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Influence of Inflammation on Metabolism in Transition Cows

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SUMMARY

- Transition dairy cows are of concern because of high disease incidence, both infectious and metabolic.
- Inflammation occurs during infections like mastitis and metritis, but may also be involved in disorders such as fatty liver and ketosis. All of these conditions lead to decreased productivity and productive life of dairy cattle.
- Oxidative stress, cytokines, and acute phase proteins are involved in inflammatory reactions and are proposed to promote metabolic disorders.
- Potential interventions to prevent metabolic inflammation are antioxidants, metabolic modifiers, and non-steroidal anti-inflammatory drugs.

INTRODUCTION

The multitude of disorders that dairy cows face during the transition to lactation is a perennial source of concern for dairy producers, nutritionists, and veterinarians. Total disease incidence in the several weeks after parturition accounts for a substantial proportion of all morbidity on many dairies (Ingvartsen, 2006), with particularly high rates of mastitis, metritis, milk fever, displaced abomasum, ketosis, and fatty liver, among other problems. Not surprisingly, these issues have been the focus of much research in recent decades. During that time, substantial progress has been made in some areas (e.g. milk fever); however, incidence of other disorders (e.g. displaced abomasum) may be on the rise (Goff, 2006).

It is well-documented that cows suffering from one transition disorder are at greater risk for contracting others, including such seemingly unrelated conditions as mastitis and ketosis (Goff, 2006). The transition from gestation to lactation dramatically increases requirements for energy, glucose, amino acids, and other nutrients in dairy cattle. Simultaneously, feed intake is often depressed. The resulting negative energy balance suppresses immune function and promotes
metabolic disorders, potentially explaining relationships between infectious and non-infectious transition disorders.

The most widely adopted practice to avoid metabolic disorders is the nutritional management of prepartum cows to prevent excess body condition. By limiting the pool of stored fat available for mobilization, restricting energy intake during the far-off dry period limits the increase in plasma non-esterified fatty acid (NEFA) concentrations during the transition period, resulting in lower fat storage and ketone production in the liver (Murondoti et al., 2004, NRC, 2001). However, results of controlled trials have been inconsistent with regard to nutritional management of dry cows; some studies have demonstrated a benefit from increased prepartum energy intake when body condition was not affected (Doepel et al., 2002), whereas restricting intake, even without affecting body condition, led to more favorable outcomes in other studies (Holcomb et al., 2001). These inconsistencies suggest that our understanding of metabolic disorders remains incomplete.

Recent research has highlighted the role of inflammation in infectious diseases and has suggested that inflammation is involved in metabolic diseases as well. A key role for inflammation in numerous transition cow disorders may help to explain links between these diverse conditions, and may also improve our ability to predict and prevent metabolic problems in transition cows. The aim of this article is to review findings relating to the role of inflammation in transition disorders and provide recommendations to smooth the transition to lactation.

**INFLAMMATORY RESPONSES TO INFECTION**

During infections such as mastitis or metritis, immune cells in the body recognize invading pathogens and become activated. When the infection is caused by Gram-negative bacteria, endotoxin released by the bacteria also activates immune cells. The activation of local and systemic host defense mechanisms requires cross-talk between numerous types of immune cells. One component of this response is inflammation. The host of signaling molecules released by activated immune cells includes inflammatory mediators such as nitric oxide, prostaglandins, and cytokines. While many of these molecules promote local inflammation and increased blood flow to the infected tissue, inflammatory cytokines play a key role in stimulating systemic inflammatory responses, including increased body temperature, increased heart rate, and decreased feed intake (Dantzer and Kelley, 2007). Cytokines are able to alter many physiological systems because nearly all cell types express cytokine receptors. Key inflammatory cytokines include tumor necrosis factor alpha (TNFα), interleukin 1β, and interleukin 6; these inflammatory cytokines act through many of the same signaling cascades and often produce similar responses in cells.

One effect of cytokines is to activate production of acute phase proteins. Primarily produced by the liver, this class of proteins includes haptoglobin, serum amyloid A, and C-reactive protein. Proteins that participate in the acute phase response to infection are generally found in very low abundance in the bloodstream, but are greatly elevated during systemic activation of the immune system. The importance of acute phase proteins in the response to infection is somewhat unclear, but they have gained widespread acceptance as markers of inflammation (Petersen et
al., 2004). Other proteins are known as negative acute phase proteins because their concentrations decline dramatically during the acute phase response.

It is clear that mammary and uterine infections result in both local and systemic inflammation. Coliform mastitis results in release of endotoxin into the bloodstream and increased plasma concentrations of cytokines and acute phase proteins (Hoeben et al., 2000). Likewise, metritis is associated with an acute phase response in transition cows (Huzzey et al., 2009); in fact, plasma haptoglobin is elevated prior to clinical signs of metritis.

**IS THERE A ROLE FOR INFLAMMATION IN METABOLIC DISORDERS AS WELL?**

Inflammation has been proposed as a missing link in the pathology of metabolic disorders in transition cows (Drackley, 1999). The metabolic effects of acute systemic inflammation include adipose tissue mobilization, breakdown of liver glycogen, and liver triglyceride accumulation, all of which occur during the transition period. More specifically, cytokines promote the breakdown of fat stores through decreased feed intake (Kushibiki et al., 2003), impaired insulin sensitivity, and direct stimulation of lipolysis (Kushibiki et al., 2001). All of these conditions are associated with ketosis and fatty liver in dairy cattle (Ingvarsen, 2006). Even more intriguing is the evidence that TNFα decreases liver glucose production (Kettelhut et al., 1987) and promotes triglyceride accumulation once mobilized NEFA reach the liver (García-Ruiz et al., 2006). The direct effects of cytokines on liver metabolism may play a key role in promoting metabolic disorders in transition cows, especially those already combating infectious disorders or with excessive body condition.

With the physiological stress associated with calving and the risk for infection that accompanies both calving and the initiation of lactation, immune responses are common during the transition period. Abrupt dietary shifts during the transition period can also contribute to systemic inflammation. Cows are generally fed diets with greater energy density at the onset of lactation, and if this change is too dramatic, it can result in ruminal production of endotoxin and subsequent transfer of endotoxin into the bloodstream (Khafipour et al., 2009). Furthermore, monocytes are known to become more responsive to inflammatory stimulants during the transition period, resulting in greater secretion of inflammatory cytokines when stimulated (Sordillo et al., 1995). Mastitis, metritis, and acute acidosis can therefore result in systemic inflammation, elevated cytokine concentrations, and altered liver metabolism.

Recent findings have supported previous speculation regarding the relationships between inflammatory mediators and metabolic disorders. Plasma concentrations of a number of inflammatory markers were increased in cows that developed fatty liver (Ametaj et al., 2005), and Ohtsuka and colleagues (2001) observed increased serum TNFα activity in cows with moderate to severe fatty liver. A retrospective study of cows on 3 commercial Italian dairies suggested that liver inflammation is associated with a problematic transition to lactation (Bertoni et al., 2008). Cows were classified in quartiles for degree of liver inflammation based on plasma concentrations of acute phase proteins. Those cows with the strongest inflammatory profiles were at 8-fold greater risk for experiencing one or more transition disorders, had lower
plasma calcium concentrations, took longer to re-breed, and produced less milk in the first month of lactation (Bertoni et al., 2008).

Even stronger evidence has emerged from 2 recent studies where inflammatory mediators directly induced metabolic problems. Trevisi and colleagues (2009) orally administered interferon-α (a cytokine) daily during the final 2 weeks of gestation, which caused liver inflammation and release of acute phase proteins. Compared to control cows, treated cows had significantly higher plasma ketone concentrations in the first 2 weeks after calving. Our own lab recently reported that subcutaneous injection of TNFα for 7 days doubled the amount of triglyceride in the livers of late-lactation dairy cows (Bradford et al., 2009). These results strongly support the hypothesis that inflammation disrupts normal metabolism, because although both treatments were considered low-dose and short-term, they nevertheless promoted ketosis and fatty liver.

Beyond direct effects on ketosis and fatty liver, inflammation may also impair glucose production. Endotoxin-induced mastitis was shown to alter expression of metabolic genes in the liver, including decreased expression of genes important for glucose production (Jiang et al., 2008). Our TNFα injection protocol also decreased expression of several of the same glucose synthesis genes (Bradford et al., 2009). In lactating cows, impaired glucose production would likely lead to increased adipose tissue breakdown, elevated plasma NEFA, and increased ketone production by the liver.

**RELATIONSHIPS BETWEEN OXIDATIVE STRESS AND INFLAMMATION**

Although the importance of inflammation in transition disorders is becoming clear, the pathways that cause this inflammation are less clear. Infections certainly initiate the process in some cows, but this is not likely the cause of metabolic disorders in all cows. In particular, the dramatically higher incidence of transition disorders in cows with excessive body condition (Morrow, 1976) is difficult to attribute exclusively to infections.

In addition to acute inflammatory events, chronic low-grade inflammation may play a role in transition disorders. In the early 1990’s, it was discovered that adipose tissue is capable of producing inflammatory cytokines such as TNFα (Hotamisligil et al., 1993). With the extensive list of “adipokines” discovered in the ensuing 15 years, human metabolic disorders are increasingly being viewed as products of low-grade adipose tissue inflammation induced by obesity. Adipose tissue is now recognized as an important source of circulating TNFα, and plasma TNFα concentrations are increased in obese individuals in a number of species, including sheep (Daniel et al., 2003). Based on these findings, infection is no longer a required component of an inflammation-based etiology for metabolic disorders in the transition period. Low-grade inflammation associated with obesity may help to explain “fat cow syndrome” (Morrow, 1976).

Lipid peroxides are also emerging as likely mediators linking plasma lipids to inflammation (Pessayre et al., 2004). Lipid peroxides are produced when intracellular lipids encounter reactive oxygen species (ROS) such as hydrogen peroxide. Some ROS are always produced in the liver; however, events occurring in early lactation likely contribute to enhanced ROS
production. One adaptation to increasing delivery of NEFA to the liver in early lactation is an increase in the capacity of peroxisomal oxidation (Grum et al., 1996), an alternative pathway for fatty acid oxidation. Enhanced peroxisomal oxidation increases total oxidative capacity of the cell, but the first step in this pathway produces hydrogen peroxide rather than NADH (Drackley, 1999), and therefore it contributes to ROS production to a greater extent than mitochondrial oxidation.

Increased ROS production in early lactation cows, coupled with increased NEFA concentration, increases lipid peroxide formation; both the transition to lactation and high body condition are associated with increased plasma markers of lipid peroxidation (Bernabucci et al., 2005). Lipid peroxides activate inflammatory cascades, which in turn alter nutrient metabolism (Pessayre et al., 2004). In addition, ROS are especially harmful to immune cells and can decrease the ability of the immune system to respond to infections (Spears and Weiss, 2008).

In summary, a new model is emerging to explain the development of numerous transition disorders. A combination of insults, including infection, chronic inflammation in obese cows, and lipid peroxide formation, promotes systemic inflammation during the transition period. Inflammation impairs immune function, making cows more susceptible to infectious disorders, and causes maladaptive shifts in metabolism, increasing the risk of metabolic disorders.

**POTENTIAL INTERVENTIONS**

An inflammation-based understanding of transition disorders opens the door for novel strategies to address these problems. The complex interactions of oxidative stress, inflammatory cascades, and metabolic pathways allow for a broad array of potential treatments to prevent transition disorders, including antioxidants, metabolic modifiers, and anti-inflammatory drugs.

**Antioxidants**

Dietary antioxidants, notably vitamin E and selenium, are important for their ability to contribute to ROS neutralization, thereby impeding the progression toward inflammation. Interestingly, plasma concentrations of α-tocopherol (vitamin E) decrease through the transition period (Weiss et al., 1990), and low antioxidant status is associated with transition cow disorders (LeBlanc et al., 2004, Mudron et al., 1997). Supplementing vitamin E prepartum improves antioxidant status (Weiss et al., 1990). Given the importance of antioxidants in modulating inflammation, it is not surprising that multiple studies have shown that supplementing vitamin E in excess of traditional recommendations decreases the incidence and severity of clinical mastitis (Smith et al., 1984, Weiss et al., 1990). Recently, a meta-analysis showed that supplemental vitamin E is also effective at preventing retained placenta (Bourne et al., 2007).

Low plasma vitamin E concentrations are associated with increased incidence of fatty liver and displaced abomasum (Mudron et al., 1997). Surprisingly, no published studies have evaluated the effects of supplemental vitamin E on liver metabolism or incidence of metabolic disorders. Given that supplemental vitamin E can decrease inflammatory cytokine production (Poynter and Daynes, 1998) and improve liver antioxidant status in mice with fatty liver (Soltys et al., 2001),
supplemental vitamin E may improve liver function in transition cows. With its demonstrated effects on immune function and its potential to benefit liver function, it is recommended that vitamin E be supplemented at a rate of at least 1,500 IU/day for close-up dry cows.

Selenium is the other antioxidant of greatest importance in dairy rations. Although responses to selenium are most dramatic when vitamin E status is marginal, selenium has unique roles in ROS neutralization and must be considered independently to achieve optimal health. The FDA restricts selenium supplementation in dairy rations to 0.3 ppm, and most farms supplement at that level, limiting the attention paid to selenium in transition health strategies. Feeding selenium yeast rather than inorganic selenium sources is a common and effective means of increasing selenium status of animals that already receive the legal limit of selenium (Salman et al., 2009). However, most evidence suggests a threshold response to selenium; once a minimum plasma concentration is reached, there may be no benefit of further increases, and in many cases, that threshold seems to be reached with inorganic selenium sources (Spears and Weiss, 2008). Nevertheless, the use of organic selenium may be worth considering in areas with selenium-deficient soils.

Beta carotene, a precursor of vitamin A, can also function as an antioxidant (Spears and Weiss, 2008), and concentrations of both vitamin A and β-carotene typically decrease during the transition period (LeBlanc et al., 2004). Although supplementing vitamin A at concentrations above current recommendations has improved udder health in some studies (NRC, 2001), in a head-to-head comparison, supplementation of β-carotene during the transition period significantly decreased incidence of both metritis and retained placenta compared to vitamin A supplementation (Michal et al., 1994). Cows fed 600 mg/day of β-carotene had equivalent plasma retinol concentrations to those supplemented with 120,000 IU/day of vitamin A (Michal et al., 1994). Replacing vitamin A supplements in transition rations with relatively high concentrations of β-carotene may be beneficial for transition cow health.

Metabolic Modifiers

Agonists for peroxisome proliferator-activated receptors (PPAR) can improve liver metabolism through several mechanisms. PPARγ agonists (primarily targeting peripheral organs) can decrease plasma NEFA concentration, whereas those targeting PPARα (the primary isoform in liver) promote fatty acid oxidation in liver, limiting triglyceride accumulation and production of lipid peroxides (Kota et al., 2005). One PPARγ agonist (2,4-thiazolidinedione) has been evaluated in transition cows, with positive effects on metabolic health and reproductive performance (Smith et al., 2007; Smith et al., 2009). Unfortunately, PPAR agonists are unlikely to be approved for use on dairies in the near future.

Choline is a nutrient that may limit oxidative stress, although like PPAR agonists, it does not directly neutralize ROS. Rather, choline likely limits lipid peroxide formation by decreasing plasma NEFA concentration and promoting clearance of triglycerides from the liver (Cooke et al., 2007). As a result, supplemental rumen-protected choline has been shown to increase plasma α-tocopherol concentration during the transition period (Pinotti et al., 2003), presumably contributing to immune function and modulation of inflammation.
Anti-Inflammatory Agents

Direct inhibition of inflammation through the use of non-steroidal anti-inflammatory drugs (NSAIDs) has shown promise for treatment of metabolic disorders in laboratory animals. Indomethacin prevented hypoglycemia after administration of inflammatory cytokines (Kettelhut et al., 1987) and mice with induced fatty liver had decreased liver triglyceride content when treated with two NSAIDs (Yu et al., 2006), among other findings. Low blood glucose and fatty liver are related problems that many early lactation cows face, and these findings in rodents suggest that NSAIDs may be useful in transition cows, as well.

Non-steroidal anti-inflammatory drugs are classified into 5 broad subclasses; salicylic acid and its derivatives, propionic acid and derivatives, pyrazole derivatives, aniline derivatives, and oxicam derivatives (Gallo et al., 2008). Each class functions slightly differently. The drugs discussed in this paper are classified as followed: meloxicam (oxicam), flunixin (aniline), carprofen and ketoprofen (propionic acid), and salicylate (salicylic acid). Since each subclass has a slightly different mode of action, they likely offer different potencies for combating acute and chronic inflammation. Not surprisingly, different results have been observed in regard to transition disorders.

Several NSAIDs in the propionic acid and oxicam subclasses have been used effectively in treating mastitis. In one study, carprofen had limited ability to suppress inflammation, but was shown to partially alleviate the decrease in ruminal contractions during mastitis (Vangroenweghe et al., 2005), which could help prevent a subsequent displaced abomasum. Ketoprofen, a similar compound, promoted ruminal contractions and was also effective at decreasing inflammatory responses to mastitis (Banting et al., 2008). In a recent report, meloxicam treatment lowered somatic cell count and a reduced the number of cows removed (culled) from the herd after mastitis (McDougall et al., 2009). To our knowledge, these classes of NSAIDs have not been tested for prevention or treatment of metabolic disorders.

Flunixin meglumine, on the other hand, has been evaluated for use in transition cows. One study indicated that uterine involution was accelerated by flunixin meglumine treatment for metritis (Amiridis et al., 2001), but another showed no beneficial effects, either systemically or in the reproductive tract (Drillich et al., 2007). Two more recent publications are especially interesting because they involved treatment of transition cows prior to any disease diagnosis to assess whether flunixin might prevent disorders. Schwartz and colleagues (2009) showed no benefit to administration of flunixin meglumine for the first 3 days of lactation. In fact, this treatment depressed feed intake and milk yield over the first week of lactation. In a much larger study, Duffield and coworkers (2009) demonstrated that flunixin injections in the first 2 days postpartum significantly increased the risk of retained placenta and metritis. This negative finding may be due to the importance of inflammatory pathways for expulsion of the placenta; regardless, at this point it appears that flunixin is not a promising candidate for treatment of transition cows.

Salicylates have been evaluated for use in the treatment of mastitis, and in general they are effective at reducing body temperatures, but do not appear to decrease the severity of the infection (Morkoc et al., 1993). However, this class of NSAIDs shows some promise in regard
Inflammation is a key component of the immune system’s attempt to fight off the invading pathogen, and anti-inflammatory treatments may increase the risk of infectious disorders. Eight NSAIDs tested in vitro suppressed phagocytosis by neutrophils (Paape et al., 1991), a key component of the immune response to bacterial infection. However, not all NSAIDs have the same suppressive effects on immune response. For example, aspirin was found to increase phagocytosis by bovine neutrophils in vitro (Bas et al., 1998, Paape et al., 1991). Bas and coworkers (1998) also observed an increase in microbicidal activity in response to low levels of aspirin. Based on promising results with aspirin, we are planning additional research to evaluate the effects of salicylate on transition cow disorders. If a compound such as salicylate can inhibit metabolic inflammation without suppressing immune responses, short-term NSAID treatment may provide an effective means to prevent metabolic disorders in the transition period.

CONCLUSIONS

Growing evidence suggests that inflammation may be a key factor in the development of many transition disorders. Because it results in suppressed immune function and altered nutrient metabolism, inflammation may provide a novel link between infectious and metabolic disorders that are common during the transition period. This model suggests that dietary antioxidants in dry cow rations should be re-evaluated on farms struggling with transition cow disorders. Additional steps, such as the incorporation of β-carotene or rumen-protected choline, may also help to prevent oxidative stress and subsequent inflammation. Future research may provide additional tools to directly combat inflammation in transition cows. Hopefully, continued progress on the pathology of transition disorders will help dairy producers to decrease the number of early lactation cows leaving the dairy herd.

REFERENCES

