Effect of Ethyl Alcohol on the Vitamin A Status of Holstein Heifers¹,²

R. W. MILLER¹ and R. W. HEMKEN
University of Maryland, College Park 20742

and

D. R. WALDO and L. A. MOORE
Dairy Cattle Research Branch, Animal Husbandry Research Division
USDA, Beltsville, Maryland 20705

Abstract

Two trials were conducted to test the effect of ethanol on the vitamin A status of cattle. In the first trial, 12 Holstein heifers in a split-plot experimental design were fed ethanol at levels of 0, 1, or 3% of the daily ration (alfalfa hay and vitamin A-fortified grain) at one morning feeding. Blood samples were drawn at 0, 4, 12, and 24 hours post-feeding. Heifers, when fed 3% ethanol, had a 37% increase in plasma vitamin A concentrations between the zero- and four-hour sampling which persisted through the 12-hour sampling. The 1% level of ethanol did not increase plasma vitamin A concentrations.

In the second trial, initial liver biopsy and jugular-blood samples were taken from the same heifers. They were then randomly assigned to receive ethanol at zero, 1, or 3% of their ration (same as first trial) for a 21-day period. At the end of the period, blood and liver biopsy samples were again taken. Final liver vitamin A concentrations were higher (P < .01) than the initial ones at each level of ethanol fed; however, the increase in the heifers fed 3% ethanol was only 13%, as compared to 50% for heifers fed zero, and 37% for the heifers which received 1% alcohol.

Introduction

In 1953, Crowley and Allen (5) reported that calves fed ethyl alcohol had a transient increase in plasma vitamin A levels. It was indicated

that this increase in plasma vitamin A concentration was due to increased mobilization of liver vitamin A.

Since the early 1960's there have been numerous reports (8, 9, 11-13) indicating that cattle fed corn silage rations showed vitamin A deficiency symptoms or at least had decreased levels of liver vitamin A reserves. It was generally postulated that this was due to a decreased utilization of corn silage carotene. However, since corn silage may contain significant quantities of alcohol (7), this experiment was conducted to try to determine if levels of alcohol that might be consumed by cattle on a high corn silage ration would mobilize liver vitamin A reserves, thereby explaining the decreased liver levels previously mentioned.

Experimental Procedures

Twelve Holstein heifers weighing an average of approximately 300 kg were fed 1.5 kg of grain mixture (44% corn meal, 22% wheat bran, 22% crimped oats, 11% soybean meal, 1% iodized salt) twice daily at 1000 and 1600 hours. At 1700 hours the heifers received alfalfa hay at the rate of 1.5% body weight. Before the start of the experiment all heifers were fed 200,000 IU of preformed vitamin A per day for four days and then the daily concentrate ration was fortified with 33,000 IU of vitamin A per kilogram. The fortified concentrate was fed for one week before the start of the experiment and continued throughout the experiment.

The experiment was conducted as two trials. In the first, or 24-hr trial, a split-plot design with three periods was used. Alcohol levels were the main plot, with sampling times as subplots. At the start of the first period at 1000 hours blood samples were taken by jugular puncture from all animals. After blood sampling was completed the heifers received their morning grain allowance. Four heifers received the grain without any additive, four received the grain mixed with absolute ethyl alcohol which was calculated to be 1% of the total daily ration, and the other four were supplemented with absolute ethyl alcohol calculated to be 3%...
of the total daily ration. Four, 12, and 24 hr after the morning feeding blood samples were taken from all animals.

Periods 2 and 3 were conducted three and seven days after Period 1 in a similar manner, with the exception that the treatments were switched each period so that every animal received each treatment.

Three days after the end of Period 3, the second trial (21-day) was initiated. The same heifers used in the previous trial were randomly assigned to receive no alcohol, 1% absolute ethyl, or 3% absolute ethyl alcohol. On the day of the start of the experiment, jugular blood and liver biopsy samples were taken from all heifers before the morning feeding. For the next 21 days the heifers on the alcohol treatments received the designated amounts of alcohol at each grain feeding. Before the morning grain feeding on Day 21 jugular blood and liver biopsy samples were obtained and the experiment terminated.

Blood samples from both phases of the experiment were analyzed for carotene and vitamin A by the method of Kimble (10). Hexane was used instead of petroleum ether for the extractions. Liver biopsy samples from the second trial were analyzed for carotene and vitamin A by a modification of the method of Ames et al. (1) to obtain carotene values. Plasma and liver carotene and vitamin A values obtained in this experiment were analyzed statistically by least-squares analysis of variance.

Results and Discussion

Table 1 shows the effect of alcohol on plasma vitamin A levels. When the heifers were fed 0 or 1% alcohol there was a small drop or no change in plasma vitamin A between the 0- and 4-hr sampling times. However, 3% alcohol produced a marked increase (interaction of time with alcohol levels significant \( P < .01 \)) in plasma vitamin A concentrations over the same period. This maximum level of plasma vitamin A was maintained through the 12-hr sampling time, then decreased before 24 hr. These results are in good agreement with those of Crowley and Allen (5). It is of interest to observe the increase in plasma vitamin A values across all treatments at the 12-hr sampling time, then decreased before 24 hr. These results are in good agreement with those of Crowley and Allen (5).

It is of interest to observe the increase in plasma vitamin A values across all treatments at the 12-hr sampling time. These samples were taken at 2200 hours. Whether this increase is due to diurnal variation or some other factor is not known.

Plasma carotene values are presented in Table 2. No significant treatment \( \times \) time interactions existed. This was not unexpected, since alcohol has not been reported to mobilize liver carotene, as it apparently does liver vitamin A. As with plasma vitamin A, plasma carotene values were higher at the 12-hr sampling time.

In this trial there was a difference \( (P < .01) \) between periods for plasma vitamin A values. The period means were 34.2, 34.2, and 26.0 \( \mu g \) per 100 ml for Periods 1, 2, and 3, respectively. This difference appeared to be an analytical artifact rather than a biological result as the hexane source was changed for the Period 3 samples. The reason this change in hexane so markedly affected plasma vitamin A values is not understood. However, earlier this laboratory found the Carr-Price reaction to be markedly influenced by the source of antimony trichloride.

Plasma carotene and vitamin A levels for the second trial are presented in Table 3. Although there are no significant differences due to the effect of alcohol, it is of interest to note that in heifers fed 3% alcohol plasma vitamin A levels remained essentially the same; whereas, the two other groups decreased.

Table 1. Plasma vitamin A levels of heifers on 24-hour trial.

<table>
<thead>
<tr>
<th>Alcohol level</th>
<th>Hours post alcohol feeding</th>
<th>Levela</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of ration</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>(( \mu g/100 ) ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>27.8b</td>
<td>25.7</td>
</tr>
<tr>
<td>1</td>
<td>32.7</td>
<td>32.5</td>
</tr>
<tr>
<td>3</td>
<td>26.9</td>
<td>36.8</td>
</tr>
<tr>
<td>Time meansc</td>
<td>29.1</td>
<td>31.7</td>
</tr>
</tbody>
</table>

a No difference \( (P > .10) \) among levels. se = 1.9.

b Interaction of time with alcohol levels significant \( (P < .01) \). se = 0.9.

c Times different \( (P < .01) \). se = 0.5.
TABLE 3. Plasma carotene and vitamin A levels of heifers on 21-day experiment.

<table>
<thead>
<tr>
<th>Alcohol level</th>
<th>Carotene</th>
<th>Vitamin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of ration</td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>0</td>
<td>234</td>
<td>250</td>
</tr>
<tr>
<td>1</td>
<td>300</td>
<td>282</td>
</tr>
<tr>
<td>3</td>
<td>256</td>
<td>241</td>
</tr>
<tr>
<td>se of a mean</td>
<td>28</td>
<td>1.6</td>
</tr>
</tbody>
</table>

There was a marked increase in liver vitamin A values during this experiment (Table 4). Although the treatment X time interaction was not significant, heifers fed 0 or 1% alcohol increased their liver vitamin A stores by 50 and 37%, respectively; whereas, the increase in the 3% alcohol heifers was only 13%. This would appear to indicate that a depletion of vitamin A from the liver might have been expected in the 3% level heifers, if the high levels of vitamin A supplement were not being fed during the experiment.

When this research was initiated it was assumed that corn silage could contain up to 0.5% alcohol on a fresh basis (7). On a dry matter basis this figure would be approximately 1.5 to 2.0% alcohol. Thus, if a ration on a dry matter basis for a dairy or beef animal contained from one-half to two-thirds of corn silage containing such a concentration of alcohol, the alcohol on a total ration basis would be approximately 1%. Thus, one of the levels of alcohol the heifers were fed was 1%. The 3% level was chosen as a level more comparable to those that Crowley and Allen (5) had shown would mobilize liver vitamin A in calves. Similar levels have also been shown to mobilize liver vitamin A in dogs (3), humans (4), and rats (2).

Since this research was completed, data have been received from Fenner (6) indicating that corn silage may contain up to 1.5% alcohol on a fresh basis. In the corn silage feeding trials previously mentioned (8, 9, 13) plasma vitamin A levels remained essentially the same or increased, while liver vitamin A concentrations dropped markedly. It is, therefore, postulated that perhaps alcohol in the corn silage could have caused an increase in the mobilization of liver vitamin A stores and resulted in the decreased liver vitamin A levels.

References