

# A New Understanding of the Causes of Fatty Liver in Dairy Cows

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## ■ Take Home Messages

- ▶ Fatty liver is an important economic disease because cows that develop fatty liver are affected by multiple metabolic and infectious diseases; they reduce milk production, and are frequently culled.
- ▶ We identified an inflammatory response in cows with fatty liver. This indicates the presence of an inflammatory agent in the blood of transition dairy cows. These new observations prompted us to suggest a novel contributing agent in development of fatty liver.
- ▶ Feeding cows high proportions of concentrate around parturition results in release of great amounts of endotoxin by rumen bacteria. Endotoxin is transferred into the blood where it stimulates immune defenses. Endotoxin also binds lipoproteins and endotoxin-lipoprotein complexes are rapidly removed from the blood by the liver, causing rapid accumulation of lipids and development of fatty liver.

## ■ What is Fatty Liver

Fatty liver is a metabolic disease of transition dairy cows affecting almost half of the herd immediately after calving (Jorritsma et al., 2000). Fatty liver is characterized by the build-up of fat (lipid) in the liver. The amount of fat accumulated in the liver in the first 10 days of calving ranges from 60 to 125 g per day (Ametaj et al., 2002). Lipids accumulated in the liver occupy from 12 to 25% of the liver wet weight. This gives the liver a yellowish color and a sponge-like appearance under the microscope. Fatty liver may lead to inflammation of the liver causing scarring and hardening of the liver and death of the cow as a consequence. Without treatment of the disease mortality can be as high as 25%.

## ■ **Diagnosis of Fatty Liver**

Fatty liver is usually diagnosed by taking a liver sample through biopsy and then measuring the amount of total lipids in the liver. Because liver biopsy is an invasive technique and can be associated with hemorrhage, infection, or death of the cow, researchers (including the author – Dr. Burim Ametaj) at Iowa State University have worked to develop a new technology using ultrasound images for non-invasive diagnosis of fatty. Results of a recent study (data unpublished) indicate a relationship between the amount of total lipids in the liver and the texture of the ultrasound images. The results will be used to develop software for diagnosis of fatty liver simply by taking liver images using the same ultrasound instrument that veterinarians use to diagnose pregnancy in dairy cows.

## ■ **Relation of Fatty Liver to Other Metabolic Diseases**

Even though there are no specific symptoms characterizing fatty liver, cows with fatty liver are the cows that start having problems from the beginning of lactation. Attention is usually drawn to cases of fatty liver as a result of the presence of other diseases. The incidence of diseases such as ketosis, milk fever, mastitis, metritis, displaced abomasum, and retained placenta is more frequent in cows with fatty liver (Markusfeld, 1985; Rukkwamsuk et al., 1999; Sweeney, et al., 1988; West et al., 1990). All these diseases have a tendency to be more severe and less responsive to treatment in those cows. Fatty liver cows are the milk fever cows that relapse and become downers, the ketotic cows that don't respond to treatment, the chronic mastitis cows, and the repeat breeders that defy all treatments. They often relapse or go from one disease to another, they reduce drastically milk production, and they are the cows that are frequently culled.

## ■ **The Conventional View on Fatty Liver**

The conventional view on the cause of fatty liver is the negative energy balance caused by a reduction in feed intake in the late dry period and early lactation. The drop in feed intake during this period can reach as high as 30% (Coppock et al., 1972). Initiation of lactation also imposes a high demand for energy. Cows go into a negative energy balance (NEB) immediately after calving if the energy requirements for milk production are not met. The energy deficiency causes cows to mobilize large quantities of fatty acids from fat deposits resulting in an increased concentration of non-esterified fatty acids (NEFA) in the blood (Herdt, 1988). The NEFA are used by the mammary gland as an energy source and are also incorporated into milk fat. They also are extracted by the liver from the circulation in proportion to their concentration in blood

(Herdt, 1988; Emery, 1992). In the liver, the NEFA are either used for energy production or synthesized into triacylglycerols (TAG). The TAG then are incorporated and released into blood in the form of very low density lipoproteins (VLDL) or stored in the liver (Grummer, 1993). The ruminant liver has an inherent inability to oxidize fatty acids or secrete TAG incorporated into VLDL (Herdt, 1988). Because mobilization and accumulation of fatty acids from adipose tissue is greater than the capacity of the liver to secrete or burn fatty acids, TAG accumulate in the liver and fatty liver develops.

### ■ Does NEB Fully Explain the Cause of Fatty Liver?

The proposed role of NEB in the development of fatty liver is plausible; however, it leaves several observations unanswered:

- Negative energy balance develops in all cows around parturition; however, only half of the cows are affected by fatty liver. What makes the other half resistant to fatty liver?
- Fatty liver is observed in different metabolic diseases such as ketosis, displaced abomasum, milk fever, retained placenta, infertility, downer syndrome, mastitis, and metritis. Diseases such as mastitis, metritis, and milk fever are not related to NEB. So, what is the cause of fatty liver in those diseases?
- Feeding diets with greater energy content ( $>1.65$  Mcal of  $NE_L$ /kg DM) during the far-off dry period is associated with a higher incidence of fatty liver. Attempts to provide more energy to cows in NEB do not seem to prevent fatty liver; on the contrary, increase the incidence of fatty liver.

All these observations prompted us to search for additional contributing agents with regard to the development of fatty liver.

### ■ Attempts to Find a Common Causal Agent of Fatty Liver

Because fatty liver is a common finding in so many different metabolic diseases scientists have made attempts to identify a common causal agent for the development of fatty liver in the aforementioned diseases. Katoh (2002) suggests that disturbances of lipoprotein metabolism are the main causal factor of fatty liver. He considers metabolic diseases of transition cows as fatty liver-related diseases or diseases that are derived from development of fatty liver. He proposes that disturbances of the metabolism of proteins produced by the liver such as apolipoprotein (apo) B-100, apo A-I, apo C-III as well as acute phase proteins such as haptoglobin and serum amyloid A are the main

disturbed blood variables that determine development of fatty liver and liver-related diseases. Katoh (2002) proposes that accumulation of fat in the liver hampers production of all these proteins and therefore compromises export of fat out of the liver. He also supports the role of NEB as the initial stimulus for activation of a cascade of responses leading to development of fatty liver and fatty liver-related diseases.

## ■ New Observations

Based on the observation that fatty liver is associated with a high incidence of infectious diseases such as mastitis, metritis, and complicated lameness the author and his colleagues at Iowa State University (Ametaj et al., 2002) tested the hypothesis that activation of the immune system might exacerbate fatty liver. Fatty liver was induced in Holstein cows by overfeeding 5 kg of cracked corn on top of a TMR (3 kg of cracked corn already contained in the TMR) during the last month of gestation. Blood and liver samples were obtained at 4 d before the expected day of calving as well as on days 3, 8, 12, 14, 22, 27, and 36 after calving. Liver samples were analyzed for the amount of total lipids, whereas blood samples were analyzed for concentrations of tumor necrosis factor-alpha (TNF-alpha), serum amyloid A (SAA), haptoglobin, calcitonin gene-related peptide (CGRP), TAG, glucose, and total cholesterol.

Results of this study (Ametaj et al., 2002) showed that cows that developed fatty liver had an average of 10.9, 11.2, and 11.4% (wet weight) total lipids in their liver on days 3, 8 and 12 after calving (Figure 1A). Results also indicated that cows with fatty liver produced less milk during the first 6 weeks of lactation (8-9 kg less milk during week 5 and 6 of lactation than control cows) (Figure 1B). They also had lower body condition score (Figure 2A) and DMI (kg/day) (Figure 2B) compared to control cows. Fatty liver cows had higher concentrations of TNF-alpha (Figure 3A) before calving compared with cows that did not develop fatty liver. More remarkable was the strong correlation between concentration of TNF-alpha in blood at 4 d before calving (correlation coefficient ( $r$ ) = 0.96) and the amount of total lipids in the liver. Also, plasma concentrations of the two main acute phase proteins, SAA and haptoglobin, were greater in cows with fatty liver immediately after parturition (Figure 3B, Figure 4A). Fatty liver cows also had higher NEFA (Figure 5A) and lower CGRP (Figure 4B), total cholesterol and glucose (Figure 6A, Figure 6B) in plasma compared to control cows. Concentration of TAG in plasma dropped sharply in both groups immediately after parturition (Figure 5B), whereas those of total cholesterol increased steadily in both groups after calving (Figure 6B). Plasma total cholesterol was lower in fatty liver cows than control cows.

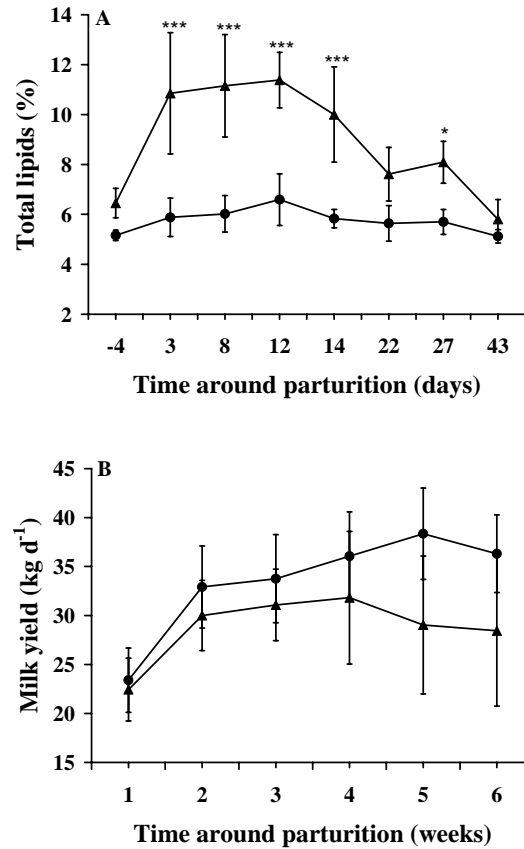


Figure 1. Liver lipids and milk yield. A. Liver total lipids (% wet weight basis) before and after calving (days -4, 3, 8, 12, 14, 22, 27, and 36), B. Milk yield (kg/day) during week 1 to 6 after calving in control (●) and fatty liver (▲) cows (n = 4 in each group; \* $P < 0.1$ , \*\*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.01$ ).

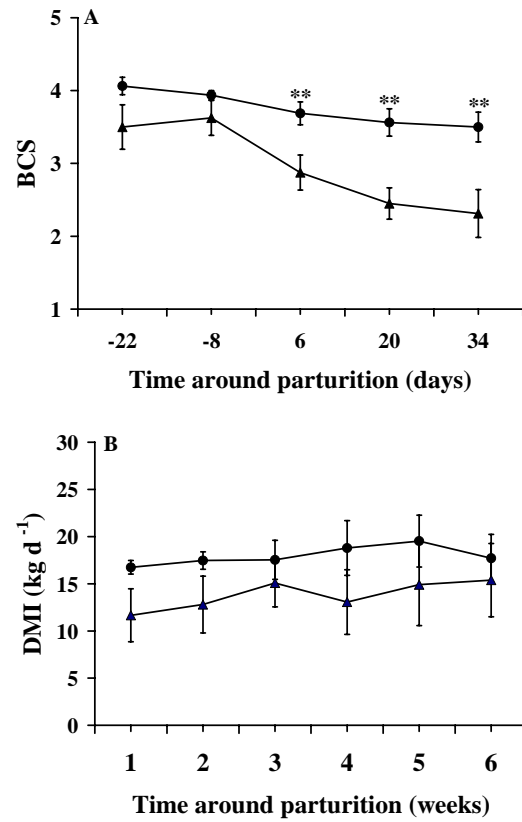
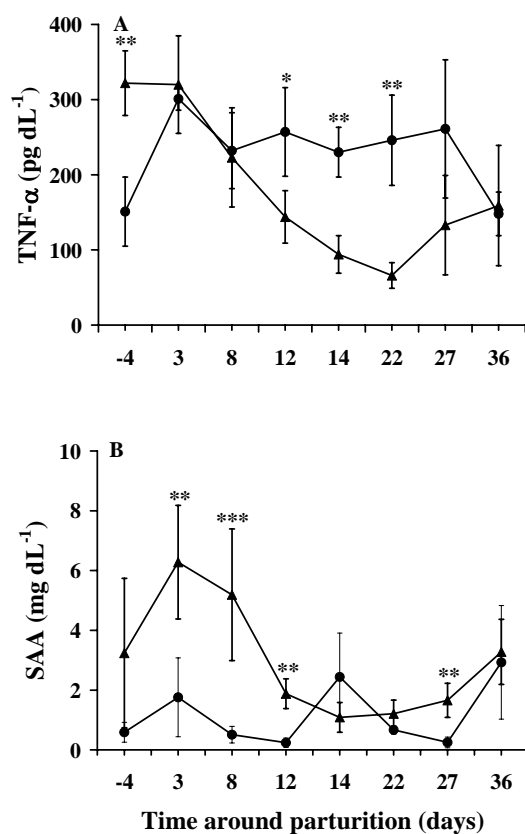


Figure 2. Body condition score and feed intake. A. Body condition score (BCS) at days 22 and 8 before calving as well as at days 6, 20, and 34 after calving, and B. DMI (kg/day) during week 1 to 6 in control (●) and fatty liver (▲) cows (n = 4 in each group; \* $P < 0.1$ , \*\*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.01$ ).



**Figure 3. Plasma acute phase proteins. A. Tumor necrosis factor-alpha (TNF- $\alpha$ ) (pg/dL), B. Serum amyloid A (SAA) (mg/dL), before and after parturition (days -4, 3, 8, 12, 14, 22, 27, and 36) in normal (●) and fatty liver (▲) cows (n = 4 in each group; \* $P < 0.1$ , \*\*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.01$ ).**

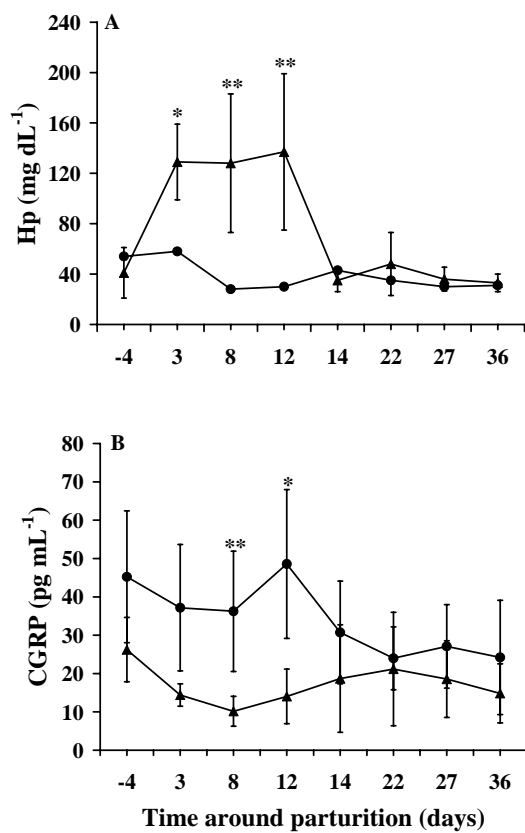
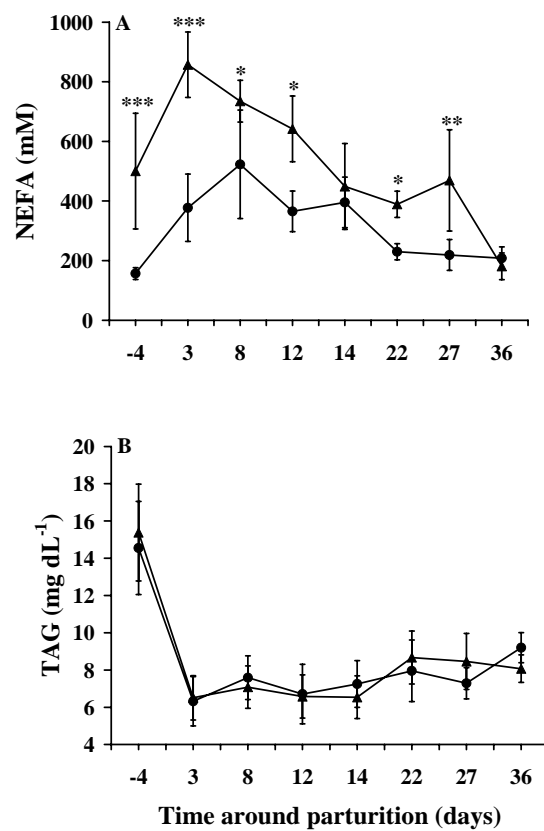
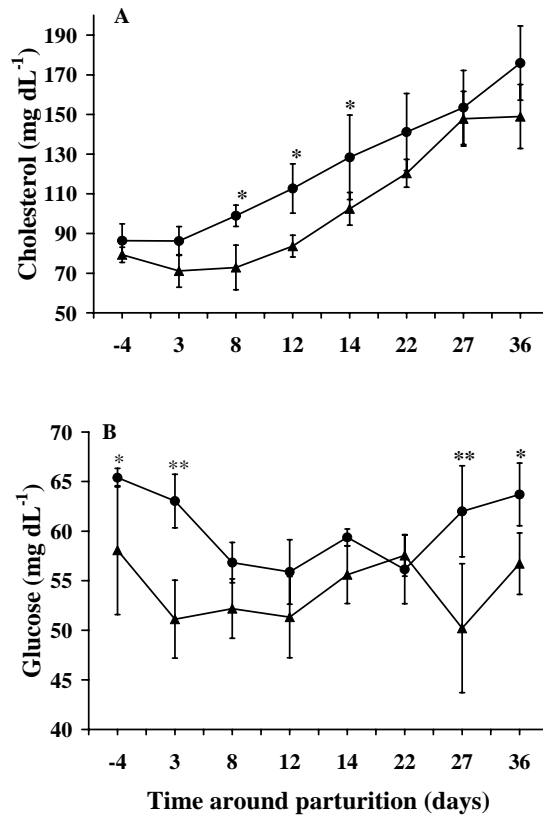


Figure 4. Plasma acute phase proteins. A. Haptoglobin (Hp) (mg/dL), B. Calcitonin gene-related peptide (CGRP) (pg/mL) before and after parturition (days -4, 3, 8, 12, 14, 22, 27, and 36) in normal (●) and fatty liver (▲) cows (n = 4 in each group; \* $P < 0.1$ , \*\*  $P \leq 0.05$ ; \*\*\*  $P \leq 0.01$ ).





**Figure 5. Plasma lipids. Concentration of A. Non-esterified fatty acids (NEFA) (mM), B. Triacylglycerols (TAG) (mg/dL), before (-4 days) and after parturition (days 3, 8, 12, 14, 22, 27, and 36) in normal (●) and fatty liver (▲) cows (n = 4 in each group; \*P < 0.1, \*\* P < 0.05, \*\*\* P ≤ 0.01).**



**Figure 6. Plasma cholesterol and glucose. A. Total cholesterol (mg/dL), and B. Glucose (mg/dL) before (-4 days) and after parturition (days 3, 8, 12, 14, 22, 27, and 36) in normal (●) and fatty liver (▲) cows (n = 4 in each group; \* $P < 0.1$ , \*\*  $P < 0.05$ , \*\*\*  $P \leq 0.01$ ).**

## ■ Inflammatory Conditions and Acute Phase Response

Acute phase response is a general non-specific immune response of the host to different stimuli such as infection, tissue injury, and trauma. The role of acute phase response is to isolate and neutralize pathogens and prevent further pathogen entry, to minimize tissue damage and promote repair processes as well as to restore normal physiological function (Kushner, 1982). The host response to pathogens or injury starts through activation of tissue (e.g., liver) macrophages at the site of the inflammatory stimulus. Activated macrophages release a variety of products known as cytokines, the most important of which

are TNF- $\alpha$ , interleukin-1 (IL-1), and IL-6. Among other functions, TNF- $\alpha$ , IL-1, and IL-6 promote production of a variety of proteins in liver such as SAA and haptoglobin (Edbrooke et al., 1991; Kushner, 1988). Serum amyloid A has been reported to increase in the plasma of dairy cows immediately after calving as well as after intravenous administration of endotoxin (Alsemgeest et al., 1993; Werling et al., 1996). Serum amyloid A associates with plasma high density lipoproteins (HDL) during the acute phase response (Hoffman and Benditt, 1982). Haptoglobin is another acute phase protein produced by the liver and has been reported to be high in cows with mastitis, metritis, and other inflammatory conditions (Hirvonen et al., 1996, 1999). The main function of haptoglobin is to bind hemoglobin released in plasma from red blood cells during hemolytic conditions.

Calcitonin gene-related peptide (CGRP) is another protein reported to increase in plasma during the acute phase response (Joyce et al., 1990). Administration of CGRP to rodents is associated with increased plasma glucose (Yamaguchi et al., 1990). The acute phase response also is associated with changes in lipid metabolism. For example, concentrations of cholesterol in plasma decrease in humans and rodents during inflammatory conditions.

## ■ Interpretation of New Findings: An Additional Contributing Agent of Fatty Liver

### A Potential Role for High Grain Diets in Development of Fatty Liver

Dairy cows as ruminants have evolved with the ability to digest roughage. By continuous genetic improvements they have been selected to achieve high milk production, which in turn requires high intake of dry matter very rich in energy. The main sources of energy for dairy cows are concentrates (i.e., grains). On the other hand, the ruminant digestive system is not developed to digest high amounts of grain (starch). Several epidemiological studies have shown a strong correlation between the amount of concentrate fed and the occurrence of acidosis, fatty liver, ketosis, left displaced abomasum, and laminitis in dairy cows (Coppock, 1972; Dougherty, 1976; Nagaraja, 1978c).

### A Challenging and Still Unanswered Question in the Dairy Industry Is How High Proportions of Grain Cause Metabolic Disorders in Early Lactation Cows.

Grains are feedstuffs that are very rich in starch. Feeding rations high in starch is associated with a shift of rumen bacterial populations from cellulolytic (cellulose-digesting) dominating species of bacteria to amylolytic (starch-digesting) dominating species of bacteria (Dehorty, 2003). Most of the known

starch digestors are Gram-negative bacteria (Dehorty, 2003). Thus, as a result of feeding high levels of concentrates to support high milk production the growth and multiplication of these bacteria is enhanced in the rumen. It has been demonstrated that these population changes are associated with an increase of up to 20-fold in the amount of lipopolysaccharides (endotoxin) in the rumen (Nagaraja et al., 1978a, 1978b; Andersen et al., 1994). Endotoxin is a constituent of the membrane of all Gram-negative bacteria. Endotoxin is believed by several investigators to play a role in development of grain-related metabolic diseases such as fatty liver, left displaced abomasum, and laminitis (Dougherty et al., 1975; Aiumlamai et al., 1992; Andersen et al., 1994). However, the precise role of endotoxin in these important disorders of dairy cattle has not yet been determined.

### **Negative Energy Balance Around Calving Is Related to Presence of Endotoxin in Blood.**

We fed cows high proportions of grain before calving and we found that concentrations of TNF-alpha in plasma were greater in the blood of cows with fatty liver the week before parturition. TNF-alpha is produced by liver macrophages in response to the presence of endotoxin in blood. Administration of TNF-alpha in cows also is associated with decreased appetite and feed intake as well as with increased release of NEFA into blood from fat deposits (Kushibiki et al., 2000). Indeed, cows with fatty liver had lower DMI compared to normal cows and greater NEFA concentrations. Therefore, the state of low appetite and decreased feed intake in cows (NEB) with fatty liver around parturition might be related to transfer of endotoxin in blood. Cows with fatty liver also had lower milk production and lower body condition score compared to normal cows. All together these results indicate that the decreased appetite and NEB established around parturition might be a consequence of feeding diets with high proportions of grain to dairy cows around parturition and transfer of endotoxin into blood.

### **Endotoxin, an Additional Contributing Agent to Development of Fatty Liver**

Endotoxins released by Gram-negative bacteria in the rumen enter into blood and are cleared by liver macrophages. Activated macrophages release a variety of products such as TNF-alpha, IL-1, and IL-6. The latter stimulate liver hepatocytes to release acute phase proteins such as SAA and haptoglobin as well as NEFA from fat deposits. The host also activates a second line of defense to clean endotoxin from circulation, lipoproteins. Endotoxin is bound and neutralized by SAA, which is associated with plasma lipoproteins (mostly HDL). Endotoxin-SAA-lipoprotein complexes are removed rapidly from circulation by liver cells. Other lipoproteins such as chylomicrons, VLDL and LDL also bind and neutralize endotoxin and are removed from circulation by the

liver (Harris et al., 2002). Rapid removal of lipoproteins, which are rich in lipids, increases the amount of fat taken by the liver contributing to development of fatty liver.

We found higher haptoglobin in cows with fatty liver (Ametaj et al., 2002). Increased production of haptoglobin by the liver is important in the host's fight against infections. Increased production of haptoglobin has two main functions. First, haptoglobin has antibacterial capability (Wassell, 2000); second, haptoglobin binds hemoglobin and prevents iron utilization from bacteria (Wassell, 2000). Presence of endotoxin in blood is known to increase bacterial translocation into circulation. Bacteria need essential nutrients such as iron for their growth and multiplication; however, during activation of immune response the host decreases iron availability.

We also measured CGRP for the first time in dairy cows around calving (Ametaj et al., 2002). This acute phase protein, released from the liver (Russwurm, 2001), is important in stimulating the release of glucose from liver stores and in protecting cows from endotoxin challenge. Infusion of glucose intravenously in rodents challenged with endotoxin improved their survival (Hinshaw et al. 1974). However, concentrations of this protein in plasma were lower in cows with fatty liver and so were concentrations of glucose indicating suppression of production of this important protein (Ametaj et al., 2002).

Even though plasma cholesterol increased in both groups of cows after calving, concentrations were lower in cows with fatty liver than normal cows (Ametaj et al., 2002). Cholesterol is the precursor to production of bile acids. Bile acids, which are secreted by the liver into the small intestine and have detergent like capabilities, play a pivotal role in digestion of lipids and thus degradation of endotoxin, which is a lipid-containing compound (lipopolysaccharide).

All together our results indicate activation of acute phase response in cows with fatty liver and suggest that mediators of acute phase response may be involved in development of fatty liver in transition dairy cows.

## ■ Conclusions

Recent observations indicate activation of immune system in cows with fatty liver. Based on those observations the author (Dr. Burim Ametaj) proposes a new contributing agent in development of fatty liver, endotoxin. Feeding high proportions of grain around parturition is associated with the release of great amounts (>20-fold) of endotoxin in the rumen. Endotoxin absorbed into circulation is removed from the blood by liver macrophages; however, a second line of defense involving plasma lipoproteins is also activated by the host. Even though lipoproteins are mostly known for their function as lipid-carriers, they also bind and neutralize endotoxin. The new hypothesis proposes that fatty liver

is exacerbated during activation of immune responses because of the rapid removal of endotoxin-lipoprotein complexes by the liver resulting in accumulation of great amounts of lipids in the liver.

### **Future Implications**

If endotoxin released in the rumen during feeding of high proportions of concentrates around parturition proves to play a significant role in the development of fatty liver in dairy cows then efforts for prevention of fatty liver should focus on establishing the optimal amount of concentrate to feed cows around calving to (1) minimize endotoxin production in the rumen and (2) to minimize absorption of endotoxin from the gastrointestinal tract.

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