The what, why, and physiologic cost of leaky gut syndrome

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Abstract

Heat stress and metabolic maladaptation to lactation (ketosis) are 2 economically devastating hurdles to profitability. These stressors affect herds of all sizes and almost every dairy region in the world. The biology of heat stress and ketosis has been studied for almost a half century, but the negative impacts of both are as evident today as they were 30 years ago. Our recent discoveries suggest that endotoxin is the common culprit in both disorders and the intestine appears to be the etiological origin of both metabolic disorders. Endotoxin stimulates the immune system and activated leukocytes switch their metabolism away from oxidative phosphorylation to rely more on aerobic glycolysis. In multiple species, we estimate that immune activation consumes about 0.45 g glucose/lb (1 g/kg) $BW^{0.75}$ or about 0.9 lb (2 kg) glucose/day in an adult lactating dairy cow. Thus, an activated immune system reprioritizes nutrient partitioning away from the synthesis of economically valuable products.

Key words: cow, dairy, leaky gut, metabolism, LPS

Résumé

Le stress dû à la chaleur et les troubles métaboliques associés à la lactation (acidose) représentent deux écueils économiquement désastreux à la profitabilité. Ces deux sources de stress affectent les troupeaux de toutes tailles dans presque toutes les régions laitières du monde. Les fondements biologiques du stress dû à la chaleur et de l'acidose ont été étudiés depuis plus d'un demi-siècle mais leurs impacts négatifs sont aussi évidents aujourd'hui qu'ils ne l'étaient il y a 30 ans. Nos découvertes récentes suggèrent qu'une endotoxine est un facteur commun aux deux conditions et l'intestin semble être l'origine étiologique des deux troubles métaboliques. L'endotoxine stimule le système immunitaire et les leucocytes activés s'éloignent de leur métabolisme basé sur la phosphorylation oxydative et se tournent davantage vers la glycolyse aérobie. Pour plusieurs espèces, nous estimons que l'activation immunitaire consume à peu près 0.45 g glucose/lb (1g/kg) PC0.75 ou près de 0.9 lb (2 kg) de glucose par jour chez une vache laitière adulte en lactation. Ainsi, l'activation du système immunitaire redistribue la division des nutriments et l'éloigne de la synthèse de produits économiquement valables.

Introduction

Suboptimal milk yield limits the USA 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 preliminary data strongly implicating intestinally derived lipopolysaccharide (LPS) as a culprit in these situations.

Heat Stress

Heat stress (HS) affects blood flow which is diverted from the viscera to the periphery in an attempt to dissipate heat leading to intestinal hypoxia.[26] Enterocytes are particularly sensitive to hypoxia and nutrient restriction,[70] resulting in ATP depletion and increased oxidative and nitrosative stress.[27] This contributes to tight junction dysfunction and gross morphological changes that ultimately reduce intestinal barrier function,[47,66] resulting in increased passage of luminal content into portal and systemic blood.[27,66] Endotoxin, otherwise referred to as 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.[5,19,78] 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.[12] For a detailed description of how livestock and other species detoxify LPS see our recent review.[56] Endotoxin infiltration into the bloodstream during HS, which was first observed by Graber et al,[22] is common among heat stroke patients[49] 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.[9,18] 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.[53] Intramammary LPS infusion increased (~2 fold) circulating insulin in lactating cows.[82] In addition, we intravenously infused LPS into growing calves and pigs and demonstrated >10-fold increase in circulating insulin.[42,45,68] 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[7] suggesting LPS stimulates insulin secretion, either directly or via GLP-1.[36] The possibility that LPS increases insulin secretion likely explains the hyperinsulinemia we have repeatedly reported in a variety of HS agriculture models.[4] Again, the increase in insulin in both models is energetically difficult to explain as feed intake was 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.[2,6,32,61] Presumably, the inflammatory state following calving disrupts normal nutrient partitioning and is detrimental to productivity,[6,54] and this assumption was recently reinforced when TNFα infusion decreased productivity (albeit without overt changes in metabolism).[58,85]

Additionally, in late-lactation cows, injecting TNFα increased (>100%) liver TAG content without a change in circulating NEFA.[8] Our recent data demonstrates 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 postpartum acute phase proteins such as LBP, serum amyloid A, and haptoglobin were also increased[1] (Figure 1). Endotoxin can originate from a variety of locations, and obvious sources in transitioning dairy cows include the uterus (metritis) and mammary gland (mastitis).[56] 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.

Rumen and Hindgut Acidosis

A transitioning dairy cow undergoes a post-calving diet shift from a mainly forage-based to a high-concentrate ration. 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.[16,62] 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 lami-
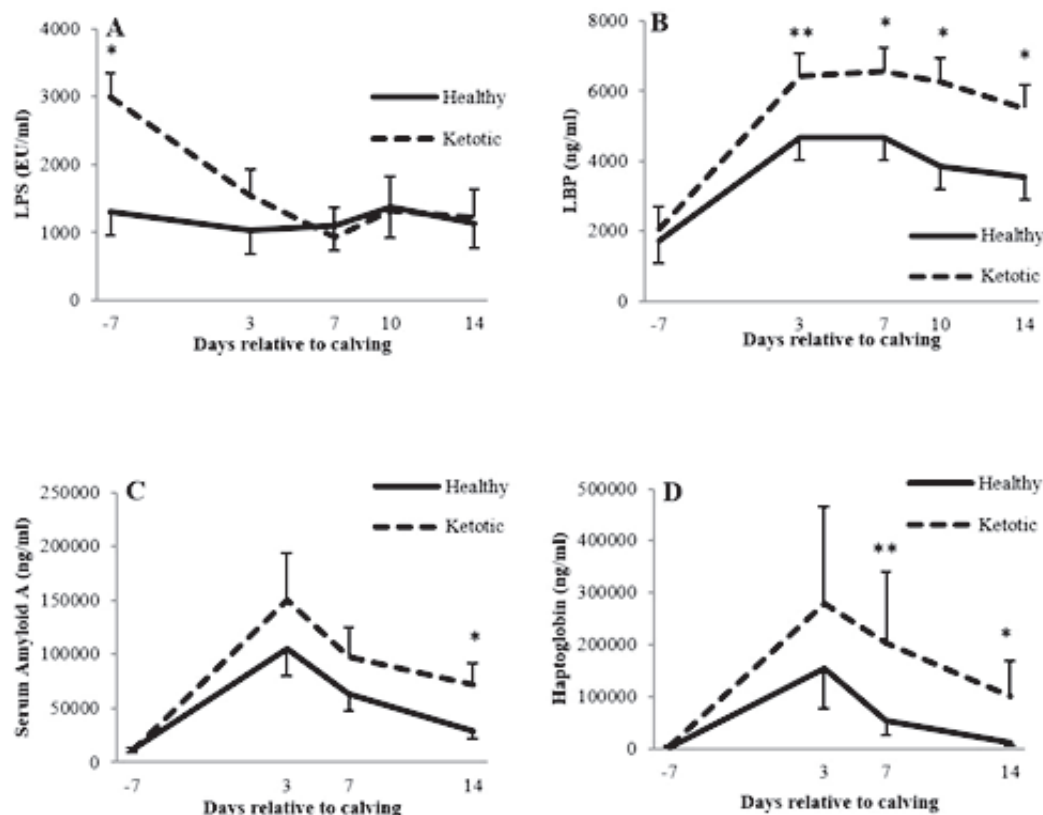

Figure 1. Markers of inflammation in healthy (solid line) and ketotic (dashed line) transition cows.

nitis, liver abscesses, and potentially death.[40,62] 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.[21,?] Although many hypothesize LPS translocation occurs at the rumen epithelium directly,[25,60] others point towards LPS translocation in the hindgut to be a potential source of peripheral inflammation.[51] 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 2 treatments.[37,38] 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[64] or even 8.8 lb (4 kg)/d of purified corn starch (Abeyta and Baumgard, unpublished). 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.[37,38,51]

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[13] and is associated with gastrointestinal permeability. In fact, we have repeatedly reported reduced intestinal barrier integrity in pigs pair-fed to their HS counterparts.[66,72] Furthermore, we recently demonstrated shortened ileum villous height and crypt depth, indicating reduced intestinal health, in cows fed 40% of ad libitum intake.[46] Recent literature indicates that the corticotropin releasing factor (CRF) system may be the mechanism involved in stress-induced leaky gut.[79,83] The CRF and other members of the CRF signaling family, including urocortin (1, 2, and 3) and their G-protein couple receptors CRF_1 and CRF_2, 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[69]). These alterations appeared to be regulated in large part by intestinal mast cells.[71] 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.[3,74] 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.[14] Excessive mast cell degranulation plays

an important role in the pathogenesis of different intestinal inflammatory disorders.[71,74] A better understanding of the role psychosocial stress plays on the initiation of different intestinal disorders in livestock is of great interest for animal agriculture systems.

Metabolism of Inflammation

Inflammation induced by LPS has an energetic cost that redirects nutrients away from anabolic processes that support milk and muscle synthesis (see review by Johnson[34,35]) and thus compromises productivity. Upon activation, immune cells become obligate glucose utilizers via a metabolic shift from oxidative phosphorylation to aerobic glycolysis, a process known as the Warburg effect (Figure 2). This metabolic shift allows for rapid ATP production and synthesis of important intermediates which support proliferation and production of reactive oxygen species.[10,65] In an effort to facilitate glucose uptake, immune cells become more insulin sensitive and increase expression of GLUT3 and GLUT4 transporters,[57,63] whereas peripheral tissues become insulin resistant.[52,67] 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 3; Wannemacher et al[84]), and hypertriglyceridemia[17,48,59,84] occur. Interestingly, despite hypertriglyceridemia, circulating BHB often decreases following LPS administration.[23,43,80,81] The mechanism of LPS-induced decreases in BHB has not been fully elucidated but may be explained by increased ketone oxidation by peripheral tissues.[86] In addition to changes in circulating metabolites, LPS has been shown to increase liver lipid accumulation, both directly through changes in lipid oxidation and transport enzymes, and indirectly through increases in circulating NEFA.[8] Collectively, these metabolic alterations are presumably employed to ensure adequate glucose delivery to activated leukocytes.

Energetic Cost of Immune Activation

Immunoactivation leads substantial energetic costs, 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 2.2 lb (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, 1.0, and 1.1 g glucose/kg (2.2 lb) $BW^{0.75}$/h, respectively.[28,42,44,45] 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
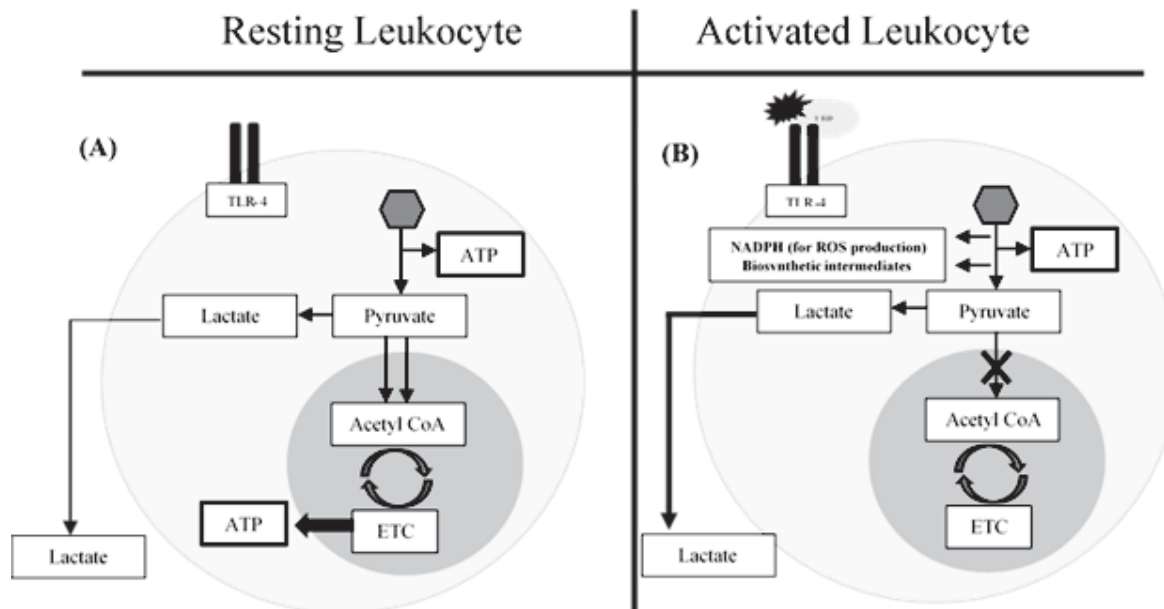
Figure 2. Metabolic pathway of a resting (A) vs activated (B) leukocyte.
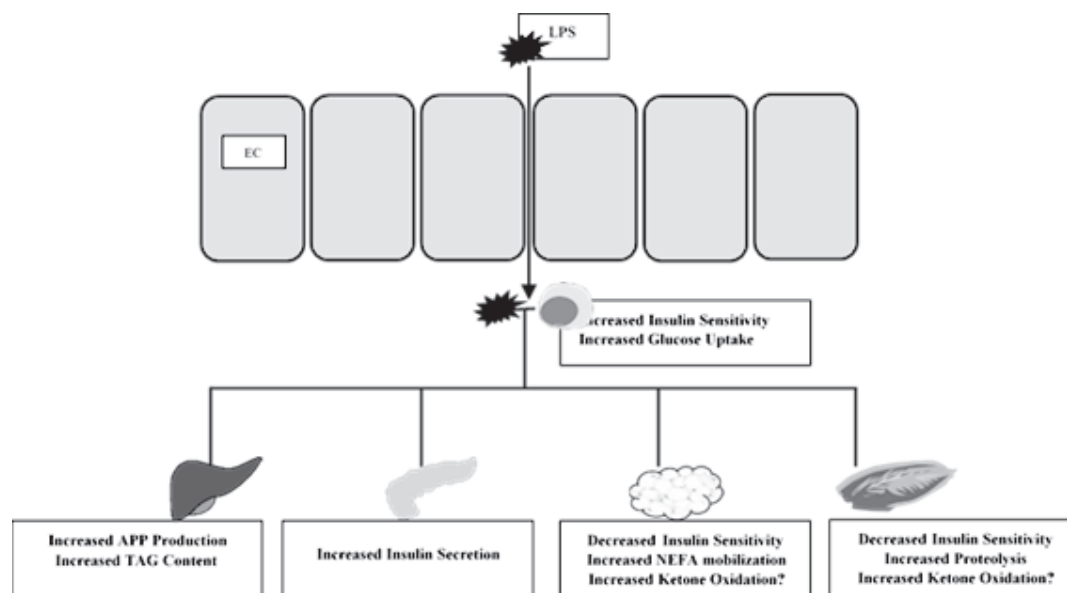

Figure 3. LPS induced alterations in peripheral metabolism.

and gluconeogenic rates have been shown to be increased during infection.[75,80] Furthermore, we have observed both increased circulating glucagon and cortisol (indirect markers of hepatic glucose output) following LPS administration[29,30] 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 2.2 lb (1 kg) milk.

Increased immune system glucose utilization occurs simultaneously with infection-induced decreased feed intake: this coupling of enhanced nutrient requirements with hypophagia obviously decreases the amount of nutrients available for the synthesis of valuable products (milk, meat, fetus, wool). 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 and initiates an immune response. This activated systemic immune response reprioritizes the hierarchy of glucose utilization, and milk synthesis is consequently deemphasized.

Conclusion

In an cow's life there are various situations that hinder animal 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. Furthermore, it is of interest to further elucidate the contribution of inflammation to subclinical hypocalcemia frequently observed in postpartum cows.

Acknowledgement

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