Leaky Gut's Contribution to Inefficient Nutrient Utilization

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Take Home Messages

- Ketosis and heat stress are two hurdles to profitable dairying.
- Both ketosis and heat stress are characterized by increased inflammation.
- Evidence suggests endotoxin originating from the gut as the underlying cause for both disorders.
- Immune system activation has important metabolic consequences that negatively affect production.

Introduction

There are a variety of situations in an animal's life when nutrient utilization is reprioritized from productive towards agriculturally unproductive purposes. Two well-known examples that markedly reduce production are heat stress and ketosis. Decreased feed intake, experienced during both disorders, is unable to fully explain production losses. Additionally, both disorders are characterized by negative energy balance, body weight loss, inflammation, and liver fat accumulation. While the metabolism of ketosis and heat stress has been thoroughly studied for the last 40 years, the initial insult in the cascade of events ultimately reducing productivity in both heat-stressed and ketotic cows has not been identified. To that end, we have generated preliminary data strongly implicating a metabolic disruptor, endotoxin, as the underlying cause in each case.

Heat Stress

Heat stress (HS) negatively impacts a variety of production parameters and is a significant financial burden (~$900 million/year for dairy in the U.S. alone;

St. Pierre et al., 2003). Heat stress affects productivity indirectly by reducing feed intake; however, direct mechanisms also contribute, as we have shown reduced feed intake only explains approximately 50% of the decreased milk yield during HS (Baumgard et al., 2012, 2013). Direct mechanisms contributing to milk yield losses during HS involve an altered endocrine profile, including reciprocal changes in circulating anabolic and catabolic hormones (Baumgard and Rhoads, 2012, 2013). Such changes are characterized by increased circulating insulin concentration and lack of adipose tissue (i.e. backfat) mobilization. Liver and skeletal muscle cellular bioenergetics also exhibit clear differences in carbohydrate production and use, respectively, due to HS. Thus, the HS response markedly alters carbohydrate, fat, and protein metabolism through coordinated changes in fuel supply and utilization across tissues in a manner distinct from commonly recognizable changes that occur in animals on a reduced plane of nutrition (Baumgard and Rhoads, 2013). The result of HS is underachievement of an animal's full genetic potential.

Ketosis

The transition period is associated with substantial metabolic changes involving normal metabolic adaptations to support milk production. Unfortunately, a disproportionate amount of herd culling occurs before cows reach 60 days in milk. Ketosis is arbitrarily defined as an excess of circulating ketone bodies (β-hydroxybutyrate [BHBA] and/or acetoacetate), and is characterized by decreases in feed intake and milk production, and increased risk of developing other transition-period diseases (Chapinal et al., 2012). About 20% of transitioning dairy cows clinically experience ketosis (BHBA > 3.0 mM; Gillund et al., 2001) while the incidence of subclinical ketosis (>1.2 mM BHBA) is thought to be much higher (> 40%; McArt et al., 2012). Ketosis is a costly disorder (estimated at ~$300 per case; McArt et al., 2015) and thus it represents a major hurdle to farm profitability. Traditionally, ketosis is thought to result from excessive fat mobilization (Baird, 1982), which in turn contributes to fatty liver and excessive ketone body synthesis.

Heat Stress Etiology

Mechanisms responsible for altered nutrient partitioning during HS are not clear; however, they might be mediated by HS effects on gastrointestinal health because HS compromises intestinal barrier function (Pearce et al., 2013; Sanz-Fernandez et al., 2014). During HS, blood flow is diverted from the internal organs to the skin in an attempt to dissipate heat, leading to reduced oxygen flow to the intestine (Baumgard and Rhoads, 2013). Unfortunately, for a variety of reasons, intestinal cells are very sensitive to reduced blood flow and their "barrier function" is quickly compromised. As a

result, HS increases the infiltration of potentially harmful intestinal molecules into circulation (Pearce et al., 2013).

Endotoxin, also known as lipopolysaccharide (LPS), is a glycolipid embedded in the outer membrane of Gram-negative bacteria, which are abundant and prolific in luminal content, and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966). Immune system activation occurs when LPS binding protein (LBP) binds LPS for removal and detoxification; thus, LBP is frequently used as a biomarker for LPS infiltration (Ceciliani et al., 2012). For a detailed description of how livestock and other species detoxify LPS see our review (Mani et al., 2012). Endotoxin infiltration during HS into the bloodstream is common among heat stroke patients (Leon, 2007) and is thought to play a central role in heat stroke pathophysiology, because survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979). It is remarkable how animals suffering from heat stroke or severe endotoxemia share many physiological and metabolic similarities to HS, such as an increase in circulating insulin (Lim et al., 2007). Infusing LPS into the mammary gland increased (~2 fold) circulating insulin in lactating cows (Waldron et al., 2006). In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10-fold increase in circulating insulin (Stoakes et al., 2015a; Kvidera et al., 2016). Interestingly, increased insulin occurs before increased inflammation and the temporal pattern agrees with our previous *in vivo* data and a recent *in vitro* report (Bhat et al., 2014) suggesting LPS stimulates insulin secretion, either directly or via other endocrine mediators (Kahles et al., 2014). The possibility that LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of heat-stressed animal models (Baumgard and Rhoads, 2013). Again, the increase in insulin is energetically difficult to explain as feed intake is severely depressed during both HS and endotoxemia.

■ Transition Period Inflammation

Recently, the concept that LPS impacts normal nutrient partitioning and potentially contributes to metabolic maladaptation to lactation has started to receive attention. Although LPS itself has not been the primary causative focus, general inflammation has been the topic of investigations. Increased inflammatory markers following parturition have been reported in cows (Bertoni et al., 2008). Presumably, the inflammatory state following calving disrupts normal nutrient partitioning and is detrimental to productivity (Bertoni et al., 2008). This assumption was recently reinforced when infusion of an inflammatory cytokine decreased productivity (albeit without overt changes in metabolism; Martel et al., 2014). Additionally, in late-lactation cows, injecting the same inflammatory cytokine increased (>100%) liver lipid content without a change in circulating non-esterified fatty acids (NEFA; Bradford et al., 2009). Our recent data demonstrate increased inflammatory markers in cows

diagnosed with ketosis only and no other health disorders. In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and post-partum acute phase proteins such as LPS-binding protein, serum amyloid A, and haptoglobin were also increased (Figure 1; Abuajamieh et al., 2016). Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus (metritis), mammary gland (mastitis) and the gastrointestinal tract (Mani et al., 2012). However, we believe intestinal permeability may be responsible for inflammation observed in the transition dairy cow. A transitioning dairy cow undergoes a post-calving diet shift from a mainly forage-based ration to a high concentrate ration. This has the potential to induce rumen acidosis, which can compromise the gastrointestinal tract barrier (Khafipour et al., 2009).

In order to further investigate the effects of intestinal permeability on production and inflammation, we intentionally induced intestinal permeability in mid-lactation dairy cows using a gamma secretase inhibitor (GSI), a compound that causes “leaky gut” (van Es et al., 2005). We anticipated feed intake of GSI-administered cows would decrease, so we pair-fed controls in order to eliminate the confounding effect of feed intake. Administering GSI decreased feed intake and altered jejunum structure consistently with characteristics of leaky gut (shortened crypt depth, decreased villus height, decreased villus height to crypt depth ratio). Circulating insulin and LBP were increased in GSI cows relative to controls. Interestingly in our GSI model, the acute phase proteins—serum amyloid A and haptoglobin—increased for both treatments over time, indicating inflammation was occurring in pair-fed controls as well (Stoakes et al., 2014). This is not surprising, as pair-fed controls were receiving only ~20% (an 80% reduction in feed intake) of their ad libitum intake and decreased feed intake has been shown to increase intestinal permeability in feed restricted rodents and humans (Rodriguez et al., 1996) and pigs (Pearce et al., 2013; Sanz-Fernandez et al., 2014). Recently, we confirmed the detrimental effects of feed restriction in mid-lactation cows by demonstrating a linear increase in circulating acute phase proteins and endotoxin with increasing severity of feed restriction. Furthermore, cows fed 40% of ad libitum intake had shortened ileum villus height and crypt depth, indicating reduced intestinal health (Stoakes et al., 2015b). In summary, inflammation is present during the transition period and likely contributes to changes in whole-animal energetics.

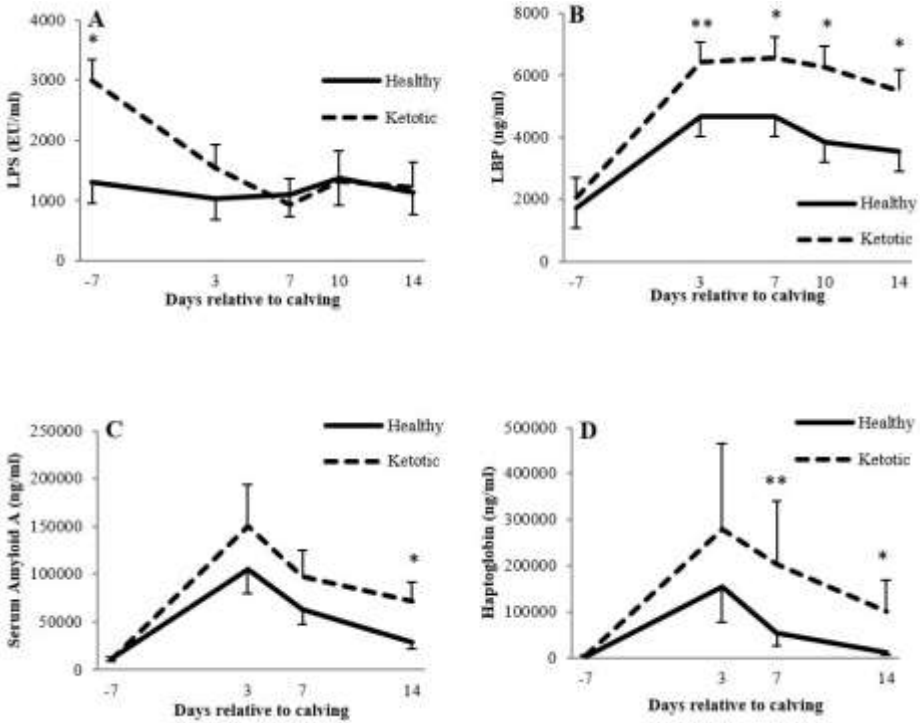

Figure 1. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows.

■ Metabolism of Inflammation

LPS-induced inflammation has an energetic cost, which redirects nutrients away from anabolic processes that support milk and muscle synthesis (see review by Johnson, 1997) and thus compromises productivity and efficiency. Interestingly, immune cells become more insulin sensitive and consume large amounts of glucose upon activation in order to support rapid proliferation and biosynthetic processes (Calder et al., 2007). In contrast, inflammation induces an insulin resistant state in skeletal muscle and adipose tissue (Liang et al., 2013). Recent data has also demonstrated a decrease in ketone oxidation during LPS infiltration (Suagee et al., 2011), which we believe may partly explain increased ketone body concentrations during the transition period.

Endotoxin has previously been recognized to be involved with metabolic dysfunction. In humans, both obesity and high fat diets are linked to endotoxemia (Cani et al., 2007). Furthermore, LPS is involved with the development of fatty liver (Ilan, 2012), and cytokines are linked to lipid accumulation and cholesterol retention (Ma et al., 2008). Experimentally-induced endotoxemia in dairy cattle has been linked to several metabolic and

endocrine disturbances including decreased circulating glucose, termination of pregnancy, leukopenia, disruption of ruminal metabolism, and altered calcium homeostasis (Griel et al., 1975; Waldron et al., 2003). The aforementioned pathological conditions are likely mediated by LPS-induced inflammation and the subsequent changes in nutrient partitioning caused by immune system activation.

Energetic Cost of Immune Activation

An activated immune system requires a large amount of energy and the literature suggests that glucose homeostasis is markedly disrupted during an endotoxin challenge. Upon immune system activation, immune cells switch their metabolism from oxidative phosphorylation to aerobic glycolysis, causing them to become obligate glucose utilizers (Vander Hiden et al., 2009). Our group recently quantified the energetic cost of an activated immune system by infusing exogenous glucose to maintain normal blood glucose levels during LPS-induced hypoglycemia (i.e., LPS-euglycemic clamp). Using this model, we estimated approximately 1 kg of glucose is used by the immune system during a 12-hour period in lactating dairy cows. Interestingly, on a metabolic body weight basis the amount of glucose utilized by LPS-activated immune system in lactating cows, growing steers and growing pigs were 0.64, 1.0, and 1.1 g glucose/kg $BW^{0.75}$/h, respectively; Stoakes et al., 2015a,c; Kvidera et al., 2016). Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake. This decreases the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool). We and others have now demonstrated that both heat-stressed and ketotic animals have increased circulating markers of endotoxin and inflammation. We believe the circulating LPS in both maladies originates from the intestine and thus both likely have an activated immune system. This activated systemic immune response reprioritizes the hierarchy of glucose utilization, and milk synthesis is consequently deemphasized.

■ Conclusion

Ketosis and heat stress are two of the most economically important pathologies that severely jeopardize the competitiveness of animal agriculture. Heat stress and ketosis affect herds of all sizes and every dairy region in the country. The biology of ketosis and heat stress has been studied for almost a half century, but the negative impacts of both are as severe today as they were 30 years ago. We suggest, based upon the literature and on our supporting evidence, that LPS is the common culprit for both metabolic disorders. Taken together, our data and the literature suggest that LPS markedly alters nutrient partitioning and is a causative agent in metabolic disruption during heat stress and ketosis.

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