INOSITOL AS A LIPOTROPIC AGENT IN DAIRY CATTLE DIETS

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Summary

Fatty liver syndrome or hepatic lipidosis (HL) is a condition thought to contribute to an increased incidence of peripartum disease, reduced response to therapy and decreased fertility in dairy cows. This syndrome is characterized by excess triglyceride (TG) accumulation in the liver and apparent decreased hepatic lipoprotein output. In lactating rats, a similar condition results from feeding an inositol-deficient diet. It is also characterized by excess hepatic TG accumulation and decreased hepatic lipoprotein output. Myo-inositol is a necessary component of the phospholipid phosphatidylinositol, which is an important membrane constituent. Myo-inositol occurs in feed mainly as the inositol hexaphosphate phytic acid. Phytic acid is undigestible by the monogastric but rumen phytases are assumed to adequately hydrolyze it. In early lactation dairy cows, lipid mobilization is intense, and the myo-inositol requirement may exceed the dietary supply or availability. Myo-inositol is being tested in a field trial as a potential lipotropic agent for dairy cows. Preliminary results suggest no lipotropic benefit from added myo-inositol.

(Key Words: Fat Liver Syndrome, Hepatic Lipidosis, Myo-inositol, Phosphatidylinositol, Phytic Acid.)

Introduction

Fat cow syndrome, fatty liver syndrome and hepatic lipidosis (HL) are terms that have been used to describe a disease syndrome in periparturient dairy cows. Cows calve and develop a variety of health problems thought to be associated with hepatic insufficiency due to large amounts of liver lipid accumulation (Morrow, 1976; Morrow et al., 1979; Reid et al., 1979a,b; Reid, 1980a,b; Deem, 1980). The condition is frequently associated with feeding practices in which excess energy is consumed by low producing late lactation cows or dry cows. They are frequently fed as groups and inadequately separated from high producers or fed free choice. This may result in obese animals. Symptoms are frequently associated with the onset of lactation (Morrow et al., 1979; Reid et al., 1979b); however, the time of onset of hepatic lipid accumulation has not been determined and the etiology is only partly understood.

Myo-inositol is classified in the group of B-vitamins and is associated with positive lipotropic activity in a number of species (Holub, 1982). A deficiency is associated with a variety of lipid metabolic disturbances (Reed et al., 1968; Wells and Burton, 1978; Chu and Hegsted, 1980) and an inverse association between liver inositol and liver fat has been reported in the bovine (Gerloff et al., 1981; Liesman et al., 1981). To our knowledge, myo-inositol as a positive lipotropic agent in the ruminant has not been investigated.

A field study has been initiated in an attempt to evaluate two objectives: 1) to more precisely determine the time of onset of hepatic lipidosis, factors contributing to it and health consequences to the animal and 2) to determine if supplemental myo-inositol will help to reduce hepatic fat accumulation in the dairy cow. Preliminary results from this trial are included in this paper.

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Fat Cow Syndrome. Fat cow syndrome was reported by Morrow (1976) to contribute to increased disease incidence and death loss. With this syndrome, grossly overweight cows calve and then develop a wide variety of diseases such as retained placenta, metritis, ketosis and displaced abomasum. Response to treatment for these diseases is poor and increased death loss results (Morrow et al., 1979). In the fat cow syndrome, the most striking necropsy lesion is severe hepatic lipid accumulation. Livers may float in water due to their high lipid content and histologically severe centrilobular fatty infiltration is present. It is believed that hepatic insufficiency due to lipidosis may contribute to many of the symptoms of fat cow syndrome (Morrow et al., 1979; Deem, 1980).

In addition to the severe HL of grossly obese cows, milder degrees of liver fat infiltration may occur and be associated with reduced fertility. Reid (1980a,b) has reported a 66% incidence of fatty liver in Friesian cows 1 wk postpartum. A longer calving interval was associated with moderate and severe HL (Morrow, 1976; Morrow et al., 1979; Reid et al., 1979b; Reid, 1980b).

The pathogenesis of HL is thought to be associated with two contributory mechanisms (figure 1). The first is an increased delivery of nonesterified fatty acids (NEFA) to the liver, due to the negative energy balance and various hormonal stimuli of early lactation dairy cows. This has been indicated by elevated NEFA in cows with HL (Morrow et al., 1979; Reid et al., 1979b; Reid, 1980b) and suggested in one report where development of HL was associated with greater loss of body fat as measured by condition score postpartum (Reid, 1980a).

The second mechanism apparently contributing to TG accumulation is reduced hepatic TG rich lipoprotein secretions. Total circulating TG are decreased in cows with HL (Morrow et al., 1979) and serum dextran precipitable lipids are decreased in cows with excess hepatic lipid (Gerloff et al., 1981; Liezman et al., 1981). Additionally, in the fatty liver of fasted cows, which has been proposed as a model for HL, hepatic output of lipids is decreased (Reid et al., 1979a). The lipid accumulating in bovine HL is nearly exclusively TG (Collins and Reid, 1980).

Myo-inositol and Lipid Disturbances. Early researchers suggested that myo-inositol was helpful in preventing development of fatty livers in experimental animals, but results were often confusing and inconclusive (Gavin and McHenry, 1941; Best et al., 1951).

More recently, a severe fatty liver could be produced in lactating rats by feeding an inositol-deficient diet (Burton and Wells, 1976). The diets were prepared to contain adequate protein, essential fatty acids, choline and other B-vitamins and .5% phthalylsulfathiazole to depress myo-inositol synthesizing intestinal microorganisms. These fatty livers could be produced only in lactating rats, suggesting an increased requirement for inositol with lactation. The fatty livers that developed had excess TG accumulation and depressed liver TG output in the very low density lipoprotein (VLDL) and high density lipoprotein (HDL) fractions (Burton and Wells, 1977). These characteristics are similar to the dairy cow with HL.

Several other lipid disturbances may be related to myo-inositol metabolism. A fatty liver syndrome in laying hens has been described in which decreased egg production is associated with excess lipid accumulation in the livers (Couch, 1956). Some reports have suggested a positive response to inositol supplementation at 1 g/kg as measured by increased egg production and reduced liver fat (Reed et
al., 1968). Other reports have shown no beneficial effect of added inositol at 1 g/kg (Leville and Bray, 1970).

Female gerbils fed a myo-inositol-deficient diet develop a severe intestinal lipodystrophy characterized by intestinal TG accumulation and hypolipidemia (Chu and Hegsted, 1980). Dietary myo-inositol or myo-inositol injection reverses this lipodystrophy and is associated with an increased plasma chylomicron concentration (Chu and Geyer, 1981). These results suggest myo-inositol is necessary for normal lipoprotein and chylomicron production.

**Function of Myo-inositol**. Inositol is the name applied to the isomers of hexahydroxy-cyclohexane. Myo-inositol is the only isomer that appears to have vitamin-like properties (figure 2). A recent review has dealt extensively with the topic (Holub, 1982). Myo-inositol appears to be important because of its incorporation into the phospholipid, phosphatidylinositol (PI; figure 3). Phosphatidylinositol is an important membrane constituent and probably exerts its lipotropic activity as an important component of lipoproteins. Quantitatively, phosphatidylcholine and phosphatidylethanolamine comprise a major portion of the membranes, with PI constituting approximately 10% of the phospholipids in the rat liver membrane (Wells and Burton, 1978).

In addition to its role as a lipotrope, inositol, as PI, is associated with several other important phenomena. With appropriate stimulation an initial degradation of membrane phosphatidylinositol occurs, followed by a rapid reformation of phosphatidylinositol with phosphatidic acid as an intermediate (figure 4). This "phosphatidylinositol effect" has been demonstrated in a wide variety of tissues under appropriate stimuli. Acetylcholine stimulates phosphatidylinositol turnover in nerve ending fractions of guinea pig cortex (Hawthorne and Pickard, 1979) and vasopressin stimulation of hepatocytes enhances PI turnover (Kirk et al., 1979). Histamine release by rat peritoneal mast cells is associated with enhanced PI metabolism (Cockroff and Gomperts, 1979) as is insulin release by rat pancreatic islets (Freinkel et al., 1975;
Phytic Acid

elements and Rhoten, 1976). This "phosphatidylinositol effect" may help regulate cell surface Ca\(^2+\) permeability and intracellular Ca\(^2+\) concentrations (Michell, 1975). It has also been suggested that enhanced PI turnover may be related to prostaglandin metabolism due to the specific molecular composition of PI. It is found primarily as the 1-stearoyl, 2-arachidonyl species (Holub, 1978) and receptor stimulation may trigger arachidonic acid release, one of the rate-limiting factors in cyclooxygenase activity.

**Phytic Acid.** In the ruminant diet, myo-inositol is present as phytate, or phytic acid, the inositol hexaphosphate (figure 5). It is often present as phytin, the Ca-Mg salt of phytic acid. Phytic acid is found in relatively high concentrations in the seed fraction of cereal grain and lower concentrations in the stem and leaf portion (Nelson et al., 1976).

In order for the phosphorus as well as the myo-inositol to be available to the animal, digestive phytase activity is necessary. Reid et al. (1947) reported that phytate phosphorus was utilized in sheep and that hydrolysis occurred in the rumen. Nelson et al. (1976) reported that no phytate phosphorus was present anywhere in the digestive tract of 9 mo-old steers fed a corn and soybean meal diet, implying that the phytate was hydrolyzed rapidly in the rumen. Under normal conditions, the myo-inositol present as phytic acid should be fully available to the ruminant. If rumen passage time or microflora were altered, though, it may not be available. It was anticipated that oral myo-inositol would escape rumen degradation because it is not degraded after 40 h of acid digestion at 100 C during the assay procedure.

**Materials and Methods**

In order to evaluate the two objectives of 1) more precisely characterizing time of onset of bovine HL and factors contributing to it and 2) determining if supplemental myo-inositol does reduce liver fat accumulation in dairy cows, a field trial was initiated. One-hundred cows from 10 Michigan dairy herds were utilized. Beginning 1 mo prepartum until 1 mo postpartum, 50 cows received .34 kg of a corn-based supplement containing 5% myo-inositol daily in addition to their regular diet. This provided 17 g of nonphytate myo-inositol daily (approximately .1% dry matter of the diet). The remaining 50 cows received .34 kg of a corn-based supplement with no additional myo-inositol. Before initiating feeding of the supplement and approximately every 2 wk thereafter until 1 mo postpartum, liver biopsies and blood samples were obtained.

Liver biopsies were obtained percutaneously through the ninth intercostal space using a commercial biopsy needle\(^6\). At each sampling, three liver specimens were obtained. Two were frozen at -30 C and the third was fixed in formalin. One of the frozen specimens was used for TG determination, extracting the lipid by the method of Hara and Radin (1978) and using a colorimetric triglyceride assay technique (Sigma Chemical Co., 1977). Total myo-inositol content was determined on the second frozen specimen by the method of Wells et al. (1965).

When biopsies were taken, blood samples were also obtained from the coccygeal vein. Blood was allowed to clot and stored at 10 C for a maximum of 5 h when it was centrifuged and the serum stored at -30 C. Insulin concentrations of serum are being determined by radioimmunoassay\(^7\). As an indicator of obesity, condition score of each cow was also assessed, based on the method of National Institute for Research in Dairying (Mulvany, 1977).

Preliminary data from three herds were analyzed by comparing means between inositol-

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\(^6\) Trucut\(^\circ\) Travenol Labs, Inc., Deerfield, IL.

\(^7\) Courtesy of Eli Lilly and Co., Indianapolis, IN.
<table>
<thead>
<tr>
<th>Approximate sampling time</th>
<th>Liver inositol (μmol/g)</th>
<th>Liver TG (% wet wt)</th>
<th>Serum insulin (μunits/ml)</th>
<th>Body condition score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supplemented</td>
<td>Unsupplemented</td>
<td>Supplemented</td>
<td>Unsupplemented</td>
</tr>
<tr>
<td>1 mo prepartum</td>
<td>3.44 ± .69 (8)</td>
<td>2.70 ± .63 (11)</td>
<td>4.39 ± 2.30 (10)</td>
<td>3.36 ± 1.61 (12)</td>
</tr>
<tr>
<td>10 d prepartum</td>
<td>2.19 ± .32 (8)</td>
<td>2.33 ± .56 (8)</td>
<td>5.56 ± 2.87 (8)</td>
<td>7.91 ± 4.79 (8)</td>
</tr>
<tr>
<td>10 d postpartum</td>
<td>2.52 ± .63 (6)</td>
<td>2.61 ± .37 (13)</td>
<td>4.40 ± 1.58 (7)</td>
<td>2.71 ± .89 (13)</td>
</tr>
<tr>
<td>1 mo postpartum</td>
<td>2.19 ± .52 (9)</td>
<td>2.01 ± .22 (12)</td>
<td>6.52 ± 2.55 (8)</td>
<td>3.42 ± .74 (13)</td>
</tr>
</tbody>
</table>

*Denotes number of observations.

b Means differ (P<.05).
supplemented and nonsupplemented groups using a two-tailed t-test.

Results and Discussion

Preliminary analysis of a limited number of animals indicates that additional inositol supplementation has no effect on liver TG accumulation, serum insulin levels, body condition score, or total liver inositol. These preliminary results are presented in table 1. Due to death loss and an occasional inability to obtain liver biopsies, number of observations/group are not constant over time.

Liver inositol did not differ between groups and concentrations are in agreement for liver inositol content of other species (Wells et al., 1965). In the liver, myo-inositol was present almost totally in the lipid-extractable fraction as the phospholipid (Wells et al., 1965), so total liver inositol was an indicator of PI. Inositol content or availability was not limiting in the herds analyzed for this investigation, as indicated by similar concentrations of inositol in liver of both groups. In the unsupplemented diet, myo-inositol was present as phytic acid. These results suggest that this myo-inositol is available to the animal in adequate amounts or that adequate myo-inositol is synthesized by intestinal or ruminal microorganisms. As expected if myo-inositol is not limiting, there was also no difference between groups in liver triglyceride content. Serum insulin concentrations did differ at the prepartum sampling, but at no other sampling time. Body condition score decreased after calving, but did not differ between groups, suggesting similar degrees of fat mobilization.

These results are very incomplete and conclusions drawn from them should be very tentative. In addition to the factors already measured, serum dextran sulfate precipitable TG will also be measured as an indicator of VLDL content of the blood. Serum aspartate aminotransferase activity will be determined also. Aspartate aminotransferase is the liver enzyme whose serum concentration is most consistently correlated with liver fat content (Reid, 1980b; Gerloff et al., 1981).

More complete analysis of data will include herd as a fixed variable and examine herd x diet interactions.

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