Causes and Physiological Consequences of Intestinal Barrier Dysfunction

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Introduction

Suboptimal milk yield limits the U.S. dairy industry's productive competitiveness, marginalizes efforts to reduce inputs into food production, and increases animal agriculture's carbon footprint. There are a variety of circumstances in a cow's life which result in hindered productivity including heat stress, ketosis, rumen and hindgut acidosis, feed restriction, and psychological stress associated with normal animal practices (i.e., pen changes, weaning, shipping). Although these insults have different origins, a commonality among them is increased production of inflammatory biomarkers and markedly altered nutrient partitioning. We and others have generated convincing data strongly implicating intestinally derived lipopolysaccharide (LPS) as sometimes being the culprit in these situations.

Heat Stress

During heat stress (HS), blood flow is diverted from the viscera to the periphery to dissipate heat, and this leads to intestinal hypoxia (Hall et al., 1999). Enterocytes are particularly sensitive to hypoxia and nutrient restriction (Rollwagen et al., 2006), resulting in ATP depletion and increased oxidative and nitrosative stress (Hall et al., 2001). This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013), resulting in increased passage of luminal content into portal and systemic blood (Hall et al., 2001; Pearce et al., 2013). Endotoxin, otherwise referred to as LPS, is a glycolipid embedded in the outer membrane of Gram-negative bacteria, which is abundant and prolific in luminal content, and is a well-characterized potent immune stimulator in multiple species (Berczi et al., 1966; Giri et al., 1990; Tough et al., 1997). Immune system activation occurs when LPS binding protein (LBP) initially binds LPS and together with CD14 and TLR4 delivers 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 recent review (Mani et al., 2012). Endotoxin infiltration into the bloodstream during HS, which was first observed by Graber et al. (1971), is common among heat stroke patients (Leon, 2007) and is thought to play a central role in heat stroke pathophysiology as survival increases when intestinal bacterial load is reduced or when plasma LPS is neutralized (Bynum et al., 1979; Gathiram et al., 1987). 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). Intramammary LPS infusion 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 (Rhoads et al., 2009; Kvidera et al., 2016, 2017c). Interestingly, increased insulin occurs prior to 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 GLP-1 (Kahles et al., 2014). The possibility that LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of HS agriculture models (Baumgard and Rhoads, 2013). Again, the increase in insulin during both HS and immunoactivation is energetically difficult to explain as feed intake is severely depressed in both experiments.

Ketosis and the Transition Period

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 (Ametaj et al., 2005; Bionaz et al., 2007; Bertoni et al., 2008; Humblet et al., 2006; Mullins et al., 2012). Presumably, the inflammatory state following calving disrupts normal nutrient partitioning and is detrimental to productivity (Bertoni et al., 2008), and this assumption was recently reinforced when TNFα infusion decreased productivity (albeit without overt changes in metabolism; Yuan et al., 2013; Martel et al., 2014). Additionally, in late-lactation cows, injecting TNFα increased (>100%) liver TAG content without a change in circulating NEFA (Bradford et al., 2009). Our recent data demonstrates increased inflammatory markers in cows diagnosed with ketosis only and no other health disorders (i.e. the inflammation was not apparently due to mastitis or metritis). In comparison with healthy controls, ketotic cows had increased circulating LPS prior to calving and post-partum acute phase proteins such as LBP, serum amyloid A, and haptoglobin were also increased (Abuajamieh et al., 2016a). However, even seemingly healthy cows experience some degree of inflammation postpartum (Humblet et al., 2006). The magnitude and persistency of the inflammatory response seems to be predictive of transition cow performance (Bertoni et al., 2008; Bradford et al., 2015; Trevisi and Minuti, 2018). Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus (metritis) and mammary gland (mastitis) (Mani et al., 2012). Additionally, we believe intestinal hyperpermeability may also be responsible for periparturient inflammation in dairy cows as many of the characteristic responses (rumen acidosis, decreased feed intake, and psychological stress) occurring during this time can compromise gut barrier function.

As aforementioned, mild inflammation is observed even in cows which seemingly complete the transition period successfully, suggesting that some level of inflammation plays an important role in cow health. In fact, previous reports have demonstrated that blocking endogenous inflammation (via administration of non-steroidal anti-inflammatory drugs [NSAID]) can increase the incidence of negative health outcomes (i.e., fever, stillbirth, retained placenta, metritis) and reduce productivity (Schwartz et al., 2009; Newby et al., 2013, 2017). Beneficial effects of NSAIDs have been observed on production performance (Carpenter et al., 2016a), but inconsistencies exist (Priest et al., 2013; Meier et al., 2014) including how NSAIDs seemingly work better in

specific parities (Farney et a., 2013) and interfere with fiber digestion (Carpenter et al., 2016b) and compromise feed intake (Carpenter et al., 2017). Although NSAIDs may be an effective prophylactic strategy during the periparturient period, further research is necessary to determine the timing of administration and type and dose of NSAID that is most effective at improving health. Alternatively, administrating a chemokine (anti or even pro-inflammatory) may hold promise in improving transition cow performance.

Rumen and Hindgut Acidosis

A transitioning dairy cow undergoes a dietary shift from a high forage to a high concentrate ration post-calving. This has the potential to induce rumen acidosis (RA) as increases in fermentable carbohydrates and DMI stimulate the buildup of short chain fatty acids and lactic acid (Nocek, 1997; Enemark, 2008). Rumen acidosis has direct and ancillary consequences accompanied by various production issues (decreased DMI, reduced milk yield, milk fat depression) and health challenges such as laminitis, liver abscesses, and potentially death (Nocek, 1997; Kleen, 2003). The mechanisms linking RA and the development of health disorders are not entirely clear, however, recent literature has indicated that inflammation associated with epithelial damage and consequential LPS translocation are at least partially responsible for production losses associated with RA (Gozho, et al., 2005; Khafipour, et al., 2009). Although many hypothesize LPS translocation occurs at the rumen epithelium directly (Guo et al., 2017; Minuti et al., 2014), others point towards LPS translocation in the hindgut to be a potential source of peripheral inflammation (Li et al., 2012). Interestingly, when RA was induced using either alfalfa pellets or high-grain diets, increased peripheral inflammation was only observed in the high-grain group, irrespective of rumen acidotic conditions being similar between the two treatments (Khafipour et al., 2009a,b). It was hypothesized that the grain supplemented group likely had increased starch flow to the hindgut, and therefore, increased fermentation that could potentially lead to hindgut acidosis and LPS translocation across the large intestine. However, we were unable to recreate production losses and systemic inflammation when we abomasally infused 500 g/d of resistant starch (Piantoni et al., 2018) or even 4 kg/d of purified corn starch (Abeyta et al., 2019). Both of our aforementioned experiments were accompanied with marked reductions in fecal pH so it is unlikely that large intestinal acidosis per se is the specific reason for systemic inflammation described in the previous reports (Li et al., 2012, Khafipour et al., 2009a,b). Regardless, we recently reported that cows with the largest decrease in fecal pH post-calving consumed less feed, produced less milk, had a larger acute phase protein response and had increased NEFA and BHB compared to cows that had a mild decrease in fecal pH following parturition (Rodriguez-Jimenez et al., 2019). Clearly, our current understanding of how hind-gut acidosis impacts the immune system and ultimately periparturient productivity is woeful.

Feed Restriction and Psychological Stress

Stress associated with feed restriction along with several other regular production practices (e.g., heat stress, weaning, transportation, overcrowding, restraint, social isolation/mixing) is frequently encountered in animal agriculture (Chen et al., 2015) and is associated with gastrointestinal hyperpermeability. In fact, we have repeatedly reported reduced intestinal

barrier integrity in pigs pair-fed to their HS counterparts (Pearce et al., 2013; Sanz-Fernandez et al., 2014). Furthermore, we recently demonstrated shortened ileum villous height and crypt depth (Kvidera et al., 2017d) as well as increased appearance of the intestinal permeability marker Cr-EDTA (Horst and Baumgard, unpublished), indicating reduced intestinal health in cows fed 40% of ad libitum intake. Recent literature indicates that the corticotropin releasing factor (CRF) system may be the mechanism involved in stress-induced leaky gut (Wallon et al., 2008; Vanuytsel et al., 2014). The CRF and other members of the CRF signaling family including urocortin (1, 2, and 3) and their G-protein couple receptors CRF1 and CRF2, have been identified as the main mediators of the stress-induced intestinal changes including inflammation, altered intestinal motility and permeability, as well as shifts in ion, water, and mucus secretion and absorption (as reviewed by Rodiño-Janeiro et al., 2015). These alterations appear to be regulated in large part by intestinal mast cells (Santos et al., 2000). Mast cells are important mediators of both innate and adaptive immunity and express receptors for the neuropeptides CRF1 and CRF2, which may in part explain the association between emotional stress and intestinal dysfunction (Smith et al., 2010; Ayyadurai et al., 2017). Furthermore, mast cells synthesize a variety of pro-inflammatory mediators (i.e., IFN-γ and TNF-α) that are released upon activation, mainly via degranulation (de Punder and Pruimboom, 2015). Excessive mast cell degranulation plays an important role in the pathogenesis of different intestinal inflammatory disorders (Santos et al., 2000; Smith et al., 2010). A better understanding of the role psychosocial stress plays on the initiation of different intestinal disorders in livestock is of obvious interest for multiple animal agriculture systems.

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, 1998) and thus compromises productivity. Upon activation, most immune cells become obligate glucose utilizers via a metabolic shift from oxidative phosphorylation to aerobic glycolysis (not anaerobic glycolysis typically learned about in biochemistry classes), a process known as the Warburg effect.

This metabolic shift allows for rapid ATP production and synthesis of important intermediates which support proliferation and production of reactive oxygen species (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). In an effort to facilitate glucose uptake, immune cells become more insulin sensitive and increase expression of GLUT3 and GLUT4 transporters (Maratou et al., 2007; O'Boyle et al., 2012), whereas peripheral tissues become insulin resistant (Poggi et al., 2007; Liang et al., 2013). Furthermore, metabolic adjustments including hyperglycemia or hypoglycemia (depending upon the stage and severity of infection), increased circulating insulin and glucagon, skeletal muscle catabolism and subsequent nitrogen loss (Figure 1; Wannemacher et al., 1980), and hypertriglyceridemia (Filkins, 1978; Wannemacher et al., 1980; Lanza-Jacoby et al., 1998; McGuinness, 2005) occur. Interestingly, despite hypertriglyceridemia, circulating BHB often decreases following LPS administration (Waldron et al., 2003a,b; Graugnard et al., 2013; Kvidera et al., 2017a). The mechanism of LPS-induced decreases in BHB has not been fully elucidated, but may be explained by increased ketone

oxidation by peripheral tissues (Zarrin et al., 2014). Collectively, these metabolic alterations are presumably employed to ensure adequate glucose delivery to activated leukocytes.

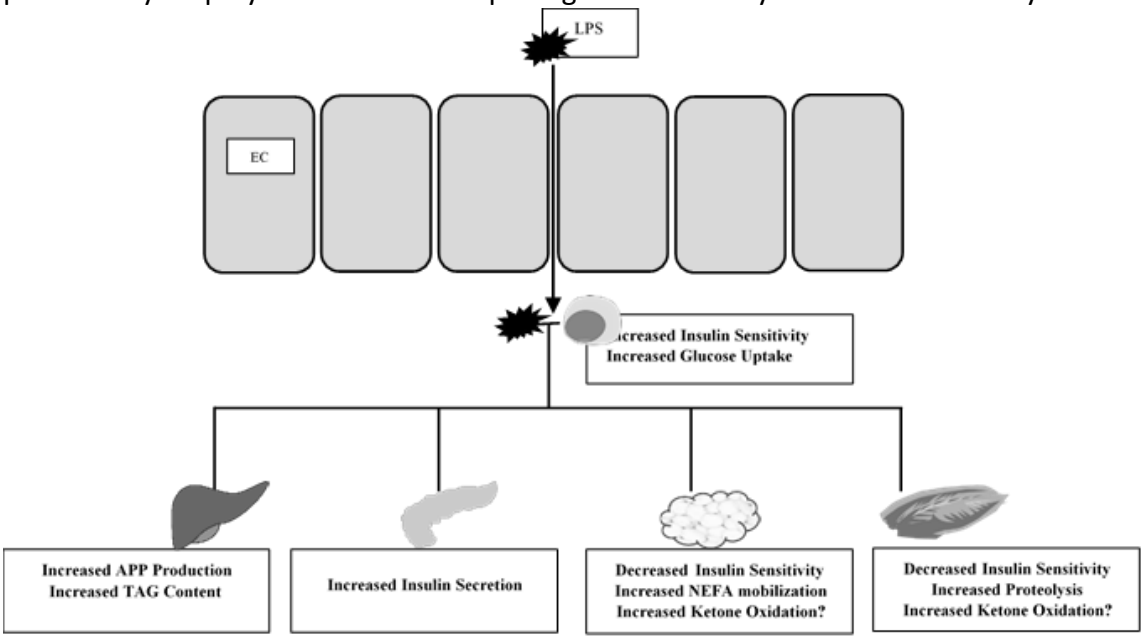

Figure 1. LPS induced alterations in peripheral metabolism.

Energetic Cost of Immune Activation

The energetic costs of immunoactivation are substantial, but the ubiquitous nature of the immune system makes quantifying the energetic demand difficult. Our group recently employed a series of LPS-euglycemic clamps to quantify the energetic cost of an activated immune system. Using this model, we estimated approximately 1 kg of glucose is used by an intensely activated 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 mid- and late-lactation cows, growing steers and growing pigs were 0.64, 1.0, 0.94, 1.0, and 1.1 g glucose/kg $BW^{0.75}$/h, respectively; Kvidera et al., 2016, 2017b,c, Horst et al., 2018, 2019). A limitation to our model is the inability to account for liver's contribution to the circulating glucose pool (i.e., glycogenolysis and gluconeogenesis). However, both glycogenolytic and gluconeogenic rates have been shown to be increased during infection (Spitzer et a., 1985; Waldron et al., 2003b) and Waldron et al. (2006) demonstrated that ~87 g of glucose appeared in circulation from these processes. Furthermore, we have observed both increased circulating glucagon and cortisol (stimulators of hepatic glucose output) following LPS administration (Horst et al., 2019) suggesting we are underestimating the energetic cost of immunoactivation. The reprioritization of glucose trafficking during immunoactivation has particular consequences during lactation as it requires ~72 g of glucose for synthesizing 1 kg milk (Kronfeld, 1982).

Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decrease the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool, etc.). We and others have now demonstrated that HS, rumen acidosis, and

psychological stress increase circulating markers of endotoxin and inflammation. We believe that the circulating LPS originates from the intestine (small or large) and initiates an immune response. This activated systemic immune response reprioritizes the hierarchy of glucose utilization and milk synthesis is consequently deemphasized.

Nutritional Mitigation Strategies: The Role of Zinc (Zn) Supplementation

Potential dietary mitigation strategies aimed at improving gut health are currently of great interest, especially considering the numerous stressors (i.e., heat stress, feed restriction, acidosis) that potentially impact intestinal permeability. Zinc is an essential nutrient which is crucial for maintaining epithelial integrity (i.e., mammary, uterine, intestinal) and regulating the renewal of damaged epithelium (Alam et al., 1994). Zinc was first demonstrated to improve intestinal "health" in human leaky gut models (Alam et al., 1994; Rodriguez et al., 1996; Sturniolo et al., 2001), and we extended this to improved metrics of intestinal permeability in a variety of farm animal stress models including heat stress (Sanz-Fernandez et al., 2014; Pearce et al., 2015; Abuajamieh et al., 2016b) and feed restriction (Horst and Baumgard, unpublished using Zn hydroxychloride). Additionally, we observed altered febrile, cytokine, and acute phase protein responses during heat stress (Sanz-Fernandez et al., 2014; Pearce et al., 2015; Abuajamieh et al., 2016b; Mayorga et al., 2018) and in response to LPS administration (Horst et al., 2019) with dietary Zn supplementation. Presumably the aforementioned changes in inflammatory variables are indicative of a blunted immune response (because of improved intestinal barrier function). Therefore, Zn as a dietary supplement appears to be a promising avenue to improve gut health and to ameliorate alimentary canal associated inflammation.

Conclusion

There are various situations in an animal's life that hinder production performance (i.e., heat stress, feed restriction, rumen acidosis, etc.) and we suggest, based upon the literature and on our supporting evidence, that LPS (of intestinal origin) may be the common culprit in these circumstances. Immune activation in response to LPS markedly alters nutrient partitioning as a means of fueling the immune response. More research is still needed to understand the mechanisms and consequences of intestinal permeability and associated inflammation in order to provide foundational information for developing strategies aimed at maintaining productivity.

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