for each age group (2 years, 3 years, and older) on each treatment (27). There was no significant (P > .05) age effect or age by treatment interaction in any of the variables of interest. Therefore, to have more degrees of freedom associated with the error mean square, treatment differences were tested by an analysis of variance in a completely random design.

Results and Discussion

Differences between the two treatments (control and restricted-roughage) in mean daily hay and grain dry matter intake are illustrated in Figure 1. From the tenth through the thirty-fourth weeks of lactation cows on the restricted-roughage treatment consumed an average of 4 kg or less of hay while control cows consumed 12 kg or more. Since the difference in grain dry matter intake was not of this magnitude, the total dry matter intake was significantly greater (P<.01) on the control treatment (Table 2). The much higher proportion of hay in the control diet resulted in significantly higher fiber intakes. The cobalt intake of cows on the restricted-roughage treatment (31 mg) was significantly higher than that of controls (18 mg).

TABLE 2. Mean daily intake of feed components.

<table>
<thead>
<tr>
<th>Intake</th>
<th>Control</th>
<th>Restricted roughage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dry matter (kg)</td>
<td>17.9a</td>
<td>13.8b</td>
</tr>
<tr>
<td>Dry matter/kg body wt (kg)</td>
<td>3.2</td>
<td>2.5b</td>
</tr>
<tr>
<td>Neutral-detergent fiber (kg)</td>
<td>7.4</td>
<td>3.0b</td>
</tr>
<tr>
<td>Acid-detergent fiber (kg)</td>
<td>.9</td>
<td>.3b</td>
</tr>
<tr>
<td>Lignin (kg)</td>
<td>17.8</td>
<td>31.0b</td>
</tr>
</tbody>
</table>

a All values are the average of eight cows over 34 weeks of lactation.
b Treatment means significantly different (P<.01).
These are both well above deficiency levels and well below toxicity levels (26).

Since rumen fluid samples were by stomach tube, differences in absolute concentrations of vitamin B\textsubscript{12} were considered of questionable significance. Expression of vitamin B\textsubscript{12} concentration by \textit{Ochro\textsubscript{monas}} assay as a percentage of that by radioisotope dilution assay (hereafter referred to as the O/R ratio) reflects the relative proportion of the total activity represented by vitamin B\textsubscript{12}, independent of concentration. At 3, 16, and 34 weeks postpartum the mean O/R ratios for controls were 42, 53, and 41\% whereas those for restricted roughage were 21, 23, and 34\%. A greater percent of total vitamin B\textsubscript{12} activity in rumen fluid on the restricted roughage treatment, therefore, consisted of analogues. The differences in O/R ratio were significant (P<.01) during the first two sampling periods, and they are in general accord with the preliminary observations of Elliot et al. (11) on sheep. Dawbarn et al. (6) reported that sheep grazing pasture had a larger proportion of rumen vitamin B\textsubscript{12} activity in a form which could be utilized by \textit{Ochro\textsubscript{monas}} than did sheep receiving cobalt-deficient wheaten-hay chaff and gluten with added cobalt. \textit{Ochro\textsubscript{monas}}-active material represented 28 to 42\% of the \textit{Escherichia coli} plate activity of rumen contents on various diets in the studies of Dryden and Hartman (9).

Gawthorne (14) noted that as cobalt intake was reduced to low or deficient levels, a larger percentage of the B\textsubscript{12} activity in sheep rumen contents was vitamin B\textsubscript{12} itself. It appears unlikely that the higher cobalt intake of the restricted roughage group was responsible for the lower O/R ratio in the present study since within treatment the O/R ratio was positively, although not significantly, correlated (r = .31) with cobalt intake. Also, a similar O/R pattern with the reverse cobalt pattern was observed in abomasal contents of sheep (11). A more likely explanation lies in the difference in the type of substrate supplied to the rumen microorganisms. This is supported by the recent report of Sutton and Elliot (28).

Serum vitamin B\textsubscript{12} activity trends are illustrated in Figure 2. Two apparently anomalous prepartum values (3.7 and 15.9) were omitted from the mean in plotting the data since their validity is questionable and their inclusion would have masked the trend evident in the rest of the data. Serum vitamin B\textsubscript{12} activity increased in early lactation in both groups but by 16 weeks had declined considerably in the cows on the control treatment. At 16 weeks postpartum serum levels were significantly higher (P<.01) on the restricted-roughage treatment and at 24 weeks postpartum the difference was still significant (P<.05). By 34 weeks the means had converged to a point where the difference was no longer significant. Serum B\textsubscript{12} reported here agrees generally with values reported by Corse and Elliot (5) with the same radioisotope assay. These levels are also in the range of blood values for liberally-fed dairy cows reported by Anthony et al. (1) using \textit{Lactobacillus} (ATCC 4797) as an assay method but are higher than those of preparturient and early lactation cows reported by Elliot et al. (10), using \textit{Lactobacillus} (ATCC 7830). The results of different assays of the same samples suggest that cattle and sheep blood is essentially free of analogues (7, 14, 20) although at least one observation (8) in sheep suggests their presence.

In many respects the urine vitamin B\textsubscript{12} trends (Fig. 2) paralleled those of serum. Urine vitamin B\textsubscript{12} increased to very high levels in early lactation, thereafter tended to decrease. Except at the 8-week sampling, mean B\textsubscript{12} content of the urine of cows on the restricted roughage was higher than that of controls. The differences were significant at 5 weeks (P<.05), 16 weeks (P<.01) and 24 weeks (P<.05). Since total urine output was not measured, it cannot be established that greater total losses of B\textsubscript{12} occurred via the kidney with roughage restriction.

Mean vitamin B\textsubscript{12} concentrations of liver samples are in Table 3. Whether expressed per gram of wet tissue or per gram of protein, mean concentration of B\textsubscript{12} in the liver of cows assigned to each treatment was not different at 3 weeks prepartum. By 5 weeks postpartum, cows on restricted roughage had significantly
VITAMIN B₁² AND ROUGHAGE

TABLE 3. Liver vitamin B₁² concentration.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sampling period</th>
<th>Wet liver (µg/g)</th>
<th>Protein (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3 Weeks prepartum</td>
<td>.73 ± .05ᵃ</td>
<td>4.85 ± .33</td>
</tr>
<tr>
<td></td>
<td>5 Weeks postpartum</td>
<td>1.20 ± .06ᵇ</td>
<td>6.72 ± .32ᵇ</td>
</tr>
<tr>
<td></td>
<td>24 Weeks postpartum</td>
<td>1.36 ± .09ᵇ</td>
<td>7.81 ± .47ᵇ</td>
</tr>
<tr>
<td>Restricted roughage</td>
<td>3 Weeks prepartum</td>
<td>.76 ± .04</td>
<td>4.70 ± .21</td>
</tr>
<tr>
<td></td>
<td>5 Weeks postpartum</td>
<td>.83 ± .06</td>
<td>5.14 ± .25</td>
</tr>
<tr>
<td></td>
<td>24 Weeks postpartum</td>
<td>.78 ± .04</td>
<td>5.08 ± .22</td>
</tr>
</tbody>
</table>

ᵃ Each value is the mean of eight observations ± SE.  
bTreatments significantly different at this period (P<.01).

less (P<.01) than controls which had increased by some 60% (wet basis) in the interval between the samples. At 24 weeks postpartum this difference was even more apparent; all cows on the control treatment had liver vitamin B₁₂ greater than 1 µg/g wet tissue whereas all cows on the restricted-roughage treatment had less than 1 µg/g. These data suggest that roughage restriction largely inhibits increase in liver B₁₂ which would normally occur during lactation. Lower liver levels of the restricted roughage group are associated with higher serum values. An examination of the individual observations on rumen B₁₂ indicated a correlation (r = .54; P<.05) between the O/R ratio at 3 weeks postpartum and the change in liver B₁₂ concentration between the prepartum and first postpartum samples.

Trends in mean milk vitamin B₁₂ activity are illustrated in Figure 3. Roughage restriction resulted in lower milk vitamin B₁₂. Although there was great variation within diets and cows, as previously observed by Gregory et al. (16), the difference between treatments was significant (P<.05) at 24 weeks postpartum. The mean B₁₂ content of milk and the lactational trends are in general agreement with previous observations (17) although some have not reported the increasing levels we observed in later lactation. Miller et al. (24) have reported significant differences in total milk vitamin B₁₂ production associated with type of feeding regime of cows at three experiment stations.

The fact that rumen vitamin B₁₂ activity, as measured by radioassay, was significantly correlated (r = .48; P<.01) with serum B₁₂ activity while that measured by _Ochromonas_ assay was not (r = −.08; P>.05), suggests that B₁₂ analogues may in some way influence serum activity. A similar picture was evident in correlations between measures of rumen B₁₂ activity and urine B₁₂ activity. The lack of a significant relationship (r = −.12; P>.05) between serum and liver B₁₂ confirms an earlier observation (32) and raises a question concerning the value of serum level as an indicator of vitamin B₁₂ status. The data suggest the hypothesis that roughage restriction, with a concomitant increase in concentrate intake, results in the absorption of substantial amounts of B₁₂ analogues which are bound by the serum and excreted in urine but are not taken up by the liver or secreted in milk, and may in fact interfere with the uptake of the vitamin by the liver and mammary gland. Unfortunately the serum samples collected were insufficient in quantity to permit their assay for evidence of analogues. Eight liver samples subjected to both assays resulted in data which supported the assumption that most or all of the liver activity was due to the vitamin itself. Of eleven milk samples similarly tested, two of seven from cows on the restricted-roughage ration gave evidence of the presence of analogues. Kon (20) has presented evidence of traces of Factor A in milk. The nature of
the B₁₂ activity of urine was not determined in the present study, but the presence of analogues has been reported by others (20). It was demonstrated by Coates et al. (3), that pseudovitamin B₁₂ and Factor B, when fed, were antagonistic to vitamin B₁₂ uptake by the liver of the chick. Similarly Factor B reduced the B₁₂ content of the liver and kidney of the sheep (4).

The results of the present study support the hypothesis under test and indicate that, in the presence of adequate cobalt, differences in type of diet can, in some manner not clearly understood, exert a strong influence on vitamin B₁₂ synthesis or availability to the dairy cow.

Acknowledgments

The assistance of Dr. T. M. Butler, A. L. Sutton and Mrs. Te-hui Chen is gratefully acknowledged.

References


