FIELD STUDY OF
BOVINE HEPATIC LIPIDOSIS

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ABSTRACT

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Eighty pluriparous dairy cows from nine Michigan herds were sampled over the peripartum period. Thirty-nine cows received 17 g supplemental myo-inositol daily and 41 cows received a placebo supplement without inositol. Liver biopsies were obtained percutaneously and analyzed for TG and inositol content. Blood samples were obtained and serum analyzed for free and total thyroxine (T4) and triiodothyronine (T3), insulin, nonesterified fatty acid (NEFA) and dextran sulfate precipitable cholesterol concentrations.

Most variables were affected strongly by time from calving. Liver TG concentrations usually increased in the immediate postpartum period, although in two herds hepatic TG concentrations were greatest prepartum. Serum insulin, dextran precipitable cholesterol, T3 and T4 concentrations declined during the peripartum period. Free concentrations of T3 and T4 paralleled the changes in total concentrations, and the fraction of thyroid hormone free did not change in the time period studied. Serum NEFA concentrations increased postpartum, and were the most highly correlated serum measurement with hepatic TG content (r=.34).

Inositol supplementation had no lipotropic effect. Cattle supplemented with myo-inositol did have lower serum T3 and free T3 concentrations, however.

Cattle were retrospectively divided into three groups bsed on degree of hepatic TG accumulation: mild, moderate or severe hepatic lipidosis (HL). 35% of cattle sampled had moderate or severe HL. Cattle with severe HL had an increased death and cull rate and had a tendency to have an increased rate of disease. Cattle with moderate HL had increased days open, and tended to have higher milk production than other groups.

It was concluded that hepatic TG accumulation is most closely related to serum NEFA concentrations and the influx of fatty acids. Severe HL has detrimental effects on health and productivity, but the increased days open associated with moderate HL is likely more closely related to other factors beside hepatic TG content, possibly milk production. Inositol supplementation was ineffective as a lipotropic agent but did result in lower serum T3 and free T3 concentrations.

DEDICATION

I was once told that if I were one-half the person either of my parents were, then that was pretty special. I agree, and would like to dedicate this thesis to them.

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To acknowledge all of the people that have assisted with my career to date is impossible; I would like to especially recognize a few, however.

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INTRODUCTION

The dairy cow in early lactation must overcome severe metabolic demands. High milk production is sustained at a time when dietary nutrients cannot fulfill production requirements. In addition, the additional requirements of having a fertile estrus and conceiving are imposed at this time of severe metabolic demand. This early lactation period is the time when most management attention is focused on the cow and when disease is most likely to occur. This time period has been actively investigated in an attempt to more clearly understand the metabolic changes which are occurring during the transition from the nonlactating to lactating state and the consequence of those changes to health and fertility.

The metabolic changes occurring in the dairy cow during the periparturient period have been observed to result in an accumulation of hepatic triglyceride (TG). Severe cases of hepatic TG accumulation have been observed in obese animals and labeled fat cow syndrome or fatty liver syndrome. This syndrome has been associated with increased rates of disease and death, although the health consequences of HL have not been clearly determined. The metabolic disease ketosis frequently occurs concurrently with excessive hepatic TG accumulation as well.

A milder, subclinical hepatic lipidosis (HL) has also been reported in the postpartum period. Dairy cattle with moderate HL have been reported to have increased days open as well as delayed first ovulation and return to first estrus. The cause of this association between hepatic TG accumulation and reproductive performance is not well understood.

The addition of myo-inositol to the diet has been observed to reduce hepatic TG accumulation in several species and we observed a negative association between hepatic inositol and TG content in postpartum dairy cattle. The use of myo-inositol as a feed additive in cattle has not been investigated.

Changes in serum lipid and hormonal concentrations around the time of calving have been described by many investigators. Intensive monitoring of changes in large numbers of cows, and assessing the association of these changes with hepatic TG accumulation has not been done as thoroughly, however.

A field study was undertaken to attempt several objectives. Changes in hepatic TG content in large numbers of Michigan dairy cattle over the peripartum period were to be determined. The degree of HL and the number of cattle which developed HL were to be determined, as well as possible consequences of HL to health and fertility. Changes in serum thyroxine and tri-iodothyronine (bound and free), insulin, dextran sulfate precipitable cholesterol and nonesterified fatty acid concentrations were also monitored over the periparturient

period, and their relationship to hepatic TG content investigated.

Myo-inositol was fed to one-half the cattle in an effort to determine if it exerted a positive lipotropic effect in normal ruminant diets.

Results of this field investigation comprise the major portion of this thesis.

REVIEW OF LITERATURE

The liver is one of the primary organs involoved in fat metabolism and in regulating lipid flux from and to the tissues. In normal liver in man the amount of lipid is approximately 5% by weight (Hoyumpa et al., 1975; Alpers and Sabesin, 1982). These lipids include triglycerides, fatty acids, phospholipids, cholesterol and cholesterol esters. Phospholipids comprise the greatest proportion. The lipid which accumulates in most types of hepatic lipidosis (HL) is primarily triglyceride (TG); the amounts of other lipids remain fairly constant, although their relative percentages decrease (Reid and Collins, 1980; Alpers and Sabesin, 1982). Maximum lipid concentrations reported have been approximately 40% to 50% by weight, or > 80% by space occupying volume (Leevy et al., 1953).

The amount of TG in the liver is regulated and determined by a variety of factors. The availability of non-esterified fatty acids (NEFA) for esterification into triglycerides has a major effect on the accumulation of hepatic TG. Under normal conditions, most of the TG synthesized in the liver is removed as very low density lipoproteins (VLDL). VLDL are formed by the assembly of TG, apoproteins, phospholipids, cholesterol, and cholesterol esters. The balance between TG synthesis and VLDL output determines the amount of hepatic lipid accumulation. An increase in TG synthesis rate without a corresponding increase in VLDL output will result in fat accumulation. Similarly, a decrease in hepatic VLDL ouput, if unaccompanied by an equal decrease in TG synthesis will also result in fatty liver. A combination of these two mechanisms is also possible.

Fatty acid metabolism.

Fatty acids for esterification or oxidation in the liver can come from three sources: 1) circulating NEFA derived from adipose tissue; 2) de novo hepatic synthesis and 3) chylomicrons containing fat derived from the diet (Hoyumpa et al., 1975; Alpers and Sabesin, 1982). In the ruminant, unlike many nonruminant species, very little fatty acid synthesis occurs in the liver (Ballard et al., 1969). In many nonruminants, de novo synthesis of fatty acids occurs from acetyl coenzyme A (AcCoA) derived primarily from decarboxylation of pyruvate within the mitochondria - thus dependent on a source of pyruvate such as glucose, glycogen or lactate. The translocation of AcCoA from the mitochondria to the cytoplasm, the site of fatty acid synthesis, occurs via the citrate - cleavage pathway. Intramitochondrially, AcCoA is condensed with oxaloacetate to form citrate, which is translocated into the cytoplasm. There it is cleaved by citrate cleavage enzyme in an ATP dependent reaction to re-form AcCoA and oxaloacetate. The AcCoA can then be utilized for fatty acid synthesis. The carboxylation of AcCoA to form malonyl CoA is the first committed step of fatty acid synthesis.

It is catalyzed by AcCoA carboxylase. Elongation of the fatty acid then occurs by addition of successive acetyl units from malonyl CoA and is catalyzed by the fatty acid synthase complex of enzymes in an NADPH requiring reaction. (See Ballard et al., 1969; Bell, 1979; Vernon, 1980, for review.)

Ruminant liver contains only limited amounts of AcCoA carboxylase, and very low activity of the citrate - cleavage pathway has been reported (Ballard et al., 1969). NADP malate dehydrogenase is a major source of reducing equivalents for fatty acid synthesis in nonruminant liver. Very low activity has been observed in ruminant liver (Hanson and Ballard, 1967; Ballard et al., 1972; Pearce, 1983), further substantiating the conclusion that very little de novo hepatic fatty acid synthesis occurs. This likely represents a metabolic adaptation of the ruminant to its dietary peculiarities. Glucose synthesis and export by the liver is required continuously because of its relative scarcity in the portal circulation. It would be inefficient to use glucose to generate AcCoA for fatty acid synthesis. Support for this interpretation is suggested by the observation that in sheep fed on a high carbohydrate diet beyond the normal weaning age, or infused with glucose intra-abomasally or intravenously, there was increased hepatic lipogenesis from glucose and acetate. Large increases in activities of citrate - cleavage enzyme and NADP malate dehydrogenase occurred. Activities were still very much lower than for the rat, however (Ballard et al., 1972).

Most ruminant diets contain very small amounts of fat (Palmquist and Jenkins, 1980) so dietary lipid is quantitatively a very minor source of fatty acids for oxidation or esterification in the ruminant liver. By far the most significant source of fatty acids for hepatic metabolism in the ruminant is from NEFA mobilized from adipose tissue. In late gestation and early lactation (Bauman and Currie, 1980) and during fasting (Radloff et al., 1966) NEFA concentrations in serum rise in the bovine. Hormone sensitive lipase (HSL) stimulates the liposysis of TG stores in adipose tissue and the release of NEFA and glycerol into the circulation. HSL is activated by cyclic adenosine monophosphate (cAMP) dependent protein kinase and is stimulated by treatment with epinephrine, norepinephrine, possibly growth hormone, glucocorticoids and glucagon. (See Vernon, 1980.) Insulin inhibits this activation. Long chain NEFA are water insoluble and are transported in the blood stream bound to albumin. Kinetic analysis of ^{14}C-labeled oleate binding to bovine serum albumin suggests that each albumin molecule has six high energy binding sites for NEFA - three primary and three secondary sites. In addition, as many as 63 weak tertiary binding sites may also exist. Between six and 13 moles of NEFA may bind to one mole of bovine albumin (Spector et al, 1969). The NEFA are transported in the blood, and are efficiently extracted from the circulation by hepatic tissues. The fractional clearance rate of NEFA from blood by the liver has been reported from 10% to 40% in various species (Katz and Bergman, 1969; Basso and Havel, 1970; Bell, 1979; Kushlan et al., 1981). In many species a fractional clearance rate of NEFA from the circulation by the liver of approximately 25% has been suggested (Basso and Havel, 1970; Bell, 1979); however, one estimate in ruminants reported a clearance rate of only 10% (Katz and Bergman, 1969). There is some evidence that isolated perfused livers from females clear NEFA at a greater rate than those from males (Kushlan et al., 1981). At physiological NEFA concentrations up to 2 to 3 mM, hepatic uptake of NEFA appears to be linear and a function of the concentration of NEFA in plasma (Katz and Bergman, 1969).

It has been suggested that the first step in hepatic fatty acid uptake is a physical adsorption to the cell membrane followed by the dissociation of NEFA and albumin. The limiting factor for uptake may be "the kinetic rate of equilibrium between the associated and dissociated form of free fatty acid and albumin" (Soler-Argilaga et al., 1974). Rates of NEFA uptake by perfused rat liver varied inversely with chain length, which corresponded to their affinity to albumin (Soler-Argilaga et al., 1973). Further evidence suggests that uptake is mediated by albumin receptors on the liver plasma membrane. Uptake into hepatocytes showed saturation kinetics as the concentration of albumin and oleate increased, but not when the concentration of oleate only was increased, suggesting that albumin receptors were saturable and rate limiting at high albumin concentrations (Weisiger et al., 1981). Uptake into adipocytes was not dependent on albumin concentrations (Abumrad et al., 1984), suggesting other cells may lack these albumin receptors. Hepatocyte receptors may contribute to efficient hepatic extraction of NEFA and other organic anions from the circulation (Weisiger et al., 1981).

Non-esterified fatty acids are transferred across the plasma membrane by an unknown process, possibly simple diffusion (Degrella and Light, 1980). A saturable transfer mechanism, probably protein in nature has also been suggested (Abumrad et al., 1981, 1984). Fatty acids are bound to fatty acid binding protein (FABP) in the cytosol. Significant correlations between cytosolic concentration of FABP and flux of NEFA into the cell have been reported (Kushlan et al., 1981; Ockner et al., 1982). This FABP also has a strong affinity for other organic anions such as bilirubin, cholesterol, heme, bromsulphthalein (BSP) and indocyanine green. It may be the same binding protein previously reported as Z-protein, sterol carrier protein or C-protein (Ockner et al., 1982). Elevated concentrations of FABP have been reported in female rat liver compared to male (Kushlan et al., 1981) and may tend to favor esterification of fatty acids at the expense of oxidation

due to the spatial location or orientation of this protein at the endoplasmic reticulum (Wu-Rideout et al., 1976).

Utilization of fatty acids by the hepatic cell requires activation to thiol esters by acyl-CoA synthetases, which generally are specific for short, medium or long chain fatty acids (Bell, 1979). Long chain acyl CoA synthetase is localized in the endoplasmic reticulum and outer mitochondrial membrane (Groot et al. 1976). It has not been measured directly in ruminant liver, but is believed to be similar to other species. Hepatic activity exceeds rates of beta oxidation (Pande, 1971) and esterification with alpha-glycerophosphate (Lloyd-Davies and Brindley, 1973), suggesting that activation of long chain fatty acids is not the rate limiting step in the metabolism of NEFA by the liver.

The two main pathways for acyl CoA within the hepatic cell are 1) oxidation and possibly ketogenesis within the mitochondria or 2) esterification to TG, phospholipid or cholesterol ester within the microsomal fraction (Mayes, 1976). The relative activities of these pathways are controlled by a variety of substrate, metabolite and hormonal effects, and have been reviewed extensively elsewhere (Hubscher, 1970; Mayes, 1976; McGarry and Foster, 1980; Haagsman and Van Golde, 1984). Triglyceride synthesis in the ruminant liver, similar to other species, occurs primarily by the glycerol phosphate pathway; however, alternative pathways may be present (Benson and

Emery, 1971; Bell, 1979). The enzymes responsible for the acylation of glycerol-3-phosphate (G3P) are localized on the outer mitochondrial membrane and the endoplasmic reticulum (Hubscher, 1970). Activity of this pathway is determined primarily by substrate availability and by modulation of activity of competitive pathways (Hubscher, 1970). Availability of G3P has been thought to be one of the controls of esterification rates (Mayes, 1976). Liver G3P concentrations were decreased in fasted or epinephrine treated rats, where rates of fatty acid esterification were decreased (Tzur, 1964). Glycerol added to the media of isolated hepatocytes from fasted rats increased esterification at the expense of oxidation without altering fatty acid uptake (Lund et al., 1980). However, increasing G3P concentrations above that in normal fed rat liver had no effect on fatty acid esterification rates. It was suggested that although G3P availability can influence esterification rates, it is probably not the major factor in their regulation (Pikkugangas et al., 1982). Probably the major determinant of the amount of acyl CoA esterified is the amount present, and the rate of hepatic oxidation of fatty acids. The metabolism of various concentrations of ^{14}C-labeled oleate was compared in perfused livers from fed and starved rats. At each oleate concentration, livers from fed rats esterified more fatty acid and oxidized less. In fed rats as oleate concentrations were increased, the fractional rate of oxidation increased and the fractional

rate of esterification decreased (although total esterification increased). In livers from starved rats the pattern was different. At low oleate concentrations, most of the fatty acid was oxidized; with increasing oleate a constant proportion of fatty acid was esterified and oxidized, so total esterification also increased (Mayes and Felts, 1967). In perfused livers from starved rats, where most NEFA are normally oxidized, by blocking fatty acid oxidation with (+)-decanoylcarnitine, virtually all the fatty acids were esterified (McGarry et al., 1973). These observations suggest that the amount of esterification is determined primarily by the amount of free fatty acids available which are not being oxidized. McGarry and Foster (1980) have proposed an elegant system of regulation of hepatic fatty acid oxidation. The primary site of beta-oxidation of long chain acyl CoA and ketogenesis is intramitochondrial, although some oxidation of NEFA probably occurs in the peroxisomes. The inner mitochondrial membrane is impermeable to long chain acyl CoA derivatives, and translocation from the cytosol is dependent on successive conversion to acyl carnitine and back to acyl CoA, catalyzed by carnitine acyl transferase I (CAT I) and carnitine acyl transferase II (CAT II), respectively. These two enzymes are located at the inner and outer surface of the inner mitochondrial membrane, respectively. The regulatory scheme suggests the control point for oxidation and ketogenesis is the transport of acyl CoA into the mitochondria at the level of CAT I. This enzyme is carnitine dependent.

Malonyl CoA formed by AcCoA carboxylase is the first intermediate in fatty acid synthesis and is a very strong competitive inhibitor of CAT I. Conditions which favor AcCoA carboxylase activity, such as high insulin concentrations or elevated citrate, result in increasing malonyl CoA concentrations, inhibition of CAT I, reduced transport and oxidation of acyl CoA and thus more acyl CoA available for esterification. Inhibition of AcCoA carboxylase results in a declining concentration of malonyl CoA, increased activity of CAT I and greater oxidation of acyl CoA intramitochondrially.

Even under conditions in which oxidation is maximally stimulated, increasing NEFA concentrations will increase hepatic esterification to TG, due probably to greater amounts of fatty acids available for esterification (Pelech et al., 1983). This situation probably occurs in ketotic dairy cows and diabetic humans where high rates of fatty acid oxidation and ketogenesis are accompanied by HL (Saarinen and Shaw, 1950; Alpers and Sabesin, 1982; Gröhn et al., 1983).

Generally the activity of the enzymes of esterification and glyceride synthesis are stimulated by an increase in insulin concentrations, a decrease in glucagon concentrations, or an increase in the insulin:glucagon ratio. The rate of esterification in ruminant liver has generally been reported to be lower than in other species (Benson and Emery, 1971; Payne and Masters, 1971). In ruminants, most fatty acids esterified are incorporated into phospholipids in the normal fed state (Bell, 1979). Diacylglycerol acyltransferase catalyzes the only reaction unique to TG synthesis, and is generally accepted as being a major control point between phospholipid and TG synthesis. Control of the relative flux between these two esterification pathways is poorly understood (Bell, 1979). It may be that enzymes for phospholipid formation are saturable at lower fatty acid concentrations and less subject to regulation, due to their essential nature in membrane formation. It has been observed that under conditions of high exogenous free fatty acid availability, TG synthesis increased to a much greater extent than phospholipid synthesis (Groener and Van Golde, 1978; Haagsman and Van Golde, 1984).

Relative rates of oxidation and esterification are determined by regulation of CAT I and activity of the alpha-glycerol-phosphate pathway. These pathways may be modulated by hormonal effects, primarily insulin and glucagon, as well as availability of substrates. At elevated NEFA concentrations, TG esterification increases dramatically, despite rapid rates of fatty acid oxidation.

Lipoprotein output.

A comprehensive discussion of lipoprotein metabolism is not intended, but a brief review will be attempted.

Several complete reviews of human and rat lipoprotein metabolism are available (Alaupovic, 1981; Eisenberg, 1983) as well as more detailed discussions of ruminant lipoprotein metabolism (Griel and McCarthy, 1972; Palmquist, 1976; Puppione, 1978; Kris-Etherton and Etherton, 1982).

Plasma lipoprotein molecules consist of a neutral lipid core containing TG and cholesterol ester and an outer shell containing the bipolar phospholipids, cholesterol and apoproteins. Human lipoproteins are divided into classes based on density differences using ultracentrifugation separation techniques (Havel et al., 1955). The relative densities are determined by the lipid:protein ratio, the amount of neutral lipid in the core and relative amounts of phospholipid and apoprotein in the outer layer. Very low density lipoprotein (VLDL) and chylomicrons ($d < 1.006$) are large, TG rich particles secreted primarily by the liver (VLDL) or intestine (chylomicrons). Low density lipoproteins (LDL) are of intermediate density ($1.006 < d < 1.063$) and relatively rich in cholesterol ester. Intermediate density lipoproteins (IDL) are a further subgroup of LDL ($1.006 < d < 1.023$) and are believed to be products of VLDL catabolism in the circulation. High density lipoproteins (HDL) ($d > 1.063$) are higher in phospholipid and apoprotein and lower in neutral lipid content than the other lipoproteins. On electrophoresis, HDL demonstrate alpha migration while LDL undergo beta migration. These respective lipoprotein classes are also referred to as alpha and beta lipoproteins, and VLDL are referred to as pre-beta lipoproteins. Chylomicrons remain at the origin. In man, these general lipoprotein classes have been further subdivided into additional classes - HDL_1, HDL_2, HDL_3, etc. (Eisenberg, 1983). Each class of lipoproteins can also be characterized to some degree by the specific apoprotein(s) present in its outer layer. These specific apoproteins have effects on plasma enzymes and interact with tissue receptor sites to control the fate and flux of the lipoproteins and their components within the circulation. In addition, they may determine the surface to core ratio and the relative amounts of different lipid components present (Alaupovic, 1981; Eisenberg, 1983).

Chylomicrons are secreted by the intestine and are the largest, least dense of the lipoprotein particles. Their core is composed largely of TG derived from fatty acids absorbed from the intestinal tract. Their surface contains apoproteins B, C, E, and A. Very low density lipoproteins are similar in structure to chylomicrons. They also contain apoproteins B, C and E and have a large TG - rich core. They are smaller and denser than chylomicrons, however, and their TG is of endogenous origin rather than dietary. They are secreted predominantly by the liver, but under some circumstances significant intestinal secretion may occur (Kalopissis et al., 1982). Apoprotein C-II

appears to be transferred from HDL to the surface of the VLDL following their release into plasma. Apoprotein C-II is an activator of lipoprotein lipase (LPL), an enzyme found on the endothelial walls of the capillaries. Lipoprotein lipase hydrolyzes the fatty acids from TG in the lipoprotein core, thus increasing local NEFA concentration at the tissue level. This results in the transport of NEFA into the tissues. A steady depletion of core TG occurs and results in the collapse of the VLDL or chylomicron outer membrane. An unstable chylomicron remnant or IDL particle results. These are cleared rapidly from the circulation by the liver. The apoprotein E on these remnants appears to interact with hepatic receptors, chylomicron remnants being cleared more efficiently than IDL (Brown et al., 1981). If not cleared, continued TG hydrolysis results in a further depletion of VLDL (and possibly chylomicron) core of TG until virtually only cholesterol esters remain. Associated with this core TG depletion is a removal of apoprotein A, C, E and phospholipid from the surface. A LDL particle remains with apoprotein B as the only apoprotein constituent. Apoprotein B is apparently the only apoprotein not transferred from molecule to molecule in the circulation. In man, LDL is thus formed totally as a catabolic product of VLDL within the circulatory system (Alaupovic, 1981; Eisenberg, 1983), although in other species direct synthesis of LDL may occur (Swift et al., 1982). Low density lipoprotein interacts with extrahepatic tissue receptors and is removed from the circulation relatively slowly. Low density lipoproteins are believed to function primarily as a transport source of cholesterol to extrahepatic tissues.

High density lipoproteins contain the relatively greatest amount of protein and phospholipids and least amount of TG. "Nascent" HDL are discoidal shaped bundles of cholesterol, apoprotein and phospholipid with very little cholesterol ester and TG. They appear to form by one of two possible mechanisms. As the VLDL and chylomicron structures collapse due to the action of LPL, surface constituents of apoproteins C, E, and A and phospholipid and cholesterol may bud out and be pinched off, forming a nascent HDL particle (Alaupovic, 1981; Eisenberg, 1983). These nascent discoidal particles may also be secreted directly by the liver (Hamilton et al., 1976), and intestine (Glickman and Green, 1977). Nascent HDL particles are converted to the more numerous spherical HDL by the action of plasma lecithin cholesterol acyl transferase (LCAT). LCAT transfers an acyl group from phosphatidylcholine to cholesterol, forming cholesterol ester. LCAT is activated by apoprotein A-1, found on the surface of the HDL particle. Its activity results in the accumulation of cholesterol ester by HDL. The movement of these hydrophobic cholesterol ester molecules to the center of the lipoprotein results in the HDL becoming spherical in shape. HDL are believed to function in the clearance of cholesterol from the bloodstream, and accept cholesterol from various tissues (Eisenberg, 1983). They also may deliver cholesterol for use by extrahepatic tissues, at least in the rat (Andersen and Dietschy, 1978).

Ruminant and bovine lipoproteins generally are believed to function in an analagous fashion to human (Palmquist, 1976; Puppione, 1978; Kris-Etherton, 1982). Several differences exist, though. Unlike the situation in man, the majority of bovine lipids are in the HDL fraction ($d > 1.063$) with low VLDL and chylomicron concentrations. The importance of chylomicrons in bovine sera has been questioned (Griel and McCarthy, 1969) but their low concentration is generally felt to reflect a relative lack of lipid in the diet, rather than an inability of the intestine to synthesize chylomicron (Bell, 1979). Low circulating VLDL TG concentrations in lactating cattle may reflect increased removal by action of mammary LPL (Bell, 1979) or reduced output by the liver.

Using human density criteria for differentiating lipoproteins in cattle has resulted in some confusing and conflicting data. In the range for human LDL ($1.006 < d < 1.063$) several investigators have observed alpha migration on electrophoresis (Puppione et al., 1972; Raphael et al., 1973; Stead and Welch, 1975). In normal human serum, only beta migration occurs in lipoproteins of this density range. Stead and Welch (1975) subfractionated their LDL into density subfractions LDL_1 (1.019 < d < 1.039) and LDL_2 (1.039 < d < 1.060) with LDL_1 having primarily alpha migration and LDL_2 primarily beta migration. This conflicted with observations of Raphael et al. (1973) in which the denser subfraction of this density range had predominantly alpha migration. Immunologically the subfraction in the density range 1.040 < d < 1.063 did cross react with antibodies to the beta and alpha fractions (Puppione et al., 1970). More recent investigations utilizing filtration gel chromatography have suggested the fraction 1.040 < d < 1.060 contains both LDL and HDL components, and in cattle human density divisions are not necessarily appropriate for separating lipoprotein classes (Ferrari and Gloeckler, 1979; Grummer et al., 1983). Sample handling may also significantly alter results. Puppione et al. (1982) have suggested cooling ruminant lipoproteins alters their density properties because of crystallization of the large numbers of saturated fatty acids in the hydrophobic core. It is clear that much work remains to better characterize lipoprotein metabolism in ruminants.

Further confusion in interpreting experimental results in cattle has resulted from the wide variety of techniques used to separate the lipoproteins. These techniques have included ultracentrifugation, electrophoresis, precipitation by sulfated polysaccharides and gel filtration techniques (See Puppione, 1978 for review). Good

comparative data on large numbers of cows across lactation and gestation is not available, so conclusions remain somewhat tentative.

Hepatic Assembly of Lipoprotein

Very low density lipoproteins are the major source of plasma TG and the major lipoprotein secretory product of the liver, although HDL may originate there as well (Hamilton et al., 1976). Within the liver there appears to be two distinct TG pools - a large, inert "floating fat" fraction composed of lipid droplets (Stein and Shapiro, 1959) and a small precursor pool actively involved in TG synthesis. The small precursor pool, located in the microsomal fraction, consists of newly synthesized TG. It is this newly synthesized TG which is preferentially incorporated into VLDL for secretion (Mayes, 1976; Ide and Ontko, 1981). Mobilization of TG from hepatic lipid deposits depends on lipolysis of the floating fat fraction followed by either oxidation or re-esterification into the precursor pool, from which VLDL synthesis occurs (Bar-On et al., 1971). Lipolysis of the hepatic TG stores requires the action of a hepatic TG lipase (HTGL). In man, rats and chickens there appear to be two distinct hepatic lipases: 1) an extracellular, heparin-releasable enzyme with alkaline pH optimum mainly associated with the exterior surface of the plasma membrane and 2) an intracellular enzyme with acid pH optimum associated with the lysosomes (Debeer et al., 1982). The intracellular, lysosome

associated enzyme is apparently responsible for hepatic lipolysis, and is probably mediated by autophagocytosis (Debeer et al., 1982).

Triglyceride, either re-esterified following lipolysis of hepatic stores or newly esterified from glycerol and NEFA at the smooth endoplasmic reticulum (ER), combines with apoprotein and phospholipid synthesized at the rough ER. From there it is transported to the Golgi and secreted into the space of Disse from where it enters the plasma (Stein and Stein, 1967). The process requires approximately 20 minutes for labeled fatty acids to appear in the circulation as VLDL. The exact sequence and control of this packaging and secretory process is not clearly determined. Some investigators have suggested that phospholipid and apoproteins are added to the TG core in the smooth ER, through which they travel to the Golgi. There they are concentrated in secretory vesicles and secreted by exocytosis (Claude, 1970, Glauman et al., 1975). Tracing protein rather than lipid, Alexander et al (1976) suggested that the TG rich particle is synthesized in the smooth ER. At the junction of the smooth and rough ER, apoprotein is added. Specialized tubules then transport these nascent lipoproteins to the Golgi, where concentration occurs in secretory vesicles. These secretory vesicles migrate to the surface and release their contents by exocytosis. Synthesis and(or) release of VLDL is dependent on normal microtubule function, as it is

inhibited by colchicine treatment (LeMarchand et al., 1973). In a recent experiment, apoprotein was labeled with ^{3}H-leucine and phospholipid and TG were labeled with ^{3}H-glycerol in cultured chick hepatocyes. Results suggested at least some of the phospholipid is added to the lipoprotein after most of the assembly has occurred, immediately before release from the liver (Janero and Lane, 1983). B apoprotein is an integral part of the lipoprotein complex and may be necessary for secretion to occur (Alexander et al., 1976). Glycosylation of the apoproteins occurs at the Golgi and is probably necessary for normal secretion (Alpers and Sabesin, 1982).

The synthesis, deposition and secretion of TG from the liver is a complex process subject to a variety of hormonal and substrate controls. A number of nutritional, metabolic or toxic states may alter the normal balance between oxidation, esterification and VLDL formation, resulting in an accumulation of fat or HL.

Fatty liver in man

Several reviews on the clinical aspects of HL in man are available (Leevy, 1962; Hoyumpa et al., 1975; Alpers and Sabesin, 1982). Leevy (1962) discussed the historical background and suggested that fatty liver in man was first recognized with the early development of post mortem dissection. Hepatic lipidosis was associated with diabetes, obesity and some infections. It was also felt to be related to alcoholism. In 1870 it was demonstrated that

ethanol induced an increase in liver fat in dogs, which could be modulated by diet. With the development of percutaneous needle biopsy techniques (Billing et al., 1953), ante mortem diagnosis of fatty liver in humans was possible, and more detailed information has accrued.

Health consequences which have been associated with HL in man have included progression to cirrhosis (Leevy, 1962), acute death, possibly due to fat emboli (Kramer et al., 1968) and menstrual irregularites (Marres et al., 1973).

Histological changes present in HL include an engorgement of the cytoplasm with TG droplets. These droplets can be selectively stained by fat stains such as oil-red-O or toluidine blue. These lipid droplets appear to be vesiculations of the ER, and coalesce into larger and larger fat droplets as TG accumulates (Alpers and Sabesin, 1982). Previously, fatty livers were characterized as to whether small, microvesicular or large cutoplasmic droplets were present (Leevy, 1962). Now these histologic differences are believed to represent stages of progression of HL (Alpers and Sabesin, 1982). Hepatic lipidosis of different etiologies results in a characteristic initial pattern of lobular infiltration. In mild cases, TG infiltration is characterized as centrilobular or periportal; with increasing severity the distribution becomes generalized (Leevy, 1962). The explanation for and significance of these characteristic patterns of distribution is not well understood. Other ultrastructural changes may include enlarged mitochondria (Gresham, 1961). Hepatic cirrhosis may result from connective tissue infiltration after rupture of fatty cysts (Leevy, 1962), but it is probable that acute necrosis must accompany fatty change for cirrhosis to develop (Hoyumpa et al., 1975; Alpers and Sabesin, 1982).

Quantitative classifications of severity of HL have varied from author to author, although generally similar group divisions have been proposed. Based on histological fat content, divisions have been proposed of mild, moderate and severe fatty metamorphosis with 10% to 30%, 30% to 80%, and > 80% of a section consisting of fat, respectively (Leevy et al., 1953). Alternative proposed divisions have been minimal, moderate and marked HL with < 10%, 10% to 50%, and > 50% lipid, respectively, based on histologic assessment (Holtzbach et al., 1974). Marres et al. utilized divisions of minimal, mild, moderate and marked fat infiltration with criteria being < 5%, 5% to 25%, 25% to 50%, or > 50% of hepatocytes with fat droplets, respectively. There is a good correlation between liver lipid or TG accumulation and histological fat content (Billing et al., 1953; Herdt et al., 1982; Gaal et al., 1983a) and divisions based on biochemical quantitation of lipids have also been proposed. Billing et al. (1953) described slight, moderate and severe HL corresponding to hepatic lipid content of < 8%, 8% to 16%, and > 16% lipid on a wet weight basis, respectively. Leevy et al. (1953) correlated histologic appearance with biochemical lipid content, and using the same mild, moderate and severe categories reported divisions of 8% to 14%, 14% to 25%, and 25% to 40% hepatic lipid, respectively. In man, less than 5% to 8% lipid by weight, or less than 10% by histological content are felt to represent normal hepatic lipid concentrations. Designations as to what constitutes mild, moderate and severe fatty liver are more arbitrary, but recent investigators appear close to the criteria of Marres et al. (1973).

In the bovine, Reid (1980) has proposed a classification of hepatic lipid content in postpartum dairy cows based on a quantitative, stereological determination of space occupied by lipid. Categories of mild, moderate, and severe fatty liver with fat content of < 20%, 20% to 40%, and > 40%, respectively, have been proposed. These quantities of fat have been correlated with biochemical determinations of hepatic lipid content, and closely correspond to liver TG concentrations of < 50, 50 to 100, and > 100 mg TG / g liver wet weight for the categories of mild, moderate and severe fatty liver, respectively (Gaal et al., 1983a). These categories are similar to those proposed by Marres et al. (1973) for human samples.

Clinical and laboratory features

Leevy (1962), in his report of 270 cases of biopsy confirmed fatty liver, indicated common clinical pathologic tests used to diagnose liver disease were not well correlated with HL. This lack of correlation of amount of fat in the liver with degree of abnormality of liver function tests has been consistently reported (Brados et al., 1963; Hoyumpa et al., 1975; Alpers and Sabesin, 1982). Hepatomegaly has been reported as a consistent sign, found in 75% of cases (Leevy, 1962). Newer techniques such as computerized tomography and Xenon-133 retention have shown better correlation than liver function tests with degree of HL (Alpers and Sabesin, 1982).

An alteration in quantity or composition of serum protein has been the most frequently noted biochemical abnormality. There is frequently a lowered albumin fraction and an increased gamma globulin fraction. No correlation of severity of hepatic lipidosis with serum protein pattern was detected, however (Leevy, 1962). Abnormal BSP retention was felt to provide "the best index to the presence of fatty liver;" however, correlation with the amount of lipid was still not strong, and abnormal values were found in only 54% of patients with fatty liver (Leevy, 1962). Retention of BSP is no longer commonly used as a diagnostic criteria in human medicine so recent reports have not referred to this test. In most cases of fatty liver, serum cholesterol is increased (Leevy, 1962), but in significant numbers of patients, notably those with protein-calorie malnutrition induced fatty liver, serum cholesterol and TG concentrations are decreased (Cravioto

et al., 1959; Leevy, 1962; Troswell et al., 1967; Alpers and Sabesin, 1982). Mild increases in serum bilirubin and aspartate transaminase concentrations have also been reported in significant numbers of fatty liver patients (Leevy, 1962; Hoyumpa et al., 1975; Alpers and Sabesin, 1982).

Fatty liver syndromes

As stated earlier, HL is caused by one of two possible mechanisms or combinations of them. These mechanisms are: 1) increased fatty acid supply and enhanced TG synthesis, and(or) 2) decreased synthesis or release of lipoproteins.

In man, fatty liver from alcoholism has probably been the most thoroughly studied syndrome, but the mechanism of induction is still not clearly understood. It is associated with an increase in serum VLDL concentrations, so the cause is presumably increased synthesis of TG. It has been suggested that the "primary abnormality which leads to TG accumulation ... is impaired oxidation of fatty acids." (Hoyumpa et al., 1975). This is possibly due to an increase in the NADH:NAD ratio during alcohol metabolism (Lieber, 1974; Hoyumpa et al., 1975), but seems more likely due to a toxic effect of the metabolic byproduct of ethanol metabolism on the mitochondria, which may impair mitochondrial oxidation of fatty acids (Lieber and Schmid, 1961). Acute alcohol administration has also been shown to increase the conversion of ^{14}C-palmitate to TG in rat liver microsomes (Schieg and Isselbacher, 1965), and increase free fatty acids in serum (Schapiro et al., 1965). Both of these mechanisms could contribute to increased hepatic TG formation. Chronic alcohol consumption may also result in a starvation-like syndrome, which may contribute to HL.

Obesity is associated with increased circulating VLDL TG and cholesterol and is frequently accompanied by mild HL (Kern et al., 1973; Andersen, 1984; Andersen et al., 1984).The exact mechanism is uncertain, but may be due to increased supply of NEFA to the liver due to an increased mass of adipose tissue (Alpers and Sabesin, 1982). Fatty liver following jejunoileal bypass surgery for obesity is, however, a significant clinical syndrome. The HL of obesity worsens in nearly all patients following bypass surgery (Piepkorn et al., 1977). Increased mobilization of adipose tissue and delivery of NEFA to the liver has been suggested as the primary cause (Alpers and Sabesin, 1982); however, dieting or gastric bypass operations do not usually result in increased hepatic fat deposition. Protein malnutrition, resulting in decreased apoprotein synthesis has also been postulated as the major cause (Moxley et al., 1974) as have toxic effects of bile salts (Mangla et al., 1974) due to altered bile acid metabolism (Piepkorn et al., 1977). Supporting this hypothesis is the observation that hepatic necrosis and fibrosis frequently accompany the lipidosis (Alpers and Sabesin, 1982).

Diabetes is frequently accompanied by HL and was found at autopsy in 51% of patients with diabetic ketoacidosis

(Reinberg and Lipson, 1950). Hepatic lipidosis is more common in adult onset diabetics than juvenile diabetics (Alpers and Sabesin, 1982) and is in direct proportion to the degree of obesity (Wasastjema et al., 1972). Thus it may be another manifestation of HL due to obesity. If so, it probably results from increased NEFA delivery to the liver, without any alteration in lipoprotein output. This is supported by the observation that circulating lipids in diabetics usually increase (Sosenko et al., 1980). Insulin deficiency does result in reduced lipoprotein clearance due to decreased heparin stimulated lipase activity (Bagdade et al., 1967), which could increase serum lipid concentrations. In rats, diabetes results in reduced lipoprotein output (Heimberg et al., 1967; Berry et al., 1981) and decreased hepatic protein synthesis (Pilkis and Korner, 1971) which could result in an increased accumulation of hepatic TG. Fatty livers occurred more frequently in patients not on insulin regulation than in those taking insulin (Wasastjema et al., 1972).

Fatty liver is a common finding with kwashiorkor (protein malnutrition) or protein - calorie malnutrition. It is accompanied by very low serum TG and VLDL concentrations (Troswell, 1967), and reduced output of hepatic VLDL due to defective apoprotein synthesis is felt to be the major problem (Waterlow and Alleyne, 1971). Serum NEFA levels are elevated, which may contribute to the HL (Lewis et al., 1964). In marasmus (energy malnutrition) there are also increased serum NEFA concentrations, but serum lipids are elevated and fatty liver is rare or absent (Lewis et al., 1964).

Idiopathic fatty liver of pregnancy and fatty liver due to tetracycline administration are two rare, poorly understood clinical syndromes which have a very high mortality rate. Etiology is poorly understood in both cases (Alpers and Sabesin, 1982). Profound hypoglycemia and multiple organ system failure has been associated with fatty liver of pregnancy (Alpers and Sabesin, 1982). A rat model for HL from tetracycline administration suggests that impaired release of VLDL is the primary cause (Breen et al., 1972).

Hepatic lipidosis in domestic species

Fatty liver in other species has usually been associated with deficiency induced lesions. Depancreatized dogs develop a severe HL and numerous lipotropic factors (which either prevent or reduce the severity of TG accumula-tion) have been investigated with this model (McHenry and Patterson, 1944). Even when these dogs are maintained with insulin, fatty liver develops.

Lecithin was discovered to be highly effective at preventing these fatty livers (Hershey, 1930), and it was determined that choline could replace lecithin and effectively prevent HL (Best et al., 1932). Simpler models were developed to study HL, depancreatized dogs being expensive to maintain. Normal rats and mice fed high fat diets may develop HL, and this model was frequently used to evaluate lipotropic factors.

In an early review McHenry and Patterson (1944) suggested that Leathes and Raper (1925) were among the earliest to recognize that phospholipids were involved in fat transport from the liver. It was subsequently determined that the component of lecithin preventing HL in dogs and rats was choline (Best et al., 1932). Further observations suggested that the deposition and accumulation of hepatic fat was affected by the type and amount of dietary protein. Casein appeared to be the most lipotropic of the proteins examined (Best et al., 1935, 1936). Subsequently, the effect of casein was explained due to its methionine content (McHenry and Patterson, 1944). The action of methionine appeared to be due to its ability to donate methyl groups for choline synthesis (du Vigneaud et al., 1940). Choline does not prevent the HL of starvation or toxicosis, but may help reduce hepatic TG more quickly following the insult (McHenry and Patterson, 1944). In a clinical report of a HL-like syndrome in cattle, choline treatment resulted in miracle-like cures (Lewis and Price, 1957).

Another phospholipid precursor identified as a lipotropic factor is myo-inositol (McHenry and Patterson, 1944). More complete reviews of the function of inositol are available (Holub, 1982; Wells, 1984) and a more detailed discussion is included in this thesis. (See

Appendix B.) In rats, HL could be produced which could be prevented only by the addition of both choline and inositol to the diet (Engel, 1942). These results were confirmed by other early investigators (Gavin et al., 1943). Inositol is required in such small quantities in the diet that it is very difficult to produce experimental deficiencies. It is synthesized from glucose in the testes, so intact males usually do not become deficient. Normal intestinal microflora probably synthesize an adequate amount in females. A deficiency has been produced in the lactating rat treated with small doses of sulfa (Burton and Wells, 1976) which results in a severe HL. This HL is characterized by early centrilobular fat infiltration. As expected with inadequate phospholipid formation, it is characterized by reduced circulating lipoproteins representing reduced VLDL output (Burton and Wells, 1977). With cessation of lactation or addition of .05% myo-inositol to the diet, the HL resolves. Fatty liver syndrome of laying hens is a spontaneous HL which has been reported to be responsive to dietary inositol supplementation in some but not all trials (Reed, 1968; Leveille and Bray, 1970).

Fatty liver in rats has also been produced by excess supplementation of the B-vitamins thiamine, riboflavin, pantothenic acid, pyridoxine and biotin (Gavin and McHenry, 1941). The mechanism of this fatty liver is not well understood.

Feeding 1% orotic acid to rats will induce HL. This model of fatty liver is associated with a specific defect in lipoprotein synthesis (Roheim et al., 1966; Windmueller and Levy, 1967). Protein synthesis is not impaired (Roheim et al., 1966) and ultrastructural studies suggest there is a secretory block within the Golgi apparatus (Sabesin et al., 1977). B- and C- apoproteins are present in the hepatic liposomes which accumulate in orotic acid fed rats (Pottenger et al., 1971), so the secretory defect resulting in HL is presumably at another point in the process. A defect in the glycosylation of the apoproteins within the Golgi may be responsible for the TG accumulation (Alpers and Sabesin, 1982).

Ethionine treatment has also been used as a means of experimentally inducing HL in rats. Ethionine lowers ATP concentrations, impairs protein synthesis, reduces lipoprotein output and results in TG accumulation. The experimental fatty liver resulting from impaired protein synthesis by agents such as ethionine, puromycin or cycloheximide is slight when compared with other mechanisms (Alpers and Sabesin, 1982) and is not frequently utilized as a model for HL.

Spontaneous fatty liver occurs in obese ponies and, to a lesser extent, horses which are fasted, especially due to illness (Schotman and Wagenaar, 1969). This HL is associated with profound lipemia and increased pre-beta serum lipoproteins (Morris et al., 1972), suggesting there is no defect in lipoprotein output. It is probably a manifestation of increased delivery of NEFA to the liver. In ponies it was usually fatal (Schotman and Wagenaar, 1969).

In addition to the fatty liver syndrome of laying hens already mentioned, poultry are subject to another apparent HL syndrome. Fatty liver and kidney syndrome (FLKS) of young growing chicks is a clinical syndrome resulting in central nervous system disturbances and sudden death. At necropsy, liver and kidney are frequently highly infiltrated with lipid. It is apparently caused by a biotin deficiency (Pearson et al., 1976). The clinical signs are attibutable to hypoglycemia due to decreased pyruvate carboxylase activity, for which biotin is a cofactor (Hood et al., 1976). The TG infiltration may be related to massive NEFA mobilization which may occur with the onset of hypoglycemia. Another explanation is also possible. Hood et al. (1976) presented evidence suggesting that pyruvate carboxylase is extremely sensitive to a marginal biotin deficiency. Pyruvate accumulates, which results in additional synthesis of AcCoA. This AcCoA in turn stimulates hepatic fatty acid synthesis. Although AcCoA carboxylase is also a biotin dependent enzyme, it may be less sensitive to biotin deficiency than pyruvate carboxylase. The imposition of stress on the hypoglycemic animal with a fatty liver results in acute death with fatty livers and kidneys at necropsy (Hood et al., 1976).

Hepatic lipidosis in cattle

An associaton of excessive grain and(or) corn silage feeding prepartum with increased death and disease rates has been reported by several groups of investigators (Emery et al., 1969; Morrow et al., 1969; Coppock et al., 1972; Fronk et al., 1980). A possible explanation for these observations is that these high dietary energy intakes resulted in obesity. Morrow (1976) described a clinical syndrome in which obese dairy cattle develop severe health problems at parturition and labeled the condition "fat cow syndrome". Cattle developing the problem were obese at parturition. Increased numbers of these animals developed such periparturient diseases as parturient paresis, retained placenta, mastitis, ketosis and displaced abomasum. Response to therapy was poor and increased death loss occurred (Morrow et al., 1979). Severe HL was a consistent necropsy finding and it has been hypothesized that this lipid infiltration interfered with normal hepatic function and contributed to the disease syndrome (Morrow et al., 1979; Deem, 1980). More recent reports have referred to fatty liver syndrome nearly synonymously with fat cow syndrome, and clinical reports linking disease outbreaks with HL have been published recently (Doxey and Scott, 1983; Higgins and Andersen, 1983; Gerloff and Herdt, 1984).

Hepatic lipidosis is associated with bovine ketosis (Saarinen and Shaw, 1950; Baird, 1982; Gröhn et al., 1983) and sheep with pregnancy toxemia (Bell, 1979) and mild degrees appear to be normal in most dairy cows in the postpartum period (Ford, 1959; Reid, 1980).

A moderate, subclinical HL occurring during the early postpartum period has also been reported (Reid, 1980). Although described as a subclinical condition, it has been associated with impaired reproductive performance (Roberts et al., 1979; Reid, 1983; Reid et al., 1983a; Reid and Roberts, 1983) and possibly increased susceptibility to mastitis (Reid and Roberts, 1983).

The impaired reproductive performance has been measured as increased days open. It has been characterized as including increased days postpartum to first ovulation, first estrus and first service (Reid et al., 1983a). Another study failed to show a difference in time to first ovulation, although cattle with moderate HL tended to have lower serum LH and progesterone concentrations than those with mild HL (Watson and Harwood, 1984).

This moderate HL appears to be part of a more generalized fat mobilization syndrome occurring in early lactation and significant lipid deposition occurs in other organs in addition to the liver (Roberts et al., 1981, 1983). Condition score loss, rather than initial condition score is associated with the degree of lipid infiltration which occurs (Reid, 1980; Reid and Roberts, 1983). Cattle with HL have increased concentrations of circulating NEFA (Morrow et al., 1979; Roberts et al., 1979; Reid et al., 1983b). Reduced circulating lipids have also been observed in cattle with HL (Morrow et al. 1979; Reid et al., 1983b). Herdt et al. (1983b) observed reduced total TG, cholesterol and phospholipids and reduced dextran precipitable lipid fractions in cattle with HL compared to controls with normal hepatic lipid concentrations. These observations suggest the HL results from both an increased influx of NEFA from adipose mobilization and a reduced output of hepatic TG as lipoprotein. This condition may be similar to the fatty liver produced in fasted cows. In this model, there is an increased delivery of NEFA from the peripheral tissues and the liver changes from a state of net output of TG to one of net uptake (Reid et al., 1979). Starvation induced ketosis and HL were also associated with a reduction in dextran sulphate precipitable lipoprotein concentrations (Brumby et al., 1975) and the histologic appearance of the liver was characterized by large accumulations of lipid droplets within the Golgi, characteristic of a defect in lipoprotein secretion (Reid, 1973).

Clinical pathological changes in HL may include a depression in white blood cell counts (Morrow et al., 1979) and serum albumin (Morrow et al., 1979; Reid et al., 1983b) as well as the changes in the serum lipid concentrations already described. BSP retention time has been suggested as a reliable method of making a diagnosis (Deem, 1980). While it may be elevated in very severe cases of HL, BSP retention time was poorly correlated with degree of HL (Herdt et al., 1982) similar to other serum enzyme and liver function tests (Herdt et al., 1982; Gröhn et al., 1983). Aspartate transaminase has shown the most consistent relationship to degree of HL in cattle, but the relationship is still weak (Herdt et al., 1982; Reid et al., 1983b) Serum NEFA concentration has been the most highly correlated blood measurement with accumulation of hepatic TG, but the ability to correctly discriminate among cows with HL based on serum NEFA concentrations is limited (Reid et al., 1983b). B-hydroxy-butyrate concentrations have also been ineffective in differentiating among cows with or without moderate HL (Gaal et al., 1983b; Gröhn et al., 1983) and the most reliable diagnostic technique is a liver biopsy. A convenient technique for estimating hepatic lipid content in small samples based on differential flotation in copper sulfate solutions has been described (Herdt et al.,1983a) and should be suitable for on farm diagnostic use.

Epidemiological reports have indicated as many as 60% of cattle may have moderate or severe HL in the first week postpartum (Reid, 1980). With more extensive sampling, that rate has been revised downward to approximately 35% (Reid and Roberts, 1983). These incidence rates were determined in Great Britain, but reports from other locations are rare. Reports of the precise time in relation to parturition when the TG accumulates are rare, also, although by eight weeks postpartum hepatic lipid content was reported back to normal (Reid, 1980).

EFFECT OF INOSITOL SUPPLEMENTATION AND TIME FROM PARTURITION ON LIVER AND SERUM LIPIDS IN DAIRY CATTLE.

Summary

Percutaneous liver biopsies and blood samples were obtained from 80 multiparous dairy cows in nine Michigan herds. Biopsies and samples were obtained serially over the peripartum period. Thirty-nine cattle received 17 g of supplemental myo-inositol in the diet to test its use as a possible lipotropic substance and 41 received a placebo. Liver biopsies were assayed for triglyceride (TG) and total myo-inositol content. Serum was assayed for dextran precipitable cholesterol, non-esterified fatty acids (NEFA), insulin and bound and free thyroxine and triiodothyronine. Results of hormone assays are reported in an accompanying paper.

Inositol supplementation had no effect on any of the variables reported in this paper. There was a significant herd effect on liver inositol, serum dextran precipitable cholesterol and NEFA concentrations. Serum NEFA and liver TG concentrations increased in the immediate postpartum period, while dextran precipitable cholesterol decreased. A significant herd by period interaction existed for liver TG and dextran preciptable cholesterol concentrations. Hepatic TG and serum NEFA concentrations were positively correlated. Excessive infiltration of bovine liver with

lipid at calving appears to be an exaggerated manifestation of normal metabolic changes.

INTRODUCTION

Fat cow syndrome has been described as a condition in which obese dairy cows develop severe health problems in the periparturient period. These include retained placenta, mastitis, metritis, ketosis and displaced abomasum. Response to therapy is poor and mortality is high (Morrow, 1976; Morrow et al., 1979). At necropsy one of the most striking lesions is the tremendous amount of fat accumulation in the liver (Morrow et al., 1979). The syndrome has been referred to as fatty liver syndrome or hepatic lipidosis (HL) (Deem, 1980).

Subclinical HL has more recently been described as a common postpartum problem in high producing dairy cattle (Reid, 1980; Reid and Roberts, 1983) and has been associated with decreased reproductive performance (Reid, 1983). Poor fertility has also been felt to contribute to severe HL (Morrow, 1976).

Some degree of HL apparently develops in most dairy cows postpartum (Ford, 1959) and is a consequence of metabolic changes occurring with the initiation of lactation. Hepatic lipidosis has also been associated with ketosis in dairy cattle (Saarinen and Shaw, 1950; Gröhn et al., 1983). The lipid which accumulates consists primarily of TG (Brumby et al., 1975; Reid and Collins, 1980; Herdt et al., 1982). In the fasted cow the pathogenesis of HL appears to result from increased hepatic uptake of NEFA due to increased mobilization from adipose tissue, and decreased output of TG as lipoprotein (Reid et al., 1979). Naturally occuring HL is associated with increased concentrations of NEFA (Reid et al., 1983b) and reduced circulating TG and cholesterol (Herdt et al., 1983b). The pathogenesis is thought to be similar to HL of fasted cattle.

Myo-inositol is generally classified as a B-vitamin. In the lactating rat, HL similar to that observed in dairy cattle develops in animals fed a myo-inositol deficient diet (Burton and Wells, 1977). Female gerbils fed a myo-inositol deficient diet develop a severe intestinal lipodystrophy. Supplementation of the diet with myo-inositol reverses this lipodystrophy and is accompanied by increased serum chylomicron concentration (Chu and Geyer, 1981). Fatty liver syndrome in laying hens has been reported to respond favorably to dietary inositol supplementation as well (Reed et al., 1968), and a negative correlation was observed between hepatic TG and inositol in cows with naturally occurring HL (Gerloff et al., 1981). These observations suggested that myo-inositol supplementation might prove to be of benefit in reducing hepatic TG accumulation in the peripartum bovine. A more detailed discussion of the potential use of inositol in dairy cattle diets was presented in a preliminary report of this trial (Gerloff et al., 1984b).

Moderate HL at one week postpartum was resolved by eight weeks postpartum (Reid, 1980) but observations of changes in hepatic TG concentrations over shorter time intervals have been lacking. Objectives of this study were to examine changes in hepatic TG concentrations in peripartum dairy cattle and to examine the relationships between serum metabolites, hormone concentrations and hepatic TG content. The effect of myo-inositol supplementation under field conditions on these variables was also investigated.

MATERIALS AND METHODS

Eighty pluriparous dairy cattle in nine Michigan dairy herds were sampled from September, 1981 to March, 1984. Eight herds were referred to the study by Michigan veterinarians suspecting a clinical problem of HL within the herd. The Michigan State University dairy herd was the ninth herd. Pluriparous cattle were used because previous reports indicated HL was rare in first lactation cattle (Reid, 1980). Seven to ten cattle from each herd were randomly assigned to one of two treatment groups. Thirty-nine 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 non-phytate myo-inositol, approximately .1% of estimated dry matter intake. A dietary myo-inositol concentration of .05% is necessary to prevent HL in the lactating rat (Burton and Wells, 1976). Forty-one cows received the same supplement without myo-inositol.

The initial samples from each cow were obtained approximately 1 m prior to expected parturition. Inositol supplementation was then initiated and continued until cattle had been sampled at least twice after parturition. Samples were obtained every 2 to 3 wk until approximately 1 m after parturition, so most cattle were sampled four or five times. Percutaneous liver biopsies were obtained through the ninth or tenth intercostal space using a 15 cm commercial biopsy needle.[1] Biopsy specimens were placed into pre-weighed vials. Vials were weighed and the specimen weight obtained by difference. Samples ranged in weight from 20 to 50 mg and were frozen at -30 C until assays were performed. At the same time blood samples were obtained from the coccygeal artery or vein, transported on ice and serum separated by centrifugation. Serum was also stored at -30 C until assays could be performed.

Body condition scores were assigned to each animal at the time of sampling, using a scale of 1 to 5, 1 being very thin and 5 very obese (Mulvaney, 1977). Milk and milkfat production values for each herd were obtained from Michigan Dairy Herd Improvement Association records. Number of animals, diet characteristics and housing facilities were recorded for each herd. Basal dietary myo-inositol intakes

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[1] Tru-cut, Travenol Labs, Deerfield, IL.

were calculated for each herd utilizing published values of phytate content of dietary feedstuffs (Nelson et al., 1968, 1976).

Liver biopsies were assayed for TG and myo-inositol concentration. For TG determination, lipid was extracted from one biopsy specimen by the method of Hara and Radin (1978), except 9 ml of 3:2 hexane:isopropanol was used when homogenizing the tissue, 3 ml of 6.7% aqueous sodium sulfite was added to separate the non-lipid portion, and the lipid phase was evaporated under N_2 and resuspended in 10 ml of 3:2 hexane:isopropanol. Triglyceride concentrations were determined in duplicate using 2 ml of extract in a commercial colorimetric assay (Sigma Chemical Co., 1977) and expressed as mg TG/g liver wet weight.

Total myo-inositol content of liver specimens were determined by a modification of the gas chromatographic method of Wells et al. (1965). The liver specimens were hydrolyzed in 2 ml of 6N HCl heated in a sealed glass tube for 40 hr at 110 C. Prior to deionization on an Amberlite MB-3 column an internal standard of 25 ug α-methylmannoside was added to each sample. Trimethyl silylation reagents were obtained from a commercial source.[2] Gas chromatography was done on a Hewlett Packard Model 5830A gas chromatograph. The column was a commercially availabe dimethyl silicone suspended on an 80/100 mesh support.[3] Oven temperature was 185 C and injection temperature 250 C. Nitrogen flow rate was 32 ml/min. A standard containing 25 ug myo-inositol and 25 ug methyl-mannoside was prepared, and total myo-inositol content of samples determined by comparison with the standard. Myo-inositol content was expressed as umoles/ g liver.

Dextran sulphate precipitation of lipoproteins in serum was as previously described (Herdt et al., 1983b) except 3 ml of serum were used and amounts of reagent were halved. Cholesterol concentration was determined on the resuspended precipitate by an automated method.[4] Serum NEFA concentrations were determined according to the method of Brunk and Swanson (1981).

An analysis of variance was performed for each trait measured using a general linear model procedure available in a standard statistical computer program (SAS Institute, 1979). A split-plot design was used and the statistical model was:

$$Y_{ijkl} = u + H_i + T_j + C_{(ij)k} + P_l + HT_{ij} + HP_{il} + TP_{jl} + E_{ijkl}$$

where Y_{ijkl} is the ijklth observation of the variable measured; u is the overall mean; H_i is the effect of ith herd (i=1,...9); T_j is the effect of the jth treatment (j=1,2); $C_{(ij)k}$ is the effect of the kth animal (k=1,...80)

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[2] Pierce Chemical Company, Rockford, IL

[3] 3% OV-1 on 80/100 mesh Chromasorb WHP, Supelco, Bellefonte, PA

[4] Flexigem Centrifugal Analyzer, Electro-Nucleonics, Inc., Fairfield, NJ

nested within herd and treatment; P_l is the effect of the lth period (l=1,...4); HT, HP and TP are two way interactions of main effects; and E_{ijkl} represents the random residual associated with each observation. Periods 1, 2, 3, and 4 were designated >3 wks prepartum, 0 to 3 wks prepartum, 0 to 3 wks postpartum, and >3 wks postpartum, respectively. The mean square of $C_{(ij)k}$ was used to test significance of herd and treatment effects. Mean square of the residual error was used to test significance of split-plot effects. Three way interaction was non-significant and included in the residual. An analysis of covariance on serum NEFA and dextran precipitable cholesterol concentrations was also performed, adding liver TG concentration as a covariable to the above model.

Because of death, culling and early parturition, not all animals had four observations; some least square means were non-estimable. True means were used to make specific comparisons, using Scheffé's test for contrasts determined a posteriori to evaluate significance of differences.

RESULTS

There was no significant effect of inositol supplementation (treatment) on any of the variables reported in this paper. (See Table 1.) This is consistent with preliminary results reported earlier (Gerloff et al., 1984b). Hepatic myo-inositol content did vary ($p<.05$) between herds (Table 1). Mean liver inositol

Table 1. Analysis of Variance for Liver Triglyceride and Inositol and Serum Dextran Precipitable Cholesterol and Nonesterified Fatty Acids

Variable		Liver TG		Liver Inositol		Serum Dextran P'ppt Cholesterol		Serum	NEFA
Effect	df	Mean Square	f Ratio	Mean Square	f Ratio	Mean Square	f Ratio	Mean Square (10^5)	f Ratio
Herd	8	21.32	.74	4.14	4.43**	951.5	4.82**	3.35	3.26**
Treatment	1	1.04	.04	2.19	2.35	421.5	2.13	.56	.54
Herd x Treatment	8	14.37	.50	.80	.86	273.5	1.39	.40	.39
Error 1 (Animal)	62	28.94	-	.93	-	197.4	-	1.02	-
Period	3	51.79	4.52**	.40	.50	3280.1	66.11***	10.6	15.75***
Herd x Period	24	23.81	2.08**	1.23	1.52	156.5	3.15***	.99	1.47
Treatment x Period	3	6.10	.53	.17	.21	77.5	1.56	1.05	1.56
Error 2 (Residual)	180	11.47	-	.81	-	49.6	-	.67	-
R^2 of Model		.60		.51		.82		.60	

**p<.01
***p<.001

concentrations are reported in Table 2, along with characteristics of the diets from the herds. Herd 5 had lower mean liver inositol content than Herd 2 ($p<.05$).

There were no significant effects of herd or treatment on hepatic TG content (Table 1). Individual animal variation was responsible for much of the differences in peripartum TG accumulation. There were significant period and herd - period interaction effects on hepatic TG content (Table 1). A plot of hepatic TG concentration over time within individual herds is shown in Figure 1. Although differences within herds were not all significant, in Herds 3 to 9 mean hepatic TG concentration was greatest in the immediate postpartum period (Period 3) and nearly returned to prepartum levels by the time of the final sample. Mean liver TG concentrations in herds 1 and 2 followed a different pattern, with highest hepatic TG concentrations observed prepartum rather than postpartum. Prepartum condition scores and management characteristics of the herds are presented in Table 3.

There were significant effects of herd and period on serum NEFA concentrations (Table 1). A plot of serum NEFA concentrations vs period for each herd is shown in Figure 2. Serum NEFA concentrations were greatest during the first postpartum sampling period ($p<.05$). Four of nine herds had increasing NEFA concentrations 0 to 3 wks prepartum (Period 2) as well.

Table 2. Mean Liver Inositol Content and Estimated Dietary Inositol Intake

		Nonlactating Cattle		Lactating Cattle	
Herd	Mean Liver Inositol Content (umoles/g)	Feedstuffs	Daily Inositol Intake (g)	Feedstuffs	Daily Inositol Intake (g)
1	2.15 ± .22	Mixed hay, DSC*	5-10	Mixed hay, alfalfa haylage DSC	25-30
2	2.56 ± .26	Corn silage, mixed hay, DSC	15-20	Corn silage, alfalfa haylage, DSC, HMSC**	30-35
3	2.22 ± .28	Corn silage, alfalfa haylage, dry ear corn	5-10	Corn silage, alfalfa hay and haylage, dry ear corn	30-35
4	1.92 ± .23	Corn silage, pasture	5-10	Corn silage, mixed hay and haylage, DSC	30-35
5	1.31 ± .11	Oat hay	0-5	Corn silage, alfalfa hay and haylage, DSC	25-30
6	1.80 ± .14	Corn silage, oat hay	10-15	Corn silage, alfalfa hay and haylage, HMSC	25-30
7	1.86 ± .13	Corn silage, alfalfa hay and haylage, HMSC	5-10	Corn silage, alfalfa hay and haylage, HMSC	25-30
8	2.24 ± .14	Corn silage, mixed hay	10-15	Corn silage, alfalfa haylage, HMSC	25-30
9	1.75 ± .15	Mixed haylage, hay, dry shelled corn	5-10	Mixed haylage, alfalfa hay, DSC	25-30

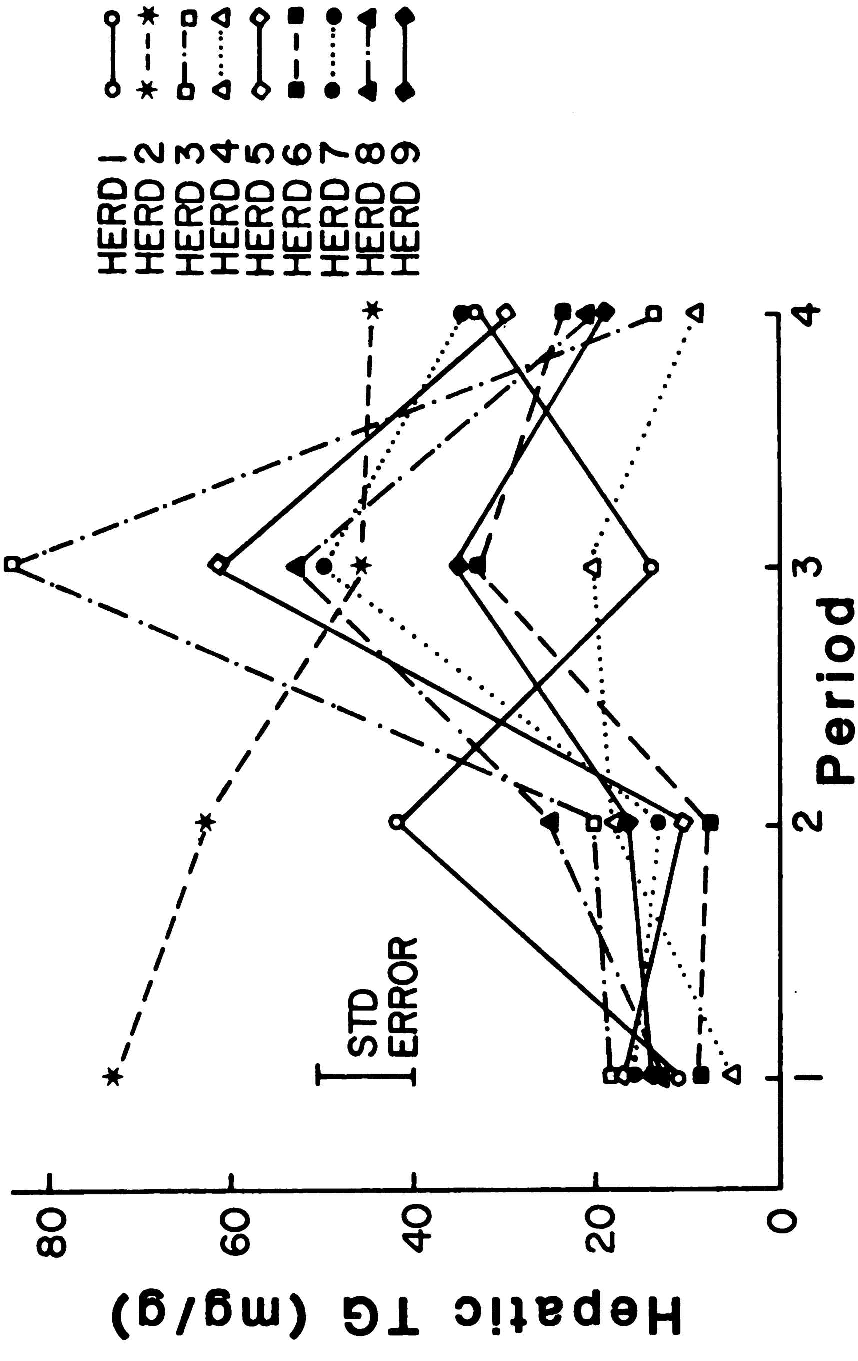
* DSC - Dry Shell Corn

** HMSC - High Moisture Shell Corn

Table 3. Management Information about Participating Herds

Herd	Housing	Feeding System	Individual Maternity Stall	Initial Condition Score	No. Cows	Rolling Herd Average Milk (kg)	%Fat	Butterfat (kg)
1	Stanchions	Individual	Yes	3.06±.21	46	8293	3.5	292
2	Free Stalls	Group	No	3.25±.15	120	8875	4.1	361
3	Stanchions	Individual	No	3.17±.17	98	8228	3.5	288
4	Stanchions	Individual	Yes	2.75±.25	103	7585	3.7	281
5	Tie Stalls	Individual	Yes	3.30±.13	53	9012	3.7	334
6	Free Stalls	Group	Yes	2.80±.27	98	7215	4.1	293
7	Free Stalls	Group	No	2.65±.18	115	6640	3.8	255
8	Free Stalls	Group	No	3.45±.12	112	7020	3.8	267
9	Free Stalls	Individual	Yes	2.79±.10	49	7579	3.4	255

Figure 1: Mean liver TG concentrations within each herd. Period 1 corresponds to > 3 weeks prepartum; Period 2, 0 to 3 weeks prepartum; Period 3, 0 to 3 weeks postpartum; and Period 4, > 3 weeks postpartum.


Hepatic TG (mg/g)
0
20
40
60
80
Period
1
2
3
4
STD ERROR
HERD 1
HERD 2
HERD 3
HERD 4
HERD 5
HERD 6
HERD 7
HERD 8
HERD 9

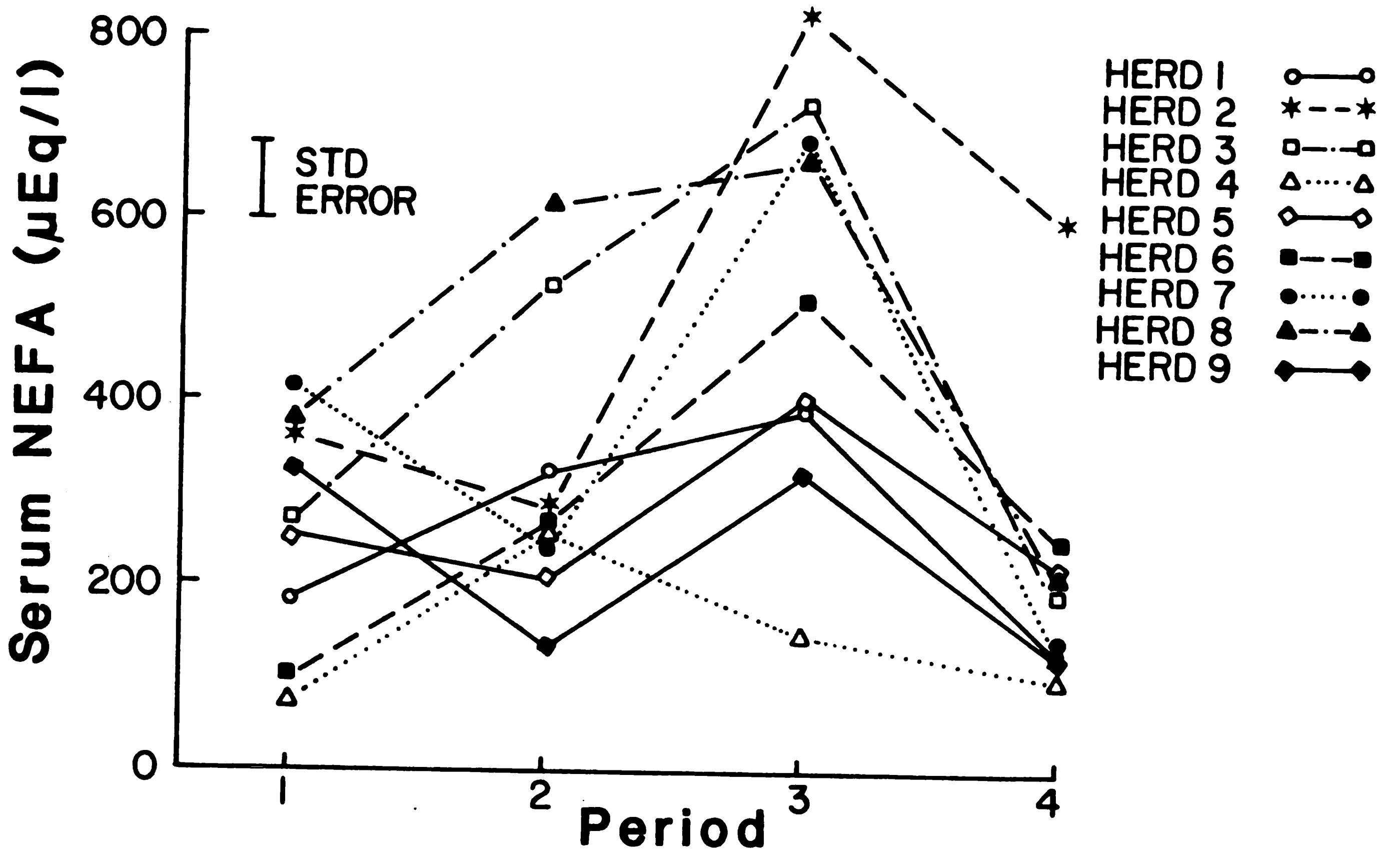


FIGURE 2: Mean Serum NEFA concentrations within each herd.

Adding liver TG as a covariable to the original statistical model resulted in an improvement in R^2 by 35% for NEFA. Results of an analysis of covariance are presented in Table 4. The correlation of NEFA and hepatic TG concentration determined by the reduction in sums of squares accounted for by the covariate hepatic TG was .34 ($p<.05$).

Significant effects of herd, period, and period - herd interaction were demonstrated on serum dextran precipitable cholesterol concentrations. (Table 1) There were large differences among cows as well. Mean dextran precipitable cholesterol concentrations in each period are plotted for each herd in Figure 3. Dextran precipitable cholesterol concentrations begin declining 0 to 3 wks prepartum (Period 2) and were lowest during the first postpartum period in most herds. By 3 to 5 wks postpartum they have returned to or exceeded concentrations observed during the non-lactating period.

Hepatic TG content was negatively correlated with serum dextran precipitable cholesterol concentration, and adding liver TG as a covariable to the original statistical model improved the R^2 for dextran precipitable cholesterol slightly ($p<.05$) (Table 4).

No consistent difference due to inositol supplementation on serum dextran precipitable cholesterol concentration was observed. Results are shown in Figure 4. In herds 3, 6, and 9 inositol supplemented animals had

Table 4. Relationship of Serum Lipid Measurements to Hepatic Triglyceride

Variable	Serum Dextran Precipitable Cholesterol	Serum NEFA
R^2 of Original Statistical Model	.82	.60
R^2 After Adding Liver TG as Covariate	.87	.80
Correlation with Liver TG	-.18*	.34*

*$p<.05$

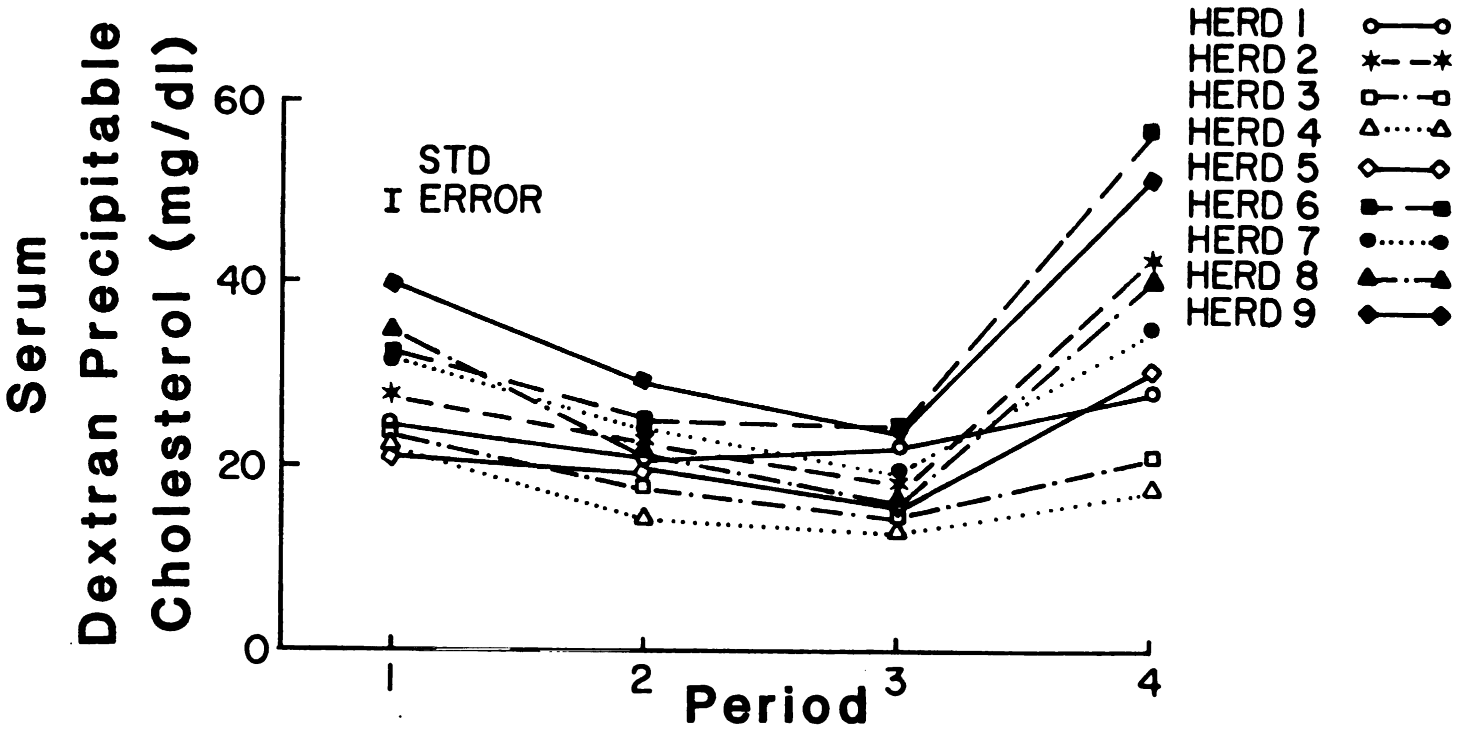


Figure 3: Mean serum dextran precipitable cholesterol concentration within each herd.

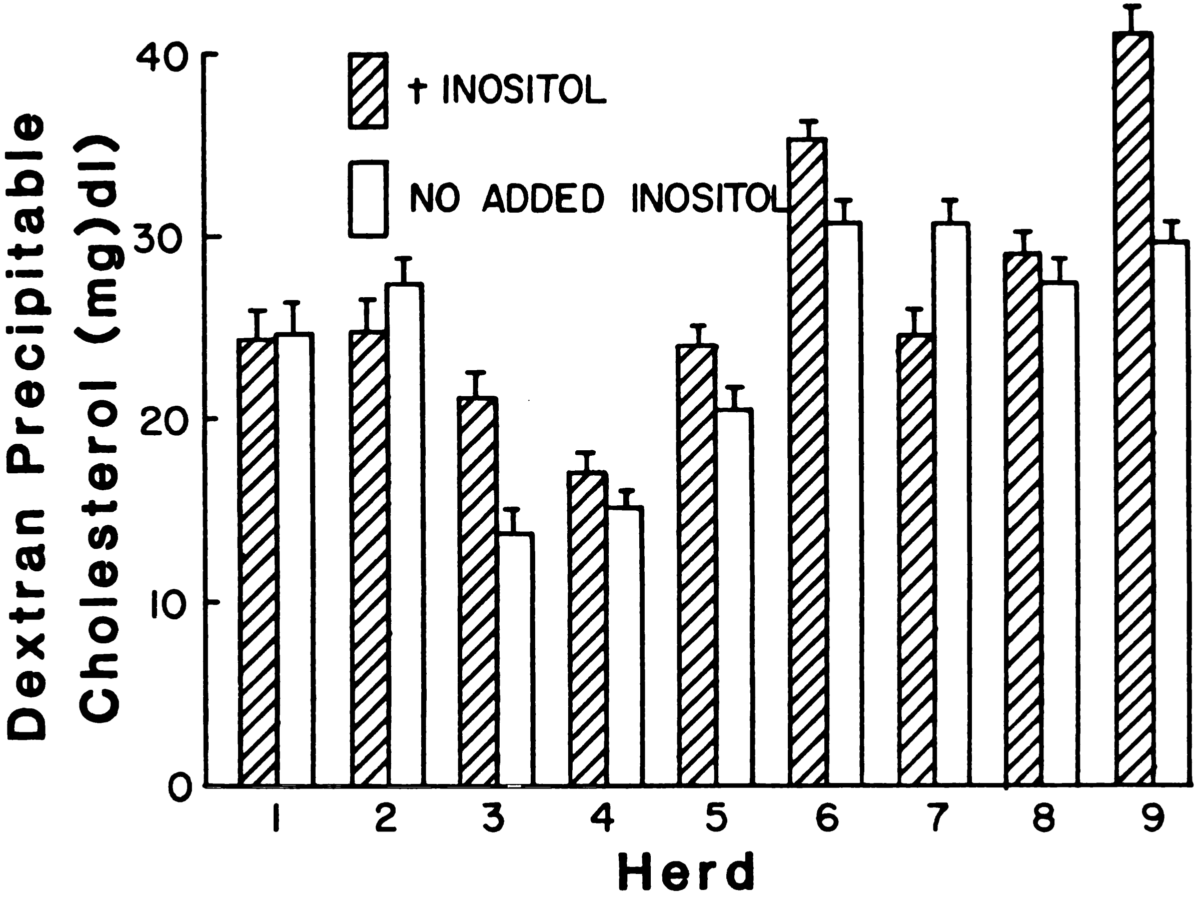


Figure 4: Mean serum dextran precipitable cholesterol concentrations in cattle receiving supplemental myo-inositol or a placebo.

greater dextran precipitable cholesterol concentrations than controls; in herd 7 they had lower. ($p<.05$) In herds 1, 2, 4, 5, and 8 there were no differences between supplemented and unsupplemented animals in serum dextran precipitable cholesterol concentrations.

DISCUSSION

It was anticipated that myo-inositol supplementation would increase hepatic myo-inositol concentration and phosphatidylinositol formation. This would result in increased hepatic TG output as VLDL, and lower liver TG concentrations. This did not occur, suggesting that myo-inositol content of the basal diet, and(or) synthesis by gastrointestinal microorganisms were more than adequate in relation to the inositol requirement for hepatic lipoprotein output. Herd differences in hepatic inositol content may be partially explained by dietary differences. There are fewer apparent differences between herds in the lactating cow diets than in the non-lactating cow diets; most of the lactating cow diets were based on a mixture of corn silage, alfalfa, and corn grain, while the non-lactating animals had a wide variety of diets. Cattle in herd 5, with the lowest mean liver inositol concentrations also had the lowest basal dietary intake of inositol (as phytic acid) during the non-lactating period. The cattle in herd 2, with the highest mean liver inositol content, also had the highest basal inositol intake during the non-lactating period. Corn silage furnished the most significant portion of the roughage in herd 2. These observations suggest that hepatic myo-inositol concentrations may reflect dietary inositol intake, but the supplemental inositol did not affect hepatic inositol content. A herd effect on liver TG concentration inversely related to liver inositol content was not present, further suggesting that within the ranges present here, hepatic myo-inositol content did not influence lipoprotein output and liver TG content. In lactating rats when myo-inositol deficiency resulted in hepatic lipidosis, total liver inositol content declined to 1.007 umoles/g (Burton and Wells, 1976).

The postpartum increase in hepatic TG concentration observed in most herds is consistent with the observations of others (Reid, 1980; Herdt et al., 1982). The degree of hepatic TG accumulation postpartum varied considerably among herds. The three herds with the greatest postpartum liver TG concentrations (>50mg/g) all had an initial mean condition score greater than 3.0, suggesting a greater degree of obesity in these herds. This relationship is not absolute, however, as herd 3 had the highest postpartum liver TG content, but herd 8 had the highest condition scores. (Table 3)

The change over time in mean hepatic TG content in cows in Herds 1 and 2 followed a different pattern than that of the other herds. This is apparently responsible for the majority of the herd by period interaction (Figure 1). In Herd 1, one animal developed a severe kidney infection, resulting in severe weight loss prepartum. Her prepartum liver TG concentration was 280 mg/g, which may have skewed the mean in Period 2 in that herd. Herd 2 had consistently higher liver TG concentrations than most of the other herds, with the prepartum samples being especially higher. The initial mean condition score of 3.25 was not as high as in several of the other herds, so degree of obesity is not a likely explanation for the elevated liver TG concentrations during the non-lactating period. As mentioned earlier, the non-lactating cow diet of herd 2 consisted of large amounts of corn silage, which may have supplied excessive energy and contributed to excessive hepatic TG deposition during the prepartum period. This was the only herd in which the owners complained of symptoms similar to those associated with fat cow syndrome in previous years, suggesting in this herd animals had been obese for longer than one year. It may be possible that duration of obesity (rather than degree of obesity only) results in significant hepatic lipid accumulation. Duration of obesity has been reported to increase hepatic lipid content in man in some studies (Zelman, 1952), but not others (Halloran et al., 1974; Andersen et al., 1984).

Serum NEFA concentrations increase during periods of negative energy balance in cattle (Radloff et al., 1966; Brumby et al., 1975; Bell, 1979; Vernon, 1980). This is consistent with the increase in serum NEFA concentrations we observed in early lactation, a period in which feed intake is usually inadequate to meet the energy demands of lactation (Bauman and Currie, 1980). The increase in NEFA concentrations preceded the usual occurrence of peak lactation (Vernon, 1980; Butler et al., 1981), but may coincide with the period of greatest negative energy balance. Our observations are consistent with others who have reported peak plasma NEFA concentrations in dairy cattle at or shortly after parturition (Radloff et al., 1966; Vernon, 1980). Herd differences in NEFA concentrations probably represent a variety of factors and their interactions. One of these factors may be genetic differences among herds. Increased concentrations of NEFA result from the hormonal changes occurring in the cow at or near the time of parturition and the demand to partition nutrients from body reserves to the mammary gland (Bauman and Currie, 1980). The contribution of endocrinological changes to adipose mobilization, such as the decline in insulin and increase in growth hormone, may represent genetic differences between cattle (Hart et al., 1978a). The two herds with the highest peak NEFA concentrations (2 and 3) were among those with the highest production, suggesting high genetic potential. Management differences between herds may also result in significant differences in mobilization and serum concentrations of NEFA. Husbandry at calving and immediately following may affect the stress level associated with parturition. Catecholamines stimulate lipolysis in vitro (Yang and Baldwin, 1973) and increase plasma NEFA concentrations in vivo (Sidhu and Emery, 1972). Herds 2, 3, 7 and 8, with highest peak NEFA concentrations, moved cattle immediately after calving from the non-lactating group or maternity pen to a lactating cow lot. The remaining herds housed and fed the recently parturient cows in an individual box stall for several days postpartum (Herds 1, 4, 5, 6 and 9). Elevated NEFA concentrations in the first group of herds may reflect greater stress due to the changes in the social environment or a larger negative energy balance from reduced food intake due to increased competition postpartum. Intake at calving is also affected by season, temperature and photoperiod (Erb et al., 1982), and in this study herd effects were confounded with seasonal effects.

The positive correlation of serum NEFA and hepatic TG concentrations is consistent with the observation that dairy cattle with postparturient fatty livers have elevated plasma NEFA concentrations (Morrow et al., 1979; Roberts et al., 1979, 1981; Reid et al., 1983b) and the conclusion that the accumulation of hepatic TG results primarily from increased delivery of NEFA to the liver.

Dextran precipitable cholesterol presumably corresponds to the cholesterol in the TG rich lipoprotein fractions secreted by the liver. Lipoproteins precipitated by the dextran reagent should have a high lipid:protein ratio (Puppione, 1978) and represent an estimation of VLDL, chylomicron and LDL in the circulation.

Variations in individual animals and herd differences in dextran precipitable cholesterol concentrations may partially result from genetic differences. Serum cholesterol concentrations in beef cattle have been estimated to be highly heritable (Stufflebean and Lasley, 1969), and somewhat less heritable in dairy cattle (Arave et al., 1975). Diet may have some effect on serum cholesterol concentrations. Increasing dietary fat content results in increased serum cholesterol concentrations in dairy cattle (Yang et al., 1978), presumably due to increased intestinal chylomicron production. In the present study, fat content of diets was not controlled, so herd differences may reflect some differences in dietary lipid content. Other differences may be present as well.

Dextran precipitable cholesterol concentrations begin declining 0 to 3 wks prepartum (Period 2) and continue to decline during the first postpartum period. By 3 to 5 wks postpartum they have returned to or exceeded levels observed during the non-lactating period. As early as 1931, blood cholesterol concentrations were observed to be lowest at the beginning of lactation (Maynard et al., 1931). Recent reports have demonstrated the decline and subsequent increase in serum total cholesterol concentrations which occur at the time of parturition (Blum et al., 1983; Kappel et al., 1984). Bertoni et al. (1984) observed declining total cholesterol and HDL cholesterol beginning 9 d prepartum. Lowest concentrations of all fractions occurred at parturition, with gradually increasing cholesterol concentrations up to 49 d postpartum. We extend these observations to the cholesterol concentrations in the dextran precipitable fraction of serum, indicating a reduction in all lipoproprotein fractions in bovine serum over the peripartum period. Total and VLDL TG concentrations in bovine blood are lowest in the postpartum period, when milk production is greatest (Varman and Schultz, 1968; Raphael et al., 1973). This has been hypothesized to be the result of increased activation of mammary lipoprotein lipase at the initiation of lactation, resulting in a more rapid clearance of triglyceride from the circulation (Bell, 1979). In addition, we suggest reduced hepatic production of VLDL is another contributing mechanism. The decline in dextran precipitable cholesterol should not result from increased uptake by the mammary gland, as arterio-venous differences of cholesterol across the mammary gland are inconsequential in both the lactating and non-lactating condition (Varman and Schultz, 1968). Dextran precipitable cholesterol concentrations may be a more reliable indicator of triglyceride-rich lipoprotein production than actual TG concentration in the presence of high lipoprotein lipase activity.

An increase in LDL and remnant uptake and catabolism may also result in decreased serum dextran precipitable cholesterol concentrations. Laarveld et al. (1982) suggested that estrogens result in increased hepatic output of VLDL and may result in increased cholesterol clearance in the lactating dairy cow. The high concentrations of estrogen metabolites in the blood during late gestation may increase cholesterol clearance, resulting in lower serum concentrations. A persistent effect of estrogen on cholesterol clearance would be required to explain the decreased postpartum concentrations of cholesterol observed, when estrogen concentrations have declined.

If significant chylomicron, VLDL, or LDL cholesterol production is occurring in the intestine, reduced hepatic lipoprotein output may not explain our observations; this seems unlikely due to the low amounts of chylomicron production in adult ruminants not fed supplemental fat (Palmquist, 1976), although other density lipoproteins could be of intestinal origin.

The negative correlation between serum dextran precipitable cholesterol and hepatic TG concentrations observed is in agreement with Reid et al. (1983b) who observed a similar negative correlation of liver fat with total cholesterol. Herdt et al. (1983b) observed reduced total and dextran precipitable lipids, including cholesterol, in dairy cattle with HL. Other clinical investigations have also observed lower serum cholesterol concentrations in cows with clinical and subclinical indications of HL (Morrow et al., 1979).

The lack of a consistent effect of inositol supplementation on dextran precipitable cholesterol concentrations further suggests that in this study addition of myo-inositol to the diet did not increase hepatic lipoprotein output.

CONCLUSIONS

Myo-inositol supplementation of dairy cattle diets at the level used in this study was ineffective in altering hepatic inositol concentrations or improving hepatic lipoprotein output as indicated by a reduction in liver TG content or an increase in circulating dextran precipitable cholesterol. Liver inositol concentrations may reflect basal dietary inositol content, however.

Normal metabolic changes near the time of parturition result in increased mobilization of NEFA from adipose tissue and may result in decreased hepatic lipoprotein output as indicated by a reduction in serum dextran precipitable cholesterol. Development of excessive hepatic lipidosis or fatty liver probably represents an exaggeration of the normal physiological events in which excessive fat mobilization is superimposed on a liver with reduced ability to secrete lipoproteins. Factors contributing to this may include obesity, management practices and genetic predisposition.

EFFECT OF INOSITOL SUPPLEMENTATION AND TIME FROM PARTURITION ON SERUM INSULIN, THYROXINE, AND TRI-IODOTHYRONINE AND THEIR RELATIONSHIP TO SERUM AND HEPATIC LIPIDS IN DAIRY CATTLE

Summary

Percutaneous liver biopsies and blood samples were obtained from 80 dairy cows in 9 Michigan herds over the peripartum period. Thirty-nine cows were fed 17 g of supplemental inositol and 41 fed a placebo. Liver biopsies were assayed for total myo-inositol and triglyceride (TG) concentrations. Blood samples were assayed for serum dextran precipitable cholesterol , non-esterified fatty acids (NEFA), insulin, thyroxine (T4), free thyroxine (FT4), tri-iodothyronine (T3), and free T3 (FT3) concentrations. Effects of treatment, herd and time from calving on the various hormone concentrations and their relationship to serum and hepatic lipid measurements are discussed in this paper. Serum concentrations of insulin and the thyroid hormones decreased near parturition, with lowest concentrations occurring in the immediate postpartum period. Concentrations of T3 correlated well with T4, and the concentrations of free hormones reflected concentrations of total thyroid hormones. The percentage of hormone in the free fraction remained constant over time. Serum insulin, T3, and T4 were negatively correlated with serum NEFA and hepatic TG concentrations. Thyroid hormones were positively correlated with serum dextran precipitable cholesterol concentrations. Inositol supplementation was

associated with reduced circulating T3 and FT3 concentrations, but not T4 and FT4 concentrations. Changes in hormone concentrations at parturition, and their relationship to liver TG and serum NEFA concentrations are consistent with a metabolic adaptation by the dairy cow to the negative energy balance of early lactation.

INTRODUCTION

Major metabolic changes occur over the periparturient period. Decreasing serum concentrations of insulin and thyroid hormones have been observed (Schwalm and Schultz, 1976a; Blum et al., 1983). The liver is the central organ of metabolism and these hormonal changes occur concurrently with an accumulation of hepatic TG which occurs in most cattle at this time (Ford, 1959; Reid, 1980).

Endocrinological effects have been postulated to contribute to the development of bovine ketosis, and ketosis is frequently accompanied by hepatic lipidosis (HL) (Bell, 1979; Gröhn et al., 1983). Decreased insulin concentrations may be associated with ketosis in dairy cattle (Baird, 1982), and diabetes results in HL in many species, including ruminants (Schwalm and Schultz, 1976b). Additionally, cattle with moderate HL postpartum have been observed to have decreased blood insulin concentrations (Reid et al., 1983b).

Dairy cattle with ketosis have also been observed to have lower serum protein bound iodine or thyroxine concentrations than normal cattle (Robertson et al., 1957; Heitzman and Mallinson, 1972); however, cattle supplemented with thyroid hormones had a higher incidence of ketosis than controls (Emery and Williams, 1964). The important role of the liver in thyroid metabolism is well known. It is the primary site of conversion of T4 to T3 as well as the principal site of thyroid binding globulin synthesis (Chopra et al., 1978). Changes in hepatic function related to TG accumulation peripartum may be closely related to thyroid activity and function.

The metabolic activity of T3 and T4 is determined by the fraction of these hormones free in the plasma. Fluctuations in total hormone concentration may not always reflect changes in FT3 or FT4 concentrations. Recent reports of peripartum changes in T3 and T4 have been published (Blum et al., 1983; Refsal et al., 1984), but published values of FT4 and FT3 concentrations in ruminants are rare.

In this paper we report the association of hepatic TG and serum lipid components measured with serum insulin, T4, T3, FT4, and FT3 concentrations. The effect of inositol supplementation and time from parturition on serum concentrations of these hormones is also examined.

MATERIALS AND METHODS

Eighty pluriparous dairy cattle from nine Michigan dairy herds were sampled as described in the preceeding paper.

Serum insulin concentrations were determined by a double antibody radioimmunoassay.[1] Intra- and inter-assay coefficients of variation were 2.0% and 5.7%, respectively. Serum T3 concentrations were determined according to the method of Refsal et al. (1984), except T3 antibody and ^{125}I-T3 tracer were obtained from different sources.[2] Serum T4 was determined by a commercial solid phase radioimmunoassay[3] with several modifications. A sample size of 30 ul was used instead of 10 ul, and incubation at 37 C was for 2 hr instead of .5 hr. In addition, 25 mg 8-anilino-1-naphthalene sulfonic acid[4] was added to each 110 ml bottle of tracer. Sensitivity of the assay was 1.5 ng/ml and intra- and inter- assay coefficients of variation were 4.8% and 6.5%, respectively.

Free T3 and FT4 concentrations were determined using the respective commercial solid phase radioimmunoassay kit.[3] Intra- and inter- assay coefficients of variation

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[1] Courtesy of Eli Lilly Co., Indianapolis, IN

[2] Miles Laboratories, Inc., Elkhart, IN and New England Nuclear, New Billerica, MA, respectively.

[3] Becton Dickinson Immunodiagnostics, Orangeburg, NY

[4] Sigma Chemical Co., St. Louis, MO

for FT3 were 4.7% and 16%, respectively. For FT4, intra- and inter assay coefficients of variation were 5.4% and 8.0%, respectively.

Hepatic TG, serum NEFA and serum dextran precipitable cholesterol concentrations were determined as described in the accompanying paper.

Variance was analyzed according to the statistical model described in the previous paper. Main effects of herd, treatment and period and all two - way interactions were tested for significance using a split - plot design. Significance of differences between means were evaluated using Scheffê's test. Additionally, Pearson product moment correlation coefficients between all variables were determined.

RESULTS

Results of the analysis of variance of serum hormone concentrations are presented in Table 5. In addition to large individual animal variation, there was a significant period and herd by period effect on serum insulin concentrations; values for each herd are shown in Figure 5. Generally, insulin concentrations declined immediately postpartum (Period 3) compared to prepartum values and remained decreased through Period 4. In contrast, one herd (herd 8) had a slight increase in mean insulin concentrations postpartum, and herds 1, 3 and 7 had increasing

Table 5. Analysis of Variance for Serum Hormone Concentrations

Variable			Insulin	T_4	T_3	FT_4	FT_3
Effect	df						
Herd	8	Mean Square	220.00	287.30	.056	143.20	5.08
		f ratio	1.95	2.26	.46	6.46*	3.43*
Treatment	1	Mean Square	1.11	340.00	.598	20.52	8.27
		f ratio	.010	2.66	4.85*	.92	5.58*
Herd x Treatment	8	Mean Square	50.20	69.60	.103	3.13	.75
		f ratio	.44	.55	.84	.14	.51
Error 1 (Animal)	62	Mean Square	112.70	127.30	.123	22.14	1.48
Period	3	Mean Square	276.40	3049.40	.690	81.45	6.61
		f ratio	6.75***	50.42***	11.87***	38.04***	10.82***
Herd x Period	24	Mean Square	83.30	62.90	.092	2.04	1.16
		f ratio	2.03**	1.04	1.59*	.95	1.91*
Treatment x Period	3	Mean Square	59.60	53.30	.042	.67	.23
		f ratio	1.45	.88	.042	.67	.23
Error 2 (Residual)	180	Mean Square	40.90	60.50	.058	2.14	.61
R^2 of Model			.62	.71	.60	.89	.67

* $p<.05$
** $p<.01$
*** $p<.001$

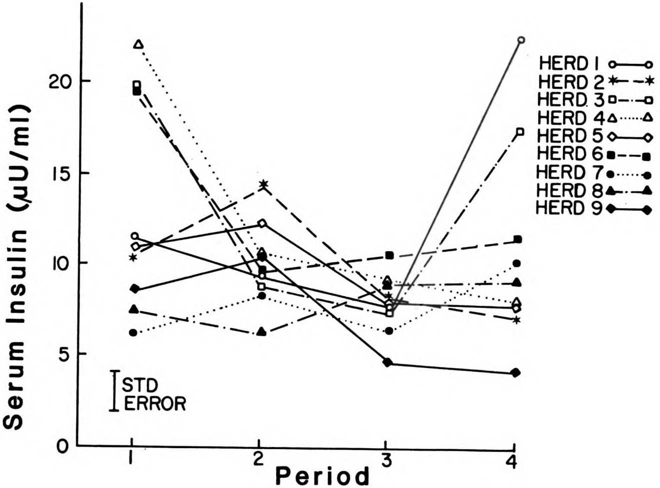

Figure 5: Mean serum insulin concentrations within each herd.

insulin concentrations in the final postpartum period (Period 4).

Correlation coefficients between serum NEFA, dextran precipitable cholesterol, liver TG and the serum hormone concentrations measured are shown in Table 6. Serum insulin concentrations were negatively correlated with liver TG and serum NEFA concentrations and positively correlated with serum T4.

Serum T4 values were significantly affected by herd and time from parturition (period) and the free fraction followed a similar pattern (Table 5). Plots of changes in T4 and FT4 are shown in Figures 6 and 7, respectively. Total and free T4 concentrations were highest in Period 1, declining to their lowest concentrations immediately postpartum (Period 3), and increasing slightly in Period 4. Herds 5 and 6 had consistently higher FT4 concentrations than the other 7 herds ($p<.05$).

T3 and FT3 concentrations generally showed a similar pattern, with 7 of 9 herds having decreased concentrations immediately postpartum, although in herds 1 and 8 T3 and FT3 concentrations increased immediately postpartum. In three herds the postpartum decline was significant ($p<.05$). T3 and FT3 results are shown in Figures 8 and 9, respectively.

T4 and T3 concentrations were highly correlated with the concentrations of their respective free fractions (Table 6), suggesting that the fraction of hormone bound vs

Table 6. Pearson Correlation Coefficients Between Measured Traits

Variable	Serum $[T_3]$	Serum $[T_4]$	Serum [Insulin]	Serum [NEFA]	Hepatic [TG]
Serum Dextran Precipitable Cholesterol	.19***	.19***	-.03	-.22***	-.18**
Hepatic [TG]	-.16**	-.27***	-.15**	.34***	
Serum [NEFA]	-.33***	-.37***	-.31***		
Serum [Insulin]	.14	.17**			
Serum $[T_4]$	.60***				
Serum $[FT_3]$	.71***	.38***			
Serum $[FT_4]$	.36***	.64***			

**p<.01
***p<.001

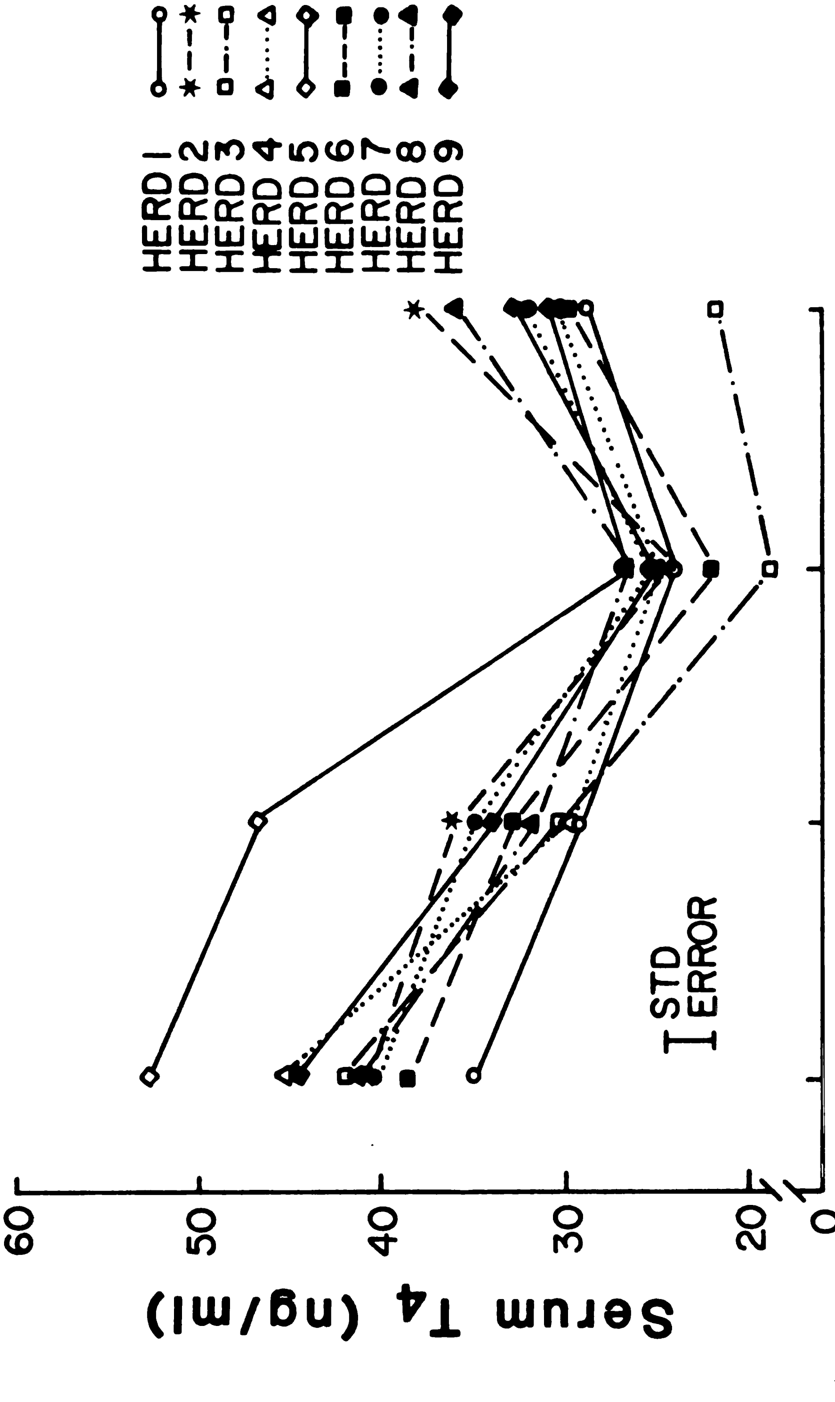

Figure 6: Mean serum T_4 concentrations within each herd.

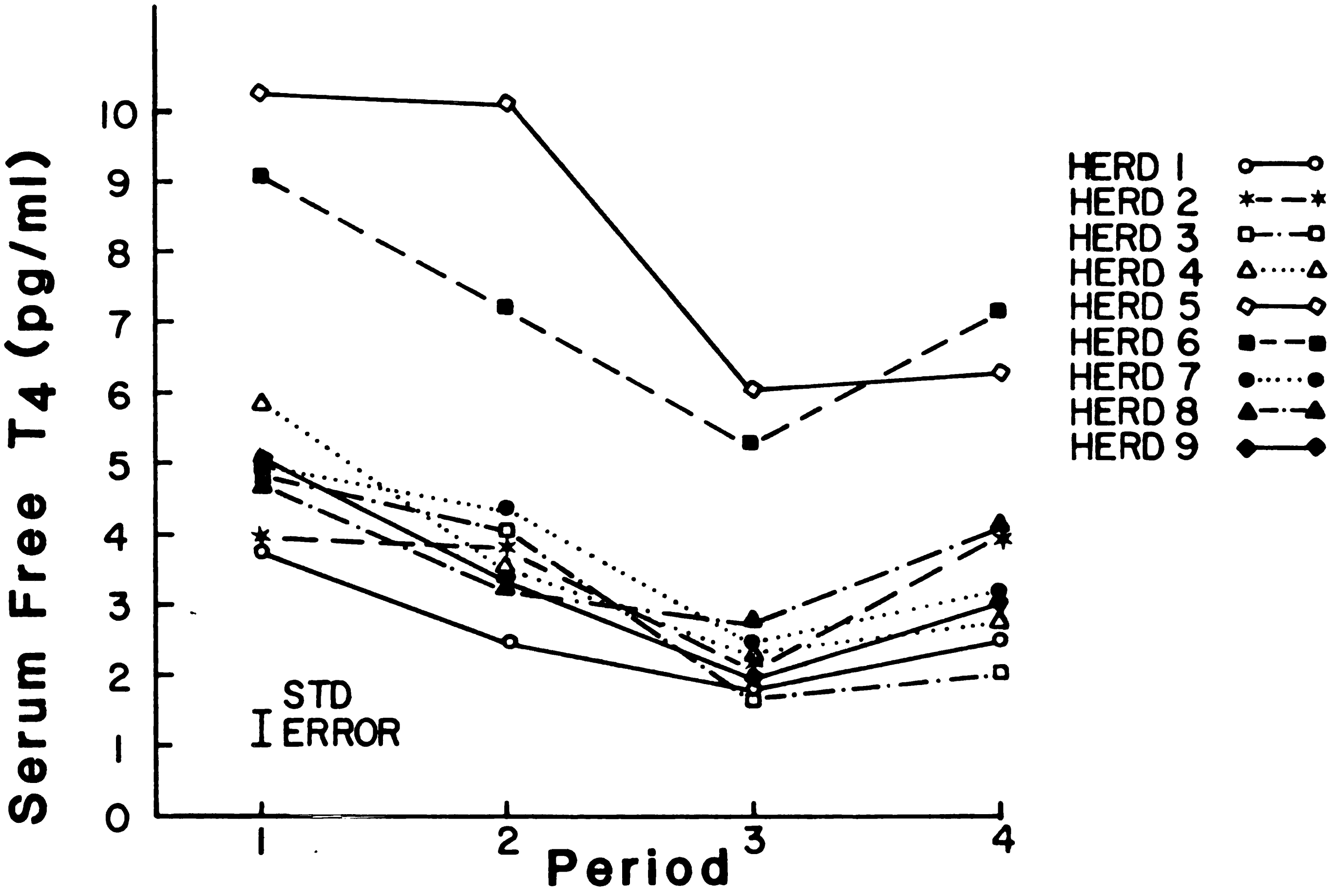

Figure 7: Mean serum FT_4 concentrations within each herd.

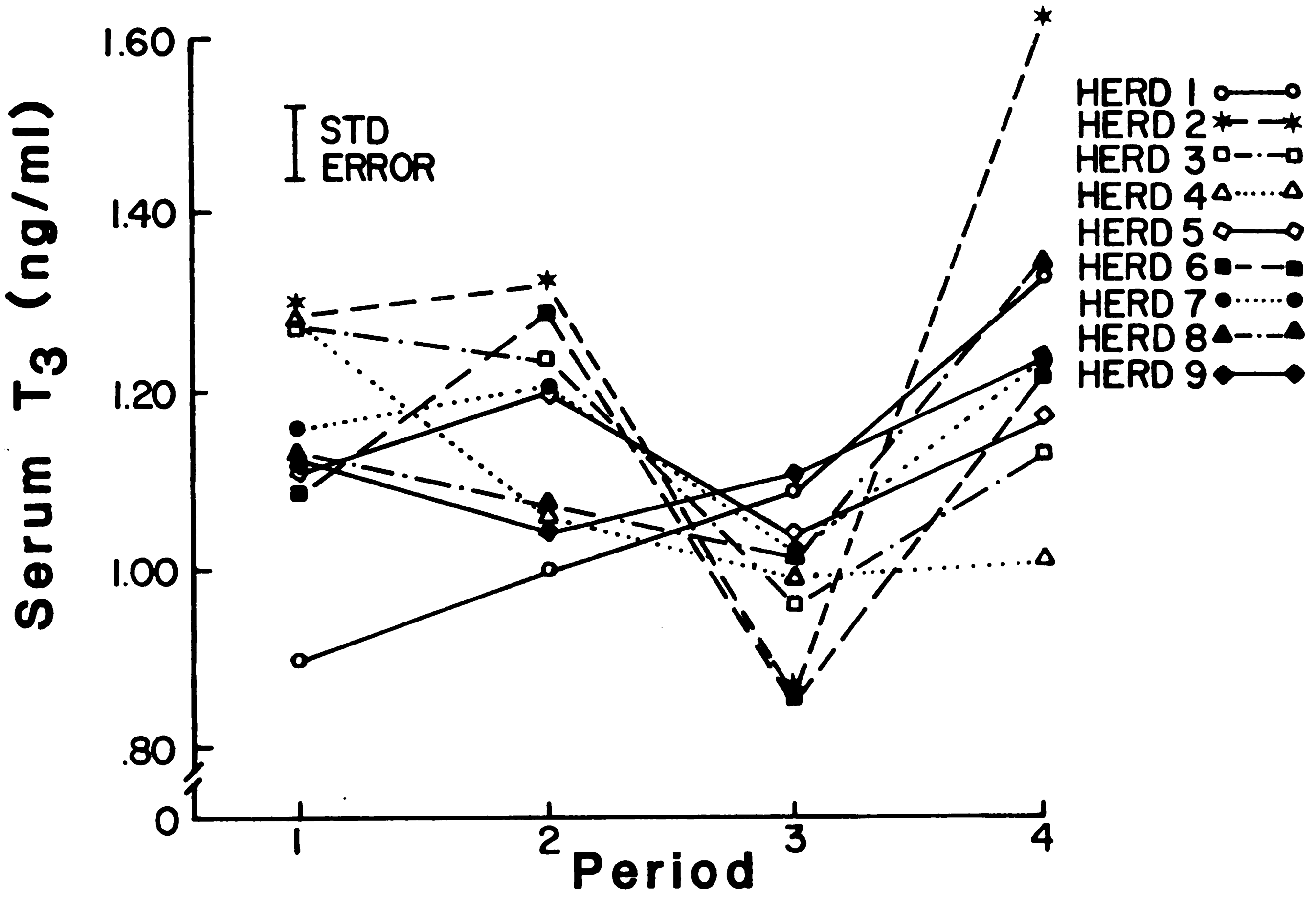

Figure 8: Mean serum T_3 concentrations within each herd.

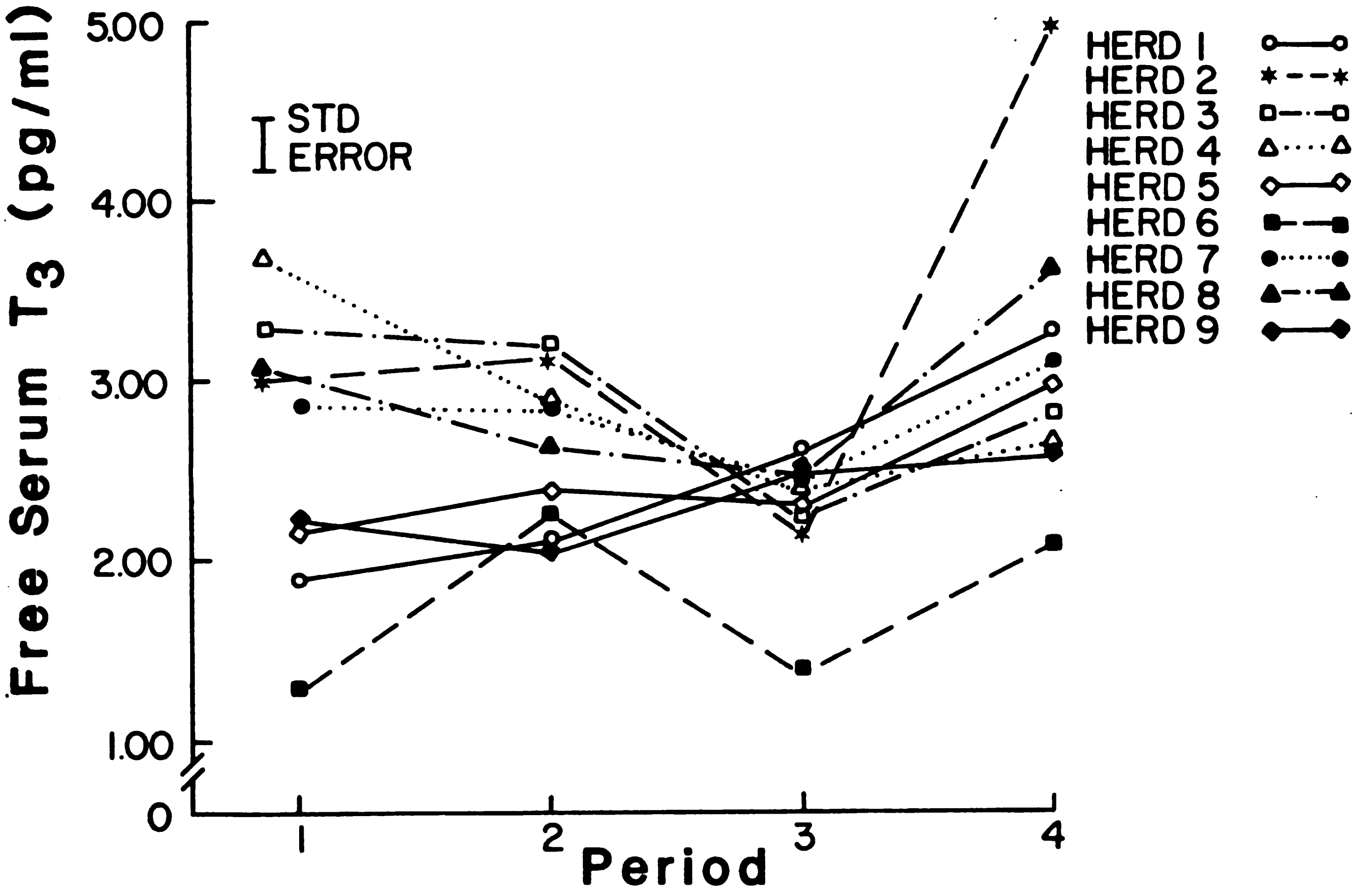

Figure 9: Mean serum FT_3 concentrations within each herd.

free remained constant. This was further substantiated by calculation of the fraction of T3 and T4 present as free hormone in each time period. Results are shown in Table 7. There were no significant differences for percentage of T4 as FT4 or T3 as FT3 between periods.

Serum T4 and T3 concentrations were positively correlated to each other, negatively correlated with serum NEFA and liver TG, and positively correlated with serum dextran precipitable cholesterol concentrations. (See Table 6)

There was a treatment effect on T3 and FT3 concentrations, but no effect of treatment on T4 or FT4 concentrations. Mean serum concentrations of T4 and T3 in each treatment group in each herd are listed in Table 8. With inositol supplementation, serum T3 concentrations were lower ($p<.05$).

DISCUSSION

Changes in serum insulin concentrations over the peripartum period are in agreement with numerous other reports showing a decline in insulin concentration at calving, (Koprowski and Tucker, 1973; Hove, 1974; Schwalm and Schultz, 1976a; Hart et al., 1978a) and increasing insulin concentration with time postpartum (Walsh et al., 1980). The decrease in insulin concentration at parturition is consistent with the metabolic response of the lactating dairy cow to partition nutrients to the mammary gland at this time (Bauman and Currie, 1980). Lower

Table 7. Fraction of Thyroid Hormones Circulating as Free Hormone

Period (No. obs.)	Percent of Hormone As Free Fraction	
	T_4	T_3
1 (66)	.014 ± .004	.218 ± .008
2 (77)	.012 ± .004	.226 ± .007
3 (76)	.012 ± .004	.223 ± .007
4 (64)	.011 ± .004	.245 ± .008

Table 8. Effect of Inositol Supplementation on Serum Thyroid Hormone Concentrations

	Serum [T_4] (ng/ml)		Serum [T_3] (ng/ml)	
Herd	+ Inositol	Without Inositol	+Inositol	Without Inositol
1	29.7	29.6	1.03	1.09
2	29.0	37.1	1.02	1.35
3	30.3	20.9	1.21	.89
4	31.5	30.1	1.03	1.08
5	37.8	41.4	1.09	1.17
6	29.5	32.5	1.02	1.27
7	30.9	34.3	1.08	1.20
8	29.8	36.7	1.04	1.20
9	32.0	38.2	1.04	1.23
Overall Mean	31.4 ± .9	34.5 ± .9	1.07 ± .03[a]	1.19 ± .03[a]

[a] Means differ, p .05

insulin concentrations appear to augment the utilization of nutrients by the mammary gland. This results in increased rates of basal and stimulated lipolysis and decreased activity of lipid synthesis pathways in adipose tissue (Sidhu and Emery, 1972; Shirley et al., 1973). The negative correlation between serum insulin and NEFA concentrations in this and other studies (Schwalm and Schultz, 1976a) is consistent with this hypothesis. Changes in insulin concentrations may reflect the altered energy balance associated with the initiation of lactation, as negative energy balance is associated with reduced circulating insulin (Blum and Kunz, 1981).

Variations in insulin concentrations among herds may be due to a number of factors. Insulin concentrations are known to be related to feeding time (Hove, 1974) and to show variable changes over a 24 hr period (Vasilatos and Wangsness, 1981). Although an attempt was made to sample each herd at the same time in relation to feeding, due to the distance of the herds from Michigan State University this was not always possible. Variations in feeding time may account for some of the differences observed in insulin concentrations. During early lactation, however, fluctuations in insulin concentrations appear minimal compared to later in lactation (Vasilatos and Wangsness, 1981) possibly due to reduced insulin response in early lactation (Lomax et al., 1979). Genetic differences may also result in differences in insulin concentrations. High yielding cattle may have reduced circulating insulin during lactation compared to low yielding cattle at the same stage of lactation (Hart et al., 1978a), but it is difficult to separate this effect from effects of energy balance.

Serum insulin concentrations were negatively correlated with liver TG concentration, in agreement with others who have observed reduced insulin concentrations in cattle with moderate fatty liver (Reid et al., 1983b). This correlation was not strong, and may reflect the significant association of both serum insulin and liver TG with serum NEFA concentrations. Alternatively, lower insulin may directly increase hepatic TG accumulation due to increased NEFA input into the liver and reduced hepatic output of lipoprotein. Hepatic lipoprotein output may be suppressed in rats under conditions of insulin deficiency (Berry et al., 1981), although this was not observed in cultured hepatocytes (Patsch et al., 1983). The low correlation between insulin and dextran precipitable cholesterol concen-trations in this study and insulin and total cholesterol in ketosis-prone cows (Schwalm and Schultz, 1976a) further suggests that decreased insulin is not directly related to lipoprotein production. Possible effects of insulin on cholesterol clearance may affect conclusions about hepatic output of lipoproteins based on serum cholesterol concentrations. In man, poorly regulated diabetes mellitus is associated with hypercholesterolemia (Sosenko et al., 1980). As lipoprotein synthesis is generally felt to be reduced in diabetes, this increase in cholesterol concentration is likely due to decreased clearance of LDL cholesterol during insulin deficiency (Brown and Ginsberg, 1982). Fibroblast LDL receptor activity, as measured by LDL degradation, has been shown to be insulin dependent (Chait et al., 1979).

T4 and T3 are secreted by the thyroid gland and have a multitude of actions. Their effects are involved in growth and differentiation and are linked with an increase in respiration and oxygen consumption (Oppenheimer, 1979). T4 is considered to function primarily as a precursor to T3, which is responsible for the majority of the tissue effects. T3 is formed by the deiodination of T4 (Chopra et al., 1978). In cattle, as in other species, the major site of T4 deiodination is apparently the liver with significant deiodination also occuring in the kidney (Kahl et al., 1984). Other T4 deiodination products with very little if any peripheral activity, such as reverse T3 (rT3) are also formed (Chopra et al., 1978). Total serum hormone concentrations do not necessarily reflect thyroid status. The active hormone is that portion not bound to protein, or in the free fraction. In assessing thyroid status it is important to know concentrations of the free hormones.

Serum T4 and T3 concentrations decreased at parturition and increased with time postpartum. This is in agreement with the observations of Blum et al.(1983) over the peripartum period. The observations of others showing a

positive effect of stage of lactation on T4 and T3 concentrations (Vanjonack and Johnson, 1975; Hart et al., 1978a; Walsh et al., 1980; Refsal et al., 1984) are also in agreement with the increase in serum T4 and T3 concentrations we observed in Period 4. Changes in concentrations of FT4 and FT3 were highly correlated with total hormone concentrations, suggesting thyroid binding capacity of serum hormones did not appreciably change over time. The percentage of T3 and T4 in the free fraction also remained constant over the time period observed. This is in agreement with observations in which free hormone concentrations showed similar changes to total hormone concentrations during late pregnancy and early lactation or in lactating cows (Hart et al., 1978b; Kunz and Blum, 1981). Refsal et al. (1984) concluded that the pregnancy associated rise in thyroid binding globulin observed in women did not occur in dairy cattle, and Etta and Reinecke (1971) observed no change in thyroid binding capacity at peak lactation. Therefore, serum concentrations of T4 and T3 probably reflect concentrations of FT4 and FT3 in the normal dairy cow.

The thyroid gland appears to be necessary for maintenance of lactation in ruminants (Tucker, 1974) and exogenous thyroid hormone or iodinated casein will stimulate milk production over short time periods but not over an entire lactation (Schmidt et al., 1971). Despite this, a consistent negative relationship between T4 and T3 and milk production has been observed by many investigators (Vanjonack and Johnson, 1975; Hart et al., 1978a; Walsh et al., 1980; Blum et al., 1983; Refsal et al., 1984). Fasting induces a reduction in T3 concentration in many species (Chopra et al., 1978; Balsam et al., 1981; Slebodzinski et al., 1982) including cattle (Heitzman and Mallinson, 1972; Blum and Kunz, 1981; Tviet and Larsen, 1983). In cattle there is an apparent reduction in T4, T3 and rT3 (Blum and Kunz, 1981). This reduction in thyroid state has been hypothesized as a defense mechanism of the organism to reduce metabolic demand in situations where catabolic functions are high. A similar mechanism may be functioning in cattle in early lactation; the decline in serum thyroid hormone concentrations is likely the result of the hormonal changes and negative energy balance induced by the initiation of lactation. That the reduced T4 and T3 concentrations we and others have observed in early lactation likely represents a response to the conditions of negative energy balance rather than a cause of many of the metabolic changes occurring is suggested by several observations. Declining T4 and T3 concentrations were associated with an elevation in serum NEFA concentrations. In contrast, when T4 is administered exogenously to dairy cattle an elevation in serum NEFA concentration occurs (Heitzman et al., 1971). Thyroid hormones apparently augment catecholamine stimulated lipolysis (Fisher and Ball, 1967). Similarly, bovine ketosis is characterized by reduced T4 concentrations (Heitzman and Mallinson, 1972), yet administration of thyroid hormones increases the incidence of ketosis (Emery and Williams, 1964). Emery and Williams (1964) suggested hypothyroidism may be one of the dairy cow's compensation mechanisms to prevent clinical ketosis. A similar compensation mechanism may be functioning in all cows in early lactation.

The reduction in circulating T3 and T4 should be associated with reduced metabolic activity by peripheral tissues. In agreement with this, glucose oxidation to CO_2, expressed as total glucose oxidized or percentage of glucose output oxidized, decreases dramatically with the onset of lactation (Bartley and Black, 1966; Bennink et al., 1972), although changes in oxidation rates of other substrates are not well established. The observation that energy is more efficiently used for weight gain during lactation than in the non-lactating condition (Moe et al., 1970) may be related to decreased thyroid hormone concentration during lactation and more efficient peripheral tissue metabolism. If the mammary gland is relatively insensitive to the decrease in serum T3 and T4 occurring in early lactation, or exhibits a selective uptake and utilization of T3 and T4 compared to peripheral tissues, the decline in T3 and T4 would effectively aid in the shunting of nutrients to the udder for milk production by reducing peripheral demand. Reports examining T4 secretion rate in relation to lactation are variable, but generally

suggest an elevation in T4 secretion rate associated with lactation (Mixner et al., 1962; Swanson, 1972; Fish and Swanson, 1983). Reconciling these observations with the reduction in serum thyroid hormones observed suggests an increased loss of T4 and T3 in heavy lactation, possibly in the mammary gland. The lowered serum T3 and T4 concentrations observed at parturition suggest a metabolic adaptation to conditions of nutrient deprivation in early lactation.

Serum T4 and T3 concentrations were negatively associated with liver TG concentrations. This probably reflects the common association of both low serum thyroid hormones and elevated liver TG concentrations with elevated serum NEFA concentrations as these characteristic changes all occurred at the same time in relation to parturition. However, livers from T3 treated rats did synthesize less TG from NEFA precursors (Olubadewo et al., 1983), and perfused livers from hypo- and hyperthyroid rats increased and decreased TG formation, respectively (Keyes and Heimberg, 1979), suggesting decreased thyroid status may result in increased hepatic TG formation. In intact animals, thyroidectectomy resulted in decreased TG synthesis (Dory et al., 1981), so the effect of thyroid status on hepatic TG synthesis is not clear. The parallel changes in T3 and T4 concentrations and similar relationship of T4 and T3 to liver TG concentrations suggest hepatic deiodination of T4

to T3 was not significantly impaired by elevated liver TG concentrations during the postpartum period.

T4 and T3 concentrations were positively correlated with serum dextran precipitable cholesterol concentrations. This is likely due to the similar changes in all near the time of parturition. In man, hyperthyroidism is associated with hypocholesterolemia, and hypothyroidism with hypercholesterolemia. This is probably due to greater cholesterol clearance in the hyperthyroid state (Abrams and Grundy, 1981). Thyroid hormones do stimulate cholesterol synthesis, however (Lipsky et al., 1955; Miettenen, 1968; Abrams and Grundy, 1981). The positive correlation between T4 and T3 and serum dextran precipitable cholesterol concentrations may reflect a stimulation of lipoprotein synthesis and output by thyroid hormones.

The significant herd variation in concentrations of thyroid hormones which was present may be due to a number of factors. Herd effects were confounded by season effects, and significant variations in thyroid hormone concentration due to season and temperature have been reported previously (Vanjonack and Johnson, 1975; Magdub et al., 1982; Refsal et al., 1984). There may also have been genetic differences between herds which affected thyroid hormone metabolism. Increased serum T4 during gestation has been associated with reduced genetic potential for milk production in dairy heifers (Bitman et al., 1984). An additional effect confounded with herd may be diet - both the energy content and, possibly, the amount of carbohydrate or glucose precursors in the diet. Carbohydrate content independent of energy content has a significant effect on thyroid metabolism in other species (Chopra et al., 1978). In one study in cattle, however, fiber content of the diet did not affect serum thyroid concentrations (Magdub et al., 1982).

The small but significant reduction in serum T3 concentrations with inositol supplementation was unexpected. There was no effect of myo-inositol supplementation on hepatic inositol content where the majority of inositol occurs in the lipid bound form (see accompanying paper). There may, however, have been an effect of supplementation on free concentrations of myo-inositol, and an effect on tissues other than liver. In a rat study using radio-labeled myo-inositol, the thyroid glands had the greatest uptake of myo-inositol per g tissue of any tissues examined (Lewin et al., 1976), suggesting a possible role in thyroid hormone metabolism for inositol.

CONCLUSIONS

In this, as in most studies of metabolite and hormonal changes, it is difficult to attribute a causal relationship among the various factors measured. Associations can be reported and possible explanations discussed.

Serum thyroid hormone and insulin concentrations decreased in the immediate postpartum period and thyroid concentrations increased with time postpartum. They were negatively associated with liver TG and serum NEFA concentrations, and probably represent a metabolic adaptation to the negative energy balance of the immediate postpartum period.

Changes in the free fractions of T4 and T3 paralleled changes in the total T4 and T3 concentrations, and the percentage bound in the serum did not appear to change, suggesting in the normal dairy cow total T4 and T3 accurately reflect thyroid status.

Inositol supplementation resulted in a reduction in serum T3 concentrations. The explanation, consequences and implications of this are not apparent at this time.

THE RELATIONSHIP OF HEPATIC LIPIDOSIS TO HEALTH AND PERFORMANCE IN DAIRY CATTLE

Summary

In a field study of 80 cows in nine Michigan dairy herds serial liver biopsies were obtained over the peripartum period to determine incidence of hepatic lipidosis (HL). Cattle were separated into categories of mild, moderate, and severe HL based on maximal amounts of liver triglyceride (TG) which accumulated during this period. Number of cattle with mild, moderate, and severe HL were 52, 16, and 12, respectively. Cattle with severe HL had greater concentrations of liver TG prior to calving and postpartum, and greater serum non-esterified fatty acid (NEFA) concentrations and condition loss postpartum than cattle with mild HL. Incidence of disease and culling and death rate due to disease were greater in cattle with severe HL. Cattle with severe HL had reproductive performance equal to normal cattle; however, cattle with moderate HL had increased days open, possibly related to greater milk production.

INTRODUCTION

A clinical condition in which obese dairy cattle developed severe health problems at parturition was described and labeled "fat cow syndrome" by Morrow (1976). Prior to this time there had been reports linking liberal grain and(or) corn silage feeding prepartum with increased death and disease loss (Morrow et al., 1969; Emery et al., 1969; Coppock et al., 1972). Necropsy lesions of cattle with fat cow syndrome include severe fatty infiltration of the liver (Morrow et al., 1979) and this HL may be responsible for many of the clinical signs (Deem, 1980). Other clinical reports associating disease outbreaks with HL or fatty liver in cattle have been published (Doxey and Scott, 1983; Higgins and Anderson, 1983; Gerloff and Herdt, 1984).

A subclinical, moderate HL developing in dairy cattle in early lactation has also been reported (Reid, 1980). This moderate HL has been linked to decreased reproductive performance as measured by days open and and increased incidence of mastitis (Reid and Roberts, 1983).

Elevated serum NEFA concentrations (Reid et al., 1983b) and decreased serum TG and cholesterol concentrations (Morrow et al., 1979; Herdt et al., 1983b) have been observed in cattle with HL. The accumulation of fat in the liver is thought to be due to an increased influx of NEFA from lipolysis of adipose tissue near parturition and a decreased output of lipoprotein by the liver. Maximum lipid accumulation has been reported to occur 1 to 2 wks postpartum, with hepatic lipid concentrations returned to prepartum levels by 8 wks postpartum. Precise characterization of the time when HL develops has not been reported, however.

The purpose of this investigation was to obtain information on the occurrence of HL in Michigan dairy cattle, particularly at what time in relation to parturition it develops and how widespread a clinical problem it may be. The effect of HL on subsequent health, productive and reproductive performance was also investigated. Selected serum lipid and hormone concentrations were also compared in cattle with differing degrees of HL in an effort to further characterize the syndrome.

MATERIALS AND METHODS

Eighty pluriparous cows from nine Michigan dairy herds were sampled over the peripartum period. Herds were referred to the study by practicing veterinarians suspecting a clinical problem with. In addition, the Michigan State University dairy herd was used as one of the herds sampled. Myo-inositol was added to the diet of 39 of the cows to investigate its potential use as a lipotropic agent. There were no differences in hepatic TG accumulation between cattle supplemented or not supplemented with inositol, so all cattle were grouped together for comparisons in this report. A preliminary report on the effects of inositol supplementation has been published (Gerloff et al., 1984b).

Percutaneous liver biopsies were obtained through the ninth or tenth intercostal space using a commercial biopsy needle[1]. At each sampling period one biopsy was weighed and frozen at -30 C until TG concentrations could be determined (Gerloff et al., 1984b). A second was fixed in

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[1] Tru-cut, Travenol Labs, Deerfield, IL

formalin, sectioned and stained with hematoxylin and eosin for histologic evaluation. Blood samples were obtained from the coccygeal artery or vein and allowed to clot. Serum was separated by centrifugation and frozen at -30 C until assays for NEFA (Brunk and Swanson, 1981) and dextran precipitable cholesterol (Herdt et al., 1983b) concentrations could be determined. Insulin concentrations in serum were determined by a double antibody radioimmunoassay[2]. Sampling began approximately 1 m prior to expected parturition and occurred every 2 to 3 wks until 1 m postpartum. Observations were grouped into four periods. Periods 1, 2, 3, and 4 corresponded to > 3 wks prepartum, 0 to 3 wks prepartum, 0 to 3 wks postpartum, and > 3 wks postpartum, respectively.

At each visit cattle were assigned a body condition score from 1 to 5, 1 being very thin and 5 very obese (Mulvaney, 1977). At the time of each postpartum sampling, cattle were palpated per rectum and cervical diameter recorded. Disease information was recorded by the owner and milk production in the current lactation obtained from Dairy Herd Improvement Association records. Milk production was expressed as mature equivalent deviation from herdmates. If available, completed records were used. If cattle left the herd prior to completing a lactation, but after 120 days of lactation, projected records were

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[2] Coutesy of Eli Lilly and Co., Indianapolis, IN

used. If culling or death occurred prior to 120 days of lactation, production records for that animal were not used in the comparisons. Days open for cattle were recorded as well. Animals culled because of infertility were assigned a days open value of 200 days. A preliminary report of the association of HL and reproductive performance in this study has been published (Gerloff et al., 1984a).

Cattle were retrospectively divided into three groups based on maximum hepatic TG concentrations. Cattle with mild HL maintained hepatic TG concentrations of < 50mg / g liver. Cattle with moderate HL had maximum hepatic TG concentrations of 50 to 100 mg / g liver, and cattle with severe HL had hepatic TG concentrations of > 100 mg / g liver. A one-way analysis of variance was performed, and significance of differences between groups were determined by using Scheffé's test for contrasts selected a posteriori. An analysis of covariance, utilizing milk production as the covariable was also performed on days open. Significance of disease and death rates were assessed by use of the chi-square test.

RESULTS

Twenty percent of cattle were classified as having moderate HL and 15% severe HL. Classification of cattle within each herd is shown in Table 9. Three herds (Herds 2, 5, and 8) were responsible for most of the cattle classified with severe HL.

Table 9. Distribution of Cattle with Hepatic Lipidosis Within Herds Sampled

Herd	Mild HL (n)	Moderate HL (n)	Severe HL (n)
1	5	3	1
2	6	1	3
3	4	1	1
4	8	0	0
5	5	2	3
6	7	3	0
7	7	2	1
8	5	2	3
9	5	2	0
Total	52	16	12

Percentage of cattle dying or being culled due to disease was greater in cows with severe HL than normal cows ($p<.10$). Rates were 15%, 31% and 42% for mild, moderate and severe HL, respectively. Differences between groups in percent of cows requiring treatment for disease approached significance ($p=.14$) and were 40%, 31.3% and 66.7% for cattle with mild, moderate and severe HL, respectively.

Cattle with severe HL had days open equal to controls; however, cattle with moderate HL had increased days open in the current lactation (Figure 10). ($p<.10$) Mean milk production, as measured by mature equivalent differences from herdmates, tended to be greater for cattle with moderate HL, although because of a large standard error, differences between groups were not statistically significant. Milk production levels for each group are shown in Figure 11. When days open were adjusted for milk production, differences between groups were no longer significant ($p>.10$). Mean cervical diameters 3 to 4 wks postpartum were 4.1, 3.8 and 4.3 cm for cattle with mild, moderate and severe HL, respectively, and did not differ between groups.

Liver TG concentrations for each group at each sampling period are shown in Figure 12. Cattle with severe HL had greater hepatic TG concentrations during all sampling periods, with the immediate pre- and postpartum samples having the greatest elevation in TG content ($p<.05$). Cattle with moderate hepatic lipidosis had

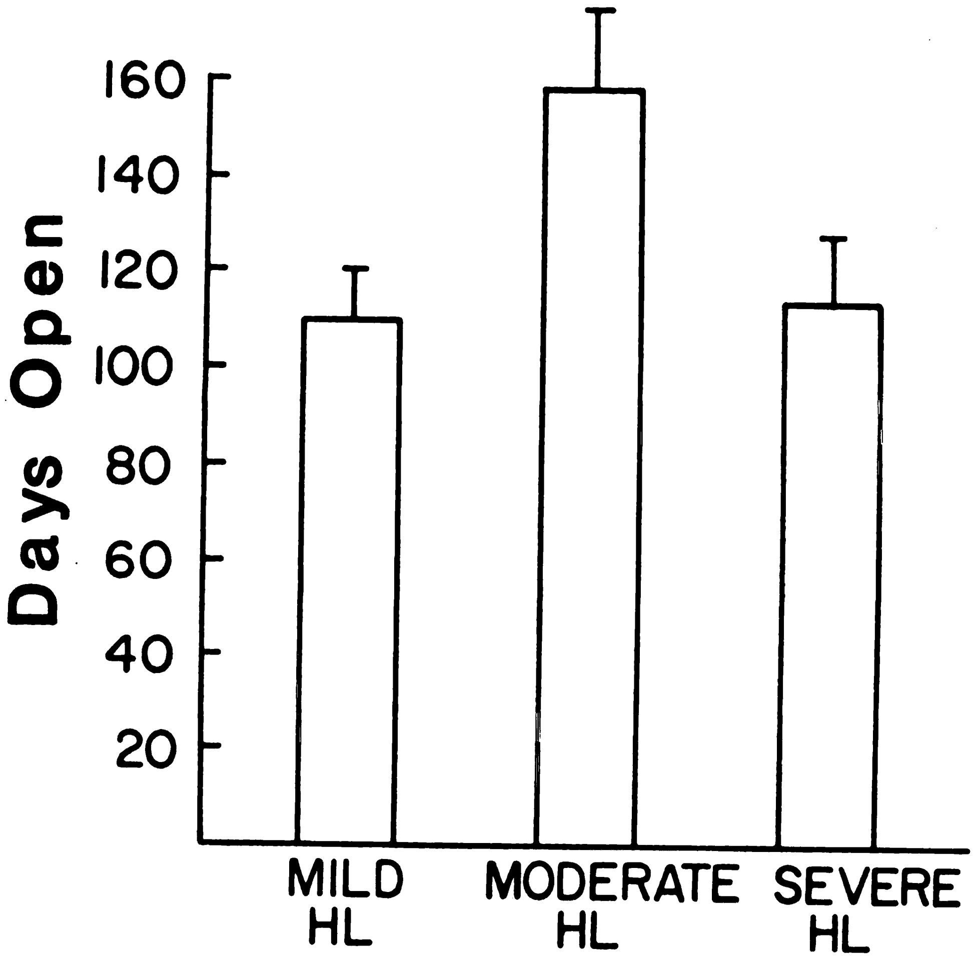


Figure 10: Days open in cattle grouped according to hepatic TG content.

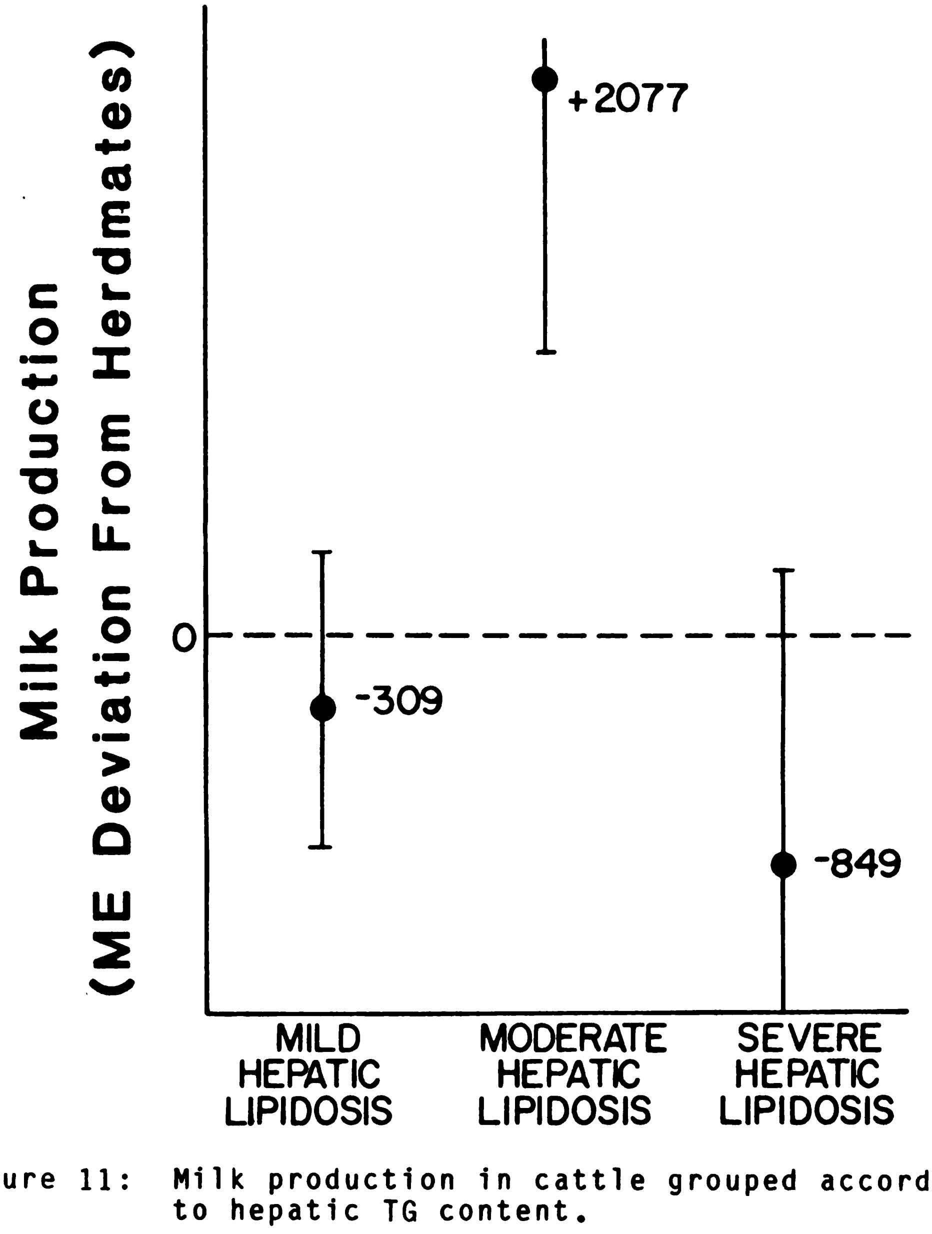


Figure 11: Milk production in cattle grouped according to hepatic TG content.

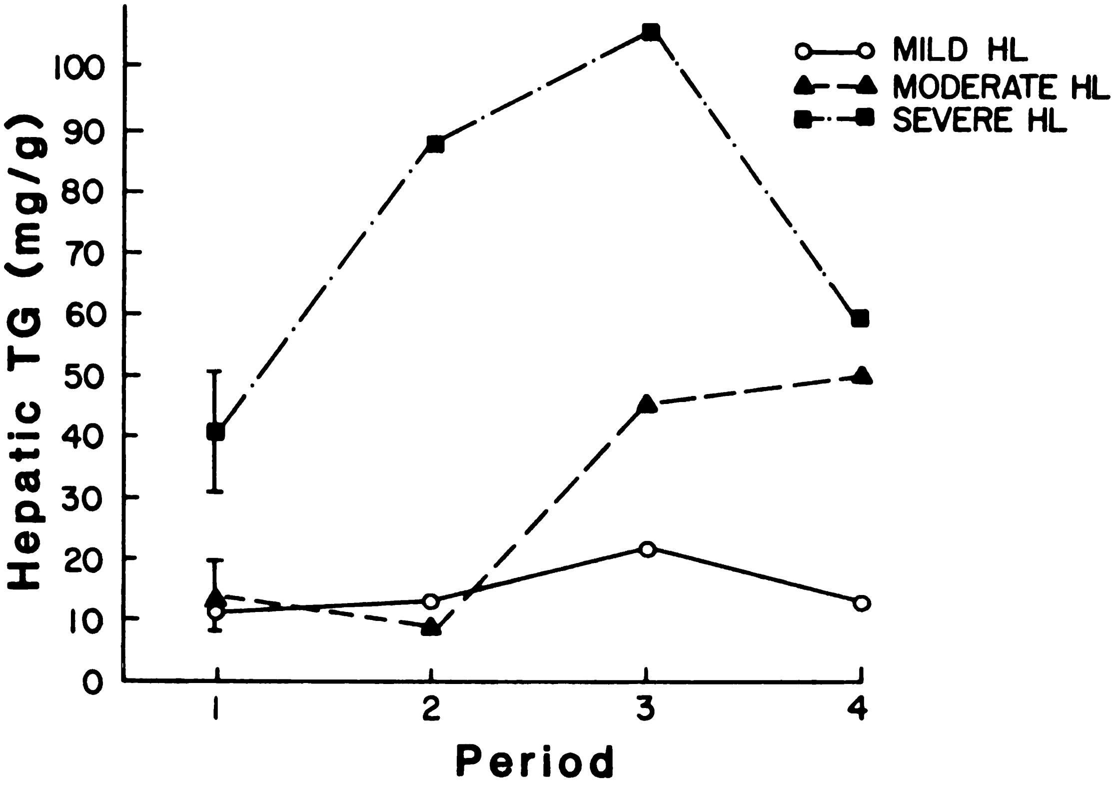


Figure 12: Hepatic TG content in cattle with mild, moderate and severe HL. Period 1, > 3 weeks prepartum; Period 2, 0 to 3 weeks prepartum; Period 3, 0 to 3 weeks postpartum; and Period 4, > 3 weeks postpartum.

significantly greater liver TG concentrations than normal cows during the postpartum period only. ($p<.05$)

Serum NEFA concentrations for the 3 groups are shown in Figure 13. Cattle with severe HL had the greatest serum NEFA concentrations at all time periods with the difference between cattle with mild and severe HL in postpartum concentrations significant ($p<.05$).

Mean serum dextran precipitable cholesterol and insulin concentrations for each group are shown in Figures 14 and 15, respectively. Most differences were not statistically significant, although cattle with severe hepatic lipidosis tended to have lower serum values of dextran precipitable cholesterol and insulin. Cattle with severe HL did have significantly lower dextran precipitable cholesterol concentrations at the initial sample, however ($p<.05$). Condition scores prepartum did not differ between groups, although cattle with severe HL had significantly greater condition score loss during the sampling period than cattle with mild HL (Figure 16).

DISCUSSION

Gaal et al. (1983a) have proposed classifying cattle into three groups based on histological liver fat content at 1 wk postpartum. Histological liver fat content is highly correlated with liver TG content measured chemically and we have utilized these proposed classifications of HL in this study. Photomicrographs of liver biopsies from

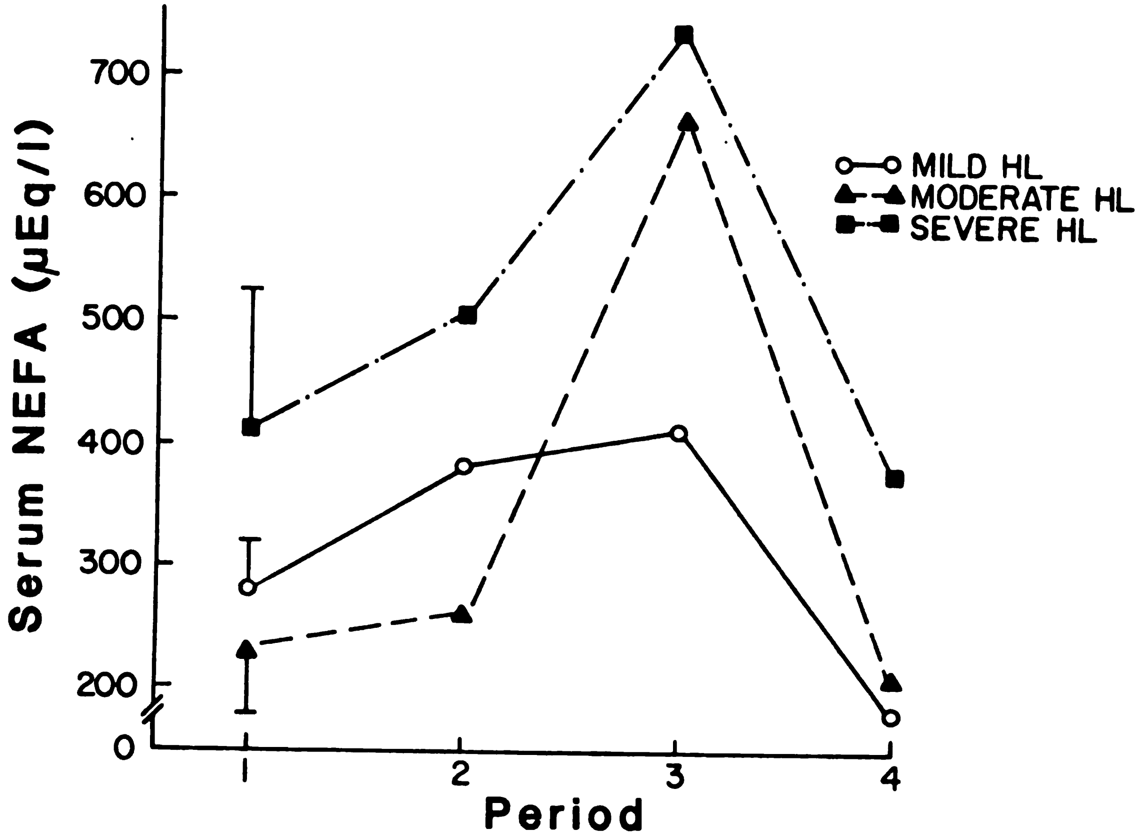


Figure 13: Serum NEFA concentrations in cattle with mild, moderate and severe HL.

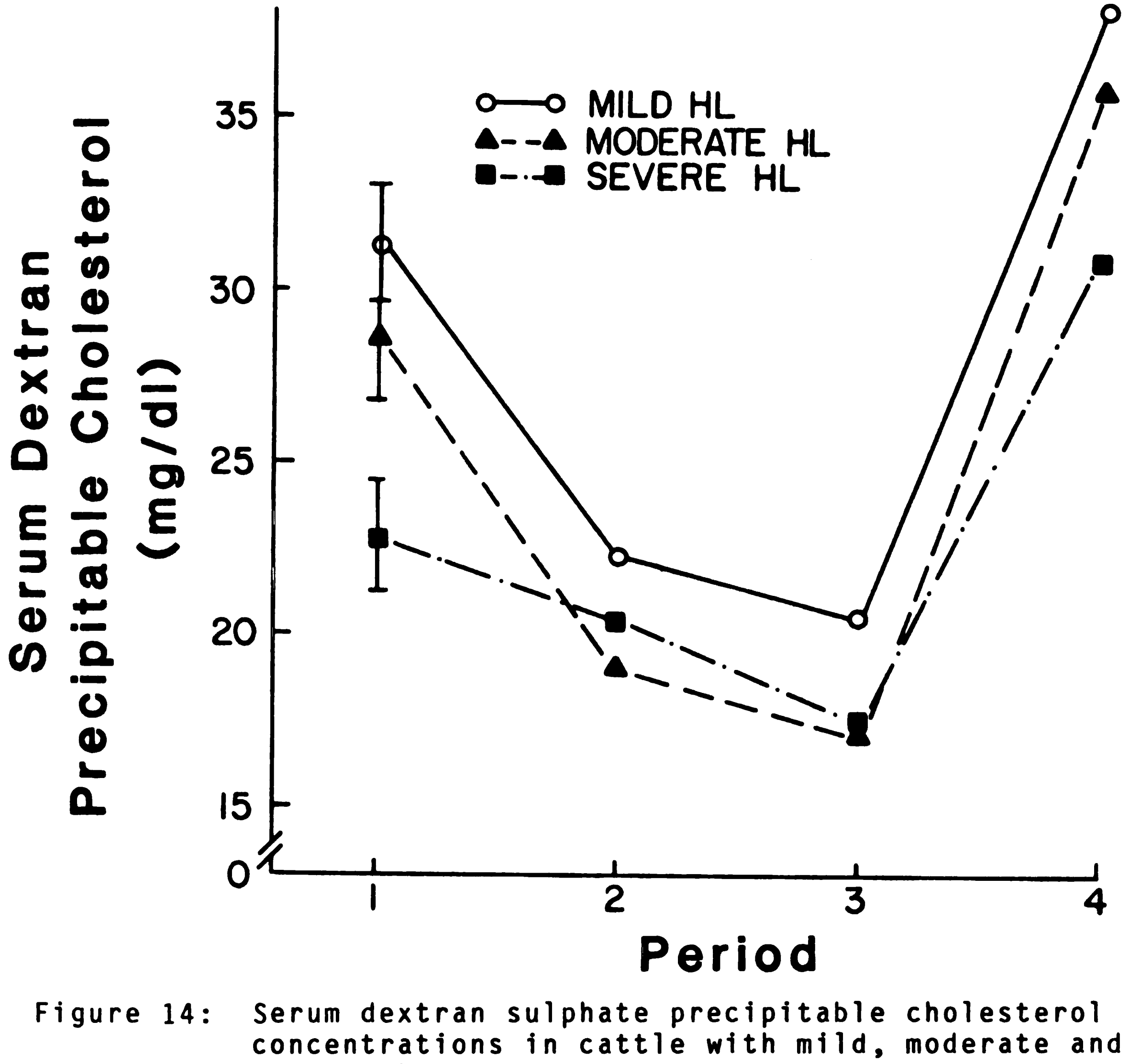


Figure 14: Serum dextran sulphate precipitable cholesterol concentrations in cattle with mild, moderate and severe HL.

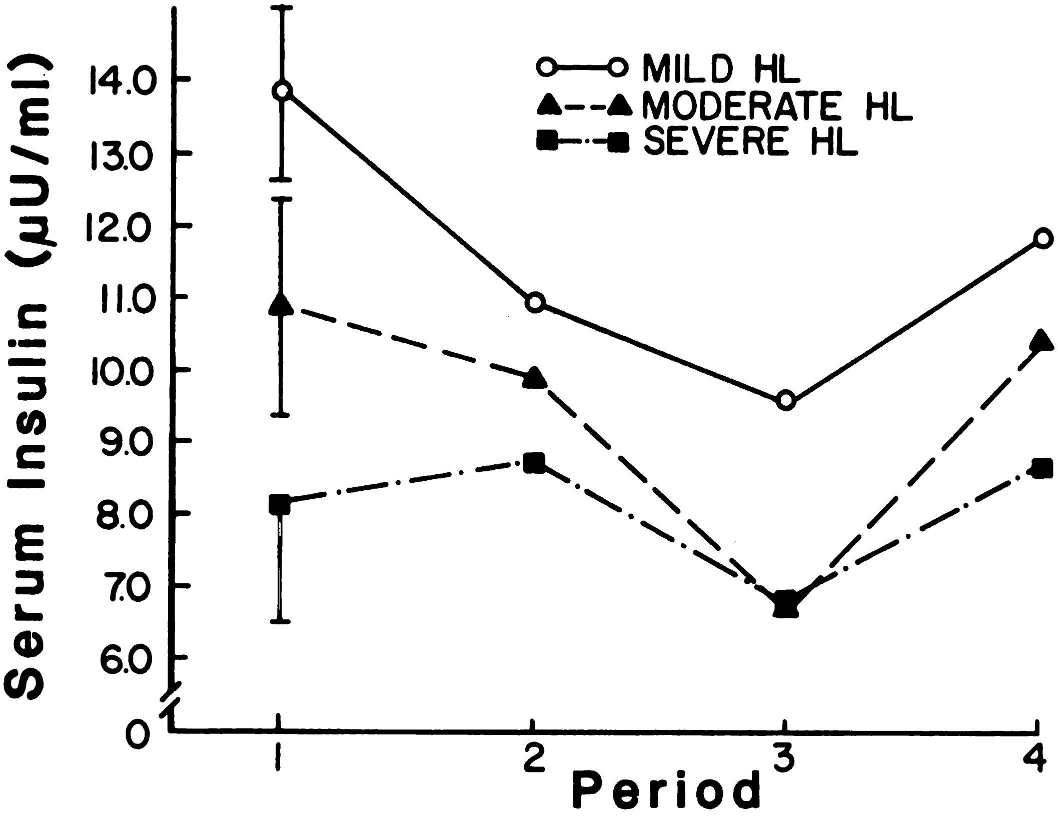


Figure 15: Serum insulin concentrations in cattle with mild, moderate and severe HL.

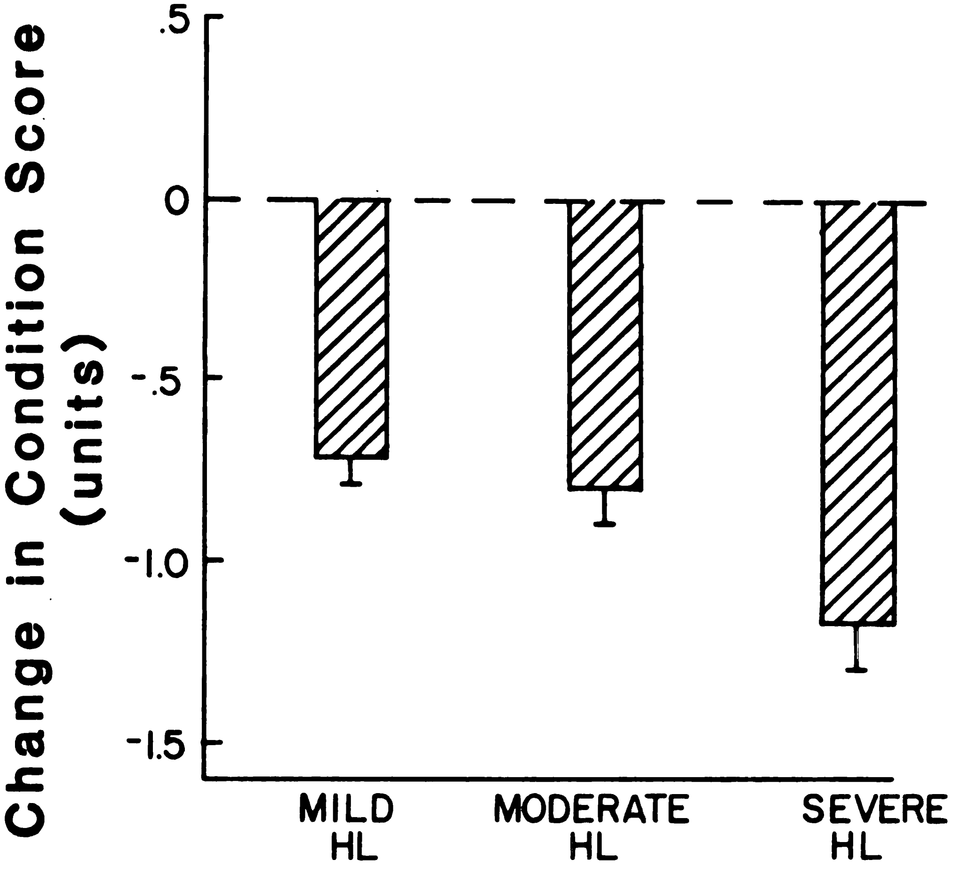


Figure 16: Condition score loss postpartum in cattle with mild, moderate and severe HL.

cattle classified into groups with mild, moderate and severe HL are presented in Figures 17, 18 and 19, respectively.

The bovine liver synthesizes very little fatty acid (Hanson and Ballard, 1967), so TG which accumulates there must result from re-esterification of NEFA mobilized from adipose tissue (Baird, 1982). Hepatic lipidosis in the periparturient dairy cow appears to result from the influx of large amounts of NEFA mobilized at the onset of parturition. Non-esterified fatty acid concentrations were greater in cows with moderate and severe HL in this study and others (Morrow et al., 1979; Roberts et al., 1981; Gaal et al., 1983b; Reid et al., 1983b) and significant lipid deposition may occur in other organs as well (Morrow et al., 1979; Roberts et al., 1981, 1983). An increase in serum NEFA concentrations occurs in most cows at this time, as indicated by the parallel rise in NEFA concentrations for all groups (Figure 13) and is consistent with the metabolic changes occurring to support lactation. In fasted cows hepatic TG secretion as lipoprotein is reduced (Reid et al., 1979), and a reduction in TG output has been thought to contribute to the accumulation of liver lipid in HL. Reduced circulating TG and cholesterol concentrations have been observed in cattle with HL (Morrow et al., 1979; Herdt et al., 1983b) and lipotropic agents such as choline have been advocated to increase hepatic output of lipoproteins (Morrow, 1976; Deem, 1980). Serum dextran

Figure 17: Photomicrograph of a liver biopsy from a cow with mild HL. This corresponds to < 50 mg TG/g liver, or < 20% of the volume occupied by fat, and would be considered normal postpartum. (H & E stain, magnified 125X)

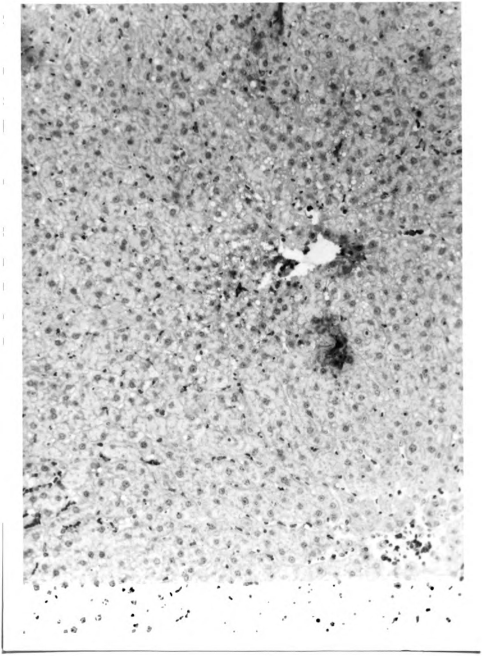


Figure 18: Photomicrograph of a liver biopsy from a cow with moderate HL. The fat is distributed centrilobularly. This corresponds to 50 to 100 mg TG/g liver, or 20% to 40% fat by volume.
(H & E stain, magnified 125X)

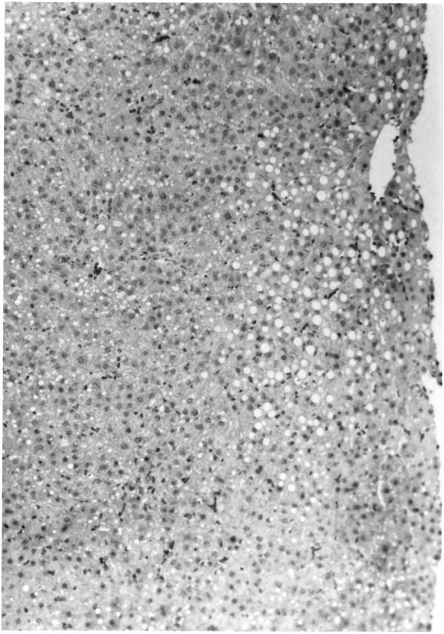
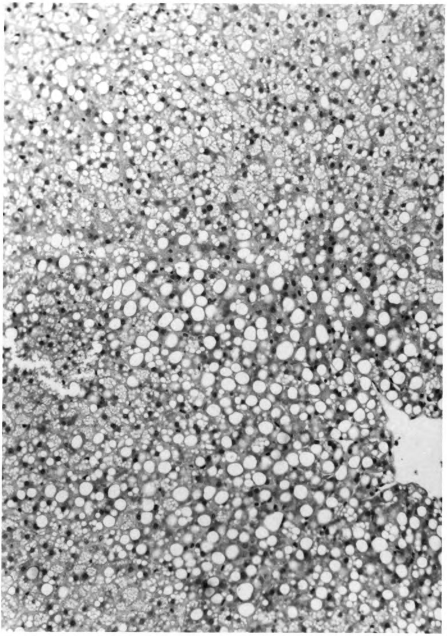


Figure 19: Photomicrograph of a liver biopsy from a cow with severe HL. Fat is now distributed more diffusely. TG content is > 100 mg/g liver, or > 40% fat by volume.
(H & E stain, magnified 125X)



precipitable cholesterol concentrations were used to provide a measure of the lower density lipoproteins in serum. It is this fraction which transports most of the TG in blood. A decrease in hepatic output is thought to contribute to TG accumulation in the bovine liver. Concentrations declined in all cows near parturition (Figure 14), and total cholesterol has been observed to follow a similar pattern (Blum et al., 1983; Kappel et al., 1984), suggesting lipoprotein production is reduced in all cattle at this time. Differences between groups were not significant in Periods 2, 3, and 4 although there was a tendency for cows with severe HL to have lower serum dextran precipitable cholesterol, and this difference was significant in the first prepartum period (Period 1). Sommer (1975) has suggested dairy cattle with decreased cholesterol concentrations prepartum have a higher incidence of health problems after calving, and they may be at increased risk of HL.

Serum insulin concentrations declined in all cows immediately postpartum (Figure 15), as has been observed by others (Schwalm and Schultz, 1976a). The differences between groups were not significant, but there was a tendency for cows with moderate and severe HL to have lower serum insulin concentrations. This is consistent with a more severe negative energy balance and increased lipolysis and circulating NEFA concentrations in these cattle, and has been observed by others (Reid et al., 1983b).

The major metabolic difference between cows with severe and mild HL appears to be degree of fat mobilization, as indicated by differences in circulating NEFA concentrations postpartum. Therapeutic and prophylactic measures should attempt to reduce lipolysis over the peripartum period . Catecholamines induce lipolysis in ruminants as in other species (Vernon, 1980) and stress at calving may contribute significantly to elevated NEFA concentrations. Attempts to reduce stress and competition for feed in the immediate postpartum period by having ample feed available, especially high quality roughage, are indicated. Immediate introduction into the milking herd may increase stress due to social interactions and should probably be avoided for several days, if possible. Niacin supplementation at 6 to 12 g per head per day has been shown to decrase lipolysis in dairy cattle (Fronk and Schultz, 1979), and can be started 2 to 3 wks prepartum as a prophylactic measure .

Condition score prepartum did not differ between groups, but there were no thin cows (condition score < 2.5) that developed severe HL. Many obese cattle (condition score $\geq$ 3.5) did not develop moderate or severe HL. Condition score loss was significantly greater in cows with severe HL, as has been previously observed (Reid, 1980), emphasizing that adipose mobilization is an important contributing factor in development of HL. Maximizing dry matter and energy intake as rapidly as possible postpartum without causing indigestion and anorexia is desirable. This is more difficult in obese animals, as the normal reduction in dry matter intake near the time of calving appears to be greater (Garnsworthy and Topps, 1982), as does NEFA mobilization and incidence of metabolic disorders in these animals (Fronk et al., 1980). Animals with higher condition scores at calving have produced more milk in some studies, however (Jaster et al., 1983), so avoiding excessively thin cows as well as obese animals is desirable .

The percentages of cows classified with moderate or severe HL in this study were similar to percentages reported by other investigators (Reid and Roberts, 1983). It was expected that more cattle would have HL because the herds were referred to the study by veterinarians diagnosing HL based on clinical signs. In only three herds (Herds 2, 5 and 8) did severe HL appear to be a significant problem. This indicates clinical diagnosis alone may be unreliable. Blood samples tested for hepatic enzyme concentrations and liver function tests have been shown to be poorly related to hepatic fat content in man as well as cattle (Gröhn et al., 1983; Reid et al., 1983b; Andersen et al., 1984) with aspartate amino transferase concentrations having the highest correlation (Reid et al., 1983b; Herdt et al., 1982). A method of classifying cattle as to mild or excessive hepatic TG accumulation based on a linear combination of serum NEFA, aspartate amino transferase and glucose concentrations postpartum has been suggested (Reid et al., 1983b). Even with this method it was possible to assign only 60% of the cattle sampled with a high degree of probability. The reliable method to diagnose HL remains a liver biopsy, although clinical signs and serum chemistries may be suggestive of the condition. A convenient method of estimating lipid content in small samples, based on density, has been reported (Herdt et al., 1983a).

A greater percentage of animals with severe HL were removed from the herds by death or culling, although cattle with moderate HL did not differ from cattle with mild HL. Disease rate in cattle with severe HL also tended to be greater than in the other two groups. This is consistent with the observations that moderate HL is a subclinical entity (Reid and Roberts, 1983) and more severe increases in liver TG accumulation are necessary before liver function as measured by serum enzyme and function tests is significantly impaired (Herdt et al., 1982). Moderate HL may be associated with decreased protein synthetic capacity, however (Reid and Collins, 1980). Severe HL may be associated with a reduced immune competence, and is associated with reduced numbers of circulating neutrophils (Morrow et al., 1979). Disease itself may have caused more severe HL by diminishing appetite, which would result in greater mobilization of NEFA from adipose tissue. In this study that possibility cannot be excluded.

An extended calving interval has been associated with moderate HL in dairy cattle (Reid, 1983), and may be associated with an increase in time to first ovulation and first estrus in these cows (Reid et al., 1983a). Serum luteinizing hormone and progesterone concentrations following the first postpartum ovulation tended to be lower in cattle with moderate hepatic lipidosis (Watson and Harwood, 1984). Other investigators have failed to observe an association between hepatic lipidosis and reduced fertility (Perkins et al., 1983). In our study, cattle with moderate HL did have increased days open compared to cattle with mild hepatic TG accumulation; however, cattle with severe HL did not differ from cattle with mild HL in days open. A direct effect of liver TG accumulation on reproductive performance seems unlikely. Previously cited reports relating liver fat accumulation to reproductive performance included only one of 80 cows classified as having severe HL. Cattle with moderate HL have a tendency to produce more milk than herdmates (Reid, 1983; Reid and Roberts, 1983) and this was true in the present study. Cattle with severe HL had mean production below herdmates, although the difference was not statistically significant. Lowered production may be caused by the increased rate of disease, and is consistent with the decreased production and increased disease obseved by others in overconditioned cattle (Fronk et al., 1980). Moderate HL, as postulated by Reid and Roberts (1983), is likely a manifestation of the transition from the non-lactating to lactating condition and consequent NEFA mobilization. The association with decreased reproductive performance may merely reflect the association of moderate HL with high milk production and negative energy balance postpartum, which also may affect reproductive function (Butler et al., 1981). In this and other studies, increased days open in cattle with moderate HL may also represent a management decision to delay rebreeding cattle at higher production levels. When days open were adjusted for milk production, differences between groups were no longer significant. Cervical diameter 3 to 4 wks postpartum, which is related to reproductive performance (Oltenacu et al., 1983), did not differ between groups.

As indicated by disease and death rates, cattle with severe HL probably represent the clinically significant group. These animals resemble those with fat cow syndrome. Hepatic TG concentrations in these cattle tended to be higher than those with mild HL at all sampling periods, especially immediately pre- and postpartum (Periods 2 and 3). Cattle with severe HL may have elevated liver TG concentration during the non-lactating period and may be more susceptible to severe TG accumulation occurring near the time of parturition. For prevention of clinical signs of severe HL in obese cattle, this should be recognized, and preventive measures be initiated longer than 2 wks prepartum.

Division of cattle into classifications of mild, moderate and severe HL in this and other studies is an arbitrary procedure, but consistent classifications facilitate comparisons between investigators. Differences between individuals within groups were in many cases greater than differences between groups. Interpretations about individual animals based on liver TG content at a single sampling time should be made with caution, but nonetheless may provide some useful information.

CONCLUSIONS

Cattle with severe HL had a tendency to increased disease rates and a greater rate of loss due to death or culling . Liver TG concentrations were higher prepartum as well as postpartum in cattle with severe HL and were associated with greater serum NEFA concentrations and increased loss of condition.

Cattle with moderate HL had greater days open than other cattle, but probably the difference did not reflect a direct effect of hepatic TG on reproductive function. It may have reflected an association of moderate HL with increased milk production.

Clinical diagnosis of HL within a herd was not consistently accurate, and liver biopsies should be utilized for a definitive diagnosis.

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APPENDIX A

APPENDIX A

Participating Herds

Herd Number	Owner	Location
1	Roger Fritz	Owendale, MI
2	Bruce and Gary Protzman	Pigeon, MI
3	Robert Mortenson	Kent City, MI
4	Michigan State Univ.	East Lansing, MI
5	Richard Gilna	Corunna, MI
6	Al and Duane Stuever	Capac, MI
7	Jerry Van Polen	Marion, MI
8	L.D. Hesselink	Tustin, MI
9	Bernard DeYarmand	Laingsburg, MI

APPENDIX B

INOSITOL AS A LIPOTROPIC AGENT IN DAIRY CATTLE DIETS[1,2]

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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; Liesman 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

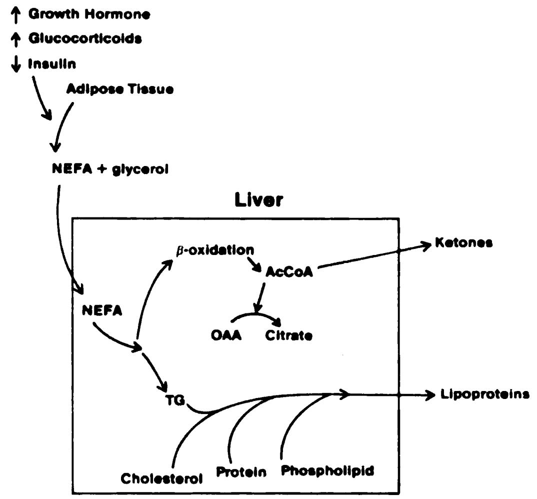

Figure 1. Increased fat mobilization of early lactation results in increased delivery of nonesterified fatty acids (NEFA) to the liver. Nonesterified fatty acids can be oxidized and form ketones or completely oxidized in the Kreb's cycle. Alternatively the NEFA can be reesterified and the TG can be resecreted as lipoproteins. In HL there is apparently an increase in NEFA influx and a decrease in lipoprotein output by the liver.

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 hexahydroxycyclohexane. 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 pancratic islets (Freinkel et al., 1975;

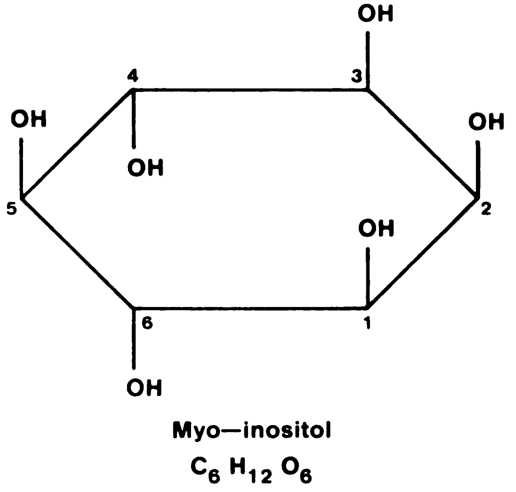

Figure 2. Myo-inositol, the isomer of hexahydroxycyclohexane which has vitamin-like properties.

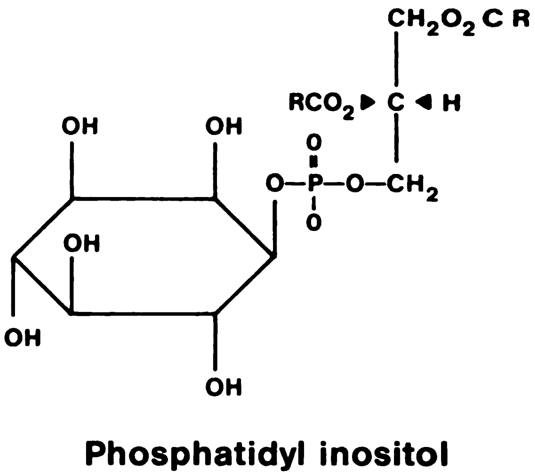

Figure 3. Phosphatidylinositol is the phospholipid form of myo-inositol.

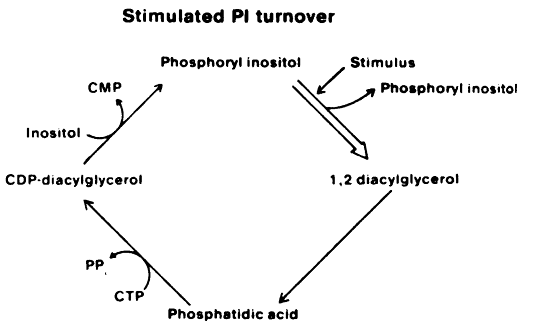

Figure 4. An appropriate stimulus results in phosphoryl-inositol release from PI. Phosphatidylinositol is then regenerated by incorporating phosphate and inositol. This PI turnover may be associated with Ca^{2+} permeability and(or) prostaglandin metabolism.

Clements 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).

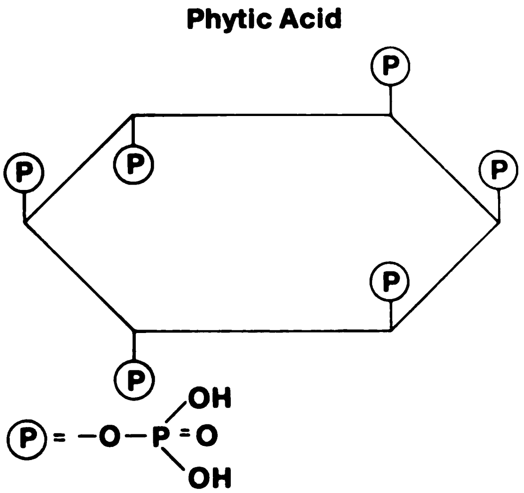

Figure 5. Phytic acid, the inositol hexaphosphate.

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® Travenol Labs, Inc., Deerfield, IL.

[7] Courtesy of Eli Lilly and Co., Indianapolis, IN.

TABLE 1. MEAN VALUES (± SE) FOR COWS WITH AND WITHOUT SUPPLEMENTAL INOSITOL AT FOUR SAMPLING PERIODS

	Liver inositol (μmol/g)		Liver TG (% wet wt)		Serum insulin (μunits/ml)		Body condition score	
Approximate sampling time	Supplemented	Unsupplemented	Supplemented	Unsupplemented	Supplemented	Unsupplemented	Supplemented	Unsupplemented
1 mo prepartum	3.44 ± .69 (8)[a]	2.70 ± .63 (11)	4.39 ±2.30 (10)	3.36 ±1.61 (12)	17.58 ±2.91 (12)	14.56 ±3.60 (14)	3.16 ± .14 (12)	3.18 ± .15 (14)
10 d prepartum	2.19 ± .32 (8)	2.33 ± .56 (8)	5.56 ±2.87 (8)	7.91 ±4.79 (8)	8.83[b] ±1.13 (12)	15.95[b] ±3.45 (13)	2.81 ± .07 (12)	2.90 ± .17 (13)
10 d postpartum	2.52 ± .63 (6)	2.61 ± .37 (13)	4.40 ±1.58 (7)	2.71 ± .89 (13)	9.42 ±2.43 (11)	8.32 ±1.93 (13)	2.31 ± .10 (11)	2.23 ± .11 (12)
1 mo postpartum	2.19 ± .52 (9)	2.01 ± .22 (12)	6.52 ±2.55 (8)	3.42 ± .74 (13)	13.42 ±1.98 (11)	18.28 ±5.86 (13)	2.29 ± .13 (11)	2.18 ± .37 (13)

[a] 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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