Liver Vitamin \( B_{12} \) Status of the Lactating Dairy Cow

K. A. WILSON, J. M. ELLIOT, and M. M. MATHIAS

Department of Animal Science, Cornell University, Ithaca, New York

Abstract

Liver homogenates, centrifuged to remove nuclei and cell debris, were assayed for vitamin \( B_{12} \) activity. Forty-one samples from 31 dairy cows were included in the study. Vitamin \( B_{12} \) values ranged from 2.8 to 6.6 \( \mu g \) per g of protein. A significant (\( P < .05 \)) part of the variation was accounted for by the multiple regression of liver vitamin \( B_{12} \) on days fresh, milk production to date during lactation, daily milk yield, and the interactions of these variables. Of seven cows sampled during early lactation, and again at variable intervals but within the first 180 days of lactation, five showed a higher liver vitamin \( B_{12} \) level at the second sampling. Liver and blood vitamin \( B_{12} \) levels were poorly correlated in the dairy cow.

In a previous report from this laboratory (2), data on blood vitamin \( B_{12} \) levels in Holstein cows at various stages of lactation were presented. Significant differences among cows and among stages of lactation were shown to occur when cows were consuming rations considered adequate in cobalt. There remained the question whether changes in blood levels of vitamin \( B_{12} \) reflected similar changes in liver storage. An opportunity to accumulate information on liver vitamin \( B_{12} \) levels in the dairy cow presented itself in connection with another experiment, in which liver samples were being taken for enzyme studies. This report presents the data obtained.

Materials and Methods

Forty-one liver samples obtained by biopsy from 31 dairy cows in the Cornell University herds are included in the study. With one exception (Jersey) all samples were from Holsteins. Eighteen blood samples were drawn from 14 cows at the time liver samples were obtained. The cows, when sampled, represented a wide range of physiological conditions. Daily milk yield ranged from 0 to 36 kg; 15 were pregnant and 19 were not; age ranged from 2 to 12 yr; 13 either were consuming or had previously been on rations in which roughage was restricted; two cows had ketosis when first sampled and two others had a recent history of ketosis. All concentrates being fed were supplemented with cobalt, and in some cases trace-mineralized salt containing cobalt was also available on a free-choice basis.

The liver samples were transported to the laboratory in ice-cold 0.25 m sucrose, blotted with filter paper, weighed, and homogenized at a dilution of approximately 1:10 in a Potter-Elvehjem glass homogenizer with a hypotonic solution consisting of 0.05 m tris-HCl buffer (pH 8.0) and 1.0 mm cysteine. In a few cases the sucrose solution was omitted. The homogenates were centrifuged at 600 \( \times g \) for 15 min to remove nuclei and cell debris. A portion of the supernatant was stored at \(-20^\circ C\) for subsequent extraction and assay; a second portion was employed for protein determination by the method of Lowry et al. (4), with minor modifications. With 12 samples a portion of the homogenate prior to centrifugation was saved for comparison with the supernatant. Venous blood samples, with heparin as anticoagulant, were stored at \(-20^\circ C\) for subsequent extraction and assay.

Liver extracts for vitamin \( B_{12} \) assay were prepared by diluting the supernatant or homogenate 1:10 with 0.2 \( m \) sodium acetate buffer (pH 4.6), adding a drop of NaCN solution (2 mg/ml), mixing thoroughly, and allowing it to stand at room temperature for one hour before heating to 96 \( ^\circ C \) for 30 min. The pH was then adjusted to approximately 7 with 0.1 \( N \) NaOH, the solution brought to standard volume with distilled water, and filtered. The filtrate was stored at \(-20^\circ C \) and appropriately diluted further with distilled water at the time of assay.

Samples of whole blood were diluted (1:4:1) with distilled water and 0.2 \( m \) sodium acetate buffer (pH 4.6); a drop of NaCN solution was added and mixed thoroughly. The samples were held at room temperature for one hour, then autoclaved at 121 \( ^\circ C \) for 30 min. They were then centrifuged and the supernatant was adjusted to pH 7, made to appropriate volume and filtered. The filtrates were stored for assay in

Received for publication February 27, 1967.

1 Present address: Department of Dairy, Michigan State University, East Lansing, Michigan.

2 Present address: U. S. Army Medical Research and Nutrition Laboratory, Fitzsimons General Hospital, Denver, Colorado.
LIVER VITAMIN B₁₂ LEVELS

the same manner as the liver filtrates.

The organism employed in the assay was *Lactobacillus leichmannii* 7830 (ATCC). The vitamin B₁₂ standard and general assay method were those previously described (2). Each liver assay, however, was set up as five dilutions in quadruplicate; each blood assay as five dilutions in triplicate, with a series of uninoculated tubes to permit correction of the blank for the red-dis color in the blood extracts (2). Liver vitamin B₁₂ levels were expressed as micrograms per gram of protein in the supernatant preparation.

Simple and multiple regression analyses were conducted to study relationships between vitamin B₁₂ levels and such other variables as stage of lactation, daily milk yield, production to date during the lactation, and two-way interactions among these variables. It was necessary to estimate from subsequent production the total milk production of four cows during the first six weeks of lactation, because it was not recorded at each milking during this period. Likewise, the daily yield just prior to biopsy was estimated on one cow.

Results and Discussion

Comparison of the vitamin B₁₂ values of the whole liver and supernatant preparations from 12 samples revealed that the supernatants were consistently lower, averaging 67 ± 4% of the values for the whole liver. This indicates that the intracellular distribution of vitamin B₁₂ is not uniform in bovine liver, and suggests that a greater concentration of the vitamin is to be found in association with nuclear than non-nuclear protein. The physiological significance of this observation is not known.

Liver samples were obtained from seven cows within the first 45 days and again within the first 180 days of lactation (Table 1). Five of the seven had a higher vitamin B₁₂ level at the second sampling, and only one was appreciably lower. This suggests that, in general, liver vitamin B₁₂ levels, like blood levels (2), are lower in early lactation than at subsequent stages. When liver and blood levels (18 pairs of samples) were correlated, however, the coefficient was relatively low (r = .34), and not statistically significant (P < .05). This does not rule out the possibility that a close relationship exists on a within-cow basis, but it clearly indicates that cows differ considerably in their liver-blood vitamin B₁₂ relationship.

The range in observed vitamin B₁₂ values was 2.8-6.6 μg per g of protein, with a mean of 4.2. The low value represents an estimated 0.7 μg per g of whole liver on a wet basis. All values, therefore, were considerably above those which have been associated with cobalt deficiency in sheep (1, 5) or cattle (6). Hedbom (3) reported an average of 1.34 (range 1.12-1.54) μg per g for 15 samples of bovine liver assayed with the organism used in the present study.

Since in the data there appeared to be some evidence of an effect of roughage restriction, the relationship of liver vitamin B₁₂ to other variables was studied, both including and excluding samples from cows subjected to such diets (Table 2). Most correlation coefficients were higher when these samples were excluded and several achieved statistical significance (P < .05). While this may indicate that a dietary effect was indeed present, observations on the low-roughage diets were not uniformly distributed across stages of lactation or levels of milk production and, therefore, should be interpreted with caution. Of the variables studied, the two most closely correlated with liver vitamin B₁₂ were milk production to date during the lactation, and days fresh. Daily milk yield at time of sampling was negatively correlated with liver vitamin B₁₂. A preliminary

**TABLE 1**

Liver vitamin B₁₂ levels of cows as affected by days fresh

<table>
<thead>
<tr>
<th>Cow</th>
<th>Days fresh</th>
<th>Sample 1 B₁₂*</th>
<th>Days fresh</th>
<th>Sample 2 B₁₂*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>16</td>
<td>3.10</td>
<td>150</td>
<td>3.82</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>3.37</td>
<td>180</td>
<td>5.20</td>
</tr>
<tr>
<td>14</td>
<td>13</td>
<td>4.50</td>
<td>65</td>
<td>2.85</td>
</tr>
<tr>
<td>19</td>
<td>43</td>
<td>3.11</td>
<td>83</td>
<td>4.88</td>
</tr>
<tr>
<td>20</td>
<td>34</td>
<td>3.21</td>
<td>67</td>
<td>4.40</td>
</tr>
<tr>
<td>25</td>
<td>15</td>
<td>3.84</td>
<td>60</td>
<td>4.19</td>
</tr>
<tr>
<td>26</td>
<td>23</td>
<td>4.04</td>
<td>63</td>
<td>2.91</td>
</tr>
</tbody>
</table>

*Expressed as μg per g protein in the supernatant preparation.

**TABLE 2**

Correlation of liver vitamin B₁₂ and other variables

<table>
<thead>
<tr>
<th></th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (41)</td>
</tr>
<tr>
<td>Liver vitamin B₁₂ level and</td>
<td></td>
</tr>
<tr>
<td>Days fresh</td>
<td>.20</td>
</tr>
<tr>
<td>Days in calf</td>
<td>.13</td>
</tr>
<tr>
<td>Daily milk yield</td>
<td>-.09</td>
</tr>
<tr>
<td>Production to date</td>
<td>.24</td>
</tr>
<tr>
<td>Age</td>
<td>-.16</td>
</tr>
</tbody>
</table>

* Significant P < .05.
** Significant P < .01.

*J. DAIRY SCIENCE* Vol. 50, No. 8
multiple regression analysis indicated that these three variables were the most important of those studied, and an analysis of the three and their first order interactions (Table 3) revealed that they accounted for a significant ($P < .05$) part of the variation in liver vitamin $B_{12}$ levels. The

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
 & All & Normal diet observations only (28) \\
\hline
Multiple correlation coefficient & .59 & .76 \\
\hline
Standard partial regression coefficients & & \\
Days fresh & $-1.32$ & $-1.66$ \\
Daily milk & $-.80$ & $-.81$ \\
Production to date & $-2.09$ & $-1.48$ \\
Days fresh $\times$ daily milk & $-1.03$ & $-.62$ \\
Days fresh $\times$ prod'n to date & $2.80$ & $2.86$ \\
Daily milk $\times$ prod'n to date & $2.00$ & $1.67$ \\
Mean squares & & \\
Regression & $2.0797^{**}$ & $1.9885^{*}$ \\
Residual & $0.4479$ & $0.6681$ \\
\hline
\end{tabular}
\caption{Summary of multiple regression analysis of liver vitamin $B_{12}$ on lactation variables}
\end{table}

largest standard partial regression coefficient was that of the interaction of days fresh and production to date. The data presented support the hypothesis that the vitamin $B_{12}$ status of the dairy cow varies with stage of lactation, with tissue levels rising as lactation progresses.

Acknowledgment

The authors gratefully acknowledge the assistance of Dr. H. F. Tyrrell with statistical analysis of the data.

References


