The Role of Inflammation in Metabolic Disorders

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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, lipopolysaccharide (**LPS**) 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, and 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 (**IL**) 1β, and IL-6; which 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 inflammation. 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).

It is clear that mammary and uterine infections result in both local and systemic inflammation. Coliform mastitis results in release of LPS 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. 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 and metritis can therefore result in systemic inflammation.

IS THERE A ROLE FOR INFLAMMATION IN METABOLIC DISORDERS?

Inflammation has been proposed as a missing link in the pathology of metabolic disorders in transition cows (Drackley, 1999). Recent findings have documented relationships between inflammatory mediators and metabolic disorders. Plasma concentrations of haptoglobin and serum amyloid A 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). These correlations have driven strong interest in potential mechanisms underlying an inflammation-based pathogenesis of transition cow disorders.

INFLAMMATORY PATHWAYS THAT ALTER NUTRIENT METABOLISM

The following discussion is intended to highlight mechanisms that may be important in promoting inflammatory effects on metabolism in periparturient or lactating dairy cows, and is not comprehensive.

Inflammatory cytokines

Consistent with their role in responses to infection, cytokines generally have catabolic effects on metabolism. 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 (Ingvartsen, 2006). Inflammatory cytokines also directly alter metabolic function of the liver. For example, TNFα decreases liver glucose production in some scenarios (Kettelhut et al., 1987) and promotes triglyceride accumulation once mobilized NEFA reach the liver (García-Ruiz et al., 2006). Triglyceride accumulation is likely due in part to decreased FA oxidation in the liver after exposure to TNFα (Nachiappan et al., 1994). Tumor necrosis factor alpha also decreased production of apolipoproteins (Ettinger et al., 1994), which may impair triglyceride export in very low-density lipoproteins (**VLDL**) and contribute to hepatic triglcyeride accumulation. 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.

Oxidative stress

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 FA oxidation. Enhanced peroxisomal oxidation increases total oxidative capacity of the hepatocyte, 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).

In vivo observations support a role for oxidative stress in metabolic disorders. Dairy cows with fatty liver have lower antioxidant status and higher hepatic lipid peroxide concentrations than healthy cows (Mudron et al., 1999). Despite these data suggesting a metabolic effect of oxidative stress, transition cow studies employing antioxidants as treatments have looked almost exclusively at effects on infectious disorders such as mastitis and metritis. In rodent models, however, studies have demonstrated that antioxidants improve metabolic function in animals challenged with high-fat diets (Mao et al., 2010), ischemia (Soltys et al., 2001), and endotoxin (Sakaguchi and Furusawa, 2006). In a recent phase 3 clinical trial, vitamin E supplementation significantly improved liver health in steatohepatitis patients compared to placebo (Sanyal et al., 2010).

Toll-like receptor 4

Toll-like receptor 4 (**TLR4**) was initially identified as a protein expressed in immune cells that is critical for responses to LPS (Poltorak et al., 1998). Binding of LPS to TLR4 promotes inflammatory responses, and there is now growing recognition that TLR4 is expressed in many cell types, including hepatocytes (Galloway et al., 2008).

Activation of TLR4-dependent pathways by LPS has numerous effects on metabolic function. Toll-like receptor 4 activation decreases insulin sensitivity in adipose tissue and liver (Shi et al., 2006). Additional studies have demonstrated that LPS signaling via TLR4 increases adipose tissue lipolysis (Zu et al., 2009) and decreases fatty acid (**FA**) oxidation in muscle (Frisard et al., 2010). In addition, despite relative insulin resistance in liver, LPS activation of this pathway can also suppress hepatic glucose production (Carl et al., 2009). Collectively, these effects have many similarities to the fatty liver / ketosis complex in transition cows.

It has long been debated whether acidosis promotes release and translocation of LPS from the rumen and into the bloodstream. Khafipour et al. (2009) nicely demonstrated that induction of sub-acute ruminal acidosis increased both ruminal and plasma LPS concentrations. This treatment also significantly elevated plasma concentrations of acute-phase proteins, presumably mediated by TLR4 sensing of the translocated LPS. Although no indices of hepatic metabolism were measured in this study, it is likely that if LPS was sufficiently elevated to induce an acute phase response, expression of metabolic genes was also altered. Studies in other species suggest that numerous physiological stressors, including heat stress, can disrupt tight junctions between gastrointestinal epithelial cells and allow translocation of LPS (Lambert, 2009). If this phenomenon is common in dairy cattle, it may play a significant role in metabolic responses to parturition, heat stress, diet transitions, and other stressors.

Activation of hepatic TLR4 may be an important component of the metabolic response when LPS concentrations are elevated. However, TLR4 is a promiscuous receptor with the ability to bind and respond to more than 10 classes of naturally-occurring compounds (Gay and Gangloff, 2007). In fact, saturated FA can also activate inflammatory pathways via TLR4 (Lee et al., 2001). This raises the possibility that elevated NEFA, especially in transition dairy cows, may alter immune (Sordillo et al., 2009) and hepatic function through TLR4 activation.

NET EFFECTS OF INFLAMMATION ON METABOLISM OF LACTATING COWS

Strong 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 wk 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 wk after calving. Our own lab recently reported that subcutaneous injection of TNFα for 7 d doubled liver triglyceride content in late-lactation dairy cows (Bradford et al., 2009). We also observed changes in mRNA abundance consistent with transcriptionally-mediated increases in FA uptake and esterification and decreased FA oxidation. These results strongly support the hypothesis that inflammation disrupts normal metabolism; because although both of the above treatments were considered low-dose and short-term, they nevertheless promoted ketosis and fatty liver, respectively.

Beyond direct promotion of ketosis and fatty liver, hepatic 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 early lactatation cows, impaired glucose production would likely lead to increased adipose tissue breakdown, elevated plasma NEFA, and increased ketone production by the liver.

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.

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., 1990a), 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., 1990b). 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., 1990b). Additionally, a meta-analysis showed that supplemental vitamin E is effective at preventing retained placenta (Bourne et al., 2007).

Although much of the literature on antioxidants in transition cows demonstrates positive effects, these nutrients must be used with caution. In an effort to maximize the odds of observing a response, most studies are designed with rather dramatic treatments; for example, the classic Weiss study cited above (1990b) compared vitamin E intakes of 574 IU/d (no supplemental vitamin E) to 1474 IU/d (supplementing 40 IU/kg dry matter). In many such scenarios, the control group is fed a diet that is marginally deficient in the nutrient of interest. On most dairies, this is not the case. As a result, adding large amounts of vitamin E, for example, can sometimes push the supply of the nutrient high enough to cause mild toxicity. Supplementing 3000 IU/d vitamin E to transition cows with adequate vitamin E status resulted in pro-oxidant responses, increasing markers of lipid peroxidation and the incidence of mastitis (Bouwstra et al., 2010). Any treatment that alters oxidative balance should be evaluated carefully.

Non-steroidal anti-inflammatory drugs (NSAIDs)

Flunixin meglumine was evaluated in 2 recent studies in which transition cows were treated prior to any disease diagnosis to assess whether flunixin might prevent disorders. Shwartz and colleagues (2009) showed no benefit to administration of flunixin meglumine for the first 3 d 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 d postpartum significantly increased the risk of retained placenta and metritis. This negative finding may be due to the ability of flunixin to inhibit cyclooxygenase enzymes, suppressing prostaglandin synthesis and slowing uterine contractions necessary for expulsion of the placenta.

Salicylates have shown some promise in regard to metabolic inflammation. Cows treated with acetyl-salicylate (aspirin) for the first 5 d of lactation had significantly lower plasma concentrations of acute phase proteins and tended to have greater peak milk production than controls (Bertoni et al., 2004). In a similar study, aspirin treatment for 5 d postpartum improved milk yield in the first 2 mo of lactation and increased first service conception rates (Trevisi and Bertoni, 2008). A relatively small number of cows was included in the study (23/treatment); however, ketosis incidence appeared to decrease with aspirin treatment (4.4 vs. 22.7 %) while metritis incidence appeared to increase (30.4 vs. 13.6 %). These results point to potential tradeoffs between metabolic and immune function associated with decreased inflammation.

Omega-3 fatty acids

A class of long-chain FA, omega-3 FA include alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. Although these FA are typically found in low abundance in ruminant diets, a number of recent studies have evaluated the use of flaxseed or fish oil-derived products to increase dietary supply of omega-3 FA. One reason for the

interest in these compounds is that they can suppress inflammatory pathways.

Thatcher and colleagues have attempted to promote immune function in the transition period by supplementing omega-6 FA compared to omega-3 FA, supplied in the form of Ca salts of FA. Although this form of FA protection does not make the FA inert in the rumen, biohydrogenation is slowed enough for these supplements to alter FA composition of tissues. Increasing the ratio of omega-6 to omega-3 FA increased the production of hydrogen peroxide and phagocytosis of bacteria by neutrophils (Thatcher et al., 2010), which could be due to increased supply of omega-6 precursors of inflammatory compounds and/or decreased supply of anti-inflammatory omega-3 FA. This treatment also increased plasma concentrations of 2 acute phase proteins (Thatcher et al., 2010), indicating a more inflamed state of the liver during the transition period. While the observed effects on neutrophil function would be expected to improve the ability of the immune system to ward off infection, liver inflammation is associated with impaired metabolic function (Bertoni et al., 2008; Bradford et al., 2009). Like the other strategies discussed above, the potential benefits of such an approach may depend on the incidence of metabolic vs. infectious diseases on a given farm, the metabolic state of the cows in question, and even the diet to which the FA supplement is added.

Immunomodulary peptides

The next generation of immune modulators may be peptide-based treatments. Peptides designed to mimic endogenous host defense peptides have improved immune response to some pathogenic challenges, and have advantages over antibiotics in terms of their lack of residue in food products as well as consumer acceptability. However, host defense peptides can cause non-specific inflammation and some may not be good tools for use in transition cows. A novel class of small cationic peptides, coined *immunomodulary peptides*, was designed to promote immune function without the inflammatory effects of host defense peptides. One such peptide was shown to improve survival of mice exposed to either gram-negative or gram-positive pathogens, primarily by increasing the effectiveness of macrophages (Scott et al., 2007). Remarkably, the peptide not only failed to induce non-specific inflammation, it actually decreased inflammatory responses to LPS in monocytes, decreasing expression of TNFα and IL-6 and increasing IL-10 expression. In the future, technologies promoting improved clearance of pathogens with limited inflammation may help cows to navigate the transition period with lower risk of both infectious and metabolic disease.

CONCLUSIONS

In summary, a new model is emerging to explain the development of numerous transition disorders. A combination of insults, including infection, translocation of LPS from the gut, and lipid peroxide formation, can promote systemic and hepatic inflammation during the transition period. Inflammation causes maladaptive shifts in metabolism, increasing the risk of metabolic disorders. This model suggests that anti-inflammatory treatments, antioxidants, or even anti-lipolytic agents may help to limit hepatic inflammation and improve metabolic function. 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.

LITERATURE CITED

Ametaj, B. N., B. J. Bradford, G. Bobe, R. A. Nafikov, Y. Lu, J. W. Young, and D. C. Beitz. 2005. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. Can. J. Anim. Sci. 85:165-175.

Bernabucci, U., B. Ronchi, N. Lacetera, and A. Nardone. 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J. Dairy Sci. 88:2017-2026.

Bertoni, G., E. Trevisi, X. Han, and M. Bionaz. 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J. Dairy Sci. 91:3300-3310.

Bertoni, G., E. Trevisi, and F. Piccioli-Cappelli. 2004. Effects of acetyl-salicylate used in post-calving of dairy cows. Vet. Res. Commun. 28:217-219.

Bourne, N., R. Laven, D. C. Wathes, T. Martinez, and M. McGowan. 2007. A meta-analysis of the effects of vitamin E supplementation on the incidence of retained foetal membranes in dairy cows. Theriogenology 67:494-501.

Bouwstra, R. J., M. Nielen, J. R. Newbold, E. H. J. M. Jansen, H. F. Jelinek, and T. V. Werven. 2010. Vitamin E supplementation during the dry period in dairy cattle. Part II: Oxidative stress following vitamin E supplementation may increase clinical mastitis incidence postpartum. J. Dairy Sci. 93:5696-5706.

Bradford, B. J., L. K. Mamedova, J. E. Minton, J. S. Drouillard, and B. J. Johnson. 2009. Daily injection of tumor necrosis factor-α increases hepatic triglycerides and alters transcript abundance of metabolic genes in lactating dairy cattle. J. Nutr. 139:1451-1456.

Carl, F. R., L. B. Natasha, J. M. Alderman, S. M. Kelli, A. H. Peter, K. Simon, A. Shizuo, E. B. James, S. B. Albert, M. Nigel, and P. C. Terry. 2009. Lipopolysaccharide inhibition of glucose production through the Toll-like receptor-4, myeloid differentiation factor 88, and nuclear factor κB pathway. Hepatology 50:592-600.

Dantzer, R., and K. W. Kelley. 2007. Twenty years of research on cytokine-induced sickness behavior. Brain. Behav. Immun. 21:153-160.

Doepel, L., H. Lapierre, and J. J. Kennelly. 2002. Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. J. Dairy Sci. 85:2315-2334.

Drackley, J. K. 1999. ADSA foundation scholar award. Biology of dairy cows during the transition period: the final frontier? J. Dairy Sci. 82:2259-2273.

Duffield, T. F., H. Putnam-Dingwell, D. Weary, A. Skidmore, L. Neuder, W. Raphael, S. Millman, N. Newby, and K. E. Leslie. 2009. Effect of flunixin meglumine treatment following parturition on cow health and milk production. J. Dairy Sci. 92(E-Suppl. 1):117 (Abstr.).

Ettinger, W. H., V. K. Varma, M. Sorci-Thomas, J. S. Parks, R. C. Sigmon, T. K. Smith, and R. B. Verdery. 1994. Cytokines decrease apolipoprotein accumulation in medium from Hep G2 cells. Arterioscler. Thromb. 14:8-13.

Frisard, M. I., R. P. McMillan, J. Marchand, K. A. Wahlberg, Y. Wu, K. A. Voelker, L. Heilbronn, K. Haynie, B. Muoio, L. Li, and M. W. Hulver. 2010. Toll-like receptor 4 modulates skeletal muscle substrate metabolism. Am. J. Physiol. Endocrinol. Metab. 298:E988-998.

Galloway, E., T. Shin, N. Huber, T. Eismann, S. Kuboki, R. Schuster, J. Blanchard, H. R. Wong, and A. B. Lentsch. 2008. Activation of hepatocytes by extracellular heat shock protein 72. Am. J. Physiol. Cell Physiol. 295:C514-520.

García-Ruiz, I., C. Rodríguez-Juan, T. Díaz-Sanjuan, P. del Hoyo, F. Colina, T. Muñoz-Yagüe, and J. A. Solís-Herruzo. 2006. Uric acid and anti-TNF antibody improve mitochondrial dysfunction in ob/ob mice. Hepatology 44:581-591.

Gay, N. J., and M. Gangloff. 2007. Structure and function of Toll receptors and their ligands. Annu. Rev. Biochem. 76:141-165.

Goff, J. P. 2006. Major advances in our understanding of nutritional influences on bovine health. J. Dairy Sci. 89:1292-1301.

Grum, D. E., J. K. Drackley, R. S. Younker, D. W. LaCount, and J. J. Veenhuizen. 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850-1864.

Hoeben, D., C. Burvenich, E. Trevisi, G. Bertoni, J. Hamann, R. Buckmaier, and J. W. Blum. 2000. Role of endotoxin and TNF-alpha in the pathogenesis of experimentally induced coliform mastitis in periparturient cows. J. Dairy Res. 67:503-514.

Holcomb, C. S., H. H. Van Horn, H. H. Head, M. B. Hall, and C. J. Wilcox. 2001. Effects of prepartum dry matter intake and forage percentage on postpartum performance of lactating dairy cows. J. Dairy Sci. 84:2051-2058.

Huzzey, J. M., T. F. Duffield, S. J. LeBlanc, D. M. Veira, D. M. Weary, and M. A. G. von Keyserlingk. 2009. Short communication: Haptoglobin as an early indicator of metritis. J. Dairy Sci. 92:621-625.

Ingvartsen, K. L. 2006. Feeding- and management-related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. Anim. Feed Sci.Tech. 126:175-213.

Jiang, L., P. Sorensen, C. Rontved, L. Vels, and K. Ingvartsen. 2008. Gene expression profiling of liver from dairy cows treated intra-mammary with lipopolysaccharide. BMC Genomics 9:443.

Kettelhut, I. C., W. Fiers, and A. L. Goldberg. 1987. The toxic effects of tumor necrosis factor in vivo and their prevention by cyclooxygenase inhibitors. PNAS 84:4273-4277.

Khafipour, E., D. O. Krause, and J. C. Plaizier. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92:1060-1070.

Kushibiki, S., K. Hodate, H. Shingu, Y. Obara, E. Touno, M. Shinoda, and Y. Yokomizo. 2003. Metabolic and lactational responses during recombinant bovine tumor necrosis factor-α treatment in lactating cows. J. Dairy Sci. 86:819-827.

Kushibiki, S., K. Hodate, H. Shingu, Y. Ueda, Y. Mori, T. Itoh, and Y. Yokomizo. 2001. Effects of long-term administration of recombinant bovine tumor necrosis factor alpha on glucose metabolism and growth hormone secretion in steers. Am. J. Vet. Res. 62:794-798.

Lambert, G. P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J. Anim Sci. 87(14_suppl):E101-108.

LeBlanc, S. J., T. H. Herdt, W. M. Seymour, T. F. Duffield, and K. E. Leslie. 2004. Peripartum serum vitamin E, retinol, and beta-carotene in dairy cattle and their associations with disease. J. Dairy Sci. 87:609-619.

Lee, J. Y., K. H. Sohn, S. H. Rhee, and D. Hwang. 2001. Saturated fatty acids, but not unsaturated fatty acids, induce the Expression of cyclooxygenase-2 mediated through Toll-like Receptor 4. J. Biol. Chem. 276:16683-16689.

Mao, G., G. A. Kraus, I. Kim, M. E. Spurlock, T. B. Bailey, Q. Zhang, and D. C. Beitz. 2010. A mitochondria-targeted vitamin E derivative decreases hepatic oxidative stress and inhibits fat deposition in mice. J. Nutr. 140:1425-1431.

Mudron, P., J. Rehage, K. Qualmann, H. P. Sallmann, and H. Scholz. 1999. A study of lipid peroxidation and vitamin E in dairy cows with hepatic insufficiency. Zentralbl. Veterinarmed. A 46:219-224.

Mudron, P., J. Rehage, H. P. Sallmann, M. Mertens, H. Scholz, and G. Kovac. 1997. Plasma and liver alpha-tocopherol in dairy cows with left abomasal displacement and fatty liver. Zentralbl. Veterinarmed. A 44:91-97.

Murondoti, A., R. Jorritsma, A. C. Beynen, T. Wensing, and M. J. H. Geelen. 2004. Unrestricted feed intake during the dry period impairs the postpartum oxidation and synthesis of fatty acids in the liver of dairy cows. J. Dairy Sci. 87:672-679.

Nachiappan, V., D. Curtiss, B. E. Corkey, and L. Kilpatrick. 1994. Cytokines inhibit fatty acid oxidation in isolated rat hepatocytes: synergy among TNF, IL-6, and IL-1. Shock 1:123-129.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. National Research Council. Natl. Acad. Sci., Washington, DC.

Ohtsuka, H., M. Koiwa, A. Hatsugaya, K. Kudo, F. Hoshi, N. Itoh, H. Yokota, H. Okada, and S. Kawamura. 2001. Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver. J. Vet. Med. Sci. 63:1021-1025.

Pessayre, D., B. Fromenty, and A. Mansouri. 2004. Mitochondrial injury in steatohepatitis. Eur. J. Gastroenterol. Hepatol. 16:1095-1105.

Petersen, H. H., J. P. Nielsen, and P. M. Heegaard. 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35:163-187.

Poltorak, A., X. He, I. Smirnova, M.-Y. Liu, C. V. Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton, and B. Beutler. 1998. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr Mice: Mutations in Tlr4 Gene. Science 282:2085-2088.

Sakaguchi, S., and S. Furusawa. 2006. Oxidative stress and septic shock: metabolic aspects of oxygen-derived free radicals generated in the liver during endotoxemia. FEMS Immunol. Med. Microbiol. 47:167-177.

Sanyal, A. J., N. Chalasani, K. V. Kowdley, A. McCullough, A. M. Diehl, N. M. Bass, B. A. Neuschwander-Tetri, J. E. Lavine, J. Tonascia, A. Unalp, M. Van Natta, J. Clark, E. M. Brunt, D. E. Kleiner, J. H. Hoofnagle, and P. R. Robuck. 2010. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N. Engl. J. Med. 362:1675-1685.

Scott, M. G., E. Dullaghan, N. Mookherjee, N. Glavas, M. Waldbrook, A. Thompson, A. Wang, K. Lee, S. Doria, P. Hamill, J. J. Yu, Y. Li, O. Donini, M. M. Guarna, B. B. Finlay, J. R. North, and R. E. W. Hancock. 2007. An anti-infective peptide that selectively modulates the innate immune response. Nat Biotech 25:465-472.

Shi, H., M. V. Kokoeva, K. Inouye, I. Tzameli, H. Yin, and J. S. Flier. 2006. TLR4 links innate immunity and fatty acid-induced insulin resistance. J. Clin. Invest. 116:3015-3025.

Shwartz, G., K. L. Hill, M. J. VanBaale, and L. H. Baumgard. 2009. Effects of flunixin meglumine on pyrexia and bioenergetic variables in postparturient dairy cows. J. Dairy Sci. 92:1963-1970.

Smith, K. L., J. H. Harrison, D. D. Hancock, D. A. Todhunter, and H. R. Conrad. 1984. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. J. Dairy Sci. 67:1293-1300.

Soltys, K., G. Dikdan, and B. Koneru. 2001. Oxidative stress in fatty livers of obese Zucker rats: Rapid amelioration and improved tolerance to warm ischemia with tocopherol. Hepatology 34:13-18.

Sordillo, L. M., G. A. Contreras, and S. L. Aitken. 2009. Metabolic factors affecting the inflammatory response of periparturient dairy cows. Animal Health Research Reviews 10:53-63.

Sordillo, L. M., G. M. Pighetti, and M. R. Davis. 1995. Enhanced production of bovine tumor necrosis factor-alpha during the periparturient period. Vet. Immunol. Immunopathol. 49:263-270.

Thatcher, W. W., F. T. Silvestre, J. E. P. Santos, and C. R. Staples. 2010. Nutritional modulation of immune-function and compatability with pregnancy. *In:* Proc. SW. Nutr. Mgmt. Conf. Tempe, AZ. Pp 25-42.

Trevisi, E., M. Amadori, A. M. Bakudila, and G. Bertoni. 2009. Metabolic changes in dairy cows induced by oral, low-dose interferon-alpha treatment. J. Anim Sci. 87:3020-3029.

Trevisi, E., and G. Bertoni. 2008. Attenuation with acetylsalicylate treatments of inflammatory conditions in periparturient dairy cows. *In:* Aspirin and Health Research Progress. P. I. Quinn, ed. Nova Science Publishers. Pages 22-37

Weiss, W. P., J. S. Hogan, K. L. Smith, and K. H. Hoblet. 1990a. Relationships among selenium, vitamin E, and mammary gland health in commercial dairy herds. J. Dairy Sci. 73:381-390.

Weiss, W. P., D. A. Todhunter, J. S. Hogan, and K. L. Smith. 1990b. Effect of duration of supplementation of selenium and vitamin E on periparturient dairy cows. J. Dairy Sci. 73:3187-3194.

Zu, L., J. He, H. Jiang, C. Xu, S. Pu, and G. Xu. 2009. Bacterial endotoxin stimulates adipose lipolysis via toll-like receptor 4 and extracellular signal-regulated kinase pathway. J. Biol. Chem. 284:5915-5926.

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