LIVER VITAMIN A SLOW RELEASE SYNDROME IN CATTLE WITH A MULTIPLE NUTRIENT IMBALANCE

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Summary

Data on liver vitamin A concentrations in malnourished, debilitated, down cattle in tropical, northern Australia support the hypothesis that a 12% annual cattle mortality was due, in part, to a slow release of liver vitamin A. High Ca and low Zn levels in the legume forage apparently contributed to the slow release. The cattle showed marked sensitivity to sunlight and exhibited problems of sight. The malnourished yearling steers averaged 183.3 µg vitamin A/g wet liver vs 152.3 µg for steers slaughtered off good green wet season forage. Indications of a slow release of liver vitamin A were that: (1) only 4 to 7 µg vitamin A/g liver were in the alcohol fraction or release form; (2) after adjustments for decreases in liver and blood volume in starving animals, blood vitamin A was lowered to 18 µg/100 ml, which was low in relation to the adjusted liver vitamin A level of 91.7 µg/g, and (3) after adjustments, the liver had released only 1,667 units of vitamin A/day in the dry season, or about 1/4 of maintenance needs. The cattle were grazing a legume forage pasture containing 7.1% protein and no measurable carotene. The forage was deficient in Zn (25 ppm), which would slow the release of liver vitamin A. High Ca levels in the legume forage (.4 to .54%) in combination with low P levels (.11 to .18%) would further aggravate the low Zn level.

(Key Words: Beef Cattle Mortality, Liver Vitamin A, Carotene, Mineral Malnutrition, Calcium, Zinc.)

Introduction

Vitamin A status was studied as a potential problem in cattle under tropical long drought situations by Ryley et al. (1960), Ryley and Gartner (1962) and Gartner and Ryley (1962), but in each case they found that cattle had high levels of liver vitamin A. Wesley-Smith (1972) reported that, at the end of a long drought, starving cows had an average of 438 µg vitamin A/g wet liver. Gartner and Alexander (1966) reported that in Queensland cows in terminal stages of malnutrition because of drought had an average of 341 µg vitamin A/g wet liver. In contrast, Gartner et al. (1968) reported yearly averages for normal beef cows in Queensland of 232 µg vitamin A/g liver one year and 281 the next.

This study involved the collection of vitamin A liver and blood data in conjunction with information on possible associative factors in forages on a 350,000-ha ranch in tropical northern Australia.

Materials and Methods

Starving cattle were observed during the long dry season from 1972 through 1979 in the Upper Adelaide River District in the Northern Territory of Australia. The cattle selected were in what was considered from a nutritional standpoint as the best available legume paddock and were stocked so that ample forage was available. A pooled sample of the Townsville Stylo (Stylosanthes humilis IFN 2-30-262) legume forage, similar to the forage the cattle were eating, was taken from eight areas of the paddock for chemical analysis. On November 1, 1978, the Australian Northern Territory Veterinary Group examined and posted yearling steers that were weak and down in the selected paddock and took various diagnostic samples. The liver samples were frozen and stored in darkness until analyzed. Liver samples (positive controls) were taken from the carcasses of apparently well-fed yearling steers of about the same weight from the same herd on the same ranch in April of 1979, near the end of the 5-month wet season. Blood vitamin A

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TABLE 1. BLOOD AND LIVER VITAMIN A ACTIVITY ANALYSIS OF CATTLE EXAMINED POSTMORTEM ON NOVEMBER 1, 1978

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Steer 1</th>
<th>Steer 2</th>
<th>Steer 3</th>
<th>Steer 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood vitamin A, µg/100 ml a</td>
<td>29</td>
<td>27</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Liver vitamin A, µg/g b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ester form c</td>
<td>176.3</td>
<td>175.5</td>
<td>275.3</td>
<td>83.1</td>
</tr>
<tr>
<td>Alcohol form</td>
<td>4.3</td>
<td>5.9</td>
<td>7.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Liver carotene, µg/g b</td>
<td>3.8</td>
<td>5.0</td>
<td>9.5</td>
<td>3.8</td>
</tr>
</tbody>
</table>

a Courtesy of R. Wesley-Smith and NT Administration, Darwin, Northern Territory, Australia.
b Conducted by Roche Pty, Ltd., Dee Why, NSW, Australia.
c The ester form is converted and expressed in terms of vitamin A alcohol potency of .3 µg vitamin A/IU.

was determined by colorimetric assay. Liver vitamin A was extracted by the method of Kimble (1939) and determined by AOAC (1975) methods.

Additional pooled samples of dry season legume forage (eight areas of a paddock for each sample) were taken for analysis in October 1979, and a pooled sample of new growth legume was collected in November 1979, after three rains totaling 65 millimeters. Carotene, N and P were determined by AOAC (1975) methods. Ca was determined by the method of Gehrke (1961). Zn was determined by atomic absorption spectrophotometry (Anonymous, 1976).

Results and Discussion

Results of the blood and liver vitamin A activity analyses are presented in table 1. At a time when the liver was being called on to supply essentially all of the daily metabolic vitamin A needs, an average of 97% of the liver vitamin A was in the ester or storage form, and only 3% was in the alcohol fraction or release form (table 1). This finding contrasts with the report by Tomlinson et al. (1974), who found that 7 to 8% of the liver vitamin A was in the alcohol or release form (table 1). This finding contrasts with the report by Tomlinson et al. (1974), who found that 7 to 8% of the liver vitamin A was in the alcohol or release form. Those workers reviewed four other papers in which the proportions of vitamin A alcohol and vitamin A ester were similar to those they had observed in their own studies. Thus, data in table 1 give an indication of a rather slow release of vitamin A from the liver.

Liver vitamin A levels near the end of the wet season in good condition, apparently healthy slaughter steers are summarized in table 2. Unadjusted data give the impression that steers at the end of a 200-day dry season had liver vitamin A concentrations 20% higher than those of comparable yearling steers grazing good green forage near the end of the wet season. Such an inference is not plausible, however. Based on textbook estimates (Best and Taylor, 1945; Fulton, 1949; Moore, 1957) that about half of the liver weight is lost during starvation, values were computed to adjust the liver vitamin A levels to a basis comparable to that of normal liver weights (third column of table 2). These results showed that about 40% of the liver vitamin A was used during the long dry season (152.3 vs 91.7 µg vitamin A/g wet liver).

Similarly, reports show that about 28% of blood volume is lost during starvation (Best and Taylor, 1945; Macfarlane et al., 1961; Weeth et al., 1967). Adjustments of the blood vitamin A levels shown in table 1 by a factor of .72 indicated an average vitamin A level of 18.2 µg/100 ml blood. This adjusted blood level was low in relation to the adjusted liver vitamin A level (Ralston and Dyer, 1959), suggesting that vitamin A was being released slowly.

Comparable liver levels of vitamin A from table 2 can be expressed in more comprehensible terms if we assume that these 150-kg yearling steers had normal liver weights of 1.1% of body weight (Morris and Gartner, 1967) during both the dry season and the wet season. Thus, the April 1979 wet season yearling steers would have averaged 837,650 units of activity from vitamin A in the liver, and the adjusted November 1, 1978 dry season yearlings about 504,350 units of activity. The decrease of 333,300 units over the 200-day dry season...
TABLE 2. LIVER VITAMIN A LEVELS IN STEERS NEAR THE END OF THE WET SEASON AND IN YEARLING STEERS DYING AT THE END OF THE DRY SEASONa

<table>
<thead>
<tr>
<th>Vitamin A, µg/g wet liver</th>
<th>November 1, 1978 dry season yearlings dying</th>
<th>Adjusted for loss in liver weightb</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 1979 wet season yearlings</td>
<td>110.6</td>
<td>180.6</td>
</tr>
<tr>
<td></td>
<td>214.6</td>
<td>181.4</td>
</tr>
<tr>
<td></td>
<td>187.5</td>
<td>282.3</td>
</tr>
<tr>
<td></td>
<td>96.6</td>
<td>89.0</td>
</tr>
<tr>
<td>Avg</td>
<td>152.3</td>
<td>183.3</td>
</tr>
</tbody>
</table>

aLiver vitamin A levels listed in equivalence of vitamin A alcohol, where .3 µg = 1 IU of vitamin A activity.
bAdjusted for a liver weight loss of 50% during starvation.

represents a release of 1,667 units of vitamin A activity from the liver per steer daily. This amount is only 28% of the NRC (1976) recommended daily vitamin A maintenance requirement for cattle of this size. These data indicate a slow release of liver vitamin A for cattle during the long dry season when there was no measurable carotene in the forage at the end of that dry season.

Activity of the yearling steers observed during the last week of October 1978, just before posting, was indicative of a possible shortage of vitamin A in some of the cattle. From 2 to 4% of the more than 1,000 total head in the paddock exhibited marked sensitivity to sunlight. They stayed in shaded areas near the water trough. These steers would not pass through the open sunny area on the way to the back side of the paddock, where the best remaining forage was located. They would follow the herd in the wooded areas, but would stop and go back when the main herd came to and proceeded through the sunny areas. They also appeared to have trouble seeing, because they stumbled over variations in the terrain. All steers that were posted were down and in a debilitated condition, but they were not necessarily the thinnest cattle in the paddock. They failed "blink" tests in which a finger was jabbed almost into the eye and a blow to the eye was faked with the flat side of the hand. The steers would look directly and very alertly at the observer, but when the observer moved very quietly, it became obvious that they had been looking in the direction of sound.

Analysis of the Townsville Stylo legume forage, comparable to that the yearling steers were consuming, is shown in table 3. On the basis of the protein level of the forage, the steers should have been receiving a maintenance diet, but because of the lack of carotene in the forage, essentially all their daily needs for vitamin A would have been from liver storage.

Legume forage taken at the end of the dry season in October 1979 was analyzed and compared with new wet season legume forage taken in November of 1979. Results are shown in table 4. The Townsville Stylo sample was a pooled sample from the same eight areas of the same paddock as the sample taken a year earlier (table 3), but there had been less grazing this time, and, thus, a higher leaf content contributed to the higher protein (table 4). These data showing mineral content seemed important in view of recent work indicating that Zn deficiency greatly retards the release of liver vitamin A (J. C. Smith et al., 1973; J. E. Smith et al., 1974).

Carotene analysis (table 4) showed that cattle could have received only a fraction of their daily vitamin A activity needs from either of the two legume dry season forages, whereas they would have received many times their daily requirement from the early wet season forage. Under these conditions, in which the burden of the cattle's dry season needs for vitamin A fell mainly on available liver supplies, the borderline low levels of Zn in both dry season

TABLE 3. ANALYSIS OF TOWNSVILLE STYLO FORAGE

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %a</td>
<td>7.1</td>
</tr>
<tr>
<td>Carotene, ppmb</td>
<td>&lt;.4</td>
</tr>
</tbody>
</table>

aCourtesy of Northern Territory Laboratory at Berrimah Experiment Station, Darwin, Northern Territory, Australia.
bWalnut Grove Laboratory, Atlantic, IA; assay procedure measures to .4 ppm.
forages would have been expected to slow the release of liver vitamin A, on the basis of work by Arora et al. (1968, 1973), Saraswat and Arora (1972), E. R. Smith et al. (1973), J. C. Smith et al. (1973) and J. E. Smith et al. (1974).

Ca levels in both dry season forages (table 4) were sufficient to supply more than twice the maintenance requirements of these cattle, and, thus, the extra Ca may have aggravated the low Zn level to further inhibit the release of liver vitamin A (Haaranen, 1963).

The low and borderline P concentrations shown in table 4 also would have had an adverse effect in this multiple nutrient imbalance, contributing additionally to an inhibited release of liver vitamin A. For instance, because of its close relationship to Ca, P could have helped to counterbalance the high Ca level if P itself had not been borderline low (NRC, 1976).

In essence, the ratios of Ca, P and Zn appeared to be important. For example, the NRC (1976) indicates that the Zn requirement of cattle appears to be between 20 and 30 mg/kg of diet. Haaranen (1963) showed that the Zn requirement increased with increased Ca to 56 ppm with .4% Ca in the diet. In the present study, dietary Ca was higher yet, and, in addition, the P concentration was below normal, so the Zn requirement was apparently considerably higher than the Zn level in the forage. Blood and Henderson (1968) refer to the "relative" deficiency of Zn with excess Ca. They reported that low dietary Zn levels of 34 to 44 ppm favored deficiency, while the addition of 100 ppm of Zn prevented deficiency.

Although protein levels in dry season legumes (table 4) appeared to be adequate for cattle, the other indicated multiple nutrient imbalances raise concern about the adequacy of vitamin A metabolism. The suggested imbalance started with the low dietary carotene, which shifted emphasis to the liver as the primary source for daily vitamin A needs. The liver would have been hampered in supplying the needed vitamin A because of the low Zn level that was aggravated by the Ca level of more than twice maintenance and further stressed by the low P level.

Results of all the other diagnostic tests on various body tissues; teeth; bones; ruminal, reticulum and intestinal contents, and blood vitamin B12 and paddock herbage samples did not reveal any other deficiency, any plant toxicity, any worm problem or any possibility of infection.

Development of the theory of a liver vitamin A slow release syndrome logically starts with the basic information of the wide individual variation among animals in the rate of release of vitamin A from the liver. Calculations from data published by Gartner and Ryley (1962) show not only a several-fold variation in the rate of release of liver vitamin A among individual cows, but also that the rate of release in some animals is below that required (range of release rates was from 5,000 to 39,000 units of vitamin A/nursing Hereford cow/day).

The next consideration in the development of the slow vitamin A release theory is the large variation in published results on the length of time for half the liver vitamin A to be released. Findings range from 28 to 184 days (Frey and Jensen, 1947; Church et al., 1956; Hayes et al., 1966, 1967; Sewell et al., 1966; Mitchell, 1967; Swanson et al., 1968; Kohlmeier and Burroughs, 1970; Meacham et al., 1970). In an

<table>
<thead>
<tr>
<th>TABLE 4. TOWNSVILLE STYLO AND CALOPO FROM END OF DRY SEASON VERSUS NEW WET SEASON CALOPO (10/22 THROUGH 11/13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Criterion</strong></td>
</tr>
<tr>
<td><strong>Townsville</strong></td>
</tr>
<tr>
<td>Carotene, ppm</td>
</tr>
<tr>
<td>Crude protein, %</td>
</tr>
<tr>
<td>Ca, %</td>
</tr>
<tr>
<td>P, %</td>
</tr>
<tr>
<td>Zn, ppm</td>
</tr>
</tbody>
</table>
experiment in which rats were given exceptionally high levels of vitamin A, Davies and Moore (1935) found that it took only a little less than 14 days for the liver to be depleted of half of its vitamin A.

The thyroid gland also has an effect on the rate at which vitamin A is released from the liver. Indications are that, under conditions of rising and hot temperatures and suboptimum feed intake, such as occurs at the end of each dry season in the Northern Territory of Australia, the thyroid effect would be a slower release of liver vitamin A (Moore, 1957; G. S. Smith et al., 1964).

Malnutrition also slows the rate of release of vitamin A from the liver (Guilbert and Harr, 1934; Roels and Mack, 1972; J. C. Smith et al., 1973). In addition, a high roughage diet causes a slower release of liver vitamin A than does a high energy diet (Hale et al., 1961; Tillman, 1962).

Heat stress may cause slow release due to impaired liver function (Aron et al., 1946). Page et al. (1959) proposed that high environmental temperatures may increase the vitamin A requirement for cattle. Certainly, there are many reports indicating that environmental temperatures have a marked effect on vitamin A metabolism (Jones et al., 1943; Moore and Sharman, 1951; Marion, 1961; Perry et al., 1962; Roussel et al., 1963; Beeson et al., 1964; Hansard et al., 1971). Jones et al. (1943) noted that when cattle have marginal vitamin A intakes, they manifest hypersensitivity to sunlight by making frantic efforts to reach the shade after having been forced into the sun.

The effect of Zn deficiency is dramatic in slowing the release of vitamin A from the liver. J. C. Smith et al. (1973) demonstrated the great tenacity of the liver in holding its vitamin A in cases of Zn deficiency.

Data accumulated over the past several years in the Upper Adelaide River District of the tropical Northern Territory of Australia confirm high levels of vitamin A in livers of malnourished cattle. Data and observations support a theory of slow release of liver vitamin A. The slow release is accentuated by a multiple nutrient imbalance starting with low carotene, followed by high Ca, low P and relatively low Zn levels in the dry season forage. The imbalance culminates with the apparent liver vitamin A slow release syndrome, wherein the rate of release is well below the NRC (1976) recommended level for maintenance.

These findings indicate that a component of vitamin A should be included whenever cattle are supplemented for any reason in the mid to late dry season while grazing sun-bleached forage containing very little carotene. Specifically, the normal tendency not to use vitamin A on the rationale that there is sufficient vitamin A in the liver should be avoided. Further, in considering the use of injectable vitamin A, the producer should recognize the importance of using a several-fold increase in dosage to compensate for the slow release of liver vitamin A.

If slow release of liver vitamin A is a problem, the use of legumes to improve protein nutrition could be wasted to some degree if the high Ca level in the legumes causes enough of an uncorrected vitamin A problem to offset the beneficial effects of more protein. In such a situation, the question logically arises as to how much P is needed to counteract the known adverse effects of high Ca when Zn and carotene intake are both low. If any appreciable P is used to counteract the multiple nutrient imbalance, then both the high cost of P and the scarcity of this nutrient would stimulate efforts to solve the problem with the less costly and less scarce oral vitamin A and(or) Zn.

If slow release of liver vitamin A can be a problem under certain conditions in the tropical Northern Territory of Australia, then it might also be a problem in other tropical areas of the world. It might be a problem in nontropical areas as well, especially where legumes are used extensively. In such cases, if verified, it would be important to consider the possibility that the slow vitamin A release syndrome might affect other species of animals, especially humans.

**Literature Cited**


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