

New Insights into Fatty Liver and Ketosis

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Introduction

Fatty liver and ketosis are interrelated disturbances of energy metabolism that impact dairy cows during the periparturient or transition period. The syndromes can directly decrease performance and adversely impact cow well-being; in addition, they can predispose cows to infectious diseases and other metabolic disorders. Both situations have been extensively researched during the last several decades and the scientific literature is plentiful – yet the disorders continue to plague dairy farmers worldwide. While the descriptive pathology and metabolic characteristics have been thoroughly described by these research efforts, the mechanism or mechanisms that trigger clinical disease and the cellular processes responsible for the resultant pathologies have remained elusive. Moreover, optimal management strategies for minimizing incidence and impact are still debated.

Given the central importance of management to minimize metabolic disorder during the transition period in determining profitability and cow well-being, the objective of this presentation is to review some key questions. What are fatty liver and ketosis and how common are they? When and how do fatty liver and ketosis develop? How do fatty liver and ketosis affect health and productivity? What nutrition and management practices predispose to fatty liver and ketosis, and how can management prevent them? The focus will be on new information and theories surrounding development and consequences of ketosis and fatty liver, with emphasis on recent findings from our research program. For additional information, readers may refer to several comprehensive reviews on various aspects of transition cow biology and management (Drackley, 1999; Drackley et al., 2001, 2005; Grummer et al., 2004; Overton and Waldron, 2004; Drackley and Andersen, 2006; Goff, 2006; Drackley and Dann, 2008).

Negative Energy Balance and the Ketosis-Fatty Liver Complex

Dairy cows undergo tremendous changes during the transition from late gestation to early lactation. The fundamental driver of the physiological changes is to ensure provision of adequate nutrients for the calf, both prenatally and postnatally. Dairy production has capitalized on this metabolic drive by selecting for ever-higher milk production. After parturition, nutrient demand is not able to be met through feed intake alone because the rate of dry matter intake (DMI) increase is slower than the rate of milk energy output. As a result, the magnitude of metabolic challenge faced by modern dairy cows is almost staggering. As demonstrated in Table 1, requirements for net energy of lactation (NE_L) essentially double “overnight” as cows calve and commence lactation.

The fact that many cows are able to meet this challenge without difficulty speaks to the fact that the metabolic adaptations necessary to support milk production are a component of the

factors being genetically selected. The nutrient and energy deficits after parturition are met by mobilization of body reserves and by decreasing nonessential use of glucose in non-mammary tissues. Metabolic adaptations are mediated by an exquisite pattern of hormonal shifts and

Table 1. Calculated net energy of lactation (NE_L) requirements (Mcal/d) for dairy cows and heifers 2 days before vs. 2 days after parturition (Drackley et al., 2005).

Function	1600-lb cow		1250-lb heifer	
	Pre-fresh	Fresh	Pre-fresh	Fresh
Maintenance	11.2	10.1	9.3	8.5
Pregnancy	3.3	---	2.8	---
Growth	---	---	1.9	1.7
Milk production	---	18.7	---	14.9
Total	14.5	28.8	14.0	25.1

Calculated from NRC (2001). Assumes precalving body weight with average decrease for calf and fluid loss at calving, milk production of 55 lb/d for cow and 45 lb/d for heifer, each containing 4% fat.

factors being genetically selected. The nutrient and energy deficits after parturition are met by mobilization of body reserves and by decreasing nonessential use of glucose in non-mammary tissues. Metabolic adaptations are mediated by an exquisite pattern of hormonal shifts and changes in tissue responsiveness to those hormones. For example, growth hormone (GH) is increased around parturition and in early lactation (Grum et al., 1996), which increases responsiveness of adipose tissue to lipolytic signals such as norepinephrine and decreases sensitivity of insulin-dependent tissues to the action of insulin, whose concentration also decreases because of negative energy balance (NEB). The resulting increase of nonesterified fatty acids (NEFA) from adipose tissue are used as alternate fuels for much of the rest of the body, and are also converted by the liver to ketone bodies. The ketones serve as alternate water-soluble fuels that can replace glucose in many tissues, thus conserving glucose for milk synthesis.

While a majority of cows are able to weather the metabolic challenges associated with initiation of milk synthesis, the fact that, on average, roughly 1 in 2 cows succumbs to some health problem during the transition period (Ferguson, 2001) underscores the fragility of the system. The etiology of most of the periparal health disorders is directly or indirectly associated with NEB (Drackley et al., 2005). Increasingly strong evidence indicates that clinical and subclinical ketosis and fatty livers are key underlying factors in development of other early

lactation disorders and disease (Duffield, 2000; Bobe et al., 2004), as well as impaired fertility (Rukkwamsuk et al., Walsh et al., 2007a,b).

Metabolic Changes during the Transition Period

As calving approaches, concentrations of progesterone in blood decrease and those of estrogen remain high or increase (Grummer, 1995). The high circulating estrogen may be one factor that contributes to decreased DMI around calving (Grummer, 1993). During the last 3 wk of pregnancy, nutrient demands by the fetal calf and placenta are at their greatest (Bell, 1995). At the same time, depending on diet composition, DMI may be decreased by 10 to 30% compared with intake during the early dry period. This in itself may not be cause for alarm, as decreased food or feed intake around parturition is a common finding in many mammalian species (Frignens, 2003). Maintaining good appetites through and after calving are likely more important.

Cows are unable to consume sufficient energy-yielding nutrients from voluntary DMI after calving to meet energetic requirements for milk production. Consequently, a state of NEB almost always occurs for a period of days to weeks in early lactation. Postpartal NEB clearly results from initiation of milk synthesis because mastectomy before parturition greatly blunts the increased concentration of NEFA released from adipose tissue at and after calving (Goff et al., 2002). However, the degree of NEB in individual cows is poorly related to milk production, or more correctly, milk energy secretion. The depth and duration of NEB is highly related to DMI (Zurek et al., 1995; Drackley et al., 2005), and thus factors that impact appetite and consumption of feed after calving are critical.

Like most mammals, a number of physiological adaptive mechanisms are invoked to allow the cow to cope with the challenges induced by NEB. Cows mobilize stored triglycerides (TG) in adipose tissues as an energy supply for milk production and maintenance functions. Glycerol released from lipolysis is used by the liver for glucose synthesis in the pathway of gluconeogenesis. The fatty acids released circulate as NEFA and are distributed with blood flow to all body tissues (Drackley et al., 2001). When NEFA concentrations are elevated during early lactation, the mammary gland takes them up efficiently and converts them to milk fat. High milk fat concentrations, or high milk ratios of fat to protein, are useful as indicators of ketosis in dairy cows.

The liver receives about 1/3 of all blood flow from the heart. Consequently, the liver is flooded with NEFA when blood concentrations increase around calving. The liver takes up NEFA in proportion to their concentration in blood. Within liver cells, NEFA can be 1) oxidized to carbon dioxide with the generation of ATP for the liver's energy needs, 2) partially oxidized to the ketone bodies β -hydroxybutyrate (BHBA) and acetoacetate, which results in ATP for the liver and water-soluble energy sources for muscle and heart, or 3) re-converted to TG. Because ruminant animals are unable to effectively export TG out of the liver as very-low density lipoproteins, TG can accumulate and cause fatty liver. Increased ketone body production, which occurs when carbohydrate (glucose) supply is limited during NEB, can result in ketosis (Drackley et al., 2001).

Metabolic adaptations in carbohydrate and protein metabolism also occur around calving (Drackley et al., 2001, 2005). Low insulin concentrations result in decreased oxidative use of glucose by peripheral tissues, thereby sparing glucose for milk synthesis. In the liver, the efficiency of gluconeogenesis from propionate increases after parturition. Glucose synthesis from glycerol and glucogenic amino acids such as alanine increases around parturition to meet the glucose demand that cannot be supplied by propionate due to low DMI. Body protein mobilization increases during the first 3 weeks postpartum to supply amino acids for both milk protein synthesis and glucose synthesis. In practical terms, this emphasizes the importance of dietary provision of metabolizable protein. Lack of response in milk production to increased rumen-undegradable protein supplementation, except in first-calf heifers (Van Saun et al., 1993; Santos et al., 2001), has been common in the literature (see Bell et al., 2000) and in our own experience (Underwood et al., 2001). Nevertheless, the importance of maintenance of maternal stores of protein on long-term health, productivity, and reproduction is backed by strong indirect evidence (Bell et al., 2000).

The onset of milk synthesis creates a large drain on the blood pool of free calcium, resulting in sometimes-marked decreases in blood calcium concentration. Hypocalcemia can be classified as subclinical (blood Ca between 8 and 5.5 mg/dL) or clinical. While the incidence of clinical hypocalcemia or milk fever is low, subclinical hypocalcemia is much more prevalent and insidious. Subclinical hypocalcemia is associated with decreased smooth muscle function, which manifests as decreased motility of the rumen and abomasum (which increases risk of displaced abomasum) and decreased contraction of the teat sphincter, thus increasing risk of mastitis (Goff, 2006). Decreased feed intake (thus increasing risk of subclinical ketosis) and decreased immune cell function also result from hypocalcemia (Kimura et al., 2006). Because of these widespread effects of hypocalcemia and the decrease in smooth muscle function in the uterus, reproductive performance may be impaired.

Hypocalcemia is usually assessed by sampling blood during the first 24 hours after calving, when blood Ca is at its nadir. Hypocalcemia can persist during the first several days postpartum, which can contribute to sluggish starts to lactation and increased incidence of secondary health problems such as ketosis and fatty liver. Until the ability of the digestive tract to absorb calcium can increase, calcium must be obtained by breaking down bone. Metabolic acidosis caused by a negative dietary cation-anion difference (DCAD) favors mobilization of calcium from bone, whereas high dietary potassium concentrations and positive DCAD suppress this process (Horst et al., 1997).

Metabolic adaptations are directed by endocrine and central nervous system controls that to some (as yet unknown) extent monitor and respond to the degree of NEB (Drackley et al., 2001, 2005). Major endocrine changes include increased secretion of somatotropin, decreased insulin and insulin-like growth factor-1 (IGF-1), and increased glucagon. Lipolysis in adipose tissue is stimulated mainly by the sympathetic nervous system in the presence of low insulin concentrations. The sympathetic nervous system responds to energy shortage or chronic stressors with greater activity. Although stressors and severe limitations in DMI can lead to NEB before calving, the degree is much less than the severe NEB that can occur following parturition. Severe or prolonged NEB can lead to increased TAG deposition in liver, increased

ketone bodies such as BHBA and acetoacetate in blood, and depletion of body reserves of energy (largely fat) and protein.

Function of the immune system also is depressed during the transition period (Mallard et al., 1998). The degree of immunosuppression seems to be greater in overconditioned cows (Lacetera et al., 2005) and in cows with fatty liver (Bobe et al., 2004). Decreased ability of the immune system to respond to infectious challenges likely is responsible for the high incidence of environmental mastitis around calving, as well as the high incidence of metritis. Reasons for the decreased immune function are not well understood. Vitamins A and E as well as a number of the trace minerals (selenium, copper, zinc) play a role in enhancing immune function. Recent evidence suggests that NEB or negative protein balance may be a major contributing factor (Goff, 1999). This finding relates well to the common observation that cows which seem to be the most stressed by nutrition and environmental factors, as judged by excessive loss of body condition, are the most likely to become ill. In particular, an inadequate supply of metabolizable protein has been related to impaired function of the immune system (Houdijk et al., 2001). Recent evidence linking retained placenta to a malfunction of the immune system (Kimura et al., 2002) suggests that protein nutrition also might impact the incidence of retained placenta.

Fatty liver

Fatty liver is defined as an accumulation of TG (the same kind of fat as found in adipose tissue, milk fat, or vegetable oils) in the liver. Fatty liver does not occur when cows are in positive energy balance and gaining weight because the liver is not a place of storage for fat like the adipose or fat tissues throughout the body (backfat, organ fat, etc.). Dietary fats provided from oilseeds such as canola or soybeans, animal fats, or commercial fat supplements are handled by the cow differently than NEFA and largely bypass the liver. In situations where milk fat is depressed by feeding excessive supplemental fats and oils perhaps with monensin, the decreased milk fat results mainly from inhibition of fatty acid synthesis in the udder by CLA and other *trans*-fatty acids produced in the rumen. Therefore, there should be little concern about dietary fats or milk fat-depressing diets leading to fatty liver development.

While the technical name is “hepatic lipidosis”, it is also called “fatty liver syndrome”, “fat cow syndrome”, or “lipomobilization syndrome”. The term “fatty liver syndrome” is probably the best name because it indicates that many other organ systems are affected besides the liver. Fatty liver is caused by excessive uptake of nonesterified fatty acids (NEFA) mobilized from body fat (adipose tissues) in response to NEB. All cows go through some degree of NEB after calving, and essentially all cows have body fat to mobilize. Thus, all cows are at risk for developing fatty livers, even those in thin body condition. However, overconditioning certainly is a major risk factor for development of fatty livers, because fat cows do not eat as well as thinner cows and easily break down body fat to NEFA.

Fatty liver usually develops after calving, with peak incidence at about 10 days in milk (Figure 1). One research study from Michigan State University found that cows which developed severe fatty livers after calving actually had significant fat accumulation in the liver during the last 3 wk before calving. However, no other studies have confirmed this pre-calving fatty liver. Fatty liver can develop quickly after calving. Estimates of rates of body fat

breakdown and liver conversion of NEFA to triglyceride indicate that a severe fatty liver could develop within one to two days after calving (Drackley et al., 2001).

Unfortunately, there are no reliable methods for diagnosis of fatty liver except for liver biopsy. Ultrasound techniques are being developed but are not yet reliable. Determination of liver TG content by small-needle biopsy is a useful and accurate indicator of the degree of metabolic disturbance faced by individual cows (Jorritsma et al., 2001). Biopsies obtained between 8 and 14 days postpartum are most useful in classifying cows. Liver lipid content can be estimated microscopically as the volume of hepatocytes occupied by lipid or chemically as the concentration of TG (Table 2). Concentrations of TG <5.0% and 5.0 to <10.0% are classified as mild and moderate fatty liver, respectively, whereas severe fatty liver is considered to be >10.0% TG (Gaal et al., 1983). Severe fatty liver likely is the only form that is clinically relevant (Gerloff et al., 1986), although moderate fatty liver is also associated with greater incidences of disease and impaired reproduction (Rukkwamsuk et al., 1999; Jorritsma et al., 2000). Surveys indicate that between 25% and 65% of cows have moderate or severe fatty liver in the first weeks after calving (Bobe et al., 2004). Measurement of glycogen in liver in addition to TG increases the usefulness of the information, as concentrations of TG and glycogen vary inversely during the early postpartum period (Figure 1; Drackley et al., 2005). However, liver biopsy is an invasive and time-consuming process, and analytical determination of TAG and glycogen is not well-adapted to rapid testing.

Table 2. Classification of fatty liver by concentration of triglyceride (TG) or by cell volume occupied by lipid

Category	TG (% wet liver)	Proportion of volume as lipid
Normal healthy	<1.0	<0.05
Mild fatty liver	1.0 – 5.0	0.05 - 0.20
Moderate fatty liver	5.0 – 10.0	0.20 - 0.40
Severe fatty liver	>10.0	>0.40

Adapted from Gaal et al., 1983

Maintaining optimal liver function is central to the ability of cows to make a smooth transition into heavy milk production. As the degree of fatty infiltration increases, normal functions of the liver are affected adversely. Cows with fatty liver have a greater degree of immune suppression around calving (Bobe et al., 2004), which may make them more susceptible to developing infectious diseases such as mastitis and metritis. Severe fatty liver usually appears as part of a multi-factor disease complex. Fat infiltration impairs the ability of the liver to detoxify ammonia to urea (Strang et al., 1998). Ammonia decreases the ability of the liver to convert propionate to glucose (Overton et al., 1999), thus linking fat accumulation to impaired gluconeogenesis in liver (Drackley et al., 2001).

Fatty liver also impairs the ability of the liver to detoxify endotoxin, and thereby renders the cow extremely sensitive to endotoxic shock and death (Andersen et al., 1996). In severe fatty liver, normal functions of the liver are severely depressed, which results in the condition of "fatty liver syndrome" or "clinical fatty liver" (Morrow, 1976). Feed intake and carbohydrate status of

the cow are important in determining the extent of body fat mobilization, fatty liver, and ketone body production in the liver. Fat infiltration per se evidently does not lead to liver failure (Rehage, 1996) but may be a contributing factor at least in some circumstances.

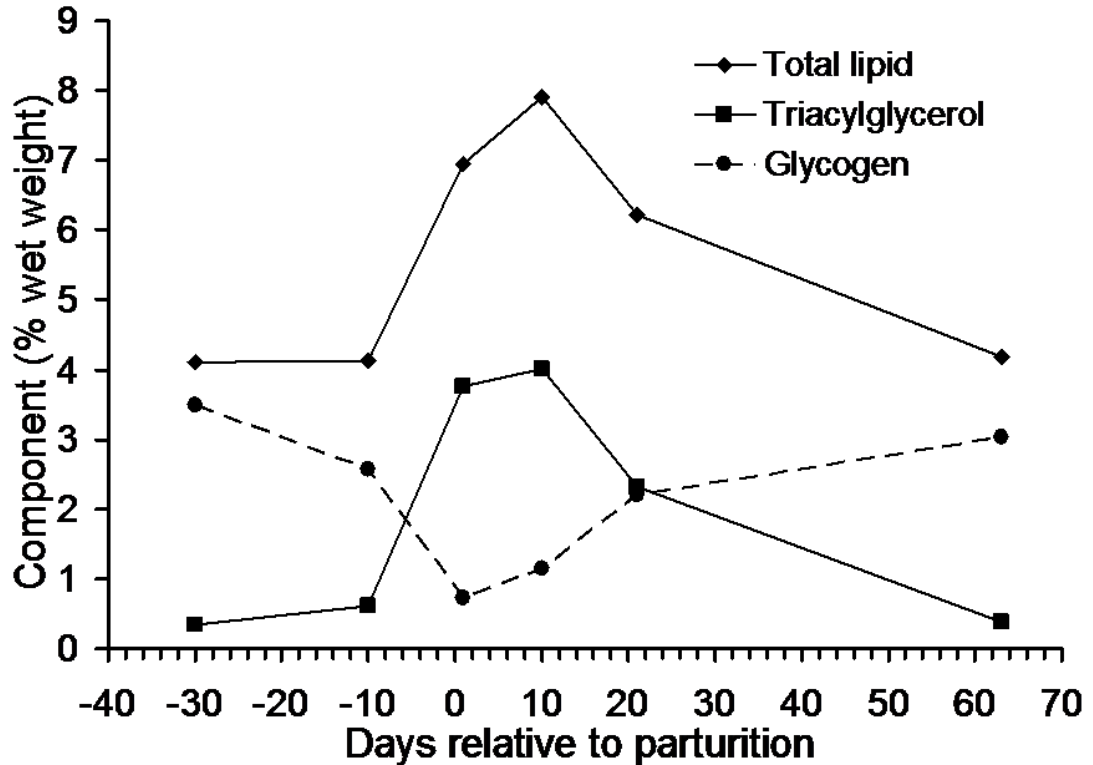


Figure 1. Concentrations of total lipid, triacylglycerol (triglyceride), and glycogen in liver during the periparturient period. (From Drackley et al., 2005)

Fatty liver is negatively associated with fertility (Rukkwamsuk et al., 1999). This association may be an indirect effect of the extreme NEB that exists in these cows. However, there also may be direct negative effects of hepatic lipid infiltration on reproduction. Blood flow through the liver may be altered by fat accumulation expanding cell volume and compressing hepatic sinusoids. Alternately, lipid accumulation may decrease the ability of the liver to metabolize or clear reproductive hormones, thus altering the normal signaling to reproductive tissues and the pituitary.

While effects of fatty liver per se on milk production are less clear, overall the evidence for impairment of dairy cow health and productivity is strong.

Ketosis

Original research summarized by Kronfeld (1982) resulted in his classification of ketosis in dairy cows into four types: 1) primary underfeeding ketosis – results because “the cow is not offered enough acceptable feed,” 2) secondary underfeeding ketosis – results because “the cow’s

voluntary feed intake is diminished by disease,” 3) alimentary or ketogenic ketosis – results because “the cow is consuming excessive amount of highly ketogenic feed,” and 4) spontaneous ketosis – results under a condition when the diet appears nutritionally adequate. More recently, reference to type I and type II ketosis has been made (Holtenius and Holtenius, 1996), which appears to be a much more functional differentiation in modern dairy production (Herdt, 2000a). Type I ketosis, also known as spontaneous ketosis, occurs when the demand for glucose is greater than the liver capacity for gluconeogenesis (Herdt, 2000a). This occurs when the supply of glucose precursors is limiting for maximal glucose production when the gluconeogenic pathways are at maximal capacity (Herdt, 2000a). During type I ketosis, concentrations of glucose and insulin in blood typically are low, the insulin to glucagon ratio low, concentrations of blood ketones are elevated, a high rate of mitochondrial uptake of NEFA exists, and severe fatty liver is absent (Herdt, 2000a).

Fatty liver is characteristically associated with so-called “type II ketosis”. Type II ketosis occurs when large amounts of NEFA are delivered to the liver when gluconeogenesis and ketogenesis are not maximally stimulated (Herdt, 2000a). Holtenius and Holtenius (1996) suggest that type II ketosis is associated with hyperglycemia, hyperinsulinemia prior to clinical signs, insulin resistance, lower concentrations of ketone bodies in blood, and higher concentrations of glucose in blood than in type I ketosis. This is the type of ketosis that results from overfeeding during the dry period and having overconditioned cows that are set up for liver function impairment due to their high depression of DMI around calving. Ketogenic grass silages that contain large amounts of butyric acid may be a contributing factor to fatty liver development as well as butyric acid (alimentary) ketosis.

The reported prevalence of ketosis varies among farms worldwide. Over the last 10 years, ketosis (particularly type II) has become the number one reason for farm consultations by the University of Wisconsin clinical veterinary staff (G. Oetzel and K. Nordlund, personal communication, 2007). Duffield (2000) reported that subclinical ketosis prevalence worldwide varied from 8.9% to 34% for cows during the first two months of lactation. Clinical ketosis prevalence was lower and varied from 2% to 15%. Both subclinical and clinical ketosis result in economic losses for dairy producers due to decreased milk yield and poorer reproductive performance. Cows with clinical ketosis had a loss of milk of 2.5 to 3.5 kg/d in the month following diagnosis, 0.3 to 0.7 kg/d during the following month, and no long-term loss of milk (Fourichon et al., 1999). Cows with clinical ketosis had more days to first service, a lower conception rate, and increased days open (Fourichon et al., 2000).

Primary ketosis can be either subclinical or clinical (Baird, 1982). Clinical signs include decreased appetite, decreased milk production, loss of BW, hypoglycemia, and hyperketonemia. Ketone bodies (BHBA and acetoacetate) are found in blood, milk, and urine. Subclinical ketosis is diagnosed at serum BHBA concentrations above 10.4 mg/dL (1000 μ mol/L) and clinical ketosis is diagnosed at BHBA concentrations above 27 mg/dL (2600 μ mol/L; Duffield, 2000). Duffield (2000) pointed out that the values used to set the threshold for subclinical ketosis are arbitrary and that clinical signs of ketosis can occur with a large range of serum BHBA concentrations.

Triggers for Clinical Disease

An age-old question and problem in the study of ketosis and fatty liver is why some cows eventually go on to develop signs and clinical pathology whereas others do not, despite roughly similar clinical biochemistry values (i.e., serum NEFA and BHBA concentrations, etc.). One of the most exciting recent areas of research is the study of gene expression in liver, using “microarray” techniques. These procedures allow quantification of the mRNA expression of thousands of gene sequences simultaneously on a microscope slide-sized glass plate. We have conducted several studies that have provided new insights into the molecular mechanisms of ketosis and fatty liver development, as well as the potential triggers for clinical disease (Loor et al., 2005, 2006, 2007).

Briefly, we have uncovered evidence for several potential disease-causing mechanisms in cows subjected to a feed restriction protocol to induce ketosis in early lactation (Dann et al., 2005; Loor et al., 2007). These cows had elevated BHBA concentrations in blood and increased TG in liver, typical of ketosis. The theme areas for which evidence was obtained for impaired liver cellular functions included: 1) down-regulation of genes responsive to oxidative stress (e.g., *SOD1*, *ATOX1*, *PRDS2*, *GLRX*, *GSTA4*, et al.), but increase of potential sources of oxidants (e.g., *ACOX1*, *UCPI*); 2) up-regulation of genes indicating enhanced DNA fragmentation and apoptosis; 3) blunted repair response to DNA damage; 4) down-regulation of components of oxidative phosphorylation, suggesting that the liver’s ability to generate ATP for its functions eventually may be impaired; 5) down-regulation of enzymes in cholesterol synthesis (e.g., *HMGCR*), which may impair VLDL export and contribute to greater TG accumulation; 6) down-regulation of growth hormone signaling mechanism (e.g., *GHR*, *IFG1*, *SOCS2*), which may disrupt normal homeostatic and homeorhetic controls; 7) down-regulation of ubiquinone synthesis and ubiquitination activity, which are involved in targeting damaged or aberrant proteins for degradation; and 8) down-regulation of components of the immune system.

Our working hypothesis in this line of investigation, then, is that as the pathophysiology of NEB continues and worsens, clinical disease eventually is triggered when accumulative cell damage in liver and other tissues prevents maintenance of homeostasis. Key functions that are compromised may include mitochondrial energy status, accumulation of aberrant proteins that cannot be properly cleared, and induction of apoptosis (programmed cell death).

Risk Factors for Fatty Liver and Ketosis

Several factors increase the likelihood of cows developing fatty livers and ketosis. Among these may be individual herd-specific factors, parity, body condition score, genetics, and season (Duffield, 2000; Bobe et al., 2004). As mentioned earlier, overconditioning is a clear risk factor. Cows with long calving intervals or long dry periods thus may be at greater risk. Even cows that are not overconditioned but are fed diets too rich in energy for long periods in the dry period are at greater risk to develop fatty livers after calving than are cows fed to meet their requirements during the dry period.

An interesting risk factor identified in recent studies is that DMI and feeding behavior during the last 2 weeks before calving are highly predictive of health problems in individual

cows (Urton et al., 2005; Hammon et al., 2006; Huzzey et al., 2007). In these studies, cows that were diagnosed with clinical metritis after parturition showed decreased feeding time and DMI before calving, when no metritis should have been present. Technology for measurement of feeding behavior in group settings is not yet commercially available. Farms on which individual cows can be assessed may be able to identify cows with less aggressive appetites before calving, which might be more likely to develop problems after calving.

A major risk area has been more clearly delineated in recent years: stressors in the animal's environment. These stressors may be heat stress, overcrowding, too frequent moves, poorly designed or maintained stalls, lack of water, or suboptimal feeding areas among others. These stressors can contribute to development of fatty liver and ketosis because they decrease DMI and signal breakdown of body fat at high rates around calving.

The importance of the general area known as "cow comfort" has emerged over the last two decades. The type of system (individual tie stalls vs. free stalls; pasture-based vs. confinement) may affect transition success, or may interact with nutrition to determine transition success (Cook and Nordlund, 2004). Within types of systems, the quality of facilities and their management becomes a huge source of difference among studies or farms implementing various nutritional programs (Grant and Albright, 1995). A large number of studies has been conducted in recent years addressing aspects of cow behavior and its interactions with feeding systems (Endres et al., 2005), feeding space (DeVries et al., 2004), stall design and size (Tucker et al., 2003, 2004); flooring surface (Tucker et al., 2006), bedding materials (Cook et al., 2004; Fregonesi et al., 2007), grouping size (Grant and Albright, 2001), and movements between groups too frequently or during the period from 3 – 9 days before calving (Cook and Nordlund, 2004). Lameness is a major factor that may be aggravated during the transition period and contribute to both metabolic disturbances and poor fertility (Cook and Nordlund, 2007). Together, these data indicate that non-nutritional aspects of dry period and transition management may be more important than diet amount or composition in determining occurrence of ketosis and fatty liver in many instances.

Diagnostic Indicators for Fatty Liver and Ketosis

Given that liver biopsy is more problematic procedure for routine diagnostic use, there is interest in using metabolites in blood as indicators of metabolic disturbance. Although responses to NEB largely are related to glucose supply and demand, the concentration of glucose in blood is regulated tightly by the cow and therefore glucose is an insensitive indicator of degree of NEB postpartum. In contrast, the concentration of NEFA is highly related to adipose tissue lipolysis, which increases in response to NEB. Concentrations of NEFA > 0.4 mM prepartum and >0.7 mM after the first week postpartum indicate problems with energy balance (Herdt, 2000b; Oetzel, 2004). Use of NEFA is a better indicator of energy imbalance prepartum than BHBA, but BHBA is more useful postpartum (Duffield, 2000).

Increased NEFA concentrations in the 2 weeks before calving are associated with 2 to 4 times increased risk of displaced abomasum (Cameron et al., 1998; LeBlanc et al., 2005), 2 times greater risk for retained placenta (Kaneene et al., 1997; LeBlanc et al., 2004), and 2 times greater risk of being culled before 60 days in milk (Duffield et al., 2005). Higher concentrations of

NEFA at 3 days postpartum (0.75 mM vs. 0.50 mM) were associated with decreased likelihood of pregnancy (Burkhart et al., 2005). Studies have shown that high prepartum NEFA also is associated with greater risk of ketosis (Drackley et al., 1992; Herdt, 2000b; Oetzel, 2004) and metritis (Kaneene et al., 1997; Hammon et al., 2006).

Measurement of BHBA before calving is of little use because concentrations rarely begin to increase appreciably until calving. The concentration of BHBA in blood postpartum is an effective indicator of NEB and metabolic disturbance, with concentrations $> 1.2 - 1.4$ mM indicating presence of subclinical ketosis (Duffield, 2000; Oetzel, 2004). Subclinical ketosis during the first 2 weeks postpartum has been associated with a 4 – 8 times greater risk for displaced abomasum (LeBlanc et al., 2005), decreased conception at first breeding (Walsh et al., 2007a,b), decreased milk production (Duffield, 2000), and increased duration and severity of mastitis (Suriyasathaporn et al., 2000).

As BHBA concentration increases in blood during ketosis, the concentration of the other ketone bodies (acetoacetate and acetone) also increase in blood, as does the decarboxylation product of BHBA, isopropanol (Andersson, 1984). The concentration of ketone bodies also increases in urine and milk, which makes it possible to qualitatively measure BHBA, acetoacetate, or acetone in milk or urine. A recent comparison of milk acetoacetate test powder, milk test strips for BHBA, and urine test strips for acetoacetate found that the milk powder had the lowest sensitivity, with the urine and milk test strips showing approximately the same sensitivity and specificity (Carrier et al., 2004). Use of urine or milk test strips is satisfactory for routine on-farm ketosis monitoring if used in a consistent manner, with samples being read at the same time after wetting the strip (usually 5 seconds).

Blood samples for NEFA and BHBA determination should be obtained from the tail vein or jugular vein, and not the mammary veins. Our recent studies have documented that either serum or plasma is acceptable for measurement of NEFA concentrations; either heparin or EDTA is acceptable as an anticoagulant (unpublished data). For herd-level diagnostics, 12 cows from the population at risk (cows from 3-14 d prepartum for NEFA; cows from 3-21 d postpartum for BHBA) should be sampled (Oetzel, 2004).

A number of acute-phase proteins induced (or suppressed) during inflammatory conditions may have merit as diagnostics of metabolic disturbance (Calamari et al., 1994; Cairoli et al., 2006). Haptoglobin is a good indicator of inflammatory disease (Crawford et al., 2005). The transition period has been associated with alterations in metabolic indicators of inflammation (Calamari et al., 1994; Ametaj et al., 2005), and the extent of inflammatory response may be related to both health problems and reproductive success (Bertoni et al., 1998). Recently, paraoxanase, another positive acute phase protein, was shown to be a potential indicator of health and inflammatory conditions (Bionaz et al., 2007). A combination or panel of metabolic indicators, resulting in an index, may offer greater utility but also increases diagnostic costs (Herdt, 2000b).

Nutrition to Prevent Fatty Liver and Ketosis

Nutrition is critically important to transition success and avoiding metabolic disorders. Over the last 20 years it has become common practice to increase energy density of diets during

the “close-up” or “pre-fresh” period by supplementing cereal grains. The preponderance of research that has examined the benefits of such rations has demonstrated little effect in decreasing disease or increasing postpartum performance. Recent studies suggest that feeding so that nutrient supply is constant over the dry period and approximately equal to requirements is the best practice (e.g., Holcomb et al., 2001; Agenäs et al., 2003; Dann et al., 2006; Janovick Guretzky et al., 2006). Where corn silage is the major forage, it must be diluted with lower-energy feeds such as cereal straw or grass hays.

Overconditioning is a major risk factor for disease and impaired reproduction, as these cows have poor appetites and extensive rates of body fat mobilization (Drackley et al., 2005). However, our recent research suggests that even over-feeding relative to requirements during the dry period predisposes cows to periparturient health problems, even if cows do not appear to be overconditioned (Dann et al., 2006; Douglas et al., 2006; Janovick Guretzky et al., 2006). Strategies by which energy intake can be limited include limit-feeding (Kunz et al., 1985; Holcomb et al., 2001; Douglas et al., 2006; Winkleman et al., 2007) and increasing bulky roughages (such as cereal straw) into the diet (Dann et al., 2006; Janovick Guretzky et al., 2006). These low-starch –high-NDF diets will limit total energy intake while allowing cows to consume feed for ad libitum intake. Proper processing of the straw or other bulky roughage is critical to prevent sorting (Drackley and Janovick Guretzky, 2007). This topic is addressed by the author in another article in this proceedings volume.

Some feed additives can be beneficial in helping cows through problem situations. Notable among these are monensin and rumen-protected choline, which has shown benefit in decreasing liver lipid accumulation (Piepenbrink and Overton, 2003) and increasing milk production (Pinotti et al., 2003), particularly in overconditioned cows (Zahra et al., 2006). Even the most abundant and important nutrient, water, can lead to metabolic disorders if it is not freely available or if waterers are contaminated. High-moisture grass silages (>70% moisture) are at great risk for clostridial fermentations that lead to butyric acid production, as well as various amines that may inhibit feed intake. Such feeds are best not fed to dry cows or fresh cows.

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