

2017

Causes and consequences of immune activation and its effect on metabolic and energetic status in production animals

Sara Kay Stoakes Kvidera
Iowa State University

Follow this and additional works at: <https://lib.dr.iastate.edu/etd>

 Part of the [Agriculture Commons](#), [Animal Sciences Commons](#), and the [Veterinary Medicine Commons](#)

Recommended Citation

Stoakes Kvidera, Sara Kay, "Causes and consequences of immune activation and its effect on metabolic and energetic status in production animals" (2017). *Graduate Theses and Dissertations*. 15344.
<https://lib.dr.iastate.edu/etd/15344>

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

Causes and consequences of immune activation and its effect on metabolic and energetic status in production animals

by

Sara Kay Stoakes Kvidera

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Nutritional Sciences (Animal Nutrition)

Program of Study Committee:

Lance H. Baumgard, Major Professor

Donald C. Beitz

Nicholas K. Gabler

Patrick J. Gorden

Howard D. Tyler

The student author and the program of study committee are solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2017

Copyright © Sara Kay Stoakes Kvidera, 2017. All rights reserved.

DEDICATION

This dissertation is written in honor of my parents, Dennis and Sharon Stoakes. Dad, thanks for being the first person to teach me how cows work and for being a perfect example of quiet strength. Mom, thanks for reading to me every night growing up. Learning to love reading took me places I'd never imagined. You both are the epitome of love, hardwork, and dedication to your work, to your children, and to each other. Christ's love shines through you, and I love you very much.

Sara Kay

TABLE OF CONTENTS

DEDICATION	ii
TABLE OF CONTENTS.....	iii
LIST OF FIGURES	vi
LIST OF TABLES	ix
ACKNOWLEDGEMENTS	xi
ABSTRACT.....	xiii
CHAPTER 1: LITERATURE REVIEW	15
Intestinal Permeability	15
Anatomy and Physiology of the Intestinal Epithelium	15
Situations of Increased Intestinal Dysfunction	18
Modeling Intestinal Permeability in Ruminants	24
Strategies to Mitigate Intestinal Permeability	25
Lipopolysaccharide.....	27
Structure and Origin.....	27
Innate Immune Response to Lipopolysaccharide	29
Immune System Energetics	35
Immunometabolism	35
Whole-body Response to Inflammation	46
Conclusion	53
CHAPTER 2: INTENTIONALLY INDUCED INTESTINAL BARRIER DYSFUNCTION CAUSES INFLAMMATION, AFFECTS METABOLISM, AND REDUCES PRODUCTIVITY IN LACTATING HOLSTEIN COWS	55
Abstract.....	55
Introduction.....	57
Materials and Methods.....	59
Animals and Sampling.....	59
Tissue Collection	61
Blood Analyses	62
Liver Analyses	62
Histological Analyses	63
Immunofluorescence Staining	64
Statistical Analyses	65
Results.....	66

Discussion.....	68
Conclusions.....	75
CHAPTER 3: CHARACTERIZING EFFECTS OF FEED RESTRICTION AND GLUCAGON-LIKE PEPTIDE 2 ADMINISTRATION ON BIOMARKERS OF INFLAMMATION AND INTESTINAL MORPHOLOGY	
	86
Abstract.....	86
Introduction.....	88
Materials and Methods.....	90
Animals and Sampling	90
Tissue Collection	92
Laboratory Analyses	93
Histological Analysis	93
Immunofluorescence Staining	95
Statistical Analyses	95
Results.....	96
Discussion.....	99
Conclusions.....	106
CHAPTER 4: TECHNICAL NOTE: ESTIMATING GLUCOSE REQUIREMENTS OF AN ACTIVATED IMMUNE SYSTEM IN HOLSTEIN STEERS	
	116
Abstract.....	116
Introduction.....	118
Materials and Methods.....	119
Results.....	122
Discussion.....	123
CHAPTER 5: ESTIMATING GLUCOSE REQUIREMENTS OF AN ACTIVATED IMMUNE SYSTEM IN LACTATING HOLSTEIN COWS.....	
	134
Abstract.....	134
Introduction.....	136
Materials and Methods.....	138
Animals and Experimental Design	138
Calculations and Statistical Analyses	141
Results.....	142
Discussion.....	145
Conclusion	154

CHAPTER 6: EFFECTS OF CONTINUOUS AND INCREASING LIPOPOLYSACCHARIDE INFUSION ON BASAL AND STIMULATED METABOLISM IN LACTATING HOLSTEIN COWS	165
Abstract	165
Introduction	167
Materials and Methods	168
Animals and Experimental Design	168
Metabolic Challenges	170
Laboratory Analyses	171
Calculations and Statistical Analyses	171
Results	172
Discussion	173
Conclusion	179
CHAPTER 7: INTEGRATIVE SUMMARY	186
REFERENCES	198
APPENDIX A: ESTIMATING GLUCOSE REQUIREMENTS OF AN ACTIVATED IMMUNE SYSTEM IN GROWING PIGS	245
APPENDIX B: EFFECTS OF REPEATED LIPOPOLYSACCHARIDE INFUSION ON HEMATOLOGIC, METABOLIC, AND INTESTINAL HISTOLOGY PARAMETERS	249
APPENDIX C: EFFECT OF SUPPLEMENTAL CITRULLINE ON THERMAL, PRODUCTION, AND INTESTINAL HEALTH PARAMETERS DURING HEAT STRESS AND NUTRIENT RESTRICTION IN GROWING PIGS	254

LIST OF FIGURES

Figure 1.	A summary of intestinal dysfunction (Yu et al., 2016).....	17
Figure 2.	Effects of gamma-secretase inhibitor or pair-feeding on dry matter intake, milk yield, and manure score.....	78
Figure 3.	Effects of gamma-secretase inhibitor or pair-feeding on circulating metabolites in cows.....	79
Figure 4.	Effects of gamma-secretase inhibitor or pair-feeding on circulating acute phase proteins	80
Figure 5.	Effects of gamma-secretase inhibitor or pair-feeding on intestinal morphology	81
Figure 6.	Correlation between jejunum villus height-to-crypt depth ratio and the change in plasma lipopolysaccharide binding protein	82
Figure 7.	Effects of gamma-secretase inhibitor or pair-feeding on liver triglyceride content	83
Figure 8.	Representative images of periodic acid-Schiff stained intestinal segments in pair-fed or gamma-secretase inhibitor-administered cows ...	84
Figure 9.	Representative image of PCNA protein intensity in intestinal segments in pair-fed or gamma-secretase inhibitor-administered cows ...	85
Figure 10.	Effects of incremental feed restriction and glucagon-like peptide 2 treatment dry matter intake and milk yield	109
Figure 11.	Effects of incremental feed restriction and glucagon-like peptide 2 treatment on circulating metabolites	110
Figure 12.	Effects of incremental feed restriction and glucagon-like peptide 2 treatment on circulating inflammatory biomarkers.....	111
Figure 13.	Effects of incremental feed restriction and glucagon-like peptide 2 treatment on intestinal morphology	112
Figure 14.	Effects of incremental feed restriction and glucagon-like peptide 2 treatment on liver triglyceride content	113
Figure 15.	Representative images of periodic acid-Schiff stained intestinal segments in feed restricted and glucagon-like peptide 2- treated cows..	114

Figure 16.	Representative image of PCNA protein intensity in intestinal segments in feed restricted and glucagon-like peptide 2-treated cows...	115
Figure 17.	Circulating glucose, insulin, and rate of glucose infusion in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged steers	131
Figure 18.	Circulating inflammatory markers in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged challenged steers	132
Figure 19.	Rectal temperature in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged cows	156
Figure 20.	Circulating glucose and rate of glucose infusion in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged cows	157
Figure 21.	Milk yield and milk and/or total glucose deficit in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged cows	158
Figure 22.	Circulating metabolites in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged cows.....	159
Figure 23.	Circulating inflammatory markers in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged cows.....	160
Figure 24.	Circulating leukocytes in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged cows.....	161
Figure 25.	Daily dose schedule of lipopolysaccharide administered intravenously over 8 days in cows	169
Figure 26.	Circulating glucose, non-esterified fatty acids, and insulin in pair-fed and continuously lipopolysaccharide-infused cows.....	182
Figure 27.	Circulating β -hydroxybutyrate, L-lactate, and urea nitrogen in pair-fed and continuously lipopolysaccharide-infused cows	183
Figure 28.	Circulating glucose and insulin response to a glucose tolerance test in pair-fed and continuously lipopolysaccharide-infused cows.....	184
Figure 29.	Circulating glucose and non-esterified fatty acids response to an epinephrine challenge in pair-fed and continuously lipopolysaccharide-infused cows	185
Figure 30.	Rectal temperature, circulating glucose, and rate of glucose infusion in growing pigs challenged with a lipopolysaccharide-euglycemic clamp.	247

Figure 31. Effect of citrulline supplementation on rectal temperature, skin temperature, respiration rate, and circulating lipopolysaccharide binding protein during heat stress or pair-feeding 257

LIST OF TABLES

Table 1.	Mechanisms of intestinal barrier maintenance	18
Table 2.	Potential strategies to ameliorate intestinal permeability	27
Table 3.	Effect of immune activation on glucose utilization in leukocyte in vitro/ex vivo and in various tissues in vivo	45
Table 4.	The effects of endotoxin on circulating metabolites and regulatory hormones in various species	47
Table 5.	Ingredients and composition of diet for the intentionally induced intestinal dysfunction project.....	76
Table 6.	Effects of gamma-secretase inhibitor or pair-feeding on production variables and vital measurements	77
Table 7.	Effects of gamma-secretase inhibitor or pair-feeding on liver and spleen measurements post-mortem and body weight change	77
Table 8.	Ingredients and composition of diet for the progressive feed restriction/glucagon-like peptide 2-administration project.....	107
Table 9.	Effects of incremental feed restriction and glucagon-like peptide 2-treatment on milk and health parameters	108
Table 10.	Circulating parameters in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged steers	133
Table 11.	Ingredients and composition of diet for the lipopolysaccharide-euglycemic clamp in lactating cows project	162
Table 12.	Milk composition parameters in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged cows	163
Table 13.	Complete blood count parameters in lipopolysaccharide (LPS) and LPS-euglycemic clamp challenged cows	164
Table 14.	Ingredients and composition of diet for the 7 day lipopolysaccharide infusion project in cows	180
Table 15.	Effects a lipopolysaccharide-euglycemic clamp challenge on circulating metabolites and inflammatory markers in pigs.....	248

Table 16.	Effects of a lipopolysaccharide-euglycemic clamp challenge on complete blood count parameters in pigs.....	248
Table 17.	Effects of 5 day lipopolysaccharide-administration on rectal temperature and circulating metabolic and inflammatory parameters in pigs.....	251
Table 18.	Effects of 5 day lipopolysaccharide-administration on blood gases, hematology, and electrolytes in pigs.....	251
Table 19.	Effects of 5 day lipopolysaccharide administration on complete blood count parameters in pigs	252
Table 20.	Effects of 5 day lipopolysaccharide administration on intestinal morphology in pigs	253
Table 21.	Effects of citrulline supplementation on production parameters during heat stress or pair feeding in pigs	258

ACKNOWLEDGEMENTS

I would first like to acknowledge my major professor, Dr. Lance Baumgard, who took a chance on a girl from northeast Iowa whose main life skill at the time was running in circles. I have endless gratitude for your training in how to think critically, challenge dogmas, and formulate ideas. I have never regretted the day I “traded my track spikes for a lab coat” and that is mostly because of you, your encouragement, and your dedication to my success. You are not only a great scientist and teacher, but also a great life coach.

I would also like to acknowledge the continual support and guidance provided by my dissertation committee members: Dr. Don Beitz, Dr. Nick Gabler, Dr. Pat Gorden, and Dr. Howard Tyler. It has been an honor and a privilege to work with such a distinguished group of scientists. Each one of you has contributed uniquely to this dissertation, and I appreciate the variety of advice and perspective you have all given me.

This dissertation would not have been possible without the hardwork and support of my colleagues, especially Dr. Maria Victoria Sanz Fernandez, Erin Horst, Dr. Mohannad Abuajamieh, Mackenzie Dickson, Johanna Mayorga, Carrie Shouse, Mohammad Al-Qaisi, and Dr. Jay Johnson. They say “many hands makes light work,” but I thought grad school was still really hard, so I’ll say instead that your frequent help and company kept me sane throughout the process, and I think we even had a little fun along the way. Thank you for all your help and intellectual and moral support over the years.

I am very grateful to the two institutions that fueled my passion for science. To begin with, I am grateful to Kansas State and their faculty, especially Dr. Barry Bradford and Dr. Tim Rozell for sparking my initial interest in physiology and graduate school. Next, I am grateful for Iowa State University’s outstanding Animal Science Department and their

faculty and staff, including Dr. Maynard Hogberg, Dr. Don Beermann, Dr. Joan Cunnick, Dr. Aileen Keating, Dr. Jason Ross, Donna Nelson, Keri Morris, Joe Detrick, Trey Faaborg, Mary Healey, Samantha Lei, and Jane Linn who have all set aside time to help me in some way during my studies here. I'm also very grateful for the many resources Iowa State has provided with their research facilities, IT resources, and statistical consulting. Great universities like Iowa State and Kansas State are the reason agriculture is a progressive and innovative sector.

Major acknowledgments go to my big brothers William and John Stoakes for helping me become the person I am today and for continually checking up on me to make sure I'm okay. Corindy and Dawn, thanks for being the sisters I never had and for always being a source of moral support I know I can turn to at any time. I'm also grateful to my grandparents, Duane and Marlene Ingle as well as Robert and Nadine Stoakes. In various ways, they forged the way for me to have the opportunities available to me today.

I have a great set of friends who supported me throughout the dissertation writing process. Patrick, Mara, Kyle, Jodi, Curtis, Leah, and Josh, I'm forever grateful for your moral support and the constant reminder to keep God at the center of everything I do.

Most importantly, my deepest appreciation and love goes to my husband, Taylor Kvidera. Thank you for staying by my side, always. You have been the absolute best encouragement and support a wife could ever need, and I see God's unconditional love through you. I am excited to continue with the many adventures that await us!

ABSTRACT

Nutrient and energy partitioning toward productive processes (i.e., growth, reproduction, and milk production) is key to economically profitable agriculture. During infection, nutrients and energy are drawn away from profitable purposes to support the immune system. Immunological costs contribute to the economic consequences of sickness that include decreased growth, inefficient feed utilization, poor reproduction, and increased health care costs. Animals experience frequent immune challenges, as bacterial insults can originate from many different scenarios. Understandably, the intestinal epithelium is a focal point of bacterial translocation due to its “gate-keeping” role of simultaneously facilitating nutrient absorption while preventing bacterial infiltration into portal and systemic circulation. Immune insults originating from intestinal bacteria have become of interest as heat stress, feed restriction, and peripheral inflammation contribute to intestinal permeability to luminal content. Additionally, metabolic maladaptation to lactation following calving (i.e., ketosis) in dairy cattle is associated with inflammation, and intestinal-derived endotoxin may contribute to this metabolic disorder. Sickness or suboptimal health, regardless of origin, is an animal welfare issue and an economic burden to animal agriculture. The activated immune system employs a unique metabolism to strategically utilize substrates and ensure its quick and effective response. An activated immune system requires a large amount of energy, and systemic glucose homeostasis is markedly disrupted during endotoxemia. Specifically, activated macrophages and other immune cells become obligate glucose utilizers and hypoglycemia and hyperlactemia are characteristic hallmarks. The increased glucose requirement by the immune system occurs simultaneously with hypophagia and thus reduced intestinally derived carbohydrate

precursors. Consequently, there is a negative glucose balance that likely limits the immune response and certainly prevents optimal productivity. Having a better understanding of the nutrient requirements of an activated immune system will provide foundational information for developing strategies aimed at minimizing productivity losses during infection. Furthermore, understanding the etiology of immune system activation in various animal agricultural situations can aid in developing strategies to maximize protection against immune system over-activation.

CHAPTER 1: LITERATURE REVIEW

Intestinal Permeability

Anatomy and Physiology of the Intestinal Epithelium

The intestinal epithelium serves a dual purpose of nutrient absorption and protection from numerous pathogens and other antigens present in the gastrointestinal tract lumen. Intestinal barrier integrity is maintained by physical and chemical means as well as an extensive mucosal immune system (summarized in Table 1 and Figure 1). One of the first physical lines of defense against foreign antigens is mucus, produced by goblet cells, which provides a barrier between intestinal microbiota and the mucosal epithelium and also acts as lubricant to keep digesta and microbes flowing continuously (McCauley and Guasch, 2015). There are over 20 different types of mucins produced throughout the gastrointestinal tract (Corfield, 2015), and absence of the major intestinal mucin, MUC2, can result in severe local inflammation and growth retardation (Van der Sluis et al., 2006). There are two distinct mucus layers in the large intestine; the inner layer is firmly attached to the epithelium and is mostly impenetrable to bacteria while the outer layer serves as a hospitable environment for some bacteria (Johansson et al., 2011). Similar to the bacterial load, proportions of goblet cells increase from the proximal to the distal intestine (Cheng and Leblond, 1974). Mucin is the glycoprotein constituent of mucus, and its secretion occurs in response to neuroendocrine signals, inflammatory mediators, and bacteria (Laboisse et al., 1996; Deplancke and Gaskins, 2001). Physiological stressors such as ischemia, nutrient restriction, and certain microbial enzymes can deplete the mucus layer

(Sherman et al., 1985; Grootjans et al., 2013; Kumar et al., 2014). Tight junctions (**TJ**) are another important aspect of the epithelial physical barrier that prevents pathogen infiltration while simultaneously allowing selective passage of ions and water (see review by Suzuki, 2013). Tight junction proteins are composed of four essential transmembrane proteins: occludin, claudin, junctional adhesion molecule, and tricellulin (Furuse et al., 1993, 1998; Martín-Padura et al., 1998; Ikenouchi et al., 2005). These transmembrane proteins are anchored to the cytosolic actomyosin rings via the scaffold protein zonula occludens (Fanning et al., 2002). Tension and contraction of the actomyosin ring is dependent on myosin light chain activity, and phosphorylation by myosin light chain kinase results in increased paracellular permeability (Cunningham and Turner, 2012). A number of cytokines and growth factors increase TJ permeability, including IFN- γ , TNF- α , IL-6, and IL-1 β (Bruewer et al., 2005; Ma et al., 2005; Al-Sadi and Ma, 2007; Suzuki et al., 2011). Conversely, there are cytokines and growth factors that aid in decreasing TJ permeability, including IL-10, TGF- β , and epidermal growth factor (Madsen et al., 1997; Howe et al., 2005; Geng et al., 2013). Ultimately, TJs and their associated intracellular complexes play a crucial role in maintaining barrier function, and inflammatory cytokines can cause alterations resulting in intestinal dysfunction.

Interestingly, organized immune tissues were first identified in the gut of primitive fish, indicating the gut as the original immune system with spleen and lymph nodes occurring as later specializations (Matsunaga and Rahman, 2001). The gut-associated lymphoid tissue includes Peyer's patches, isolated lymphoid follicles, intraepithelial lymphocytes, lymphocytes within the lamina propria, and plasma cells (Murphy, 2012). Peyer's patches are covered by specialized M-cells which lack microvilli and are instead

characterized by microfolds on the apical membrane. These M-cells have a much thinner layer of mucus relative to enterocytes and therefore purposely endocytose more antigens from the lumen. In the subepithelial dome of Peyer's patches, dendritic cells phagocytose, process, and present antigens to T-cells, which aids in activation and differentiation (Murphy, 2012). The majority of these interactions result in anti-inflammatory cytokine production by both macrophages and T-cells. However, when dysbiosis or pathogen infiltration occurs, dendritic cells switch into their pro-inflammatory phenotypes, initiating a pro-inflammatory response to clear invading pathogens (Murphy, 2012).

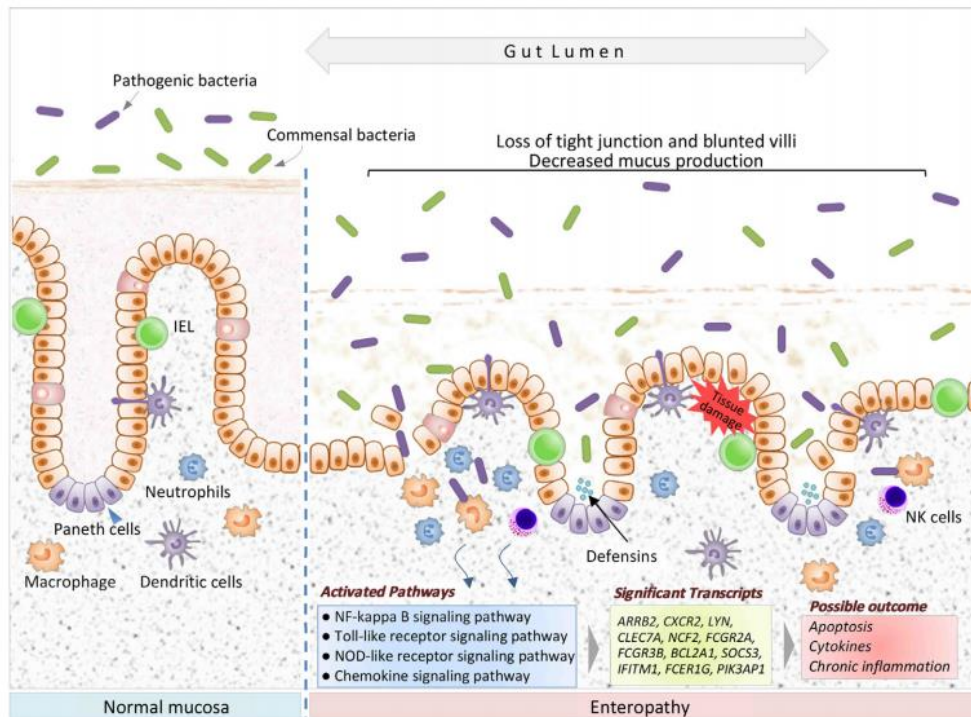


Figure 1. A summary of intestinal dysfunction (Yu et al., 2016)

Synthesis and secretion of secretory immunoglobulin A (sIgA) is another important component of mucosal immunity. Plasma cells of the mucosal immune system synthesize and secrete sIgA into the gut lumen that binds and neutralizes antigens within the mucus

to inhibit propagation of an immune response (Mantis and Forbes, 2010). In addition, sIgA can facilitate antigen uptake into lymphoid compartments (Kadaoui and Chorthésy, 2007). A synergistic relationship exists between sIgA-secreting B cells and innate immune components of the gut associated lymphoid tissue, and knockout of either system results in enhanced stimulation of the other and enteric dysfunction (Slack et al., 2009; Shulzhenko et al., 2011).

Table 1. Mechanisms of intestinal barrier maintenance (summarized from Murphy, 2012)

	Defense Mechanisms	Cell Types
Mechanical	low pH, mucins, tight junction proteins	parietal cells, goblet cells, epithelial cells
Chemical	lysozymes, phospholipase A2, C-type lectins, defensins, cathelicidins, histatins	Paneth cells, epithelial cells
Immune system	cytotoxicity, IgA, phagocytosis, degranulation, pathogen detection	intraepithelial lymphocytes, plasma cells, macrophages, neutrophils, dendritic cells

Failure in one or more of the aforementioned mechanisms of barrier function can lead to increased intestinal permeability to luminal contents and subsequent activation of the immune system in order to detoxify invading pathogens (see ‘Detoxification’) and is associated with a large energetic cost (see ‘Immune System Energetics’).

Situations of Increased Intestinal Dysfunction

Human diseases

There are a variety of human diseases, albeit with etiological differences, where the common dominant pathology is impaired intestinal barrier function (i.e., leaky gut), including Crohn’s disease, inflammatory bowel syndrome, celiac disease, and alcoholism

(Draper et al., 1983; Bargiggia et al., 2003; Ludvigsson et al., 2007; McGowan et al., 2012). Furthermore, increased intestinal permeability has been associated with the development of type 1 diabetes (Bosi et al., 2006; Li and Atkinson, 2015). Incidentally, intestinal endotoxin infiltration contributes to hepatic steatosis (Nolan, 1975; Bargiggia et al., 2003; Ilan, 2012; Damms-Machado et al., 2017), and thus the aforementioned human diseases are often characterized by increased hepatic lipid content. In addition to the diseases mentioned above with various underlying etiologies, psychological stress has been implicated as an inducer of increased gastrointestinal tract barrier permeability (de Punder and Pruijboom, 2015). In fact, public speaking increases intestinal permeability, and these stress-induced increases in permeability are likely mediated by corticotropin-releasing hormone activation of mast cells (Wallon et al., 2008; Vanuytsel et al., 2014). Interestingly, a variety of models have demonstrated inflammation originating from non-intestinal sources can also impact intestinal health. Hemorrhagic shock, human immunodeficiency virus, burn injury, and intravenous lipopolysaccharide (**LPS**) all increase gastrointestinal tract barrier permeability (Ziegler et al., 1988; Russell et al., 1995; Hietbrink et al., 2009; Epple and Zeitz, 2012). Presumably this systemic effect is mediated via inflammatory cytokine activation of myosin light chain kinase and altered TJ proteins (Bruewer et al., 2003; Cunningham and Turner, 2012). Thus, intestinal barrier dysfunction transcends species and is implicated in a variety of human disorders. While the physiology remains the same, the cause-effect relationship of intestinal permeability and other disorders remains somewhat vague in humans because of the large variation in genetic, diet, and lifestyle factors existing in human studies.

Rumen acidosis

The shift from a high-forage to high-concentrate diet is common practice for both beef and dairy cattle to provide a concentrated energy source to maximize production. These grains can be rapidly fermented in the rumen at a rate that exceeds the removal of the resulting volatile fatty acids, resulting in their accumulation and subsequent depressed ruminal pH (Owens et al., 1998). Broadly, rumen acidosis is a maladaptation to dietary changes, and the arbitrary threshold for non-physiological rumen pH is 5.5 and is defined as subacute rumen acidosis (**SARA**; Nordlund et al., 1995). Prevalence of SARA varies depending on herd management, but studies in Wisconsin indicate approximately 20% of tested commercial dairy cows had a ruminal pH below 5.5, and similar results have been obtained in German and Polish dairy herds (Oetzel et al., 1999; Kleen et al, 2013; Stefańska et al., 2016). Rumen acidosis is characterized by several negative consequences, including decreased dry matter intake, laminitis, liver abscesses, diarrhea, and milk fat depression (Kleen et al., 2003).

Perhaps the earliest and most well-studied consequences of rumen acidosis are liver abscesses, which were first connected with rumen lesions by H. A. Smith in 1944 (reviewed by Nagaraja and Chengappa, 1998). Recently, there has been a renewed interest in rumen acidosis effects on epithelial barriers and consequences of bacterial translocation extending beyond the liver to systemic inflammation. Inducing SARA causes rapid bacterial proliferation and endotoxin release in the rumen (Gozho et al., 2005; Khafipour et al., 2009a,b), and several authors have shown systemic inflammation occurs in experimentally-induced SARA, attributable to changes in gastrointestinal tract permeability and bacterial endotoxin translocation (Gozho et al., 2005; Emmanuel et al., 2007b; Minuti et al., 2014;

Khafipour et al., 2009a). While effects of rumen acidosis on gastrointestinal permeability and subsequent inflammation are becoming well-established, the exact site of gastrointestinal permeability remains less clear. Rumen biopsies from SARA-induced cattle demonstrated deteriorated epithelial structural suggestive of rumen bacterial translocation (Steele et al., 2011). In vitro, Emmanuel and colleagues (2007a) demonstrated both endotoxin and low pH increased permeability of rumen and colon tissues, however, the concentration of LPS used to induce this change in permeability likely exceeded physiological concentrations. On the other hand, Khafipour et al. (2009a,b) demonstrated systemic inflammation occurred only when SARA was induced using a grain-based model and not when SARA was induced using alfalfa pellets. They speculated elevated inflammation in the grain-based model was due to hindgut acidosis, as high dietary starch levels can cause some starch to bypass pre-gastric fermentation and small intestinal enzymatic hydrolysis (Reynolds, 2006). Further data generated by Li et al., (2012) demonstrated a grain-induced model of SARA increased cecal LPS concentration and induced a systemic inflammatory response more than alfalfa-induced SARA; however, both grain- and alfalfa-based models of SARA induce similar decreases in cecal digesta pH, suggesting rapid bacterial proliferation and increased LPS concentrations, not altered pH, may be the cause of hindgut permeability. The rumen epithelium consists of four distinct layers (basale, spinosum, granulosum, and corneum) which together make up an ~85 μm thick epithelial layer (Steele et al., 2016). The outer two layers of epithelial cells include the stratum granulosum and keratinized stratum corneum which work together to prevent permeability (Graham and Simmons, 2005; Elias, 2005; Steele et al., 2016). In contrast, the large intestine has a single layer of columnar epithelial cells ~20 μm thick and

is protected by an inner and outer layer of mucus, the latter of which supports microbial habitation (Steele et al., 2016). Due to the lack of protozoa and salivary bicarbonate, the large intestine also possesses less buffering capacity than the rumen, despite similar bacterial concentrations and capacity for carbohydrate fermentation (Gressley et al., 2011). These differences suggest relative fragility in the hindgut compared to the rumen.

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 reduced feed intake only explains approximately 50% of the decreased milk yield during heat stress (Rhoads et al., 2009; Wheelock et al., 2010; Baumgard et al., 2011). During HS, blood flow is diverted from the viscera to the periphery in an attempt to dissipate heat leading 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 TJ dysfunction and gross morphological changes that ultimately reduce intestinal barrier function (Lambert et al., 2002; Pearce et al., 2013a). As a result, HS increases the passage of luminal content into portal and systemic blood (Hall et al., 2001; Pearce et al., 2013a). Endotoxin infiltration during HS into systemic circulation was first observed by Graber et al. (1971) and is now commonly reported in 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).

Feed restriction

There are a variety of occasions when feed intake is decreased, including gestation, heat stress, overcrowding, drought, shipping, poor management, and spoiled feed among others (Ingvarlsen et al., 1992; Grant and Albright, 1995; Swanson and Morrow-Tesch, 2001; Baumgard and Rhoads, 2013; Proudfoot et al., 2014; Hirata et al., 2015). Additionally, the periparturient period is a critical time of increased energy requirements, yet is a well-documented period of inadequate feed intake (Ingvarlsen et al., 2000). While overlying conditions responsible for reduced feed intake can markedly affect metabolism and inflammation, reduced feed intake has a direct effect on intestinal integrity in pigs (Pearce et al., 2013a) and cattle (Zhang et al., 2013). Malnutrition-induced changes in intestinal permeability has been previously observed in rats, guinea pigs, and humans (Rodriguez et al., 1996; Welsh et al., 1998; Boza et al., 1999). Mechanistically, fasting reduces mucosal surface area, villus height, cell numbers, cell proliferation, and cell migration rates and is coupled with increased rates of cell loss, apoptosis, and mucin depletion, altogether contributing to increased intestinal permeability (Sherman et al., 1985; Chappell et al., 2003; Ferraris and Carey, 2000). Although altered morphology does not prove increased permeability, malnutrition and intestinal permeability have been documented in humans and rodents (Welsh et al., 1998; Boza et al., 1999), and structural changes in villi morphology (i.e., decreased villus height, crypt depth, and their ratio) can be reflective of increased intestinal permeability (Ford et al., 1985; Pearce et al., 2013a). Numerous feed restriction models demonstrate detrimental effects on intestinal histology, including reduced villous height in feed restricted pigs (Carey et al., 1994; Pearce et al., 2013a), chickens (Yamauchi et al., 1996), mice (Ueno et al., 2011), and rats (Holt et al.,

1986). However, some feed restriction models indicate no effect or even an increase in villi height (Holt et al., 1986; Chappell et al., 2003; Hodin et al., 2011; Tůmová et al., 2015). Reasons for discrepant reports could be due to severity and duration of feed restriction as well as animal age. In particular, the effects of feed restriction in ruminants are difficult to assess because rumen fermentation continues for > 48 h following feed restriction (Bergman, 1990), and it can take over 4 d to truly fast a ruminant (Blaxter and Wainman, 1966). Others have demonstrated an acute phase protein response to short-term feed restriction in beef cattle, suggesting that feed and water deprivation are the main contributors to the acute phase response in receiving feedlot cattle (Cappelozza et al., 2011; Marques et al., 2012). The severity and duration of feed restriction in the literature vary considerably and effects on gut health are not extensively studied in the ruminant animal, ergo it is not clear what magnitude of feed restriction is required to dependably compromise intestinal barrier function and cause an inflammatory response in cattle.

Modeling Intestinal Permeability in Ruminants

A variety of situations in animal agriculture can reduce intestinal barrier function, the consequences of which likely stimulate the immune system, cause inflammation, and eventually compromise production. Example circumstances in addition to those previously mentioned include weaning (Boudry et al., 2004; Moeser et al., 2007) and the periparturient period (Abuajamieh et al., 2016). However, a myriad of behavioral, metabolic, and endocrine events accompanying the aforementioned situations make it difficult to study the consequences of poor intestinal barrier health in isolation. Presumably an impaired intestinal barrier will negatively impact economically important phenotypes. However,

directly studying post-absorptive and production consequences of leaky gut is difficult as conditions thought to be responsible for reducing intestinal barrier integrity also affect multiple tissues and systems. Obvious examples of biologically confounding situations include the periparturient period and heat stress, both of which are accompanied by marked homeorhetic adaptations to support a new dominant physiological state (Bauman and Currie, 1980; Baumgard and Rhoads, 2013).

Evaluating the metabolic, endocrine, inflammatory, and production consequences of intestinal dysfunction in isolation would provide insight into its direct impact on the pathophysiology of common on-farm disorders. Thus, there is a need to develop appropriate models to test potential leaky gut mitigation strategies. There are several chemically induced models of leaky gut (e.g., non-steroidal anti-inflammatory drugs, gamma-secretase inhibitors, dextran sodium sulfate; Fortun and Hawkey, 2007; Wirtz et al., 2007), but these approaches are associated with side effects or are logistically difficult to deliver, particularly in ruminants. Feed restriction provides an inviting alternative because it has previously been shown to cause intestinal dysfunction in a variety of species, including ruminants (see 'Feed Restriction' section), but a consistent severity and duration of feed restriction which causes negative effects on gut health is yet to be established.

Strategies to Mitigate Intestinal Permeability

The aforementioned situations of intestinal permeability demonstrate the fragility of intestinal barrier function. There are various direct strategies which have shown to improve intestinal barrier function, and these are highlighted in Table 2. In addition to these strategies specifically aimed at increasing gut barrier integrity, a number of tactics may be

employed to prevent the initial onset of intestinal barrier dysfunction. Rumen acidosis can be prevented through nutritional strategies such as bicarbonate, yeast, direct fed microbials, or ionophores (reviewed by Lean et al., 2014); therefore these strategies may benefit gastrointestinal health. Secondly, maximizing feed intake can be accomplished via feeding chromium or through electrolyte supplement (West et al., 1991; Hayirli et al., 2001) and this may help to mitigate feed restriction-induced changes in gut integrity. Both prevention of rumen acidosis and maximized feed intake can also be accomplished through proper feeding management (Zebeli et al., 2015), and an unpredictable feeding environment is associated with inflammation (Proudfoot et al., 2014). Lastly, improving immune function through immunomodulation may prevent inflammatory-induced changes in gut barrier function and help with efficient pathogen clearance. Dietary chromium and β -glucans modulate the immune system and exhibit beneficial effects on immune function (Borgs and Mallard, 1998; Murphy et al., 2010). Circulating immune cell numbers and function can also be improved with granulocyte colony-stimulating factor administration in cattle (Kehrli et al., 1991; Kimura et al., 2014). Dexamethasone is synthetic anti-inflammatory corticosteroid with the potential to reduce inflammation-induced gut barrier dysfunction (Chen et al., 1996). Preventing intestinal permeability should alleviate the negative effects; however, further research is needed on the gut-specific effects of these indirect, prevention-based strategies.

Table 2. Potential strategies to ameliorate intestinal permeability

Strategy	Presumed Mechanism of Action	References
Glucagon-like peptide 2	↑ intestinal integrity	1,2,3,27
Glutamine	↑ intestinal integrity	4,5,6
Zinc	↑ intestinal integrity	7,8,9,28
Dairy products	↑ intestinal integrity	10,11,12
Betaine	↑ intestinal integrity	13
Butyrate	↑ intestinal integrity	14,15,16
Vitamin A	antioxidant	17,18,19,20
Vitamin C	antioxidant	21,22,23
Vitamin E	antioxidant	24,25
Selenium	antioxidant	25,26
¹ Boushey et al., 1999	¹¹ Prosser et al., 2004	²⁰ Elli et al., 2009
² Cameron and Perdue, 2005	¹² Hering et al., 2011	²¹ Buffinton and Doe, 1995
³ Walker et al., 2015	¹³ Cronjé, 2005	²² Inoue et al., 1998
⁴ Van der Hulst et al., 1993	¹⁴ Ferreira et al., 2012	²³ Hosseinpour et al., 2012
⁵ Wang et al., 2015a	¹⁵ Wang et al., 2012	²⁴ Singh et al., 2012
⁶ Shu et al., 2016	¹⁶ Huang et al., 2015	²⁵ Liu et al., 2016
⁷ Sanz-Fernandez et al., 2014	¹⁷ McCollough et al., 1999	²⁶ Oztürk et al., 2002
⁸ Song et al., 2015	¹⁸ Warden et al., 1997	²⁷ Connor et al., 2017
⁹ Jafarpour et al., 2015	¹⁹ Thurnham et al., 2000	²⁸ Pearce et al., 2015
¹⁰ Playford et al., 1999		

Lipopolysaccharide

Structure and Origin

A Gram-negative bacterial cell is protected by an envelope consisting of two phospholipid membranes. The outermost facing membrane leaflet contains a special kind of glycolipid known as lipopolysaccharide (**LPS**), which is composed of three regions: lipid A, a core polysaccharide, and an O polysaccharide. Lipid A is the toxic portion of LPS that interacts with receptors to induce an immune response (Munford, 2005). Characteristics of lipid A acyl chains, such as number and length as well as phosphorylation state, contribute to its degree of toxicity, and the particular structure of lipid A in

Escherichia coli enables maximal activation with its receptor (Erridge et al., 2002). Interestingly, Gram-negative bacteria can modulate their lipid A-synthesizing enzyme expression in response to inflammation in order to reduce immune system activation and provide resistance to antimicrobial peptides (Cullen et al., 2015). The O polysaccharide region is associated with LPS virulence. The O polysaccharide can contain up to 200 sugars and extends beyond the cell which aids in phagocytosis resistance by white blood cells (Slonczewski and Foster, 2011). Changes in these sugar sequences result in different degrees of virulence, likely due to several factors including: adherence to certain tissues (i.e., epithelial tissue), resistance to phagocytes, serving as a water-solubilizing carrier for lipid A, protection from immune system components aimed at deactivating LPS, and antigenic variation allowing multiple opportunities for a pathogen to infect its host (Erridge et al., 2002).

Lipopolysaccharide is present in all Gram-negative bacterial species; however, *Escherichia coli* is of particular interest in nutritional physiology because of its abundant presence in the intestinal lumen. Humans live continuously with over 1 g of LPS within the gut (Erridge et al., 2007), and bacterial load increases from the proximal to distal intestine (Donaldson et al., 2016). The gut lumen contains more bacteria than the human body has eukaryotic cells, and these bacteria play an intricate role in nutrient digestion and utilization (Marchesi et al., 2016). Importance of bacterial contribution to nutrient utilization, and therefore exposure to LPS, is certainly greater in ruminant than monogastric animals due to pre-gastric fermentation. Lipopolysaccharide is shed from bacterial cells during normal growth and division (Crutchley et al., 1968; Tsuji and Harrison, 1978), and administering antibiotics can also induce LPS shedding due to bacterial death (van

Langevelde et al., 1998). Thus, natural exposure to endotoxin is dynamic and variable. Yet, one of the most common ways to model immune activation and inflammation is through bolus administration of LPS, which elicits a well-characterized and robust immune response (van Miert and Frens, 1968; Lohuis et al., 1988a,b). Human data suggest continuous LPS infusion prompts a less aggressive but more sustained release of inflammatory cytokines, likely more akin to natural LPS exposure (Taudorf et al., 2007). Continuous low-dose LPS exposure is not well-studied, particularly in ruminants, and having a better understanding how low-dose continuous LPS infusion affects metabolism and the immune response is critical to minimizing productivity losses when physiological states or environmental conditions naturally increase LPS exposure.

Innate Immune Response to Lipopolysaccharide

Recognition

Lipopolysaccharide elicits an immune response via its recognition by Toll-like receptor (TLR) 4. The TLR4 complex is present on various cells including leukocytes, myocytes, adipocytes, and intestinal and mammary epithelial cells (Lin et al., 2000; Ibeagha-Awemu et al., 2008; Abreu, 2010; Frisard et al., 2010; Murphy, 2012) and elicits a well-characterized toxic immune response in animals (Berczi et al., 1966). Though TLR is present in intestinal epithelial cells, its expression is much lower in comparison with other tissues in order to maintain a more tolerant environment (Abreu, 2010). Lipopolysaccharide binding protein (LBP) facilitates the interaction of LPS and free or membrane-bound cluster of differentiation 14 (CD14) that in turn aids interaction of LPS with the TLR4 receptor complex. Jack and colleagues (1997) used LBP knockout mice to

demonstrate LBP's crucial role in mounting an inflammatory reaction necessary to control live bacterial infection. The trans-membrane TLR4 and extracellular myeloid differential protein 2 (**MD2**) work together to recognize LPS, resulting in TLR4 dimerization and signal transduction using the intracellular adaptor protein myeloid differentiation factor 88 (**MyD88**) and IL-1 receptor-associated kinase. This ultimately results in nuclear transcription factor kappa-B (**NFκB**) activation and an increase in inflammatory cytokine transcription to promote immune cell responses (Lu et al., 2008b). Alternatively, Kawai and colleagues (1999) found MyD88-independent responses to TLR4 dimerization that still led to NFκB activation in MyD88-knockout mice. However, this MyD88-independent pathway resulted in hyporesponsiveness to LPS; similar to the response of endotoxin tolerant animals (Kawai et al., 1999; see 'Endotoxin Tolerance' section). Ultimately, inflammatory cytokines produced from this activation process are what affect local and systemic physiology, resulting in fever, acute phase protein production, and/or metabolic alterations.

Liver Response

Traditionally, the liver is characterized as a metabolic organ; however, its role in immunity and endotoxin detoxification is extremely important (Strnad et al., 2017). Within 10 min, more than 60% of i.v. injected bacteria can be found in the liver (Yan et al., 2014). Upon immunoactivation, the liver decreases synthesis of some proteins. These are termed negative acute phase proteins and include albumin, paraoxonase, and retinol binding protein. Positive acute phase proteins such as haptoglobin (**Hp**), serum amyloid A (**SAA**), and LBP aid in immune system propagation and endotoxin detoxification; thus, hepatic production of these proteins increases during the acute phase response (Ceciliani et al.,

2012). Circulating major acute phase proteins can increase several-fold during immune system activation, making them attractive markers of inflammatory response. Haptoglobin is secreted mainly by hepatocytes and functions to bind hemoglobin released from red blood cell lysis (Eaton et al., 1982; Bertaggia et al., 2014). In doing so, Hp is thought to reduce iron availability to invasive and proliferating bacteria. Interestingly, Hp can also modulate the immune response through inhibiting neutrophil respiratory burst and may also prevent muscle atrophy during exercise or high fat diet induced oxidative stress, making its role in immunity complex (Oh et al., 1990; Bertaggia et al., 2014). Serum amyloid A has been suggested to play a role in cholesterol clearance from lysed cells, promotion of immune cell infiltration into tissue, upregulation of mucin expression, and opsonization of bacteria such as *E. coli* (Manley et al., 2006; Shah et al., 2006; Shigemura et al., 2014; Ye and Sun, 2015; Gouwy et al., 2015). Acute-phase SAA is produced in several isoforms by the liver (SAA1 and SAA2) as well as extrahepatic sources (SAA3) including adipose and mammary tissue (Ceciliani et al., 2012).

Lipopolysaccharide binding protein was first characterized in 1986 by Tobias and colleagues after they noticed the rate at which LPS binds to high density lipoprotein was decreased in acute phase serum relative to normal serum, and that LPS-protein precipitate from normal serum dissolves readily in saline whereas LPS in precipitate from acute phase serum does not. Production of LBP mainly occurs in the liver, but it can also be synthesized by lung or intestinal epithelial cells, the kidney, and the reproductive tract (Wang et al., 1998; Vreugdenhil et al., 1999; Malm et al., 2005). The primary purpose of LBP is to transfer LPS to the TLR4/CD14+ receptor on various immune cells in order to actuate an immune response (Lu et al., 2008b). Interestingly, LBP can either play a pro- or anti-

inflammatory role following LPS activation (Gutsmann et al., 2001). Constitutive levels of LBP promote inflammation (Dentener et al., 1993) while acute phase levels of LBP inhibit LPS induced cytokine response in both mice and humans (Lamping et al., 1998; Zweigner et al., 2001). The anti-inflammatory effects of LBP are mediated by facilitating the transfer of LPS to lipoproteins which can clear LPS via non-inflammatory routes (Wurfel et al., 1994). This makes LBP a particularly useful protein because it prevents toxic shock during extensive LPS infiltration and induces inflammation to control moderate bacterial infection (Kitchens et al., 2005). Interestingly, Jack et al., (1997) demonstrated LBP knockout mice administered a high i.p. LPS dose have a 100% survival rate while wild-type controls die quickly from septic shock. On the other hand, when gram-negative bacteria were injected i.p., LBP knockout mice were unable to inhibit bacterial proliferation and died within 5 d while most wild-type controls survived. These results indicate the significance of LBP in immunoactivation to clear invading pathogens and is an important concept to remember when considering models of immune challenges as LBP can appear detrimental in one model and protective in another.

Detoxification

Several different mechanisms exist for an organism to eliminate LPS. At the gut level, enterocytes can secrete alkaline phosphatase, both into the lumen and into portal circulation, which dephosphorylates LPS and renders it immunologically inert (Eliakim et al., 1991; Goldberg et al., 2008). Furthermore, dephosphorylated LPS still binds to TLR4 (without initiating inflammation) and thus competitively inhibits intact LPS from binding (Lallès, 2014). Another enzyme important to LPS detoxification is acyloxyacyl hydrolase (**AOAH**), which is produced by macrophages, neutrophils, and dendritic cells (Munford

and Hall, 1986; Katz et al., 1999; Lu et al., 2003). Acyloxyacyl hydrolase detoxifies LPS by cleaving a secondary acyl group from LPS, rendering it inert and ~1% as immunogenic as fully acylated LPS (Munford and Hall, 1986; Lu et al., 2005). Interestingly, both LBP and sCD14 can rearrange LPS to better expose the lipid A portion for detoxification by AOA (Gioannini et al., 2007). As stated previously, the liver's role in immunity and endotoxin detoxification is extremely important (Strnad et al., 2017), and the majority of infiltrating pathogens end up in the liver relatively quickly (Yan et al., 2014) or originate from portal circulation (Jacob et al., 1977). The majority of LPS is cleared via resident liver macrophages (i.e., Kupffer cells) via both inflammatory and non-inflammatory mechanisms (which are not well-defined) and excreted back into the gastrointestinal tract lumen via bile (Mani et al., 2012; Guerville and Boudry, 2016). Interestingly, transition metals such as manganese, zinc, chromium, and copper may aid in the anti-inflammatory clearance by binding directly to LPS and preventing a cytokine response without affecting endocytosis (Thomas et al., 2008). Another major route of LPS detoxification is via lipoproteins, which sequester LPS quickly and are subsequently cleared via tissues expressing lipoprotein receptors (Munford et al., 2005). Lipopolysaccharide neutralization is affected by lipid emulsions (Read et al., 1995), chylomicrons (Vreugdenhil et al., 2003), very low density lipoproteins (**VLDL**; Harris et al., 1990), low density lipoproteins (**LDL**; Morel et al., 1986), high density lipoproteins (**HDL**; Guo et al., 2013), apolipoprotein B (Vreugdenhil et al., 2001), and apolipoprotein E (Rensen et al., 1997). Transfer of LPS from HDL to LDL can also occur, facilitated by LBP and other transfer proteins, but this transfer causes HDL to lose its cholesterol binding abilities and can cause dyslipidemia (Levels et al., 2005). Additionally, LBP and transfer proteins (e.g., phospholipid transfer

protein) can transfer LPS directly from bacterial membranes to lipoproteins (Vreugdenhil et al., 2003). The lipoprotein sequestration of LPS prevents TLR4 binding and immune system activation (Van Amersfoort et al., 2003), and infusing lipoproteins protects animals from the lethal effects of an LPS challenge (Harris et al., 1990, Casas et al., 1995; Van Amersfoort et al., 2003). Thus, LPS detoxification can occur via a variety of ways (both inflammatory and anti-inflammatory) with the ultimate end goal of neutralizing any infiltrating pathogens without excessive inflammation.

Endotoxin Tolerance

Endotoxin tolerance is defined as a reduced inflammatory response to LPS that arises from repeated LPS exposure. It was first characterized in rabbits by Beeson (1946) who reported a diminishing fever response upon repeated *E. typhosa* injection. A plethora of modern literature exists as well; for example, rats repeatedly injected with LPS before subsection to a cecal ligation model of sepsis demonstrated reduced mortality relative to rats who were not pre-exposed to LPS (Shi et al., 2011). Tolerance is characterized by decreased pro-inflammatory cytokines and TLR expression as well as increased anti-inflammatory cytokines (Biswas and Lopez-Collazo, 2009). Additionally, LPS tolerance increases expression of some genes termed “non-tolerizable” which are thought to protect against systemic infection, although this branch of tolerance is not well-studied and remains debated (Seeley and Ghosh, 2017). Induced tolerance in monocytes abolishes aerobic glycolysis normally induced during activation (Cheng et al., 2014; see ‘Immune System Energetics’ section), suggesting a link between tolerance, metabolic function, and immunity. The cellular mechanisms responsible for inducing tolerance are not well-defined and are currently being investigated, but may include: transcription factor and histone

modification, nucleosome remodeling, DNA methylation, CpG island promoters, micro RNAs, and long noncoding RNAs (Seeley and Ghosh, 2017). Daily LPS injections induce a greater degree of tolerance than weekly injections, although weekly injections are still effective (Beeson, 1947). Monocytes can develop LPS tolerance in as little as 1 h in vitro (del Fresno et al., 2009); however, endotoxin tolerance in vivo takes 24-36 h to develop. Once induced, tolerance can last several weeks (Beeson, 1947). How this occurs is also unclear, but may involve circulating microRNAs or alteration of the microbiome (Vatanen et al., 2016; Seeley and Ghosh, 2017). Tolerance has been described in several different species (van Miert and Frens, 1968) and appears to be an evolutionary advantage to limiting mortality due to septic shock. However, septic patient mortality is correlated with immunosuppression (Munoz et al., 1991; Boomer et al., 2011), and therefore contributes to confusion within the field.

Immune System Energetics

Immunometabolism

The Warburg effect

In normal resting cells, energy is most efficiently produced via the combined processes of glycolysis, the tricarboxylic acid (**TCA**) cycle, and oxidative phosphorylation which generates approximately 36 to 38 adenosine triphosphate (**ATP**) per molecule of glucose. In this process, glucose is catabolized via glycolysis to pyruvate, then to acetyl-CoA using the enzyme pyruvate dehydrogenase (**PDH**). Acetyl-CoA enters the TCA cycle as citrate by combining with oxaloacetate to eventually produce ATP via oxidative and

substrate-level phosphorylation. During hypoxia, cells shunt pyruvate away from mitochondrial oxidative phosphorylation toward lactate production; this is known as anaerobic glycolysis (Berg et al., 2002). Glycolysis converts glucose into two pyruvate molecules, each of which are reduced to lactate molecules by the enzyme lactate dehydrogenase. This reaction takes place in the cytosol, generating a net two molecules of ATP. Regeneration of NAD⁺ also takes place and allows glycolysis to proceed since it needs NAD⁺ to convert glyceraldehyde 3-phosphate to 1,3 biphosphoglycerate. Interestingly, a switch from oxidative phosphorylation to glycolysis, even in the presence of oxygen, occurs in cancer cells (Warburg, 1927), preimplantation embryonic development (Seshagiri and Bavister, 1991; Redel, 2012), and immune cells (Warburg, 1958; Sbarra and Karnovsky, 1959; Vander Heiden et al., 2009). This is known as the “Warburg Effect.”

The Warburg Effect is named for Otto Warburg who was the first to characterize this metabolic phenomenon (1927) in cancer cells. Through his work, Warburg provided evidence that under normal conditions with adequate oxygen supply, cancer cells produced lactate from glucose. This phenomenon is in contrast to what was previously described in normal resting cells which prefer the efficiency of the TCA cycle for maximal ATP production. Warburg noted later (1958) that this also occurred in activated lymphocytes. Today, we know that the Warburg effect is not limited to cancer cells. Rapidly proliferating cells often utilize the Warburg effect to support rapid growth. An initial thought was that rate of ATP production was greater in aerobic glycolysis compared to oxidative phosphorylation (Nakashima et al., 1984), and is therefore able to provide a source of “quick energy.” However, Lunt and Vander Heiden (2011) argued that the main purpose

for the adjustment to glycolysis is to generate increased concentrations of glycolytic intermediates to support cellular anabolic processes. In a proliferating cell, approximately 85% of glucose goes into glycolysis, 5% into oxidative phosphorylation for energy extraction, and 10% of the glucose is diverted into biosynthetic pathways before pyruvate production (Vander Heiden et al., 2009). Thus, glucose is an important substrate because its glycolytic intermediates are used to synthesize proteins, lipids, nucleic acids, and NADPH via the pentose phosphate pathway crucial for various immune cell functions (Palsson-McDermott and O'Neill, 2013). The ability to continually proliferate despite hypoxic environments is another advantage cells acquire from using glycolysis as it does not require oxygen consumption. Cancer cells create hypoxic environments due to rapid proliferation and inadequate vascularization. Further, sites of infection and inflammation may become nutrient or oxygen deficient due to oxygen consumption by neutrophils, increased metabolic activity of cells at the site, or necrosis of infected cells (Pearce et al., 2013b). Hypoxia-inducible factor 1 (**HIF-1**) is a transcription factor that responds to decreased oxygen availability and is also a major regulator of the Warburg effect (Semenza et al., 1994). Expression of glycolytic enzymes as well as glucose transporters, GLUT1 and GLUT3, are induced and increased via HIF-1 (Berg et al., 2002), allowing glycolysis to be more effective. Inducing HIF-1 occurs as a mediator of the Warburg effect in cancer (Maynard and Ohh, 2007) and immune cells (Imtiyaz and Simon, 2010). Even in normoxic conditions, succinate stabilizes HIF-1 (Selak et al., 2005) allowing HIF-1 to continue glycolytic regulation. A combination of all aforementioned advantages to utilizing the glycolytic pathway make the Warburg effect an operational method of energy extraction for proliferating cells independent of oxygen availability.

Active immune cells in both the innate and adaptive systems demonstrate the metabolic change from oxidative phosphorylation to glycolysis, including: dendritic cells, neutrophils, macrophages, T lymphocytes, and B lymphocytes (Kellett, 1966; van Raam et al., 2006; Krawczyk et al., 2010; Wang et al., 2011; Blair et al., 2012). The innate immune system is the first line of defense. Pattern recognition receptors, either membrane bound or in the cytosol, detect pathogen-associated molecular patterns (**PAMPs**) or damage-associated molecular patterns. Binding of a ligand to the receptor causes a signaling cascade to occur resulting in transcription factor activation (e.g. NF- κ B). Dendritic cells, important in cross talk between innate and adaptive immune systems, use a TLR agonist upon activation to change their metabolism from oxidative phosphorylation to glycolysis using the P13/Akt pathway, allowing for increased cellular function and synthesis and secretion of immune mediators (Krawczyk et al., 2010). Neutrophils are also dependent on aerobic glycolysis as they consume little oxygen and have few mitochondria (van Raam et al., 2006). Neutrophils increase NADPH generation via aerobic glycolysis and the pentose phosphate pathway for production of hydrogen peroxide for microbicidal function (Dale et al., 2008). In addition, macrophages exhibit the Warburg effect (Kellett, 1966). Interestingly, macrophages are able to differentiate into one of two types: M1 (classical) or M2 (alternative). The M1 macrophage is a “killer macrophage” stimulated by INF γ or particular TLR ligands. Differentiation of a macrophage to the M1 phenotype induces the switch to glycolysis. In contrast, the M2 macrophage only appears after cytokines such as IL-4, IL-13, or TGF β stimulate development and does not switch metabolism to glycolysis (Palsson-McDermott and O’Neill, 2013). The M2 phenotype is a convalescent macrophage

which produces the anti-inflammatory cytokine IL-10 and is responsible for constructive processes such as wound healing (Mills, 2012).

Primary cells of the adaptive immune system are B and T lymphocytes. T lymphocytes differentiate into regulatory cells, such as helper (**Th**) and regulatory (**Treg**), or effector cells, such as cytotoxic T (**Tc**), to help to provide immunity. Helper T cells activate the immune response and prompt differentiation of B cells. Once activated, B cells produce antibodies unique to the presented antigen, allowing them to bind to the antigen and remove it via immune effector cells and molecules. Helper T cells also stimulate differentiation of T lymphocytes into Tc cells utilizing cytotoxic proteins to kill infected cells. These Tc cells also release $INF\gamma$ to inhibit viral replication and activate macrophages. Immune system regulation and suppression occurs via Treg cells secreting the anti-inflammatory cytokine IL-10 to inhibit proliferation and activation of lymphocytes and macrophages (Borth, 2011).

Glycolysis is necessary for B cell survival *ex vivo* and is stimulated via the B cell antigen receptor with protein kinase $C\beta$ as a critical component (Blair et al., 2012). Metabolic reprogramming of T cells involving suppression of β -oxidation and stimulation of glycolysis is dependent on the Myc transcription factor, and may involve HIF-1 activation to maintain glycolytic activities after initiation of the cell cycle (Wang et al., 2011). T cells require glycolysis to be effective as a signaling mechanism to control cellular function through production of $IFN\gamma$. Changes in available GAPDH caused by glycolysis regulate cytokine mRNA translation otherwise inhibited by GAPDH binding to cytokine mRNA (Chang et al., 2013). This post-transcriptional control of T cell function by aerobic

glycolysis is a great example of how metabolism can have a profound impact on cell signaling.

Alternative Role for the TCA Cycle

Warburg's suggestion that mitochondrial metabolism is negligible during the shift to glycolytic pathway has recently been challenged (Koppenol et al., 2011). In fact, when the Warburg effect is exhibited in cancer and immune cells, the TCA cycle can become an anabolic instead of catabolic process. Intermediates of the TCA cycle, such as citrate and succinate, have significant roles in macrophages during an LPS challenge (Infantino et al., 2011; Selak et al., 2005). These intermediates provide substrate for biosynthesis of several different molecules and play an important role in inflammatory signaling (Palsson-McDermott and O'Neill, 2013). The efflux of TCA cycle intermediates for biosynthesis is termed "cataplerosis" while the replenishing of intermediates is termed "anaplerosis." Pyruvate, amino acids, and glutamine all contribute to anaplerosis (Owen et al., 2002).

Recently, citrate has demonstrated importance in macrophage function. The work of Infantino and colleagues (2011) has demonstrated LPS-stimulated macrophages increase mitochondrial citrate carrier (**CIC**) to facilitate citrate transport from the mitochondrial matrix to the cytosol in exchange for malate. Upon citrate transportation into the cytosol, it is cleaved into acetyl-CoA and oxaloacetate by the enzyme citrate lyase. Fatty acid biosynthesis can then utilize acetyl-CoA for prostaglandin synthesis while oxaloacetate produces NADPH and H⁺ needed for nitric oxide (**NO**) generation and reactive oxygen species (**ROS**) formation via NADPH oxidase. Further, work by Infantino and colleagues (2011) demonstrated that CIC gene silencing and CIC inhibition reduces the production of nitric oxide, reactive oxygen species, and prostaglandins necessary for macrophage

immune function. Consequently, citrate is critical for macrophage activation by LPS and plays an important part in immune function.

Macrophages stimulated by LPS also demonstrate an increase in the TCA cycle intermediate succinate (Tannahill et al., 2013). In addition, increased circulating succinate is present in rodent models of hypertension and metabolic disease (Sadagopan et al., 2007). Sources of succinate arise either from anaplerosis using glutamine as a substrate or generation through the γ -aminobutyric acid shunt (Palsson-McDermott and O'Neill, 2013). Succinate can be exported from the mitochondria into the cytosol in exchange for inorganic phosphate through the dicarboxylate carrier (Berg et al., 2002). Work done by Selak et al. (2005) demonstrates elevated succinate levels create negative feedback via product inhibition on prolyl hydroxylases (**PHDs**). Inhibiting PHDs stabilizes HIF-1, preventing its hydroxylation. Stabilization of HIF-1 allows it to continue down regulating oxidative phosphorylation by inducing pyruvate dehydrogenase kinase 1 and 3 that both inactivate PDH (Kim et al., 2006; Lu et al., 2008a; Papandreou 2006), discouraging entry of pyruvate into the TCA cycle and allowing for continued HIF-1 stabilization. Tannahill and colleagues (2013) also demonstrated HIF-1 stabilization and induction of inflammatory cytokine IL-1 β due to succinate signaling. Increases in succinate also lead to a posttranslational modification known as protein lysine-succinylation (Zhang, et al., 2011). Not much is known about the effects of succinylation; however, it is a widespread posttranslational modification dependent on succinyl-CoA concentration and there is frequent overlap between succinylation and acetylation (Weinert et al., 2013). Succinate also has the ability to act as a signaling molecule in dendritic cells through the receptor GPR91 acting with TLRs to induce production of inflammatory cytokines (Rubic et al.,

2008). In summary, succinate can act as a stabilizer of HIF-1, demonstrate protein succinylation, and activate GPR91 to induce an inflammatory response.

Metabolism includes multifaceted and complex interactions between multiple systems, tissues, and organs. The Warburg effect, induced in inflammation and cancer, proves that metabolic pathways can be used to serve anabolic process in unexpected ways. Increased rates of glycolysis observed in cancer and immune cells help them not only to generate energy swiftly, but also to synthesize biomolecules needed for proliferation and other tasks. Any hypoxic environment experienced by cancer or immune cells is also neutralized by their ability to generate energy and molecules without oxygen. In addition, immune cells displaying the Warburg effect such as T cells rely on its characteristics for inflammatory signaling. Inhibiting the Warburg effect in inflammatory diseases is an exciting field of opportunity because it allows for many potential points of regulation. Better knowledge of the Warburg effect and its consequences on metabolism are necessary to developing novel approaches to mitigate inflammatory disorders.

Glucose consumption

Due to the shift in metabolism described in the previous sections, various in vitro experiments demonstrate a substantial increase in glucose consumption by activated immune cells as glucose is their primary fuel and an important biosynthetic precursor (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). Insulin, a major glucoregulatory hormone, positively affects function and/or glucose uptake in lymphocytes, neutrophils, and monocytes (Strom et al., 1975; Helderman, 1981; Daneman et al., 1992; Walrand et al., 2004). Furthermore, activated monocytes, T- and B-lymphocytes, and neutrophils demonstrate increased GLUT1, GLUT3, and GLUT4 cell

surface expression, and insulin augments expression of GLUT3 and GLUT4 (Maratou et al., 2007). Monocytes from lactating dairy cows also have increased gene expression of GLUT3 and GLUT4 upon endotoxin stimulation in vitro (O'Boyle et al., 2012), although others have observed no effect (Eger et al., 2016). Both glucose and insulin levels are low during the periparturient period in dairy cows, but how this directly affects immune function is not yet clear. Kimura et al. (1999) demonstrated metabolic stress of milk production may contribute to immune cell dysfunction as mastectomized cows recovered neutrophil function more quickly than lactating cows. In vitro data suggest glucose supplementation to neutrophils from early and mid-lactation cows increases phagocytosis but has few other effects (Garcia et al., 2015).

Previous estimates of glucose utilization in various immune cells and tissues both in vitro and in vivo and are summarized in Table 3. These studies firmly established that activated leukocytes primarily oxidize large quantities of glucose. Furthermore, the in vitro tissue studies firmly established tissues with a large immune compartment markedly increase glucose utilization. However, the extent of in vivo glucose consumption by the activated immune system is difficult to assess due to the ubiquitous and fluctuating distribution of immune cells and organ specific changes in insulin sensitivity. Furthermore, in vivo data are difficult to interpret due to different experimental procedures and conditions. Whole-body glucose utilization has been estimated using 2-deoxyglucose tracers in rodents by Lang et al. (1991, 1993) and in monkeys by Wannemacher et al. (1980). However, whole body glucose disposal measurements are governed by two opposing influences: tissues which increase glucose uptake and tissues which decrease glucose uptake. Glucose dynamics during immunoactivation involve orchestrated systemic

changes including peripheral insulin resistance and increased hepatic glucose output which successfully provides glucose in excess of immune cell utilization during the hyperglycemic phase of immunoactivation. Lipopolysaccharide-induced hypoglycemia represents the inability of glucose sparing mechanisms (reduced glucose uptake by insulin sensitive tissues and increased hepatic rates of glycogenolysis and gluconeogenesis) to keep pace with glucose utilization of the activated immune system. Thus, tissue specific rates of glucose utilization are currently much more informative than whole-body glucose disposal, and there is a need for an accurate way to specifically quantify whole-body immune cell glucose consumption in vivo. Furthermore, rodent models of glucose disposal may differ from most other species, as rodents do not seem to consistently exhibit hyperinsulinemia during immunoactivation (Lang et al., 1993; Table 4). Better understanding the impact of immunoactivation on whole-animal physiological glucose consumption has practical implications to animal agriculture as glucose is an important fuel for productive purposes. Whether glucose can become a limiting factor in the immune response is not clear and thus the benefits of supplemental glucose to sick animals remains unknown. However, from an animal production perspective, infection and inflammation noticeably redirect resources toward the immune system and away from utilization and synthesis of economically relevant products. Having a better understanding of the energetic and nutrient requirements of the immune response is critical to developing strategies to minimize productivity losses when physiological states or environmental conditions activate the immune system.

Table 3. Effect of immune activation on glucose utilization in leukocyte in vitro/ex vivo and in various tissues in vivo

Leukocytes in vitro/ex vivo				
Leukocyte	Response	Average % Change	% Change Range	Reference
T-lymphocytes	↑	+2,296%	100-5,153%	1,2,18
B-lymphocytes	↑	+858%	150-1,400%	15,17,19
Neutrophils	↑	+248%	142-339%	6,9,10
Macrophages	↑	+219%	49-600%	4,5,6,7,8,9,10,16
Dendritic cells	↑	+71%	67-80%	20,21,22
Tissues in vivo				
Tissue	Response	Average % Change	% Change Range	Reference
Lung	↑	+84%	60 – 140%	7,9,11,12,13,14
Liver	↑	+153%	60 – 400%	7,9,11,12,13,14
Ileum	↑ / =	+62%	40 – 80%	7,11,12,13,14
Spleen	↑	+92%	52 – 150%	7,9,11,12,13,14
Skin	↑ / =	+76%	50 – 125%	7,11,12,13,14
Muscle ^b	↑ / ↓ / =	+54% / -44%	-44 – 75%	3,7,9,11,12,13,14
Kidney	↑ / ↓ / =	75 % / -20%		11,13,14
Adipose ^c	↓ / =	-40%		3,11,13,14
Brain	=			11,12
Testis	=			7,9,11

^aMethods of immune activation include-

leukocytes in vivo/ex vivo: concanavalin A, anti-Ig, lipopolysaccharide, insulin, tumor necrosis factor, granulocyte macrophage colony-stimulating factor, latex

tissues in vivo: tumor necrosis factor, cecal ligation puncture, granulocyte macrophage colony-stimulating factor, live *E. coli*, lipopolysaccharide

^bGastrocnemius

^cWhite adipose tissue

¹Brand, 1985

²Greiner et al., 1994

³Stevens et al., 2017

⁴Costa Rosa et al., 1995

⁵Spolarics et al., 1991a

⁶Spolarics et al., 1991b

⁷Spolarics et al., 1991c

⁸Spolarics et al., 1992a

⁹Spolarics et al., 1992b

¹⁰Mészáros et al., 1991

¹¹Lang et al., 1990

¹²Lang et al., 1991

¹³Lang et al., 1993

¹⁴Maitra et al., 2000

¹⁵Caro-Maldonado et al., 2014

¹⁶Fukuzumi et al., 1996

¹⁷Doughty et al., 2006

¹⁸Hume et al., 1978

¹⁹Dufort et al., 2007

²⁰Jantsch et al., 2008

²¹Krawczyk et al., 2010

²²Everts et al., 2012

Whole-body Response to Inflammation

The energetic response to inflammation has been studied for centuries. As early as the 1830s, medical doctors such as Robert James Graves (for whom Graves' disease is named) began teaching about the importance of maintaining energy intake during illness (Graves, 1842). Ever since, there has been accumulating appreciation for the complex and dynamic metabolic response to inflammation. Some metabolic effects of immune activation via endotoxin administration are highlighted in Table 3. However, it is important to understand metabolic responses generally exhibit a biphasic pattern during the course of infection. Interpreting the summary of studies listed in Table 1 is complicated by the fact that measurements were from samples obtained across various time points during or after immunoactivation. Metabolic changes are best described as a series of longitudinal measurements, and it is important to avoid characterizing samples obtained from one phase of sepsis as the overall metabolic response to sepsis. Certainly, metabolic responses and timing also depend largely upon the model of immunoactivation used. Lipid, protein, and carbohydrate metabolism are markedly altered by immunoactivation, and this is discussed below.

Table 4. The effects of endotoxin on plasma metabolites and regulatory hormones in various species

Metabolite	Species	Response	Reference
Insulin	Bovine	↑ / ↓	3,4,11,19,22,23,27 / 28
	Dogs	↑	20
	Horses	↑	9
	Pigs	↑ / ↓	36 / 8,36
	Rodent	= / ↑↓	38,40 / 39
	Sheep	↑	10,16,18
Glucagon	Bovine	↑	3,4
	Dogs	↑	20
	Rodents	↑	38,39,40
	Sheep	↑	16
Epinephrine	Bovine	↑	31
	Human	↑	25
	Pigs	↑	26
	Rodent	↑	39
Cortisol	Bovine	↑	1,3,4,11,22,23,24,27,31,33
	Human	↑	25
	Pig	↑	8,26
	Sheep	↑	10
Glucose	Bovine	↑↓ / ↑ / ↓	1,2,3,19,23,24,27,34 / 4,6,22 / 21,28,29
	Dogs	↓	17,20
	Pigs	↑↓ / ↓	36 / 7,8
	Rodents	↑↓	38,39
	Sheep/Goats	↑↓ / ↓ / ↑	15,16,32 / 10,18,30 / 30
NEFA	Bovine	↑ / = / ↓	2,3,5,6,23,28 / 4,19 / 11,22
	Pigs	↑	8,36
	Rodents	↓	37
	Sheep	↓ / =	15 / 30
BHB	Bovine	↓	3,4,5,6,22,23,24
	Rodent	↓	37
	Sheep	↓	15
L-lactate	Bovine	= / ↑	3/ 1,21,23,24,34
	Pigs	↑	7
	Rodent	↑	40
	Sheep	↑ / =	16 / 15
Calcium	Bovine	↓	2,12,14,34
	Dogs	↓	17
	Horses	↓	9
	Pigs	↓	13
	Sheep	↓	15
BUN	Bovine	↑ / ↓	2,19,27,28,33 / 4
	Goat	=	30,35
	Pigs	↑	7,36
1 Giri et al., 1990	11 Vernay et al., 2012	21 Gerros et al., 1995	31 Burdick et al., 2011
2 Elsasser et al., 1996	12 Waldron et al., 2003b	22 Waldron et al., 2006	32 Wang et al., 2015b
3 Waldron et al., 2003a	13 Carlstedt et al., 2000	23 Steiger et al., 1999	33 Waggoner et al., 2009a
4 Zarrin et al. 2014	14 Griel et al., 1975	24 Werling et al. 1996	34 Tennant et al., 1973
5 Graunard et al., 2013	15 Naylor and Kronfeld, 1986	25 Richardson et al., 1989	35 van Miert et al., 1983
6 Moyes et al., 2014	16 Naylor and Kronfeld, 1985	26 Williams et al., 2009	36 Myers et al., 1997
7 Bruins et al., 2003	17 Holowaychuk et al., 2012	27 Waggoner et al., 2009b	37 Kaminski et al., 1979
8 Leininger et al., 2000	18 Southorn and Thompson, 1986	28 Ballou et al., 2008	38 Yamashita et al., 2015
9 Toribio et al., 2005	19 Burdick Sanchez et al., 2014	29 Bieniek et al., 1998	39 Maitra et al., 2000
10 Yates et al., 2011	20 Blackard et al., 1976	30 Wang et al., 2016	40 Lang et al., 1991

Carbohydrate Metabolism

The earliest observation of altered carbohydrate metabolism during sickness occurred in 1926 when Francis Hector observed decreased glucose clearance following a glucose tolerance test in diphtheria patients (Hector, 1926). Similarly in 1928, Sweeney recognized impaired glucose disposal during endotoxemia. Since then, it has been well-established that inflammatory conditions cause whole-body insulin resistance (McGuinness, 2005) and this has been confirmed in mid-lactation dairy cows (Vernay et al., 2012). Specifically, peripheral insulin resistance occurs in skeletal muscle and adipose tissue, the two largest “sinks” of insulin-stimulated glucose disposal (Lang et al., 1990; Song et al., 2006). However, some studies observe an increase in adipose tissue glucose utilization (Maitra et al., 2000). This is complicated by the fact that the immune system is ubiquitous and dynamic with leukocytes infiltrating various tissues during systemic inflammation (Peterson et al., 2003; Cani et al., 2007; Caesar et al., 2012; Pillon et al., 2013). Furthermore, macrophages can make up to 40% of adipose tissue cells in obese individuals (Weisberg et al., 2003). To highlight cell type differences within a tissue, Mészáros and colleagues (1991) examined different cell fractions within the liver after an i.v. LPS challenge and demonstrated glucose uptake did not change in parenchymal cells but markedly increased in Kupffer cells (~7-fold) and neutrophils (~5-fold). In general, tissues with a large immune compartment (spleen, liver, lung, and ileum) increase glucose utilization following LPS administration (Lang et al., 1993). Collectively, this data suggests immune cells markedly increase glucose utilization following immunoactivation and changes in whole body glucose disposal are primarily due to insulin resistance in tissues with the largest mass (i.e., adipose and muscle). Additionally, activated immune

cells exploit aerobic glycolysis in order to utilize glucose, and this results in increased circulating lactate (see 'Immune System Energetics' and Table 4). Further research highlighting glucose utilization of different cell types within various tissues would be useful in better characterizing glucose utilization during immunoactivation. Synchronously with peripheral insulin resistance, hepatic glucose output increases via both glycogenolysis and gluconeogenesis, depending on the stage and severity of the infection (Filkins, 1978; McGuinness, 1994; Waldron 2003a). This is facilitated by characteristic increases of glucagon, epinephrine, and cortisol (see Table 4). Interestingly, following glucose infusion, there is impaired suppression of hepatic glucose output during sepsis, endotoxemia, and burn injuries (Long et al., 1976; Lang et al., 1987b; Wolfe and Burke, 1978). However, the liver is less sensitive to epinephrine-induced increases in glucose turnover (Hargrove et al., 1989), likely because high epinephrine exposure during immunoactivation down-regulates adrenergic receptors and desensitizes tissues to subsequent re-exposure (Gurr and Ruh, 1980; Snavely et al., 1985). Interestingly, increased circulating insulin is observed during immunoactivation. This is peculiar because glucose and glucagon typically act contrariwise and predominance of one over the other depends upon the prandial state. Endotoxin-induced hyperinsulinemia has been described in a variety of animal models (see Table 4). Additionally, glucose infusion can exacerbate this hyperinsulinemia (Blackard et al., 1976), suggesting an interaction between LPS and glucose infusion as recently proposed (Baumgard et al., 2016). However, it is not likely LPS-induced hyperglycemia is the sole cause of hyperinsulinemia during immunoactivation as prevention of hyperglycemia by fasting (thus depletion of hepatic glycogen storage) does not prevent increased circulating insulin (Hand et al., 1983; Stoakes et al., 2015a). The exact cause of

increased insulin secretion remains unclear, but is likely due to direct action of LPS on the pancreas (Vives-Pi et al., 2003; Bhat et al., 2014) or LPS-stimulated secretion of the insulin secretagogue glucagon-like peptide 1 (Nguyen et al., 2014). Rationale for why circulating insulin increases are not clear, but insulin becomes important for development and sensitivity to glucose uptake in immune cells during activation (Shimizu et al., 1983; Helderman, 1984; Calder et al., 2007; Maratou et al., 2007). Therefore, hyperinsulinemia coupled with peripheral insulin resistance may be a homeorhetic strategy employed to ensure adequate glucose delivery to the immune system. Despite the aforementioned efforts to spare glucose, hypoglycemia often develops following an LPS challenge, likely because the rate of glucose utilization by the immune system exceeds the orchestrated capacity of the liver to export glucose and insulin sensitive tissues to reduce glucose disposal (McGuinness, 2005).

Protein Metabolism

In 1857, Parkes first demonstrated negative nitrogen balance during immune activation, and Voit furthered this knowledge in 1895 by showing dietary carbohydrates alleviate nitrogen loss in endotoxic dogs (Beisel and Wannemacher, 1980). We now know that skeletal muscle is catabolized during infection to provide amino acids as substrates for gluconeogenesis (Wannemacher et al., 1980) as well as to provide amino acids for synthesis of leukocytes and acute phase proteins (Iseri and Klasing, 2013). In humans, it has been estimated up to 850 mg acute phase proteins are synthesized per kg body weight in a typical acute phase response, and the mismatch in amino acid composition between muscle and acute phase proteins means more than 1,900 mg of skeletal muscle protein must be mobilized to provide a sufficient quantity of phenylalanine to support synthesis of the

acute phase proteins (Reeds et al., 1994). Thus, blood urea nitrogen consistently increases in monogastric models of endotoxemia (see Table 4) due to increased protein catabolism. Results in ruminants generally agree, but are more variable, as circulating BUN is complicated by the contribution of rumen urea flux and endotoxin-induced hypophagia reduces substrate delivery and rumen microbiota and function (Galyean et al., 1981; Jing et al., 2014), with the potential to mask catabolic-induced changes in BUN. Thus, it is important in ruminant studies to utilize pair-feeding models in order to account for decreased feed intake. This is again complicated by the rumen, as it takes 4 or more days to truly fast ruminants (Blaxter and Wainman, 1966).

Lipid Metabolism

Circulating levels of triglycerides and VLDLs are increased during endotoxemia in several monogastric species and calves (Kaufmann et al., 1976; Lanza-Jacoby et al., 1998; Ballou et al., 2008) due to increased hepatic triglyceride secretion or reduced lipoprotein lipase activity in adipose and muscle, depending on the LPS dose (Feingold et al., 1992). Conversely, decreased triglyceride levels in response to LPS infusion have been observed in heifers and goats (Steiger et al., 1999; Wang et al., 2017), although endotoxin-induced circulating triglyceride data in mature ruminants is scarce. Decreased circulating triglycerides in ruminants relative to non-ruminants may be due to the limited capacity for hepatic synthesis and secretion of VLDL in ruminant animals (Kleppe et al., 1988). Adipose tissue lipolysis during immunoactivation is variable. Some report increased circulating NEFA while others report decreases (see Table 4). In general, it seems circulating NEFA increase over time in immunoactivated animals, but this response is blunted when compared to pair-fed animals. Hyperinsulinemia likely contributes to

situations of decreased plasma NEFA observed in LPS-administered animals as insulin is a potent antilipolytic hormone (Vernon, 1992). Furthermore, increased circulating lactate during endotoxemia may contribute to the blunted lipolytic response because it mediates insulin's antilipolytic effects by binding to the adipocyte G protein-coupled receptor GPR81 (Ahmed et al., 2010). Despite decreased circulating NEFA relative to pair-fed animals, NEFA accumulation into hepatic triglycerides is accelerated during immunoactivation (Guckian, 1973; Fiser et al., 1974; Lanza-Jacoby and Tabares, 1990) and a variety of infection/inflammation models have demonstrated fatty liver as a consequence (Hirsch et al., 1964; Ilan, 2012; Fukunishi et al., 2013; Wenfeng et al., 2014; Damms-Machado et al., 2017). Despite accelerated hepatic triglyceride accumulation, ketogenesis is compromised during infection in rodent models (Neufeld et al., 1976; Takeyama et al., 1990; Gitomer et al., 1995). Circulating insulin may play an important role in inhibiting ketogenesis during sepsis. Neufeld et al., (1980) demonstrated low blood ketones in infected rats was abolished when insulin production was inhibited via streptozotocin injection. Several ruminant models have demonstrated reduced circulating BHB following endotoxin administration (Waldron et al., 2003a; Graugnard et al., 2013), but reasons for decreased circulating ketones are less clear because a majority of circulating BHB during positive energy balance originates from the rumen epithelium as opposed to the liver (Pennington, 1952). Therefore, decreased BHB may simply be due to illness-induced hypophagia, and this explains decreases in pair-fed animals as well. Administering LPS also decreases rumen motility (Jing et al., 2014), and whether or not this gastrointestinal stasis affects ketone synthesis is not known. Waldron et al., (2003a) observed no LPS effects on ketone synthesis in incubated liver slices obtained from

lactating cows, indicating ketogenesis remains functional. Additionally, an increased rate of BHB infusion was required to maintain hyperketonemia in lactating cows challenged with LPS (Zarrin et al., 2014), indicating increased circulating BHB clearance and peripheral tissue utilization could help explain the decrease as this may be a strategy utilized to spare glucose for immune cells. Furthermore, we have shown increased BHB levels in cows infused continuously with LPS for 7 d relative to pair-fed controls (see chapter 6). Because ruminant lipid metabolism differs markedly from rodents and humans, a better understanding of how endotoxemia specifically influences ruminant metabolism has obvious practical implications to a variety of dairy cow pathological conditions.

Conclusion

Immunoactivation can be caused by a variety of situations, but intestinal permeability is gaining rapid attention, as a wide variety of situations can lead to compromised intestinal barrier function and subsequent endotoxin translocation into portal and systemic circulation. Immune insults originating from intestinal bacteria have become of interest as heat stress, feed restriction, and peripheral inflammation contribute to increased intestinal permeability. While much attention has been paid to obvious sources of inflammation, relatively little is known about intestinal dysfunction and its contribution to immunoactivation, particularly in ruminants. A partial objective of this dissertation was to determine, in lactating dairy cows, if feed restriction causes intestinal dysfunction and to determine metabolic consequences of intentionally-induced intestinal dysfunction (Chapter 2 and 3).

Intestinal dysfunction can result in translocation of PAMPs which activate the immune system and cause an inflammatory situation. This has obvious implications to animal agriculture, as homeorhetic adaptation in support of an immune response takes precedence over energy directed towards economically productive purposes. Glucose metabolism is especially altered during inflammation, and this is mainly due to the increased requirement by immune cells coupled with peripheral insulin resistance. Accurately quantifying the amount of glucose the immune system diverts from milk or lean tissue synthesis would provide a greater understanding of practical dietary requirements and management strategies to better maintain glucose homeostasis in immune-challenged animals. Ultimately, the experiments within this dissertation will provide valuable and pragmatic information enabling us to model dietary requirements during times of infection. This will allow nutritionists and producers to more efficiently predict and compile least costs rations to maximize productivity and profitability while simultaneously promoting optimal animal health. Therefore, another objective of this dissertation was to determine the glucose requirements of an activated immune system (Chapter 4 and 5). Finally, current knowledge of endotoxin effects on metabolism are limited to bolus or short term LPS infusion models, yet during natural infection, endotoxin release is concomitant with dynamics of bacterial growth and proliferation. Key knowledge gaps remain in distinguishing how accurate single or repeated LPS boluses mimic chronic, naturally-occurring exposure and therefore a final objective to this dissertation was to observe if chronically administered endotoxin would also result in detrimental metabolic changes similar to bolus models (Chapter 6).

**CHAPTER 2: INTENTIONALLY INDUCED INTESTINAL BARRIER
DYSFUNCTION CAUSES INFLAMMATION, AFFECTS METABOLISM, AND
REDUCES PRODUCTIVITY IN LACTATING HOLSTEIN COWS**

A paper published in 2017 by Journal of Dairy Science

(In Press) doi: <http://doi.org/10.3168/jds.2016-12349>

S. K. Kvidera,* M. J. Dickson,* M. Abuajamieh,* D. B. Snider,* M. V. Sanz Fernandez,*
J. S. Johnson,* A.F. Keating,* P. J. Gorden,† H. B. Green,‡ K. M. Schoenberg,‡ and L. H.
Baumgard*

*Department of Animal Science, Iowa State University, Ames 50011

†College of Veterinary Medicine, Iowa State University, Ames 50011

‡Elanco Animal Health, Greenfield, IN 46140

Abstract

Study objectives were to evaluate the effects of intentionally reduced intestinal barrier function on productivity, metabolism, and inflammatory indices in otherwise healthy dairy cows. Fourteen lactating Holstein cows (parity 2.6 ± 0.3 ; 117 ± 18 days in milk) were enrolled in two experimental periods. Period 1 (P1; 5 d) served as the baseline for period 2 (P2; 7 d) during which cows received one of two i.v. treatments twice daily: 1) sterile saline or 2) gamma-secretase inhibitor (GSI; 1.5 mg/kg body weight). Gamma-secretase inhibitor reduces intestinal barrier function via inhibiting crypt cell differentiation into absorptive enterocytes. During P2, control cows receiving sterile saline were pair-fed

(PF) to the GSI-treated cows and all cows were euthanized at the end of P2. Administering GSI increased goblet cell area 218, 70, and 28% in jejunum, ileum, and colon, respectively. In the jejunum, GSI-treated cows had increased crypt depth and reduced villus height, villus height-to-crypt depth ratio, cell proliferation, and mucosal surface area. Plasma lipopolysaccharide binding protein increased with time, and tended to be increased 42% in GSI-treated cows relative to PF-controls on d 5-7. Circulating haptoglobin and serum amyloid A concentrations increased (585 and 4.4 fold, respectively) similarly in both treatments. Administering GSI progressively reduced dry matter intake (66%) and, by design, the pattern and magnitude of decreased nutrient intake was similar in PF-controls. A similar progressive decrease (42%) in milk yield occurred in both treatments, and there were no treatment effects detected on milk components. Cows treated with GSI tended to have increased plasma insulin (68%) and decreased circulating non-esterified fatty acids (29%) compared to PF cows. For both treatments, plasma glucose decreased with time while β -hydroxybutyrate progressively increased. Liver triglycerides increased 221% from P1 to sacrifice in both treatments. No differences in liver weight, liver moisture, or body weight change were detected. In summary, intentionally compromising intestinal barrier function caused inflammation, altered metabolism and markedly reduced feed intake and milk yield. Further, we have demonstrated that progressive feed reduction appears to cause leaky gut and inflammation.

Keywords: inflammation, insulin, intestinal integrity, lipopolysaccharide

Introduction

There is an increasing appreciation for the importance of proper intestinal barrier function in domestic farm animals. The gastrointestinal tract's (GIT) luminal content technically remains extrinsic to the animal, and thus the GIT serves a dual role of absorbing valuable nutrients while simultaneously preventing infiltration of unwanted compounds and molecules (Mani et al., 2012). The human GIT has a surface area of ~400 m², which is 200 times greater than skin (Murphy, 2012), and is continuously subjected to potentially pathogenic microorganisms and toxins (Mani et al., 2012). Barrier importance is heightened in cattle as both the size of the GIT and potential toxin exposure are more extensive in ruminants due to pre-gastric fermentation compartments. Therefore, it is not surprising that a large majority of the immune system resides in the splanchnic bed (van der Heijden et al., 1987).

There are a variety of diseases, albeit with etiological differences, where the common dominant pathology is impaired intestinal barrier function (i.e., leaky gut), including Crohn's disease, inflammatory bowel syndrome, celiac disease, and alcoholism (Draper et al., 1983; Bargiggia et al., 2003; Ludvigsson et al., 2007; McGowan et al., 2012). Recognized circumstances in animal agriculture where GIT barrier function is compromised include weaning (Boudry et al., 2004; Moeser et al., 2007), heat stress (Baumgard and Rhoads, 2013; Pearce et al., 2013a), and rumen acidosis (Emmanuel et al., 2007a,b; Khafipour et al., 2009; Minuti et al., 2014). Additionally, reduced feed intake has been shown to decrease barrier integrity in humans (Welsh et al., 1998) and farm animals (Pearce et al., 2013a; Zhang et al., 2013; Stoakes et al., 2015b). Further, we have preliminary evidence strongly implicating leaky gut as the etiological origin of ketosis in

transitioning dairy cows (Abuajamieh et al., 2016). Accordingly, there are multiple situations experienced by farm animals with the potential to induce leaky gut.

Presumably an impaired intestinal barrier will negatively impact economically important phenotypes. However, directly studying post-absorptive and production consequences of leaky gut is difficult as conditions thought to be responsible for reducing intestinal barrier integrity also affect multiple tissues and systems. Obvious examples of biologically confounding situations include the periparturient period and heat stress, both of which are accompanied by marked homeorhetic adaptations to support a new dominant physiological state (Bauman and Currie, 1980; Baumgard and Rhoads, 2013). Evaluating the metabolic, endocrine, inflammatory, and production consequences of leaky gut in isolation would provide insight into its direct impact on the pathophysiology of common on-farm disorders.

We hypothesized that intestinal tract barrier dysfunction (in apparently otherwise healthy animals) would detrimentally affect production parameters, metabolic variables, and inflammatory indices, and that these post-absorptive consequences would resemble characteristic biomarkers in the aforementioned disorders. To test this, we utilized gamma-secretase inhibitor (**GSI**) to decrease intestinal barrier integrity. Administering GSI causes intestinal metaplasia of mucus secreting goblet cells from crypt cells via Notch pathway interference (Milano et al., 2004; van Es et al., 2005), which is necessary for normal absorptive enterocyte maturation and proliferation (Okamoto et al., 2009). Disrupting ordinary crypt cell differentiation using GSI severely damages intestinal structures (Wong et al., 2004) and inhibiting the Notch pathway decreases epithelial cell turnover and increases intestinal permeability (Obata et al., 2012).

Materials and Methods

Animals and Sampling

The Institutional Animal Care and Use Committee at Iowa State University (**ISU**) approved all procedures involving animals. Fourteen lactating Holstein cows (117 ± 18 DIM; 666 ± 14 kg BW; parity 2.6 ± 0.3) were housed at the ISU Dairy Farm and enrolled in two experimental periods. Period 1 (**P1**) lasted 4-5 d and served as the baseline (data generated for covariate analysis) for period 2 (**P2**). Period two lasted 7 d during which cows received one of two i.v. treatments twice daily at 0600 and 1800 h: 1) control (1 L sterile saline; $n = 7$) or 2) GSI (1.5 mg/kg BW semagacestat dissolved in 1 L sterile saline; LY-450139; Eli Lilly and Company, Indianapolis, IN; $n = 7$). The GSI dose was selected from a preliminary dose response trial where 1 mg/kg/d BW did not induce overt phenotypic responses while 6 mg/kg/d caused a severe and rapid decrease in feed intake (data not shown). Control animals were pair-fed (**PF**) to the GSI-treated cows to eliminate the confounding effects of dissimilar nutrient intake as we have previously described (Wheelock et al., 2010).

All cows were individually fed a TMR once daily at 0800 h, and orts were recorded daily prior to feeding. The TMR was formulated by Nutrition Professionals, Inc. (Neenah, WI) to meet or exceed the predicted requirements of energy, protein, minerals, and vitamins (NRC, 2001; Table 5). Reductions in daily feed intake by GSI-treated cows in P2 were determined as a percentage of their mean daily ad libitum intake during P1. Initiation of P2 for the PF cows occurred 1 d later to allow for pair-feeding calculations and

implementation. For tissue collection consistency, PF and GSI cows were euthanized after morning blood samples of the same calendar day (P2D8 for GSI-treated cows and P2D7 for PF cows).

Throughout the experiment, cows were milked twice daily at 0500 and 1700 h, and yields were recorded at each milking. Individual milk samples for composition analysis were obtained daily during both periods at the AM milking. Samples were stored at 4°C with a preservative (bronopol tablet; D & F Control System, San Ramon, CA) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC approved infrared analysis equipment and procedures.

Heart rate, respiration rate, rectal temperature, and manure score were measured twice daily at 0700 and 1900 h. Heart rate and respiration rate were measured as beats or flank movements during a 15 s interval and were later transformed to beats/min and breaths/min, respectively. Rectal temperatures were measured using a digital thermometer (GLA M700, San Luis Obispo, CA). Manure score for each animal was determined based on a scale of 1 (liquid) to 5 (firm) using the manure scoring system by Skidmore and colleagues (1996). Body weights were recorded on d 5 of P1 to calculate GSI dosage and again immediately preceding sacrifice.

Jugular catheters were inserted into all cows as previously described (Baumgard et al., 2002b) prior to P1 for blood sampling and a second catheter was inserted 1 d before P2 for GSI or saline administration. Serum and plasma samples were collected daily at 0500 h during both periods using an empty glass tube and a glass tube containing 50 µL sterile heparin (Sagent Pharmaceuticals, Schaumburg, IL), respectively. Pyrogen free serum samples were collected on d 3 and 5 of P1 and d 1, 3, 5, and 7 of P2. Prior to collection,

the coccygeal area was scrubbed with alcohol soaked gauze and the sample was collected via venipuncture into evacuated sterile serum collection tubes (BD Vacutainer, Franklin Lakes, NJ) and handled in sterile conditions from then on. Serum samples were allowed to clot for one hour at room temperature. Plasma and serum were harvested following centrifugation at 1500 x g for 15 min at 4°C, and subsequently frozen at -20°C until analysis.

Tissue Collection

Liver biopsies were collected on d 3 of P1 and d 5 of P2 as previously described (Rhoads et al., 2004). Briefly, the area was shaved, disinfected, and locally anesthetized using lidocaine before performing a percutaneous biopsy with a trocar. Four samples (~20 mg each) were collected, snap frozen in liquid nitrogen and stored at -80°C until analyzed. Incisions were then sutured and topically treated with AluShield™ (Neogen Corp., Lexington, KY).

Cows were euthanized with a CASH Special captive bolt gun (Accles & Shelvoke Ltd., Sutton Coldfield, West Midlands) using a large animal charge followed by exsanguination completed by severing the carotid arteries bilaterally. Liver and intestinal tissues were harvested immediately and liver and spleen weights were recorded. A liver sample was collected from ~20 cm dorsal to the ventral liver margin (a location anticipated to be where the liver biopsies were obtained) and was snap frozen in liquid nitrogen and stored at -80°C until further analysis. A jejunum segment was collected ~5.5 m proximal to the ileocecal junction. An ileum segment was collected immediately proximal to the ileocecal junction. A colon segment was collected ~1.5 m proximal to the pelvic inlet.

Jejunum, ileum, and colon segments (~20-30 cm) were flushed with cold PBS to remove intestinal content. A transversal section was collected from each sample and subsequently fixed in 10% neutral buffered formalin for later histological analysis.

Blood Analyses

Plasma insulin, non-esterified fatty acids (**NEFA**), glucose, BHB, LPS-binding protein (**LBP**), serum amyloid A (**SAA**), haptoglobin, and BUN concentrations were determined using commercially available kits validated for use in our laboratory (insulin, Mercodia AB, Uppsala, Sweden; NEFA, Wako Chemicals USA, Richmond, VA; glucose, Wako Chemicals USA Inc., Richmond, VA; BHB, Pointe Scientific Inc., Canton, MI; LBP, Hycult Biotech, Uden, Netherlands; SAA, Tridelata Development Ltd., Kildare, Ireland; haptoglobin, Immunology Consultants Laboratory Inc., Portland, OR; BUN, Teco Diagnostics Anaheim, CA). The inter- and intra-assay coefficients of variation for insulin, NEFA, glucose, BHB, SAA, LBP, and BUN assays were 6.7 and 17.5%, 7.1 and 7.3%, 6.9 and 5.9%, 14.3 and 4.6%, 27.3 and 5.3%, and 5.8 and 1.9%, respectively. Serum LPS was analyzed in triplicate using sterile procedures and a PyroGene endotoxin detection assay (Lonza, Walkersville, MD); inter- and intra-assay coefficient were 64.9 and 29.7%, respectively.

Liver Analyses

Portions of the liver samples (~5.0 g) were weighed, dried in an oven for 18 h at 102°C, and reweighed after cooling in order to determine liver moisture percentage. To determine liver triglyceride content, a portion (~20 mg) was weighed and homogenized

with 500 μ L chilled PBS. The homogenate was then centrifuged at 8,000 $\times g$ for 2 min at 4°C. Free glycerol was immediately determined using 10 μ L of supernatant via enzymatic glycerol phosphate oxidase method (Sigma-Aldrich, St. Louis, MO). An additional 300 μ L of supernatant was removed and incubated with 75 μ L lipase (MP Biomedicals, Solon, OH) at 37°C for 16 h before determining total glycerol using the same enzymatic glycerol phosphate oxidase method. Free glycerol (before lipase digestion) was subtracted from total glycerol (after lipase digestion) in order to determine triglyceride content, and this was expressed as a percentage of wet weight of the original sample before homogenization. The intra-assay coefficient of variation for free glycerol and total glycerol was 1.9 and 0.8%, respectively.

Histological Analyses

For histological analysis, 10% neutral buffered formalin-fixed jejunum, ileum, and colon were sectioned and periodic acid-Schiff (**PAS**) stained at the ISU Veterinary Diagnostic Laboratory for morphological and goblet cell area quantification. One slide per cow per tissue was generated. For histological and goblet cell analysis, a microscope (Leica® DMI3000 B Inverted Microscope, Bannockburn, IL) with an attached camera (QImaging® 12-bit QICAM Fast 1394, Surrey, BC) was used to obtain images from five non-overlapping fields per slide at 100X magnification. All morphological measurements were obtained using ImageJ (1.48b, National Institutes of Health, USA). For villus measurements, one villus per image was measured for a total of five measurements per cow. Total mucosal area was determined after subtracting luminal area, and area of PAS stain was measured using the ImageJ color deconvolution tool with H PAS vector. Goblet

cell area was expressed as percentage of the total mucosal area stained by PAS. Villus height was measured from the villus tip to the villus-crypt interface. Villus width was measured at mid-villus height. Crypt depth was measured from the villus-crypt opening to the laminae propria. Crypt width was measured at the villus-crypt interface level. A mucosal surface area estimate was obtained using the mucosal-to-serosal amplification ratio *M* as previously reported by Kisielinski and colleagues (2002), where:

$$M = \frac{(villus\ width \times villus\ length) + \left(\frac{villus\ width}{2} + \frac{crypt\ width}{2}\right)^2 - \left(\frac{villus\ width}{2}\right)^2}{\left(\frac{villus\ width}{2} + \frac{crypt\ width}{2}\right)^2}$$

Immunofluorescence Staining

For immunofluorescence analysis, 10% neutral buffered formalin-fixed jejunum, ileum, and colon were sectioned at 5 microns thickness and mounted in the histology laboratory at the ISU Veterinary Diagnostic Laboratory. Slides were deparaffinized using Citrisolv™ Hybrid Solvent (Fisher Scientific), rehydrated by incubation for 3 min twice in 100% ethanol, 95% ethanol for 1 min, 80% ethanol for 1 min, followed by rinsing in distilled and deionized water. Antigen retrieval was performed by incubating slides in citrate buffer (Sodium Citrate, Citric Acid, Tween20, water) at 95°C for 30 min. Tissue sections were blocked in 5% BSA (Sigma Aldrich) in PBS for 90-180 min. Primary antibody against proliferating cell nuclear antigen (**PCNA**; PC10 mAb, Cell Signaling Technology #2586; 1:200 dilution), a marker of cellular proliferation, was applied to each section and incubated overnight at 4°C. Slides were washed thrice in PBS and incubated in fluorescent secondary antibody (anti-mouse IgG (H+L) F(ab')₂ Fragment, AlexaFluor® 488 conjugate, Cell Signaling Technology #4408; 1:1000 dilution) for 1 h at room

temperature. Slides were washed thrice in PBS and 4',6-diamidino-2-phenylindole stain was applied to each section. Slides were stored in the dark at 4°C to prevent fading. To decrease variability due to staining day, each tissue type was stained on the same day, and slides were imaged within a 6 h timeframe. For each animal, five images from each tissue were collected on a Leica fluorescent microscope at 100X magnification. To ensure antibody binding specificity, negative controls omitted either the primary antibody, secondary antibody, or both. Mouse IgG was used in place of the primary antibody to control for antibody isotype nonspecific binding. The fluorescence signaling was obtained for one individual villi per image in the jejunum and ileum samples while the total fluorescence of each colon image was performed using integrated density quantification in ImageJ software.

Statistical Analyses

For production and blood parameters, effects of treatment, day, and treatment by day interaction were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC) using repeated measures analysis with an autoregressive covariance structure and day as the repeated effect. Each specific variable's P1 value (when available) served as a covariate. For post-mortem data, the effect of treatment was assessed using PROC MIXED. Immunofluorescence staining data was analyzed using the unpaired t-test function in GraphPad Prism. Irrespective of treatment, Pearson correlation analysis between intestinal parameters and the change in circulating inflammatory parameters (P2D5-P2D7 average minus P1 average) was conducted using PROC CORR of SAS. Data are reported as LSmeans and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.15$.

Results

During P2, GSI administration progressively decreased DMI ($P < 0.01$; Figure 2A), and feed intake was reduced 66% by the end of the study. By experimental design, the pattern and extent of feed intake reduction was similar in PF cows ($P = 0.96$; Figure 2A). There was a similar and progressive decline in milk yield for both treatments during P2 ($P < 0.01$; Figure 2B), whereby milk yield was reduced 42% by the end of the study. There were no differences in milk components between treatments; however, there was a tendency for milk SCC and MUN to be increased in GSI-treated cows (144 and 22%, respectively, $P \leq 0.08$; Table 6). Milk fat content increased (45%) over time for both PF and GSI treatments ($P = 0.05$; Table 6).

Manure score was decreased 29% in GSI-treated cows ($P = 0.03$; Figure 2C) due to a progressive reduction that did not occur in PF-controls. Overall, GSI-treated cows had slightly increased heart rate and respiration rate in comparison with PF cows (8 beats/min and 7 breaths/min, respectively; $P \leq 0.01$; Table 6). There was a tendency for GSI-treated cows to have an increased rectal temperature compared to PF-controls (38.6 vs. 38.4°C; $P = 0.06$; Table 6).

Plasma glucose concentrations decreased slightly with time ($P = 0.05$; Figure 3A) in both treatments. Cows treated with GSI tended to have increased plasma insulin (68%) and decreased circulating NEFA (29%) compared to PF-controls ($P \leq 0.07$; Figure 3B and 3C). Circulating NEFA increased with time in both treatments ($P < 0.01$; Figure 3C) whereby at the end of P2, NEFA were increased 5.2 and 2.2 fold relative to baseline values in PF and GSI-treated cows, respectively. There was a tendency for a treatment by day

interaction in plasma BHB due to a more pronounced increase in GSI-treated cows (124% increase on d 7 from baseline; Figure 3D) compared to PF controls (81% increase on d 6 from baseline) over time. No differences in circulating BUN were detected (data not shown). Cows treated with GSI tended to have a 70% decrease in circulating LPS on d 5 and 7 relative to PF cows ($P = 0.12$; Figure 4A). Plasma LBP increased with time ($P < 0.01$; Figure 4B) and tended to be increased 42% in GSI-treated cows compared to PF-controls on d 5-7 ($P = 0.14$; Figure 4B). Haptoglobin and SAA progressively increased similarly in both treatments and were 585 and 4.4 fold higher, respectively, at the end of P2 relative to baseline ($P < 0.01$; Figure 4C and 4D).

Compared to PF controls, administering GSI increased goblet cell area 218, 70, and 28% in jejunum, ileum, and colon, respectively ($P \leq 0.02$; Figure 5A and Figure 8). Jejunum villus height, villus height-to-crypt depth ratio, and mucosal surface area were reduced (21, 36, and 21%, respectively) in GSI-treated cows while jejunum crypt depth was increased 43% compared to PF controls ($P < 0.01$; Figure 5B, 5D, and 5E). Jejunum villus width was not affected by treatment. Crypt depth tended to be increased 21% in the ileum of GSI-treated animals relative to controls ($P = 0.06$; Figure 5C), but there were no other detectable effects on ileum morphology. Intestinal cellular proliferation, as measured by PCNA, was decreased 27% in the jejunum of GSI-treated cows relative to PF controls ($P < 0.01$), but no differences were observed in the ileum or colon (Figure 5F & Figure 7).

Irrespective of treatment, change in circulating LBP (P2D5-7 average minus P1 average) was correlated with the jejunum villus height-to-crypt-depth ratio ($r = -0.89$, $P < 0.01$; Figure 6) as well as jejunum crypt depth and villus height ($r = 0.84$ and -0.69 , respectively, $P \leq 0.01$; data not shown). Change in circulating SAA was correlated with

jejunum villus width ($r = 0.64$, $P = 0.03$) and tended to be correlated with the villus height-to-crypt depth ratio and mucosal surface area ($r = -0.47$ and -0.53 , respectively, $P \leq 0.13$; data not shown). Circulating haptoglobin change tended to be correlated with jejunum villus width and jejunum mucosal surface area ($r = 0.50$ and -0.44 , $P \leq 0.15$; data not shown). In the ileum, there were no correlations between changes in acute phase proteins and intestinal morphology parameters.

Liver triglyceride content was increased 221% at sacrifice relative to baseline in both treatments ($P < 0.01$; Figure 7). There were no treatment differences in liver weight, liver moisture percentage, liver or spleen weight as a percentage of BW, or change in overall BW (Table 7).

Discussion

A variety of situations in animal agriculture can reduce intestinal barrier function—the consequences of which likely stimulate the immune system, cause inflammation, and eventually compromise production. Example circumstances include weaning (Boudry et al., 2004; Moeser et al., 2007), heat stress (Baumgard and Rhoads, 2013; Pearce et al., 2013a), rumen acidosis (Emmanuel et al., 2007a,b; Khafipour et al., 2009; Minuti et al., 2014), and the periparturient period (Abuajamieh et al., 2016). However, a myriad of behavioral, metabolic, and endocrine events accompanying the aforementioned situations make it difficult to study the consequences of poor intestinal barrier health in isolation. To our knowledge, a controlled experiment intentionally reducing intestinal barrier integrity has never been performed in ruminants. Our objectives were to elucidate the inflammatory,

production, and metabolic consequences of intestinal barrier dysfunction in otherwise healthy mid-lactation dairy cows.

Administering GSI in the current experiment disturbed intestinal architecture, changes likely reflective of leaky gut (Ford et al., 1985; Pearce et al., 2013a). Crypt cell Notch signaling is inhibited by GSI (De Strooper et al., 1999), and altering this pathway increases intestinal permeability (Obata et al., 2012). Furthermore, GSI decreases absorptive enterocyte proliferation and causes goblet cell hyperplasia (van Es et al., 2005), which was evident in the current study by the decrease in jejunal cell proliferation and increase in goblet cell area in jejunum, ileum, and colon. Effects of GSI were particularly evident in the jejunum as changes in villus height, crypt depth and their ratio, and mucosal surface area are indicative markers of damaged epithelium (van der Hulst et al., 1998; Pearce et al., 2013a). It is unknown why the jejunum was more sensitive to GSI than the ileum and colon, but a plausible explanation is the larger proportion of absorptive enterocytes in jejunum villi relative to other segments of the GIT (Cheng and Leblond, 1974). Additionally, goblet cell hyperplasia likely contributed to the progressive decrease in manure scores (indicative of increased fecal moisture) due to increased mucus production.

The direct consequence of intestinal barrier dysfunction is the increased leakage of luminal antigens into the blood stream with the potential to initiate an inflammatory response. Elevated rectal temperature, heart rate, and respiration rate are indicative of immunoactivation (Jacobsen et al., 2005), and these were increased in cows administered GSI, suggesting a greater degree of inflammation. To further assess inflammation, we measured three acute phase proteins (**APP**): haptoglobin, SAA, and LBP. The liver

produces APP as a secondary (non-local) response to toxic stimuli, and they have been widely acknowledged as markers of systemic inflammation (Ceciliani et al., 2012). Admittedly, APP production is a non-specific response to endotoxin infiltration which could originate from a wide variety of sources (e.g., uterus, mammary, etc.). However, in the present study, because of the localized intestinal effects of GSI and lack of overt extra-intestinal infection, intestinal barrier dysfunction is the ostensible source of the APP response. All three APP measured increased from P1 to P2 in both GSI and PF cows. The increased circulating APP in PF cows was not unexpected as reduced nutrient intake has been reported to compromise intestinal integrity in rodent, pig, and human models (Rodriguez et al., 1996; Welsh et al., 1998; Pearce et al., 2013a), and we have previously demonstrated reduced feed intake negatively affects ileum morphology in lactating cows (Stoakes et al., 2015b). After 5 d of treatment, LBP levels tended to be increased in GSI-treated cows compared to PF-controls (Figure 4B). Interestingly, the magnitude of increased circulating LBP in GSI-infused cows mirrors the increase observed in cows with ketosis and rumen acidosis (Abuajamieh et al., 2016; Khafipour et al., 2009), indicating a similar inflammatory response to seemingly differing and unconnected disorders. Incidentally, this is also the period of time (near the end of P2) when treatment differences in LPS were largest (Figure 4A). Binding of LBP to LPS is essential for the activation of its receptor TLR-4 and the subsequent production of inflammatory mediators (Lu et al., 2008b). Therefore, LPS clearance partially depends on LBP and interpreting changes in both variables can be difficult when considered independently. Changes in circulating LBP were well-correlated with jejunal intestinal morphology parameters of villus height, crypt depth, and the villus height-to-crypt depth ratio, suggesting changes in circulating LBP are

responsive to the degree of intestinal damage and decreased barrier function (i.e., ~79% of the variation in circulating LBP changes can be explained by the jejunum villus height-to-crypt depth ratio). Reasons why LBP could be a more sensitive marker of leaky gut-induced inflammation than the other APPs measured remains unknown, but is likely due to the fact the LBP is specific for LPS clearance while SAA and haptoglobin play more broad roles in combating infection (Ceciliani et al., 2012). The increased LBP levels during the end of P2 coupled with the histological data suggests GSI treatment had a more detrimental effect on intestinal integrity, resulting in increased luminal content leakage into the portal blood, and perhaps a more robust response to detoxify LPS compared to PF cows.

Utilizing the pair-feeding design allows us to discriminate between direct effects of GSI and indirect effects of GSI-mediated reduced DMI. Hence, the GSI-treated cows had a progressive and marked decrease in DMI and, by design, the PF-controls had a similar pattern and magnitude of feed intake reduction. The GSI treatment induced a steady decrease in milk yield, but this appeared to be mostly related to inadequate feed consumption as the PF cows had a comparable decrease in both pattern and severity. For the current experiment, we assume decreased DMI is a consequence of GSI-induced leaky gut, but we acknowledge GSI may alter appetite by some extra-intestinal unknown mechanism. Regardless, decreased feed intake and/or milk yield is characteristic of inflammatory situations such as weaning, heat stress, transition period disorders, and rumen acidosis. While the magnitude of decreased DMI and milk yield in the current study is more extensive than the majority of these circumstances, the post-absorptive metabolism and inflammatory responses underpinning the aforementioned disorders share similarities to what is described herein.

The metabolic profile of the current model resembles conditions involving immune system activation, further evidence supporting the hypothesis that leaky gut is the cause of many on-farm disorders. For example, despite marked reductions in feed intake, plasma insulin levels remained unchanged from P1 in the GSI-treated cows, but progressively decreased as expected in the PF cows. By d 5-7 of P2, insulin concentrations were increased 79% in GSI-treated cows compared to PF-controls. The increased insulin (a potent anabolic hormone) in GSI-treated cows is energetically difficult to explain as feed intake was severely depressed and cows were obviously in an intensely catabolic condition (i.e., > 40 kg BW loss). However, this unusual temporal pattern is similar to increased insulin levels experienced by a variety of species exposed to heat stress, another leaky gut-inducing and catabolic condition (Baumgard and Rhoads, 2013). The insulin differences may be partially explained by LPS, as infusing LPS into the mammary gland increased circulating insulin in lactating cows (Waldron et al., 2006), and we have demonstrated increased circulating insulin in pigs, calves, and lactating cows infused i.v. with LPS (Rhoads et al., 2009; Stoakes et al., 2015a; Kvidera et al., 2016a, 2017a). The changes in insulin were not reflected in circulating glucose, which similarly decreased over time for both treatments, suggesting that GSI-treated cows may have become slightly insulin resistant. This profile fits with a status of immunoactivation which is characterized by hyperinsulinemia and whole-body insulin resistance (Lang et al., 1990; McGuinness et al., 2005; Kvidera et al., 2016a, 2017b) which redirects glucose away from major disposal sites (i.e., muscle and adipose) towards the immune system, a substantial insulin-induced and insulin independent glucose utilizer upon activation (Lang et al., 1990; Maratou et al., 2007; Palsson-McDermott and O'Neill, 2013). We have previously established an intensely activated

immune system of a lactating dairy cow requires over 1 kg glucose in a 12 h period (Kvidera et al., 2017a), and this highlights the fact that immune stimulation comes at a huge glucose cost. Thus, the aforementioned changes in carbohydrate metabolism appear to be a strategy to both “spare glucose” (muscle and adipose tissue insulin resistance) and activate/provide fuel for the immune system (hyperinsulinemia).

As expected, considering inadequate energy intake, there was a progressive and marked increase in plasma NEFA levels during P2 in both treatment groups; however, GSI cows tended to have a 41% decrease in circulating NEFA on d 5-7 compared to PF cows. The treatment differences in insulin likely explain the differences in circulating NEFA as insulin is a potent anti-lipolytic signal (Vernon, 1992), and these insulin/NEFA idiosyncrasies mimic the metabolic fingerprint of multiple heat-stressed species (Baumgard and Rhoads, 2013). Despite tending to have decreased NEFA concentrations during the end of P2, GSI-administered cows had a higher peak BHB relative to PF-controls (124 versus 81% increase from baseline, respectively). Interestingly, during the periparturient period, NEFA and BHB are not well-correlated, and dairy cows diagnosed with ketosis have increased BHB levels despite similar NEFA concentrations (McCarthy et al., 2015; Zhang et al., 2016). Similarly, BHB are elevated in heat-stressed pigs despite a blunted response to lipolytic signals (Sanz Fernandez et al., 2015). Reasons for discordant patterns in NEFA and BHB are not clear, but increased circulating NEFA certainly cannot fully explain increased circulating BHB observed in GSI-treated cows. Despite marked treatment differences in circulating NEFA, liver triglyceride content increased similarly in both treatments from what is considered “normal” during P1 to “mildly fatty” at sacrifice (Bobe et al., 2004). Incidentally, intestinal endotoxin infiltration contributes to hepatic

steatosis (Ilan, 2012), and fatty liver is characteristic of both ketotic and heat-stressed transitioning dairy cows (Bobe et al., 2004; Bernabucci et al., 2010). Thus, it is not surprising GSI-infused cows had increased liver fat content despite ~40% less circulating NEFA.

To our knowledge, this is the first study utilizing GSI in a ruminant model. Therefore, our understanding is limited regarding its direct influence on metabolic or endocrine systems. Potential toxicity issues arise with GSI due to their inhibition of Notch cleavage which is associated with alteration of lymphopoiesis in addition to intestinal toxicity (Wong et al., 2004). However, clinical trials for the specific GSI used in the present study have been conducted successfully in mice, dogs, and guinea pigs with few negative side effects (Hyslop et al., 2004; May et al., 2004; Lanz et al., 2006), and multiple human studies have been conducted by Siemers and colleagues (2005, 2006, 2007). Furthermore, goblet cell metaplasia may be a limitation to our study as increased mucus production may have partially limited bacterial translocation into portal and systemic circulation. However, due to the temporal increase in both circulating biomarkers of leaky gut and metabolic alterations, we consider this unlikely. Lastly, our experimental design is complicated by the fact that both treatments negatively affected intestinal health during P2. Fortunately, neither treatment was implemented during P1, and each treatment's repeatedly measured parameters can be compared back to its respective baseline value. Regrettably, this type of comparison cannot be accomplished for the intestinal morphology and organ data as these variables were only measured at sacrifice. However, the detrimental effect of GSI on jejunal intestinal morphology (coupled with differences in LBP and insulin) despite similar

feed restriction to the controls indicates a more severe effect of GSI on intestinal integrity than feed restriction alone.

Conclusions

In summary, administering GSI appeared to induce intestinal damage and compromise intestinal permeability. This reduced barrier integrity had production, metabolic, and inflammatory consequences including decreased DMI, decreased milk yield, hyperinsulinemia, increased circulating ketones, and increased APP. Further, feed restriction appears to directly cause intestinal barrier dysfunction. Consequently, we now have strong evidence to suggest intestinal barrier dysfunction directly affects traditional production, metabolic, and inflammatory parameters and many of these changes are strikingly similar to the phenotypes observed in both heat-stressed and ketotic dairy cows.

Table 5. Ingredients and composition of diet¹

Composition	% of DM ²
Ingredient	
Corn Silage	33.6
Alfalfa Hay	19.8
Rolled Corn	17.1
Ground Corn	13.7
Whole Cotton	8.6
Soy Plus ^{®3}	4.2
High Protein Soybean Meal ⁴	3.0
Chemical analysis, % of DM	
CP	15.7
NDF	31.6
ADF	22.7
NE _L Mcal/kg DM	1.6

¹Values represent an average of samples collected and composited throughout the trial. Dry matter averaged 53%.

²Average nutrient levels: 5.74% Fat, 0.84% Ca, 0.34% P, 0.37% Mg, 0.19% S, 1.1% K, 0.44% Na, 0.47% Cl, 56.30 ppm of Zn, 60.08 ppm of Mn, 95.76 ppm of Fe, 17.28 ppm of Cu, 0.19 ppm of Co, 0.28 ppm Se, 43.68 ppm of I, 4475.9 IU/kg of vitamin A, 1438.8 IU/kg of vitamin D, and 26.95 IU/kg of vitamin E

³Cooker-expeller processed soybean meal produced by West Central Cooperative, Ralston, IA, containing 46.6% CP, 60% RUP (% CP); DM basis).

⁴Solvent-extracted soybean meal containing 54.5% CP, 35% RUP (% CP); DM basis).

Table 6. Effects of gamma-secretase inhibitor or pair-feeding on production variables and vital measurements

Parameter	Treatment			<i>P</i>		
	PF ¹	GSI ²	SEM	TRT ³	Day	TRT ³ x Day
Milk components						
Milk fat, %	4.2	4.4	0.4	0.65	0.05	0.51
Lactose, %	4.8	4.8	0.1	0.92	0.11	0.99
Protein, %	2.6	2.7	0.1	0.61	0.17	0.73
Total solids, %	12.4	12.9	0.2	0.28	0.18	0.37
Other solids, %	5.7	5.7	0.1	0.98	0.10	0.99
SCC, *1000/mL	54	132	27	0.07	0.32	0.30
MUN, mg/dL	11.5	14.0	0.9	0.08	0.10	0.28
Rectal temperature, °C	38.4	38.6	0.1	0.06	0.31	0.48
Respiration rate, breaths/min	33	40	2	0.01	0.01	0.36
Heart rate, beats/min	66	74	2	<0.01	<0.01	0.24

¹Cows pair-fed to gamma-secretase inhibitor treatment and administered 1 L saline twice daily

²Cows administered 1.5 mg/kg BW gamma-secretase inhibitor twice daily

³Treatment

Table 7. Effects of gamma-secretase inhibitor or pair-feeding on liver and spleen measurements post-mortem and body weight change

Parameter	Treatment		SEM	<i>P</i> Treatment
	PF ¹	GSI ²		
Liver				
Weight, kg	9.5	10.6	0.5	0.19
Liver, % of BW ³	1.6	1.8	0.1	0.42
Moisture, %	68.6	71.0	1.0	0.14
Spleen				
Weight, kg	1.20	1.05	0.05	0.73
Spleen, % of BW	0.18	0.16	0.01	0.28
Body weight				
BW change, kg ⁴	-48.2	-65.2	17.7	0.53

¹Cows pair-fed to gamma-secretase inhibitor treatment and administered 1 L saline twice daily

²Cows administered 1.5 mg/kg BW gamma-secretase inhibitor twice daily

³Percentage of BW

⁴BW change over duration of treatment administration

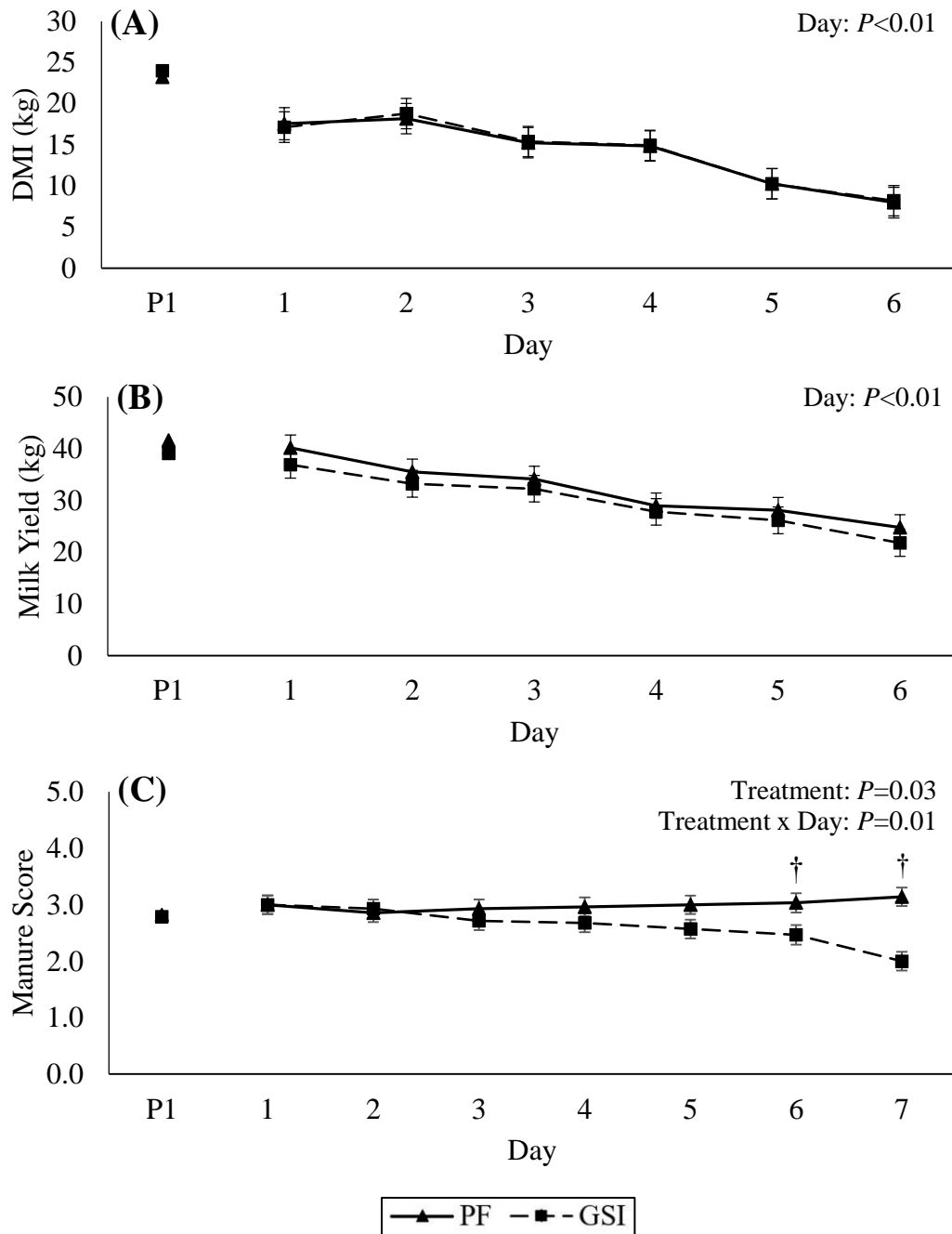


Figure 2. Effects of gamma-secretase inhibitor (GSI) or pair-feeding (PF) on (A) DMI, (B) milk yield, and (C) manure score in lactating Holstein cows. Values for P1 represent the period 1 average that was statistically used as a covariate. † Indicates a significant treatment effect ($P \leq 0.05$).

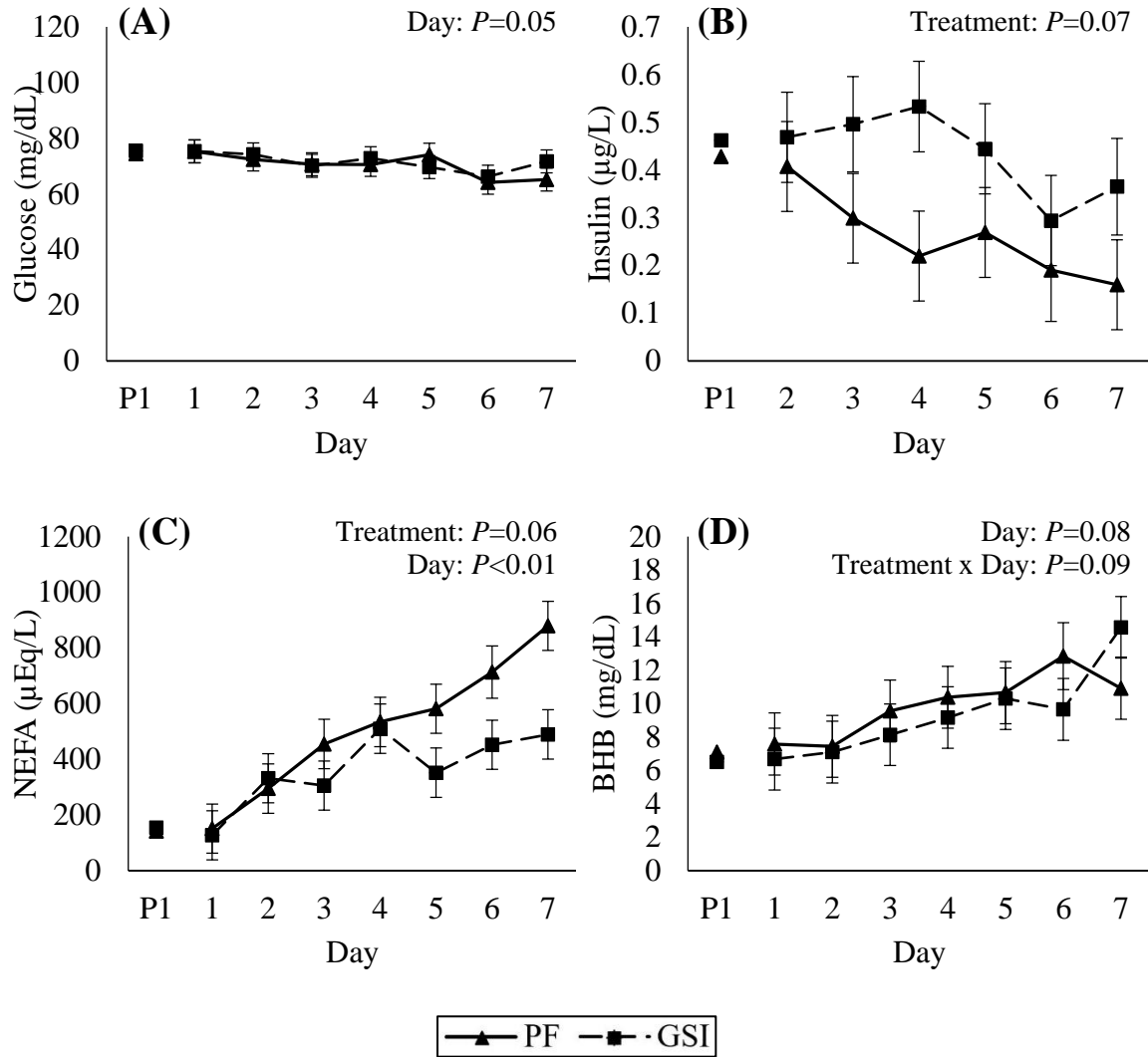


Figure 3. Effects of gamma-secretase inhibitor (GSI) or pair-feeding (PF) on plasma metabolites (A) glucose, (B) insulin, (C) non-esterified fatty acids (NEFA), and (D) BHB in lactating Holstein cows. Values for P1 represent the period 1 average that was statistically used as a covariate. †Indicates a significant treatment effect ($P \leq 0.05$).

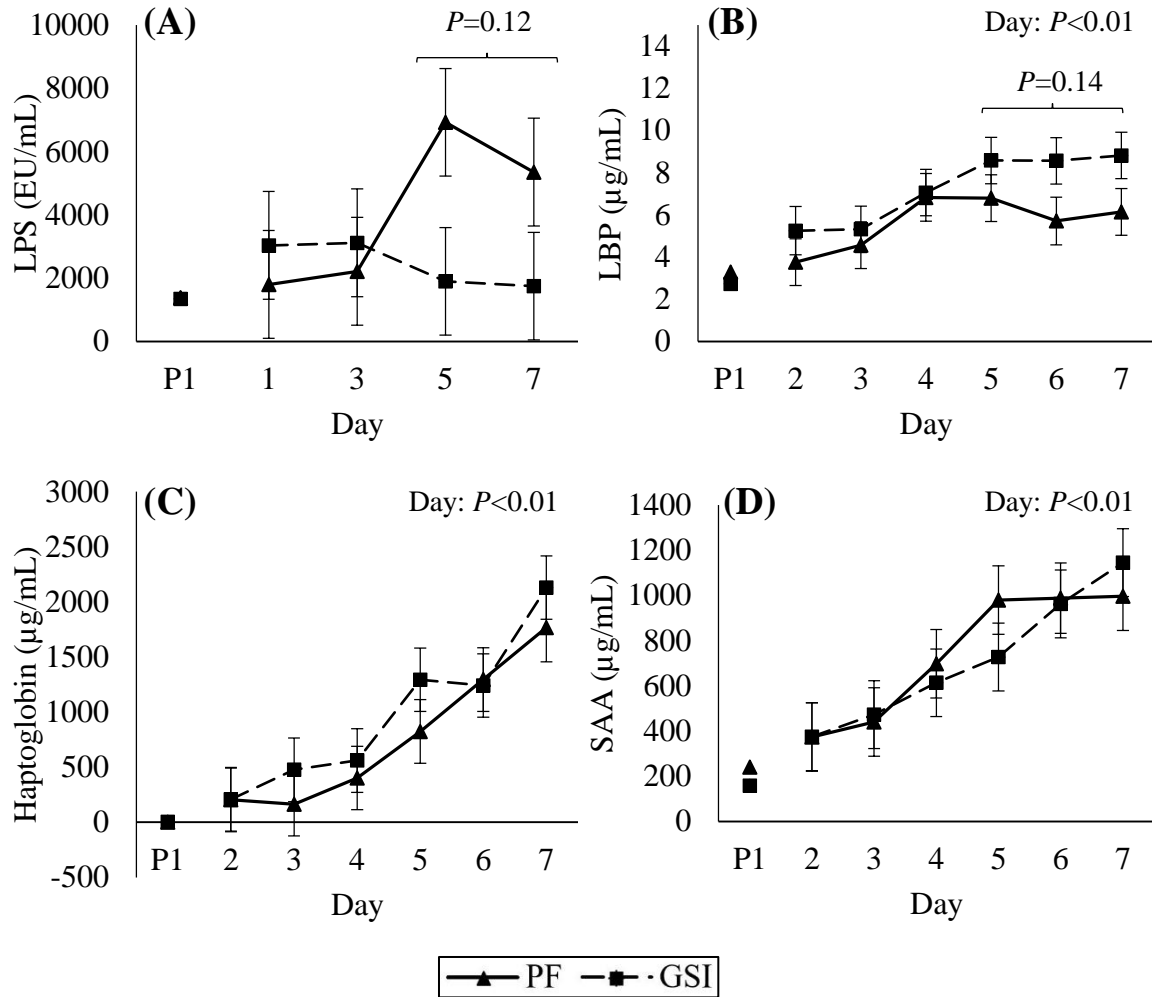


Figure 4. Effects of gamma-secretase inhibitor (GSI) or pair-feeding (PF) on blood acute phase proteins (A) lipopolysaccharide, (B) lipopolysaccharide binding protein (LBP), (C) haptoglobin, and (D) serum amyloid A (SAA) in lactating Holstein cows. Values for P1 represent the period 1 average that was statistically used as a covariate. †Indicates a significant treatment effect ($P \leq 0.05$).

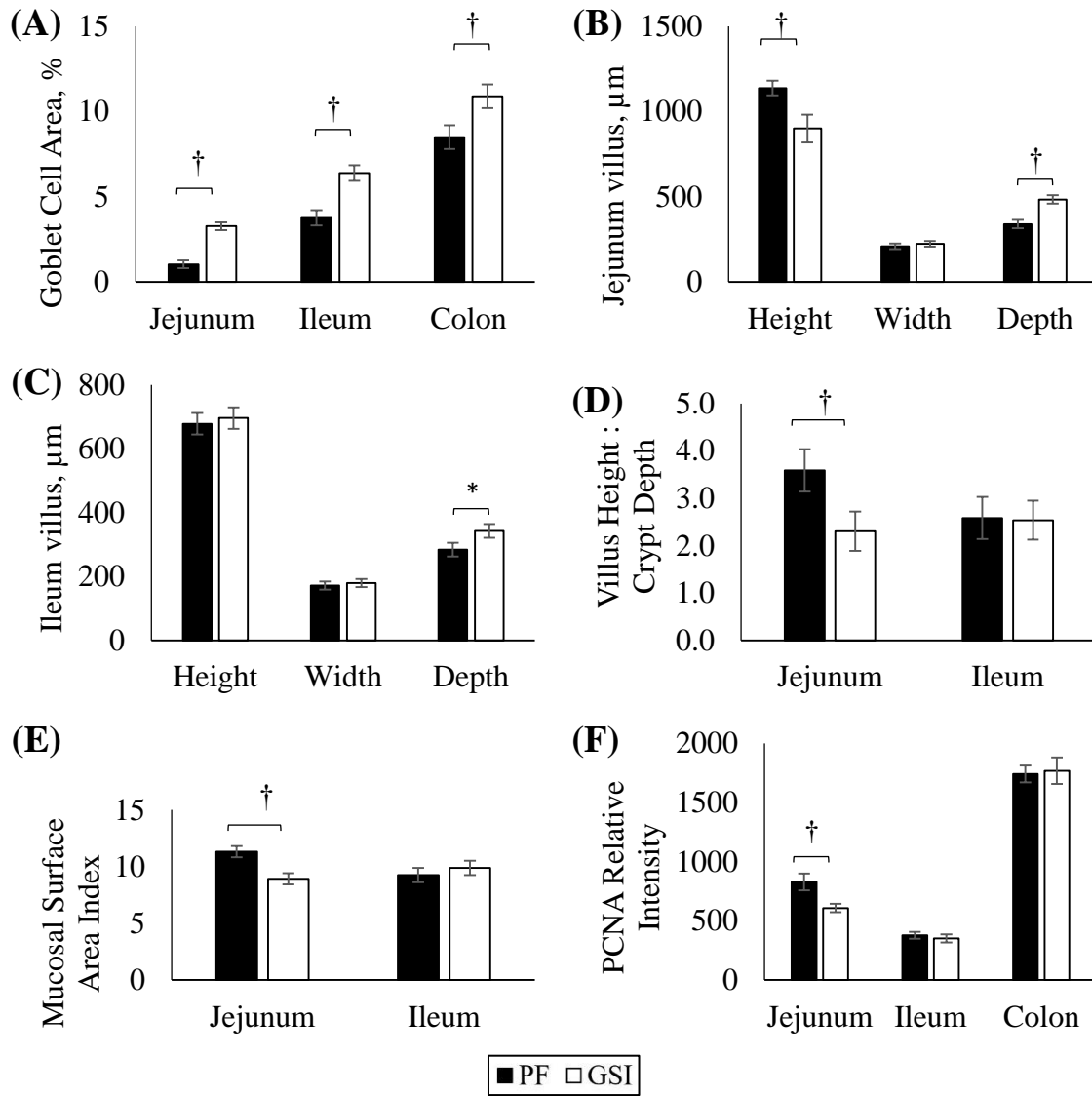


Figure 5. Effects of gamma-secretase inhibitor (GSI) or pair-feeding (PF) on (A) goblet cell area as a percentage of epithelial area in jejunum, ileum, and colon, (B) jejunum villus morphology, (C) ileum villus morphology, (D) villus height-to-crypt depth ratio in jejunum and ileum, (E) mucosal surface area in jejunum and ileum, and (F) intensity of PCNA protein in jejunum, ileum, and colon. *Indicates a tendency for a treatment effect ($P \leq 0.15$). †Indicates a significant treatment effect ($P \leq 0.05$). Mucosal surface area is expressed as an M-index as described by Kisielinski et al., 2002.

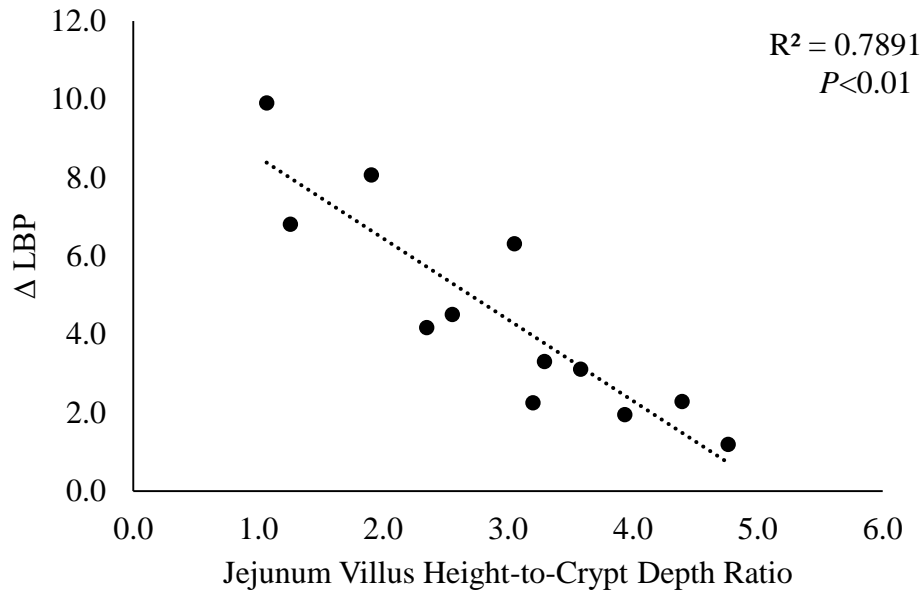


Figure 6. Correlation between the jejunum villus height-to-crypt depth ratio and the change in circulating lipopolysaccharide binding protein (LBP; P2D5-7 average minus the P1 average). Analysis includes the combination of cows administered 1.5 mg/kg BW gamma-secretase inhibitor 2x/d and cows pair-fed to gamma-secretase inhibitor treatment and administered 1 L saline 2x/d.

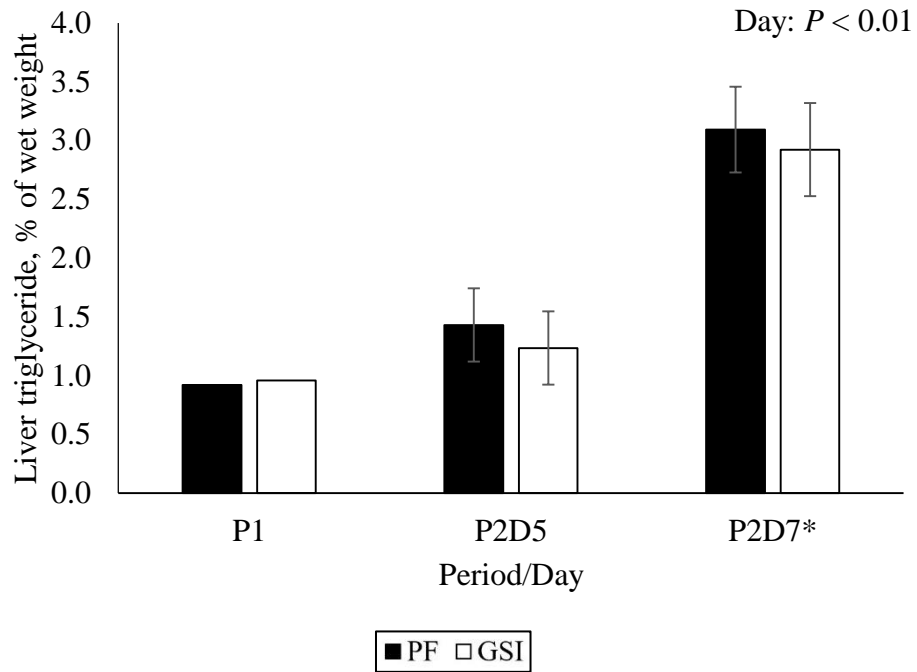


Figure 7. Effects of gamma-secretase inhibitor (GSI) or pair-feeding (PF) on liver triglyceride as a percentage of wet weight. *Due to logistical constraints, animals were sacrificed on the same calendar day (P2D7 and P2D8 for PF and GSI animals, respectively).

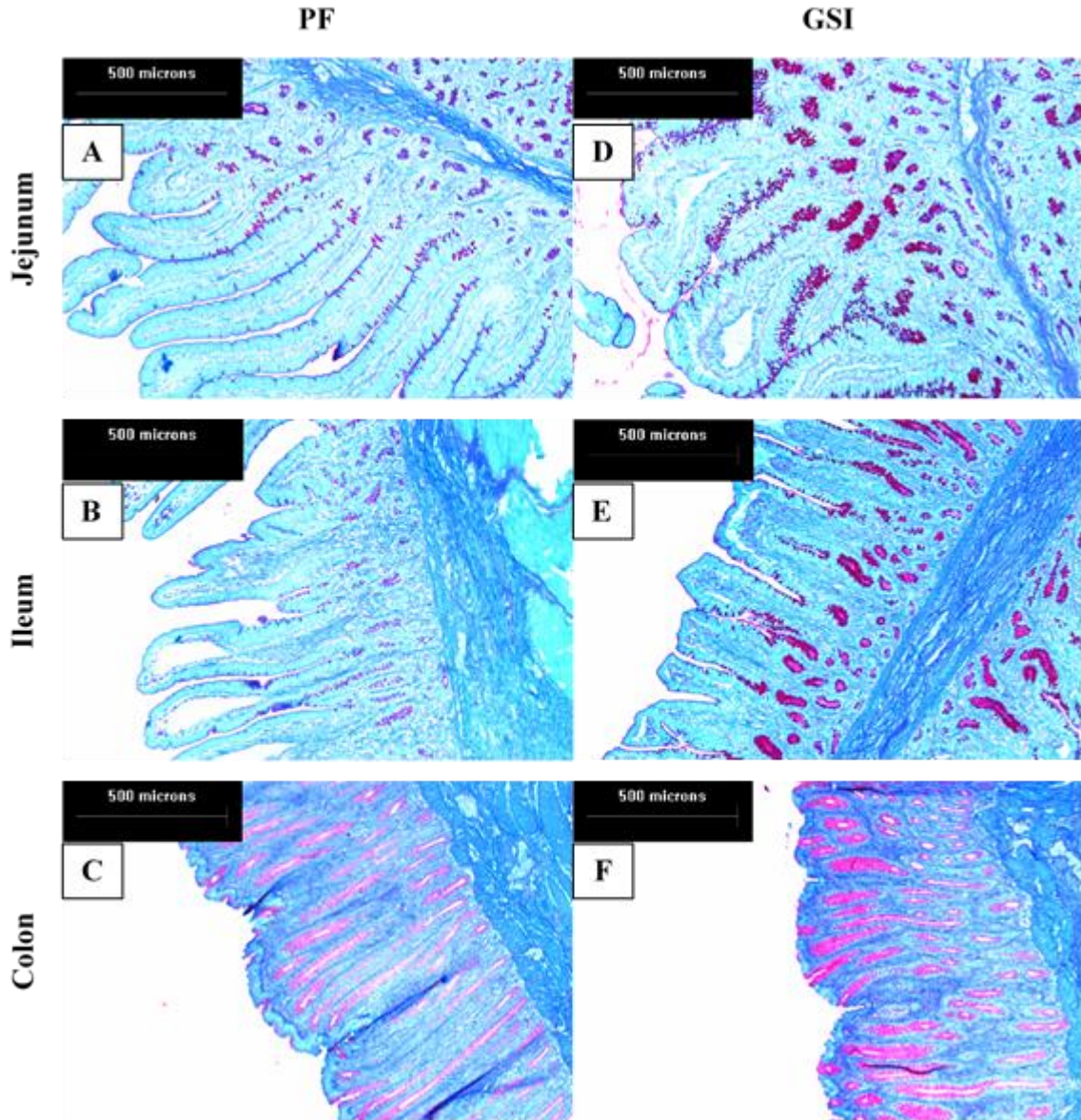


Figure 8. Representative images of periodic acid-Schiff stained (A) jejunum, (B) ileum, and (C) colon of pair-fed controls (PF) and (D) jejunum, (E) ileum, and (F) colon of cows administered gamma-secretase inhibitor (GSI). Dark purple stain is positive for mucosubstances.

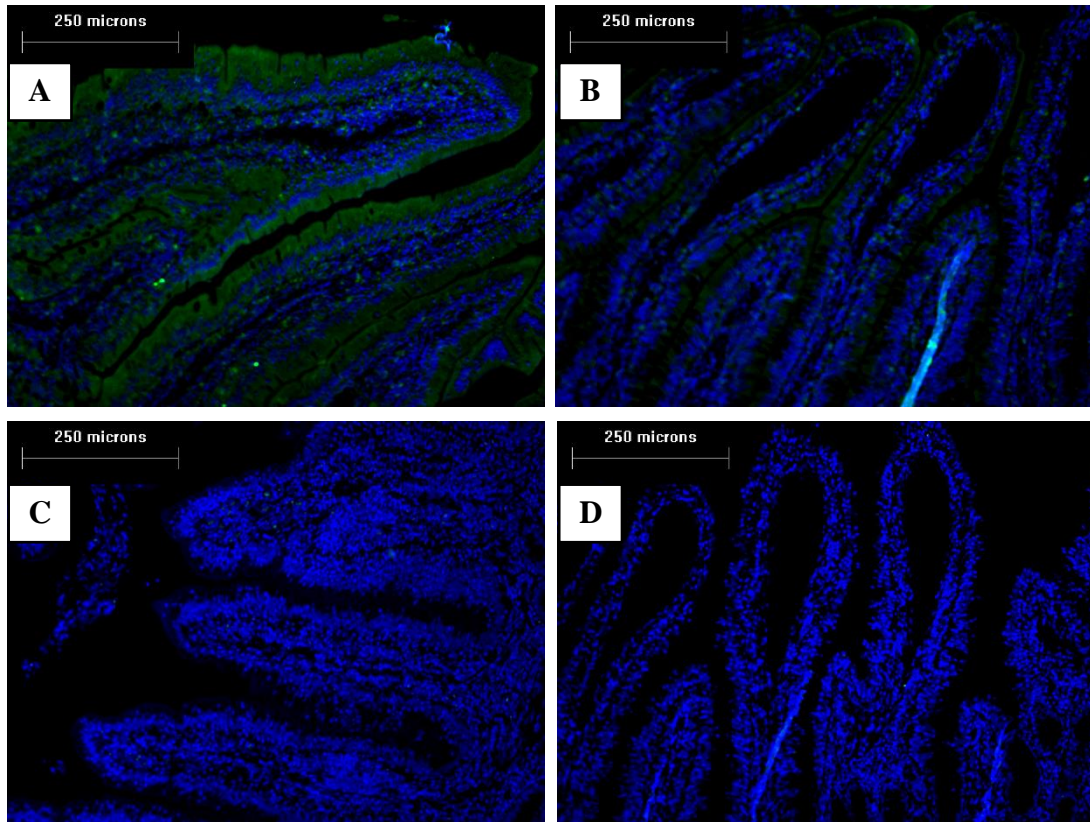


Figure 9. Representative image of PCNA protein intensity in jejunum of a pair-fed control (PF; A) and a cow administered gamma-secretase inhibitor (GSI; B). Negative controls are primary antibody omitted (C), IgG serum in place of primary antibody (D). Nuclear stain is blue while PCNA stain is green.

**CHAPTER 3: CHARACTERIZING EFFECTS OF FEED RESTRICTION AND
GLUCAGON-LIKE PEPTIDE 2 ADMINISTRATION ON BIOMARKERS OF
INFLAMMATION AND INTESTINAL MORPHOLOGY**

Paper prepared for submission to the Journal of Dairy Science

S. K. Kvidera,* E. A. Horst,* M. V. Sanz Fernandez,* M. Abuajamieh,* S. Ganesan,* P. J. Gorden,† H. B. Green,‡ K. M. Schoenberg,‡ W. E. Trout,‡ A. F. Keating,* and L. H. Baumgard*

*Department of Animal Science, Iowa State University, Ames 50011

†College of Veterinary Medicine, Iowa State University, Ames 50011

‡Elanco Animal Health, Greenfield, IN 46140

Abstract

Objectives were to characterize how progressive feed restriction (FR) affects inflammation, metabolism, and intestinal morphology, and to investigate if glucagon-like peptide 2 (GLP2) administration influences the aforementioned responses. Twenty-eight Holstein cows (157 ± 9 days in milk) were enrolled in 2 experimental periods. Period 1 (5 d of ad libitum [AL] feed intake) served as baseline for period 2 (P2; 5 d), during which cows received 1 of 6 treatments: 1) 100% of AL feed intake (AL100), 2) 80% of AL feed intake, 3) 60% of AL feed intake, 4) 40% of AL feed intake (AL40), 5) 40% of AL feed intake + GLP2 administration (AL40G; 75 $\mu\text{g}/\text{kg}$ BW s.c. 2x/d), or 6) 20% of AL feed intake

(AL20). As the magnitude of FR increased, body weight and milk yield decreased linearly. Blood urea nitrogen and insulin decreased while non-esterified fatty acids and liver triglyceride content increased linearly with progressive FR. Circulating endotoxin, lipopolysaccharide binding protein, haptoglobin, serum amyloid A, and lymphocytes increased or tended to increase linearly with advancing FR. Circulating haptoglobin decreased (76%) and serum amyloid A tended to decrease (57%) in AL40G relative to AL40 cows. Cows in AL100, AL40, and AL40G treatments were sacrificed to evaluate intestinal histology. Jejunum villus width, crypt depth, and goblet cell area, as well as ileum height, crypt depth, and goblet cell area were reduced (36, 14, 52, 22, 28, and 25%, respectively) in AL40 cows compared to AL100 controls. Ileum cellular proliferation tended to be decreased (14%) in AL40 vs. AL100 cows. Relative to AL40, AL40G cows had improved jejunum and ileum morphology, including: increased villus height (46 and 51%), villus height to crypt depth ratio (38 and 35%), mucosal surface area (30 and 27%), cellular proliferation (43 and 36%), and goblet cell area (59 and 41%). Colon goblet cell area was also increased (48%) in AL40G relative to AL40 cows. In summary, progressive FR increased circulating markers of inflammation which we speculate is due to increased intestinal permeability as demonstrated by intestinal architecture deterioration. Furthermore, GLP2 improved intestinal morphology and ameliorated circulating markers of inflammation. Consequently, FR is a viable model to study consequences of intestinal barrier dysfunction and administering GLP2 appears to be an effective mitigation strategy to improve gut health.

Keywords: feed restriction, glucagon like-peptide 2, gut health

Introduction

There are various situations in animal agriculture where feed intake is suboptimal either due to feed unavailability (e.g., shipping, drought, overcrowding) or as a result of an adaptive response (e.g., heat stress, immunoactivation). The decrease in energy and nutrient intake constrains animal performance below its genetic potential and jeopardizes efficiency and profitability. However, inadequate feed intake might impact productivity by mechanisms other than reduced substrate availability. Specifically, feed restriction (**FR**) and malnutrition cause intestinal barrier dysfunction in rodents, chickens, pigs, and humans (Rodriguez et al., 1996; Yamauchi et al., 1996; Welsh et al., 1998; Boza et al., 1999, Pearce et al., 2013); and we and others have demonstrated that this is also the case in ruminants (Zhang et al., 2013; Kvidera et al., 2017b). Thus, FR's deleterious impact on the intestinal barrier appears to transcend species. Mechanistically, how FR negatively affects barrier integrity remains ill-defined, but fasting and malnutrition reduce intestinal epithelial cell numbers, proliferation, migration rates, and villus height as well as increase intestinal cell loss rate and apoptosis (Ferraris and Carey, 2000). Furthermore, FR decreases antimicrobial secreting Paneth cell function which compromises the gut's immune defense capacity and alters the microbiome (Hodin et al., 2011; Le Floc'h et al., 2014). Due to its impact on intestinal barrier function, FR increases the risk of endotoxin translocation from the gut lumen into systemic circulation (Deitch et al., 1990), with the potential to activate the immune system.

Endotoxin infiltration of the intestinal barrier activates the immune system and causes a well-characterized inflammatory response (van Miert and Frens, 1968; Lohuis et al., 1988) coupled with a large glucose requirement by stimulated leukocytes (Kvidera et

al., 2016, 2017a). Immunoactivation rearranges the hierarchy of nutrient partitioning away from economically important phenotypes, contributing to a reduction in profitability. Thus, there is a need to develop appropriate models to test potential “leaky gut” mitigation strategies. There are several chemically-induced models of leaky gut (e.g., non-steroidal anti-inflammatory drugs, gamma-secretase inhibitors, dextran sodium sulfate; Fortun and Hawkey, 2007; Wirtz et al., 2007; Kvidera et al., 2017b), but these approaches are associated with side effects or are logistically difficult to deliver, particularly in ruminants. Consequently, we and others have observed negative effects of FR on barrier function and inflammation (Zhang et al., 2013; Kvidera et al., 2017), making it a simple and appealing model for intestinal barrier dysfunction. The severity and duration of FR in the aforementioned literature varies considerably and its effects on gut health are not extensively studied in the ruminant animal, ergo it is not clear what magnitude of FR is required to dependably compromise intestinal barrier function. Consequently, a primary objective of the current experiment was to identify a FR level that induces an inflammatory response mirroring the physiological characteristics of on-farm situations that cause leaky gut.

A potential mitigation strategy to improve intestinal barrier function is glucagon-like peptide 2 (GLP2) administration. Endogenous GLP2 is secreted by enteroendocrine cells in response to luminal nutrients and has trophic effects on intestinal growth (Drucker and Yusta, 2014). Exogenous GLP2 administration prevents increased intestinal permeability in mouse models (Benjamin et al., 2000; Cameron and Perdue, 2005). Further, GLP2 administration improved expression of tight junction proteins, small intestine blood flow and growth, and reduced acute phase proteins in calves (Taylor-Edwards et al., 2011;

Walker et al., 2015; Connor et al., 2017). Thus, a second objective was to evaluate whether GLP2 treatment ameliorates the negative impact of FR on intestinal health and systemic inflammation.

Materials and Methods

Animals and Sampling

Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals. Twenty-eight lactating Holstein cows (157 ± 9 DIM, 713 ± 10 kg BW, parity 2 to 4) were utilized in an experiment conducted in two replications. Cows were housed in individual box-stalls (4.57 m by 4.57 m) at the Iowa State University Dairy Farm. Throughout the experiment, cows were milked twice daily (0600 and 1800 h) and yields was recorded at each milking. Cows were individually fed a TMR formulated by Nutrition Professionals, Inc. (Neenah, WI) to meet or exceed the predicted requirements (NRC, 2001) of energy, protein, minerals and vitamins (Table 1). Energy balance was calculated using the following equations: $EBAL = \text{energy intake} - \text{energy output}$, where $\text{energy intake} = 1.6 \text{ Mcal/kg} \times \text{DMI}$ and $\text{energy output} = (\text{NEM} = 0.08 \text{ Mcal/kg} \times \text{BW}^{0.75}) + [\text{NEL} = \text{milk yield} \times (0.029 \times \text{fat \%} + 0.0547 \times \text{protein \%} + 0.0395 \times \text{lactose \%})]$.

The trial consisted of two experimental periods within each replication. Period 1 (**P1**) lasted 5 d and served as the baseline which yielded data for covariate analysis. During P1, cows were fed ad libitum; feed was distributed once daily (0800 h) and orts were collected before the AM feeding. Period 2 (**P2**) lasted 5 d during which cows were allocated to one of six FR treatments: 1) ad libitum feed intake (**AL100**; n = 3), 2) 80% of P1 feed intake (**AL80**; n = 5), 3) 60% of P1 feed intake (**AL60**; n = 5), 4) 40% of P1 feed intake +

saline administration (**AL40**; ~ 3 mL s.c. at 0700 and 1900 h; n = 5), 5) 40% of P1 feed intake + GLP2 administration (**AL40G**; 75 µg bovine GLP2/kg BW at 0700 and 1900 h; Elanco Animal Health, Greenfield, IN; n = 5), or 6) 20% of P1 feed intake (**AL20**; n = 5).

Daily feed intake during P2 was determined by applying the desired percentage of FR to each cow's mean daily intake during P1. The calculated amount of feed was divided into three equal portions during P2 (0800, 1300, and 1800 h) in order minimize metabolic variation due to gorging. During both P1 and P2, vitals were recorded twice daily at 0700 and 1900 h. Heart rate (**HR**) and respiration rate (**RR**) were measured as beats or flank movements during a 15 s interval and were later transformed to beats/min and breaths/min, respectively. Rectal temperatures (**Tr**) were measured using a digital thermometer (GLA M700, San Luis Obispo, CA). Individual milk samples for composition analysis were obtained daily during both periods from the PM milking. Samples were stored at 4°C with a preservative (bronopol tablet; D & F Control System, San Ramon, CA) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC approved infrared analysis equipment and procedures. Body weights determined on d 1 of acclimation were utilized for calculating the GLP2 dose. Body weights were obtained again on d 5 of P2.

A jugular catheter was implanted in all cows prior to P1. Blood samples were collected daily at 1730 h during both periods into an empty glass tube and a glass tube containing 50 µL sterile heparin (Sagent Pharmaceuticals, Schaumburg, IL). Pyrogen free serum samples were collected on d 2 and d 4 of both P1 and P2 via coccygeal venipuncture. Prior to collection, the coccygeal area was scrubbed with alcohol soaked gauze and the sample was collected into evacuated sterile serum collection tubes (BD Vacutainer, Franklin Lakes, NJ) and subsequently handled in sterile conditions. Serum samples were

allowed to clot at room temperature for one hour prior to centrifugation. Plasma and serum were harvested following centrifugation at 1,500 x g for 15 min at 4°C, and subsequently frozen at -20°C until analysis. Samples for complete blood count analysis were collected on d 2 of P1 and d 4 of P2 via coccygeal venipuncture (3 ml Vacuette® EDTA tubes; Greiner Bio-One, Monroe, NC), kept overnight at 4°C, and submitted to the Iowa State Department of Veterinary Pathology for analysis.

Tissue Collection

Liver biopsies were collected from all animals on d 2 of P1 and d 5 of P2 as previously described (Rhoads et al., 2004). Briefly, the area was shaved, disinfected, and locally anesthetized using lidocaine before performing a percutaneous biopsy with a trocar. Samples were snap frozen in liquid nitrogen and stored at -80°C until analysis. Incisions were then sutured and topically treated with AluShield (Neogen Corp., Lexington, KY).

At the end of P2, cows were either returned to the ISU dairy herd or were transported to the Iowa State Livestock Infectious Disease Isolation Facility and euthanized via captive bolt gun followed by exsanguination. A total of 13 cows from AL100 (n = 3), AL40 (n = 5) and AL40G (n = 5) were sacrificed. Liver and intestinal tissues were harvested within 15 min of euthanasia. Intestinal samples (25 cm segments) were collected as follows: jejunum was collected 1 m proximal to the ileocecal junction, ileum was collected 18 cm proximal to the ileocecal junction, and descending colon was collected 50 cm proximal to the rectum. All intestinal segments were flushed with cold PBS to remove luminal content and a 4 cm section from the middle of the segment was collected and fixed in 10% neutral buffered formalin for later histological analysis.

Laboratory Analyses

Plasma insulin, non-esterified fatty acids (**NEFA**), glucose, BHB, lipopolysaccharide-binding protein (**LBP**), serum amyloid A (**SAA**), haptoglobin, and BUN concentrations were determined using commercially available kits validated in our laboratory (insulin, Mercodia AB, Uppsala, Sweden; NEFA, Wako Chemicals USA, Richmond, VA; glucose, Wako Chemicals USA Inc., Richmond, VA; BHBA, Pointe Scientific Inc., Canton, MI; LBP, Hycult Biotech, Uden, Netherlands; SAA, Tridelta Development Ltd., Kildare, Ireland; haptoglobin, Immunology Consultants Laboratory Inc., Portland, OR; BUN, Teco Diagnostics Anaheim, CA). Pyrogen free serum samples were analyzed in duplicate using sterile procedures and a Pierce® *Limulus* Amebocyte LAL Chromogenic Endotoxin Quantitation Kit (No. 88282; Thermo Scientific, Rockford, IL).

Portions of the liver samples (~5.0 g) were weighed, dried for 18 h at 102°C, and reweighed after cooling in order to determine liver moisture percentage. Hepatic triglyceride (**TG**) content was measured using a method adapted from (Morey et al., 2011). A ~20 mg liver sample was weighed and homogenized with 500 µL chilled PBS. The homogenate was then centrifuged at 8,000 x g for 2 min at 4°C. Free glycerol was immediately determined using 10 µL of supernatant via enzymatic glycerol phosphate oxidase method (Sigma-Aldrich, St. Louis, MO). An additional 300 µL of supernatant was removed and incubated with 75 µL lipase (MP Biomedicals, Solon, OH) at 37°C for 16 h before determining total glycerol using the same method. Free glycerol (before lipase digestion) was subtracted from total glycerol (after lipase digestion) in order to determine TG content, and this was expressed as a percentage of wet weight of the original sample.

The intra-assay coefficient of variation for free glycerol and total glycerol was 1.9 and 0.8%, respectively.

Histological Analysis

For histological analysis, 10% neutral buffered formalin-fixed ileum, jejunum, and colon samples were submitted to the Iowa State University Veterinary Diagnostic Laboratory for sectioning and periodic acid-Schiff (**PAS**) staining for goblet cell area and villi morphology quantification. One slide per cow per tissue was generated. Using a microscope (Leica® DMI3000 B Inverted Microscope, Bannockburn, IL) with an attached camera (QImaging® 12-bit QICAM Fast 1394, Surrey, BC), five images per section of intestine were obtained at 50X magnification. All image processing and quantification was done using ImageJ 1.48v (National Institutes of Health, USA). PAS stain was measured using the Image J color deconvolution tool with H PAS vector. Goblet cell area was expressed as a percentage of the total mucosal area stained by PAS. For villus measurements, two villi per image were measured for a total of ten measurements per cow. Villus height was measured from the tip to the villus-crypt interface. Villus width was measured at mid height. Crypt depth was measured from the villus-crypt opening to the laminae propria. Crypt width was measured at the villus-crypt interface level. A mucosal surface area estimate was obtained using the mucosal-to-serosal amplification ratio *M* as previously reported by Kisielinski and colleagues (2002), where:

$$M = \frac{(villus\ width \times villus\ length) + \left(\frac{villus\ width}{2} + \frac{crypt\ width}{2}\right)^2 - \left(\frac{villus\ width}{2}\right)^2}{\left(\frac{villus\ width}{2} + \frac{crypt\ width}{2}\right)^2}$$

Immunofluorescence Staining

For immunofluorescence analysis, 10% neutral buffered formalin-fixed jejunum, ileum, and colon were sectioned at 5 microns thickness and mounted in the histology laboratory at the University of Iowa. Slides were deparaffinized using Citrisolv™ Hybrid Solvent (Fisher Scientific), rehydrated by 100%, 95% and 80% ethanol for 5 min each, followed by rinsing in distilled water. Antigen retrieval was performed by incubating slides in citrate buffer (Sodium Citrate, Citric Acid, Tween20, water) in a laboratory microwave for 8 min and then slides were allowed to cool down for 20 min. Tissue sections were blocked in 5% BSA (Sigma Aldrich) in PBS for 90-180 min. Primary antibody against proliferating cell nuclear antigen (**PCNA**; PC10 mAb, Cell Signaling Technology #2586; 1:200 dilution), a marker of cellular proliferation, was applied to each section and incubated overnight at 4°C. Slides were washed thrice in 1XPBS for 10 min each and incubated in fluorescent secondary antibody (anti-mouse IgG (H+L) F(ab')₂ Fragment, AlexaFluor® 488 conjugate, Cell Signaling Technology #4408; 1:1000 dilution) for 1 h at room temperature. Slides were washed thrice in 1XPBS for 10 min each and 4',6-diamidino-2-phenylindole stain was applied to each section. Slides were stained with BSA/BSA, primary antibody/BSA, BSA/secondary antibody for negative control. Images were collected on a Leica fluorescent microscope at 100X magnification. The relative intensity of the PCNA staining was analyzed (3 animals per treatment and 10 villi per animal) using integrated density quantification in ImageJ software.

Statistical Analyses

The effects of FR on most variables progressed with time, and thus data from P2 d5 were statistically analyzed using PROC MIXED of SAS (SAS Institute Inc., Cary, NC)

with extent of FR as the fixed effect. Linear and quadratic effects of AL100, AL80, AL60, AL40, and AL20 treatments (not AL40G) were analyzed using preplanned orthogonal contrasts. In addition, a preplanned contrast of AL40 and AL40G treatments was included. For endotoxin analysis, both P2D2 and P2D4 data were used but the effects of day and treatment by day interaction were not significant and were therefore removed from the model. For post-mortem organ and histology data, preplanned contrasts of AL100 versus AL40 and AL40 versus AL40G were utilized. For liver TG, effect of treatment, period, and treatment by period interaction were assessed as well as linear and quadratic effects. Effect of replicate was included in the model and was removed if not significant. Each specific variable's P1 value (when available) served as a covariate. Results are reported as least squares means and considered different when $P \leq 0.05$ and tend to differ if $P < 0.15$.

Results

As dictated by experimental design, DMI linearly declined with increasing FR ($P < 0.01$; Figure 1A). Similarly, there was a linear decrease in both milk yield (Figure 1B) and EBAL (Table 2). There were no DMI, milk yield, or EBAL differences detected between cows assigned to the AL40G and AL40 treatments ($P > 0.10$). A linear increase in milk fat content occurred with increased severity of FR ($P = 0.01$; Table 2). Advancing FR linearly decreased MUN, protein, and lactose content ($P < 0.01$; Table 2). There were no differences in milk composition between cows in the AL40 and AL40G treatments.

Heart and respiration rate declined linearly with FR ($P < 0.05$; Table 2). Cows in the AL40G treatment had increased HR (7 bpm) and Tr (0.3°C) compared to AL40 controls ($P \leq 0.05$; Table 2). Unexpectedly, AL100 cows lost weight (28 kg), but body weight loss

increased with advancing FR severity (linear: $P < 0.01$; Table 2). Body weight loss tended to be greater in AL40G relative to AL40 cows (60 vs. 40 kg; $P = 0.09$). Body weight loss data should be interpreted with caution because cows were not weighed at a similar time relative to either milking or feeding due to logistical issues.

Overall, there was no difference in circulating glucose ($P = 0.33$; Figure 2A). Both circulating insulin and BUN decreased linearly ($P < 0.01$; Figure 2B and 2C) while plasma NEFA increased linearly ($P < 0.01$; Figure 2D) with increasing FR. Circulating BHB tended to differ ($P = 0.07$) between treatments with AL20 cows having the highest levels (51% above AL100 controls; Figure 2E). Relative to AL40 cows, AL40G cows tended to have decreased circulating glucose (60 vs. 66 mg/dL; $P = 0.08$; Figure 2A) and had increased circulating BUN (27%; $P = 0.03$; Figure 2C).

Circulating endotoxin, haptoglobin, SAA, and LBP increased or tended to increase linearly with advancing FR ($P \leq 0.08$; Figure 3A-D). A quadratic effect was detected for the insulin-to-DMI ratio where AL100 and AL20 treatments did not differ and AL80 and AL60 treatments were decreased ~50% relative to AL100 and AL20 ($P < 0.01$; Figure 3E). Circulating lymphocytes increased with advancing FR ($P = 0.05$; Figure 3F), but there were no treatment or contrasts effects on any other immune cell parameters, including total white blood cells, neutrophils, monocytes, eosinophils, and basophils (data not shown). In comparison with AL40 controls, AL40G cows had similar circulating endotoxin, but had decreased haptoglobin (76%; $P = 0.01$; Figure 3B) and tended to have decreased SAA levels (57%; $P = 0.11$; Figure 3C). Circulating LBP did not differ between AL40G cows and AL40 controls ($P = 0.34$; Figure 3D).

Relative to AL100 cows, AL40 cows had decreased jejunum villus width, jejunum crypt depth, ileum villus height, and ileum crypt depth (36, 14, 22, and 28%, respectively; $P < 0.01$; Figure 4A and 4B), and ileum mucosal surface area tended to be decreased (12%; $P = 0.08$; Figure 4D). Intestinal cellular proliferation, as measured by PCNA staining, tended to be decreased 14% in the ileum of AL40 relative to AL100 cows ($P = 0.06$; Figure 4E). Goblet cell area was decreased 52 and 25% in the jejunum and ileum ($P \leq 0.01$) and tended to be decreased 17% in the colon of AL40 compared to AL100 cows ($P = 0.06$; Figure 4F). No differences in villus height to crypt depth ratio between AL100 and AL40 treatments were detected ($P > 0.10$; Figure 4C). In comparison with the AL40 treatment, cows treated with GLP2 (AL40G) had increased jejunum and ileum villus height (46 and 51%, respectively) and increased ileum crypt depth (17%; $P \leq 0.01$; Figure 4A and 4B). In both jejunum and ileum, AL40G cows had increased villus height to crypt depth ratios and mucosal surface area (38 and 35%, respectively and 30 and 29%, respectively; $P < 0.01$; Figure 4C and 4D). Relative intensity of PCNA protein was increased in both jejunum (43%) and ileum (36%) of AL40G cows relative to AL40 controls ($P < 0.01$; Figure 4E). Goblet cell area from AL40G cows was increased 59, 41, and 48% in the jejunum, ileum, and colon, respectively, relative to AL40 controls ($P < 0.01$; Figure 4F).

Liver TG did not differ during P1 ($P > 0.10$). At the end of P2, liver TG were increased linearly with increased severity of FR ($P = 0.01$), and this was mainly due to a ~88% increase in AL40 and AL20 relative to AL100 cows (Figure 5).

Discussion

There is increasing appreciation for how intestinal barrier integrity influences performance in animal agriculture. Circumstances where the gastrointestinal barrier is compromised include weaning (Boudry et al., 2004; Moeser et al., 2007), heat stress (Baumgard and Rhoads, 2013; Pearce et al., 2013), rumen acidosis (Emmanuel et al., 2007; Khafipour et al., 2009; Minuti et al., 2014), and the periparturient period (Abuajamieh et al., 2016). Thus, identifying a mitigation strategy that could improve or ameliorate leaky gut would presumably enhance production efficiency in a variety of species. However, a standardized and easily implemented model to identify potential target molecules has not been established. Reduced feed intake causes intestinal barrier dysfunction in humans (Welsh et al., 1998), rodents (Holt et al., 1986; Ueno et al., 2011), chickens (Yamauchi et al., 1996), pigs (Carey et al., 1994; Pearce et al., 2013), and ruminant models (Zhang et al., 2013). Further, our preliminary data suggest that this is also the case in lactating cows (Kvidera et al., 2017b). Therefore, our objectives were to identify a magnitude of FR that affects circulating biomarkers reflective of leaky gut, and to evaluate GLP2 administration as a potential mitigation strategy in lactating cows.

As expected, FR reduced milk yield and energy balance and increased BW loss, and this demonstrates that we successfully implemented the experimental design. Further, milk yield and energy balance decreases were similar to other studies of comparable length and degree of restriction (Carlson et al., 2006; Ferraretto et al., 2014). Metabolic changes observed during the current study are consistent with the well-known hallmarks of reduced feed intake in ruminants, including: reduced circulating insulin (de Boer et al., 1985; Carlson et al., 2006; Ferraretto et al., 2014) and BUN (Bjerre-Harpøth et al., 2012; Lérias

et al., 2015) and increased circulating NEFA (de Boer et al., 1985; Carlson et al., 2006; Ferraretto et al., 2014). Additionally, liver fat content was increased in animals restricted to 40 or 20% ad libitum intake, which agrees with similar models (Carlson et al., 2003) while less severe or more chronic FR models do not observe such effects (Gross et al., 2013).

In the current study, there were no overt health disorders, so the increase in circulating inflammatory biomarkers observed ostensibly originated from the translocation of gut luminal content into circulation due to reduced intestinal barrier integrity. Circulating endotoxin was elevated with increasing severity of FR, and levels were similar to those observed by Khafipour and colleagues (2009). However, endotoxin data should be treated with caution as the Limulus Amebocyte Lysate assay measures endotoxin biological activity and not LPS bound to inflammatory mediators such as soluble CD14 or LBP (Guerville and Boudry, 2016). Perhaps better markers of gut luminal content infiltration are acute phase proteins (**APP**), which are produced by the liver as a secondary (non-local) response to toxic stimuli and have been widely utilized as markers of systemic inflammation (Ceciliani et al., 2012). Acute phase proteins LBP, SAA, and haptoglobin were increased with progressive FR, indicating an inflammatory response. Others have demonstrated an APP response to short-term FR in beef cattle, suggesting that feed and water deprivation are the main contributors to the APP response in receiving feedlot cattle (Cappellozza et al., 2011; Marques et al., 2012). It is unclear why AL20 cows had decreased levels of APP relative to less severe restricted cows. However, the magnitude of APP production can be impaired during malnourishment (Doherty et al., 1993; Reid et al., 2002), yet remains responsive (Ling et al., 2004), and perhaps restricting feed to less than

40% of ad libitum limits the APP response. Circulating insulin is also acutely increased during times of inflammation (Baumgard et al., 2016; Kvidera et al., 2017a,b); however, differences in feed intake (and thus circulating nutrients, a major driver of insulin secretion) make this interpretation difficult. Utilizing an insulin-to-DMI ratio allows us to quantify the amount of circulating insulin per unit of DMI. The insulin-to-DMI ratio reached its nadir in the AL60 treatment before increasing to the point where AL20 cows do not differ from the AL100 cows. In the current study, the quadratic effect of FR on the insulin-to-DMI ratio further confirms the most severely feed restricted treatments were experiencing inflammation.

Reduced feed intake has deleterious effects on intestinal health (Ferraris and Carey, 2000). In the current study, FR to 40% of ad libitum intake for 5 d negatively impacted intestinal architecture, particularly reducing the villus height and crypt depth in the ileum. The decreased villus height is likely due to the 14% decrease in cellular proliferation (Figure 4E). Numerous other FR models demonstrate detrimental effects on intestinal histology, including reduced villus height in feed restricted pigs (Carey et al., 1994; Pearce et al., 2013), chickens (Yamauchi et al., 1996), mice (Ueno et al., 2011), and rats (Holt et al., 1986). However, some FR models indicate no effect or even an increase in villi height (Holt et al., 1986; Chappell et al., 2003; Hodin et al., 2011; Tůmová et al., 2015). Reasons for discrepancy between results could be due to severity and duration of FR as well as the intestinal segment examined and its relative importance to that species. Mechanistically, fasting reduces intestinal epithelial cell numbers, cellular proliferation, and cellular migration rates, and this is coupled with increased rates of cellular loss and apoptosis, altogether contributing to changes in intestinal architecture and increased epithelial

permeability (Chappell et al., 2003; Ferraris and Carey, 2000). Although altered morphology does not prove decreased barrier function, malnutrition and increased intestinal permeability have been reported in numerous species (Holt et al., 1986; Welsh et al., 1998; Boza et al., 1999). Further, altered intestinal barrier function has been demonstrated in studies where structural changes in villi morphology were similar to those observed in the current study (Ford et al., 1985; Pearce et al., 2013).

The importance of proper barrier function cannot be understated as the intestine is continuously exposed to potential pathogens and toxins (Mani et al., 2012). The human gastrointestinal tract has a surface area of $\sim 400\text{m}^2$ (Murphy, 2012) and is continuously subjected to potentially pathogenic microorganisms and toxins (Mani et al., 2012). Pathogen exposure is certainly more extensive in ruminants due to pre-gastric fermentation compartments and the relative size of the gastrointestinal tract. The direct consequence of intestinal barrier dysfunction is increased leakage of luminal antigens into the submucosa and portal blood stream with the potential to initiate an inflammatory response. This can undoubtedly compromise production as immunoactivation is an energetically expensive process which redirects energy away from agriculturally productive purposes and compromises animal welfare (Kvidera et al., 2017a). Therefore, researching molecules or nutritional strategies to ameliorate gut barrier dysfunction, including exogenous GLP2, has been gaining recent attention in production agriculture (Thymann et al., 2014; Connor et al., 2016). Endogenous GLP2 is a proglucagon-derived peptide secreted by enteroendocrine L cells in response to luminal nutrients (Drucker and Yusta, 2014). The trophic effects of GLP2 on intestine are mediated through increased mesenteric blood flow, greater nutrient uptake, and release of growth factors such as IGF-1, IGF-2, keratinocyte

growth factor, and epidermal growth factor-ErbB family members (Connor et al., 2016). Exogenous GLP2 administration reduced intestinal permeability, hepatic bacterial cultures, and intestinal crypt apoptosis in stressed mouse models (Boushey et al., 1999; Cameron and Perdue, 2005). The authors are unaware of previous literature examining GLP2's effect in lactating dairy cattle; however, GLP2 receptors have been characterized in the lactating cow gastrointestinal tract (Connor et al., 2010). In calves, GLP2 administration improved small intestine tight junction protein expression, blood flow, and growth and reduced circulating acute phase proteins (Taylor-Edwards et al., 2011; Connor et al., 2013; Walker et al., 2015; Connor et al., 2017). In the current study, administering GLP2 increased ileum villus height above the ad libitum controls. Negative effects of FR on ileum mucosal area and goblet cell area of all three segments were also rescued or even improved by GLP2 administration. Though FR did not affect villus height to crypt depth ratios, jejunum villus height, or jejunum mucosal surface area, GLP2 increased each of these parameters ~30-50% above ad libitum control cows. Increased villus height in animals administered GLP2 is likely explained by the 43 and 36% increase in cellular proliferation observed in both the jejunum and ileum. It is unclear why FR impacted the ileum more than the jejunum, but may be due to diminished nutrient availability from the proximal to distal intestine. Subtle changes in jejunum intestinal architecture also may not be detected due to the low number of cows utilized in this study. Regardless, these data clearly demonstrate the beneficial effects of GLP2 on the intestine in both stressed and non-stressed sections of the intestine (i.e., both jejunum and ileum), which agrees with Hu et al. (2010) who also observed GLP2's trophic effects in both heat-stressed and healthy chickens. Further, administering GLP2 lowered circulating haptoglobin and serum amyloid A 76 and 57%,

respectively. A reduced circulating serum amyloid A response to coccidial infection was also observed in calves administered GLP2 (Connor et al., 2017). Despite not being able to establish causation, we hypothesize GLP2's positive effects on intestinal integrity and subsequent prevention of endotoxin infiltration play a role in the APP reduction. Glucagon like peptide 2 may also have a direct role in inhibiting inflammation because it stimulates antimicrobial peptide secretion by Paneth cells in the intestinal epithelium (Lee et al., 2012) which may reduce microbial load and subsequent microbial component leakage into systemic circulation. Furthermore, GLP2 has the ability to suppress the pro-inflammatory response of macrophages to LPS (Xie et al., 2014). Collectively, the histology and changes in acute phase protein profile strongly suggest that GLP2 improved gut barrier function.

Despite its beneficial effects on intestinal health, GLP2 administration did not improve production parameters, as evidenced by similar milk yield and composition between AL40 and AL40G cows. This is not entirely surprising, as the experiment was knowingly underpowered to detect production differences. Additionally, the FR model limits any positive effects GLP2 may have on feed intake. It would be of interest to study GLP2 administration during a natural model of stress with objectives of determining if GLP2 has a beneficial effect on feed intake or production and if this further improves intestinal growth and integrity. It is unclear whether the GLP2-induced decrease in circulating glucose is of biological relevance (66 vs. 60 mg/dL), but it may be related GLP2's neuroendocrine role in the gut-brain axis which can result in suppression of hepatic glucose production and increased glucose tolerance (Guan, 2014). Injecting GLP2 did not influence circulating insulin, and this is not surprising as GLP2 does not directly modulate insulin secretion (Schmidt et al., 1985). Interestingly, GLP2 administration increased BUN

by 27%. Reasons behind this effect are not clear, but interpreting BUN in ruminants is difficult because it originates from both skeletal muscle catabolism and excessive rumen ammonia production.

Decreased intestinal barrier integrity has several negative consequences and thus research into ruminant intestinal health has gained recent attention (Connor et al., 2016; Steele et al., 2016). However, it is difficult to model leaky gut in order to test potential mitigation strategies. Various models exist to pharmaceutically induce intestinal barrier dysfunction, including non-steroidal anti-inflammatory drugs, gamma-secretase inhibitors, and dextran sodium sulfate (Fortun and Hawkey, 2007; Wirtz et al., 2007; Kvidera et al., 2017b), but these approaches are difficult to administer in ruminants and are often associated with side effects. As previously mentioned, the severity and duration of FR in literature varies considerably and its effects on gut health are not extensively studied in the ruminant animal, ergo it is not clear what magnitude of FR is required to dependably compromise intestinal barrier function. In the current study, animals restricted to 40% of their ad libitum intake for 5 days exhibited a well-defined increase in inflammatory parameters. Deterioration of intestinal architecture accompanied this inflammation, giving us confidence that intestinal dysfunction was likely the cause. One drawback to our model is the limitation of beneficial effects a mitigation strategy may have on feed intake. Despite this, GLP2 administration improved intestinal health and subsequently reduced inflammatory parameters independently of any changes in feed intake.

Conclusions

Increasing severity of FR reduced milk yield and increased circulating endotoxin and APP as well as liver TG. It appears that cows fed 40% of their ad libitum intake were most affected, and this treatment showed obvious signs of intestinal dysfunction, including reduced ileum villus height, mucosal surface area, and cellular proliferation as well as reduced goblet cell area in all three intestinal sections. Administering GLP2 had a substantial effect on intestinal growth, morphology, and reduced circulating APP in comparison with cows on a similar plane of nutrition. In conclusion, FR had detrimental effects on intestinal barrier integrity and administering GLP2 alleviates these negative effects.

Table 8. Ingredients and composition of diet

Ingredient	% of DM ²
Corn Silage	42.8
Alfalfa Hay	19.7
Rolled Corn	18.1
Whole Cotton	8.6
High Protein Soybean Meal ³	5.4
Soy Plus ^{®4}	3.0
Ground Corn	2.3
Chemical analysis, % of DM	
CP	16.7
NDF	32.1
ADF	21.8
NE _L Mcal/kg DM	1.6

¹Values represent an average of ration nutrient summary reports collected throughout the trial. Diet moisture averaged 43.56%.

²Average nutrient levels: 4.41% Fat, 0.84% Ca, 0.47% P, 0.37% Mg, 0.20% S, 1.20% K, 0.45% Na, 0.48% Cl, 56.58 ppm of Zn, 64.85 ppm of Mn, 136.46 ppm of Fe, 17.91 ppm of Cu, 0.22 ppm of Co, 0.27 ppm Se, 0.85 ppm of I, 4402.2 IU/kg of vitamin A, 1438.8 IU/kg of vitamin D, and 24.2 IU/kg of vitamin E

³Solvent-extracted soybean meal containing 54.5% CP, 35% RUP (% CP; DM basis).

⁴Cooker-expeller processed soybean meal produced by West Central Cooperative, Ralston, IA, containing 46.6% CP, 60% RUP (% CP; DM basis).

Table 9. Effects of 5 d of incremental feed restriction and GLP2 treatment on milk and health parameters

Parameter	Treatment ¹						SEM	<i>P</i> Treatment	Contrasts		
	AL100	AL80	AL60	AL40	AL40G	AL20			Linear ²	Quadratic ²	AL40 v AL40G
Milk parameters											
Fat, %	3.9	4.3	4.1	4.4	4.6	5.4	0.3	0.07	0.01	0.22	0.68
Lactose, %	4.8 ^a	4.6 ^{ab}	4.6 ^{ab}	4.5 ^{ab}	4.4 ^{bc}	4.2 ^c	0.1	0.01	<0.01	0.69	0.47
Protein, %	3.2	3.0	3.0	2.9	2.8	2.9	0.1	0.08	0.04	0.25	0.36
TS, %	12.8	13.0	12.5	12.7	13.1	13.4	0.4	0.55	0.41	0.24	0.49
MUN, mg/dL	14.6 ^a	14.1 ^{ab}	12.7 ^{bc}	11.6 ^c	12.2 ^c	11.3 ^c	0.5	<0.01	<0.01	0.76	0.41
Milk SCC, *1000/mL	82	84	256	130	153	411	92	0.12	0.03	0.48	0.86
Health parameters											
EBAL, Mcal/d	11.2 ^a	2.6 ^b	0.0 ^b	-5.0 ^c	-6.4 ^c	-10.5 ^d	1.2	<0.01	<0.01	0.32	0.49
Tr ³ , °C	38.3	38.5	38.3	38.1	38.4	38.3	0.1	0.24	0.17	0.99	0.05
RR ⁴ , breaths/min	42	45	39	38	43	36	3	0.13	0.04	0.69	0.15
Heart rate, beats/min	71 ^a	69 ^{ab}	64 ^b	58 ^c	65 ^{ab}	65 ^{ab}	2	0.01	0.01	0.05	0.02
BW loss, kg	28 ^c	25 ^c	36 ^c	40 ^{bc}	60 ^{ab}	69 ^a	8	<0.01	<0.01	0.09	0.09

¹Treatments: AL100 = ad libitum intake; AL80 = restricted to 80% of ad libitum intake; AL60 = restricted to 60% of ad libitum intake; AL40 = restricted to 40% of ad libitum intake + saline (3 mL s.c. twice daily); AL40G = restricted to 40% of ad libitum intake + glucagon-like peptide 2 (75 µg/kg BW s.c. twice daily); AL20 = restricted to 20% of ad libitum intake

²Assessed using AL100, AL80, AL60, AL40, and AL20 treatments

³Rectal temperature

⁴Respiration rate

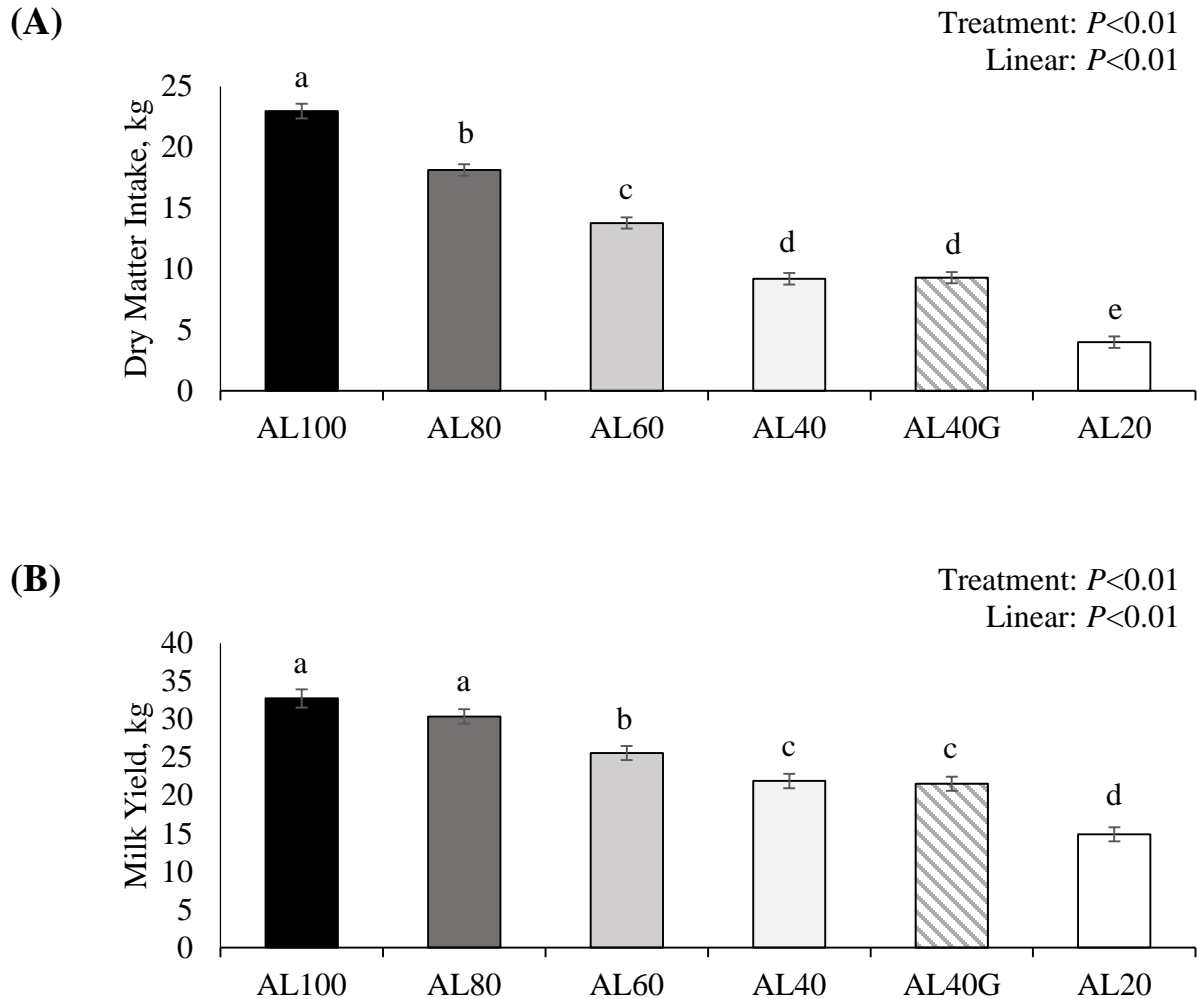


Figure 10. Effects of incremental feed restriction and GLP2 treatment on (A) dry matter intake and (B) milk yield on d 5 of treatment. Treatments include: AL100 = ad libitum intake, AL80 = restricted to 80% of ad libitum intake, AL60 = restricted to 60% of ad libitum intake, AL40 = restricted to 40% of ad libitum intake + saline (3 mL s.c. twice daily), AL40G = restricted to 40% of ad libitum intake + glucagon-like peptide 2 (75 μ g/kg BW s.c. twice daily), and AL20 = restricted to 20% of ad libitum intake. ^{a-e}Values with differing superscripts denote differences ($P \leq 0.05$) between treatments.

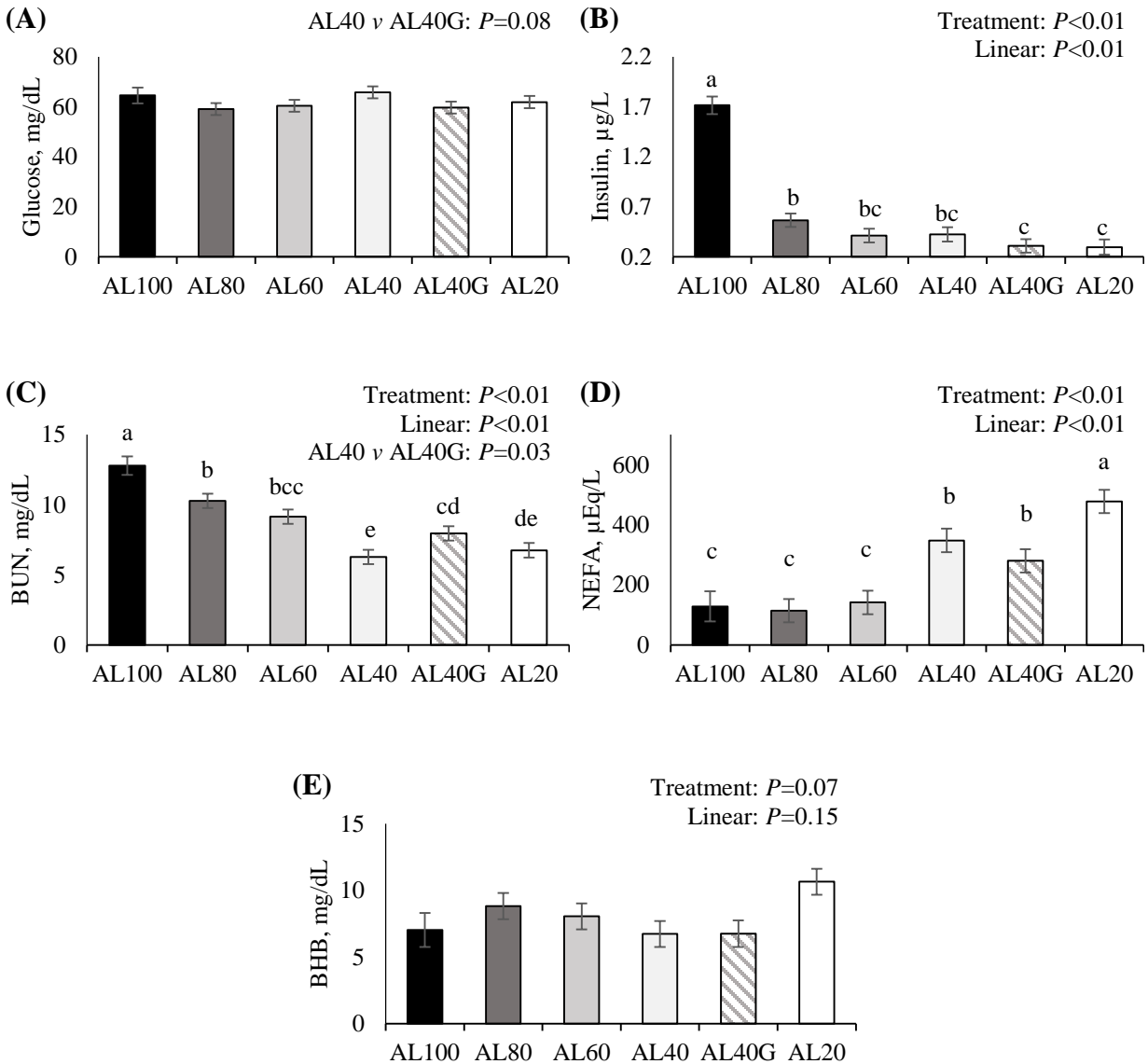


Figure 11. Effects of incremental feed restriction and GLP2 treatment on circulating (A) glucose, (B) insulin, (C) BUN, (D) non-esterified fatty acids (NEFA), and (E) BHB on d 5 of treatment. Treatments include: AL100 = ad libitum intake, AL80 = restricted to 80% of ad libitum intake, AL60 = restricted to 60% of ad libitum intake, AL40 = restricted to 40% of ad libitum intake + saline (3 mL s.c. twice daily), AL40G = restricted to 40% of ad libitum intake + glucagon-like peptide 2 (75 $\mu\text{g/kg}$ BW s.c. twice daily), and AL20 = restricted to 20% of ad libitum intake. ^{a-}

^eValues with differing superscripts denote differences ($P \leq 0.05$) between treatments.

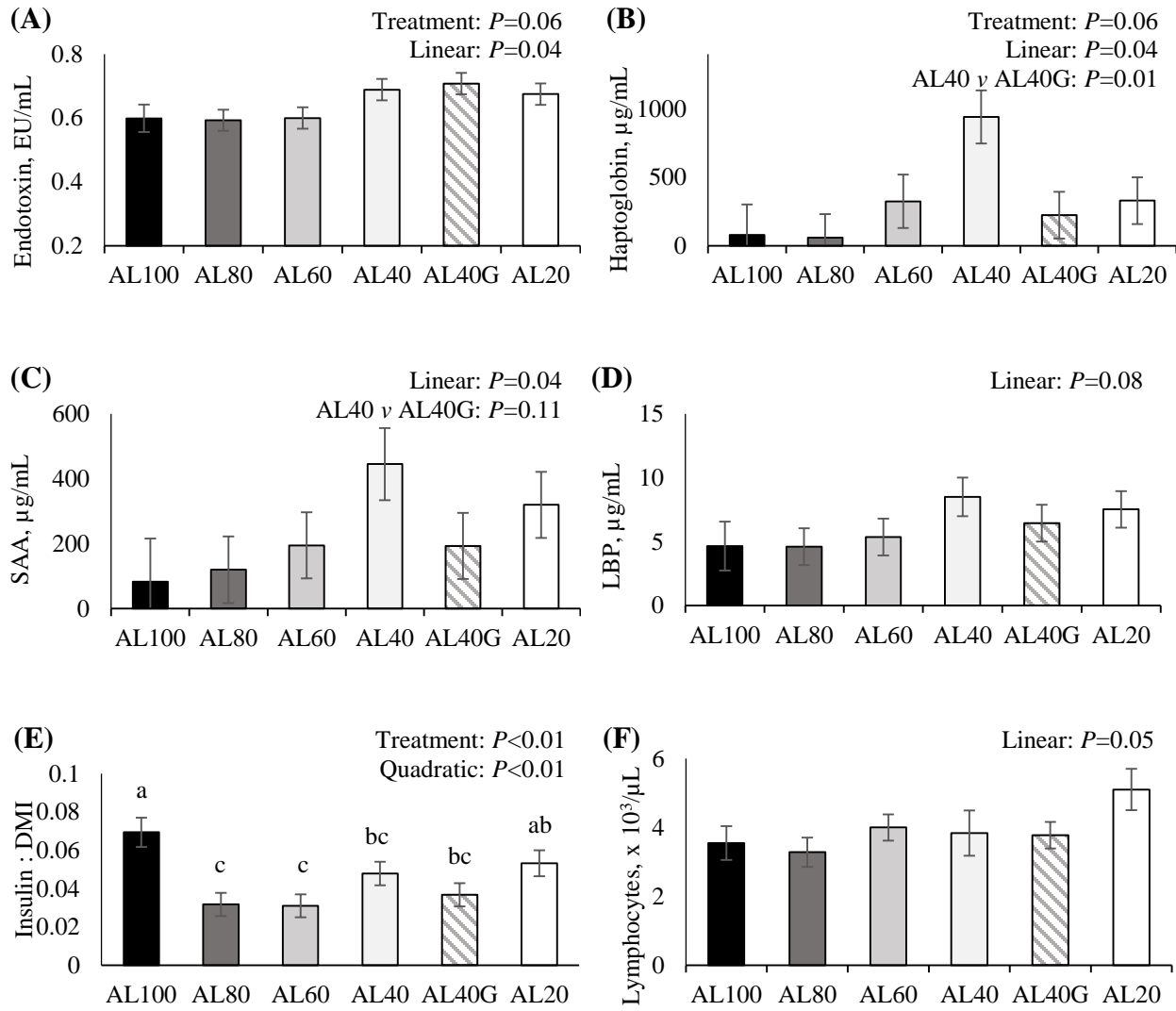


Figure 12. Effects of incremental feed restriction and GLP2 treatment on (A) circulating endotoxin, (B) haptoglobin, (C) serum amyloid A (SAA), (D) LPS-binding protein (LBP), (E) insulin-to-dry matter intake ratio (Insulin:DMI), and (F) lymphocytes. Treatments include: AL100 = ad libitum intake, AL80 = restricted to 80% of ad libitum intake, AL60 = restricted to 60% of ad libitum intake, AL40 = restricted to 40% of ad libitum intake + saline (3 mL s.c. twice daily), AL40G = restricted to 40% of ad libitum intake + glucagon-like peptide 2 (75 $\mu\text{g/kg}$ BW s.c. twice daily), and AL20 = restricted to 20% of ad libitum intake. ^{a-c}Values with differing superscripts denote differences ($P \leq 0.05$) between treatments.

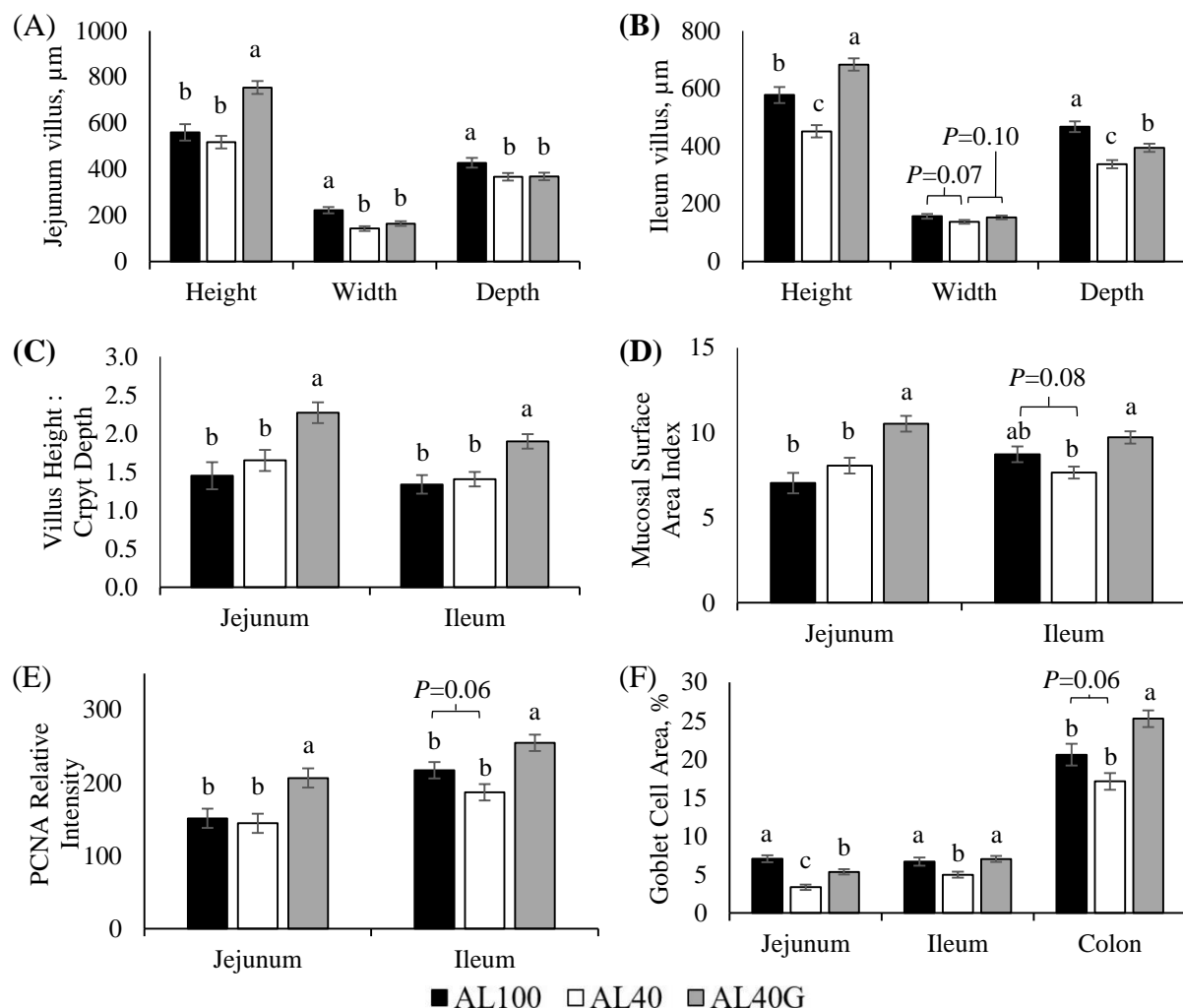


Figure 13. Effects of incremental feed restriction and GLP2 treatment on (A) jejunum villus morphology, (B) ileum villus morphology, (C) villus height-to-crypt depth ratio in jejunum and ileum, (D) mucosal surface area in jejunum and ileum, (E) intensity of PCNA protein in jejunum and ileum, and (F) goblet cell area as a percentage of epithelial area in jejunum, ileum, and colon. Treatments include: AL100 = ad libitum intake, AL40 = restricted to 40% of ad libitum intake + saline (3 mL s.c. twice daily), and AL40G = restricted to 40% of ad libitum intake + glucagon-like peptide 2 (75 μg/kg BW s.c. twice daily). ^{a-c}Values with differing superscripts denote differences ($P \leq 0.05$) between treatments. Mucosal surface area is expressed as an M-index as described by Kisielinski et al., 2002.

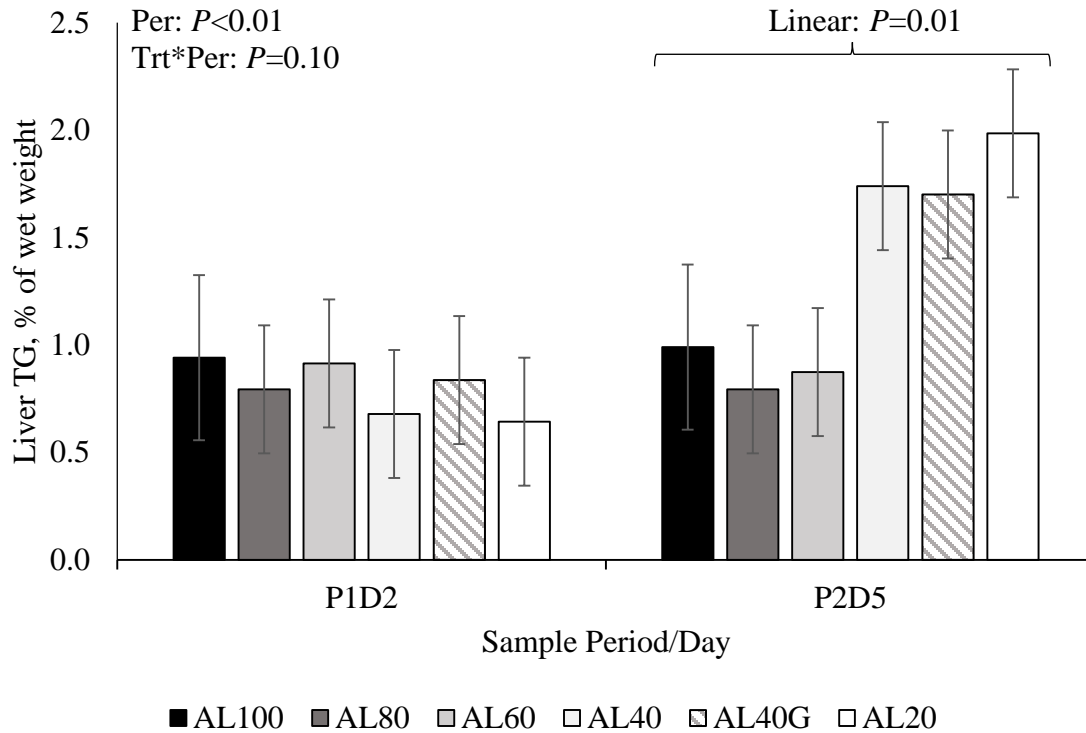


Figure 14. Effects of incremental feed restriction and GLP2 treatment on liver triglyceride (TG) content. Treatments include: AL100 = ad libitum intake, AL80 = restricted to 80% of ad libitum intake, AL60 = restricted to 60% of ad libitum intake, AL40 = restricted to 40% of ad libitum intake + saline (3 mL s.c. twice daily), AL40G = restricted to 40% of ad libitum intake + glucagon-like peptide 2 (75 $\mu\text{g}/\text{kg}$ BW s.c. twice daily), and AL20 = restricted to 20% of ad libitum intake.

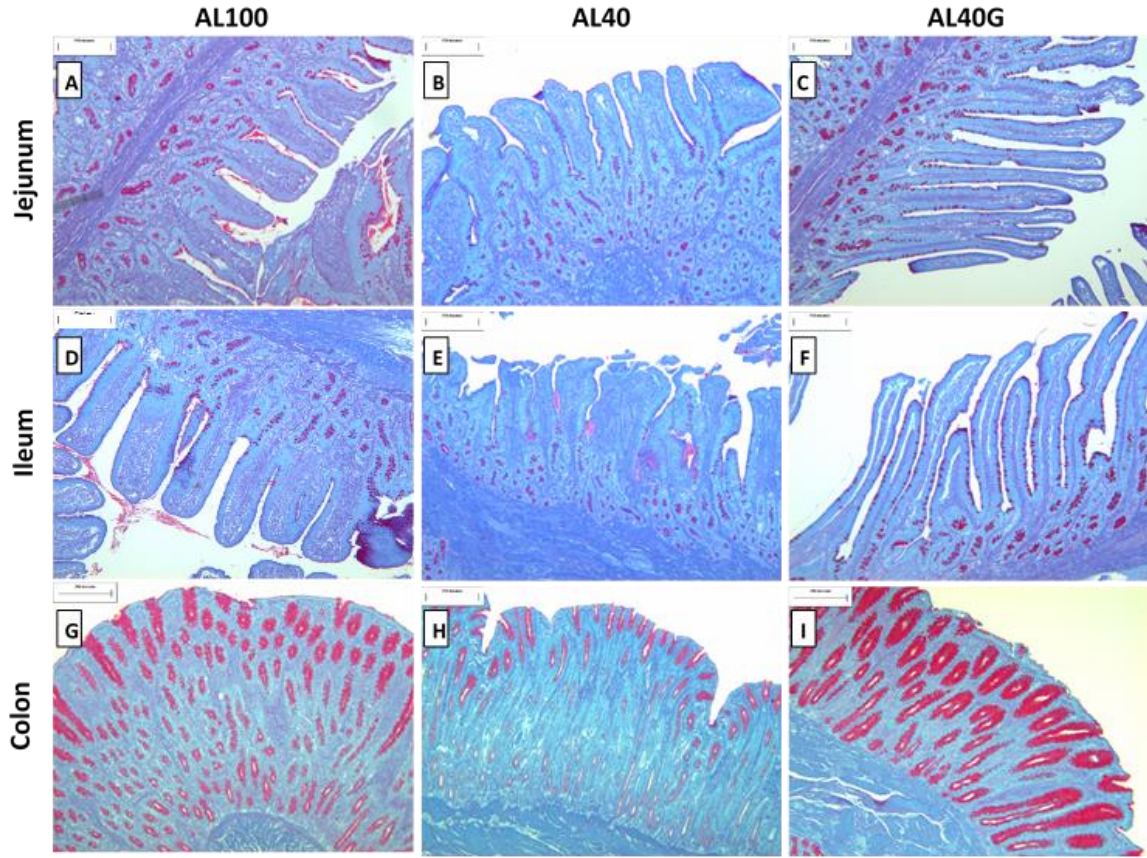


Figure 15. Representative images of periodic acid-Schiff stained (A) AL100 jejunum, (B) AL40 jejunum, (C) AL40G jejunum, (D) AL100 ileum, (E) AL40 ileum, (F) AL40G ileum, (G) AL100 colon, (B) AL40 colon, (I) AL40G colon. Dark purple stain is positive for mucosubstances.

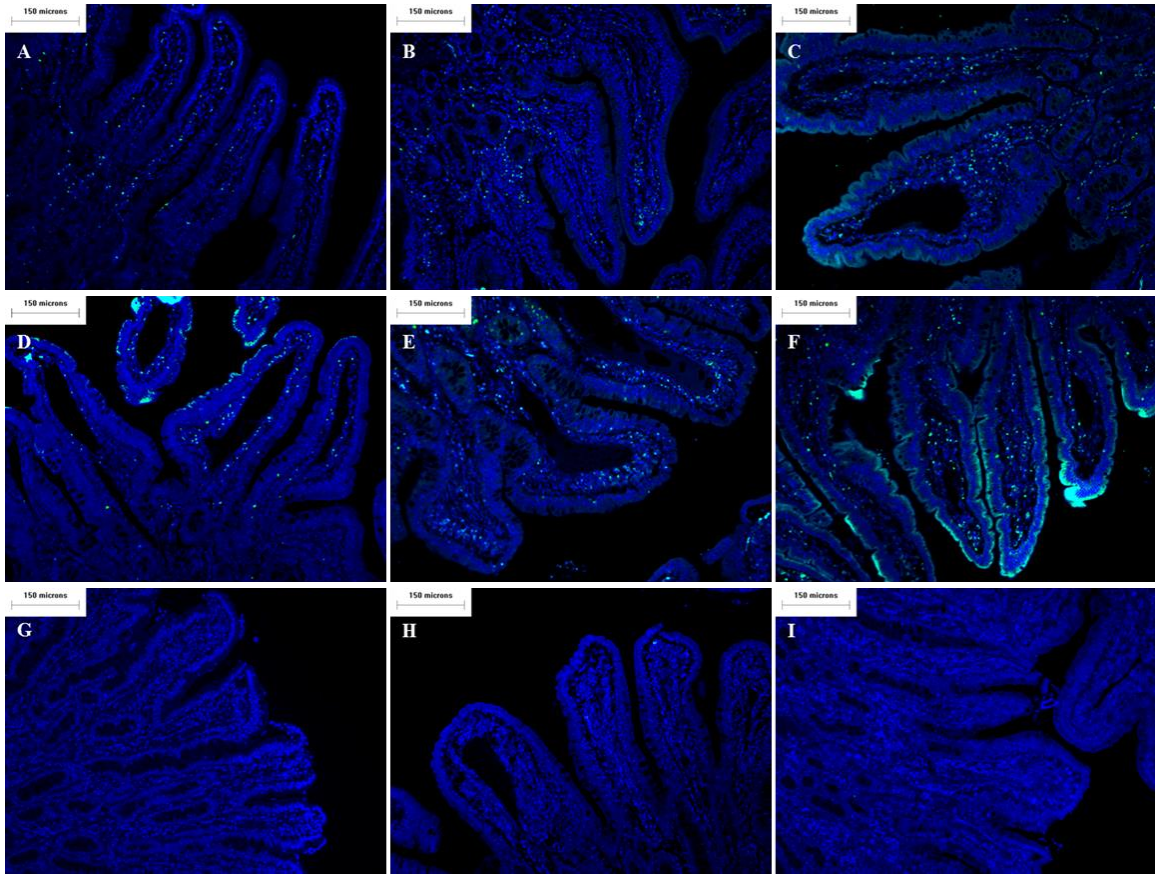


Figure 16. Representative image of PCNA protein intensity in (A) AL100 jejunum, (B) AL40 jejunum, (C) AL40G jejunum, (D) AL100 ileum, (E) AL40 ileum, (F) AL40G ileum. Negative controls are (F) BSA/BSA, (G) primary antibody alone, (H) secondary antibody alone. Nuclear stain is blue while PCNA stain is green.

**CHAPTER 4: TECHNICAL NOTE: ESTIMATING GLUCOSE
REQUIREMENTS OF AN ACTIVATED IMMUNE SYSTEM IN HOLSTEIN
STEERS**

A paper published in 2016 by Journal of Animal Science 94:4591-4599.

doi: 10.2527/jas.2016-0765

S. K. Kvidera, E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L.
H. Baumgard

Iowa State University Department of Animal Science, Ames, Iowa, 50011

Abstract

Infection and inflammation impede efficient animal productivity. The activated immune system ostensibly requires large amounts of energy and nutrients otherwise destined for synthesis of agriculturally relevant products. Accurately determining the immune system's in vivo energy needs is difficult, but a better understanding may facilitate developing nutritional strategies to maximize productivity. The study objective was to estimate immune system glucose requirements following an i.v. lipopolysaccharide (LPS) challenge. Holstein steers (148 ± 9 kg; $n=15$) were jugular catheterized bilaterally and assigned to 1 of 3 i.v. treatments: control (CON; 3 mL saline; $n=5$), LPS-administered controls (LPS-C; *E. coli* 055:B5; 1.5 $\mu\text{g}/\text{kg}$ BW; $n=5$), and LPS + euglycemic clamp (LPS-Eu; 1.5 $\mu\text{g}/\text{kg}$ BW; 50% dextrose infusion to maintain euglycemia; $n=5$). In LPS-Eu steers,

post-bolus blood samples were analyzed for glucose every 10 min. Dextrose infusion rates were adjusted to maintain euglycemia for 720 min. All steers were fasted during the challenge. Samples for later analysis were obtained at 180, 360, 540, and 720 min relative to LPS administration. Rectal temperature was increased $\sim 0.5^{\circ}\text{C}$ in both LPS treatments relative to CON steers ($P = 0.01$). Steers in both LPS treatments were hyperglycemic for ~ 3 h post-bolus; thereafter, blood glucose was markedly decreased (30%; $P < 0.01$) in LPS-C relative to both CON and LPS-Eu steers. A total of 516 ± 65 g of infused glucose was required to maintain continuous euglycemia in LPS-Eu steers. Circulating insulin increased in LPS-C and LPS-Eu steers relative to CON ($\sim 70\%$ and ~ 20 fold, respectively; $P < 0.01$). Circulating NEFA increased similarly with time for both CON and LPS-C compared to LPS-Eu steers ($\sim 43\%$; $P < 0.01$). Plasma L-lactate and LPS binding protein increased (~ 198 and $\sim 90\%$, respectively; $P < 0.01$) and ionized calcium decreased (18%; $P < 0.01$) in both LPS treatments relative to CON steers. Circulating white blood cells decreased initially in LPS-Eu and LPS-C relative to controls (180 min; 85%) followed by a progressive increase with time ($P = 0.02$). Blood neutrophils followed the same pattern, however, at 720 min, neutrophils were decreased in LPS-Eu compared to LPS-C, resulting in a decreased neutrophil-to-lymphocyte ratio (54%; $P = 0.03$). The large amount of glucose needed to maintain euglycemia indicates extensive repartitioning of nutrients away from growth and the importance of glucose as a fuel for the immune system.

Key words: glucose homeostasis, immune challenge, lipopolysaccharide

Introduction

Infection and inflammation have negative economic consequences on animal agriculture due to decreased production, inefficient feed utilization, poor reproduction, and increased health care costs. An activated immune system demands a large amount of energy and nutrients (Lochmiller and Deerenberg, 2000; Johnson, 2012) which reprioritizes the hierarchy of nutrient partitioning away from productive purposes. For instance, glucose homeostasis is markedly disrupted during an endotoxin (e.g., lipopolysaccharide; **LPS**) challenge and hypoglycemia and hyperlactemia are characteristic hallmarks (Filkins, 1978; McGuinness, 2005; Michaeli et al., 2012). In addition, in vitro experiments suggest activated immune cells experience a substantial increase in glucose consumption and utilize glucose as their primary source for the generation of energy, biosynthetic precursors, and signaling intermediates (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). The extent of immune cell glucose consumption in vivo is difficult to assess due to the ubiquitous and fluctuating distribution of immune cells and organ specific changes in insulin sensitivity; however, better understanding the impact on energy status has practical implications to animal agriculture as glucose is obviously an important fuel for productive purposes.

Despite the increase in glucose requirements, an activated immune response is often accompanied by anorexia and thus decreased diet-derived glucose or glucose precursors. To ensure an adequate nutrient supply to the immune system, hepatic glucose output can increase via both glycogenolysis and gluconeogenesis (Filkins, 1978; McGuinness, 1994). Concurrently, there is an increase in peripheral insulin resistance leading to decreased glucose uptake by skeletal muscle and adipose tissue (Lang et al., 1990; Song et al., 2006).

Despite the homeorhetic efforts to spare glucose for the immune system, hypoglycemia often develops following a LPS challenge, likely because the immune system's rate of glucose utilization exceeds the synchronized capacity of the liver to export glucose and insulin sensitive tissues to reduce glucose disposal (McGuinness, 2005). Therefore, the experimental objective was to estimate the amount of glucose needed to maintain euglycemia following an LPS challenge as a proxy for the amount of glucose required to fuel an acute immune response.

Materials and Methods

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Fifteen Holstein steers (148 ± 9 kg) were randomly assigned to individual pens at the Iowa State University Zumwalt Climatic Research Station (Ames, IA). Steers were allowed 5 d to acclimate during which they were implanted with bilateral jugular catheters and fed ad libitum once daily (0600 h) a diet formulated to meet or exceed the predicted requirements (NRC, 2001) of energy, protein, minerals, and vitamins. Steers were randomly assigned to 1 of 3 i.v. bolus treatments: control (**CON**; 3 mL sterile saline; $n=5$), LPS-administered controls in which hypoglycemia was allowed to develop (**LPS-C**; 1.5 $\mu\text{g}/\text{kg}$ BW LPS; $n=5$), and LPS-administered in which euglycemia was maintained (**LPS-Eu**; 1.5 $\mu\text{g}/\text{kg}$ BW LPS; $n=5$). The LPS dose used was selected based on Waggoner et al. (2009b) and their observation of a 30% decrease in circulating glucose 4 h post-LPS. Lipopolysaccharide (*Escherichia coli* O55:B5; Sigma Aldrich, St. Louis, MO) was dissolved in sterile saline at a concentration of 75 $\mu\text{g}/\text{mL}$ and passed through a 0.2 μm sterile syringe filter (Thermo Scientific; Waltham, MA). The total volume of LPS solution

administered was approximately 3 mL. In the LPS-Eu treatment, we performed a euglycemic clamp where 50% dextrose (VetOne, Boise, ID) was i.v. infused at a known and adjustable rate utilizing a modular pump (Deltec 3000, Deltec Inc., St. Paul, MN) in order to maintain the pre-LPS infusion blood glucose levels.

Feed was removed ~1 h prior to treatment administration and animals remained fasted during the 720 min data collection period. Baseline blood samples were obtained -30, -20, and 0 min relative to bolus administration to establish baseline glucose levels. Each respective treatment bolus was administered immediately following the 0 min blood sample collection. For LPS-Eu steers, post-bolus blood samples (1 mL) were collected every 10 min and immediately analyzed for glucose (TRUEbalance glucometer; McKesson, San Francisco, CA). Dextrose infusion began when blood glucose content declined below baseline levels, and its rate of infusion was adjusted as necessary to maintain blood glucose concentration at baseline levels ($\pm 10\%$). The rate of 50% dextrose infusion (mL/h) was transformed to rate of glucose infusion (**ROGI**; g/h). The total glucose infused for each steer was calculated using the ROGI for each 10 min interval (72 intervals in total) according the following equation:

$$\sum_{i=0}^{72} ROGI \left(\frac{g}{h} \right)_i * \frac{1 h}{60 min} * 10 min$$

Blood glucose was measured every 30 min in CON and LPS-C steers for the first 300 min and every 60 min thereafter. Additional serum and plasma samples (~10 mL each) for further analysis were collected from all treatments at -30, 0, 180, 360, 540, and 720 min relative to LPS administration. Rectal temperatures were obtained -30 and 0 min relative to bolus administration, every 30 min for the first 300 min, and every 60 min thereafter using a digital thermometer (GLA M700, San Luis Obispo, CA).

Insulin, glucose, NEFA, β -hydroxy butyrate (**BHB**), LPS binding protein (**LBP**), L-lactate, serum amyloid A (**SAA**), haptoglobin, and blood urea nitrogen (**BUN**) concentrations were determined using commercially available kits according to manufacturers' instructions (insulin, Mercodia AB, Uppsala, Sweden; glucose, Wako Chemicals USA Inc., Richmond, VA; NEFA, Wako Chemicals USA, Richmond, VA; BHB, Pointe Scientific Inc., Canton, MI; LBP, Hycult Biotech, Uden, Netherlands; L-lactate, Biomedical Research Service Center, Buffalo, NY; SAA, Tridelata Development Ltd., Kildare, Ireland; haptoglobin, Immunology Consultants Laboratory Inc., Portland, OR; BUN, Teco Diagnostics Anaheim, CA). The inter- and intra-assay coefficients for haptoglobin, insulin, NEFA, BHB, L-lactate, SAA, LBP, and BUN assays were 14.0 and 10.2%, 12.6 and 7.0%, 9.9 and 3.9%, 3.8 and 2.3%, 7.1 and 2.9%, 14.2 and 6.2%, 33.0 and 2.6%, and 12.2 and 5.2%, respectively. Ionized blood calcium was measured using an i-STAT handheld machine and cartridge (CG8+; Abbott Point of Care, Princeton, NJ). For white blood cell (**WBC**) count, a 3 mL blood sample was collected (K2EDTA; BD Franklin Lakes, NJ) and stored at 4°C for 12 h before submission to the Iowa State Department of Veterinary Pathology for complete blood count analysis.

Post-bolus blood glucose was divided into two phases: a hyperglycemic (0-180 min) and hypoglycemic (180-720 min) phase which were statistically analyzed separately. Rectal temperature and ROGI were analyzed for the entire post-bolus period. Remaining parameters were analyzed for the hypoglycemic phase (180-720 min, during which blood samples were obtained). Each animal's respective parameter was analyzed using repeated measures with an autoregressive covariance structure for blood parameters and spatial power law for rectal temperature and blood glucose. The repeated effect was minute after

bolus administration. Each specific variable's pre-bolus value served as a covariate. Effects of treatment, time, and treatment by time interaction (except for ROGI, where only the effect of time within the LPS-Eu treatment was analyzed) were assessed as a completely randomized design using PROC MIXED (SAS Inst. Inc., Cary, NC). Pre-formed contrasts were used to estimate differences between CON and LPS-infused steers (LPS-C and LPS-Eu) as well as between the two LPS-infused treatments (LPS-C vs. LPS-Eu). Data are reported as LSmeans and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results

Rectal temperature was increased $\sim 0.5^{\circ}\text{C}$ in both LPS treatments relative to CON steers ($P = 0.01$; Table 10). Both LPS-infused treatments (LPS-C and LPS-Eu), had $\sim 100\%$ increased circulating glucose for 180 min following LPS-administration relative to CON steers ($P = 0.04$; Figure 17A). From 180 to 720 min, there was a 30% decrease in blood glucose in LPS-C steers relative to CON and LPS-Eu steers ($P < 0.01$; Figure 17A). No differences in blood glucose were observed between CON and LPS-Eu treatments ($P = 0.59$) following the 180th min, indicating euglycemia was successfully maintained. Glucose infusion began 178 ± 17 min post-LPS administration (range 150-230 min). The ROGI increased with time ($P < 0.01$) and 516 ± 65 g of glucose were infused to maintain euglycemia (Figure 17B) from the 180th to 720th min.

Insulin increased 25.5-fold in LPS-Eu steers compared to CON steers ($P < 0.01$; Figure 17C). Ad hoc analysis also indicated that LPS-C steers had a 70% increase ($P < 0.05$) in circulating insulin compared to controls (data not shown). Circulating NEFA and

BHB were reduced (43 and 33%, respectively) in LPS-Eu versus CON and LPS-C steers ($P \leq 0.01$; Table 10). Compared to CON, steers in both LPS treatments had a similar increase in circulating BUN (53%; $P = 0.03$; Table 10), L-lactate (199%; $P = 0.01$; Figure 18A), LBP (90%; $P < 0.01$; Figure 18B), and a tendency for increased SAA (26%; $P = 0.10$; Table 10). Ionized calcium decreased 16% in both LPS treatments relative to CON steers ($P < 0.01$; Table 10).

There was a treatment by time interaction ($P = 0.02$; Figure 18C) for WBC counts as LPS steers experienced an initial decrease in cell number (180 min; 85%) followed by a progressive increase with time and WBC counts were similar to CON levels by 720 min. Cell types primarily contributing to the WBC temporal changes were neutrophils, lymphocytes, monocytes, and basophils which were initially reduced (9, 85, 91, and 79%, respectively; $P \leq 0.08$) at 180 min post-LPS and gradually increased thereafter (Table 10). The progressive increase in neutrophils tended to be reduced in LPS-Eu steers ($P = 0.08$; Table 10), resulting in a 63% decrease in neutrophil number by min 720 compared to LPS-C steers. This resulted in a 100% decrease in the neutrophil-to-lymphocyte ratio (NLR) in LPS-Eu relative to LPS-C steers ($P = 0.03$; Table 10). Eosinophils increased 76% and platelets decreased 52% in both LPS treatments relative to CON steers ($P < 0.05$; Table 10).

Discussion

Animal productivity is suboptimal during infection and inflammation due to increased energy requirements and subsequent nutrient partitioning towards the immune system and away from agriculturally important products (e.g., skeletal muscle, milk, fetus,

etc.). For example, skeletal muscle proteolysis is an important source of amino acids needed for acute phase protein synthesis, and this has been studied extensively (Klasing and Austic, 1984a,b; Reeds et al., 1994; Grimble and Grimble, 1998; Reeds and Jahoor, 2001; Li et al., 2007; Johnson, 2012). However, quantifying nutrient requirements of the immune system is difficult due to its dynamic and ubiquitous distribution throughout tissues. In terms of bioenergetics, both whole body energy expenditure and glucose utilization are estimated to increase approximately 50% during an infection (Lang and Dobrescu, 1991; Lang et al., 1993; Plank et al., 1998). This is likely because immune cells become substantial glucose consumers in order to support the energetic and substrate needs of activation (Calder et al., 2007; Maratou et al., 2007; Maciver et al., 2008; Palsson-McDermott and O'Neill, 2013; Kelly and O'Neill, 2015). For instance, in tissues with a large immune compartment (spleen, liver, lung, and ileum) LPS increases glucose utilization which is enhanced by euglycemia (Lang et al., 1993). Determining energetic requirements of the immune system in vivo is arduous as immune cells are present in virtually every tissue. Furthermore, the distribution of immune cells throughout different tissues is dynamic and can change rapidly as demonstrated by leukopenia observed in this and other studies (Griel et al., 1975; Bieniek et al., 1998). Despite its obvious difficulty, accurately estimating energetic and nutrient requirements of an activated immune response is essential for developing strategies to optimize productivity in immune-challenged animals. Herein, we propose using a euglycemic clamp after LPS administration in order to estimate the amount of glucose utilized during an intense immune response.

In the current study, we successfully induced an immune response by LPS injection as indicated by pyrexia and increased circulating levels of acute phase proteins LBP and

SAA (26 and 90% for SAA and LBP, respectively; Table 10 and Figure 18B). While the increase in acute phase proteins is consistent with other ruminant literature, there is substantial variation in baseline values and magnitudes of change (Carroll et al., 2009; Schroedl et al., 2001; Graugnard et al., 2013; Plessers et al., 2015). This is likely due to differences in models, route of LPS administration, and/or laboratory procedures, but differences in baseline values have even been reported within labs (Khafipour et al., 2009a,b). Severe hypoglycemia (30% decrease; Figure 17A) began ~180 min post bolus and continued through 720 min. Hyperlactemia (~200% increase; Figure 18A) was also evident in LPS-infused steers, especially at 180 min, and declined slightly with time. Additionally, circulating ionized calcium decreased (18%) which agrees with others (Griel et al., 1975; Carlstedt et al., 2000; Waldron et al., 2003b) and may be due to calcium's involvement with immune system activation (Hendy and Canaff, 2016). The aforementioned observations are indicative of an aggressive and sustained immune response and are similar to other models of endotoxemia (Filkins, 1978; Lang et al., 1993; Waldron et al., 2003b; Michaeli et al., 2012). Dextrose infusion initiation, coinciding with the development of hypoglycemia, was fairly consistent between steers (178 ± 17 min post-LPS; range 150-230 min). The ROGI increased steadily over time before plateauing at ~650 min post-LPS. From the accumulated ROGI, we estimate the activated immune system uses approximately 516 g of glucose in a 720 min period. However, as further discussed below, this calculation is likely underestimated as it does not incorporate glucose use by immune cells during the hyperglycemic phase.

Nevertheless, the current experimental design has some limitations. Firstly, the extent of peripheral tissue glucose consumption limits our capacity to accurately estimate

immune system's glucose utilization. However, many studies demonstrate reduced insulin-sensitivity and/or glucose utilization in both muscle and adipose tissue during endotoxemia both in vivo (Raymond et al., 1981; Ling et al., 1994; Poggi et al., 2007; Mulligan et al., 2012) and in vitro (Song et al., 2006; Liang et al., 2013). Lang and colleagues (1993) obtained similar results in a rodent model of endotoxemia; however, when providing glucose to maintain euglycemia, glucose utilization increased in skeletal muscle and adipose tissue. Nonetheless, in tissues with a large immune compartment (spleen, liver, lung, and ileum) glucose utilization was increased in LPS-hypoglycemic rats and the increase was even more pronounced in LPS-euglycemic conditions, acknowledging the relative importance of activated immune cells to whole-body glucose consumption. Additionally, endotoxemia likely causes macrophage infiltration into adipose and muscle (Caesar et al., 2012; Pilon et al., 2013), highlighting the difficulty to pinpoint glucose consumption by the immune system using 2-deoxyglucose, a non-metabolizable marker of glucose uptake, as immune cells are located in essentially every tissue. Immune cell distribution radically changes after LPS administration as demonstrated by leukopenia observed in this and other studies (Figure 18C; Griel et al., 1975; Bieniek et al., 1998), which is likely due to extravasation of leukocytes into tissues, especially those with important immune functions such as liver, kidney, spleen, and lung (Mészáros et al., 1991; Lang and Dobrescu, 1991). In agreement, Mészáros and colleagues (1991) examined different cell fractions within the liver after an i.v. LPS challenge and demonstrated glucose uptake did not change in parenchymal cells but markedly increased in Kupffer cells and neutrophils. A potential refinement to the current experimental design would be to incorporate tracers in order to measure glucose uptake by individual tissues during sepsis.

However, an accurate measurement would still require isolating and studying different cell types within a given tissue. The aforementioned studies demonstrate glucose incorporation into immune cells increases while extra-immune cells decrease glucose utilization during LPS infusion and supports our assumption that infused glucose was primarily utilized by immune cells rather than peripheral tissue.

A second limitation to our experimental design is the lack of hepatic glucose output measurements which prevents us from estimating the liver's contribution to the circulating glucose pool. However, increased glycogenolysis and gluconeogenesis following endotoxin administration has been reported in ruminants (Waldron et al., 2003a) and other species (Wolfe et al., 1977; Filkins, 1978; Spitzer et al., 1985; Lang et al., 1985; McGuinness, 1994; McGuinness, 2005). Hepatic glycogenolysis is likely a large contributor to the hyperglycemic period post-LPS bolus in the current study (Figure 17A), and we are unable to calculate the amount of endogenous glucose utilized by the immune system prior (~180 min) to dextrose infusion. The hyperglycemic phase is a consequence of orchestrated systemic changes including peripheral insulin resistance and increased hepatic glucose output which successfully provides glucose in excess of immune cell utilization. The LPS-induced hypoglycemia represents the inability of glucose sparing mechanisms (reduced glucose uptake by insulin sensitive tissues and increased hepatic rates of glycogenolysis and gluconeogenesis) to keep pace with the activated immune system's glucose utilization. If increased rates of hepatic glycogenolysis and gluconeogenesis described in ruminant and non-ruminant models hold true in the current model, then we are underestimating the amount of glucose entering the circulating pool and subsequently the total amount of glucose utilized by the activated immune system.

Interestingly, differences between the LPS-C and LPS-Eu steers were mostly related to metabolism rather than immunity. Particularly, the magnitude of insulin increase in LPS-Eu steers is remarkable, but not unprecedented, as similar results have been described in dogs (Blackard et al., 1976). This suggests an interaction between LPS and glucose infusion as recently proposed (Baumgard et al., 2016). Further, severe hyperinsulinemia likely contributes to the decrease in plasma NEFA and BHB observed in LPS-Eu cows, as insulin is a potent antilipolytic hormone (Vernon, 1992). Regardless of exogenous glucose infusion, hyperinsulinemia following an LPS challenge has been previously observed in cattle (Waldron et al., 2003a,b; Burdick Sanchez et al., 2013), and might be explained by LPS's amplifying role in glucose-stimulated insulin release reported in various in vitro experiments (Vives-Pi et al., 2003; Bhat et al., 2014). Furthermore, insulin becomes important for immune cell glucose uptake and development during activation (Shimizu et al., 1983; Helderman, 1984; Calder et al., 2007; Maratou et al., 2007).

Another hallmark of endotoxemia is hyperlactemia (Wolfe et al., 1977; Michaeli et al., 2012), which is likely due to the increase glucose utilization via aerobic glycolysis of immune cells after activation (Palsson-McDermott and O'Neill, 2013). In agreement, in the current study, LPS-Eu steers experienced a numerical 48% increase in plasma L-lactate compared to LPS-C steers, which likely represents an increase in glucose utilization by immune cells when glucose supply is not a limiting factor. Other potential sources include skeletal muscle, which may export L-lactate as an oxidative fuel for non-immune cells in an attempt to "spare" glucose for the immune system, a process akin to the Warburg Effect (Tannahill and O'Neill, 2011). Other adaptations to support the innate immune response

demand for energy and biosynthetic precursors include muscle proteolysis (Klasing and Austic, 1984a; Doyle et al., 2011; Michaeli et al., 2012, Johnson, 2012), which also likely occurred in the current experiment based on the increase in circulating BUN in LPS-infused steers. Interestingly, insulin normally inhibits skeletal muscle proteolysis (Allen, 1988), but insulin's inhibitory effect on muscle proteolysis is compromised during sepsis (Hasselgren et al., 1987) and may be due to LPS-induced insulin resistance and increased glucocorticoids taking primary control of muscle (Hall-Angerås et al., 1991).

Regarding immune parameters, leukopenia was evident at 180 min post-LPS and WBC count gradually increased to baseline levels by 720 min. Leukopenia has been observed in other endotoxemia studies (Griel et al., 1975; Bieniek et al., 1998) and is likely due to leukocyte extravasation into tissues. The NLR was increased in LPS-C steers and similar results were observed in LPS-infused rodents (Rose et al., 2007). The NLR is a marker of systemic inflammation as well as a predictor of mortality in various diseases involving the liver and cardiovascular system (Núñez et al., 2008; Leithead et al., 2015). Infusing glucose in LPS-Eu steers attenuated the increase in NLR and perhaps demonstrates a benefit of supplemental glucose to the innate immune system's ability to detoxify LPS.

In conclusion, our experiment demonstrates the induction of acute endotoxemia causes hypoglycemia within 3 hours and this likely results from the immune systems rate of glucose utilization exceeding whole-body glucose sparing mechanisms. From the ROGI, we estimate the activated immune system uses approximately 516 g of glucose in a 720 min period. This is ostensibly underestimated because we are unable to account for immune cell glucose utilization during the acute hyperglycemic phase and the liver's increased

contribution to the circulating pool as described in ruminant and non-ruminant models following LPS infusion. Regardless, on a metabolic bodyweight basis, the requirement for glucose is approximately $1.0 \text{ g/kg BW}^{0.75}/\text{h}$ which is comparable with data we have generated in other LPS-euglycemic clamp experiments in growing pigs and lactating cows (1.1 and $0.64 \text{ g/kg BW}^{0.75}/\text{h}$, respectively; Kvidera and Baumgard, unpublished data). The consistency in calculated glucose utilization despite different ages, physiological status, and species suggests the reprioritization and extent of fuel utilization by immune cells upon severe activation might be a conserved response. Whether glucose can become a limiting factor in immune response and the benefit of supplemental glucose is not clear. However, from an animal production perspective, infection and inflammation redirect resources toward the immune system and away from utilization and development of economically relevant tissues. Having a better understanding of the energetic and nutrient requirements of the immune response is critical in order to develop strategies to minimize productivity losses when physiological states or environmental conditions activate the immune system.

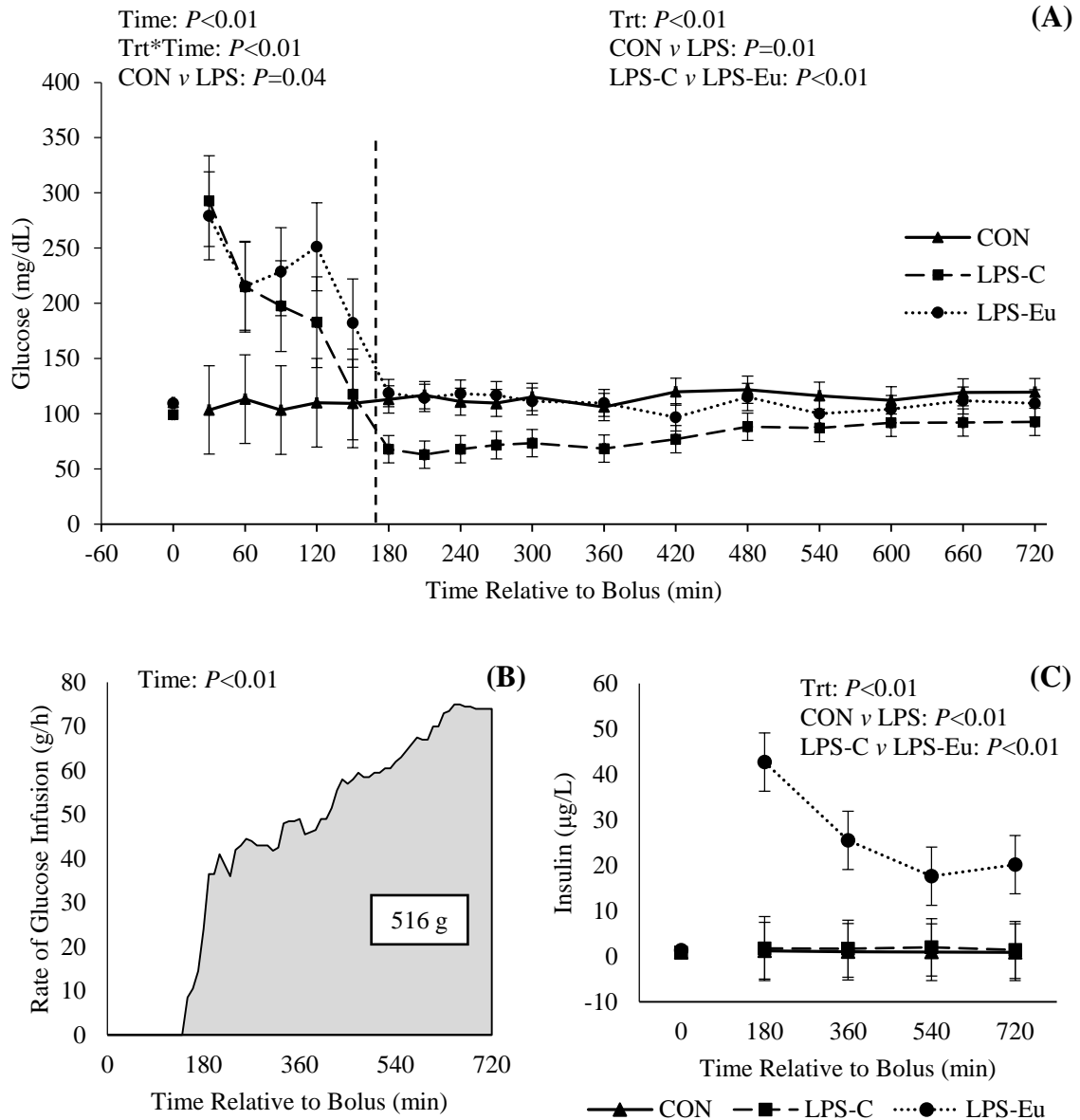


Figure 17. (A) Blood glucose levels in steers administered a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu) during both hyperglycemic (0-150 min) and hypoglycemic (180-720 min) phases, (B) the average rate of glucose infusion in LPS-Eu steers, and (C) circulating insulin levels in CON, LPS-C, and LPS-Eu steers during the hypoglycemic phase (180-720 min).

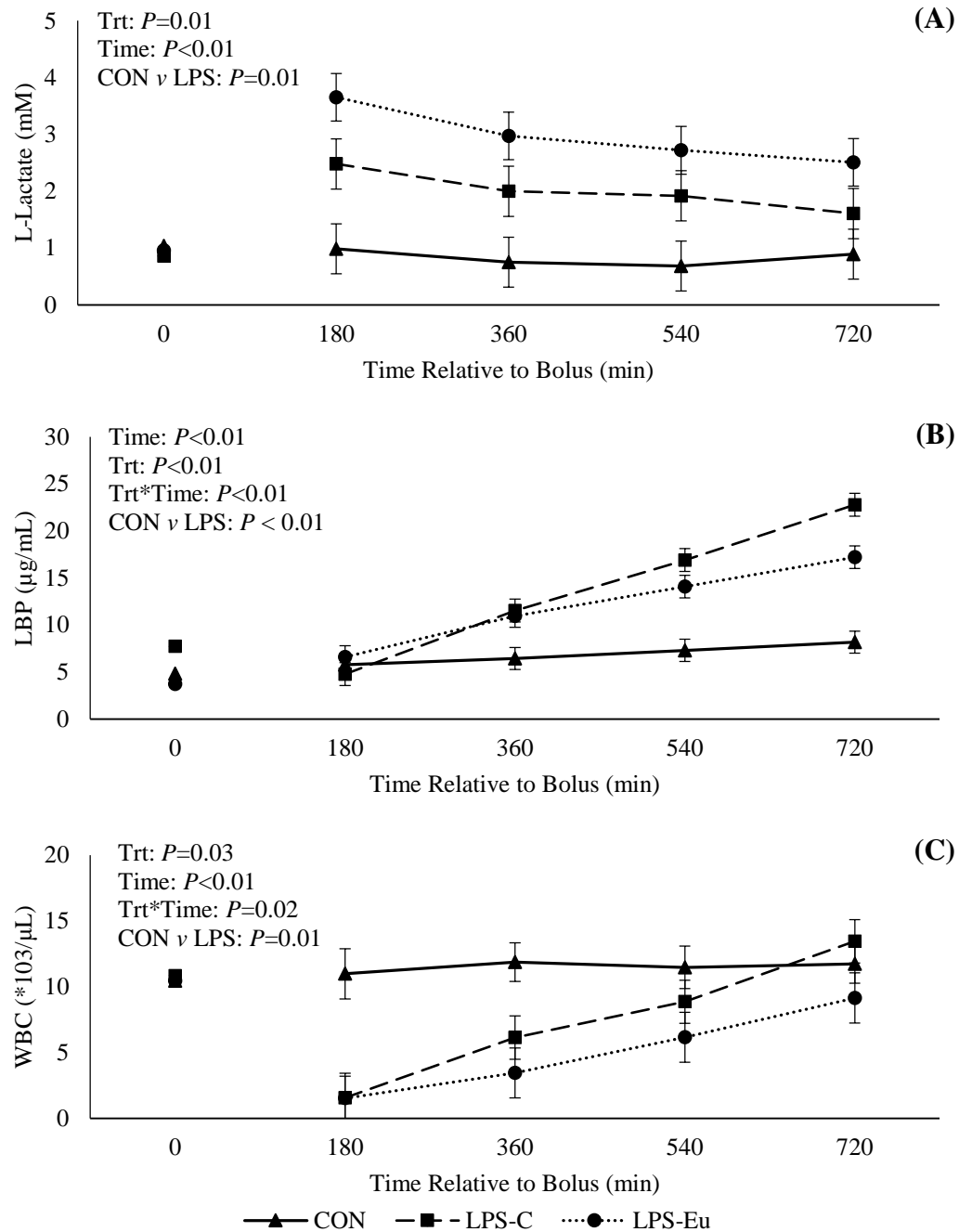


Figure 18. Circulating (A) L-lactate, (B) lipopolysaccharide binding protein (LBP), and (C) white blood cell count (WBC) during the hypoglycemic period (180-720 min) in steers given a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu).

Table 10. Blood parameters in steers given a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu) during the hypoglycemic phase 180-720 min post-LPS administration

Parameter	Treatment ¹			SEM	P-value			Contrasts	
	CON	LPS-C	LPS-Eu		Treatment	Time	Treatment x Time	CON v LPS ²	LPS-C v LPS-Eu
Rectal Temperature, °C	38.7 ^a	39.2 ^b	39.2 ^b	0.1	0.02	<0.01	0.34	0.01	0.95
Metabolites									
NEFA, mEq/L	340 ^a	352 ^a	197 ^b	28	<0.01	<0.01	<0.01	0.08	<0.01
BHB ³ , mg/dL	4.3 ^a	3.8 ^a	2.7 ^b	0.3	0.01	0.09	0.19	0.02	0.03
Blood urea N, mg/dL	6.7	10.4	10.1	1.1	0.08	0.01	0.34	0.03	0.84
Haptoglobin, µg/mL	136	170	152	65	0.95	0.19	0.41	0.75	0.87
LBP ⁴ , µg/mL	6.9 ^a	14.0 ^b	12.2 ^b	1.0	<0.01	<0.01	<0.01	<0.01	0.27
SAA ⁵ , µg/mL	87	117	103	11	0.22	<0.01	0.32	0.10	0.47
Ionized Calcium, mmol/L	1.40 ^a	1.16 ^b	1.19 ^b	0.02	<0.01	0.01	0.02	<0.01	0.38
Complete Blood Count									
Neutrophils, x10 ³ /µL	4.4	3.8	1.6	1.1	0.25	0.01	0.08	0.21	0.21
Lymphocytes, x10 ³ /µL	6.0 ^a	2.9 ^b	2.8 ^b	0.6	0.01	<0.01	0.01	<0.01	0.89
Monocytes, x10 ³ /µL	0.57 ^a	0.20 ^b	0.18 ^b	0.10	0.03	0.02	0.06	0.01	0.83
Eosinophils, x10 ³ /µL	0.29	0.56	0.46	0.06	0.06	0.05	0.17	0.03	0.27
Basophils, x10 ³ /µL	0.13	0.08	0.07	0.02	0.07	<0.01	0.04	0.03	0.53
Red blood cells, x10 ⁶ /µL	9.0	9.4	9.6	0.2	0.26	0.02	0.03	0.11	0.65
Platelets, x10 ³ /µL	553 ^a	274 ^b	256 ^b	31	<0.01	0.15	0.68	<0.01	0.71
NLR ⁶	0.8	1.2	0.6	0.2	0.07	0.12	0.24	0.81	0.03

¹CON = saline bolus; LPS-C = LPS bolus; LPS-Eu = LPS bolus and euglycemic clamp

²LPS-C and LPS-Eu treatments

³β-hydroxy butyrate

⁴Lipopolysaccharide binding protein

⁵Serum amyloid A

⁶Neutrophil-to-lymphocyte ratio

^{a-c} Means with different letters differ ($P \leq 0.05$)

CHAPTER 5: ESTIMATING GLUCOSE REQUIREMENTS OF AN ACTIVATED IMMUNE SYSTEM IN LACTATING HOLSTEIN COWS

A paper published in 2016 by Journal of Dairy Science 100:2360-2374.

doi: 10.3168/jds.2016-12001

S. K. Kvidera, E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard

Iowa State University Department of Animal Science, Ames, Iowa, 50011

Abstract

Accurately quantifying activated immune system energy requirements in vivo is difficult, but a better understanding may advance strategies to maximize animal productivity. Study objectives were to estimate whole-body glucose utilization following an i.v. endotoxin challenge. Lactating Holstein cows were jugular catheterized and assigned 1 of 3 bolus treatments: control (CON; 5 mL saline; n = 6), lipopolysaccharide (LPS)-administered (LPS-C; 1.5 μ g/kg BW; *E. coli* 055:B5; n = 6), and LPS + euglycemic clamp (LPS-Eu; 1.5 μ g/kg BW; 50% glucose solution infusion; n = 6). After LPS administration, blood glucose was determined every 10 min and glucose infusion rates were adjusted in LPS-Eu cows to maintain euglycemia for 720 min. Blood samples were obtained 180, 360, 540, and 720 min postbolus for further analysis. Cows were milked 360 and 720 min postbolus. Blood glucose was increased 84% in LPS-administered cows for

up to 150 min postbolus; thereafter, circulating glucose was decreased 30% in LPS-C relative to LPS-Eu and CON cows. Mild hyperthermia ($+0.5^{\circ}\text{C}$) occurred between 30 and 90 min postbolus in LPS-administered relative to CON cows; thereafter, rectal temperature did not differ between treatments. Milk yield and lactose percentage were decreased 80 and 11%, respectively, in LPS-administered relative to CON cows. Circulating insulin was increased 4-fold and non-esterified fatty acids, β -hydroxybutyrate, and ionized Ca were decreased $\sim 50\%$ in LPS-administered compared to CON cows. Plasma L-lactate, haptoglobin, and serum amyloid A increased ~ 160 , 260 , and 75% , respectively, in LPS-administered relative to CON cows. Overall, LPS binding protein was increased 87% in LPS-administered relative to CON cows; however, at 720 min, it was decreased 25% in LPS-Eu compared to LPS-C cows. White blood cell count decreased $\sim 90\%$ in LPS-administered cows at 180 min and progressively increased to $\sim 50\%$ of CON values by 720 min. Total glucose deficit during the 720 min following LPS administration was calculated as the decrease in the amount of glucose required to synthesize milk (due to the decrease in MY relative to prebolus levels) plus the amount of glucose infused to maintain euglycemia (in LPS-Eu cows only) and was 461, 1259, and 1553 g for CON, LPS-C, and LPS-Eu cows, respectively. Our data indicate an acutely activated immune system uses >1 kg of glucose within 720 min and maintaining euglycemia did not rescue milk synthesis.

Key words: glucose homeostasis, inflammation, insulin, lipopolysaccharide

Introduction

Maximizing nutrient and energy partitioning toward productive processes is key to economically profitable animal agriculture. Immunoactivation is characterized by decreased milk and lean tissue synthesis, inefficient feed utilization, and poor reproduction presumably due to immune system nutrient consumption (Lochmiller and Deerenberg, 2000; Johnson, 2012). Farm animals experience frequent immune challenges, and obvious sources in dairy cattle include metritis and mastitis (Sheldon et al., 2008; Ballou, 2012). A more inconspicuous source of inflammation is increased gastrointestinal permeability which can be a consequence of different stressors including dietary changes (e.g., grain-induced acidosis; Khafipour et al., 2009a), systemic inflammation (Hietbrink et al., 2009), or environmental factors like heat stress (Baumgard and Rhoads, 2013). Immunoactivation begins when molecular patterns from invading pathogens (e.g., bacteria, viruses, yeast, parasites) are recognized by immune cells, eliciting the transcription and production of inflammatory cytokines which results in an acute phase response characterized by fever, changes in circulating leukocyte numbers, and hepatic acute phase protein synthesis (Cecilian et al., 2012). Immunoactivation can be experimentally modeled by administering lipopolysaccharide (**LPS**), a cell wall component of Gram-negative bacteria which elicits a well-characterized and robust immune response (van Miert and Frens, 1968; Lohuis et al., 1988b). However, the response can vary in magnitude as repeated or continuous exposure to endotoxin results in hyporesponsiveness (i.e., tolerance; Beeson, 1947).

Immunoactivation markedly disrupts glucose homeostasis and is characterized by hypoglycemia and hyperlactemia (Filkins, 1978; McGuinness, 2005; Michaeli et al., 2012).

In vitro experiments demonstrate a substantial increase in glucose consumption by activated immune cells as glucose is their primary fuel and an important biosynthetic precursor (Calder et al., 2007; Palsson-McDermott and O'Neill, 2013). Despite the increase in glucose requirements, anorexia accompanies immunoactivation which decreases diet-derived glucose or glucose precursors. Decreased milk synthesis is one of the first observable signs of infection/inflammation in dairy cattle and this presumably represents a strategy to spare glucose for the immune system. To further ensure an adequate fuel supply for the immune system, hepatic glucose output increases via both glycogenolysis and gluconeogenesis (Filkins, 1978; McGuinness, 1994; Waldron 2003a). Synchronously, peripheral insulin resistance occurs leading to decreased glucose uptake by skeletal muscle and adipose tissue (Lang et al., 1990; Song et al., 2006). These metabolic adaptations are indicative of homeorhetic partitioning towards a new dominant physiological state of immunoactivation. Despite the aforementioned efforts to spare glucose, hypoglycemia often develops following a LPS challenge, likely because the immune system's rate of glucose utilization exceeds the orchestrated capacity of the liver to export glucose and insulin sensitive tissues to reduce glucose disposal (McGuinness, 2005).

The extent of in vivo glucose consumption by the activated immune system is difficult to assess due to the ubiquitous and fluctuating distribution of immune cells and organ specific changes in insulin sensitivity; however, better understanding its impact on bioenergetics is a prerequisite to developing strategies aimed at minimizing production losses in immunoactivated animals. Therefore, the experimental objective was to estimate the amount of glucose needed to maintain euglycemia following an LPS challenge as a proxy for the amount of glucose required to fuel an acute immune response.

Materials and Methods

Animals and Experimental Design

All procedures were approved by the Iowa State University Institutional Animal Care and Use Committee. Eighteen Holstein cows (718 ± 16 kg; 169 ± 7 DIM; parity 2 or 3) were utilized and housed in individual box-stalls (4.57 m by 4.57 m) at the Iowa State University Dairy Farm. Due to space constraints, the study was conducted in two replications occurring 14 d apart with 9 different cows in each replication. Treatment was balanced within each replication. Cows were allowed 5 d to acclimate during which they were implanted with bilateral jugular catheters. Cows were fed ad libitum once daily (0600 h) a diet formulated to meet or exceed the predicted requirements (NRC, 2001; Table 11) of energy, protein, minerals, and vitamins. Cows were milked four times daily (0000, 0600, 1200, 1800 h) throughout the experiment. During the final 3 d of acclimation, milk yields were recorded and a sample for composition analysis was obtained at each milking. To estimate the glucose requirements of an activated immune system we employed an LPS-euglycemic clamp technique as we have recently described (Kvidera et al., 2016a). Cows were randomly assigned to 1 of 3 i.v. bolus treatments administered immediately after the 0600 h milking: 1) control (**CON**; 3 mL sterile saline; $n = 6$), 2) LPS-administered controls in which hypoglycemia was allowed to develop (**LPS-C**; $1.5 \mu\text{g/kg BW LPS}$; $n = 6$), and 3) LPS-administered in which euglycemia was maintained (**LPS-Eu**; $1.5 \mu\text{g/kg BW LPS}$; $n = 6$). Selection of the LPS dose was influenced by the magnitude of hypoglycemia observed in earlier reports (Giri et al., 1990; Waldron et al., 2003a; Waggoner et al., 2009b). Lipopolysaccharide (*Escherichia coli* O55:B5; Sigma Aldrich, St. Louis, MO) was

dissolved in sterile saline at a concentration of 200 µg/mL and passed through a 0.2 µm sterile syringe filter (Thermo Scientific; Waltham, MA). The total volume of LPS solution administered was approximately 5 mL. In the LPS-Eu treatment, we performed a euglycemic clamp where a 50% glucose solution (as dextrose; VetOne, Boise, ID) was i.v. infused at a known and adjustable rate utilizing a modular pump (Deltec 3000, Deltec Inc., St. Paul, MN) in order to maintain the pre-LPS administration blood glucose concentrations.

Cows were tethered during the 12 h challenge (but allowed to stand up and lay down) to allow for frequent sampling. Water was provided ad libitum and feed was removed ~1 h prior to treatment administration and animals remained fasted during the 720 min data collection period in order to eliminate the confounding effect of dissimilar nutrient intake. Cows were milked immediately prior to administration (0600 h) and continued to be milked every 360 min (6 h) with yields and samples for composition analysis obtained at each milking. Samples were stored at 4°C with a preservative (bronopol tablet; D & F Control System, San Ramon, CA) until analysis by Dairy Lab Services (Dubuque, IA) using AOAC approved infrared analysis equipment and procedures.

Blood samples were obtained at -30, -20, and 0 min relative to LPS administration to establish baseline glucose levels. Each respective treatment bolus was administered immediately following the 0 min blood sample collection. For LPS-Eu cows, postbolus blood samples (1 mL) were collected every 10 min and immediately analyzed for glucose concentration (TRUEbalance glucometer; McKesson, San Francisco, CA). Glucose infusion began when blood glucose concentration declined below baseline levels, and its rate of infusion was adjusted as necessary to maintain blood glucose concentration at

baseline levels ($\pm 10\%$). The rate of 50% glucose solution infusion (mL/h) was transformed to rate of glucose infusion (**ROGI**; g/h). The total glucose infused for each cow was calculated using the ROGI for each 10 min interval (72 intervals in total) according to the following equation:

$$\sum_{i=0}^{72} ROGI \left(\frac{g}{h} \right)_i * \frac{1 h}{60 min} * 10 min$$

Blood glucose was measured every 30 min in CON and LPS-C cows for the first 300 min and every 60 min thereafter. Additional plasma and serum samples (~10 mL each) were collected from all treatments at -30, 0, 180, 360, 540, and 720 min relative to LPS administration. Plasma and serum were harvested following centrifugation at 1500 x g for 15 min at 4°C and were subsequently frozen at -20°C until analysis. Urine was collected at 0, 180, 360, 540, and 720 min relative to LPS administration and was frozen at -20°C until analysis. Rectal temperatures were measured at -30 and 0 min relative to LPS administration, every 30 min for the first 300 min postbolus, and every 60 min thereafter using a digital thermometer (GLA M700, San Luis Obispo, CA).

Plasma insulin, non-esterified fatty acids (**NEFA**), BHB, LPS binding protein (**LBP**), L-lactate, serum amyloid A (**SAA**), haptoglobin, and BUN as well as urine glucose concentrations were determined using commercially available kits according to manufacturers' instructions (insulin, Mercodia AB, Uppsala, Sweden; NEFA, Wako Chemicals USA, Richmond, VA; BHB, Pointe Scientific Inc., Canton, MI; LBP, Hycult Biotech, Uden, Netherlands; L-lactate, Biomedical Research Service Center, Buffalo, NY; SAA, Tridelata Development Ltd., Kildare, Ireland; haptoglobin, Immunology Consultants Laboratory Inc., Portland, OR; BUN, Teco Diagnostics, Anaheim, CA; glucose, Wako Chemicals USA Inc., Richmond, VA). The inter- and intra-assay coefficients of variation

for haptoglobin, insulin, NEFA, BHB, L-lactate, SAA, LBP, and BUN assays were 1.2 and 26.1%, 8.3 and 13.8%, 6.7 and 2.0%, 10.5 and 5.3%, 18.3 and 10.2%, 43.0 and 20.8%, 12.1 and 7.4%, and 2.8 and 3.7%, respectively. The intra-assay coefficient of variation for urine glucose was 4.9%. Blood ionized calcium was measured using an i-STAT handheld machine and cartridge (CG8+; Abbott Point of Care, Princeton, NJ). For white blood cell (WBC) count, a 3 mL blood sample was collected (K2EDTA; BD Franklin Lakes, NJ) and stored at 4°C for ~12 h before submission to the Iowa State University's Department of Veterinary Pathology for complete blood count analysis.

Calculations and Statistical Analyses

Administration of LPS decreased milk yield and therefore decreased glucose utilized for milk synthesis. The decrease in milk yield allows us to estimate the amount of glucose conserved (milk glucose deficit) and channeled toward the activated immune system in LPS-administered cows. As described in the results section, the CON cows also reduced their milk yield due to fasting, allowing us to account for the milk glucose deficit due to reduced feed intake alone. The amount of glucose utilized for milk synthesis was calculated for each milking based on Kronfeld's (1982) estimation of 72 g glucose required to synthesize 1 kg of milk. Milk glucose utilization values obtained prior to the challenge were averaged in order to establish a baseline value. Milk glucose utilization at both 360 and 720 min was subtracted from the baseline in order to calculate the milk glucose deficit. For CON and LPS-C cows, milk glucose deficit was solely used to calculate total glucose deficit. For LPS-Eu cows, milk glucose deficit plus the amount of glucose infused to maintain euglycemia were combined to obtain total glucose deficit.

The temporal pattern of postbolus blood glucose was divided into two phases: a hyperglycemic (0-150 min) and hypoglycemic (180-720 min) phase which were statistically analyzed separately. Rectal temperature and ROGI were analyzed for the entire postbolus period. Remaining parameters were analyzed for the hypoglycemic phase (180-720 min, during which blood samples, milk yield, and milk composition were obtained). Each animal's respective parameter was analyzed using repeated measures with an autoregressive covariance structure for blood and milk parameters and spatial power law for rectal temperature and blood glucose. The repeated effect was minute after LPS administration. Each specific variable's prebolus value (when available) served as a covariate. Effects of treatment, time, treatment by time interaction, and replicate (except for ROGI, where only the effect of time within the LPS-Eu treatment was analyzed) were assessed as a completely randomized design using PROC MIXED (SAS Inst. Inc., Cary, NC). Pre-formed contrasts were used to estimate differences between CON and LPS-administered cows (LPS-C and LPS-Eu) as well as between the two LPS-administered treatments (LPS-C vs. LPS-Eu). Data are reported as LSmeans and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results

Overall rectal temperature did not differ between treatments ($P = 0.94$); however, mild hyperthermia ($+0.5^{\circ}\text{C}$) was observed between 30 to 90 min postbolus in LPS-administered relative to CON cows ($P = 0.04$; Figure 19). Cows treated with LPS (LPS-C and LPS-Eu) anecdotally developed visible symptoms of immunoactivation within 120 min postbolus, including lethargy, drooping ears, and diarrhea. Cows administered LPS

had increased (84%) circulating glucose from 0-150 min postbolus relative to CON cows ($P < 0.01$; Figure 20A). From 180 to 720 min, LPS-C cows had 30% decreased blood glucose compared to CON and LPS-Eu cows. There were no differences in circulating glucose between CON and LPS-Eu cows from 180-720 min ($P = 0.26$), indicating successful maintenance of euglycemia in LPS-Eu cows. There were no treatment differences detected in urine glucose (<1.5 mg/dL; $P = 0.29$; data not shown). Glucose infusion began 143 ± 18 min post-LPS administration (range 60-170) and increased with time ($P < 0.01$; Figure 20B). An average of 265 ± 98 g of glucose were infused to maintain euglycemia during the entire 720 min clamp.

Milk yield decreased ~80% for both LPS treatments relative to CON cows at both 360 and 720 min postbolus ($P < 0.01$; Figure 21A). Relative to the baseline value, milk yield of CON cows was decreased at both 360 and 720 min (21%; $P = 0.07$ and 39%; $P < 0.01$, respectively), and this can be attributed to fasting. Milk lactose percentage decreased 11% in LPS-treated cows relative to CON cows ($P = 0.01$; Table 12). Milk fat percentage increased in all treatments with time (36%; $P = 0.02$; Table 12), and there were no effects on milk protein percentage ($P > 0.10$; Table 12). There was a progressive increase in MUN (~20%) for all treatments ($P < 0.01$; Table 12). Overall, somatic cell count increased 100% in LPS-C relative to CON cows while LPS-Eu cows did not differ from either treatment ($P = 0.03$; Table 12). The milk glucose deficit accumulated over 720 min was increased ~175% in LPS-administered cows relative to controls ($P < 0.01$; Figure 21B). Total glucose deficit accumulated over 720 min differed between all treatments with CON, LPS-C, and LPS-Eu cows having a 461, 1259, and 1553 g glucose deficit, respectively ($P < 0.01$; Figure 21B).

Insulin increased 4-fold in LPS relative to CON cows ($P = 0.01$; Figure 22A). Circulating NEFA and BHB were decreased 46 and 53%, respectively, in LPS versus CON cows ($P < 0.01$; Figure 22B and 22C). Blood urea nitrogen increased ~30% in all treatments over time ($P = 0.01$; data not shown). Ionized calcium decreased 46% in both LPS treatments relative to CON cows ($P < 0.01$; Figure 22D). Compared to CON cows, L-lactate, haptoglobin, and SAA increased ~160, 260, and 75%, respectively, in LPS-treated cows ($P \leq 0.01$; Figure 23A, 23B, and 23C). Overall, an 87% increase in LBP was observed in LPS compared to CON cows, and by 720 min, LBP was reduced 25% in LPS-Eu relative to LPS-C cows ($P < 0.01$; Figure 23D).

There was a treatment by time interaction ($P < 0.01$; Figure 24A) for WBC counts as LPS-administered cows experienced an initial decrease in cell number (180 min; 90%) followed by a progressive increase with time, and WBC counts were 54% of the CON values by 720 min. Lymphocytes, neutrophils, and monocytes primarily contributed to the WBC temporal changes as they were initially reduced (86, 98, and 85%, respectively; $P < 0.01$) at 180 min post-LPS administration and gradually increased to 50, 53, and 47% of CON values, respectively, by 720 min (Figure 24B and 24C; Table 13). The neutrophil to lymphocyte ratio (**NLR**) decreased 80% at 180 min in LPS-administered cows relative to controls and returned to baseline levels by 540 min ($P = 0.02$; Table 13). Red blood cells, hemoglobin, and hematocrit increased in LPS-administered cows relative to CON (6, 8, and 10%; $P \leq 0.01$; Table 13). Platelets were decreased 63% in LPS-administered cows relative to controls while mean platelet volume was increased 105% ($P < 0.01$; Table 13). There were no differences observed between LPS-C and LPS-Eu cows for any complete blood count parameter.

Discussion

Accurately determining nutrient requirements of the immune system is difficult due to its ubiquitous and fluctuating distribution throughout tissues. From a metabolic perspective, both whole body energy expenditure and glucose utilization markedly increase during infection (Lang and Dobrescu, 1991; Lang et al., 1993; Plank et al., 1998), ostensibly because immune cells become substantial and obligate glucose consumers to support the energetic and substrate needs of activation (Calder et al., 2007; Maratou et al., 2007; MacIver et al., 2008; Palsson-McDermott and O'Neill, 2013; Kelly and O'Neill, 2015). For instance, in tissues with a large immune compartment (spleen, liver, lung, and ileum) LPS increases glucose utilization which is enhanced by maintaining euglycemia (Lang et al., 1993). Herein, we propose using a euglycemic clamp after LPS administration in order to estimate the amount of glucose utilized during an intense immune response.

Immunoactivation was successfully induced in the current study as indicated by increased circulating levels of acute phase proteins haptoglobin, SAA, and LBP. While the increase in acute phase proteins is consistent with the literature, there is substantial variation in baseline, temporal pattern, and severity of increase (Jacobsen et al., 2004; Carroll et al., 2009; Graugnard et al., 2013; Moyes et al., 2014). This may be the result of different experimental models, route of LPS administration, animal variation, and/or laboratory procedures, but the variation (even from the same laboratory; Khafipour et al., 2009a,b) highlights why repeated sampling from the same animal pre and post-immunoactivation is necessary to put the magnitude of change into perspective and context. Ionized calcium was severely decreased (46%) following LPS administration, which

agrees with others (Griel et al., 1975; Elsasser et al., 1996; Waldron et al., 2003b), and is presumably due to calcium's involvement with immune system activation (Hendy and Canaff, 2016).

Severe hypoglycemia (30% decrease; Figure 20A) and hyperlactemia (160% increase; Figure 23A) developed ~180 min postbolus and continued through 720 min. These indications of glucose dyshomeostasis are similar to other models of endotoxemia in ruminants (Giri et al., 1990; Gerros et al., 1995; Elasser et al., 1996; Yates et al., 2012; Burdick Sanchez et al., 2013; Zarrin et al., 2014) and other species (Wolfe et al., 1977; Bruin et al., 2003; Michaeli et al., 2012). Glucose infusion initiation, coinciding with development of hypoglycemia, was fairly consistent between cows (143 ± 18 min post-LPS administration; range 60-170). Interestingly, ROGI was maximum at 210 min postbolus and decreased to ~20 g/h between 360 and 540 min before increasing steadily to a plateau of ~40 g/h at 650 min (Figure 20B). Urine was not a source of glucose disposal as it was almost similarly immeasurable (< 1.5 mg/dL) from cows in all treatments. Milk yield was acutely decreased ~80% in both LPS-administered treatments and this ostensibly represents a strategy to spare glucose for immune cell utilization. Admittedly, this is an extreme decrease, but similar magnitudes of milk synthesis cessation are frequent during *E. coli* mastitis (Gröhn et al., 2004) and the acute model was necessary to achieve appreciable hypoglycemia in order to employ the euglycemic clamp. In CON cows, the ~30% decrease in milk yield can be solely attributed to fasting. The decrease in milk yield allows us to estimate the amount of glucose conserved (milk glucose deficit) due to both reduced feed intake and glucose utilization by the immune system. The milk glucose deficit was 461, 1259, and 1288 g for CON, LPS-C, and LPS-Eu cows, respectively. In CON and

LPS-C cows, milk glucose deficit makes up the entirety of the total glucose deficit calculation. In LPS-Eu cows, the accumulated ROGI was ~265 g, which provides a total glucose deficit of 1553 g in LPS-Eu cows. Taking into account the reduction of feed intake by subtracting the 461 g deficit observed in CON cows, we estimate the activated immune system uses approximately 1092 g of glucose in a 720-min period. This calculation is likely underestimated as it does not incorporate glucose use by immune cells during the hyperglycemic phase. On a metabolic bodyweight basis, the glucose requirement is approximately 0.66 g/kg BW^{0.75}/h which is comparable with data we have generated in other LPS-euglycemic clamp experiments in growing steers and pigs (1.0 and 1.1 g/kg BW^{0.75}/h, respectively; Kvidera et al., 2016a; Kvidera and Baumgard, unpublished data). The immune system utilizes a large quantity of glucose following immunoactivation and the rate is seemingly conserved across species and life stages.

An additional reason for increased fuel oxidation (glucose in particular) following LPS administration could be to facilitate a febrile response. However, increased glucose utilization continues independently of rectal temperature during sepsis in experimental animal models (Lang et al., 1987a). In support of this, we observed only mild and transient pyrexia in LPS-administered cows (between 30 and 90 min postbolus) despite marked metabolic changes that existed for the entire experiment. This agrees with Giri et al. (1990) who also observed short-lived fever post-LPS despite large and prolonged changes in glucose homeostasis. Moreover, the fever response to LPS in ruminants is inconsistent and not dose dependent as sometimes higher doses attenuate the febrile response (Lohuis et al., 1988a; Gerros et al., 1995; Waldron et al., 2003a; Jacobsen et al., 2005), while high LPS

doses consistently causes hypoglycemia. Regardless, increased glucose utilization during this experiment does not appear to be an attempt to mount a fever.

The current experimental design has some limitations. First, the extent of peripheral tissue glucose consumption limits our capacity to accurately estimate immune system glucose utilization. However, insulin sensitivity and/or glucose utilization is reduced in both muscle and adipose tissue (typically large glucose consumers) during endotoxemia both in vivo (Raymond et al., 1981; Ling et al., 1994; Poggi et al., 2007; Mulligan et al., 2012) and in vitro (Song et al., 2006; Liang et al., 2013). As mentioned before, tissues with a large immune compartment (spleen, liver, lung, and ileum) actually increase glucose utilization following LPS administration (Lang et al., 1993). Further, Mészáros and colleagues (1991) examined different cell fractions within the liver after an i.v. LPS challenge and demonstrated glucose uptake did not change in parenchymal cells but markedly increased in Kupffer cells (i.e., resident hepatic macrophages) and neutrophils. Overall, these results highlight the relative importance of activated immune cells to tissue specific glucose consumption. In addition, immune cell distribution radically changes after LPS administration as demonstrated by leukopenia observed in this and other studies (Griel et al., 1975; Bieniek et al., 1998), which is likely due to tissue leukocyte sequestration, especially tissues with important immune functions (Mészáros et al., 1991; Lang and Dobrescu, 1991). Endotoxemia also likely causes macrophage infiltration into adipose and muscle (Caesar et al., 2012; Pillon et al., 2013). The aforesaid studies demonstrate glucose incorporation into immune cells increases while extraimmune cells decrease glucose utilization following LPS administration and this supports our assumption glucose was primarily utilized by immune cells rather than peripheral tissue.

A second drawback to our experimental model is the lack of hepatic glucose output measurements which prevents us from estimating the liver's contribution to the circulating glucose pool. However, endotoxemia-induced increased hepatic glycogenolysis and gluconeogenesis has been reported in multiple species (Wolfe et al., 1977; Filkins, 1978; Spitzer et al., 1985; Lang et al., 1985; McGuinness, 1994, McGuinness, 2005) including ruminants (Waldron et al., 2003a). Hepatic glycogenolysis is likely the primary contributor to the acute hyperglycemia observed following LPS administration in the current study (Figure 20A), but we are unable to calculate the amount of endogenous glucose utilized by the immune system prior to the initiation of glucose infusion (~180 min). The hyperglycemic phase results from orchestrated peripheral insulin insensitivity coupled with enhanced hepatic glucose production which provides glucose in surplus of immune cell utilization capacity. The hypoglycemic phase represents the inability of such glucose sparing mechanisms to keep pace with the activated immune system's glucose consumption. If increased rates of hepatic glycogenolysis and gluconeogenesis described in ruminants and nonruminants apply to the current model, then we are further underestimating the quantity of glucose entering the circulating pool and subsequently the total amount of glucose utilized by the activated immune system.

There were little or no overall metabolic, production, or immune differences detected between LPS-C and LPS-Eu cows, indicating a similar response following LPS regardless of glycemic status. Most notably, maintaining euglycemia did not rescue milk yield or lactose content, indicating that decreased milk synthesis is not due to LPS-induced hypoglycemia. It is likely LPS induces a direct effect on mammary tissue, as mammary epithelial cells express Toll-like receptor 4, an LPS receptor (Ibeagha-Awemu et al., 2008).

Alternatively, endotoxin-induced increases in proinflammatory cytokines and hormonal changes (e.g., cortisol) may indirectly inhibit milk synthesis (Verheijden et al., 1983). Interestingly, LPS reduces the integrity of the mammary epithelial barrier (Wellnitz et al., 2016) and cows from the LPS-C treatment had increased milk SCC, which agrees with a previous i.v. LPS report (Shuster et al., 1991). Further, euglycemia appeared to attenuate the LPS-induced mammary permeability as milk SCC from LPS-Eu cows was not different from either treatment. Markers of inflammation in the mammary gland post-LPS administration are increased when euglycemia is maintained compared to hypoglycemic cows (Vernay et al., 2012), suggesting glucose may be a limiting factor in mammary immune cell activation and subsequent pathogen clearance. Likewise, the reduction in circulating LBP at 540 and 720 min coupled with the increase in circulating neutrophils at 720 min in LPS-Eu relative to LPS-C cows indicates that glucose availability may be a bottleneck to mounting a full immune response.

Despite fasting, administering LPS induced acute and sustained hyperinsulinemia which has been previously observed in cattle (Waldron et al., 2003a; Burdick Sanchez et al., 2013; Zarrin et al., 2014; Kvidera et al., 2016a) and pigs (Stoakes et al., 2015a). Contrary to our previous results in growing steers (Kvidera et al., 2016a), infusing glucose did not exacerbate hyperinsulinemia to levels above LPS-C. An interaction between LPS and glucose infusion has been recently proposed (Baumgard et al., 2016) and may be due to either direct action of LPS on the pancreas (Vives-Pi et al., 2003; Bhat et al., 2014), LPS-induced hyperglycemia (Figure 20A), or LPS-stimulated secretion of the insulin secretagogue glucagon-like peptide 1 (Nguyen et al., 2014). Circulating insulin remained increased (~4 fold) during the hypoglycemic phase (180-720 min postbolus) so the acute

hepatic glycogenolysis is not likely the cause. Rationale for why circulating insulin increases are not clear, but insulin is important for immune cell glucose uptake and development during activation (Shimizu et al., 1983; Helderma, 1984; Calder et al., 2007; Maratou et al., 2007). Severe hyperinsulinemia likely contributes to decreased plasma NEFA observed in LPS-administered cows relative to controls, as insulin is a potent antilipolytic hormone (Vernon, 1992). Interestingly, despite the gradual increase in NEFA over time in all treatments (more pronounced in the controls), BHB was markedly decreased (>50%) throughout the experiment. This agrees with other ruminant models (Waldron et al., 2003a; Graugnard et al., 2013; Kvidera et al., 2016a), yet contradicts our pig results where BHB was increased following LPS administration (Stoakes et al., 2015a). Reasons for decreased circulating ketones are not clear. A majority of circulating BHB in ruminants in positive energy balance (akin to the current study) originates from the rumen epithelium as opposed to the liver (Pennington, 1952). Administering LPS decreases rumen motility (Jing et al., 2014), and whether or not this gastrointestinal stasis affects ketone synthesis is not known. An additional reason could be reduced liver production, as data from rodent models have demonstrated a decreased capacity for hepatic ketone synthesis during endotoxemia (Takeyama et al., 1990; Gitomer et al., 1995). However, Waldron et al. (2003a) observed no LPS effects on ketone synthesis in incubated liver slices obtained from lactating cows. Additionally, an increased rate of BHB infusion was required to maintain hyperketonemia in lactating cows challenged with LPS (Zarrin et al., 2014), indicating increased circulating BHB clearance and peripheral tissue utilization could help explain the decrease as this may be a strategy utilized to spare glucose for immune cells. Nevertheless, having a better understanding of how endotoxemia influences NEFA and

ketone metabolism likely has practical implications to a variety of dairy cow pathological conditions (e.g., ketosis).

Hyperlactemia is another characteristic of endotoxemia (Wolfe et al., 1977; Michaeli et al., 2012), which is likely due to enhanced glucose utilization via aerobic glycolysis in activated immune cells (Palsson-McDermott and O'Neill, 2013). In the current study, LPS-administered cows had >150% increase in circulating L-lactate. Other potential sources include skeletal muscle, which may export L-lactate as an oxidative fuel for non-immune cells in an attempt to “spare” glucose for the immune system, a process akin to the Warburg Effect (Tannahill and O'Neill, 2011). Muscle proteolysis is another adaptation employed during immunoactivation in order to support the demand for gluconeogenic precursors and amino acids for acute phase protein production (Klasing and Austic, 1984a; Doyle et al., 2011; Michaeli et al., 2012, Johnson, 2012). Similar to results of Zarrin and colleagues (2014) in lactating cows, we detected no treatment differences in circulating BUN concentrations but did observe a similar progressive increase over time. This is in contrast to findings in LPS-administered non-lactating ruminants (Elasser et al., 1996; Ballou et al., 2008; Kvidera et al., 2016a) and other species (Myers et al., 1997; Bruins et al., 2003, Rose et al, 2007), where LPS administration markedly increases BUN. Relatively little data exists on circulating BUN following endotoxin challenge in lactating animals and why there appears to be differences between growing and lactating physiological states is not clear.

Regarding immune parameters, leukopenia was evident at 180 min post-LPS and WBC count gradually increased to ~50% of CON levels by 720 min. Lymphocytes, neutrophils, and monocytes were the main contributors as they all followed the same

temporal pattern. Leukopenia has been observed in other ruminant endotoxemia studies (Griel et al., 1975; Bieniek et al., 1998; Jacobsen et al., 2005) and is likely due to accelerated leukocyte extravasation into tissues. Samples obtained beyond 720 min have observed leukocytosis following an initial period of leukopenia (Griel et al., 1975; Jacobsen et al., 2005; Horst and Baumgard, unpublished data), indicating circulating immune cells eventually exceed baseline levels. In summary, concentrations of circulating immune cells are highly dependent on time relative to immune activation. Leukopenia in the current study indicated immune cell mobilization into tissue, and the current experimental duration was likely not long enough to observe the characteristic leukocytosis in later stages of immunoactivation.

Glucose is obviously a critically important metabolite in all species, but it is especially pertinent to lactating dairy cows as ruminants rely almost exclusively on hepatic gluconeogenesis instead of dietary carbohydrate digestion/absorption to meet their glucose requirements. Further, glucose is the substrate for lactose synthesis and lactose production is the primary osmoregulator of milk yield (Chaiyabutr et al., 1980), so extramammary processes which increase glucose utilization have the potential to decrease glucose supply to the mammary gland and thus decrease productivity. Herein we describe the immune system as a substantial glucose utilizer and this has implications to several practical scenarios. Metritis and mastitis are common infections, especially in early lactation, which prevent optimal productivity. There are two less overt situations where immune system glucose utilization limits maximum milk yield. The first is heat stress as heat-stressed animals have a compromised intestinal barrier function and thus intestinal derived LPS infiltration (Baumgard and Rhoads, 2013). Immune system glucose consumption during

heat stress likely explains why decreased feed intake is only responsible for about 50% of reduced milk yield. The second is maladaptation to lactation as the transition period is associated with increased inflammatory markers (Bertoni and Trevisi, 2013; Bradford et al., 2015), although a source of blatant infection is often unclear. We have extended this and recently demonstrated increased circulating inflammatory biomarkers in periparturient cows diagnosed with ketosis and no other obvious disorder (i.e. mastitis or metritis; Abuajamieh et al, 2016). We hypothesize dietary shifts and/or inadequate feed intake associated with the transition period cause leaky gut and thus endotoxin infiltration (Abuajamieh et al., 2016), a scenario similar to heat stress (Kvidera et al., 2016b). Having a better understanding of how the immune system influences nutrient partitioning may allow for developing strategies to ameliorate production losses and reduce morbidity.

Conclusion

Our experiment demonstrates the induction of acute endotoxemia induces hypoglycemia resulting from the immune system's rate of glucose utilization exceeding whole-body glucose sparing mechanisms. From the combination of reduced milk carbohydrate output and ROGI, we estimate the activated immune system uses > 1 kg of glucose within 720 min. This is ostensibly underestimated because we are unable to account for immune system glucose utilization during the acute hyperglycemic phase and the liver's increased contribution to the circulating pool. The consistency between glucose utilization in the current experiment and other LPS-Eu clamps we have performed, despite differences in animal ages, physiological status, and species, suggests the reprioritization and extent of fuel utilization by immune cells upon activation is a conserved response.

Whether glucose can become a limiting factor in the immune response is not clear and thus the benefits of supplemental glucose to sick animals remains unknown. However, from an animal production perspective, infection and inflammation noticeably redirect resources toward the immune system and away from utilization and synthesis of economically relevant products. Having a better understanding of the energetic and nutrient requirements of the immune response is critical to developing strategies to minimize productivity losses when physiological states or environmental conditions activate the immune system.

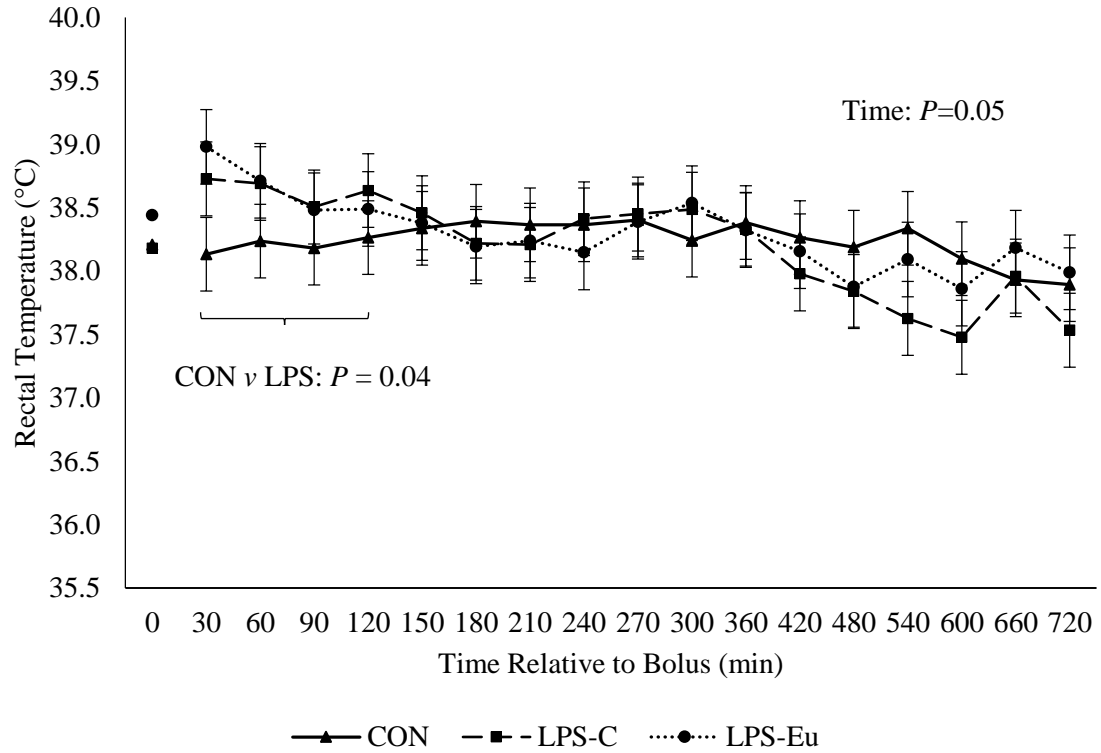


Figure 19. Rectal temperature in cows administered a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu).

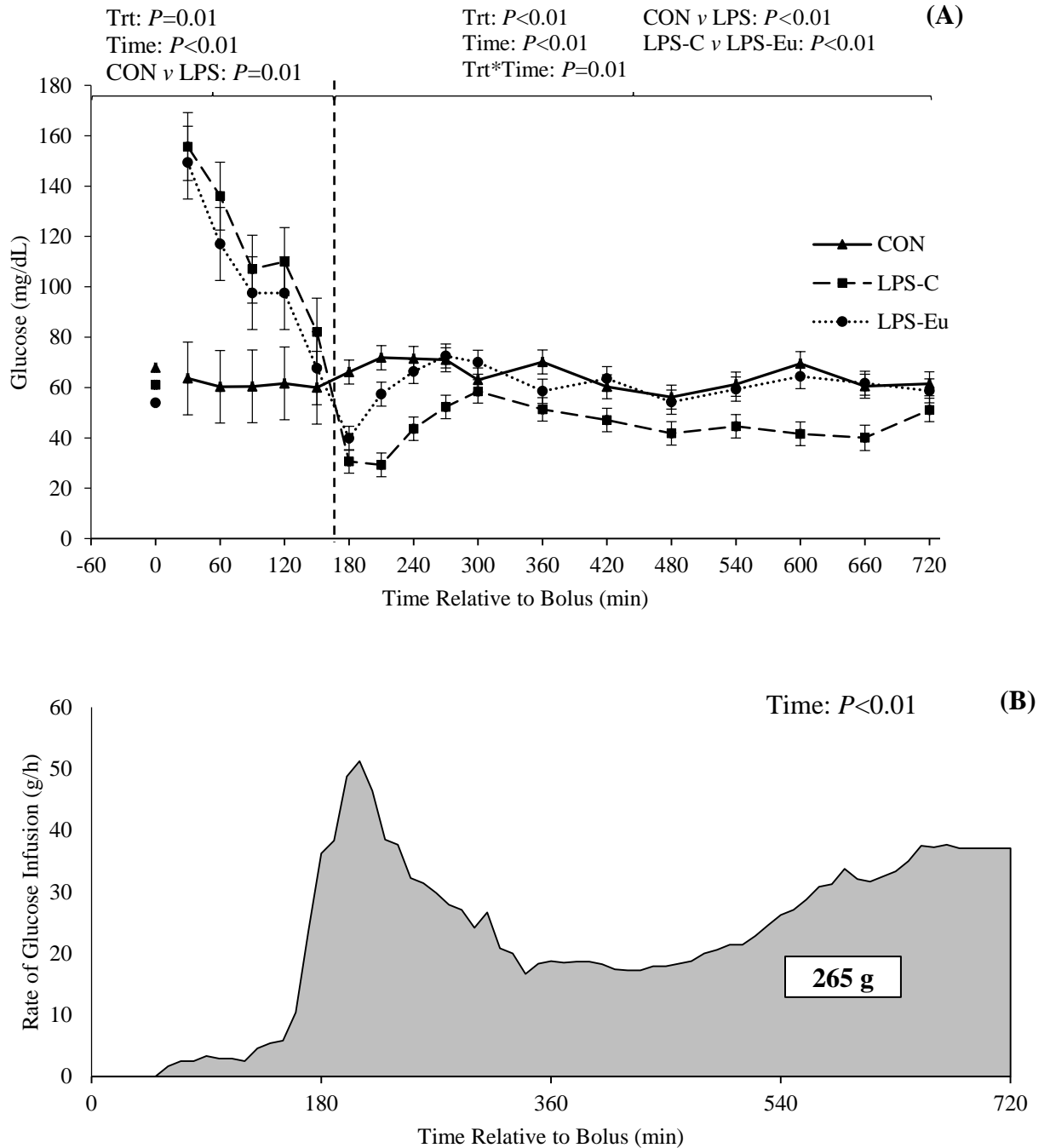


Figure 20. (A) Blood glucose levels in cows administered a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu) during both hyperglycemic (0-150 min) and hypoglycemic (180-720 min) phases and (B) the average rate of glucose infusion in LPS-Eu cows.

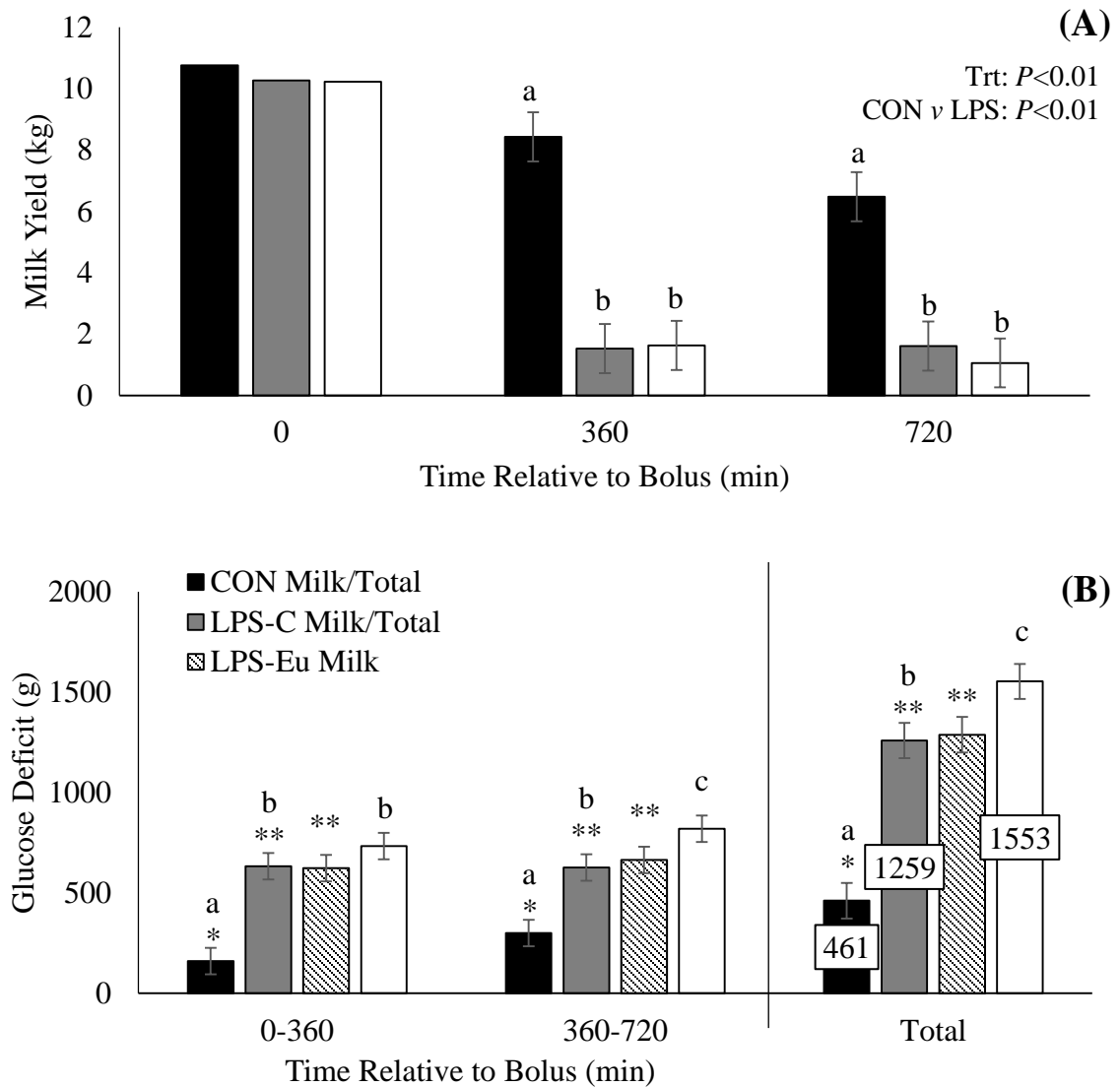


Figure 21. (A) Milk yield at 360 and 720 min postbolus and (B) milk and/or total glucose deficit from 0-360, 360-720, and accumulated over 720 min in cows administered a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu). **,*** Represent differences between milk glucose deficits ($P \leq 0.05$). ^{a-c} Represents differences between total glucose deficits ($P \leq 0.05$; total glucose deficit = milk glucose deficit in CON and LPS-C cows; total deficit = milk glucose deficit + infused glucose in LPS-Eu cows).

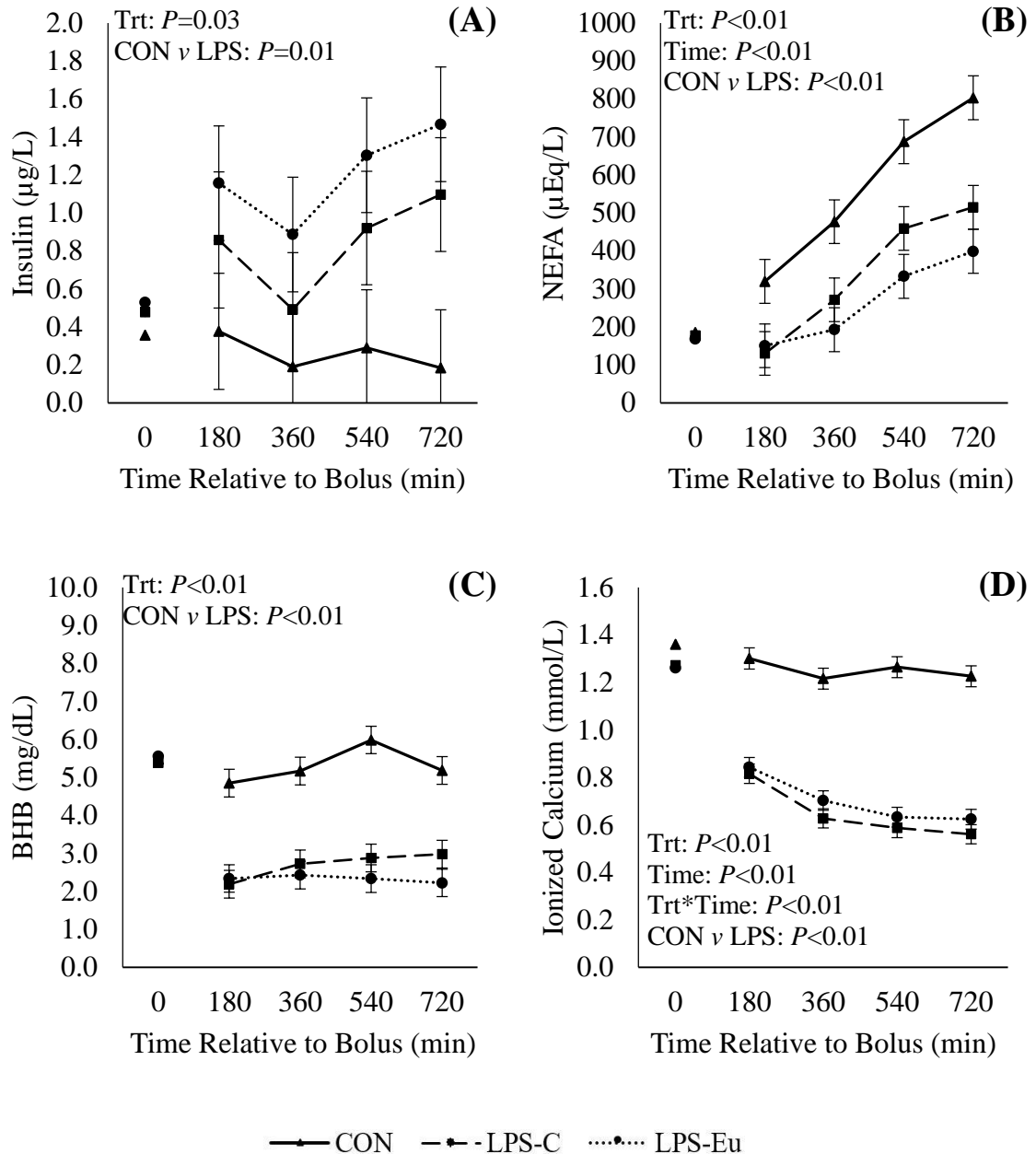


Figure 22. Circulating (A) insulin, (B) non-esterified fatty acids (NEFA), (C) BHB, and (D) ionized calcium during the hypoglycemic phase (180 -720 min) in cows administered a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu).

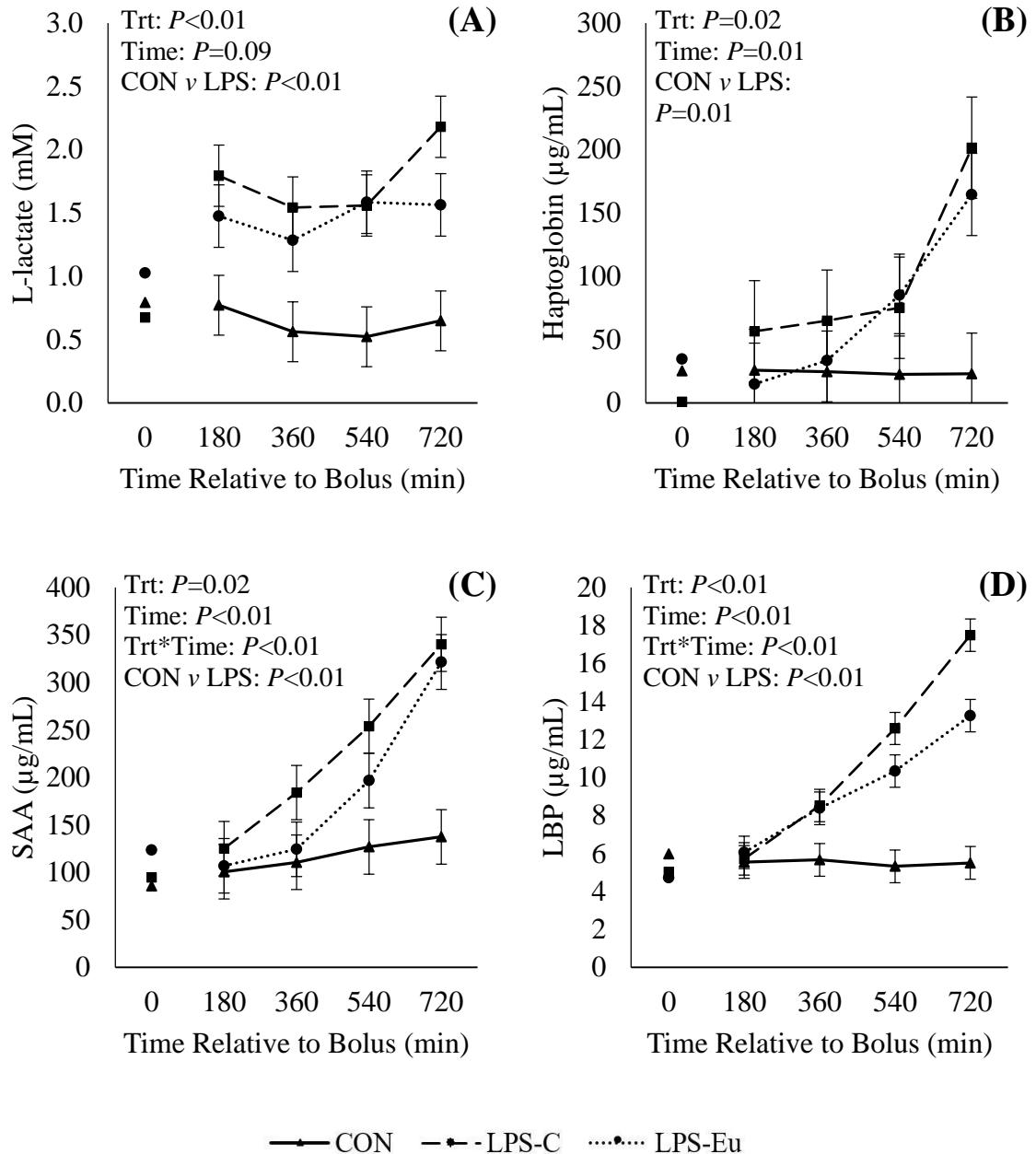


Figure 23. Circulating (A) L-lactate, (B) haptoglobin, (C) serum amyloid A (SAA), and (D) lipopolysaccharide-binding protein (LBP) during the hypoglycemic phase (180 -720 min) in cows administered a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu).

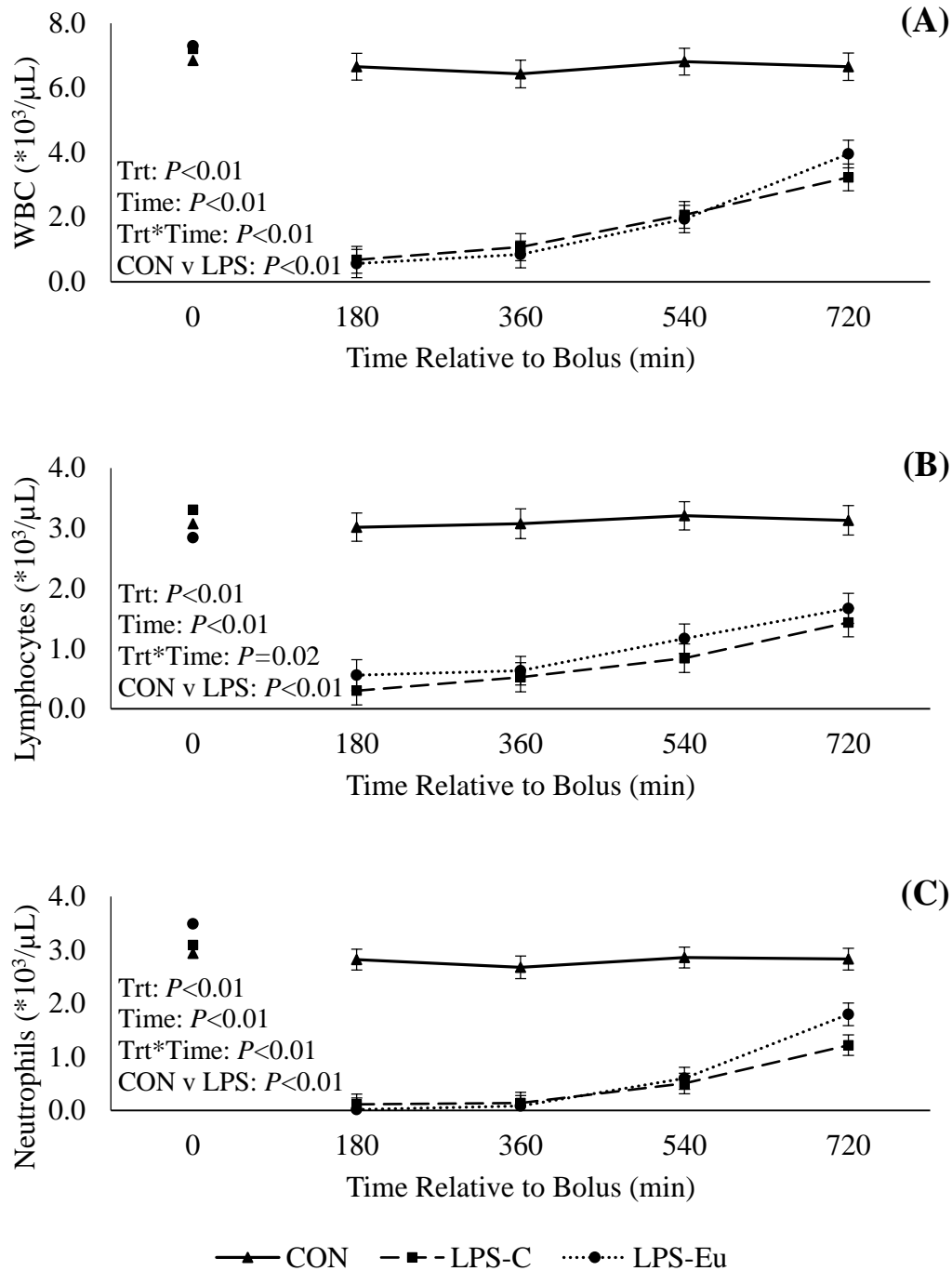


Figure 24. Circulating (A) white blood cell (WBC), (B) lymphocyte, and (C) neutrophil counts during the hypoglycemic phase (180 -720 min) in cows administered a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu).

Table 11. Ingredients and composition of diet

Ingredient	% of DM ^{1,2}
Corn Silage	20.5
Alfalfa Hay	10.2
Lactation Grain	8.9
Ground Corn	4.7
Whole Cotton	4.6
Soy Plus	1.8
Soy 48	2.3
Chemical analysis, % of DM	
Starch	25.3
CP	16.6
NDF	31.4
ADF	22.2
NE _L Mcal/kg DM	1.62

¹Diet moisture averaged 42.32%.

²Average nutrient levels: 4.72% Fat, 0.81% Ca, 0.47% P, 0.36% Mg, 0.19% S, 1.20% K, 0.45% Na, 0.47% Cl, 56.05 ppm of Zn, 62.79 ppm of Mn, 129.60 ppm of Fe, 17.54 ppm of Cu, 0.22 ppm of Co, 0.28 ppm Se, 0.84 ppm of I, 4453.3 IU/kg of vitamin A, 1190.5 IU/kg of vitamin D, and 26.5 IU/kg of vitamin E

Table 12. Milk composition parameters in cows given a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu) at 360 and 720 min post-LPS administration

Parameter ²	Treatment ¹			SEM	P-value			Contrasts	
	CON	LPS-C	LPS-Eu		Treatment	Time	Treatment x Time	CON v LPS ³	LPS v LPS-Eu
Milk solids									
Fat, %	3.4	3.4	3.2	0.3	0.91	0.02	0.86	0.81	0.71
Protein, %	3.1	2.9	3.1	0.1	0.13	0.36	0.75	0.14	0.15
Lactose, %	4.7 ^a	4.1 ^b	4.3 ^b	0.1	0.01	<0.01	0.08	0.01	0.29
TS, %	12.2	11.2	11.3	0.3	0.14	0.02	0.78	0.05	0.80
MUN, mg/dL	18.1	16.8	16.4	0.6	0.26	<0.01	0.68	0.12	0.67
Milk SCC	54 ^a	108 ^b	81 ^{ab}	13	0.03	0.01	0.23	0.02	0.15

¹CON = saline bolus; LPS-C = LPS bolus; LPS-Eu = LPS bolus and euglycemic clamp

²Data are presented as least square means from milk samples collected 360 and 720 min postbolus

³LPS-C and LPS-Eu treatments

Table 13. Complete blood count parameters in cows given a bolus of saline (CON), lipopolysaccharide (LPS-C), or lipopolysaccharide accompanied with a euglycemic clamp (LPS-Eu) during the hypoglycemic phase 180-720 min post LPS-administration

Parameter	Treatment ¹			SEM	P-value			Contrasts	
	CON	LPS	LPS-Eu		Treatment	Time	Treatment x Time	CON v LPS ²	LPS-C v LPS-Eu
Monocytes, *10 ³ /μL	0.24 ^a	0.04 ^b	0.05 ^b	0.03	<0.01	0.02	0.07	<0.01	0.80
Eosinophils, *10 ³ /μL	0.35	0.36	0.23	0.05	0.25	0.03	<0.01	0.43	0.13
Basophils, *10 ³ /μL	0.05 ^a	0.02 ^b	0.02 ^b	0.01	0.01	<0.01	0.24	<0.01	0.67
Red blood cells, *10 ⁶ /μL	6.2 ^a	6.6 ^b	6.5 ^b	0.1	0.04	0.08	0.58	0.02	0.65
Hemoglobin, gm/dL	10.8 ^a	11.9 ^b	11.5 ^b	0.2	0.02	0.04	0.36	0.01	0.23
Hematocrit, %	28.9 ^a	32.3 ^b	31.3 ^b	0.7	0.02	0.60	0.24	0.01	0.39
Platelets, *10 ³ /μL	323 ^a	136 ^b	102 ^b	15	<0.01	0.02	0.62	<0.01	0.12
Mean platelet volume, fl	6.6 ^a	13.4 ^b	13.7 ^b	1.0	<0.01	0.22	0.37	<0.01	0.84
NLR ³	1.05 ^a	0.65 ^b	0.56 ^b	0.13	0.05	<0.01	0.02	0.02	0.63

¹CON = saline bolus; LPS-C = LPS bolus; LPS-Eu = LPS bolus and euglycemic clamp

²LPS-C and LPS-Eu treatments

³Neutrophil to lymphocyte ratio

**CHAPTER 6: EFFECTS OF CONTINUOUS AND INCREASING
LIPOPOLYSACCHARIDE INFUSION ON BASAL AND STIMULATED
METABOLISM IN LACTATING HOLSTEIN COWS**

S. K. Kvidera, M. J. Dickson, E. A. Horst, C. S. Shouse, K. L. Bidne, E. J. Mayorga, M.
Al-Qaisi, H. A. Ramirez, A. F. Keating, and L. H. Baumgard

Iowa State University Department of Animal Science, Ames, Iowa, 50011

Abstract

Immunoactivation via an acute lipopolysaccharide (LPS) bolus elicits well-characterized immune and metabolic responses in experimental settings. However, conventional immunoactivation situations rarely mimic the nature of a LPS bolus. Therefore, experimental objectives were to characterize the basal and stimulated metabolic response to continuous LPS infusion in mid-lactation Holstein cows (169 ± 20 DIM; 681 ± 16 kg BW; parity 3.1 ± 0.4). Following 3 d acclimation, cows were enrolled in 2 experimental periods (P). During P1 (3 d), cows were fed ad libitum and baseline data was collected. Starting P2 (8 d), cows were assigned to 1 of 2 treatments: 1) saline-infused and pair-fed (CON-PF; 1 L i.v. sterile saline at 40 mL/h; n=6) or 2) LPS-infused and ad libitum-fed (LPS-AL; *E. coli* O55:B5; 0.017, 0.020, 0.026, 0.036, 0.055, 0.088, 0.148, and 0.148 $\mu\text{g}/\text{kg}$ BW/h for d 1 through 8, respectively; n=6). During P2, CON-PF cows were pair-fed to LPS-AL cows to eliminate confounding effects of dissimilar nutrient intake. Following the P2D8 0600h milking, feed was removed and all cows received an i.v. glucose tolerance

test (GTT) to assess glucose disposal. Feed was returned following the challenge. Four hours following the GTT feed was again removed and cows received an epinephrine challenge (EC). Blood samples for both challenges were collected -30, -20, -10, 0, 5, 10, 20, 30, 45, 60, and 90 min relative to bolus. LPS reduced DMI (28%) on d 1 but DMI gradually returned to pre-infusion levels by the end of P2, and overall milk yield was reduced similarly (17%) between treatments. Blood samples were collected on d 1 and 2 of P1 and 1, 3, 5, and 7 d of P2. There were no differences in basal circulating glucose. Plasma insulin during P2 was increased 140% in LPS-AL relative to CON-PF cows. Circulating non-esterified fatty acids were increased from d 1 to 3 and subsequently decreased from d 3 to 7 in cows from both treatments. Relative to P1, CON-PF cows had a 29% decrease in plasma β -hydroxybutyrate (BHB) while it did not change in LPS-AL cows, thus during P2, LPS-AL cows had overall increased (41%) BHB relative to CON-PF cows. Blood urea nitrogen (BUN) gradually decreased in CON-PF cows and increased in LPS-AL cows from d 1 to 5 of P2, resulting in an overall 25% increase in LPS-AL versus CON-PF cows. In response to the GTT, the glucose and insulin AUC were increased 33 and 56%, respectively in LPS-AL relative to CON-PF cows. There were no observable treatment differences in the glucose or non-esterified fatty acid response to the EC. In conclusion, chronic LPS exposure induces peripheral insulin resistance but does not affect adipocyte and hepatocyte sensitivity to a catabolic signal. Chronic LPS-induced changes in metabolism are suggestive of altered protein, lipid, and carbohydrate metabolism and overall nutrient partitioning.

Key words: inflammation, insulin, lipopolysaccharide

Introduction

An activated immune system requires a large amount of energy (Kvidera et al., 2017a), which is reallocated from profitable purposes to support the immune system, resulting in decreased growth, inefficient feed utilization, poor reproduction, and increased health care costs (Lochmiller and Deerenberg, 2000). Immunoactivation begins when pathogen associated molecular patterns (**PAMPs**) from invading pathogens (e.g., bacteria, viruses, yeast, and parasites) are recognized by leukocytes, eliciting the transcription and production of inflammatory cytokines, including tumor necrosis factor α (**TNF α**). Inflammation is further characterized by pyrexia, changes in circulating leukocyte numbers, and hepatic acute phase protein synthesis (Ceciliani et al., 2012). Metabolic disturbances also occur in response to immunoactivation including: hyperglycemia or hypoglycemia (depending upon the stage and severity of infection), increased circulating insulin and glucagon, skeletal muscle catabolism and subsequent nitrogen loss (Wannemacher et al., 1980), and hypertriglyceridemia (Filkins, 1978; Wannemacher et al., 1980; Lanza-Jacoby et al., 1998; McGuinness, 2005). These metabolic perturbations are presumably strategies employed to ensure adequate glucose delivery to the immune system, which becomes an obligate glucose utilizer following activation (Maratou et al., 2007; Palsson-McDermott and O'Neill, 2013). However, the aforementioned response to LPS can vary in magnitude as repeated or continuous exposure to endotoxin causes hyporesponsiveness (i.e., tolerance; Beeson, 1947).

Immunoactivation is often experimentally modeled by administering a bolus containing the PAMP lipopolysaccharide (**LPS**), a cell wall component of gram-negative bacteria that elicits a well-described and robust immune response (van Miert and Frens,

1968; Lohuis et al., 1988a,b). Yet during natural infection, endotoxin release is concomitant with dynamics of bacterial growth and proliferation (Crutchley et al., 1967; Tsuji and Harrison, 1978). Therefore, key knowledge gaps remain in distinguishing how accurate single or repeated LPS boluses mimic chronic, naturally-occurring pathogen exposure. Human data suggests continuous LPS infusion prompts a less aggressive and more sustained release of inflammatory mediators; likely a more appropriate model of chronic inflammation than an acute bolus (Taudorf et al., 2007). Having a better understanding of how continuous LPS infusion affects immunometabolism is critical to developing strategies to minimize production losses in naturally immunoactivated farm animals. Therefore, experimental objectives were to characterize the effect of continuous and exponentially increasing LPS infusion on basal and stimulated metabolism in order to better understand how chronic LPS affects nutrient partitioning and productivity.

Materials and Methods

Animals and Experimental Design

Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals. Twelve lactating Holstein cows (169 ± 20 DIM; 681 ± 16 kg BW; parity 3.1 ± 0.4 , range 2-5) were utilized and housed in individual box-stalls (4.57 by 4.57 m) at the Iowa State University Dairy Farm. Cows were allowed to acclimate for 3 d during which bilateral jugular catheters were implanted, and were then enrolled in two experimental periods. Period 1 (P1) lasted 3 d and served as the baseline (data generated for covariate analysis) for period 2 (P2). Period two lasted 8 d during which cows received one of two treatments 1) control (1 L sterile saline infused constantly at a rate of 40 mL/h;

jugular i.v.; n = 6) or 2) LPS-infused (0.017, 0.020, 0.026, 0.036, 0.055, 0.088, 0.148, and 0.148 $\mu\text{g}/\text{kg}$ BW/h for d 1 through 8, respectively; Figure 25; n = 6). The LPS dose and increasing daily infusion rate were selected based on data from a similar experimental design utilizing pigs (Kvidera et al., 2016b; Huntley et al., 2017). A stock solution of lipopolysaccharide (*Escherichia coli* O55:B5; Sigma Aldrich, St. Louis, MO) was made at a concentration of 300 $\mu\text{g}/\text{mL}$, passed through a 0.2 μm sterile syringe filter (Thermo Scientific; Waltham, MA), and stored in a sterile glass bottle 24 h prior to P2. Each day, the amount of stock solution needed for each cow's daily dose was calculated and injected into a 1 L bottle of sterile saline. The i.v. infusion system was incubated overnight with LPS solution (3 $\mu\text{g}/\text{mL}$) 1 d prior to initiation of infusion to saturate potential plastic LPS binding sites.

Control animals were pair-fed (PF) to the LPS-treated cows to eliminate the confounding effects of dissimilar nutrient intake as we have previously described (Wheelock et al., 2010). All cows were individually fed a TMR once daily at 0800 h, andorts were recorded prior to feeding. The TMR (consisting primarily of corn silage) was formulated by Dynamic Nutrition Systems (Pierz, MN) to meet or exceed the predicted requirements of energy, protein, minerals, and vitamins (NRC, 2001; Table 14). Reductions in daily feed intake by LPS-treated cows during P2 were determined as a percentage of their mean daily ad libitum intake during P1. Due to space constraints and to allow for pair-feeding calculations and implementation, control cows were 9 d behind LPS-cows in experimental protocol. Throughout the experiment, cows were milked twice daily at 0600 and 1800 h, and yields were recorded at each milking. Body weights were recorded on d 1 of acclimation and were used to calculate LPS dosage. Plasma samples were

collected daily at 0600 h during both P1 and P2. Whole blood was collected from the jugular catheter contralateral to the LPS-infusion catheter using EDTA as the anticoagulant (K2 EDTA, BD Vacutainer, Franklin Lakes, NJ). Plasma was harvested following centrifugation at 1500 x g for 15 min at 4°C, and subsequently frozen at -20°C until analysis.

Metabolic Challenges

For both metabolic challenges, feed was removed immediately prior to the challenge and was returned upon challenge conclusion. Lipopolysaccharide infusion was paused temporarily (~5-10 min) to administer glucose or epinephrine bolus. Prior to bolus administration, ~5 mL of blood was drawn and discarded to prevent infusion of any LPS remnant in the catheter line. Infusing LPS resumed following bolus administration, and all blood samples were collected from the contralateral catheter. On P2D8, a glucose tolerance test (**GTT**) was performed following the morning milking at 0600 h. A 50% dextrose solution bolus (VetOne; MWI Veterinary Supply, Boise, ID) was administered at 0.25 g/kg BW via the jugular catheter as previously described (Wheelock et al., 2010). Blood samples were collected at -30, -20, -10, 0, 5, 7.5, 10, 20, 30, 45, 60, and 90 min relative to glucose administration. Four hours after the conclusion of the GTT an epinephrine challenge (**EC**; 1.4 µg/kg BW; VetOne; MWI Veterinary Supply, Boise, ID) was performed as previously described (Baumgard et al., 2002a), and blood samples were collected -30, -20, -10, 2.5, 5, 7.5, 10, 20, 30, 45, 60, 90, and 120 min relative to the epinephrine administration. All blood samples were collected in disposable tubes containing EDTA as the anticoagulant (K2 EDTA, BD Vacutainer, Franklin Lakes, NJ). Plasma was harvested following

centrifugation at 1500 x g for 15 min at 4°C, and subsequently frozen at -20°C until analysis.

Laboratory Analyses

Plasma glucose, insulin, non-esterified fatty acids (**NEFA**), β -hydroxybutyrate (**BHB**), L-lactate, and blood urea nitrogen (**BUN**) were determined using commercially available kits according to manufacturers' instructions (glucose, Wako Chemicals USA Inc., Richmond, VA; insulin, Mercodia AB, Uppsala, Sweden; NEFA, Wako Chemicals USA, Richmond, VA; BHB, Pointe Scientific Inc., Canton, MI; L-lactate, Biomedical Research Service Center, Buffalo, NY; BUN, Teco Diagnostics, Anaheim, CA). The inter- and intra-assay coefficients of variation for glucose, insulin, NEFA, BHB, L-lactate, and BUN assays were 1.4 and 6.9%, 5.9 and 15.3%, 2.2 and 18.3%, 4.0 and 17.1%, 5.3 and 9.7%, and 9.1 and 10.6%, respectively.

Calculations and Statistical Analyses

Production and immunological data as well as molecular aspects of insulin action are reported elsewhere (Dickson and Keating, unpublished; Horst and Baumgard, unpublished). Metabolic responses to the GTT and EC were calculated as area under the curve (AUC) by linear trapezoidal summation between successive pairs of metabolite concentrations and time coordinates after subtracting baseline values. For the GTT, area under the curve (AUC) was calculated through the 90 and 45 min sample for glucose and insulin, respectively. For the EC, AUC was calculated through the 60 min sample for both glucose and non-esterified fatty acids. The AUC data was analyzed with SAS version 9.3 (SAS Inst. Inc., Cary, NC) using PROC MIXED and treatment as a fixed effect.

For all other measurements, each animal's respective parameter was analyzed using repeated measures with an autoregressive covariance structure. The repeated effect was day of LPS administration. Each specific variable's prebolus value (when available) served as a covariate. Effects of treatment, time, and treatment by time interaction were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC). All data are reported as LSmeans and considered significant if $P \leq 0.05$ and a tendency if $0.05 < P \leq 0.10$.

Results

Basal circulating glucose concentration did not differ between treatments ($P = 0.42$; Figure 26A). Relative to baseline values, P2 circulating insulin tended to be decreased in CON-PF cows (47%; $P = 0.08$) while LPS-AL cows did not change ($P = 0.42$). Overall, this resulted in 140% increased circulating insulin in LPS-AL versus CON-PF cows ($P = 0.01$; Figure 26B). Circulating basal NEFA were increased from d 1 to 3 and subsequently decreased from d 3 to 7 in both treatments ($P < 0.01$; Figure 26C). Relative to P1, CON-PF cows had a 29% decrease in plasma β -hydroxybutyrate (BHB; $P = 0.04$) while BHB in LPS-AL did not change ($P = 0.40$), thus LPS-AL cows had overall increased (41%) BHB relative to CON-PF cows ($P = 0.01$; Figure 27A). Circulating BUN gradually decreased in CON-PF cows and increased in LPS-AL cows from d 1 to 5 ($P = 0.04$), resulting in an overall 25% increase in LPS-AL versus CON-PF cows ($P = 0.02$; Figure 27C).

In response to the GTT, glucose AUC was increased (33%; 2523 vs. 3367 mg/dL·min) in LPS-AL cows relative to CON-PF cows ($P = 0.02$; Figure 28A). Similarly, LPS-AL cows had a 56% increase in insulin AUC relative to CON-PF cows (265 vs. 414

$\mu\text{g/L}\cdot\text{min}$; $P = 0.03$; Figure 28B). In response to the EC, neither glucose nor NEFA AUC differed between treatments ($P \geq 0.53$; Figure 29A and 29B).

Discussion

Infection and inflammation impede efficient animal agriculture, and both begin when PAMPs from invading pathogens (e.g., bacteria, viruses, yeast, parasites) are recognized by immune cells, eliciting transcription and production of inflammatory cytokines. One of the most common ways to model immune activation is through bolus LPS administration, which elicits a well-characterized and robust immune response (van Miert and Frens, 1968; Lohuis et al., 1988a,b). Another strategy is to infuse the inflammatory cytokine $\text{TNF}\alpha$, a method effectively bypassing both PAMP recognition and the signaling cascade that results in an array of inflammatory cytokine production. However, the metabolic effects of $\text{TNF}\alpha$ injection are unexplainably variable (Bradford et al., 2009; Yuan et al., 2013; Martel et al., 2014) which limits its utilization as an appropriate model. Compared to $\text{TNF}\alpha$ administration techniques, i.v. LPS infusion is a more systemic model and avoids the inflammation and cytokine production associated with surgical insertion of $\text{TNF}\alpha$ infusion pumps. Further, LPS infusion, rather than $\text{TNF}\alpha$ administration, includes PAMP recognition and $\text{NF}\kappa\text{B}$ pathway initiation which elicits production of several cytokines, including $\text{IL-1}\beta$ and IL-6 in addition to $\text{TNF}\alpha$ (Murphy et al., 2012). Additionally, each unique PAMP can produce distinctive cytokine profiles which in turn influence the pattern and production of acute phase proteins (Gabay and Kushner, 1999). Thus, the current model of continuous LPS infusion over several days is an attempt to more accurately model the inflammatory milieu that accompanies real infections and the

dynamic release of endotoxin upon bacterial overgrowth and proliferation (Crutchley et al., 1967; Tsuji and Harrison, 1978). Inherently, living microorganisms replicate exponentially, and thus in vivo endotoxin exposure can (if not controlled and remedied by the immune system) also become exponential (Goris et al., 1988). Consequently, we chose to increase the daily LPS dose exponentially in an attempt to mimic endotoxin exposure during bacterial overgrowth (Figure 25). Metabolic alterations to bolus or short-term endotoxin infusion have been well-described in cattle (Lohuis et al., 1988b; Giri et al., 1990; Waldron et al., 2003a); however, the effects of continuous and chronic systemic LPS infusion on basal and stimulated energetic metabolism in a lactating ruminant model are unknown.

Basal hyperinsulinemia was one of the most marked treatment differences observed in the current study, and it agrees with LPS bolus models in a variety of species (Toribio et al., 2005; Waggoner et al., 2009b; Yates et al., 2011; Kvidera et al., 2016a; Kvidera et al., 2017a) and when LPS is infused over 100 min (Waldron et al., 2003a). Incidentally, this agrees with long-term (10 d) continuous TNF α infusion which increases circulating insulin in rodents (Raina et al., 1994). The changes in basal insulin were not reflected by decreased circulating glucose, which suggests that long-term LPS exposure induces whole-body insulin resistance. This is corroborated by decreased glucose disposal rates following the GTT (Figure 28A). Whole-body insulin resistance following an LPS bolus has been previously reported in mid-lactation dairy cows using a hyperinsulinemic-euglycemic clamp (Vernay et al., 2012). Additionally, the 56% increase in insulin AUC in LPS-infused cows is interesting as we and others have shown that glucose infusion exacerbates LPS-induced hyperinsulinemia (Blackard et al., 1976; Kvidera et al., 2016a) and suggests an

interaction between LPS and glucose stimulated insulin secretion as recently proposed (Baumgard et al., 2016). Similarly, heat stressed animals have an increased insulin response to glucose, presumably due to heat-induced intestinal barrier dysfunction and subsequent LPS infiltration into portal circulation (Wheelock et al., 2011; Baumgard and Rhoads, 2013; Sanz Fernandez et al., 2015). Insulin becomes important for leukocyte glucose uptake and development during immunoactivation (Shimizu et al., 1983; Helderman, 1984; Calder et al., 2007; Maratou et al., 2007) and hyperinsulinemia coupled with peripheral insulin resistance may be a homeorhetic strategy employed to ensure adequate glucose delivery to the immune system. Immune cells require significant amounts of glucose upon immunoactivation due to a metabolic switch from oxidative phosphorylation to aerobic glycolysis (Palsson-McDermott and O'Neill, 2013), resulting in generation and export of lactate. Interestingly, circulating lactate levels did not differ between treatments which is surprising as immunoactivation is frequently characterized by hyperlactemia (Michaeli et al., 2012; Kvidera et al., 2017a). This may be explained by the relatively low-dose and chronic nature of the model, as LPS enhances gluconeogenesis (Filkins, 1978); thus, hepatic lactate uptake and subsequent gluconeogenesis is likely able to keep pace with the increase in lactate pool entry.

Skeletal muscle is catabolized during infection to provide amino acids as substrates for gluconeogenesis (Wannemacher et al., 1980) as well as to provide the building blocks for the synthesis of leukocytes and acute phase proteins (Iseri and Klasing, 2013). The profile of the immune system's amino acid requirement differs from that of skeletal muscle, thus surplus amino acids are mobilized and unutilized amino acids are deaminated (Reeds et al., 1994). Therefore, not surprisingly, circulating BUN is consistently increased in pig

models of endotoxemia (Myers et al., 1997; Bruins et al., 2003), presumably due to increased skeletal muscle protein catabolism. Results from ruminants generally agree (Burdick Sanchez et al., 2014), but are more variable (Waggoner et al., 2009a,b; Zarrin et al., 2014), probably because interpreting circulating BUN is complicated by the contribution of rumen derived ammonia. In addition, endotoxin-induced hypophagia reduces substrate (amino acids) delivery to the liver as changes well as rumen microbiota profile and function (Galyean et al., 1981; Jing et al., 2014), and this potentially masks any catabolic-induced changes in BUN. Thus, it is important in ruminant studies to utilize pair-feeding models to account for decreased feed intake. However, this again is complicated by the rumen, as it takes 4 or more days to truly fast a ruminant (Blaxter and Wainman, 1966). Regardless, data from the current study corroborates monogastric reports as BUN decreased gradually in CON-PF cows but increased in LPS-AL cows (Figure 27C). Thus, endotoxemia-induced increased muscle catabolism likely occurs in lactating dairy cows as well. It would be of interest to study alternative markers (e.g., 3-methylhistidine, urine creatinine, etc.) to confirm whether muscle catabolism occurs during immunoactivation in ruminants.

Patterns of unstimulated circulating NEFA were similar between LPS-infused and pair-fed animals. This is surprising as basal circulating insulin was increased 140% in LPS-animals and insulin is a potent antilipolytic hormone (Vernon, 1992). Previous studies conducted by our group demonstrated increased basal NEFA relative to baseline in LPS bolus-administered animals, but this response is blunted relative to the NEFA pattern of pair-fed animals (Kvidera et al., 2016a; Kvidera et al., 2017a). Other studies have observed an increase in NEFA following LPS bolus (Waldron et al., 2003a) while others have

reported no changes in circulating NEFA (Waldron et al., 2006; Zarrin et al., 2014). Reasons for why basal NEFA levels are inconsistent amongst studies is not clear but likely stems from differences in the dose, timing, and route of LPS administration. Circulating BHB were decreased in pair-fed cows, but were maintained at baseline levels in LPS-infused cows. Interestingly, the discordant BHB patterns occurred despite cows from both treatments having similar concentrations of circulating NEFA. The majority of circulating BHB during positive energy balance originates from the rumen epithelium as opposed to the liver (Pennington, 1952). The maintenance of ketone levels in LPS-infused cows relative to pair-fed controls could be caused by 1 of 3 factors: 1) decreased ketone utilization by peripheral tissue, 2) increased absorption efficiency by the rumen epithelium, or 3) increased hepatic ketone production. A decrease in ketone utilization is not likely, as ketones provide an energy source for non-immune tissues during LPS-induced insulin resistance. Furthermore, an increased rate of BHB infusion was required to maintain hyperketonemia in lactating cows challenged with LPS (Zarrin et al., 2014), indicating increased circulating BHB clearance. Administering LPS also decreases rumen motility and increases rumen butyrate concentrations, changes indicative of poor rumen ketone absorptive capacity (Jing et al., 2014). Lastly, Waldron and colleagues (2003a) observed no LPS effects on ketone synthesis in incubated liver slices obtained from lactating cows, indicating ketogenesis remains functional. However, other animal models demonstrate reduced hepatic ketogenesis during inflammation, attributed to either circulating insulin's lipogenic role in both hepatic cells and adipocytes (therefore reducing substrate delivery for ketogenesis; Beisel et al. 1980, Neufeld et al., 1980) or direct antiketogenic effects of inflammatory cytokines (Memon et al., 1992). We and others have shown reduced

circulating BHB following bolus endotoxin administration (Graugnard et al., 2013; Zarrin et al., 2014; Kvidera et al., 2016a; Kvidera et al., 2017a) and LPS infused over 100 min (Waldron et al., 2003a), which contrasts with the current study. One factor potentially responsible for these differences may be the LPS dose. Larger LPS doses cause more robust metabolic changes (Lohuis et al., 1988b; Waldron et al., 2003a); therefore, most bolus studies use a large LPS dose in order to accurately observe and measure changes. In the current study, a relatively low dose was initially used (0.4 $\mu\text{g}/\text{kg}$ BW over 24 h) and was gradually increased to what would be considered a high dose if given as a bolus (3.56 $\mu\text{g}/\text{kg}$ BW over 24 h on d 7 and 8) because 2.0 $\mu\text{g}/\text{kg}$ BW can be fatal in lactating cows (Waldron et al., 2003a). With this dose schedule, metabolic shifts were certainly not as dramatic, and endotoxin tolerance obviously developed, so it is very likely ketone metabolism differs in this model compared to bolus administration. Very little literature exists (in any species) on the effect of chronic inflammation on ketone metabolism over several days, and maintenance of ketone levels during continuous low-dose LPS infusion in lactating cows appears to be a novel discovery.

Previous data has demonstrated both blunted lipolysis and a blunted glucose response to adrenergic stimulation during sepsis (Hargrove et al., 1989; Forse et al., 1994). Hargrove et al. (1990) suggested this was primarily due to increased circulating insulin, which is a strong inhibitor of both adipose lipolysis and hepatic glucose output (Halter et al., 1984; Vernon, 1992). Elevated circulating insulin also occurs in heat-stressed animals, and they also have an attenuated NEFA response to lipolytic signals (Baumgard et al., 2011; Sanz Fernandez et al., 2015). The blunted response to adrenergic stimulation could also be due to desensitization of adrenergic receptors, as epinephrine is increased during

heat stress (Alvarez and Johnson, 1973) and endotoxin challenge in humans, cattle, and sheep (Richardson et al., 1989; Williams et al., 2009; Burdick Sanchez et al., 2011). Chronically increased circulating levels of epinephrine can downregulate and desensitize adrenergic receptors (Briggs et al., 1983; Snavely et al., 1985), potentially inducing tolerance to adrenergic signaling. Surprisingly, despite the LPS-induced hyperinsulinemia, no changes in adipose lipolysis or hepatic glucose output in response to the EC were detected in the current study. It would be of interest to evaluate the temporal pattern of both liver and adipose tissue responsiveness to catabolic signals during endotoxemia.

Conclusion

A variety of studies have characterized LPS-induced metabolic effects using bolus or repeated bolus models. We attempted to more accurately represent chronic in vivo infection by infusing LPS continuously with exponentially increasing doses for 7 d. The most striking effect of continuous low-dose LPS infusion was chronically elevated circulating insulin. The lack of changes in basal circulating glucose coupled with the decrease in glucose disposal following the GTT strongly suggests that chronic LPS infusion causes insulin resistance. Furthermore, because both treatments had a similar catabolic response to epinephrine despite increased insulin in LPS-infused animals, it appears chronic LPS infusion may diminish insulin's inhibitory role on both hepatic glucose output and adipose tissue lipolysis. Increased circulating BUN and BHB in LPS-infused cows also suggests protein and lipid metabolism are altered during continuous LPS exposure. Future studies delineating mechanisms regulating endotoxin-induced altered nutrient partitioning would help to understand nutrient requirements during illness.

Table 14. Ingredients and composition of diet

Ingredient	% of DM ²
Corn Silage	33.58
CGF	16.01
Alfalfa Hay	12.74
Lactation Grain	11.19
Ground Corn	9.58
Whole Cotton	5.09
QLF	2.38
Soy Plus	1.04
Soybean Meal	4.75
Chemical analysis, % of DM	
Starch	22.86
CP	16.95
NDF	31.09
ADF	20.28
NE _L Mcal/kg DM	1.44

¹Values represent an average of ration nutrient summary reports collected throughout the trial. Diet moisture averaged 52.49%.

²Average nutrient levels: 5.02% Fat, 0.91% Ca, 0.47% P, 0.36% Mg, 0.23% S, 1.32% K, 0.53% Na, 0.59% Cl, 69.39 ppm of Zn, 38.09 ppm of Mn, 3.02 ppm of Fe, 11.61 ppm of Cu, 0.65 ppm of Co, 0.28 ppm Se, 0.65 ppm of I, 6005.6 IU/kg of vitamin A, 477.9 IU/kg of vitamin D, and 15.7 IU/kg of vitamin E

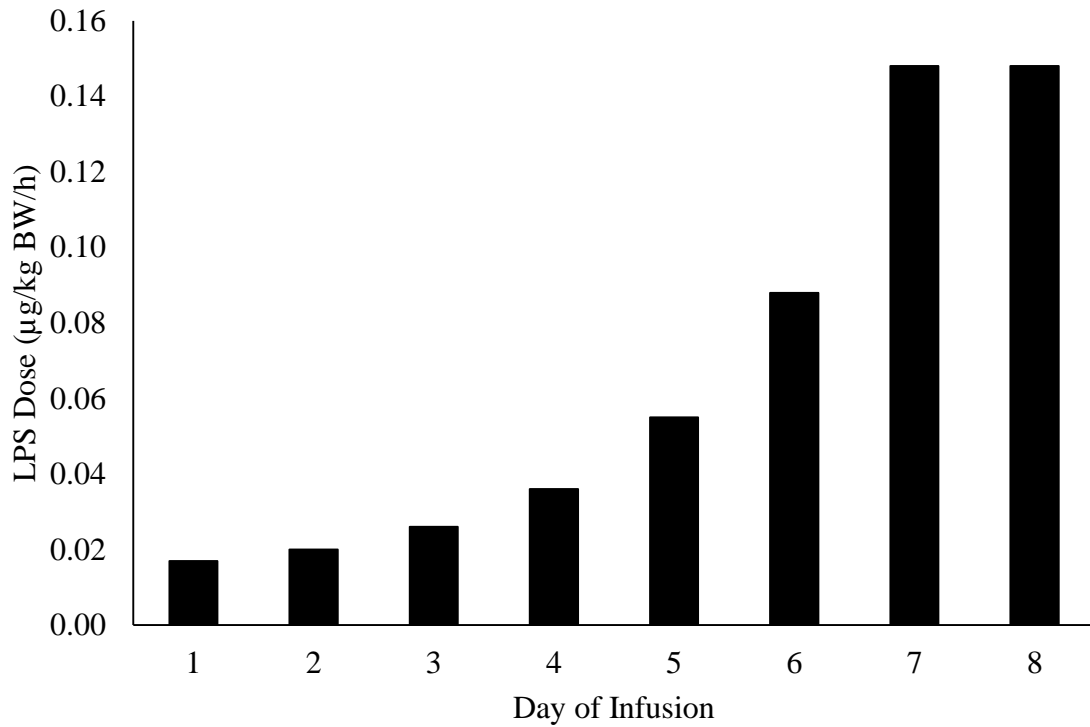


Figure 25. Daily dose schedule of lipopolysaccharide (LPS) administered intravenously via a jugular catheter in LPS-AL cows. Rate of infusion remained constant at 40 mL/h while LPS solution concentration was adjusted to achieve the predetermined daily dose. Control cows were infused with sterile saline at a rate of 40 mL/h.

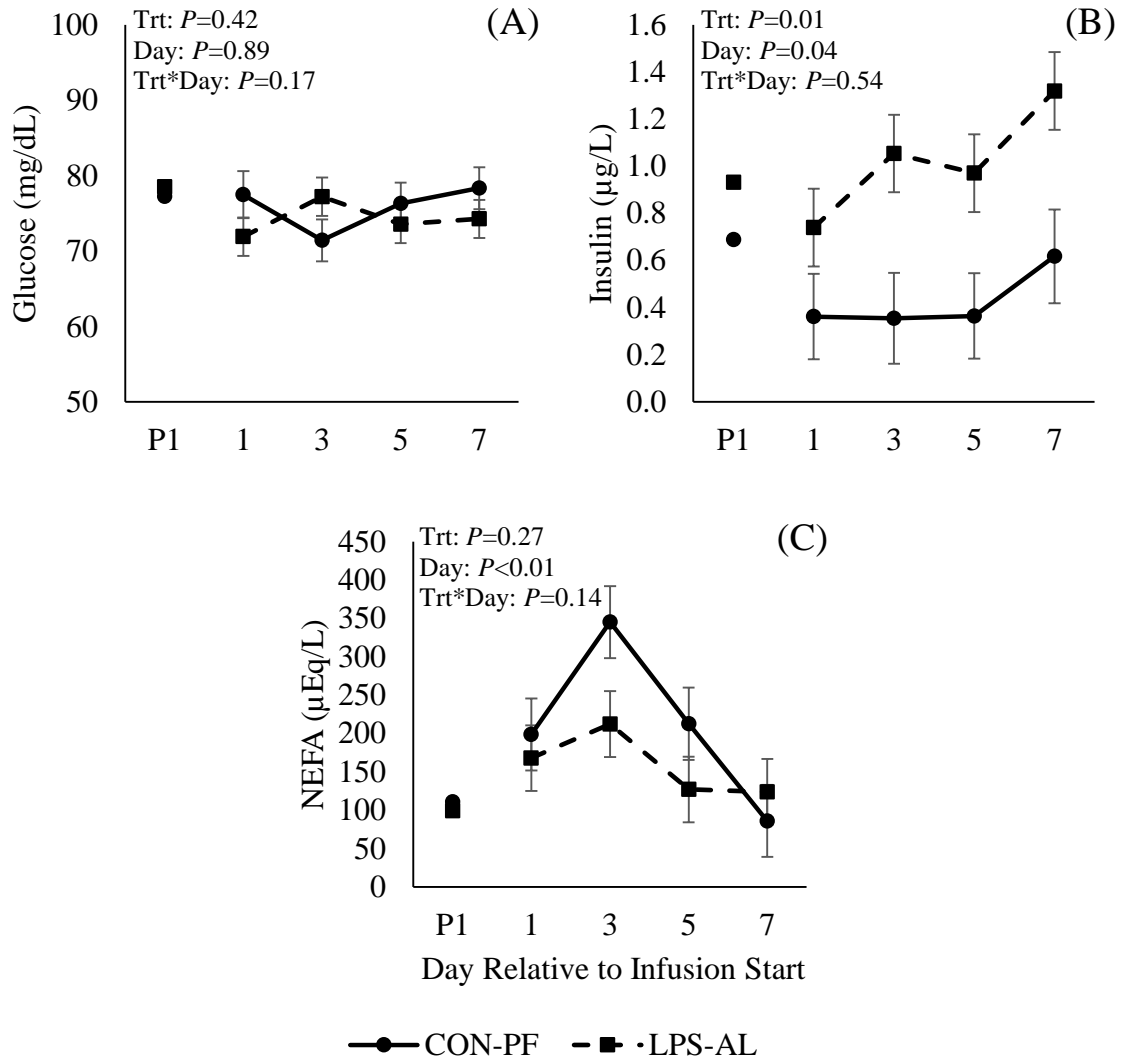


Figure 26. Circulating daily levels of (A) glucose, (B) insulin, and (C) non-esterified fatty acid in cows administered a continuous and increasing dose of lipopolysaccharide (LPS-AL) or sterile saline and pair-fed (CON-PF) to LPS-infused cows.

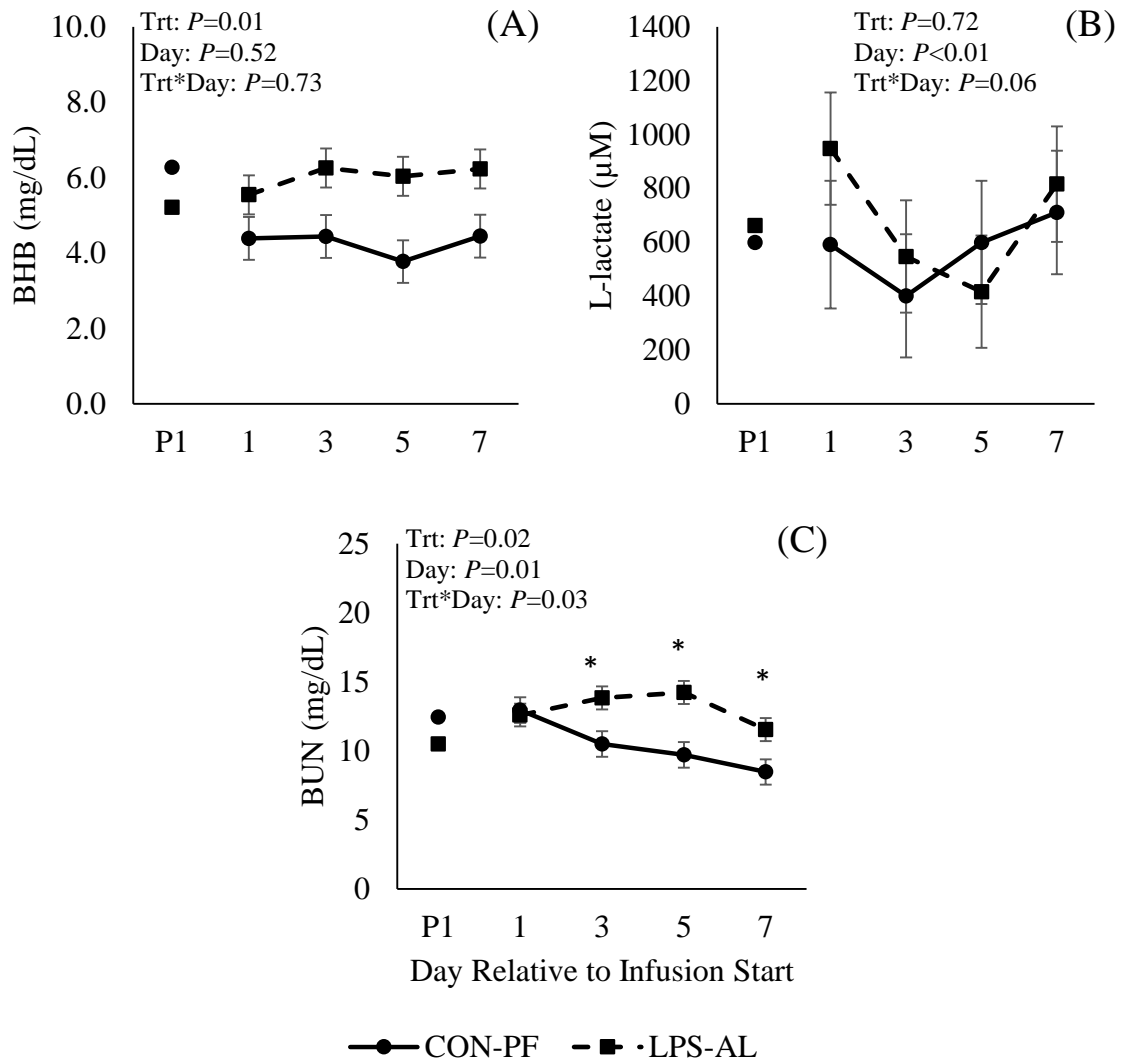


Figure 27. Circulating daily levels of (A) β -hydroxybutyrate, (B) L-lactate, and (C) blood urea nitrogen in cows administered a continuous and increasing dose of lipopolysaccharide (LPS-AL) or sterile saline and pair-fed (CON-PF) to LPS-infused cows. *Indicates treatment differences at the indicated time point ($P < 0.05$).

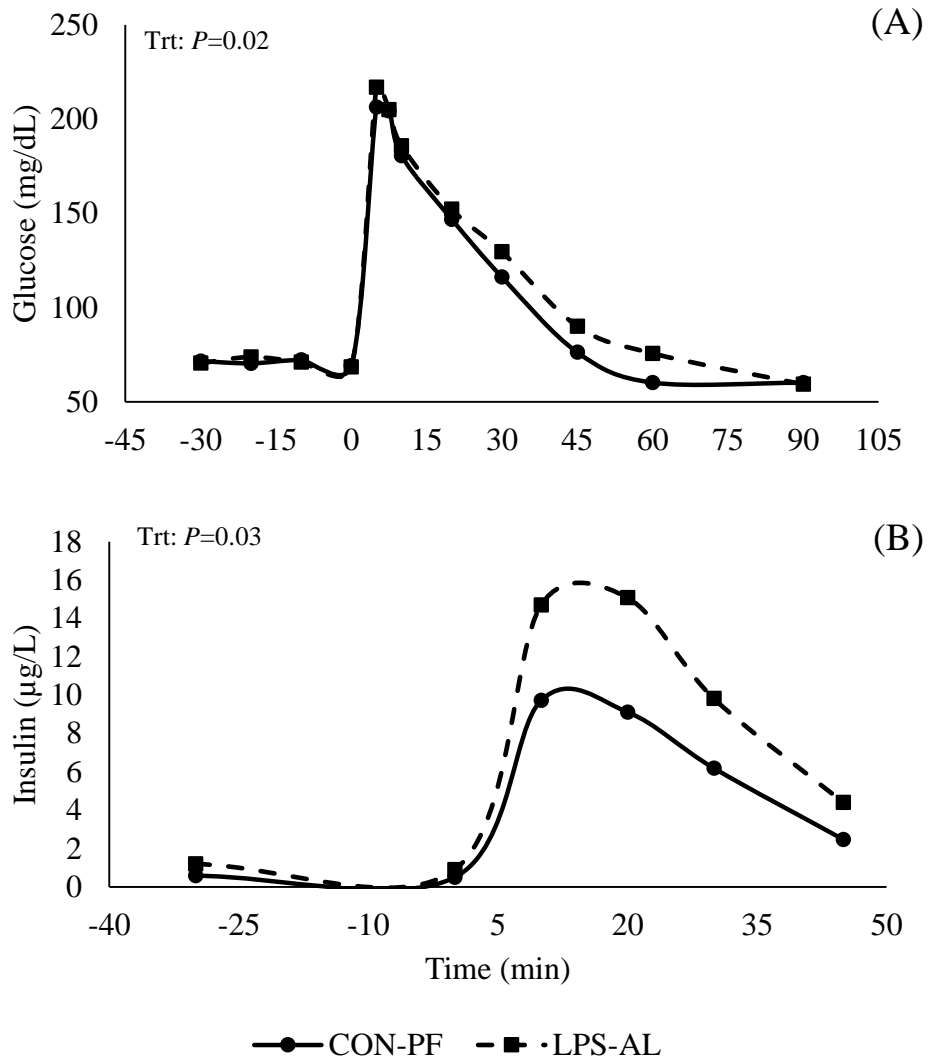


Figure 28. Circulating (A) glucose and (B) insulin response to an intravenous glucose tolerance test during day 8 of continuous and increasing lipopolysaccharide infusion (LPS-AL) or sterile saline and pair-fed (CON-PF) to LPS-infused cows.

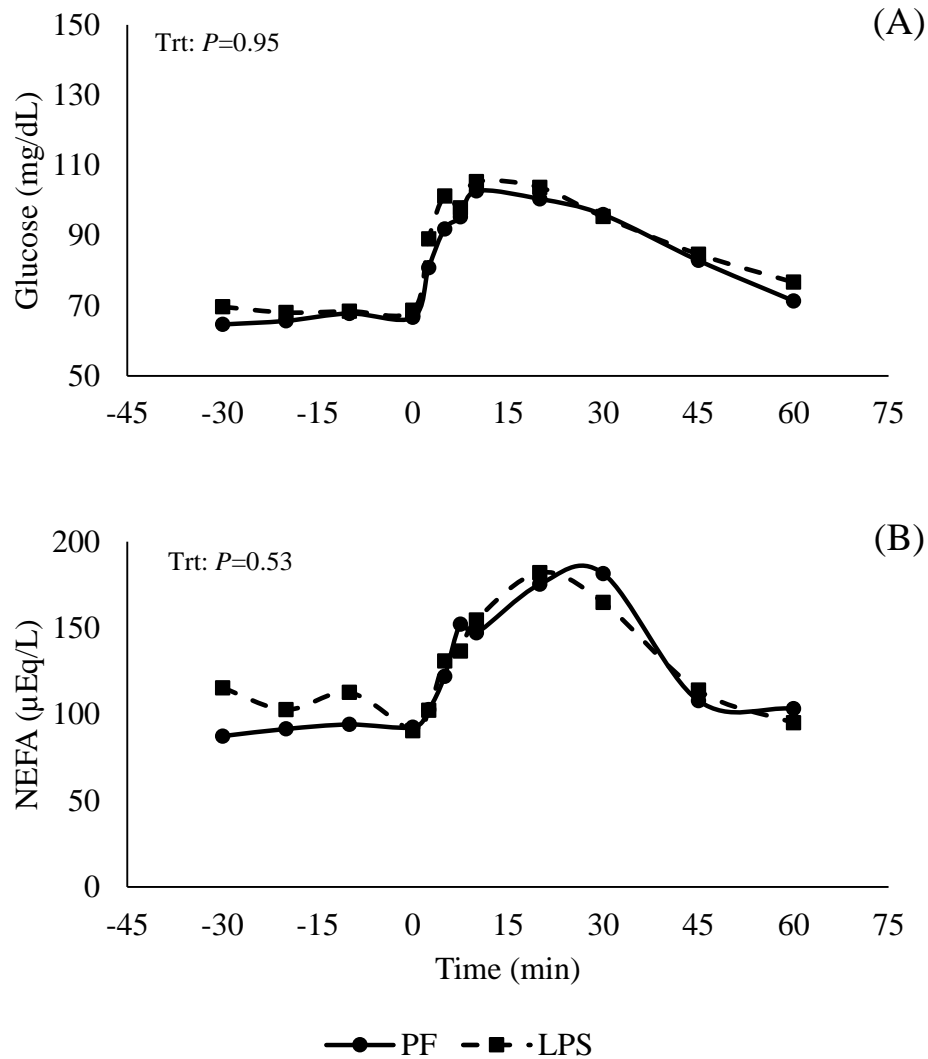


Figure 29. Circulating (A) glucose and (B) non-esterified fatty acids (NEFA) response to an intravenous epinephrine challenge during day 8 of continuous and increasing lipopolysaccharide infusion (LPS-AL) or sterile saline and pair-fed (CON-PF) to LPS-infused cows.

CHAPTER 7: INTEGRATIVE SUMMARY

Infection and inflammation are likely experienced by all animals in varying degrees at some point in their lifetime. Inflammation has negative economic consequences to animal agriculture due to decreased production, inefficient feed utilization, poor reproduction, and increased health care costs. An activated immune system demands a large amount of energy and nutrients (Lochmiller and Deerenberg, 2000; Johnson, 2012) which reprioritizes the hierarchy of nutrient partitioning away from productive purposes. For example, skeletal muscle proteolysis is an important source of amino acids for different components of the immune response. The amino acid requirement during immunoactivation has been studied extensively (Klasing and Austic, 1984a,b; Reeds et al., 1994; Grimbale and Grimbale, 1998; Reeds and Jahoor, 2001; Li et al., 2007; Johnson, 2012). Accurately quantifying energetic requirements of the immune system is more difficult because there are immune cells within virtually every tissue, and leukocyte distribution and flux changes during immunoactivation. Whole-body expenditure and glucose utilization are estimated to increase approximately 50% during an infection (Lang and Dobrescu, 1991; Lang et al., 1993; Plank et al., 1998). Recently, Huntley et al. (2017) estimated that immune system stimulation increases maintenance requirements of growing pigs by ~ 25%. Thus, it is very clear that an activated immune response increases whole-body energy consumption. Increased energy expenditure is partially explained by the febrile response which accompanies immunoactivation; however, increased glucose utilization continues independently of rectal temperature changes (Lang et al., 1987a). Therefore, we believe glucose utilization by an activated immune system is comprising a majority of this increased energy cost. As previously discussed, activated immune cells switch their

metabolism from oxidative phosphorylation to aerobic glycolysis, which substantially increases glucose consumption to support energetic and substrate demand (Calder et al., 2007; Maratou et al., 2007; MacIver et al., 2008; Palsson-McDermott and O'Neill, 2013; Kelly and O'Neill, 2015). This is especially important to the dairy industry because glucose is the precursor to lactose, the primary osmoregulator of milk yield (Chaiyabutr et al., 1980). Thus, extramammary processes that increase glucose utilization have the potential to decrease glucose supply to the mammary gland and limit milk synthesis. Elucidating the bioenergetics of an immune response is an important prerequisite to developing strategies aimed at minimizing production losses. As this research progresses, the practical implications will have clear benefits, particularly in lactating dairy cattle where glucose drives milk productivity.

Obvious sources of inflammation in dairy cattle include metritis and mastitis (Sheldon et al., 2008; Ballou, 2012). A more inconspicuous source of inflammation is increased gastrointestinal barrier dysfunction which can be a consequence of different stressors including dietary changes (e.g., grain-induced acidosis; Khafipour et al., 2009a) or environmental factors like heat stress (Baumgard and Rhoads, 2013). Additionally, we hypothesize the periparturient period is a time characterized by intestinal dysfunction (Abuajamieh et al., 2016) and have also shown in the current dissertation that feed restriction contributes to intestinal dysfunction in lactating dairy cows (chapter 3).

Gastrointestinal tract luminal content is technically extrinsic to the animal and is colonized with bacteria which increase in concentration from the proximal to distal intestine. Therefore, it is not surprising that a large majority of the immune system resides in the splanchnic bed (van der Heijden et al., 1987). Barrier importance is heightened in

cattle as both the size of the GIT and potential toxin exposure are more extensive in ruminants due to pre-gastric fermentation compartments. Exposure to immunogenic compounds and the initiation of inflammation certainly has implications on a variety of productive and metabolic aspects. However, studying the direct consequences of intestinal barrier dysfunction is difficult as the natural situations characterized by leaky gut are accompanied by a number of other behavioral and endocrine changes, which greatly increases variability and makes developing a repeatable leaky gut model arduous. Additionally, gastrointestinal dysfunction in dairy cattle remains less studied relative to other production animals. This is, in part, due to research costs and inability to perform gut health tests such as the lactulose/mannitol test due to rumen degradation of these unique sugars (Ahmed et al., 2013). Therefore, accurately characterizing the direct effects of leaky gut, developing an adequate and repeatable model, and characterizing the metabolic cost of immune activation and consequence of long-term inflammation are prerequisites for the development of strategies to mitigate intestinal dysfunction and its negative consequences.

Impaired intestinal barrier function occurs in a wide variety of human and animal diseases despite different underlying etiologies (see Chapter 1 ‘Situations of increased intestinal permeability’ section). However, discerning the direct metabolic and production consequences of leaky gut is difficult because conditions responsible for reducing intestinal barrier integrity can differ widely and affect multiple tissues and systems. For example, heat stress and the periparturient period are both situations where decreased barrier function occurs, yet both are characterized by other homeorhetic adaptations to support a new dominant physiological state (i.e., heat dissipation and milk production). Therefore, the objective of chapter 2 was to evaluate the metabolic, inflammatory, and production

consequences of intentionally-induced intestinal dysfunction in otherwise healthy mid-lactation cows. We hypothesized that intestinal tract dysfunction would negatively affect production and would alter metabolism and inflammatory parameters to resemble characteristic biomarkers during heat stress and the periparturient period. Our results confirmed our hypothesis, as intentionally damaging gut morphology reduced dry matter intake (~70%) and milk production (~40%) over time. Cows with intentionally-induced intestinal dysfunction had increased insulin, peak circulating ketones, and tended to have increased LBP relative to pair-fed cows. Interestingly, we discovered that pair-fed controls also had increased circulating acute phase proteins relative to their baseline values. This indicated that feed restriction itself causes intestinal barrier dysfunction, and this agrees with a variety of other models.

Feed intake reduction is frequently experienced by livestock either voluntarily (e.g., heat stress, immunoactivation, psychological stress, etc.) or involuntarily (e.g., shipping, poor management, drought, overcrowding, etc.). Therefore, when studying different stress situations, it is important to distinguish between their direct (i.e., the stressor) and indirect (i.e., via reduced feed intake) effects. For example, heat-stressed animals drastically decrease feed intake and exhibit reduced intestinal barrier integrity, leading to inflammation and poor performance. Similarly, we have administered gamma-secretase inhibitor in cows and observed intestinal damage and reduced intake. However, due to pair-fed controls in these experiments, we have also determined these negative effects are partially explained by reduced intake and its independent role in reducing intestinal barrier function (Pearce et al., 2013a; Baumgard and Rhoads 2013; Kvidera et al., 2017a). Therefore, objectives of chapter 3 were to determine if simply feed restricting cows would

negatively affect intestinal histology, to identify a magnitude of feed restriction that affects circulating inflammatory markers, and to assess GLP2's ability to mitigate intestinal dysfunction caused by feed restriction. We discovered that feed restriction affects ileum morphology in cows fed 40% of their ad-libitum intake. Furthermore, incremental feed restriction linearly increased circulating markers of inflammation. Administration of GLP2 in cows restricted to 40% of their ad libitum feed intake improved intestinal morphology variables above the level of control animals, clearly demonstrating a beneficial effect of GLP2 on gut health during reduced feed intake. Therefore, in this study we concluded that a simple feed restriction of $\leq 40\%$ ad libitum intake in lactating cows is a viable model for intestinal dysfunction, as it caused marked changes in intestinal architecture and increased inflammatory biomarkers. Furthermore, we successfully tested GLP2 as a mitigation strategy using this model. Administering GLP2 ameliorated intestinal damage caused by feed restriction and actually improved intestinal growth beyond ad libitum control values. Thus, future studies delineating production responses to GLP2 administration are warranted.

Chapters 2 and 3 successfully demonstrated altered metabolism and increased markers of inflammation during intestinal dysfunction. How these alterations contribute to productivity were the objectives of the remaining chapters in the dissertation. As discussed previously, estimating glucose requirements of an activated immune system is difficult due to the fluctuating nature of immune cells within immune and non-immune tissue. Therefore, the objective of chapter 4 was to describe a model where glucose requirements of an activated immune system could be estimated using an LPS bolus followed by a euglycemic clamp. Administration of the LPS bolus caused hypoglycemia (~30% below

baseline levels) in control animals within 3 h. Approximately 516 g of glucose were infused within the 12 h period in order maintain blood glucose levels. This model has obvious limitations because glucose uptake by adipose and muscle tissue, two of the largest glucose disposal sites, is unknown. However, insulin insensitivity has been well-characterized in both adipose and muscle during endotoxemia both in vivo (Raymond et al., 1981; Ling et al., 1994; Poggi et al., 2007; Mulligan et al., 2012) and in vitro (Song et al., 2006; Liang et al., 2013). Furthermore, our model is limited because we are not able to calculate hepatic glucose output, including the ~180 min period of hyperglycemia due to hepatic glycogenolysis immediately following LPS infusion. The contribution of gluconeogenesis to the glucose pool is also unclear in our model. Acute endotoxemia seems to inhibit gluconeogenesis while more chronic inflammatory situations increase gluconeogenesis (Filkins, 1978; McGuinness, 2005). Therefore, knowing the gluconeogenic contribution to our model would be of interest. The lack of endogenous glucose contribution measurements means we are underestimating the quantity of glucose entering the circulating pool and subsequently the total amount of glucose utilized by the immune system. Quantifying this would refine our model and make it more accurate.

Using this model, we quantified glucose consumption by an activated immune system in both Holstein steer calves (chapter 4) and lactating Holstein dairy cows (chapter 5). We found that glucose utilization by the activated immune system was 1.0 g/kg BW^{0.75}/h and 0.66 g/kg BW^{0.75}/h in steer calves and lactating cows, respectively. Data we have also generated in pigs (1.1 g/kg BW^{0.75}/h; Appendix A) and in a separate lactating cow study (1.0 g/kg BW^{0.75}/h; Horst and Baumgard, unpublished data) appears comparable and thus conserved across species and life stages, although this needs to be investigated

further. The aforementioned data comprises all our knowledge of glucose consumption of the immune system using this newly developed model. Therefore, it would be of interest to test the LPS-euglycemic clamp in different stages of lactation or during heat stress to see if these altered physiological states influence glucose consumption by the immune system.

Modeling immunoactivation, such as we have done in chapters 4 and 5, is frequently done using an acute LPS bolus. A high dose inducing a robust response was required for the development of hypoglycemia in order to quantify activated immune system glucose requirements; however, this acute i.v. bolus is not a great model of naturally occurring inflammation as cows would likely not experience such an intense immediate systemic exposure. During natural infection, endotoxin release is directly tied to bacterial growth and proliferation because LPS is shed through bacterial growth or death (Crutchley et al., 1967; Tsuji and Harrison, 1978). Therefore, we wanted to compare and contrast metabolic changes due to chronic versus acute LPS exposure. Similar to acute LPS exposure, we found chronic exposure increases circulating levels of insulin. In contrast, chronic exposure elevated circulating BUN and BHB levels relative to pair-fed animals. Control animals were pair-fed throughout the study, so this model helps to rid ambiguity within ruminant literature as BUN is complicated by the contribution of rumen derived ammonia. Furthermore, this data agrees with monogastric models of increased BUN following LPS exposure, likely due to muscle catabolism. However, BUN is certainly not a perfect biomarker of muscle catabolism, and investigating more specific markers of muscle catabolism is justified.

Reasons behind the circulating BHB differences in chapter 6 are unclear and certainly warrant further investigation into altered lipid metabolism following LPS exposure in both acute and chronic situations. A variety of studies have demonstrated decreased BHB following acute LPS infusion (see Table 4), including Zarrin et al. (2014) who observed significantly decreased BHB levels despite increasing BHB infusion in an attempt to maintain hyperketonemic levels. These studies suggest increased BHB utilization by peripheral tissue. Circulating BHB is also likely affected by decreased rumen motility and butyrate absorptive capacity (Jing et al., 2014). Waldron et al. (2003a) reported hepatic ketogenesis during LPS infusion remains functional in lactating cows, yet various other animal models show impaired hepatic ketogenesis due to high circulating insulin levels (Neufeld et al., 1980). Hepatic steatosis can be a consequence of infection or inflammation (Hirsch et al., 1964; Ilan, 2012; Fukunishi et al., 2013; Wenfeng et al., 2014; Damms-Machado et al., 2017) because NEFA accumulation into hepatic triglycerides is accelerated during immunoactivation (Guckian, 1973; Fiser et al., 1974; Lanza-Jacoby and Tabares, 1990). Normally, increased liver lipid content and reduced glucose availability contribute to ketogenesis in order to continue producing energy for extrahepatic tissues. Thus, the relationship between inflammation, ketone metabolism, and hepatic steatosis remain ill-defined in lactating cows and further studies investigating ketone metabolism and flux throughout tissues may be beneficial.

Interestingly, decreased circulating calcium is another hallmark of acute LPS exposure (see Table 4). Calcium is involved in immune activation (Hendy and Canaff, 2016), but where circulating calcium is redistributed or mechanistic reasons for hypocalcemia are unclear. Others have found calcium accumulates in ascites during i.v.

endotoxin infusion, and administering exogenous calcium i.v. has no benefit during sepsis (Carlstedt et al., 2008). Infusing glucose did not rescue milk yield following an acute LPS challenge (chapter 5); however, milk production is also dependent on calcium, which was reduced 46% and did not return to baseline levels within the 12 h period of the study. Perhaps infusion of both glucose and calcium could work synergistically to rescue milk yield during acute inflammation. Temporally characterizing circulating calcium following an LPS bolus would also give a better idea of how severely calcium homeostasis is affected. While calcium metabolism is not the focus of this dissertation, understanding the role inflammation and endotoxin plays in milk fever may also be of interest. Due to the magnitude of change observed by us and others, it is clear calcium homeostasis is severely affected by acute LPS and merits further research.

A portion of the current dissertation's focus was glucose consumption by the immune system. Glucose is certainly an important substrate for immune cells and glucose availability may limit the immune response (Kimura et al., 1999; Vernay et al., 2012; Garcia et al., 2015). However, in addition to glucose availability, circulating NEFA and/or BHB may affect immune function. A variety of studies have evaluated how NEFA and/or BHB affect various immune cells and their functions such as cytokine and immunoglobulin secretion, proliferation, phagocytic activity, and chemotaxis (Targowski and Klucinski, 1983; Kluciński et al., 1988; Lacetera et al., 2004; Renner et al., 2013). Contrary to these findings, some studies demonstrate no change or an enhanced immune response to increasing levels of BHB and/or NEFA (Bouchard et al., 1999; Lessard et al., 2004; Valko et al., 2007; Dänicke et al., 2012; Schulz et al., 2015). Differences in cell culture conditions and the difficulty of simulating the complex metabolic environment of a transition cow

likely contribute to these conflicting results. Of particular interest is a set of *in vitro* studies by Franklin, Nonnecke, and Young (Franklin et al., 1991; Nonnecke et al., 1992) which demonstrated no change in lymphocyte proliferation or B cell IgM secretion with glucose and ketone body concentrations representative of a clinically ketotic cow. In conclusion, the literature is conflicting and more decisive *in vivo* research is needed to fully comprehend the dynamic relationship of glucose, insulin, NEFA, and BHB with immune cell function, particularly in transitioning dairy cows.

Neutrophil function during the periparturient period is thought to be compromised, although a complete understanding of the mechanisms governing this are not entirely clear. As discussed above, postpartum immune dysfunction has been attributed in part to increased circulating metabolites such as BHB and NEFA. Furthermore, metabolic stresses associated with milk production are thought to contribute to immune cell dysfunction (Kimura et al., 1999) and cows with retained placental also exhibit depressed neutrophil function (Kimura et al., 2002). The immune cell functional assays in the aforementioned studies utilize leukocytes from peripheral circulation and do not include extravasated neutrophils or neutrophils in the margined pool (i.e., neutrophil accumulation within organs or neutrophils adhered to blood vessel endothelial cells). During acute LPS infusion, changes in leukocytes are very dynamic with leukopenia occurring immediately, ostensibly increasing the fraction extravasated and margined pool neutrophils. It would be of interest to somehow characterize function of this non-circulating fraction, although this would be difficult as the lifespan of a neutrophil within tissue is influenced by inflammation and neutrophil extracellular traps *in vitro* lead to neutrophil death within 4 h (Fuchs et al., 2007; Kolazkowska and Kubes, 2013). It may be that during parturition and peripheral

inflammatory situations (such as retained placenta), leukocyte distribution changes include functional leukocyte extravasation while less functional leukocytes remain in circulation. While we did not characterize circulating leukocyte dynamics past 12 h in the current dissertation, recent data our lab has generated indicates leukopenia is followed by leukocytosis by ~18 h (Horst and Baumgard, unpublished data). Characterizing neutrophil function at these different time points would help us to elucidate whether depressed neutrophil function during the periparturient period is related to changes in leukocyte distribution between the circulating and non-circulating pools.

Finally, animal models outside lactating dairy cows have demonstrated systemic or peripheral inflammation (Ziegler et al., 1988; Russell et al., 1995; Hietbrink et al., 2009; Epple and Zeitz, 2012) and psychological stress (Wallon et al., 2008; Vanuytsel et al., 2014) can contribute to gastrointestinal dysfunction. These aforementioned situations certainly have practical implications to dairy farm management. It would be of interest to study the effects of non-intestinal inflammation sources (i.e., mastitis, metritis, and laminitis) on intestinal health. Likewise, the social stresses of pen moves and overcrowding are frequently observed on-farm and create an unpredictable feeding environment which is associated with inflammation (Proudfoot et al., 2014). Understanding the impact of these stressful situations on intestinal health is worthy of additional study.

In conclusion, intestinal barrier dysfunction is a situation likely more ubiquitous than previously thought. Continued study of intestinal dysfunction in lactating cows is necessary in order to develop mitigation strategies to curb inflammation and prevent production losses. The current dissertation elucidated direct metabolic and inflammatory consequences and also characterized feed restriction's effects on intestinal dysfunction.

Furthermore, cost of immunoactivation in terms of glucose utilization was explicated. Future directions to expand upon this work include a greater understanding of calcium and lipid metabolism during inflammation, better characterization of immune cell function and how it is impacted by circulating metabolites and inflammation, and additional situations which may compromise intestinal integrity and cause inflammatory situations in practical dairy settings. With a greater appreciation for the metabolic and inflammatory changes, we can move forward in developing mitigation strategies aimed at increasing intestinal barrier integrity and limiting the negative influence of immunoactivation on metabolism and production.

REFERENCES

- Abreu, M. T. 2010. Toll-like receptor signaling in the intestinal epithelium: how bacterial recognition shapes intestinal function. *Nat. Rev. Immunol.* 10:131-144.
- Abuajamieh, M., S. K. Kvidera, M. V. Sanz Fernandez, A. Nayeri, N. C. Upah, E. A. Nolan, S. M. Lei, J. M. DeFrain, H. B. Green, K. M. Schoenberg, W. E. Trout, and L. H. Baumgard. 2016. Inflammatory biomarkers are associated with ketosis in periparturient Holstein cows. *Res. Vet. Sci.* 12:359-365.
- Ahmed, S., A. Minuti, and P. Bani. 2013. In vitro rumen fermentation characteristics of some naturally occurring and synthetic sugars. *Ital. J. Anim. Sci.* 6:424-426
- Al-Sadi, R. M. and T. Y. Ma. 2007. IL-1beta causes an increase in intestinal epithelial tight junction permeability. *J. Immunol.* 178:4641-4649.
- Allen, R. E. 1988. Muscle growth and development. In: C. Carlson, editor, *Designing Foods. Animal Product Options in the Marketplace.* National Academy Press, Washington, DC. p. 142-162.
- Alvarez, M. B. and H. D. Johnson. 1973. Environmental heat exposure on cattle plasma catecholamine and glucocorticoids. *J. Dairy Sci.* 56:189-194.
- Ballou, M. A., G. D. Cruz, W. Pittroff, D. H. Keisler, and E. J. DePeters. 2008. Modifying the acute phase response of Jersey calves by supplementing milk replacer with omega-3 fatty acids from fish oil. *J. Dairy Sci.* 91:3478-3487.
- Ballou, M. A. 2012. Growth and Development Symposium: Inflammation: Role in the etiology and pathophysiology of clinical mastitis in dairy cows. *J. Anim. Sci.* 90:1466-1478.
- Bargiggia, S., G. Maconi, M. Elli, P. Molteni, S. Ardizzone, F. Parente, I. Todaro, S. Greco, G. Manzionna, and G. Bianchi Porro. 2003. Sonographic prevalence of liver steatosis and biliary tract stones in patients with inflammatory bowel disease: study of 511 subjects at a single center. *J. Clin. Gastroenterol.* 36:417-420.
- Bauman, D. E. and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514-1529.
- Baumgard, L. H., B. A. Corl, D. A. Dwyer, and D. E. Bauman. 2002a. Effects of conjugated linoleic acids (CLA) on tissue response to homeostatic signals and plasma variables associated with lipid metabolism in lactating dairy cows. *J. Anim. Sci.* 80:1285-1293.

- Baumgard, L. H., W. J. Weber, H. Chester-Jones, L. B. Hansen, G. W. Kazmer, S. A. Zinn, and B. A. Crooker. 2002b. Growth hormone response to growth hormone releasing factor by Holstein calves from genetic lines selected for milk yield. *J. Dairy Sci.* 85:2529-2540.
- Baumgard, L. H., J. B. Wheelock, S. R. Sanders, C. E. Moore, H. B. Green, M. R. Waldron, and R. P. Rhoads. 2011. Postabsorptive carbohydrate adaptations to heat stress and monensin supplementation in lactating Holstein cows. *J. Dairy Sci.* 94:5620-5633.
- Baumgard, L.H and R.P. Rhoads. 2013. Effects of heat stress on postabsorptive metabolism and energetics. *Annu. Rev. Anim. Biosci.* 1:7.1-7.27.
- Baumgard, L. H., G. J. Hausman, and M. V. Sanz Fernandez. 2016. Insulin: pancreatic secretion and adipocyte regulation. *Domest. Anim. Endocrinol.* 54:76-84.
- Beeson, P. B. 1946. Development of tolerance to typhoid bacterial pyrogen and its abolition by reticulo-endothelial blockade. *Proc. Soc. Exp. Biol. Med.* 61:248-250.
- Beeson, P. B. 1947. Tolerance to bacterial pyrogens. I. Factors influencing its development. *J. Exp. Med.* 86:29-38.
- Beisel, W. R. and R. W. Wannemacher. 1980. Gluconeogenesis, ureagenesis, and ketogenesis during sepsis. *J. Parenter. Enteral. Nutr.* 4:277-285.
- Benjamin, M. A., D. M. McKay, P. C. Yang, H. Cameron, and M. H. Perdue. 2000. Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse. *Gut* 47:112-119.
- Berczi, I., L. Bertok, and T. Bereznai. 1966. Comparative studies on the toxicity of *Escherichia coli* lipopolysaccharide endotoxin in various animal species. *Can. J. Microbiol.* 12:1070-1071.
- Berg, J., J. Tymoczko, and L. Stryer. 2002. *Biochemistry*. 5 ed. W.H. Freeman, New York.
- Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* 70:567-590.
- Bernabucci, U., N. Lacetera, L. H. Baumgard, R. P. Rhoads, B. Ronchi, and A. Nardone. 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal* 4:1167-1183.

- Bertaglia, E., G. Scabia, S. Dalise, F. Lo Verso, F. Santini, P. Vitti, C. Chisari, M. Sandri, and M. Maffei. 2014. Haptoglobin is required to prevent oxidative stress and muscle atrophy. *PLoS One* 9:e100745.
- Bertoni, G. and E. Trevisi. 2013. Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds. *Vet. Clin. North Am. Food Anim. Pract.* 29:413-431.
- Bhat, U. G., V. Ilievski, T. G. Unterman, and K. Watanabe. 2014. Porphyromonas gingivalis lipopolysaccharide upregulates insulin secretion from pancreatic β cell line MIN6. *J. Periodontol.* 85:1629-1636.
- Bieniek, K., A. Szuster-Ciesielska, T. Kamińska, M. Kondracki, M. Witek, and M. Kandefer-Szerszeń. 1998. Tumor necrosis factor and interferon activity in the circulation of calves after repeated injection of low doses of lipopolysaccharide. *Vet. Immunol. Immunopathol.* 62:297-307.
- Biswas, S. K. and E. Lopez-Collazo. 2009. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends Immunol.* 30:475-487.
- Bjerre-Harpøth, V., N. C. Friggens, V. M. Thorup, T. Larsen, B. M. Damgaard, K. L. Ingvarsten, and K. M. Moyes. 2012. Metabolic and production profiles of dairy cows in response to decreased nutrient density to increase physiological imbalance at different stages of lactation. *J. Dairy Sci.* 95:2362-2380.
- Blackard, W. G., J. H. Anderson, and J. J. Spitzer. 1976. Hyperinsulinism in endotoxin shock dogs. *Metabolism* 25:675-684.
- Blair, D., F. J. Dufort, and T. C. Chiles. 2012. Protein kinase C β is critical for the metabolic switch to glycolysis following B-cell antigen receptor engagement. *Biochem. J.* 448:165-169.
- Blaxter, K. L. and F. W. Wainman. 1966. The fasting metabolism of cattle. *Br. J. Nutr.* 20:103-111.
- Bobe, G., J. W. Young, and D. C. Betiz. 2004. Invited review: Pathology, etiology, prevention, and treatment of fatty liver in dairy cows. *J. Dairy Sci.* 87:3105-3124.
- Boomer, J. S., K. To, K. C. Chang, O. Takasu, D. F. Osborne, A. H. Walton, T. L. Bricker, S. D. Jarman, D. Kreisel, A. S. Krupnick, A. Srivastava, P. E. Swanson, J. M. Green, and R. S. Hotchkiss. 2011. Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA* 306:2594-2605.
- Borgs, P. and B. A. Mallard. 1998. Immune-endocrine interactions in agricultural species: chromium and its effect on health and performance. *Domest. Anim. Endocrinol.* 15:431-438.

- Borth, C. 2011. *Essentials of Pathophysiology: Concepts of Altered Health States*. 3rd ed. Lippincott Williams & Wilkins.
- Bosi, E., L. Molteni, M. G. Radaelli, L. Folini, I. Fermo, E. Bazzigaluppi, L. Piemonti, M. R. Pastore, and R. Paroni. 2006. Increased intestinal permeability precedes clinical onset of type 1 diabetes. *Diabetologia* 49:2824-2827.
- Bouchard, L., S. Blais, C. Desrosiers, X. Zhao, and P. Lacasse. 1999. Nitric oxide production during endotoxin-induced mastitis in the cow. *J. Dairy Sci.* 82:2574-2581.
- Boudry, G., V. Péron, I. Le Huërou-Luron, J. P. Lallès, and B. Sève. 2004. Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. *J. Nutr.* 134:2256-2262.
- Boushey, R. P., B. Yusta, and D. J. Drucker. 1999. Glucagon-like peptide 2 decreases mortality and reduces the severity of indomethacin-induced murine enteritis. *Am. J. Physiol.* 277:E937-947.
- Boza, J. J., D. Möennoz, J. Vuichoud, A. R. Jarret, D. Gaudard-de-Weck, R. Fritsché, A. Donnet, E. J. Schiffrin, G. Perruisseau, and O. Ballèvre. 1999. Food deprivation and refeeding influence growth, nutrient retention and functional recovery of rats. *J. Nutr.* 129:1340-1346.
- Bradford, B. J., L. K. Mamedova, J. E. Minton, J. S. Drouillard, and B. J. Johnson. 2009. Daily injection of tumor necrosis factor-alpha increases hepatic triglycerides and alters transcript abundance of metabolic genes in lactating dairy cattle. *J. Nutr.* 139:1451-1456.
- Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *J. Dairy Sci.* 98:6631-6650.
- Brand, K. 1985. Glutamine and glucose metabolism during thymocyte proliferation. Pathways of glutamine and glutamate metabolism. *Biochem. J.* 228:353-361.
- Briggs, M. M., J. M. Stadel, R. Iyengar, and R. J. Lefkowitz. 1983. Functional modification of the guanine nucleotide regulatory protein after desensitization of turkey erythrocytes by catecholamines. *Arch. Biochem. Biophys.* 224:142-151.
- Bruewer, M., A. Luegering, T. Kucharzik, C. A. Parkos, J. L. Madara, A. M. Hopkins, and A. Nusrat. 2003. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J. Immunol.* 171:6164-6172.

- Bruewer, M., M. Utech, A. I. Ivanov, A. M. Hopkins, C. A. Parkos, and A. Nusrat. 2005. Interferon-gamma induces internalization of epithelial tight junction proteins via a macropinocytosis-like process. *FASEB J.* 19:923-933.
- Bruins, M. J., N. E. Deutz, and P. B. Soeters. 2003. Aspects of organ protein, amino acid and glucose metabolism in a porcine model of hypermetabolic sepsis. *Clin. Sci. (Lond.)*. 104:127-141.
- Buffinton, G. D. and W. F. Doe. 1995. Altered ascorbic acid status in the mucosa from inflammatory bowel disease patients. *Free Radic. Res.* 22:131-143.
- Burdick, N. C., J. A. Carroll, L. E. Hulbert, J. W. Dailey, M. A. Ballou, R. D. Randel, S. T. Willard, R. C. Vann, and T. H. Welsh. 2011. Temperament influences endotoxin-induced changes in rectal temperature, sickness behavior, and plasma epinephrine concentrations in bulls. *Innate Immun.* 17:355-364.
- Burdick Sanchez, N. C., R. Chaffin, J. A. Carroll, C. C. Chase, S. W. Coleman, and D. E. Spiers. 2013. Heat-tolerant versus heat-sensitive *Bos taurus* cattle: influence of air temperature and breed on the metabolic response to a provocative immune challenge. *Domest. Anim. Endocrinol.* 45:180-186.
- Burdick Sanchez, N. C., J. A. Carroll, R. D. Randel, R. C. Vann, and T. H. Welsh. 2014. Associations between endotoxin-induced metabolic changes and temperament in Brahman bulls. *J. Anim. Physiol. Anim. Nutr. (Berl.)*. 98:178-190.
- Bynum, G., J. Brown, D. Dubose, M. Marsili, I. Leav, T. G. Pistole, M. Hamlet, M. LeMaire, and B. Caleb. 1979. Increased survival in experimental dog heatstroke after reduction of gut flora. *Aviat. Space Environ. Med.* 50:816-819.
- Caesar, R., C. S. Reigstad, H. K. Bäckhed, C. Reinhardt, M. Ketonen, G. Lundén, P. D. Cani, and F. Bäckhed. 2012. Gut-derived lipopolysaccharide augments adipose macrophage accumulation but is not essential for impaired glucose or insulin tolerance in mice. *Gut* 61:1701-1707.
- Calder, P. C., G. Dimitriadis, and P. Newsholme. 2007. Glucose metabolism in lymphoid and inflammatory cells and tissues. *Curr. Opin. Clin. Nutr. Metab. Care* 10:531-540.
- Cameron, H. L. and M. H. Perdue. 2005. Stress impairs murine intestinal barrier function: improvement by glucagon-like peptide-2. *J. Pharmacol. Exp. Ther.* 314:214-220.
- Cani, P. D., J. Amar, M. A. Iglesias, M. Poggi, C. Knauf, D. Bastelica, A. M. Neyrinck, F. Fava, K. M. Tuohy, C. Chabo, A. Waget, E. Delmée, B. Cousin, T. Sulpice, B. Chamontin, J. Ferrières, J. F. Tanti, G. R. Gibson, L. Casteilla, N. M. Delzenne,

- M. C. Alessi, and R. Burcelin. 2007. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 56:1761-1772.
- Cappellozza, B. I., R. F. Cooke, C. Trevisanuto, V. D. Tabacow, F. N. T. Cooke, and D. W. Bohnert. 2011. Feed and water restriction elicits an acute-phase protein response in beef cattle. *J. Anim. Sci.* 89(E-Suppl. S1):269.(Abstr.).
- Carey, H. V., U. L. Hayden, and K. E. Tucker. 1994. Fasting alters basal and stimulated ion transport in piglet jejunum. *Am. J. Physiol.* 267:R156-163.
- Carlson, D. B., N. B. Litherland, H. M. Dann, J. C. Woodworth, and J. K. Drackley. 2006. Metabolic effects of abomasal L-carnitine infusion and feed restriction in lactating Holstein cows. *J. Dairy Sci.* 89:4819-4834.
- Carlstedt, F., M. Eriksson, R. Kiiski, A. Larsson, and L. Lind. 2000. Hypocalcemia during porcine endotoxemic shock: effects of calcium administration. *Crit. Care Med.* 28:2909-2914.
- Caro-Maldonado, A., R. Wang, A. G. Nichols, M. Kuraoka, S. Milasta, L. D. Sun, A. L. Gavin, E. D. Abel, G. Kelsoe, D. R. Green, and J. C. Rathmell. 2014. Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. *J. Immunol.* 192:3626-3636.
- Carroll, J. A., R. R. Reuter, C. C. Chase, S. W. Coleman, D. G. Riley, D. E. Spiers, J. D. Arthington, and M. L. Galyean. 2009. Profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge. *Innate Immun.* 15:81-89.
- Casas, A. T., A. P. Hubsch, B. C. Rogers, and J. E. Doran. 1995. Reconstituted high-density lipoprotein reduces LPS-stimulated TNF alpha. *J. Surg. Res.* 59:544-552.
- Ceciliani, F., J. J. Ceron, P. D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. *J. Proteomics* 75:4207-4231.
- Chaiyabutr, N., A. Faulkner, and M. Peaker. 1980. The utilization of glucose for the synthesis of milk components in the fed and starved lactating goat in vivo. *Biochem. J.* 186:301-308
- Chappell, V. L., M. D. Thompson, M. G. Jeschke, D. H. Chung, J. C. Thompson, and S. E. Wolf. 2003. Effects of incremental starvation on gut mucosa. *Dig. Dis. Sci.* 48:765-769.
- Chang, C. H., J. D. Curtis, L. B. Maggi, B. Faubert, A. V. Villarino, D. O'Sullivan, S. C. Huang, G. J. van der Windt, J. Blagih, J. Qiu, J. D. Weber, E. J. Pearce, R. G.

- Jones, and E. L. Pearce. 2013. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* 153:1239-1251.
- Chen, K., M. Inoue, and A. Okada. 1996. Expression of inducible nitric oxide synthase mRNA in rat digestive tissues after endotoxin and its role in intestinal mucosal injury. *Biochem. Biophys. Res. Commun.* 224:703-708.
- Cheng, H. and C. P. Leblond. 1974. Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I. Columnar cell. *Am. J. Anat.* 141:461-479.
- Cheng, S. C., L. A. Joosten, and M. G. Netea. 2014. The interplay between central metabolism and innate immune responses. *Cytokine Growth Factor Rev.* 25:707-713.
- Connor, E. E., R. L. Baldwin, A. V. Capuco, C. M. Evoke-Clover, S. E. Ellis, and K. S. Sciabica. 2010. Characterization of glucagon-like peptide 2 pathway member expression in bovine gastrointestinal tract. *J. Dairy Sci.* 93:5167-5178.
- Connor, E. E., S. Kahl, T. H. Elsasser, R. L. Baldwin, R. Fayer, M. Santin-Duran, G. L. Sample, and C. M. Evoke-Clover. 2013. Glucagon-like peptide 2 therapy reduces negative effects of diarrhea on calf gut. *J. Dairy Sci.* 96:1793-1802.
- Connor, E. E., C. M. Evoke-Clover, E. H. Wall, R. L. Baldwin, M. Santin-Duran, T. H. Elsasser, and D. M. Bravo. 2016. Glucagon-like peptide 2 and its beneficial effects on gut function and health in production animals. *Domest. Anim. Endocrinol.* 56:S56-65.
- Connor, E. E., E. H. Wall, D. M. Bravo, C. M. Evoke-Clover, T. H. Elsasser, R. L. Baldwin, M. Santín, B. T. Vinyard, S. Kahl, and M. P. Walker. 2017. Reducing gut effects from *Cryptosporidium parvum* infection in dairy calves through prophylactic glucagon-like peptide 2 therapy or feeding of an artificial sweetener. *J. Dairy Sci.* 100:3004-3018.
- Corfield, A. P. 2015. Mucins: a biologically relevant glycan barrier in mucosal protection. *Biochim. Biophys. Acta.* 1850:236-252.
- Costa Rosa, L. F., R. Curi, C. Murphy, and P. Newsholme. 1995. Effect of adrenaline and phorbol myristate acetate or bacterial lipopolysaccharide on stimulation of pathways of macrophage glucose, glutamine and O₂ metabolism. Evidence for cyclic AMP-dependent protein kinase mediated inhibition of glucose-6-phosphate dehydrogenase and activation of NADP⁺-dependent 'malic' enzyme. *Biochem. J.* 310:709-714.

- Cronje, P. 2005. Heat stress in livestock—the role of the gut in its aetiology and a potential role for betaine in its alleviation. *RAAN* 15:107-122.
- Crutchley, M. J., D. G. Marsh, and J. Cameron. 1967. Free Endotoxin. *Nature* 214:1052.
- Cullen, T. W., W. B. Schofield, N. A. Barry, E. E. Putnam, E. A. Rundell, M. S. Trent, P. H. Degan, C. J. Booth, H. Yu, and A. L. Goodman. 2015. Gut microbiota. Antimicrobial peptide resistance mediates resilience of prominent gut commensals during inflammation. *Science* 347:170-175.
- Cunningham, K. E. and J. R. Turner. 2012. Myosin light chain kinase: pulling the strings of epithelial tight junction function. *Ann. N.Y. Acad. Sci.* 1258:34-42.
- Dale, D. C., L. Boxer, and W. C. Liles. 2008. The phagocytes: neutrophils and monocytes. *Blood* 112:935-945.
- Damms-Machado, A., S. Louis, A. Schnitzer, V. Volynets, A. Rings, M. Basrai, and S. C. Bischoff. 2017. Gut permeability is related to body weight, fatty liver disease, and insulin resistance in obese individuals undergoing weight reduction. *Am. J. Clin. Nutr.* 105:127-135.
- Daneman, D., B. Zinman, M. E. Elliott, P. J. Bilan, and A. Klip. 1992. Insulin-stimulated glucose transport in circulating mononuclear cells from nondiabetic and IDDM subjects. *Diabetes* 41:227-234.
- Dänicke, S., J. Kowalczyk, L. Renner, J. Pappritz, U. Meyer, R. Kramer, E. M. Weber, S. Döll, J. Rehage, and G. Jahreis. 2012. Effects of conjugated linoleic acids fed to dairy cows during early gestation on hematological, immunological, and metabolic characteristics of cows and their calves. *J. Dairy Sci.* 95:3938-3953.
- de Boer, G., A. Trenkle, and J. W. Young. 1985. Glucagon, insulin, growth hormone, and some blood metabolites during energy restriction ketonemia of lactating cows. *J. Dairy Sci.* 68:326-337.
- de Punder, K. and L. Pruimboom. 2015. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. *Front. Immunol.* 6:223.
- De Strooper, B., W. Annaert, P. Cupers, P. Saftig, K. Craessaerts, J. S. Mumm, E. H. Schroeter, V. Schrijvers, M. S. Wolfe, W. J. Ray, A. Goate, and R. Kopan. 1999. A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature* 398:518-522.
- Deitch, E. A., W. J. Ma, L. Ma, R. D. Berg, and R. D. Specian. 1990. Protein malnutrition predisposes to inflammatory-induced gut-origin septic states. *Ann. Surg.* 211:560-567.

- del Fresno, C., F. García-Río, V. Gómez-Piña, A. Soares-Schanoski, I. Fernández-Ruíz, T. Jurado, T. Kajiji, C. Shu, E. Marín, A. Gutierrez del Arroyo, C. Prados, F. Arnalich, P. Fuentes-Prior, S. K. Biswas, and E. López-Collazo. 2009. Potent phagocytic activity with impaired antigen presentation identifying lipopolysaccharide-tolerant human monocytes: demonstration in isolated monocytes from cystic fibrosis patients. *J. Immunol.* 182:6494-6507.
- Deplancke, B. and H. R. Gaskins. 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *Am. J. Clin. Nutr.* 73:1131S-1141S.
- Dentener, M. A., E. J. Von Asmuth, G. J. Francot, M. N. Marra, and W. A. Buurman. 1993. Antagonistic effects of lipopolysaccharide binding protein and bactericidal/permeability-increasing protein on lipopolysaccharide-induced cytokine release by mononuclear phagocytes. Competition for binding to lipopolysaccharide. *J. Immunol.* 151:4258-4265.
- Doherty, J. F., M. H. Golden, J. G. Raynes, G. E. Griffin, and K. P. McAdam. 1993. Acute-phase protein response is impaired in severely malnourished children. *Clin. Sci. (Lond.)* 84:169-175.
- Doughty, C. A., B. F. Bleiman, D. J. Wagner, F. J. Dufort, J. M. Mataraza, M. F. Roberts, and T. C. Chiles. 2006. Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth. *Blood* 107:4458-4465.
- Doyle, A., G. Zhang, E. A. Abdel Fattah, N. T. Eissa, and Y. P. Li. 2011. Toll-like receptor 4 mediates lipopolysaccharide-induced muscle catabolism via coordinate activation of ubiquitin-proteasome and autophagy-lysosome pathways. *FASEB J.* 25:99-110.
- Draper, L. R., L. A. Gyure, J. G. Hall, and D. Robertson. 1983. Effect of alcohol on the integrity of the intestinal epithelium. *Gut* 24:399-404.
- Drucker, D. J. and B. Yusta. 2014. Physiology and pharmacology of the enteroendocrine hormone glucagon-like peptide-2. *Annu. Rev. Physiol.* 76:561-583.
- Dufort, F. J., B. F. Bleiman, M. R. Gumina, D. Blair, D. J. Wagner, M. F. Roberts, Y. Abu-Amer, and T. C. Chiles. 2007. Cutting edge: IL-4-mediated protection of primary B lymphocytes from apoptosis via Stat6-dependent regulation of glycolytic metabolism. *J. Immunol.* 179:4953-4957.
- Eaton, J. W., P. Brandt, J. R. Mahoney, and J. T. Lee. 1982. Haptoglobin: a natural bacteriostat. *Science* 215:691-693.

- Eger, M., J. Hussen, M. Koy, S. Dänicke, H. J. Schuberth, and G. Breves. 2016. Glucose transporter expression differs between bovine monocyte and macrophage subsets and is influenced by milk production. *J. Dairy Sci.* 99:2276-2287.
- Eliakim, R., A. Mahmood, and D. H. Alpers. 1991. Rat intestinal alkaline phosphatase secretion into lumen and serum is coordinately regulated. *Biochim. Biophys. Acta.* 1091:1-8.
- Elias, P. M. 2005. Stratum corneum defensive functions: an integrated view. *J. Invest. Dermatol.* 125:183-200.
- Elli, M., O. Aydin, S. Bilge, A. Bozkurt, A. Dagdemir, F. G. Pinarli, and S. Acar. 2009. Protective effect of vitamin A on ARA-C induced intestinal damage in mice. *Tumori.* 95:87-90.
- Elsasser, T. H., M. Richards, R. Collier, and G. F. Hartnell. 1996. Physiological responses to repeated endotoxin challenge are selectively affected by recombinant bovine somatotropin administration to calves. *Domest. Anim. Endocrinol.* 13:91-103.
- Emmanuel, D. G., K. L. Madsen, T. A. Churchill, S. M. Dunn, and B. N. Ametaj. 2007a. Acidosis and lipopolysaccharide from *Escherichia coli* B:055 cause hyperpermeability of rumen and colon tissues. *J. Dairy Sci.* 90:5552-5557.
- Emmanuel, D. G., S. Shanthipoosan, and B. N. Ametaj. 2007b. High grain diets perturb rumen and plasma metabolites and induce inflammatory responses in early lactation dairy cows. *Ital. J. Anim. Sci.* 6:424-426.
- Epple, H. J. and M. Zeitz. 2012. HIV infection and the intestinal mucosal barrier. *Ann N Y Acad Sci* 1258:19-24.
- Erridge, C., E. Bennett-Guerrero, and I. R. Poxton. 2002. Structure and function of lipopolysaccharides. *Microbes Infect.* 4:837-851.
- Erridge, C., T. Attina, C. M. Spickett, and D. J. Webb. 2007. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am. J. Clin. Nutr.* 86:1286-1292.
- Everts, B., E. Amiel, G. J. van der Windt, T. C. Freitas, R. Chott, K. E. Yarasheski, E. L. Pearce, and E. J. Pearce. 2012. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. *Blood* 120:1422-1431.
- Fanning, A. S., T. Y. Ma, and J. M. Anderson. 2002. Isolation and functional characterization of the actin binding region in the tight junction protein ZO-1. *FASEB J.* 16:1835-1837.

- Feingold, K. R., I. Staprans, R. A. Memon, A. H. Moser, J. K. Shigenaga, W. Doerrler, C. A. Dinarello, and C. Grunfeld. 1992. Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance. *J. Lipid. Res.* 33:1765-1776.
- Ferraretto, L. F., H. Gencoglu, K. S. Hackbart, A. B. Nascimento, F. Dalla Costa, R. W. Bender, J. N. Guenther, R. D. Shaver, and M. C. Wiltbank. 2014. Effect of feed restriction on reproductive and metabolic hormones in dairy cows. *J. Dairy Sci.* 97:754-763.
- Ferraris, R. P. and H. V. Carey. 2000. Intestinal transport during fasting and malnutrition. *Annu. Rev. Nutr.* 20:195-219.
- Ferreira, T. M., A. J. Leonel, M. A. Melo, R. R. Santos, D. C. Cara, V. N. Cardoso, M. I. Correia, and J. I. Alvarez-Leite. 2012. Oral supplementation of butyrate reduces mucositis and intestinal permeability associated with 5-Fluorouracil administration. *Lipids* 47:669-678.
- Filkins, J. P. 1978. Phases of glucose dyshomeostasis in endotoxicosis. *Circ. Shock* 5:347-355.
- Fiser, R. H., J. C. Denniston, and W. R. Beisel. 1974. Endotoxemia in the Rhesus monkey: alterations in host lipid and carbohydrate metabolism. *Pediatr. Res.* 8:13-17.
- Ford, R. P., I. S. Menzies, A. D. Phillips, J. A. Walker-Smith, and M. W. Turner. 1985. Intestinal sugar permeability: relationship to diarrhoeal disease and small bowel morphology. *J. Pediatr. Gastroenterol. Nutr.* 4:568-574.
- Forse, R. A., R. Leibel, J. Askanazi, J. Hirsch, and J. M. Kinney. 1987. Adrenergic control of adipocyte lipolysis in trauma and sepsis. *Ann. Surg.* 206:744-751.
- Fortun, P. J. and C. J. Hawkey. 2007. Nonsteroidal antiinflammatory drugs and the small intestine. *Curr. Opin. Gastroenterol.* 23:134-141.
- Franklin, S. T., J. W. Young, and B. J. Nonnecke. 1991. Effects of ketones, acetate, butyrate, and glucose on bovine lymphocyte proliferation. *J. Dairy Sci.* 74:2507-2514.
- 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.

- Fuchs, T. A., U. Abed, C. Goosmann, R. Hurwitz, I. Schulze, V. Wahn, Y. Weinrauch, V. Brinkmann, and A. Zychlinsky. 2007. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol* 176(2):231-241.
- Fukunishi, S., T. Sujishi, A. Takeshita, H. Ohama, Y. Tsuchimoto, A. Asai, Y. Tsuda, and K. Higuchi. 2014. Lipopolysaccharides accelerate hepatic steatosis in the development of nonalcoholic fatty liver disease in Zucker rats. *J. Clin. Biochem. Nutr.* 54:39-44.
- Fukuzumi, M., H. Shinomiya, Y. Shimizu, K. Ohishi, and S. Utsumi. 1996. Endotoxin-induced enhancement of glucose influx into murine peritoneal macrophages via GLUT1. *Infect. Immun.* 64:108-112.
- Furuse, M., T. Hirase, M. Itoh, A. Nagafuchi, S. Yonemura, and S. Tsukita. 1993. Occludin: a novel integral membrane protein localizing at tight junctions. *J. Cell Biol.* 123:1777-1788.
- Furuse, M., K. Fujita, T. Hiragi, K. Fujimoto, and S. Tsukita. 1998. Claudin-1 and -2: novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J. Cell Biol.* 141:1539-1550.
- Gabay, C. and I. Kushner. 1999. Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* 340:448-454.
- Galyean, M. L., R. W. Lee, and M. E. Hubbert. 1981. Influence of fasting and transit on ruminal and blood metabolites in beef steers. *J. Anim. Sci.* 53:7-18.
- Garcia, M., T. H. Elsasser, Y. Qu, X. Zhu, and K. M. Moyes. 2015. Glucose supplementation has minimal effects on blood neutrophil function and gene expression in vitro. *J. Dairy Sci.* 98:6139-6150.
- Gathiram, P., M. T. Wells, J. G. Brock-Utne, and S. L. Gaffin. 1987. Antilipopolysaccharide improves survival in primates subjected to heat stroke. *Circ. Shock* 23:157-164.
- Geng, Y., J. Li, F. Wang, Q. Li, X. Wang, L. Sun, and W. Li. 2013. Epidermal growth factor promotes proliferation and improves restoration after intestinal ischemia-reperfusion injury in rats. *Inflammation* 36:670-679.
- Gerros, T. C., S. D. Semrad, and R. A. Proctor. 1995. Alterations in clinical, hematological and metabolic variables in bovine neonatal endotoxemia. *Can. J. Vet. Res.* 59:34-39.

- Gioannini, T. L., A. Teghanemt, D. Zhang, P. Prohinar, E. N. Levis, R. S. Munford, and J. P. Weiss. 2007. Endotoxin-binding proteins modulate the susceptibility of bacterial endotoxin to deacylation by acyloxyacyl hydrolase. *J. Biol. Chem.* 282:7877-7884.
- Giri, S. N., P. Emau, J. S. Cullor, G. H. Stabenfeldt, M. L. Bruss, R. H. Bondurant, and B. I. Osburn. 1990. Effects of endotoxin infusion on circulating levels of eicosanoids, progesterone, cortisol, glucose and lactic acid, and abortion in pregnant cows. *Vet. Microbiol.* 21:211-231.
- Gitomer, W. L., B. C. Miller, and G. L. Cottam. 1995. In vivo effects of lipopolysaccharide on hepatic free-NAD(P)(+)-linked redox states and cytosolic phosphorylation potential in 48-hour-fasted rats. *Metabolism* 44:1170-1174.
- Goldberg, R. F., W. G. Austen, X. Zhang, G. Munene, G. Mostafa, S. Biswas, M. McCormack, K. R. Eberlin, J. T. Nguyen, H. S. Tatlidede, H. S. Warren, S. Narisawa, J. L. Millán, and R. A. Hodin. 2008. Intestinal alkaline phosphatase is a gut mucosal defense factor maintained by enteral nutrition. *Proc. Natl. Acad. Sci. U.S.A.* 105:3551-3556.
- Goris, H., F. de Boer, and D. van der Waaij. 1988. Kinetics of endotoxin release by gram-negative bacteria in the intestinal tract of mice during oral administration of bacitracin and during in vitro growth. *Scand. J. Infect. Dis.* 20:213-219.
- Gouwy, M., M. De Buck, N. Pörtner, G. Opdenakker, P. Proost, S. Struyf, and J. Van Damme. 2015. Serum amyloid A chemoattracts immature dendritic cells and indirectly provokes monocyte chemotaxis by induction of cooperating CC and CXC chemokines. *Eur. J. Immunol.* 45:101-112.
- Gozho, G. N., J. C. Plaizier, D. O. Krause, A. D. Kennedy, and K. M. Wittenberg. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *J. Dairy Sci.* 88:1399-1403.
- Graber, C. D., R. B. Reinhold, J. G. Breman, R. A. Harley, and G. R. Hennigar. 1971. Fatal heat stroke. Circulating endotoxin and gram-negative sepsis as complications. *JAMA* 216:1195-1196.
- Graham, C. and N. L. Simmons. 2005. Functional organization of the bovine rumen epithelium. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288:R173-181.
- Grant, R. J. and J. L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. *J. Anim. Sci.* 73:2791-2803.
- Graugnard, D. E., K. M. Moyes, E. Trevisi, M. J. Khan, D. Keisler, J. K. Drackley, G. Bertoni, and J. J. Loo. 2013. Liver lipid content and inflammometabolic indices

in peripartur dairy cows are altered in response to prepartur energy intake and postpartur intramammary inflammatory challenge. *J. Dairy Sci.* 96:918-935.

- Graves, R. J. 1842. Lecture IV. Pages 278-285 in *Clinical Lectures*. 2nd Edition. Barrington & Haswell.
- Greiner, E. F., M. Guppy, and K. Brand. 1994. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. *J. Biol. Chem.* 269:31484-31490.
- Gressley, T. F., M. B. Hall, and L. E. Armentano. 2011. Ruminant Nutrition Symposium: Productivity, digestion, and health responses to hindgut acidosis in ruminants. *J. Anim. Sci.* 89:1120-1130.
- Griel, L. C., A. Zarkower, and R. J. Eberhart. 1975. Clinical and clinico-pathological effects of *Escherichia coli* endotoxin in mature cattle. *Can. J. Comp. Med.* 39:1-6.
- Grimble, R. F. and G. K. Grimble. 1998. Immunonutrition: role of sulfur amino acids, related amino acids, and polyamines. *Nutrition* 14:605-610.
- Gröhn, Y. T., D. J. Wilson, R. N. González, J. A. Hertl, H. Schulte, G. Bennett, and Y. H. Schukken. 2004. Effect of pathogen-specific clinical mastitis on milk yield in dairy cows. *J. Dairy Sci.* 87:3358-3374.
- Grootjans, J., I. H. Hundscheid, and W. A. Buurman. 2013. Goblet cell compound exocytosis in the defense against bacterial invasion in the colon exposed to ischemia-reperfusion. *Gut Microbes* 4:232-235.
- Gross, J. J., F. J. Schwarz, K. Eder, H. A. van Dorland, and R. M. Bruckmaier. 2013. Liver fat content and lipid metabolism in dairy cows during early lactation and during a mid-lactation feed restriction. *J. Dairy Sci.* 96:5008-5017.
- Guan, X. 2014. The CNS glucagon-like peptide-2 receptor in the control of energy balance and glucose homeostasis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 307:R585-596.
- Guckian, J. C. 1973. Role of metabolism in pathogenesis of bacteremia due to *Diplococcus pneumoniae* in rabbits. *J. Infect. Dis.* 127:1-8.
- Guerville, M. and G. Boudry. 2016. Gastrointestinal and hepatic mechanisms limiting entry and dissemination of lipopolysaccharide into the systemic circulation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 311:G1-G15.

- Guo, L., J. Ai, Z. Zheng, D. A. Howatt, A. Daugherty, B. Huang, and X. A. Li. 2013. High density lipoprotein protects against polymicrobe-induced sepsis in mice. *J. Biol. Chem.* 288:17947-17953.
- Gurr, J. A. and T. A. Ruh. 1980. Desensitization of primary cultures of adult rat liver parenchymal cells to stimulation of adenosine 3',5'-monophosphate production by glucagon and epinephrine. *Endocrinology* 107:1309-1319.
- Gutsmann, T., M. Müller, S. F. Carroll, R. C. MacKenzie, A. Wiese, and U. Seydel. 2001. Dual role of lipopolysaccharide (LPS)-binding protein in neutralization of LPS and enhancement of LPS-induced activation of mononuclear cells. *Infect. Immun.* 69:6942-6950.
- Hall, D. M., K. R. Baumgardner, T. D. Oberley, and C. V. Gisolfi. 1999. Splanchnic tissues undergo hypoxic stress during whole body hyperthermia. *Am. J. Physiol.* 276:G1195-1203.
- Hall, D. M., G. R. Buettner, L. W. Oberley, L. Xu, R. D. Matthes, and C. V. Gisolfi. 2001. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. *Am. J. Physiol. Heart Circ. Physiol.* 280:H509-521.
- Hall-Angerås, M., U. Angerås, O. Zamir, P. O. Hasselgren, and J. E. Fischer. 1991. Effect of the glucocorticoid receptor antagonist RU 38486 on muscle protein breakdown in sepsis. *Surgery* 109:468-473.
- Halter, J. B., J. C. Beard, and D. Porte. 1984. Islet function and stress hyperglycemia: plasma glucose and epinephrine interaction. *Am. J. Physiol.* 247:E47-52.
- Hand, M. S., M. J. Fettman, L. G. Chandrasena, J. L. Cleek, R. A. Mason, and R. W. Phillips. 1983. Increased glucose uptake precedes hyperinsulinemia in awake endotoxemic minipigs. *Circ. Shock* 11:287-295.
- Hargrove, D. M., C. H. Lang, G. J. Bagby, and J. J. Spitzer. 1989. Epinephrine-induced increase in glucose turnover is diminished during sepsis. *Metabolism* 38:1070-1076.
- Hargrove, D. M., N. Skrepnik, C. H. Lang, G. J. Bagby, and J. J. Spitzer. 1990. Role of insulin in the blunted glucose metabolic response of septic rats to epinephrine. *Metabolism* 39:1180-1185.
- Harris, H. W., C. Grunfeld, K. R. Feingold, and J. H. Rapp. 1990. Human very low density lipoproteins and chylomicrons can protect against endotoxin-induced death in mice. *J. Clin. Invest.* 86:696-702.

- Hasselgren, P. O., B. W. Warner, J. H. James, H. Takehara, and J. E. Fischer. 1987. Effect of insulin on amino acid uptake and protein turnover in skeletal muscle from septic rats. Evidence for insulin resistance of protein breakdown. *Arch. Surg.* 122:228-233.
- Hayirli, A., D. R. Bremmer, S. J. Bertics, M. T. Socha, and R. R. Grummer. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. *J. Dairy Sci.* 84:1218-1230.
- Hector, F. J. 1926. Carbohydrate metabolism in diphtheria. *Lancet* 208: 642–645
- Helderman, J. H. 1981. Role of insulin in the intermediary metabolism of the activated thymic-derived lymphocyte. *J. Clin. Invest.* 67:1636-1642.
- Helderman, J. H. 1984. Acute regulation of human lymphocyte insulin receptors. Analysis by the glucose clamp. *J. Clin. Invest.* 74:1428-1435.
- Hendy, G. N. and L. Canaff. 2016. Calcium-sensing receptor, proinflammatory cytokines and calcium homeostasis. *Semin. Cell Dev. Biol.* 49:37-43.
- Hering, N. A., S. Andres, A. Fromm, E. A. van Tol, M. Amasheh, J. Mankertz, M. Fromm, and J. D. Schulzke. 2011. Transforming growth factor- β , a whey protein component, strengthens the intestinal barrier by upregulating claudin-4 in HT-29/B6 cells. *J. Nutr.* 141:783-789.
- Hietbrink, F., M. G. Besselink, W. Renooij, M. B. de Smet, A. Draisma, H. van der Hoeven, and P. Pickkers. 2009. Systemic inflammation increases intestinal permeability during experimental human endotoxemia. *Shock* 32:374-378.
- Hirata, M., Y. Matsumoto, S. Izumi, Y. Soga, F. Hirota, and M. Tobisa. 2015. Seasonal and interannual variations in feeding station behavior of cattle: effects of sward and meteorological conditions. *Animal* 9:682-690.
- Hirsch, R. L., D. G. McKay, R. I. Travers, and R. K. Skraly. 1964. Hyperlipidemia, fatty liver, and bromsulphophthalein retention in rabbits injected intravenously with bacterial endotoxins. *J. Lipid Res.* 5:563-568.
- Hodin, C. M., K. Lenaerts, J. Grootjans, J. J. de Haan, M. Hadfoune, F. K. Verheyen, H. Kiyama, E. Heineman, and W. A. Buurman. 2011. Starvation compromises Paneth cells. *Am. J. Pathol.* 179:2885-2893.
- Holowaychuk, M. K., A. J. Birkenheuer, J. Li, H. Marr, A. Boll, and S. K. Nordone. 2012. Hypocalcemia and hypovitaminosis D in dogs with induced endotoxemia. *J. Vet. Intern. Med.* 26:244-251.

- Holt, P. R., S. Wu, and K. Y. Yeh. 1986. Ileal hyperplastic response to starvation in the rat. *Am. J. Physiol.* 251:G124-131.
- Hosseinpour, M., H. Ehteram, M. Farhadi, and S. Behdad. 2012. Evaluation of dextrose water, black tea and orange juice on histopathologic recovery of surgery-induced intestinal damage in rabbits. *Trauma Mon.* 17:275-278.
- Howe, K. L., C. Reardon, A. Wang, A. Nazli, and D. M. McKay. 2005. Transforming growth factor-beta regulation of epithelial tight junction proteins enhances barrier function and blocks enterohemorrhagic *Escherichia coli* O157:H7-induced increased permeability. *Am. J. Pathol.* 167:1587-1597.
- Hu, X. F., Y. M. Guo, B. Y. Huang, S. Bun, L. B. Zhang, J. H. Li, D. Liu, F. Y. Long, X. Yang, and P. Jiao. 2010. The effect of glucagon-like peptide 2 injection on performance, small intestinal morphology, and nutrient transporter expression of stressed broiler chickens. *Poult. Sci.* 89:1967-1974.
- Huang, C., P. Song, P. Fan, C. Hou, P. Thacker, and X. Ma. 2015. Dietary Sodium Butyrate Decreases Postweaning Diarrhea by Modulating Intestinal Permeability and Changing the Bacterial Communities in Weaned Piglets. *J. Nutr.* 145:2774-2780.
- Hume, D. A., J. L. Radik, E. Ferber, and M. J. Weidemann. 1978. Aerobic glycolysis and lymphocyte transformation. *Biochem. J.* 174:703-709.
- Huntley, N. F., C. M. Nyachoti, and J. F. Patience. 2017. Immune system stimulation increases nursery pig maintenance energy requirements. *J. Anim. Sci* 95(E-Suppl. 5):145 (Abstr.).
- Hyslop, P. A., P. C. May, J. E. Audia, D. O. Calligaro, C. L. McMillian, C. O. Garner, J. W. Cramer, B. D. Gitter, W. J. Porter, and J. S. Nissen. 2004. Reduction in A-Beta 1-40 and A β -Beta 1-42 in CSF and plasma in the beagle dog following acute oral dosing of the gamma-secretase inhibitor, LY450139. *Neurobiol. Aging.* 25(Suppl. 2):S147. (Abstr.).
- Ibeagha-Awemu, E. M., J. W. Lee, A. E. Ibeagha, D. D. Bannerman, M. J. Paape, and X. Zhao. 2008. Bacterial lipopolysaccharide induces increased expression of toll-like receptor (TLR) 4 and downstream TLR signaling molecules in bovine mammary epithelial cells. *Vet. Res.* 39:11.
- Ikenouchi, J., M. Furuse, K. Furuse, H. Sasaki, and S. Tsukita. 2005. Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. *J. Cell Biol.* 171:939-945.

- Ilan, Y. 2012. Leaky gut and the liver: a role for bacterial translocation in nonalcoholic steatohepatitis. *World J. Gastroenterol.* 18:2609-2618.
- Intiyaz, H. Z. and M. C. Simon. 2010. Hypoxia-inducible factors as essential regulators of inflammation. *Curr. Top. Microbiol. Immunol.* 345:105-120.
- Infantino, V., P. Convertini, L. Cucci, M. A. Panaro, M. A. Di Noia, R. Calvello, F. Palmieri, and V. Iacobazzi. 2011. The mitochondrial citrate carrier: a new player in inflammation. *Biochem. J.* 438:433-436.
- Ingvarstsen, K. L., H. R. Andersen, and J. Foldager. 1992. Effect of sex and pregnancy on feed intake capacity of growing cattle. *Acta. Agric. Scand., Sect. A, Anim. Sci.* 42:40-46.
- Ingvarstsen, K. L. and J. B. Andersen. 2000. Integration of metabolism and intake regulation: a review focusing on periparturient animals. *J. Dairy Sci.* 83:1573-1597.
- Inoue, O. J., D. E. Freeman, and M. Wallig. 1998. Effects of hypochlorous acid and ascorbic acid on conductance, permeability, and structure of equine colonic mucosa in vitro. *Am. J. Vet. Res.* 59:82-87.
- Iseri, V. J. and K. C. Klasing. 2013. Dynamics of the systemic components of the chicken (*Gallus domesticus*) immune system following activation by *Escherichia coli*; implications for the costs of immunity. *Dev. Comp. Immunol.* 40:248-257.
- Jack, R. S., X. Fan, M. Bernheiden, G. Rune, M. Ehlers, A. Weber, G. Kirsch, R. Mentel, B. Fürll, M. Freudenberg, G. Schmitz, F. Stelter, and C. Schütt. 1997. Lipopolysaccharide-binding protein is required to combat a murine gram-negative bacterial infection. *Nature* 389:742-745.
- Jacob, A. I., P. K. Goldberg, N. Bloom, G. A. Degenshein, and P. J. Kozinn. 1977. Endotoxin and bacteria in portal blood. *Gastroenterology* 72:1268-1270.
- Jacobsen, S., P. H. Andersen, T. Toelboell, and P. M. Heegaard. 2004. Dose dependency and individual variability of the lipopolysaccharide-induced bovine acute phase protein response. *J. Dairy Sci.* 87:3330-3339.
- Jacobsen, S., T. Toelboell, and P. H. Andersen. 2005. Dose dependency and individual variability in selected clinical, haematological and blood biochemical responses after systemic lipopolysaccharide challenge in cattle. *Vet. Res.* 36:167-178.
- Jafarpour, N., M. Khorvash, H. R. Rahmani, A. Pezeshki, and M. Hosseini Ghaffari. 2015. Dose-responses of zinc-methionine supplements on growth, blood

- metabolites and gastrointestinal development in sheep. *J. Anim. Physiol. Anim. Nutr. (Berl)* 99:668-675.
- Jantsch, J., D. Chakravorty, N. Turza, A. T. Prechtel, B. Buchholz, R. G. Gerlach, M. Volke, J. Gläsner, C. Warnecke, M. S. Wiesener, K. U. Eckardt, A. Steinkasserer, M. Hensel, and C. Willam. 2008. Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function. *J. Immunol.* 180:4697-4705.
- Jing, L., R. Zhang, Y. Liu, W. Zhu, and S. Mao. 2014. Intravenous lipopolysaccharide challenge alters ruminal bacterial microbiota and disrupts ruminal metabolism in dairy cattle. *Br. J. Nutr.* 112:170-182.
- Johansson, M. E., D. Ambort, T. Pelaseyed, A. Schütte, J. K. Gustafsson, A. Ermund, D. B. Subramani, J. M. Holmén-Larsson, K. A. Thomsson, J. H. Bergström, S. van der Post, A. M. Rodriguez-Piñero, H. Sjövall, M. Bäckström, and G. C. Hansson. 2011. Composition and functional role of the mucus layers in the intestine. *Cell. Mol. Life Sci.* 68:3635-3641.
- Johnson, R. W. 2012. Fueling the immune response: what's the cost? Pages 211-223 in *Feed efficiency in swine*. John Patience, ed. Wageningen Academic Publishers, The Netherlands.
- Kadaoui, K. A. and B. Corthésy. 2007. Secretory IgA mediates bacterial translocation to dendritic cells in mouse Peyer's patches with restriction to mucosal compartment. *J. Immunol.* 179:7751-7757.
- Kahl, S. and T. H. Elsasser. 2006. Exogenous testosterone modulates tumor necrosis factor-alpha and acute phase protein responses to repeated endotoxin challenge in steers. *Domest. Anim. Endocrinol.* 31:301-311.
- Kaminski, M. V., H. A. Neufeld, and J. G. Pace. 1979. Effect of inflammatory and noninflammatory stress on plasma ketone bodies and free fatty acids and on glucagon and insulin in peripheral and portal blood. *Inflammation* 3:289-294.
- Katz, S. S., Y. Weinrauch, R. S. Munford, P. Elsbach, and J. Weiss. 1999. Deacylation of lipopolysaccharide in whole *Escherichia coli* during destruction by cellular and extracellular components of a rabbit peritoneal inflammatory exudate. *J. Biol. Chem.* 274:36579-36584.
- Kaufmann, R. L., C. F. Matson, and W. R. Beisel. 1976. Hypertriglyceridemia produced by endotoxin: role of impaired triglyceride disposal mechanisms. *J. Infect. Dis.* 133:548-555.

- Kawai, T., O. Adachi, T. Ogawa, K. Takeda, and S. Akira. 1999. Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* 11:115-122.
- Kehrli, M. E., J. P. Goff, M. G. Stevens, and T. C. Boone. 1991. Effects of granulocyte colony-stimulating factor administration to periparturient cows on neutrophils and bacterial shedding. *J. Dairy Sci.* 74:2448-2458.
- Kellett, D. N. 1966. 2-Deoxyglucos and inflammation. *J. Pharm. Pharmacol.* 18:199-200.
- Kelly, B. and L. A. O'Neill. 2015. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res.* 25:771-784.
- Khafipour, E., Krause, D.O., Plaizier, J.C., 2009a. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. *J. Dairy Sci.* 92:1060-1070.
- Khafipour, E., Krause, D.O., Plaizier, J.C., 2009b. Alfalfa pellet-induced subacute ruminal acidosis in dairy cows increases bacterial endotoxin in the rumen without causing inflammation. *J. Dairy Sci.* 94:1712-1724.
- Kim, J. W., I. Tchernyshyov, G. L. Semenza, and C. V. Dang. 2006. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab.* 3:177-185.
- Kimura, K., J. P. Goff, and M. E. Kehrli. 1999. Effects of the presence of the mammary gland on expression of neutrophil adhesion molecules and myeloperoxidase activity in periparturient dairy cows. *J. Dairy Sci.* 82:2385-2392.
- Kimura, K., J. P. Goff, M. E. Kehrli, and T. A. Reinhardt. 2002. Decreased neutrophil function as a cause of retained placenta in dairy cattle. *J. Dairy Sci.* 85:544-550.
- Kimura, K., J. P. Goff, P. Canning, C. Wang, and J. A. Roth. 2014. Effect of recombinant bovine granulocyte colony-stimulating factor covalently bound to polyethylene glycol injection on neutrophil number and function in periparturient dairy cows. *J. Dairy Sci.* 97:4842-4851.
- Kisielinski, K., S. Willis, A. Prescher, B. Klosterhalfen, and V. Schumpelick. 2002. A simple new method to calculate small intestine absorptive surface in the rat. *Clin. Exp. Med.* 2:131-135.
- Kitchens, R. L. and P. A. Thompson. 2005. Modulatory effects of sCD14 and LBP on LPS-host cell interactions. *J. Endotoxin Res.* 11:225-229.
- Klasing, K. C. and R. E. Austic. 1984a. Changes in protein degradation in chickens due to an inflammatory challenge. *Proc. Soc. Exp. Biol. Med.* 176:292-296.

- Klasing, K. C. and R. E. Austic. 1984b. Changes in protein synthesis due to an inflammatory challenge. *Proc. Soc. Exp. Biol. Med.* 176:285-291.
- Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. Noordhuizen. 2003. Subacute ruminal acidosis (SARA): a review. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* 50:406-414.
- Kleen, J. L., L. Upgang, and J. Rehage. 2013. Prevalence and consequences of subacute ruminal acidosis in German dairy herds. *Acta. Vet. Scand.* 55:48.
- Kleppe, B. B., R. J. Aiello, R. R. Grummer, and L. E. Armentano. 1988. Triglyceride accumulation and very low density lipoprotein secretion by rat and goat hepatocytes in vitro. *J. Dairy Sci.* 71:1813-1822.
- Kluciński, W., A. Degórski, E. Miernik-Degórska, S. Targowski, and A. Winnicka. 1988. Effect of ketone bodies on the phagocytic activity of bovine milk macrophages and polymorphonuclear leukocytes. *Zentralbl. Veterinarmed. A* 35:632-639.
- Kolaczowska, E. and P. Kubes. 2013. Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.* 13:159-175.
- Koppenol, W. H., P. L. Bounds, and C. V. Dang. 2011. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat. Rev. Cancer* 11:325-337.
- Krawczyk, C. M., T. Holowka, J. Sun, J. Blagih, E. Amiel, R. J. DeBerardinis, J. R. Cross, E. Jung, C. B. Thompson, R. G. Jones, and E. J. Pearce. 2010. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 115:4742-4749.
- Kronfeld, D. S. 1982. Major metabolic determinants of milk volume, mammary efficiency, and spontaneous ketosis in dairy cows. *J. Dairy Sci.* 65:2204-2212.
- Kumar, P., Q. Luo, T. J. Vickers, A. Sheikh, W. G. Lewis, and J. M. Fleckenstein. 2014. EatA, an immunogenic protective antigen of enterotoxigenic *Escherichia coli*, degrades intestinal mucin. *Infect. Immun.* 82:500-508.
- Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard. 2016a. Technical note: A procedure to estimate glucose requirements of an activated immune system in steers. *J. Anim. Sci.* 94:4591-4599.
- Kvidera, S. K., E. A. Horst, M. Al-Qaisi, M. J. Dickson, R. P. Rhoads and L. H. Baumgard. 2016b. Leaky Gut's Contribution to Inefficient Nutrient Utilization.

Pages 20-26 in Four-State Dairy Nutrition and Management Conference Proc.,
Dubuque, IA.

- Kvidera, S. K., E. A. Horst, E. J. Laughlin, E. J. Mayorga, J. T. Seibert, M. Abuajamieh, M. Al-Qaisi, S. Lei, A. F. Keating, J. W. Ross, and L. H. Baumgard. 2016c. Effects of Repeated Lipopolysaccharide Infusion on Hematologic, Metabolic, and Intestinal Histology Parameters. *FASEB J.* 30:925.14.
- Kvidera, S. K., E. A. Horst, M. Abuajamieh, E. J. Mayorga, M. V. Sanz Fernandez, and L. H. Baumgard. 2017a. Glucose requirements of an activated immune system in lactating Holstein cows. *J. Dairy Sci.* 100:2360-2374.
- Kvidera, S. K., M. J. Dickson, M. Abuajamieh, D. B. Snider, M. V. Sanz Fernandez, J. S. Johnson, A. F. Keating, P. J. Gordon, H. B. Green, K. M. Schoenberg, and L. H. Baumgard. 2017b. Intentionally induced intestinal barrier dysfunction causes inflammation, affects metabolism, and reduces productivity in lactating Holstein cows. *J. Dairy Sci.* (In Press). doi: <http://doi.org/10.3168/jds.2016-12349>.
- Laboisse, C., A. Jarry, J. E. Branka, D. Merlin, C. Bou-Hanna, and G. Vallette. 1996. Recent aspects of the regulation of intestinal mucus secretion. *Proc. Nutr. Soc.* 55:259-264.
- Lacetera, N., D. Scalia, O. Franci, U. Bernabucci, B. Ronchi, and A. Nardone. 2004. Short communication: effects of nonesterified fatty acids on lymphocyte function in dairy heifers. *J. Dairy Sci.* 87:1012-1014.
- Lallès, J. P. 2014. Intestinal alkaline phosphatase: novel functions and protective effects. *Nutr. Rev.* 72:82-94.
- Lambert, G. P., C. V. Gisolfi, D. J. Berg, P. L. Moseley, L. W. Oberley, and K. C. Kregel. 2002. Selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. *J. Appl. Physiol.* 92:1750-1761; discussion 1749.
- Lamping, N., R. Dettmer, N. W. Schröder, D. Pfeil, W. Hallatschek, R. Burger, and R. R. Schumann. 1998. LPS-binding protein protects mice from septic shock caused by LPS or gram-negative bacteria. *J. Clin. Invest.* 101:2065-2071.
- Lang, C. H., G. J. Bagby, and J. J. Spitzer. 1985. Glucose kinetics and body temperature after lethal and nonlethal doses of endotoxin. *Am. J. Physiol.* 248:R471-478.
- Lang, C. H., G. J. Bagby, H. L. Blakesley, and J. J. Spitzer. 1987a. Fever is not responsible for the elevated glucose kinetics in sepsis. *Proc. Soc. Exp. Biol. Med.* 185:455-461.

- Lang, C. H., G. J. Bagby, A. Z. Buday, and J. J. Spitzer. 1987b. The contribution of gluconeogenesis to glycogen repletion during glucose infusion in endotoxemia. *Metabolism* 36:180-187.
- Lang, C. H., C. Dobrescu, and K. Mészáros. 1990. Insulin-mediated glucose uptake by individual tissues during sepsis. *Metabolism* 39:1096-1107.
- Lang, C. H. and C. Dobrescu. 1991. Sepsis-induced increases in glucose uptake by macrophage-rich tissues persist during hypoglycemia. *Metabolism* 40:585-593.
- Lang, C. H., Z. Spolarics, A. Ottlakan, and J. J. Spitzer. 1993. Effect of high-dose endotoxin on glucose production and utilization. *Metabolism* 42:1351-1358.
- Lanz, T. A., M. J. Karmilowicz, K. M. Wood, N. Pozdnyakov, P. Du, M. A. Piotrowski, T. M. Brown, C. E. Nolan, K. E. Richter, J. E. Finley, Q. Fei, C. F. Ebbinghaus, Y. L. Chen, D. K. Spracklin, B. Tate, K. F. Geoghegan, L. F. Lau, D. D. Auperin, and J. B. Schachter. 2006. Concentration-dependent modulation of amyloid-beta in vivo and in vitro using the gamma-secretase inhibitor, LY-450139. *J. Pharmacol. Exp. Ther.* 319:924-933.
- Lanza-Jacoby, S. and A. Tabares. 1990. Triglyceride kinetics, tissue lipoprotein lipase, and liver lipogenesis in septic rats. *Am. J. Physiol.* 258:E678-685.
- Lanza-Jacoby, S., H. Phetteplace, N. Sedkova, and G. Knee. 1998. Sequential alterations in tissue lipoprotein lipase, triglyceride secretion rates, and serum tumor necrosis factor alpha during *Escherichia coli* bacteremic sepsis in relation to the development of hypertriglyceridemia. *Shock* 9:46-51.
- Lean, I. J., H. M. Golder, and M. B. Hall. 2014. Feeding, evaluating, and controlling rumen function. *Vet. Clin. North Am. Food Anim. Pract.* 30:539-575.
- Le Floc'h, N., C. Knudsen, T. Gidenne, L. Montagne, E. Merlot, and O. Zemb. 2014. Impact of feed restriction on health, digestion and faecal microbiota of growing pigs housed in good or poor hygiene conditions. *Animal* 8:1632-1642.
- Lee, S. J., J. Lee, K. K. Li, D. Holland, H. Maughan, D. S. Guttman, B. Yusta, and D. J. Drucker. 2012. Disruption of the murine Glp2r impairs Paneth cell function and increases susceptibility to small bowel enteritis. *Endocrinology* 153:1141-1151.
- Leininger, M. T., C. P. Portocarrero, A. P. Schinckel, M. E. Spurlock, C. A. Bidwell, J. N. Nielsen, and K. L. Houseknecht. 2000. Physiological response to acute endotoxemia in swine: effect of genotype on energy metabolites and leptin. *Domest. Anim. Endocrinol.* 18:71-82.

- Leithead, J. A., N. Rajoriya, B. K. Gunson, and J. W. Ferguson. 2015. Neutrophil-to-lymphocyte ratio predicts mortality in patients listed for liver transplantation. *Liver Int.* 35:502-509.
- Leon, L. R. 2007. Heat stroke and cytokines. *Prog. Brain Res.* 162:481-524.
- Lérias, J. R., R. Peña, L. E. Hernández-Castellano, J. Capote, N. Castro, A. Argüello, S. S. Araújo, Y. Saco, A. Bassols, and A. M. Almeida. 2015. Establishment of the biochemical and endocrine blood profiles in the Majorera and Palmera dairy goat breeds: the effect of feed restriction. *J. Dairy Res.* 82:416-425.
- Lessard, M., N. Gagnon, D. L. Godson, and H. V. Petit. 2004. Influence of parturition and diets enriched in n-3 or n-6 polyunsaturated fatty acids on immune response of dairy cows during the transition period. *J. Dairy Sci.* 87:2197-2210.
- Levels, J. H., J. A. Marquart, P. R. Abraham, A. E. van den Ende, H. O. Molhuizen, S. J. van Deventer, and J. C. Meijers. 2005. Lipopolysaccharide is transferred from high-density to low-density lipoproteins by lipopolysaccharide-binding protein and phospholipid transfer protein. *Infect. Immun.* 73:2321-2326.
- Li, P., Y. L. Yin, D. Li, S. W. Kim, and G. Wu. 2007. Amino acids and immune function. *Br. J. Nutr.* 98:237-252.
- Li, S., E. Khafipour, D. O. Krause, A. Kroeker, J. C. Rodriguez-Lecompte, G. N. Gozho, and J. C. Plaizier. 2012. Effects of subacute ruminal acidosis challenges on fermentation and endotoxins in the rumen and hindgut of dairy cows. *J. Dairy Sci.* 95:294-303.
- Li, X. and M. A. Atkinson. 2015. The role for gut permeability in the pathogenesis of type 1 diabetes--a solid or leaky concept? *Pediatr. Diabetes* 16:485-492.
- Liang, H., S. E. Hussey, A. Sanchez-Avila, P. Tantiwong, and N. Musi. 2013. Effect of lipopolysaccharide on inflammation and insulin action in human muscle. *PLoS ONE* 8:e63983.
- Lin, Y., H. Lee, A. H. Berg, M. P. Lisanti, L. Shapiro, and P. E. Scherer. 2000. The lipopolysaccharide-activated toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes. *J. Biol. Chem.* 275:24255-24263.
- Ling, P. R., B. R. Bistrain, B. Mendez, and N. W. Istfan. 1994. Effects of systemic infusions of endotoxin, tumor necrosis factor, and interleukin-1 on glucose metabolism in the rat: relationship to endogenous glucose production and peripheral tissue glucose uptake. *Metabolism* 43:279-284.

- Ling, P. R., R. J. Smith, S. Kie, P. Boyce, and B. R. Bistrian. 2004. Effects of protein malnutrition on IL-6-mediated signaling in the liver and the systemic acute-phase response in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287:R801-808.
- Liu, F., J. J. Cottrell, J. B. Furness, L. R. Rivera, F. W. Kelly, U. Wijesiriwardana, R. V. Pustovit, L. J. Fothergill, D. M. Bravo, P. Celi, B. J. Leury, N. K. Gabler, and F. R. Dunshea. 2016. Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs. *Exp. Physiol.* 101:801-810.
- Lochmiller, R. and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *OIKOS* 88:87-98.
- Lohuis, J. A., J. H. Verheijden, C. Burvenich, and A. S. van Miert. 1988a. Pathophysiological effects of endotoxins in ruminants. 1. Changes in body temperature and reticulo-rumen motility, and the effect of repeated administration. *Vet. Q.* 10:109-116.
- Lohuis, J. A., J. H. Verheijden, C. Burvenich, and A. S. van Miert. 1988b. Pathophysiological effects of endotoxins in ruminants. 2. Metabolic aspects. *Vet. Q.* 10:117-125.
- Long, C. L., J. M. Kinney, and J. W. Geiger. 1976. Nonsuppressability of gluconeogenesis by glucose in septic patients. *Metabolism* 25:193-201.
- Lu, M., M. Zhang, R. L. Kitchens, S. Fosmire, A. Takashima, and R. S. Munford. 2003. Stimulus-dependent deacylation of bacterial lipopolysaccharide by dendritic cells. *J. Exp. Med.* 197:1745-1754.
- Lu, M., M. Zhang, A. Takashima, J. Weiss, M. A. Apicella, X. H. Li, D. Yuan, and R. S. Munford. 2005. Lipopolysaccharide deacylation by an endogenous lipase controls innate antibody responses to Gram-negative bacteria. *Nat. Immunol.* 6:989-994.
- Lu, C. W., S. C. Lin, K. F. Chen, Y. Y. Lai, and S. J. Tsai. 2008a. Induction of pyruvate dehydrogenase kinase-3 by hypoxia-inducible factor-1 promotes metabolic switch and drug resistance. *J. Biol. Chem.* 283:28106-28114.
- Lu, Y. C., Yeh, W. C., and P.S. Ohashi. 2008b. LPS/TLR4 signal transduction pathway. *Cytokine* 42:145-151.
- Ludvigsson, J. F., P. Elfström, U. Broomé, A. Ekblom, and S. M. Montgomery. 2007. Celiac disease and risk of liver disease: a general population-based study. *Clin. Gastroenterol. Hepatol.* 5:63-69.e61.

- Lunt, S. Y. and M. G. Vander Heiden. 2011. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu. Rev. Cell Dev. Biol.* 27:441-464.
- Ma, T. Y., M. A. Boivin, D. Ye, A. Pedram, and H. M. Said. 2005. Mechanism of TNF- α modulation of Caco-2 intestinal epithelial tight junction barrier: role of myosin light-chain kinase protein expression. *Am. J. Physiol. Gastrointest. Liver Physiol.* 288:G422-430.
- Madsen, K. L., S. A. Lewis, M. M. Tavernini, J. Hibbard, and R. N. Fedorak. 1997. Interleukin 10 prevents cytokine-induced disruption of T84 monolayer barrier integrity and limits chloride secretion. *Gastroenterology* 113:151-159.
- MacIver, N. J., S. R. Jacobs, H. L. Wieman, J. A. Wofford, J. L. Coloff, and J. C. Rathmell. 2008. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J. Leukoc. Biol.* 84:949-957.
- Maitra, S. R., M. M. Wojnar, and C. H. Lang. 2000. Alterations in tissue glucose uptake during the hyperglycemic and hypoglycemic phases of sepsis. *Shock* 13:379-385.
- Malm, J., E. A. Nordahl, A. Bjartell, O. E. Sørensen, B. Frohm, M. A. Dentener, and A. Egesten. 2005. Lipopolysaccharide-binding protein is produced in the epididymis and associated with spermatozoa and prostasomes. *J. Reprod. Immunol.* 66:33-43.
- Mani, V., T. E. Weber, L. H. Baumgard, and N. K. Gabler. 2012. Growth and Development Symposium: Endotoxin, inflammation, and intestinal function in livestock. *J. Anim. Sci.* 90:1452-1465.
- Manley, P. N., J. B. Ancsin, and R. Kisilevsky. 2006. Rapid recycling of cholesterol: the joint biologic role of C-reactive protein and serum amyloid A. *Med. Hypotheses* 66:784-792.
- Mantis, N. J. and S. J. Forbes. 2010. Secretory IgA: arresting microbial pathogens at epithelial borders. *Immunol. Invest.* 39:383-406.
- Maratou, E., G. Dimitriadis, A. Kollias, E. Boutati, V. Lambadiari, P. Mitrou, and S. A. Raptis. 2007. Glucose transporter expression on the plasma membrane of resting and activated white blood cells. *Eur. J. Clin. Invest.* 37:282-290.
- Marchesi, J. R., D. H. Adams, F. Fava, G. D. Hermes, G. M. Hirschfield, G. Hold, M. N. Quraishi, J. Kinross, H. Smidt, K. M. Tuohy, L. V. Thomas, E. G. Zoetendal, and A. Hart. 2016. The gut microbiota and host health: a new clinical frontier. *Gut* 65:330-339.

- Marques, R. S., R. F. Cooke, C. L. Francisco, and D. W. Bohnert. 2012. Effects of twenty-four hour transport or twenty-four hour feed and water deprivation on physiologic and performance responses of feeder cattle. *J. Anim. Sci.* 90:5040-5046.
- Martel, C. A., L. K. Mamedova, J. E. Minton, M. L. Jones, J. A. Carroll, and B. J. Bradford. 2014. Continuous low-dose infusion of tumor necrosis factor alpha in adipose tissue elevates adipose tissue interleukin 10 abundance and fails to alter metabolism in lactating dairy cows. *J. Dairy Sci.* 97:4897-4906.
- Martín-Padura, I., S. Lostaglio, M. Schneemann, L. Williams, M. Romano, P. Fruscella, C. Panzeri, A. Stoppacciaro, L. Ruco, A. Villa, D. Simmons, and E. Dejana. 1998. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J. Cell Biol.* 142:117-127.
- Matsunaga, T. and A. Rahman. 2001. In search of the origin of the thymus: the thymus and GALT may be evolutionarily related. *Scand. J. Immunol.* 53:1-6.
- May, P., Z. Yang, W. Li, P. Hyslop, E. Siemers, E. Boggs, and L. Boggs. 2004. Multi-compartmental pharmacodynamic assessment of the functional gamma-secretase inhibitor LY450139 in PDAPP transgenic and nontransgenic mice. *Neurobiol. Aging.* 25(Suppl. 2):S65. (Abstr.).
- Maynard, M. A. and M. Ohh. 2007. The role of hypoxia-inducible factors in cancer. *Cell. Mol. Life Sci.* 64:2170-2180.
- McCarthy, M. M., S. Mann, D. V. Nydam, T. R. Overton, and J. A. McArt. 2015. Short communication: concentrations of nonesterified fatty acids and β -hydroxybutyrate in dairy cows are not well correlated during the transition period. *J. Dairy Sci.* 98:6284-6290.
- McCauley, H. A. and G. Guasch. 2015. Three cheers for the goblet cell: maintaining homeostasis in mucosal epithelia. *Trends Mol. Med.* 21:492-503.
- McCullough, F. S., C. A. Northrop-Clewes, and D. I. Thurnham. 1999. The effect of vitamin A on epithelial integrity. *Proc. Nutr. Soc.* 58:289-293.
- McGowan, C. E., P. Jones, M. D. Long, and A. S. Barritt. 2012. Changing shape of disease: nonalcoholic fatty liver disease in Crohn's disease-a case series and review of the literature. *Inflamm. Bowel Dis.* 18:49-54.
- McGuinness, O. P. 1994. The impact of infection on gluconeogenesis in the conscious dog. *Shock* 2:336-343.

- McGuinness, O. P. 2005. Defective glucose homeostasis during infection. *Annu. Rev. Nutr.* 25:9-35.
- Memon, R. A., K. R. Feingold, A. H. Moser, W. Doerrler, S. Adi, C. A. Dinarello, and C. Grunfeld. 1992. Differential effects of interleukin-1 and tumor necrosis factor on ketogenesis. *Am. J. Physiol.* 263:E301-309.
- Mészáros, K., J. Bojta, A. P. Bautista, C. H. Lang, and J. J. Spitzer. 1991. Glucose utilization by Kupffer cells, endothelial cells, and granulocytes in endotoxemic rat liver. *Am. J. Physiol.* 260:G7-12.
- Michaeli, B., A. Martinez, J. P. Revely, M. C. Cayeux, R. L. Chioléro, L. Tappy, and M. M. Berger. 2012. Effects of endotoxin on lactate metabolism in humans. *Crit. Care* 16:R139.
- Milano, J., J. McKay, C. Dagenais, L. Foster-Brown, F. Pognan, R. Gadiant, R. T. Jacobs, A. Zacco, B. Greenberg, and P. J. Ciaccio. 2004. Modulation of notch processing by gamma-secretase inhibitors causes intestinal goblet cell metaplasia and induction of genes known to specify gut secretory lineage differentiation. *Toxicol. Sci.* 82:341-358.
- Mills, C. D. 2012. M1 and M2 Macrophages: Oracles of Health and Disease. *Crit. Rev. Immunol.* 32:463-488.
- Minuti, A., S. Ahmed, E. Trevisi, F. Piccioli-Cappelli, G. Bertoni, N. Jahan, and P. Bani. 2014. Experimental acute rumen acidosis in sheep: consequences on clinical, rumen, and gastrointestinal permeability conditions and blood chemistry. *J. Anim. Sci.* 92:3966-3977.
- Moeser, A. J., C. V. Klok, K. A. Ryan, J. G. Wooten, D. Little, V. L. Cook, and A. T. Blikslager. 2007. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292:G173-181.
- Morel, D. W., P. E. DiCorleto, and G. M. Chisolm. 1986. Modulation of endotoxin-induced endothelial cell toxicity by low density lipoprotein. *Lab. Invest.* 55:419-426.
- Morey, S. D., L. K. Mamedova, D. E. Anderson, C. K. Armendariz, E. C. Titgemeyer, and B. J. Bradford. 2011. Effects of encapsulated niacin on metabolism and production of periparturient dairy cows. *J. Dairy Sci.* 94:5090-5104.
- Moyes, K. M., T. Larsen, P. Sørensen, and K. L. Ingvarsen. 2014. Changes in various metabolic parameters in blood and milk during experimental *Escherichia coli*

- mastitis for primiparous Holstein dairy cows during early lactation. *J. Anim. Sci. Biotechnol.* 5:47.
- Mulligan, K. X., R. T. Morris, Y. F. Otero, D. H. Wasserman, and O. P. McGuinness. 2012. Disassociation of muscle insulin signaling and insulin-stimulated glucose uptake during endotoxemia. *PLoS ONE* 7:e30160.
- Munford, R. S. and C. L. Hall. 1986. Detoxification of bacterial lipopolysaccharides (endotoxins) by a human neutrophil enzyme. *Science* 234:203-205.
- Munford, R. S. 2005. Detoxifying endotoxin: time, place and person. *J. Endotoxin Res.* 11:69-84.
- Munoz, C., J. Carlet, C. Fitting, B. Misset, J. P. Blériot, and J. M. Cavaillon. 1991. Dysregulation of in vitro cytokine production by monocytes during sepsis. *J. Clin. Invest.* 88:1747-1754.
- Murphy, E. A., J. M. Davis, and M. D. Carmichael. 2010. Immune modulating effects of β -glucan. *Curr. Opin. Clin. Nutr. Metab. Care* 13:656-661.
- Murphy, K. 2012. *Janeway's Immunobiology*. 8th ed. Garland Science, New York, NY.
- Myers, M. J., D. E. Farrell, C. M. Evoke-Clover, M. W. McDonald, N. C. Steele. 1997. Effect of growth hormone or chromium picolinate on swine metabolism and inflammatory cytokine production after endotoxin challenge exposure. *Am. J. Vet. Res.* 58:594-600.
- Nagaraja, T. G. and M. M. Chengappa. 1998. Liver abscesses in feedlot cattle: a review. *J. Anim. Sci.* 76:287-298.
- Nakashima, R. A., M. G. Paggi, and P. L. Pedersen. 1984. Contributions of glycolysis and oxidative phosphorylation to adenosine 5'-triphosphate production in AS-30D hepatoma cells. *Cancer Res.* 44:5702-5706.
- National Research Council. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Naylor, J. M. and D. S. Kronfeld. 1985. In vivo studies of hypoglycemia and lactic acidosis in endotoxic shock. *Am. J. Physiol.* 248:E309-316.
- Naylor, J. M. and D. S. Kronfeld. 1986. Relationships between metabolic changes and clinical signs in pregnant sheep given endotoxin. *Can. J. Vet. Res.* 50:402-409.

- Neufeld, H. A., J. A. Pace, and F. E. White. 1976. The effect of bacterial infections on ketone concentrations in rat liver and blood and on free fatty acid concentrations in rat blood. *Metabolism* 25:877-884.
- Neufeld, H. A., J. G. Pace, M. V. Kaminski, D. T. George, P. B. Jahrling, R. W. Wannemacher, and W. R. Beisel. 1980. A probable endocrine basis for the depression of ketone bodies during infectious or inflammatory state in rats. *Endocrinology* 107:596-601.
- Newsholme, P., R. Curi, S. Gordon, and E. A. Newsholme. 1986. Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. *Biochem. J.* 239:121-125.
- Nguyen, A. T., S. Mandard, C. Dray, V. Deckert, P. Valet, P. Besnard, D. J. Drucker, L. Lagrost, and J. Grober. 2014. Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: involvement of the GLP-1 pathway. *Diabetes* 63:471-482.
- Nolan, J. P. 1975. The role of endotoxin in liver injury. *Gastroenterology* 69:1346-1356.
- Nonnecke, B. J., S. T. Franklin, and J. W. Young. 1992. Effects of ketones, acetate, and glucose on in vitro immunoglobulin secretion by bovine lymphocytes. *J. Dairy Sci.* 75:982-990.
- Nordlund, K.V., Garrett, E.F., and G. R. Oetzel. 1995. Herd-based rumenocentesis: a clinical approach to the diagnosis of subacute rumen acidosis in dairy herds. *Compendium Contin. Educ. Pract. Vet.* 17: S48-56.
- O'Boyle, N. J., G. A. Contreras, S. A. Mattmiller, and L. M. Sordillo. 2012. Changes in glucose transporter expression in monocytes of periparturient dairy cows. *J. Dairy Sci.* 95:5709-5719.
- Obata, Y., D. Takahashi, M. Ebisawa, K. Kakiguchi, S. Yonemura, T. Jinnohara, T. Kanaya, Y. Fujimura, M. Ohmae, K. Hase, and H. Ohno. 2012. Epithelial cell-intrinsic Notch signaling plays an essential role in the maintenance of gut immune homeostasis. *J. Immunol.* 188:2427-2436.
- Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. *Vet. Clin. North Am. Food Anim. Pract.* 20:651-674.
- Oh, S. K., N. Pavlotsky, and A. I. Tauber. 1990. Specific binding of haptoglobin to human neutrophils and its functional consequences. *J. Leukoc. Biol.* 47:142-148.

- Okamoto, R., K. Tsuchiya, Y. Nemoto, J. Akiyama, T. Nakamura, T. Kanai, and M. Watanabe. 2009. Requirement of Notch activation during regeneration of the intestinal epithelia. *Am. J. Physiol. Gastrointest. Liver Physiol.* 296:G23-35.
- Owens, F. N., D. S. Secrist, W. J. Hill, and D. R. Gill. 1998. Acidosis in cattle: a review. *J. Anim. Sci.* 76:275-286.
- Owens, F. N., R. A. Zinn, and Y. K. Kim. 1986. Limits to starch digestion in the ruminant small intestine. *J. Anim. Sci.* 63:1634-1648.
- Owen, O. E., S. C. Kalhan, and R. W. Hanson. 2002. The key role of anaplerosis and cataplerosis for citric acid cycle function. *J. Biol. Chem.* 277:30409-30412.
- Oztürk, C., D. Avlan, I. Cinel, L. Cinel, A. Unlü, H. Camdeviren, U. Atik, and U. Oral. 2002. Selenium pretreatment prevents bacterial translocation in rat intestinal ischemia/reperfusion model. *Pharmacol. Res.* 46:171-175.
- Palsson-McDermott, E. M. and L. A. O'Neill. 2013. The Warburg effect then and now: from cancer to inflammatory diseases. *Bioessays* 35:965-973.
- Papandreou, I., R. A. Cairns, L. Fontana, A. L. Lim, and N. C. Denko. 2006. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab.* 3:187-197.
- Pearce, S. C., V. Mani, T. E. Weber, R. P. Rhoads, J. F. Patience, L. H. Baumgard, and N. K. Gabler. 2013a. Heat stress and reduced plane of nutrition decreases intestinal integrity and function in pigs. *J. Anim. Sci.* 91:5183-5193.
- Pearce, E. L., M. C. Poffenberger, C. H. Chang, and R. G. Jones. 2013b. Fueling immunity: insights into metabolism and lymphocyte function. *Science* 342:1242454.
- Pearce, S. C., M. V. Sanz Fernandez, J. Torrison, M. E. Wilson, L. H. Baumgard, and N. K. Gabler. 2015. Dietary organic zinc attenuates heat stress-induced changes in pig intestinal integrity and metabolism. *J. Anim. Sci.* 93:4702-4713.
- Pennington, R. J. 1952. The metabolism of short-chain fatty acids in the sheep. I. Fatty acid utilization and ketone body production by rumen epithelium and other tissues. *Biochem. J.* 51:251-258.
- Peterson, J. M., K. D. Feedback, J. H. Baas, and F. X. Pizza. 2006. Tumor necrosis factor- α promotes the accumulation of neutrophils and macrophages in skeletal muscle. *J. Appl. Physiol.* 101:1394-1399.

- Pillon, N. J., P. J. Bilan, L. N. Fink, and A. Klip. 2013. Cross-talk between skeletal muscle and immune cells: muscle-derived mediators and metabolic implications. *Am. J. Physiol. Endocrinol. Metab.* 304:E453-465.
- Pithon-Curi, T. C., M. P. De Melo, and R. Curi. 2004. Glucose and glutamine utilization by rat lymphocytes, monocytes and neutrophils in culture: a comparative study. *Cell. Biochem. Funct.* 22:321-326.
- Plank, L. D., A. B. Connolly, and G. L. Hill. 1998. Sequential changes in the metabolic response in severely septic patients during the first 23 days after the onset of peritonitis. *Ann. Surg.* 228:146-158.
- Playford, R. J., D. N. Floyd, C. E. Macdonald, D. P. Calnan, R. O. Adenekan, W. Johnson, R. A. Goodlad, and T. Marchbank. 1999. Bovine colostrum is a health food supplement which prevents NSAID induced gut damage. *Gut* 44:653-658.
- Plessers, E., H. Wyns, A. Watteyn, B. Pardon, P. De Backer, and S. Croubels. 2015. Characterization of an intravenous lipopolysaccharide inflammation model in calves with respect to the acute-phase response. *Vet. Immunol. Immunopathol.* 163:46-56.
- Poggi, M., D. Bastelica, P. Gual, M. A. Iglesias, T. Gremeaux, C. Knauf, F. Peiretti, M. Verdier, I. Juhan-Vague, J. F. Tanti, R. Burcelin, and M. C. Alessi. 2007. C3H/HeJ mice carrying a toll-like receptor 4 mutation are protected against the development of insulin resistance in white adipose tissue in response to a high-fat diet. *Diabetologia* 50:1267-1276.
- Proudfoot, K., S.J. LeBlanc, D. Weary, B. Bradford, L. Mamedova, N. von Keyserlingk. 2014. A competitive and unpredictable feeding environment precalving increases inflammation and endometritis in Holstein dairy cows. *J. Dairy Sci.* 97(E-Suppl. 1):51. (Abstr.).
- Prosser, C., K. Stelwagen, R. Cummins, P. Guerin, N. Gill, and C. Milne. 2004. Reduction in heat-induced gastrointestinal hyperpermeability in rats by bovine colostrum and goat milk powders. *J. Appl. Physiol.* 96:650-654.
- Raina, N., J. Matsui, S. C. Cunnane, and K. N. Jeejeebhoy. 1995. Effect of tumor necrosis factor-alpha on triglyceride and phospholipid content and fatty acid composition of liver and carcass in rats. *Lipids* 30:713-718.
- Raymond, R. M., J. M. Harkema, and T. E. Emerson. 1981. In vivo skeletal muscle insulin resistance during E coli endotoxin shock in the dog. *Circ. Shock* 8:425-433.

- Read, T. E., C. Grunfeld, Z. Kumwenda, M. C. Calhoun, J. P. Kane, K. R. Feingold, and J. H. Rapp. 1995. Triglyceride-rich lipoproteins improve survival when given after endotoxin in rats. *Surgery* 117:62-67.
- Redel, B. K., A. N. Brown, L. D. Spate, K. M. Whitworth, J. A. Green, and R. S. Prather. 2012. Glycolysis in preimplantation development is partially controlled by the Warburg Effect. *Mol. Reprod. Dev.* 79:262-271.
- Reeds, P. J., C. R. Fjeld, and F. Jahoor. 1994. Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? *J. Nutr.* 124:906-910.
- Reeds, P. J. and F. Jahoor. 2001. The amino acid requirements of disease. *Clin. Nutr.* (Suppl. 1):15–22.
- Reid, M., A. Badaloo, T. Forrester, J. F. Morlese, W. C. Heird, and F. Jahoor. 2002. The acute-phase protein response to infection in edematous and nonedematous protein-energy malnutrition. *Am. J. Clin. Nutr.* 76:1409-1415.
- Renner, L., S. Kersten, A. Duevel, H. J. Schuberth, and S. Dänicke. 2013. Effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic acid, linoleic acid, phytanic acid and the combination of various fatty acids on proliferation and cytokine expression of bovine peripheral blood mononuclear cells. *Nutrients* 5:2667-2683.
- Rensen, P. C., M. Oosten, E. Bilt, M. Eck, J. Kuiper, and T. J. Berkel. 1997. Human recombinant apolipoprotein E redirects lipopolysaccharide from Kupffer cells to liver parenchymal cells in rats in vivo. *J. Clin. Invest.* 99:2438-2445.
- Reynolds, C. K. 2006. Production and metabolic effects of site of starch digestion in dairy cattle. *Anim. Feed Sci. Technol.* 130:78–94.
- Rhoads, R. P., J. W. Kim, B. J. Leury, L. H. Baumgard, N. Segole, S. J. Frank, D. E. Bauman, and Y. R. Boisclair. 2004. Insulin increases the abundance of the growth hormone receptor in liver and adipose tissue of periparturient dairy cows. *J. Nutr.* 134:1020–1027.
- Rhoads, M., R. Rhoads, M. VanBaale, R. Collier, S. Sanders, W. Weber, B. Crooker, and L. Baumgard. 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. *J. Dairy Sci.* 92:1986-1997.
- Richardson, R. P., C. D. Rhyne, Y. Fong, D. G. Hesse, K. J. Tracey, M. A. Marano, S. F. Lowry, A. C. Antonacci, and S. E. Calvano. 1989. Peripheral blood leukocyte kinetics following in vivo lipopolysaccharide (LPS) administration to normal

- human subjects. Influence of elicited hormones and cytokines. *Ann. Surg.* 210:239-245.
- Rodriguez, P., N. Darmon, P. Chappuis, C. Candalh, M. A. Blaton, C. Bouchaud, and M. Heyman. 1996. Intestinal paracellular permeability during malnutrition in guinea pigs: effect of high dietary zinc. *Gut* 39:416-422.
- Rollwagen, F. M., S. Madhavan, A. Singh, Y. Y. Li, K. Wolcott, and R. Maheshwari. 2006. IL-6 protects enterocytes from hypoxia-induced apoptosis by induction of bcl-2 mRNA and reduction of fas mRNA. *Biochem. Biophys. Res. Commun.* 347:1094-1098.
- Rose, R., A. Banerjee, and S. K. Ramaiah. 2007. Characterization of a lipopolysaccharide mediated neutrophilic hepatitis model in Sprague Dawley rats. *J. Appl. Toxicol.* 27:602-611.
- Rubic, T., G. Lametschwandtner, S. Jost, S. Hinteregger, J. Kund, N. Carballido-Perrig, C. Schwärzler, T. Junt, H. Voshol, J. G. Meingassner, X. Mao, G. Werner, A. Rot, and J. M. Carballido. 2008. Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. *Nat. Immunol.* 9:1261-1269.
- Russell, D. H., J. C. Barreto, K. Klemm, and T. A. Miller. 1995. Hemorrhagic shock increases gut macromolecular permeability in the rat. *Shock* 4:50-55.
- Sadagopan, N., W. Li, S. L. Roberds, T. Major, G. M. Preston, Y. Yu, and M. A. Tones. 2007. Circulating succinate is elevated in rodent models of hypertension and metabolic disease. *Am. J. Hypertens.* 20:1209-1215.
- Sanz Fernandez, M. V., S. C. Pearce, N. K. Gabler, J. F. Patience, M. E. Wilson, M. T. Socha, J. L. Torrison, R. P. Rhoads, and L. H. Baumgard. 2014. Effects of supplemental zinc amino acid complex on gut integrity in heat-stressed growing pigs. *Animal* 8:43-50.
- Sanz Fernandez, M. V., J. S. Johnson, M. Abuajamieh, S. K. Stoakes, J. T. Seibert, L. Cox, S. Kahl, T. H. Elsasser, J. W. Ross, S. C. Isom, R. P. Rhoads, and L. H. Baumgard. 2015. Effects of heat stress on carbohydrate and lipid metabolism in growing pigs. *Physiol. Rep.* 3:e12315.
- Sbarra, A. J. and M. L. Karnovsky. 1959. The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. *J. Biol. Chem.* 234:1355-1362.
- Schmidt, W. E., E. G. Siegel, and W. Creutzfeldt. 1985. Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. *Diabetologia* 28:704-707.

- Schroedl, W., B. Fuerll, P. Reinhold, M. Krueger, and C. Schuett. 2001. A novel acute phase marker in cattle: lipopolysaccharide binding protein (LBP). *J. Endotoxin Res.* 7:49-52.
- Schulz, K., J. Frahm, S. Kersten, U. Meyer, D. Reiche, H. Sauerwein, and S. Dänicke. 2015. Effects of elevated parameters of subclinical ketosis on the immune system of dairy cows: in vivo and in vitro results. *Arch. Anim. Nutr.* 69:113-127.
- Seeley, J. J. and S. Ghosh. 2017. Molecular mechanisms of innate memory and tolerance to LPS. *J. Leukoc. Biol.* 101:107-119.
- Selak, M. A., S. M. Armour, E. D. MacKenzie, H. Boulahbel, D. G. Watson, K. D. Mansfield, Y. Pan, M. C. Simon, C. B. Thompson, and E. Gottlieb. 2005. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell* 7:77-85.
- Semenza, G. L., P. H. Roth, H. M. Fang, and G. L. Wang. 1994. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J. Biol. Chem.* 269:23757-23763.
- Seshagiri, P. B. and B. D. Bavister. 1991. Glucose and phosphate inhibit respiration and oxidative metabolism in cultured hamster eight-cell embryos: evidence for the Crabtree effect. *Mol. Reprod. Dev.* 30:105-111.
- Shah, C., R. Hari-Dass, and J. G. Raynes. 2006. Serum amyloid A is an innate immune opsonin for Gram-negative bacteria. *Blood* 108:1751-1757.
- Sheldon, I. M., E. J. Williams, A. N. Miller, D. M. Nash, and S. Herath. 2008. Uterine diseases in cattle after parturition. *Vet. J.* 176:115-121.
- Sherman, P., J. Forstner, N. Roomi, I. Khatri, and G. Forstner. 1985. Mucin depletion in the intestine of malnourished rats. *Am. J. Physiol.* 248:G418-423.
- Shi, D. W., J. Zhang, H. N. Jiang, C. Y. Tong, G. R. Gu, Y. Ji, H. Summah, and J. M. Qu. 2011. LPS pretreatment ameliorates multiple organ injuries and improves survival in a murine model of polymicrobial sepsis. *Inflamm. Res.* 60:841-849.
- Shigemura, H., N. Ishiguro, and Y. Inoshima. 2014. Up-regulation of MUC2 mucin expression by serum amyloid A3 protein in mouse colonic epithelial cells. *J. Vet. Med. Sci.* 76:985-991.
- Shimizu, F., C. R. Kahn, C. Garzelli, J. J. Hooks, and A. L. Notkins. 1983. The binding of insulin to mouse leucocytes during viral infections. *Diabetologia* 25:521-524.

- Shu, X. L., T. T. Yu, K. Kang, and J. Zhao. 2016. Effects of glutamine on markers of intestinal inflammatory response and mucosal permeability in abdominal surgery patients: A meta-analysis. *Exp. Ther. Med.* 12:3499-3506.
- Shulzhenko, N., A. Morgun, W. Hsiao, M. Battle, M. Yao, O. Gavrilova, M. Orandle, L. Mayer, A. J. Macpherson, K. D. McCoy, C. Fraser-Liggett, and P. Matzinger. 2011. Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. *Nat. Med.* 17:1585-1593.
- Shuster, D. E., R. J. Harmon, J. A. Jackson, and R. W. Hemken. 1991. Reduced lactational performance following intravenous endotoxin administration to dairy cows. *J. Dairy Sci.* 74:3407-3411.
- Siemers, E. R., R. A. Dean, S. Friedrich, L. Ferguson-Sells, C. Gonzales, M. R. Farlow, and P. C. May. 2007. Safety, tolerability, and effects on plasma and cerebrospinal fluid amyloid-beta after inhibition of gamma-secretase. *Clin. Neuropharmacol.* 30:317-325.
- Siemers, E., M. Skinner, R. A. Dean, C. Gonzales, J. Satterwhite, M. Farlow, D. Ness, and P. C. May. 2005. Safety, tolerability, and changes in amyloid beta concentrations after administration of a gamma-secretase inhibitor in volunteers. *Clin. Neuropharmacol.* 28:126-132.
- Siemers, E. R., J. F. Quinn, J. Kaye, M. R. Farlow, A. Porsteinsson, P. Tariot, P. Zoulnouni, J. E. Galvin, D. M. Holtzman, D. S. Knopman, J. Satterwhite, C. Gonzales, R. A. Dean, and P. C. May. 2006. Effects of a gamma-secretase inhibitor in a randomized study of patients with Alzheimer disease. *Neurology* 66:602-604.
- Singh, P. K., S. Y. Wise, E. J. Ducey, O. O. Fatanmi, T. B. Elliott, and V. K. Singh. 2012. α -Tocopherol succinate protects mice against radiation-induced gastrointestinal injury. *Radiat. Res.* 177:133-145.
- Skidmore, A., A. Brand, and C. Sniffen. 1996. Monitoring milk production: Defining preset targets and execution. Pages 223-262 in *Herd Health and Production Management in Dairy Practice*. A. Brand, J Noordhuizen, and Y Schukken, ed. Wageningen Academic Publishers, Wageningen, Netherlands.
- Slack, E., S. Hapfelmeier, B. Stecher, Y. Velykoredko, M. Stoel, M. A. Lawson, M. B. Geuking, B. Beutler, T. F. Tedder, W. D. Hardt, P. Bercik, E. F. Verdu, K. D. McCoy, and A. J. Macpherson. 2009. Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* 325:617-620.
- Slonczewski, J. L. and J. W. Foster. 2011. *Microbiology: An Evolving Science*. 2nd ed. W.W. Norton & Company, Inc., New York, NY.

- Smith, H. A. 1944. Ulcerative lesions of the bovine rumen and their possible relation to hepatic abscesses. *Am. J. Vet. Res.* 5:234-242.
- Snavely, M. D., M. G. Ziegler, and P. A. Insel. 1985. Subtype-selective down-regulation of rat renal cortical alpha- and beta-adrenergic receptors by catecholamines. *Endocrinology* 117:2182-2189.
- Song, M. J., K. H. Kim, J. M. Yoon, and J. B. Kim. 2006. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. *Biochem. Biophys. Res. Commun.* 346:739-745.
- Song, Z. H., K. Xiao, Y. L. Ke, I. F. Jiao, and C. H. Hu. 2015. Zinc oxide influences mitogen-activated protein kinase and TGF- β 1 signaling pathways, and enhances intestinal barrier integrity in weaned pigs. *Innate Immun.* 21:341-348.
- Southorn, B. G. and J. R. Thompson. 1986. Time course changes in blood metabolites during endotoxin fever in sheep. *Can. J. Vet. Res.* 50:374-379.
- Spitzer, J. A., K. M. Nelson, and R. E. Fish. 1985. Time course of changes in gluconeogenesis from various precursors in chronically endotoxemic rats. *Metabolism* 34:842-849.
- Spolarics, Z., G. J. Bagby, C. H. Lang, and J. J. Spitzer. 1991a. Up-regulation of glucose metabolism in Kupffer cells following infusion of tumour necrosis factor. *Biochem. J.* 278:515-519.
- Spolarics, Z., A. P. Bautista, and J. J. Spitzer. 1991b. Metabolic response of isolated liver cells to in vivo phagocytic challenge. *Hepatology* 13:277-281.
- Spolarics, Z., A. Schuler, G. J. Bagby, C. H. Lang, K. Mészáros, and J. J. Spitzer. 1991c. Tumor necrosis factor increases in vivo glucose uptake in hepatic nonparenchymal cells. *J. Leukoc. Biol.* 49:309-312.
- Spolarics, Z., A. Ottlakán, C. H. Lang, and J. J. Spitzer. 1992a. Kupffer cells play a major role in insulin-mediated hepatic glucose uptake in vivo. *Biochem. Biophys. Res. Commun.* 186:455-460.
- Spolarics, Z., A. Schuler, G. J. Bagby, C. H. Lang, S. Nelson, and J. J. Spitzer. 1992b. In vivo metabolic response of hepatic nonparenchymal cells and leukocytes to granulocyte-macrophage colony-stimulating factor. *J. Leukoc. Biol.* 51:360-365.
- St. Pierre, N., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. *J. Dairy Sci.* 86:E52-E77.

- Steele, M. A., J. Croom, M. Kahler, O. AlZahal, S. E. Hook, K. Plaizier, and B. W. McBride. 2011. Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300:R1515-1523.
- Steele, M. A., G. B. Penner, F. Chaucheyras-Durand, and I. L. Guan. 2016. Development and physiology of the rumen and the lower gut: Targets for improving gut health. *J. Dairy Sci.* 99:4955-4966.
- Stefańska, B., W. Nowak, J. Komisarek, M. Taciak, M. Barszcz, and J. Skomiał. 2016. Prevalence and consequence of subacute ruminal acidosis in Polish dairy herds. *J. Anim. Physiol. Anim. Nutr. (Berl.)*. <https://doi.org/10.1111/jpn.12592>
- Steiger, M., M. Senn, G. Altreuther, D. Werling, F. Sutter, M. Kreuzer, and W. Langhans. 1999. Effect of a prolonged low-dose lipopolysaccharide infusion on feed intake and metabolism in heifers. *J. Anim. Sci.* 77:2523-2532.
- Stevens, J. R., R. P. McMillan, J. T. Resendes, S. K. Lloyd, M. M. Ali, M. I. Frisard, S. Hargett, S. R. Keller, and M. W. Hulver. 2017. Acute low-dose endotoxin treatment results in improved whole-body glucose homeostasis in mice. *Metabolism.* 68:150-162.
- Stoakes, S. K., E. A. Nolan, M. Abuajamieh, M. V. Sanz Fernandez, and L. H. Baumgard. 2015a. Estimating glucose requirements of an activated immune system in growing pigs. *J. Anim. Sci.* 93(E-Suppl. S3):634. (Abstr.).
- Stoakes, S. K. E. A. Nolan, D. J. Valko, M. Abuajamieh, J. T. Seibert, M. V. Sanz Fernandez, P. J. Gorden, H. B. Green, K. M. Schoenberg, W. E. Trout, and L. H. Baumgard. 2015b. Characterizing the effect of feed restriction on biomarkers of leaky gut. *J. Dairy Sci.* 98(E-Suppl. 2):274. (Abstr.).
- Strnad, P., F. Tacke, A. Koch, and C. Trautwein. 2017. Liver - guardian, modifier and target of sepsis. *Nat. Rev. Gastroenterol. Hepatol.* 14:55-66.
- Strom, T. B., R. A. Bear, and C. B. Carpenter. 1975. Insulin-induced augmentation of lymphocyte-mediated cytotoxicity. *Science* 187:1206-1208.
- Suzuki, T., N. Yoshinaga, and S. Tanabe. 2011. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. *J. Biol. Chem.* 286:31263-31271.
- Suzuki, T. 2013. Regulation of intestinal epithelial permeability by tight junctions. *Cell. Mol. Life Sci.* 70:631-659.

- Swanson, J. C., and J. Morrow-Tesch. 2001. Cattle transport: Historical, research, and future perspectives. *J. Anim. Sci.* 79:E102-E109.
- Sweeney, J. S. 1928. Effect of toxemia on tolerance for dextrose and on the action of insulin. *Arch. Intern. Med.* 41:420-427
- Takeyama, N., Y. Itoh, Y. Kitazawa, and T. Tanaka. 1990. Altered hepatic mitochondrial fatty acid oxidation and ketogenesis in endotoxic rats. *Am. J. Physiol.* 259:E498-505.
- Tannahill, G. M. and L. A. O'Neill. 2011. The emerging role of metabolic regulation in the functioning of Toll-like receptors and the NOD-like receptor Nlrp3. *FEBS Lett.* 585:1568-1572.
- Tannahill, G. M., A. M. Curtis, J. Adamik, E. M. Palsson-McDermott, A. F. McGettrick, G. Goel, C. Frezza, N. J. Bernard, B. Kelly, N. H. Foley, L. Zheng, A. Gardet, Z. Tong, S. S. Jany, S. C. Corr, M. Haneklaus, B. E. Caffrey, K. Pierce, S. Walmsley, F. C. Beasley, E. Cummins, V. Nizet, M. Whyte, C. T. Taylor, H. Lin, S. L. Masters, E. Gottlieb, V. P. Kelly, C. Clish, P. E. Auron, R. J. Xavier, and L. A. O'Neill. 2013. Succinate is an inflammatory signal that induces IL-1 β through HIF-1 α . *Nature* 496:238-242.
- Targowski, S. P. and W. Klucinski. 1983. Reduction in mitogenic response of bovine lymphocytes by ketone bodies. *Am. J. Vet. Res.* 44:828-830.
- Taudorf, S., K. S. Krabbe, R. M. Berg, B. K. Pedersen, and K. Møller. 2007. Human models of low-grade inflammation: bolus versus continuous infusion of endotoxin. *Clin. Vaccine Immunol.* 14:250-255.
- Taylor-Edwards, C. C., D. G. Burrin, J. C. Matthews, K. R. McLeod, J. J. Holst, and D. L. Harmon. 2010. Expression of mRNA for proglucagon and glucagon-like peptide-2 (GLP-2) receptor in the ruminant gastrointestinal tract and the influence of energy intake. *Domest. Anim. Endocrinol.* 39:181-193.
- Taylor-Edwards, C. C., D. G. Burrin, J. J. Holst, K. R. McLeod, and D. L. Harmon. 2011. Glucagon-like peptide-2 (GLP-2) increases small intestinal blood flow and mucosal growth in ruminating calves. *J. Dairy Sci.* 94:888-898.
- Tennant, B., M. Reina-Guerra, and D. Harrold. 1973. Metabolic response of calves following acute experimental endotoxemia. *Ann. Rech. Veter.* 4:135-147.
- Thomas, P., H. Hayashi, D. Lazure, P. A. Burke, O. Bajenova, A. Ganguly, and R. A. Forse. 2008. Inhibition of lipopolysaccharide activation of Kupffer cells by transition metals. *J. Surg. Res.* 148:116-120.

- Thurnham, D. I., C. A. Northrop-Clewes, F. S. McCullough, B. S. Das, and P. G. Lunn. 2000. Innate immunity, gut integrity, and vitamin A in Gambian and Indian infants. *J. Infect. Dis.* 182:S23-28.
- Thymann, T., I. Le Huërou-Luron, Y. M. Petersen, M. S. Hedemann, J. Elinf, B. B. Jensen, J. J. Holst, B. Hartmann, and P. T. Sangild. 2014. Glucagon-like peptide 2 treatment may improve intestinal adaptation during weaning. *J. Anim. Sci.* 92:2070-2079.
- Tobias, P. S., K. Soldau, and R. J. Ulevitch. 1986. Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. *J. Exp. Med.* 164:777-793.
- Toribio, R. E., C. W. Kohn, J. Hardy, and T. J. Rosol. 2005. Alterations in serum parathyroid hormone and electrolyte concentrations and urinary excretion of electrolytes in horses with induced endotoxemia. *J. Vet. Intern. Med.* 19:223-231.
- Tůmová, E., Z. Volek, D. Chodová, H. Härtlová, P. Makovický, J. Svobodová, T. A. Ebeid, and L. Uhlířová. 2016. The effect of 1-week feed restriction on performance, digestibility of nutrients and digestive system development in the growing rabbit. *Animal* 10:1-9.
- Tsuji, K. and S. J. Harrison. 1978. Dry-heat destruction of lipopolysaccharide: dry-heat destruction kinetics. *Appl. Environ. Microbiol.* 36:710-714.
- Ueno, P. M., R. B. Oriá, E. A. Maier, M. Guedes, O. G. de Azevedo, D. Wu, T. Willson, S. P. Hogan, A. A. Lima, R. L. Guerrant, D. B. Polk, L. A. Denson, and S. R. Moore. 2011. Alanine-glutamine promotes intestinal epithelial cell homeostasis in vitro and in a murine model of weanling undernutrition. *Am. J. Physiol. Gastrointest. Liver Physiol.* 301:G612-622.
- Valko, M., D. Leibfritz, J. Moncol, M. T. Cronin, M. Mazur, and J. Telser. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* 39:44-84.
- Van Amersfoort, E. S., T. J. Van Berkel, and J. Kuiper. 2003. Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock. *Clin. Microbiol. Rev.* 16:379-414.
- van der Heijden, P. J., W. Stok, and A. T. Bianchi. 1987b. Contribution of immunoglobulin-secreting cells in the murine small intestine to the total 'background' immunoglobulin production. *Immunology* 62:551-555.
- van der Hulst, R. R., B. K. van Kreel, M. F. von Meyenfeldt, R. J. Brummer, J. W. Arends, N. E. Deutz, and P. B. Soeters. 1993. Glutamine and the preservation of gut integrity. *Lancet* 341:1363-1365.

- van der Hulst, R. R., M. F. von Meyenfeldt, B. K. van Kreel, F. B. Thunnissen, R. J. Brummer, J. W. Arends, and P. B. Soeters. 1998. Gut permeability, intestinal morphology, and nutritional depletion. *Nutrition* 14:1-6.
- Van der Sluis, M., B. A. De Koning, A. C. De Bruijn, A. Velcich, J. P. Meijerink, J. B. Van Goudoever, H. A. Büller, J. Dekker, I. Van Seuning, I. B. Renes, and A. W. Einerhand. 2006. Muc2-deficient mice spontaneously develop colitis, indicating that MUC2 is critical for colonic protection. *Gastroenterology* 131:117-129.
- van Es, J. H., M. E. van Gijn, O. Riccio, M. van den Born, M. Vooijs, H. Begthel, M. Cozijnsen, S. Robine, D. J. Winton, F. Radtke, and H. Clevers. 2005. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* 435:959-963.
- van Langevelde, P., K. M. Kwappenberg, P. H. Groeneveld, H. Mattie, and J. T. van Dissel. 1998. Antibiotic-induced lipopolysaccharide (LPS) release from *Salmonella typhi*: delay between killing by ceftazidime and imipenem and release of LPS. *Antimicrob. Agents Chemother.* 42:739-743.
- van Miert, A. S. and J. Frens. 1968. The reaction of different animal species to bacterial pyrogens. *Zentralbl. Veterinarmed. A* 15:532-543.
- van Miert, A. S., C. T. van Duin, J. H. Verheijden, and A. J. Schotman. 1983. Staphylococcal enterotoxin B and *Escherichia coli* endotoxin: comparative observations in goats on fever and associated clinical hematologic and blood biochemical changes after intravenous and intramammary administration. *Am. J. Vet. Res.* 44:955-963.
- van Raam, B. J., A. J. Verhoeven, and T. W. Kuijpers. 2006. Mitochondria in neutrophil apoptosis. *Int. J. Hematol.* 84:199-204.
- Vander Heiden, M. G., L. C. Cantley, and C. B. Thompson. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324:1029-1033.
- Vander Heiden, M. G., J. W. Locasale, K. D. Swanson, H. Sharfi, G. J. Heffron, D. Amador-Noguez, H. R. Christofk, G. Wagner, J. D. Rabinowitz, J. M. Asara, and L. C. Cantley. 2010. Evidence for an alternative glycolytic pathway in rapidly proliferating cells. *Science* 329:1492-1499.
- Vanuysel, T., S. van Wanrooy, H. Vanheel, C. Vanormelingen, S. Verschueren, E. Houben, S. Salim Rasoel, J. Tóth, L. Holvoet, R. Farré, L. Van Oudenhove, G. Boeckxstaens, K. Verbeke, and J. Tack. 2014. Psychological stress and

corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. *Gut* 63:1293-1299.

Vatanen, T., A. D. Kostic, E. d'Hennezel, H. Siljander, E. A. Franzosa, M. Yassour, R. Kolde, H. Vlamakis, T. D. Arthur, A. M. Hämäläinen, A. Peet, V. Tillmann, R. Uibo, S. Mokurov, N. Dorshakova, J. Ilonen, S. M. Virtanen, S. J. Szabo, J. A. Porter, H. Lähdesmäki, C. Huttenhower, D. Gevers, T. W. Cullen, M. Knip, R. J. Xavier, and D. S. Group. 2016. Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. *Cell* 165:1551.

Verheijden, J. H., A. S. Van Miert, A. J. Schotman, and C. T. Van Duin. 1983. Pathophysiological aspects of *E. coli* mastitis in ruminants. *Vet. Res. Commun.* 7:229-236.

Vernay, M. C., O. Wellnitz, L. Kreipe, H. A. van Dorland, and R. M. Bruckmaier. 2012. Local and systemic response to intramammary lipopolysaccharide challenge during long-term manipulated plasma glucose and insulin concentrations in dairy cows. *J. Dairy Sci.* 95:2540-2549.

Vernon, R. G. 1992. Effects of diet on lipolysis and its regulation. *Proc. Nutr. Soc.* 51:397-408.

Vives-Pi, M., N. Somoza, J. Fernández-Alvarez, F. Vargas, P. Caro, A. Alba, R. Gomis, M. O. Labeta, and R. Pujol-Borrell. 2003. Evidence of expression of endotoxin receptors CD14, toll-like receptors TLR4 and TLR2 and associated molecule MD-2 and of sensitivity to endotoxin (LPS) in islet beta cells. *Clin. Exp. Immunol.* 133:208-218.

Vreugdenhil, A. C., M. A. Dentener, A. M. Snoek, J. W. Greve, and W. A. Buurman. 1999. Lipopolysaccharide binding protein and serum amyloid A secretion by human intestinal epithelial cells during the acute phase response. *J. Immunol.* 163:2792-2798.

Vreugdenhil, A. C., A. M. Snoek, C. van 't Veer, J. W. Greve, and W. A. Buurman. 2001. LPS-binding protein circulates in association with apoB-containing lipoproteins and enhances endotoxin-LDL/VLDL interaction. *J. Clin. Invest.* 107:225-234.

Vreugdenhil, A. C., C. H. Rousseau, T. Hartung, J. W. Greve, C. van 't Veer, and W. A. Buurman. 2003. Lipopolysaccharide (LPS)-binding protein mediates LPS detoxification by chylomicrons. *J. Immunol.* 170:1399-1405.

Waggoner, J. W., C. A. Löest, C. P. Mathis, D. M. Hallford, and M. K. Petersen. 2009a. Effects of rumen-protected methionine supplementation and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers. *J. Anim. Sci.* 87:681-692.

- Waggoner, J. W., C. A. Löest, J. L. Turner, C. P. Mathis, and D. M. Hallford. 2009b. Effects of dietary protein and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers. *J. Anim. Sci.* 87:3656-3668.
- Waldron, M. R., T. Nishida, B. J. Nonnecke, and T. R. Overton. 2003a. Effect of lipopolysaccharide on indices of peripheral and hepatic metabolism in lactating cows. *J. Dairy Sci.* 86:3447-3459.
- Waldron, M. R., B. J. Nonnecke, T. Nishida, R. L. Horst, and T. R. Overton. 2003b. Effect of lipopolysaccharide infusion on serum macromineral and vitamin D concentrations in dairy cows. *J. Dairy Sci.* 86:3440-3446.
- Waldron, M. R., A. E. Kulick, A. W. Bell, and T. R. Overton. 2006. Acute experimental mastitis is not causal toward the development of energy-related metabolic disorders in early postpartum dairy cows. *J. Dairy Sci.* 89:596-610.
- Walker, M. P., C. M. Evoke-Clover, T. H. Elsasser, and E. E. Connor. 2015. Short communication: Glucagon-like peptide-2 and coccidiosis alter tight junction gene expression in the gastrointestinal tract of dairy calves. *J. Dairy Sci.* 98:3432-3437.
- Wallon, C., P. C. Yang, A. V. Keita, A. C. Ericson, D. M. McKay, P. M. Sherman, M. H. Perdue, and J. D. Söderholm. 2008. Corticotropin-releasing hormone (CRH) regulates macromolecular permeability via mast cells in normal human colonic biopsies in vitro. *Gut* 57:50-58.
- Walrand, S., C. Guillet, Y. Boirie, and M. P. Vasson. 2004. In vivo evidences that insulin regulates human polymorphonuclear neutrophil functions. *J. Leukoc. Biol.* 76:1104-1110.
- Wang, S. C., R. D. Klein, W. L. Wahl, W. H. Alarcon, R. J. Garg, D. G. Remick, and G. L. Su. 1998. Tissue coexpression of LBP and CD14 mRNA in a mouse model of sepsis. *J. Surg. Res.* 76:67-73.
- Wang, R., C. P. Dillon, L. Z. Shi, S. Milasta, R. Carter, D. Finkelstein, L. L. McCormick, P. Fitzgerald, H. Chi, J. Munger, and D. R. Green. 2011. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* 35:871-882.
- Wang, H. B., P. Y. Wang, X. Wang, Y. L. Wan, and Y. C. Liu. 2012. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Dig. Dis. Sci.* 57:3126-3135.

- Wang, B., G. Wu, Z. Zhou, Z. Dai, Y. Sun, Y. Ji, W. Li, W. Wang, C. Liu, F. Han, and Z. Wu. 2015a. Glutamine and intestinal barrier function. *Amino Acids* 47:2143-2154.
- Wang, L. F., G. Q. Yang, S. Yang, G. Y. Yang, M. Li, H. S. Zhu, Y. Y. Wang, L. Q. Han, R. Y. Liu, S. D. Jia, and F. Song. 2015b. Alteration of factors associated with hepatic gluconeogenesis in response to acute lipopolysaccharide in dairy goat. *J. Anim. Sci.* 93:2767-2777.
- Wang, L. F., S. D. Jia, G. Q. Yang, R. Y. Liu, G. Y. Yang, M. Li, H. S. Zhu, Y. Y. Wang, and L. Q. Han. 2017. The effects of acute lipopolysaccharide challenge on dairy goat liver metabolism assessed with (1) HNMR metabonomics. *J. Anim. Physiol. Anim. Nutr. (Berl.)*. 101:180-189.
- Wannemacher, R. W., F. A. Beall, P. G. Canonico, R. E. Dinterman, C. L. Hadick, and H. A. Neufeld. 1980. Glucose and alanine metabolism during bacterial infections in rats and rhesus monkeys. *Metabolism* 29:201-212.
- Warburg, O. 1925. Iron, the oxygen-carrier of respiration-ferment. *Science* 61:575-582.
- Warburg, O., F. Wind, and E. Negelein. 1927. The Metabolism of Tumors in the Body. *J. Gen. Physiol.* 8:519-530.
- Warburg, O., K. Gawehn, and A. W. Geissler. 1958. [Metabolism of leukocytes]. *Z. Naturforsch. B.* 13B:515-516.
- Warden, R. A., R. S. Noltorp, J. L. Francis, P. R. Dunkley, and E. V. O'Loughlin. 1997. Vitamin A deficiency exacerbates methotrexate-induced jejunal injury in rats. *J. Nutr.* 127:770-776.
- Weinert, B. T., C. Schölz, S. A. Wagner, V. Iesmantavicius, D. Su, J. A. Daniel, and C. Choudhary. 2013. Lysine succinylation is a frequently occurring modification in prokaryotes and eukaryotes and extensively overlaps with acetylation. *Cell Rep.* 4:842-851.
- Weisberg, S. P., D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel, and A. W. Ferrante. 2003. Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112:1796-1808.
- Wellnitz, O., C. Zbinden, X. Huang, and R. M. Bruckmaier. 2016. Short communication: Differential loss of bovine mammary epithelial barrier integrity in response to lipopolysaccharide and lipoteichoic acid. *J. Dairy Sci.* 99:4851-4856.

- Welsh, F. K., S. M. Farmery, K. MacLennan, M. B. Sheridan, G. R. Barclay, P. J. Guillou, and J. V. Reynolds. 1998. Gut barrier function in malnourished patients. *Gut* 42:396-401.
- Wenfeng, Z., W. Yakun, M. Di, G. Jianping, W. Chuanxin, and H. Chun. 2014. Kupffer cells: increasingly significant role in nonalcoholic fatty liver disease. *Ann. Hepatol.* 13:489-495.
- Werling, D., F. Sutter, M. Arnold, G. Kun, P. C. Tooten, E. Gruys, M. Kreuzer, and W. Langhans. 1996. Characterisation of the acute phase response of heifers to a prolonged low dose infusion of lipopolysaccharide. *Res. Vet. Sci.* 61:252-257.
- West, J. W., B. G. Mullinix, and T. G. Sandifer. 1991. Changing dietary electrolyte balance for dairy cows in cool and hot environments. *J. Dairy Sci.* 74:1662-1674.
- Wheelock, J., R. Rhoads, M. Vanbaale, S. Sanders, and L. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. *J. Dairy Sci.* 93:644-655.
- Williams, P. N., C. T. Collier, J. A. Carroll, T. H. Welsh, and J. C. Laurenz. 2009. Temporal pattern and effect of sex on lipopolysaccharide-induced stress hormone and cytokine response in pigs. *Domest. Anim. Endocrinol.* 37:139-147.
- Wirtz, S., C. Neufert, B. Weigmann, and M. F. Neurath. 2007. Chemically induced mouse models of intestinal inflammation. *Nat. Protoc.* 2:541-546.
- Wolfe, R. R., D. Elahi, and J. J. Spitzer. 1977. Glucose and lactate kinetics after endotoxin administration in dogs. *Am. J. Physiol.* 232:E180-185.
- Wolfe, R. R. and J. F. Burke. 1978. Effect of glucose infusion on glucose and lactate metabolism in normal and burned guinea pigs. *J Trauma* 18(12):800-805.
- Wong, G. T., D. Manfra, F. M. Poulet, Q. Zhang, H. Josien, T. Bara, L. Engstrom, M. Pinzon-Ortiz, J. S. Fine, H. J. Lee, L. Zhang, G. A. Higgins, and E. M. Parker. 2004. Chronic treatment with the gamma-secretase inhibitor LY-411,575 inhibits beta-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J. Biol. Chem.* 279:12876-12882.
- Wurfel, M. M., S. T. Kunitake, H. Lichenstein, J. P. Kane, and S. D. Wright. 1994. Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. *J. Exp. Med.* 180:1025-1035.
- Xie, S., B. Liu, S. Fu, W. Wang, Y. Yin, N. Li, W. Chen, J. Liu, and D. Liu. 2014. GLP-2 suppresses LPS-induced inflammation in macrophages by inhibiting ERK phosphorylation and NF- κ B activation. *Cell. Physiol. Biochem.* 34:590-602.

- Yamauchi, K., H. Kamisoyama, and Y. Isshiki. 1996. Effects of fasting and refeeding on structures of the intestinal villi and epithelial cells in White Leghorn hens. *Br. Poult. Sci.* 37:909-921.
- Yamashita, H., M. Aoyama-Ishikawa, M. Takahara, C. Yamauchi, T. Inoue, M. Miyoshi, N. Maeshige, M. Usami, A. Nakao, and J. Kotani. 2015. Endogenous interleukin 18 suppresses hyperglycemia and hyperinsulinemia during the acute phase of endotoxemia in mice. *Surg. Infect. (Larchmt.)*. 16:90-96.
- Yan, J. and S. Li. 2014. The role of the liver in sepsis. *Int. Rev. Immunol.* 33:498-510.
- Yates, D. T., C. A. Löest, T. T. Ross, D. M. Hallford, B. H. Carter, and S. W. Limesand. 2011. Effects of bacterial lipopolysaccharide injection on white blood cell counts, hematological variables, and serum glucose, insulin, and cortisol concentrations in ewes fed low- or high-protein diets. *J. Anim. Sci.* 89:4286-4293.
- Ye, R. D. and L. Sun. 2015. Emerging functions of serum amyloid A in inflammation. *J. Leukoc. Biol.* 98:923-929.
- Yu, J., M. I. Ordiz, J. Stauber, N. Shaikh, I. Trehan, E. Barnell, R. D. Head, K. Maleta, P. I. Tarr, and M. J. Manary. 2016. Environmental Enteric Dysfunction Includes a Broad Spectrum of Inflammatory Responses and Epithelial Repair Processes. *Cell. Mol. Gastroenterol. Hepatol.* 2:158-174.e151.
- Yuan, K., J. K. Farney, L. K. Mamedova, L. M. Sordillo, and B. J. Bradford. 2013. TNF α altered inflammatory responses, impaired health and productivity, but did not affect glucose or lipid metabolism in early-lactation dairy cows. *PLoS One* 8:e80316.
- Zarrin, M., O. Wellnitz, H. A. van Dorland, J. J. Gross, and R. M. Bruckmaier. 2014. Hyperketonemia during lipopolysaccharide-induced mastitis affects systemic and local intramammary metabolism in dairy cows. *J. Dairy Sci.* 97:3531-3541.
- Zebeli, Q., K. Ghareeb, E. Humer, B. U. Metzler-Zebeli, and U. Besenfelder. 2015. Nutrition, rumen health and inflammation in the transition period and their role on overall health and fertility in dairy cows. *Res. Vet. Sci.* 103:126-136.
- Zhang, Z., M. Tan, Z. Xie, L. Dai, Y. Chen, and Y. Zhao. 2011. Identification of lysine succinylation as a new post-translational modification. *Nat. Chem. Biol.* 7:58-63.
- Zhang, S., R. I. Albornoz, J. R. Aschenbach, D. R. Barreda, and G. B. Penner. 2013. Short-term feed restriction impairs the absorptive function of the reticulo-rumen and total tract barrier function in beef cattle. *J. Anim. Sci.* 91:1685-1695.

- Zhang, G., D. Hailemariam, E. Dervishi, S. A. Goldansaz, Q. Deng, S. M. Dunn, and B. N. Ametaj. 2016. Dairy cows affected by ketosis show alterations in innate immunity and lipid and carbohydrate metabolism during the dry off period and postpartum. *Res. Vet. Sci.* 107:246-256.
- Ziegler, T. R., R. J. Smith, S. T. O'Dwyer, R. H. Demling, and D. W. Wilmore. 1988. Increased intestinal permeability associated with infection in burn patients. *Arch. Surg.* 123:1313-1319.
- Zweigner, J., H. J. Gramm, O. C. Singer, K. Wegscheider, and R. R. Schumann. 2001. High concentrations of lipopolysaccharide-binding protein in serum of patients with severe sepsis or septic shock inhibit the lipopolysaccharide response in human monocytes. *Blood* 98:3800-3808.

APPENDIX A: ESTIMATING GLUCOSE REQUIREMENTS OF AN ACTIVATED IMMUNE SYSTEM IN GROWING PIGS

An abstract presented in 2015 at the ASAS-ADSA Joint Annual Meeting: J. Anim. Sci. 93(E-Suppl. S3):634.

Abstract

Activated immune cells are obligate glucose utilizers and a large lipopolysaccharide (LPS) IV dose causes severe hypoglycemia. Therefore, study objectives were to use the quantity of glucose needed to maintain euglycemia following an endotoxin challenge as a proxy of immune cell glucose requirement. Fifteen fasted crossbred gilts (30 ± 2 kg) were jugular catheterized bilaterally and assigned one of two IV bolus treatments: control (CON; 10 mL sterile saline; $n=7$) or LPS-infused + euglycemic clamp (LPS-Eu; *E. coli* 055:B5; 5 $\mu\text{g}/\text{kg}$ BW; 50% dextrose infusion to maintain euglycemia; $n=8$). Following infusion, blood glucose was determined every 10 min and dextrose infusion rates were adjusted in LPS-Eu pigs to maintain euglycemia for 8 h. Rectal temperature was increased in LPS-Eu pigs relative to control (39.8 vs 38.8°C , $P<0.01$). After 3 h, blood glucose content gradually declined for CON pigs while LPS-Eu glucose levels remained unchanged ($P=0.01$). Plasma insulin, BUN, BHB, and L-lactate were increased in LPS-Eu pigs compared to CON (69, 57, 21, and 60%, respectively; $P<0.05$). By 8 h, plasma LPS binding protein was increased 24% in LPS-Eu pigs relative to controls ($P<0.01$). Plasma NEFA increased with time in CON pigs, but remained unchanged in the LPS-Eu pigs ($P<0.01$). White blood cells, lymphocytes, monocytes, eosinophils, and basophils were decreased in LPS-Eu pigs

relative to CON ($P<0.01$). Additionally, the neutrophil-to-lymphocyte ratio was increased in LPS-Eu pigs relative to CON (72%, $P<0.01$). During the 8 h, 116 ± 8 g of infused glucose was required to maintain euglycemia. If the amount of glucose required to maintain euglycemia can be used as a proxy, then the glucose requirements of an activated immune system are approximately $1.1 \text{ g/kg BW}^{0.75}/\text{hr}$.

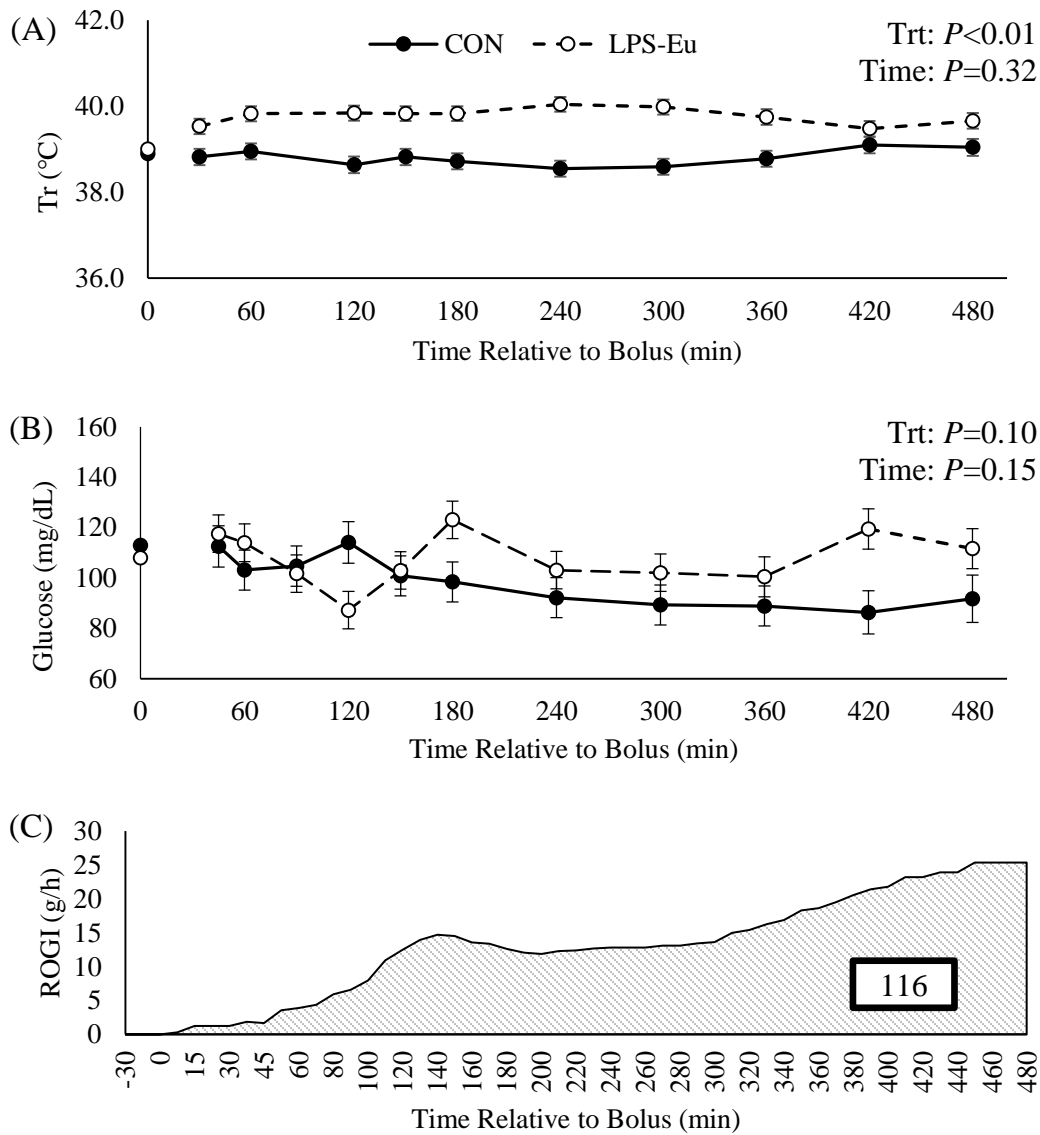


Figure 30. Effects of saline (CON; 10 mL i.v. saline bolus; $n=7$) or lipopolysaccharide infusion + euglycemic clamp (LPS-Eu; 5 $\mu\text{g}/\text{kg}$ BW lipopolysaccharide i.v. bolus; *E. coli* 055:B5 in saline; $n=8$) on (A) rectal temperature (Tr), (B) blood glucose and (C) rate of glucose infusion (ROGI) in fasted growing pigs (30.3 ± 2 kg BW)

Table 15. Effects of saline (CON) or LPS infusion + euglycemic clamp (LPS-Eu) on circulating metabolites and markers of inflammation in fasted growing pigs (30.3 ± 2 kg BW)

Parameter	Trt ¹		SEM	P		
	CON	LPS-Eu		Trt	Time	Trt x Time
Insulin, µg/L	0.04	0.13	0.02	<0.01	<0.01	<0.01
NEFA ² , µEq/L	326	147	22	<0.01	<0.01	<0.01
β-hydroxybutyrate, µmol	61.2	77.6	3.9	0.01	<0.01	0.02
L-lactate, mM	1.3	3.2	0.2	<0.01	<0.01	<0.01
LBP ³ , µg/mL	14.9	16.3	0.5	0.04	0.34	0.27
Blood urea N, mg/dL	7.3	14.0	1.6	0.01	<0.01	<0.01

¹Treatment: CON = 10 mL i.v. sterile saline bolus (n=7); LPS-Eu = 5 µg/kg BW lipopolysaccharide i.v. bolus; *E. coli* 055:B5 in saline (n=8)

²Non-esterified fatty acids

³Lipopolysaccharide binding protein

Table 16. Effects of saline (CON) or LPS infusion + euglycemic clamp (LPS-Eu) on complete blood count parameters in fasted growing pigs (30.3 ± 2 kg BW)

Parameter	Trt ¹		SEM	P		
	CON	LPS-Eu		Trt	Time	Trt x Time
White blood cells, x 10 ³ /µL	14.8	6.7	1.0	<0.01	0.01	0.03
Neutrophils, x 10 ³ /µL	5.69	4.56	0.70	0.29	<0.01	<0.01
Lymphocytes, x 10 ³ /µL	7.72	1.94	0.47	<0.01	0.94	0.71
Monocytes, x 10 ³ /µL	0.92	0.12	0.07	<0.01	0.83	0.58
Eosinophils, x 10 ³ /µL	0.23	0.07	0.03	<0.01	0.63	0.06
Basophils, x 10 ³ /µL	0.04	0.02	0.01	0.05	0.08	0.64
Unidentified cells, x 10 ³ /µL	0.20	0.05	0.03	<0.01	0.70	0.67
Red blood cells, x 10 ⁶ /µL	5.53	6.18	0.07	<0.01	<0.01	<0.01
Hemoglobin, gm/dL	9.77	10.89	0.09	<0.01	<0.01	<0.01
Hematocrit, %	30.84	34.63	0.42	<0.01	<0.01	<0.01
Platelets, x 10 ³ /µL	491	211	21	<0.01	0.39	0.38
Neutrophil:Lymphocytes	0.74	2.60	0.27	<0.01	<0.01	<0.01

¹Treatment: CON = 10 mL i.v. sterile saline bolus (n=7); LPS-Eu = 5 µg/kg BW lipopolysaccharide i.v. bolus; *E. coli* 055:B5 in saline (n=8)

**APPENDIX B: EFFECTS OF REPEATED LIPOPOLYSACCHARIDE INFUSION
ON HEMATOLOGIC, METABOLIC, AND INTESTINAL HISTOLOGY
PARAMETERS**

An abstract presented in 2016 at Experimental Biology: FASEB J. 30:925.14.

Abstract

Objectives were to characterize effects of repeated LPS infusion on circulating metabolites, white blood cells, and intestinal histology in pigs. Ten crossbred female pigs (168 ± 2 kg) were reproductively synchronized and jugular catheterized. The experimental period, during the follicular phase of the estrous cycle, lasted 5 d and pigs were assigned to either control (CON; 3 mL i.v. saline; n=4) or lipopolysaccharide (LPS; 0.1 μ g/kg BW LPS i.v.; *Escherichia coli* 055:B5 Sigma, St. Louis, Missouri; n=6) treatments administered 4x/d at 0000, 0600, 1200, and 1800h. Each animal was limit-fed 2.7 kg of feed once daily at 0600 h. Blood samples for metabolites and a complete blood count were obtained daily at 1730h. Rectal temperatures were measured 2x/d at 0530 and 1730h and condensed into daily averages. Animals were sacrificed on d 6 beginning at 0600h and segments of jejunum, ileum, and colon were collected and analyzed for morphology and were stained for myeloperoxidase (a measure of neutrophil infiltration). Circulating platelets were decreased 31% ($P<0.01$) and total white blood cell count tended to be decreased 10% ($P=0.07$) in LPS relative to CON pigs. Lipopolysaccharide binding protein was increased 37% in LPS pigs relative to CON pigs ($P=0.05$) and was increased 60% on d 1 of the experiment relative to CON pigs ($P=0.02$). There was a treatment by day interaction for

neutrophils, lymphocytes, monocytes, and eosinophils where on d 1 only, neutrophils were increased 58% in LPS pigs relative to CON ($P<0.01$) and lymphocytes, monocytes, and eosinophils were decreased by 61, 93, and 81%, respectively in LPS pigs relative to CON ($P<0.01$ for all). Likewise, treatment by day differences in blood metabolites were observed where on d 1, insulin, blood urea nitrogen, β -hydroxybutyrate, and ionized calcium increased 36, 14, 13, and 7%, respectively, in LPS pigs relative to CON pigs ($P=0.03$, $P=0.07$, $P=0.02$, and $P<0.01$, respectively). There was no treatment by day interaction for blood glucose; however, it tended to be increased 6% in LPS pigs on d 1 relative to controls (84 vs 79 mg/dL; $P=0.08$). Rectal temperature did not differ between the two treatments throughout the entire study (38.5 vs 38.6°C; $P=0.71$). In the jejunum, a tendency for reduced goblet cell area was observed (2.2 vs 5.6%; $P=0.06$) as well as an increase in myeloperoxidase stained area (1.03 vs 0.87%; $P<0.01$) in LPS pigs relative to CON. No other differences in histological measurements were observed. In conclusion, LPS only altered metabolism on d 1 of the 5 d injection period. Platelets were the only measurement consistently affected throughout the 5 d. Jejunum was the only intestinal segment which seemed to be affected by repeated LPS exposure as determined by a tendency for reduced goblet cell area and an increase in myeloperoxidase stained area.

Table 17. Effects of 5-day lipopolysaccharide or saline administration on rectal temperature and circulating metabolic and inflammatory parameters

Parameter	Treatment ¹		SEM	P		
	CON	LPS		Trt	Day	Trt x Day
Glucose, mg/dL	79	84	3	0.33	0.74	0.36
Rectal Temperature, °C	38.5	38.6	0.1	0.71	<0.01	0.68
Insulin, µg/L	0.392	0.519	0.06	0.12	0.20	0.03
NEFA ² , mEq/L	83.1	89.7	14.6	0.76	0.61	0.54
β-hydroxybutyrate, mg/dL	0.69	0.73	0.02	0.25	0.02	0.02
Blood urea nitrogen, mg/dL	9.0	9.6	0.4	0.41	0.02	0.07
L-lactate, µM	2006	1879	53	0.11	0.25	0.08
LBP ³ , µg/mL	12.5	19.9	2.3	0.05	0.01	0.02

¹Treatments: CON = control pigs receiving 3 mL saline i.v. 4x/d for 5 d; LPS = pigs receiving 0.1 µg/kg BW lipopolysaccharide *Escherichia coli* 055:B5 i.v. 4x/d for 5 d

²Non-esterified fatty acids

³Lipopolysaccharide binding protein

Table 18. Effects of 5-day lipopolysaccharide or saline administration on blood gases, hematology, and electrolytes

Parameter	Treatment ¹		SEM	P		
	CON	LPS		Trt	Day	Trt x Day
pH	7.44	7.45	0.01	0.09	<0.01	0.42
CO ₂ partial pressure, mmHg	47.2	44.9	0.6	0.03	<0.01	0.36
O ₂ partial pressure, mmHg	37.7	42.6	1.7	0.07	<0.01	0.78
Base excess, mmol/L	7.9	7.0	0.4	0.17	0.08	0.73
HCO ₃ , mmol/L	32.0	31.0	0.4	0.12	0.01	0.72
Total CO ₂ , mmol/L	33.5	32.4	0.4	0.11	0.01	0.49
O ₂ saturation, %	71.9	78.8	1.6	0.02	<0.01	0.35
Hemoglobin, g/dL	10.3	10.6	0.3	0.53	0.42	0.42
Hematocrit, %	30.4	31.2	0.8	0.52	0.42	0.71
Sodium, mmol/L	142.4	141.6	0.2	0.05	0.06	<0.01
Potassium, mmol/L	4.1	4.1	0.1	0.45	0.34	0.30
Ionized Calcium, mmol/L	1.40	1.39	0.01	0.63	<0.01	<0.01

¹Treatments: CON = control pigs receiving 3 mL saline i.v. 4x/d for 5 d; LPS = pigs receiving 0.1 µg/kg BW lipopolysaccharide *Escherichia coli* 055:B5 i.v. 4x/d for 5 d

Table 19. Effects of 5-day LPS or saline administration on complete blood count parameters

Parameter	Treatment ¹			P		
	CON	LPS	SEM	Treatment	Day	Trt x Day
White blood cells, x 10 ³ /μL	18.5	16.7	0.6	0.07	<0.01	0.14
Neutrophils, x 10 ³ /μL	4.6	5.1	0.4	0.42	<0.01	<0.01
Lymphocytes, x 10 ³ /μL	11.5	10.3	0.7	0.27	<0.01	<0.01
Monocytes, x 10 ³ /μL	0.77	0.56	0.08	0.13	<0.01	<0.01
Eosinophils, x 10 ³ /μL	1.2	0.9	0.1	0.09	0.55	<0.01
Basophils, x 10 ³ /μL	0.08	0.07	0.01	0.74	0.30	0.21
Unidentified cells, x 10 ³ /μL	0.08	0.08	0.01	0.83	0.06	0.54
Red blood cells, x 10 ⁶ /μL	6.1	6.3	0.1	0.18	0.25	0.86
Platelets, x 10 ³ /μL	292	201	20	0.01	<0.01	<0.01
Neutrophils:Lymphocytes	0.42	0.76	0.1	0.09	<0.01	<0.01

¹Treatments: CON = control pigs receiving 3 mL saline i.v. 4x/d for 5 d; LPS = pigs receiving 0.1 μg/kg BW lipopolysaccharide *Escherichia coli* 055:B5 i.v. 4x/d for 5 d

Table 20. Effects of 5-day lipopolysaccharide or saline administration on intestinal morphology

Parameter	Treatment ¹		SEM	P Trt
	CON	LPS		
Jejunum				
Crypt depth, μm	359	306	21	0.12
Villous height, μm	387	410	32	0.62
Villous width, μm	170	177	9	0.60
Goblet cell area, % ²	5.6	2.2	1.0	0.06
Mucosal surface area, M index ³	6.3	5.9	0.3	0.49
MPO Area, % ⁴	0.9	1.0	0.1	<0.01
Ileum				
Crypt depth, μm	165	179	20	0.63
Villous height, μm	330	328	23	0.95
Villous width, μm	134	132	11	0.87
Goblet cell area, % ²	6.3	4.9	1.8	0.61
Mucosal surface area, M index ³	6.4	6.3	0.4	0.92
MPO Area, % ⁴	4.9	5.0	0.3	0.85
Colon				
Goblet cell area, % ²	16.2	13.6	3.0	0.57
MPO Area, % ⁴	0.5	0.4	0.1	0.19

¹Treatments: CON = control pigs receiving 3 mL saline i.v. 4x/d for 5 d; LPS = pigs receiving 0.1 $\mu\text{g}/\text{kg}$ BW lipopolysaccharide *Escherichia coli* 055:B5 i.v. 4x/d for 5 d

²Expressed as a percentage of epithelial area

³M-index = (villous surface + unit bottom – villous bottom)/unit bottom, where villus surface = $\pi \cdot (\text{villous length} \cdot \text{villous width})$, unit bottom = $\pi \cdot (\text{villous width}/2 + \text{crypt width}/2)^2$, and villous bottom = $\pi \cdot (\text{villous width}/2)^2$ as described by Kisielinski et al., 2002.

⁴Percentage of positive myeloperoxidase relative to total stained area

APPENDIX C: EFFECT OF SUPPLEMENTAL CITRULLINE ON THERMAL, PRODUCTION, AND INTESTINAL HEALTH PARAMETERS DURING HEAT STRESS AND NUTRIENT RESTRICTION IN GROWING PIGS

A combination of two abstracts presented in 2016 at the ASAS-ADSA Joint Annual Meeting: J. Anim. Sci. 94(E-Suppl. 5):995 and in 2017 at the ASAS-ADSA Midwest Meeting: J. Anim. Sci. 95(E-Suppl. 2):20.

Abstract

Heat stress (HS) compromises intestinal barrier function, and citrulline improves intestinal health in rodent models. Therefore, objectives were to characterize effects of citrulline supplement (CIT) on physiological and production responses during HS. Supplements were fed twice daily at 0600 and 1800 h and consisted of 20 g of cookie dough without citrulline (CON) or with 0.13 g/kg BW L-citrulline (CIT; 99.3% purity; MP Biomedicals, Santa Ana, CA). Forty crossbred gilts (30 ± 2 kg) were assigned to 1 of 5 supplemental-environmental treatments: 1) thermoneutral (TN; $23.6 \pm 0.1^\circ\text{C}$) *ad libitum* feed (AL) with CON (TNAL; n=8), 2) TN pair-fed (PF) with CON (PF-CON; n=8), 3) TN PF with CIT (PF-CIT; n=8), 4) HS AL with CON (HS-CON; n=8), and 5) HS AL with CIT (HS-CIT; n=8). Acclimation lasted 4 d and all pigs received the CON supplement. During period 1 (P1; 7 d), pigs were kept in TN, fed AL, and fed respective supplemental treatments. During period 2 (P2; 60 h), HS-CON and HS-CIT animals were fed AL and exposed to cyclical HS (33.6 to 38.3°C) while TNAL, PF-CON, and PF-CIT remained in TN and were fed either AL or PF to their HS counterparts to negate the effect of dissimilar nutrient intake.

Feed intake was measured daily and BW was obtained 1 d before P1, d 7 of P1, and at P2 conclusion. Animals were sacrificed following P2 and segments of jejunum, ileum, and colon were collected and analyzed for morphology and were stained for myeloperoxidase (a measure of neutrophil infiltration). Rectal temperature (Tr), skin temperature (Ts), and respiration rate (RR) were obtained once daily at 1800 h during P1 and thrice daily at 0600, 1200, and 1800 h during P2. Pigs exposed to HS had increased Tr (0.8°C), Ts (4.7°C), and RR (47 bpm) relative to TN pigs ($P<0.01$). HS-CIT pigs had decreased RR (7 bpm, $P=0.04$) and a tendency for decreased Tr (0.1°C, $P=0.07$). Feed intake decreased ~16% in HS relative to TNAL pigs ($P<0.01$) and did not differ between HS and PF pigs ($P>0.10$). P2 ADG decreased 11 and 30% in HS and PF pigs, respectively, relative to TNAL pigs. PF-CIT pigs tended to have increased (0.12 kg/d) ADG compared to PF-CON pigs ($P=0.09$). Gain:feed was similar between TNAL and HS pigs but decreased 30% in PF relative to TNAL pigs ($P<0.01$). No effects of CIT on production variables during HS were detected. Exposure to HS decreased both jejunum villus height and mucosal surface area (12 and 15%, respectively; $P<0.01$) with no effect of CIT supplementation. In the ileum, exposure to HS decreased villus height and mucosal surface area (15 and 19 %, respectively $P<0.01$) in HS-CON relative to TNAL pigs, and supplementation with CIT ameliorated these changes ($P<0.01$). Pigs supplemented with CIT during HS had 53% increased circulating LBP concentrations relative to HS-CON ($P=0.03$). There were no treatment differences on neutrophil infiltration in the jejunum. Pigs exposed to HS tended to have increased ileum neutrophil infiltration relative to TNAL controls (30%; $P=0.09$), but did not differ from PF animals. Interestingly, PF-CON pigs had increased ileum neutrophil infiltration relative to TNAL and PF-CIT pigs (49 and 63%, respectively; $P\leq 0.02$). In the colon, HS increased

neutrophil infiltration 62% ($P=0.02$) relative to TNAL controls, but this did not differ from PF-CON pigs as colon neutrophil infiltration was also increased in PF-CON relative to TNAL and PF-CIT pigs (64 and 108%, respectively; $P\leq 0.03$). In summary, no effect of CIT supplementation on production variables during HS were detected. Supplementing CIT had minimal effects on physiological and production parameters during HS, but tended to increase ADG during limit-feeding. Exposure to HS had detrimental effects to both jejunum and ileum morphology, and CIT supplementation was only able to rescue changes in the ileum. Both HS and nutrient restriction increase neutrophil infiltration in the distal gastrointestinal tract. Supplemental citrulline did not diminish this immune response during HS but does ameliorate it under nutrient-restriction conditions. There may be beneficial effects of CIT on gut health during HS and nutrient restriction, but further investigation is needed.

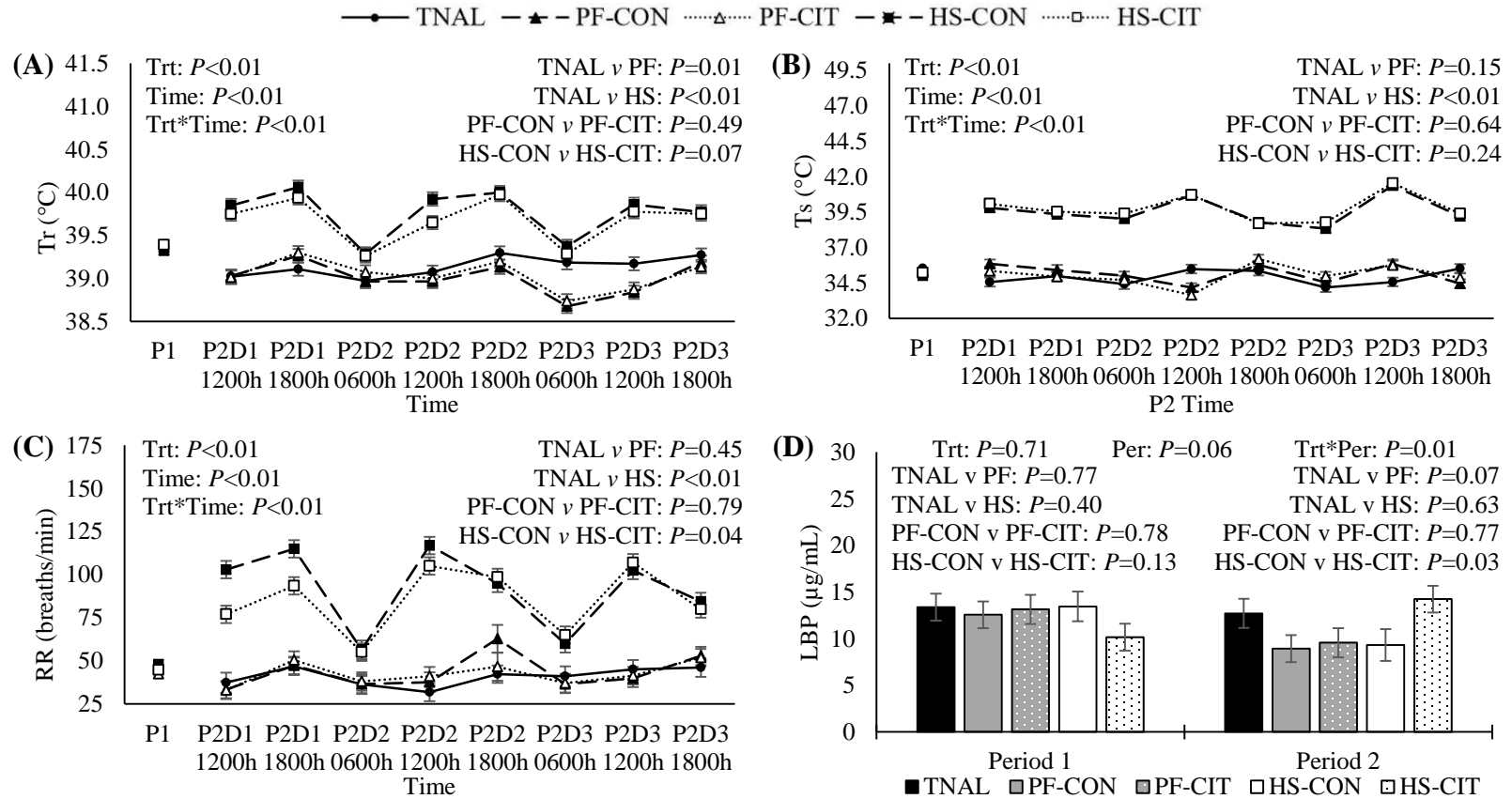


Figure 31. Effect of citrulline supplementation on (A) rectal temperature (Tr), (B) skin temperature (Ts), (C) respiration rate (RR), and (D) circulating lipopolysaccharide binding protein (LBP) during heat stress or pair-feeding

Table 21. Effects of citrulline supplementation on production parameters during heat stress or pair feeding

	Treatment ¹					SEM	P Trt	Contrasts			
	TNAL	PF-CON	PF-CIT	HS-CON	HS-CIT			TNAL v PF ²	TNAL v HS ³	PF-CIT v PF-CON	HS-CIT v HS-CON
Period 1											
End BW, kg	40.4	40.0	41.0	40.0	40.1	0.2	0.10	0.71	0.28	0.01	0.91
FI, kg/d	2.05	1.96	2.16	1.92	1.94	0.07	0.15	0.94	0.15	0.04	0.82
ADG ⁴	0.89	0.84	0.98	0.84	0.85	0.04	0.10	0.71	0.28	0.01	0.91
Gain:Feed	0.44	0.43	0.46	0.44	0.43	0.02	0.75	0.59	0.85	0.22	0.69
Period 2											
End BW, kg	43.5	41.1	42.5	42.5	42.6	0.3	<0.01	<0.01	0.02	0.01	0.86
FI, kg/60 h	5.28	4.03	4.77	4.08	4.20	0.26	<0.01	0.01	<0.01	0.05	0.72
ADG ⁴	1.01	0.36	0.48	0.82	0.83	0.10	<0.01	<0.01	0.08	0.34	0.93
Gain:Feed	0.45	0.24	0.26	0.50	0.49	0.06	0.02	0.01	0.52	0.83	0.89

¹Treatments: TNAL = thermoneutral ad-libitum; PF-CON = pair-fed control; PF-CIT = pair-fed citrulline; HS-CON = heat stress control; HS-CIT = heat stress citrulline

²PF-CON and PF-CIT pigs

³HS-CON and HS-CIT pigs

⁴Feed intake

⁵Average daily gain