Bovine Ketosis and Depressed Fat Test in Milk: A Problem of Methionine Metabolism and Serum Lipoprotein Aberration 1

During early lactation the high producing dairy cow is in a negative energy and protein balance (8, 11). The increased demands for nutrients associated with the initiation of lactation is met by an increased intake of food and also by mobilization of endogenous lipids and proteins from body tissues. We report preliminary findings on the composition of blood lipoproteins during the early weeks of lactation for normal and ketotic cows; postulations on the etiology of the disease; and, the effectiveness of treatment based on these findings. Likewise, the biochemical similarities of bovine ketosis and low fat testing milk will be discussed.

Blood samples from Holstein cows in the University Herd were obtained weekly or more often beginning with the first week of lactation and extending for as long as 16 weeks. All animals were fed the same winter ration of mixed forage with grain supplementation. Cows were clinically examined weekly or more often as needed by the onset of ketosis. \( \beta \)-lipoproteins were removed from the blood serum by the method of Sakagami and Zilversmit (24). Methods of lipid extraction and fractionation have been reported (18). The animals were classified according to their clinical condition at the time of sampling as normal, subclinical ketotic, and clinical ketotic. A cow was considered to have subclinical ketosis when showing a positive ketouria; and, clinical ketosis when showing ketouria, depressed feed intake, and reduced milk yield.

Extensive data on the fatty acid composition of blood serum \( \alpha \)- and \( \beta \)-lipoproteins of these cows are being reported (16). The neutral and polar lipid classes from several hundred samples were screened by thin-layer chromatography. All samples were plated at an equal weight of total lipid, so that visual comparisons were possible. Figure 1 shows the separation of neutral lipids associated with the alpha and beta lipoproteins of bovine blood serum. A, B, C = alpha lipoproteins of clinical ketosis (A); subclinical ketosis (B); and a normal cow (C). D, E, F = beta lipoproteins of these same animals. CE = cholesterol esters; T = triglycerides; FA = free fatty acids; C = free cholesterol; PL = total phospholipids.

Two points appear of major significance: First, stressed cows are almost completely devoid of lecithin and sphingomyelin in the \( \beta \)-lipoproteins. Second, the \( \alpha \)-lipoproteins show a large increase in the relative concentration of a fraction which we originally thought to be phosphatidyl-ethanolamine. Subsequent work with this fraction indicates it is composed of at least 90% of what we presently believe to be a peptolipid. Although the compound(s) is not yet positively identified, it appears similar to that previously reported by others (9, 10, 22). No metabolic role has yet been ascribed to this compound(s); however, with the finding of its increased concentration under stressed conditions it seems feasible to clarify its role more fully. The differences in fatty acid (16) and phospholipid composition suggest that the blood lipid transport system is seriously stressed in the ketotic situation.

In the lactating animal there is considerable demand for methionine for synthesis of milk protein and probably also as a methyl donor in the formation of phospholipids (2, 29). Because of bacterial action in the rumen (4, 20), the cow's supply of methionine from the diet will be relatively low and the increased cellular

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metabolism and mobilization of body reserves associated with the beginning of lactation will further increase demands for methionine. High milk production will also require increased synthesis, by the liver and intestinal tract wall, of serum lipoproteins for the marked increase in lipid transport. In addition to its role as a methyl donor for phospholipids, methionine has recently (26) been shown to have a special function in the binding of the lipid and protein moieties in the formation of serum lipoproteins. In view of our observed changes in lipoprotein composition, and the increased demands for methionine at the beginning of lactation, we postulated that a shortage of this amino acid may be important in the development of bovine ketosis, and the highly significant decrease in blood serum proteins, other than serum albumin, reported for ketotic cows by Pehrson (19) supports our postulate. Furthermore, treatment of a goat with ethionine, a methionine antagonist, has produced biochemical changes similar to those we have noted in primary ketosis.

In an exploratory experiment, 23 ketotic cows were treated with methionine or its analog. Eleven of the cows received one to several intravenous infusions of 20 g of L-methionine. The other cows received one 20-g infusion of L-methionine and orally each day by capsules, 30 g of DL-a-hydroxy y-methyl mercapto butyrate-calcium for one to three days. The latter approach was to explore the possibility of obtaining the ideal of one veterinarian visit per cow (14). Because of the exploratory nature of the work, we believed it was permissible to average the data for a number of cows, even though there was variation in the number of infusions and times and manner of administration. Milk production data from 19 cows were averaged to give a five-day cumulative increase of 7.95 kg over the average treatment day production. These data compare very well with the recent report of Robertson (21) showing 6.07 kg increase for cows treated with glucocorticoid plus insulin and a 4.14 kg increase in milk production for those animals treated with glucocorticoid alone. In our view, the data from four animals were unsuitable for inclusion in the mean. In one case the cow required per os treatment daily for several weeks, since she would slip into a ketotic state a few days after discontinuing the capsule. This pattern, we believe is consistent with our present theory. The case history on the second cow is complicated by ketosis, metritis, and mastitis. Three days after calving she received methionine, stilbestrol, and antibiotics. Milk production increased from 19 to 22.7 kg the following day, but appetite did not improve. She then received glucocorticoid. Six days later she was again ketotic. At this time she was treated with L-methionine intravenously and methionine analog per os for three days. The five-day increase in milk production was 20.9 kg. The third cow received glucose plus glucocorticoid after diagnosis of ketosis. The following day she had not improved and milk production was still depressed. She was then given two 20-g intravenous infusions of L-methionine four hours apart. Milk
production improved immediately, resulting in a five-day increase of 22.6 kg. The last cow was not included in the mean, since she had recently calved and her milk production on the day of diagnosis was the highest yet for the lactation. Following treatment she rapidly responded, giving a five-day increase of 71.0 kg in milk production.

The improved production response obtained with glucocorticoid plus insulin over glucocorticoids alone by Robertson (21) is consistent with our present theory. Glucocorticoids will promote the breakdown or mobilization of tissue protein (23), while insulin will suppress the formation of gluconeogenic enzymes in the liver (27) and the dual hormonal action will increase the availability of amino acids by promoting their mobilization but slowing their conversion to glucose.

Changes in the fatty acid composition of total blood serum of a cow with ketosis before and after methionine treatment are reported in Table 1. This rapid change to normal composition (16) illustrates the key participation of methionine in lipid transport. In 1946 Shaw (25) reported the unsuccessful treatment of two ketogenic cows using daily injections or oral administration of 12.5 g of DL-methionine. Treatment with acetyl-methionine of cows with ketosis has been reported (15). No data were included in this report and it appears that most cases were fasting ketosis following indigestion caused by sugar beets, although a few cases may have been primary ketosis.

The blood serum lipoproteins of cows with low fat milk are also altered, as reflected by their fatty acid composition (5, 17). In addition, what we believe to be the peptolipid type compound characteristic of blood serum of cows with ketosis appeared in the milk polar lipids of cows in herds with a low fat problem. Intramammary infusion of 2-amino-2-methylpropanol, which inhibits lecithin formation (28), produced biochemical changes in the polar lipids of milk similar to those we have noted from low testing herds. Methionine could be limiting in these situations for several reasons. First, a number of rations that produce this problem are handled in such a way as to be susceptible to oxidative deterioration. The methyl-mercapto portion of the methionine molecule would be one of the most susceptible to such attack. Secondly, the apparent influence of the plane of nutrition and nitrogen source on rumen synthesis of methionine could be an influencing factor (3). Thirdly, endogenous oxidation of mercapto groups could occur as the result of feeding high levels of unsaturated fatty acids which may initiate lipid peroxidation at sensitive cellular locales (12).

In one high producing herd, receiving excellent quality forage free-choice, a number of cows showed low milk fat tests. Low fat test, even with adequate dietary roughage, is becoming a common occurrence. After feeding the five lowest testing cows 30 g of DL-a-hydroxy γ-methyl mercapto butyrate-calcium for six days, the average fatty acid composition of the milk fat changed, as reported in Table 2. The increase in short-chain fatty acids of methionine in lipid transport. In 1946 Shaw (25) reported the unsuccessful treatment of two ketogenic cows using daily injections or oral administration of 12.5 g of DL-methionine. Treatment with acetyl-methionine of cows with ketosis has been reported (15). No data were included in this report and it appears that most cases were fasting ketosis following indigestion caused by sugar beets, although a few cases may have been primary ketosis.

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lipid and protein metabolism in ruminants will be essential to the development of high producing efficient animals.

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References


