Challenging the paradigm of metabolic diseases in the fresh period

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The origin of both heat stress (all farm animals) and ketosis (dairy cows) problems is a compromised intestinal integrity.
Lab Research Priorities: Ruminants and Pigs

- Feed Restriction
- Transition Period
- Heat Stress
- Hind Gut Acidosis

Leaky Gut

- Metabolic Response
- Immunometabolic Response
- Endocrine Response

Production
Documented Causes of Increased Intestinal Permeability ("leaky gut")

- Transition Period
- Heat Stress
- Large Intestine Acidosis
- Feed Restriction
- Psychological Stress
- Distant Inflammation
- Weaning
- Large Intestine PTN Fermentation
- Small Intestine Bacteria Overgrowth
Glucose is made from propionate
Lactose is made from glucose
Milk yield is determined by the amount of synthesized lactose
Gastro-Intestinal Tract Review
Reminder: Intestinal Functions

- GIT is a tube running from the mouth to the anus.
  - Everything inside of the tube is technically outside the body.

- Digest and absorb nutrients.
  - GIT lumen is a inhospitable environment.

- Prevent parasites, pathogens, enzymes, acids, toxins etc. From infiltrating “self”.
  - Barrier function.
Human GIT Surface Area:

That’s an enormous amount of area to “defend”!

No wonder 70% of the immune system resides in GIT

Gut Surface Area = Doubles Tennis Court
Intestinal Morphology

Thermal Neutral Well-fed  Heat Stress  Pair-fed

Pearce et al., 2011
Blood stream → Submucosa

- Lumen
- TJs
- Hypoxia → HIF-1α
- Actin, Myosin
- MLCK
- IkB
- NFκB
- PGE2
- TNFα
- IL-1β
- IL-16
- INFγ
- APP

Healthy TJs → Compromised TJs
Lipopolysaccharide (LPS) stimulates the immune system
LPS promotes inflammation production....catabolic condition
- TNFα, IL-1 etc..
  - Reduced appetite
  - Stimulates fever
  - Causes muscle breakdown
  - Induces lethargy
  - ....reduces productivity
LPS causes liver damage
Cause(s) of Leaky Gut?

- Direct affects of heat
- Hypoxia
- Osmotic stress
- Dehydration stress
- Oxidative stress
- pH stress
- Increased intraluminal endotoxin concentration
- ??
Leaky Gut and Ketosis?
Transition Period Disorders: Mediated Largely by NEFA

- Transition Period
  - Metabolic shift
  - NEBAL
  - Negative effects on future production

- Dystocia
- Milk fever
- Retained placenta
- Metritis
- Ketosis
- Displaced abomasum
- Fatty liver
- Lameness
- Death

Only 50% of cows complete the transition period without experiencing one of these problems

Drackley, 1999
Dogma

- Excess adipose tissue mobilization causes fatty liver and ketosis
- This is exacerbated in high producing cows
- Industry Goal: Reduce blood NEFA
Many studies *associate* NEFA and BHBA with:

- Increased risk of ketosis, decreased milk yield, LDA, metritis, retained placenta, laminitis, or poor reproduction
  - Chapinal et al., 2011; Huzzey et al., 2011; Ospina et al., 2010a, 2010c; Duffield et al., 2009; LeBlanc et al., 2005

- Plasma NEFA are markedly increased (>700 mEq/L) following calving in almost all cows
  - ~15-20% get clinical ketosis
  - What makes these cows more susceptible to ketosis?
    - Predisposition to developing fatty liver?
Thought Provoking Observations??

1) Associations and correlations
   - No cause and effect

2) Infusing ketones or NEFA does not cause negative outcomes

3) Ketones do not decrease feed intake
   - Otherwise a starving animal would not have an appetite

4) Infusing ketones do not increase blood ketone levels
   - In late lactation ketone removal from the circulating pool is very rapid

5) Cannot recreate ketosis during established lactation
   - Using a feed-restriction model doesn’t cause fatty liver and ketosis

6) Some females do not consume food after parturition
   - Ocean mammals
Maybe its not NEFA’s fault?

- Maybe something other than increased NEFA is responsible for fatty liver?
- High NEFA and ketones are a consequence….not the problem
- The “content” of anything is a balance between pool entry and pool removal.
- Maybe something is preventing efficient hepatic lipid export?
Human Intestinal Disorders

- Diseases associated with increased intestinal permeability (leaky gut)
  - Crohn’s disease
  - Irritable bowel syndrome
  - Inflammatory bowel syndrome
  - Celiac disease
  - Alcoholism

- Post-absorptive phenotype
  - Increased circulating lipopolysaccharide (LPS)
  - Circulating acute phase proteins
  - Fatty liver: without affiliated rise in blood non-esterified fatty acids (NEFA)
    - Inflammation can compromise the liver’s ability to export lipid and increases NEFA incorporation into hepatic triglycerides (Lanza-Jacoby and Tabares, 1990; Ma et al., 2008)
Humans with intestinal barrier dysfunction have fatty liver….but do not have increased [NEFA]
Peculiar Observations?

- Incidence of clinical ketosis in Southwest vs Midwest and Northeast
  - ~0.5% vs. 10-15%

- Heat Stress cows have increased incidence of fatty liver

- Rumen acidosis:
  - Ground grain: systemic inflammation
  - Alfalfa pellets: no inflammation
## Subacute Rumen Acidosis (SARA) and Systemic Inflammation

<table>
<thead>
<tr>
<th>SARA Inducer</th>
<th>Rumen pH &lt; 5.6 (min/d)</th>
<th>∆ LPS (EU/mL)</th>
<th>∆ LPS (EU/mL)</th>
<th>∆ SAA (µg/mL)</th>
<th>∆ Haptoglobin (µg/mL)</th>
<th>∆ LBP (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa Pellet</td>
<td>268</td>
<td>+ 60,139</td>
<td>0</td>
<td>- 15.3</td>
<td>- 29</td>
<td>- 3.8</td>
</tr>
<tr>
<td>Grain Pellet</td>
<td>279</td>
<td>+ 47,579</td>
<td>+ 0.52</td>
<td>+ 269.2</td>
<td>+ 476</td>
<td>+ 34.9</td>
</tr>
</tbody>
</table>

Adapted from Khafipour et al., 2009a,b
Cartoon created from comments made within Dr. Kees Plazier’s papers
Transition Period: Acute Phase Protein Response & Inflammation

- Associated with or partly responsible for transition period issues?
  - Hailemariam et al., 2014

- Homeorhetic adaptation to lactation?
  - Farney et al., 2013

- Inflammation source???
  - General
  - Uterus
  - Mammary
  - Gastrointestinal tract?
Reasons for increased likelihood of leaky gut in transition dairy cows???

- Dietary shift to increased concentrates
  - Rumen acidosis with grain compromises GIT barrier and increases blood LPS
    - Starch delivery to the hind-gut
  - Rumen acidosis with alfalfa pellets does not increase blood LPS
    - Plaizier et al., 2012; 2009

- Distally derived cytokines
  - Paibomesai et al., 2013
↑ Inflammatory response
↑ Acute Phase Proteins:
• Serum Amyloid A
• Haptoglobin
• LBP

Sara Stoakes
Objectives

- Measure biomarkers of leaky gut in cows that were retrospectively classified as ketotic (only diagnosed problem) and healthy herd mates
  - \( n = 8 \) ketotic cows
  - \( n = 8 \) “healthy” cows

- Initial experiment had non-ketotic objectives
  - Nayeri et al., 2015
Increased LBP in Ketotic Cows

Healthy vs. Ketotic Transition Cows

Lipopolysaccharide Binding Protein (LBP)

- Trt: $P = 0.047$
- DIM: $P < 0.01$
- Trt X DIM: $P < 0.01$

Nayeri et al., 2013
Objectives

- Confirm that the biomarkers of leaky gut increase during the transition period for clinically ketotic cows

- A compromised GIT barrier and subsequent endotoxin (LPS) infiltration may play a causative key role in ketosis development
Abuajamien et al., 2015

LPS

Trt:  P = 0.02
Day:  P = 0.26

Days relative to calving

EU/ml

Healthy
Ketotic

Trt:  P = 0.40
LBP

Days relative to calving

Healthy

Ketotic

Abuajamieh et al., 2015

Trt:  $P=0.06$
Day:  $P<0.01$
Still not causative data

Same “associative” problem that NEFA and ketones have with ketosis
Objectives

Determine if intentionally induced gastrointestinal permeability reduces productivity and alters energetic and inflammatory indices.....

in otherwise healthy dairy cows
Gamma-Secretase Inhibitor (GSI)

- Reduces crypt cell differentiation
- Decreases enterocyte migration on the microvilli
- Decreased differentiation likely compromises intestinal integrity
- Potential model for natural causes of "leaky gut" (van Es et al., 2005)

Guilmeau, 2012
Control

Stoakes et al., 2014
Stoakes et al., 2014
Stoakes et al., 2014

**Insulin**
- Treatment: $P = 0.07$
- Day: $P < 0.01$

**NEFA**
- Treatment: $P = 0.06$
- Day: $P < 0.01$

**Glucose**
- Day: $P = \text{not shown}$

**BHBA**
- Day: $P = 0.08$
- Treatment x Day: $P = 0.09$
Summary

- GSI morphologically altered villi architecture and biomarkers of the acute phase protein response: characteristics consistent with leaky gut.

- Induced leaky gut markedly affected production and metabolism: responses similar to ketosis.

- Feed restriction (by itself) negatively affected intestinal barrier function.

- Feed restriction may be a useful tool to test molecules targeting leaky gut mitigation.
Glucose is made from propionate
Lactose is made from glucose
Milk yield is determined by the amount of synthesized lactose
Evolution of the Immunometabolic Field
Review: Coordination of Nutrient Partitioning

Nutrient Flux Hierarchy

Metabolic Priority

CNS
Immune System
Reproduction
Lactation
Muscle
Adipose
Profesor Otto Warburg

First recognized the unique metabolism of cancer cells (1927)
- Large glucose consumers
- Switch from oxidative phosphorylation → aerobic glycolysis

Also observed activated lymphocytes become highly glycolytic (1958)

Mentored Hans Krebs

Drinking buddy with Albert Einstein
GLU

Resting Immune Cell

Activated Immune Cell

Warburg Effect

GLU

LPS

Carrie Shouse
Glucose and the Immune System

- At rest, immune cells can oxidize multiple fuels
- Once activated, immune cells become obligate glucose utilizers
- How much glucose does the immune system use?
- Milk synthesis is regulated by lactose synthesis….glucose is precursor to lactose
How much glucose is the entire body using??

80 years later and we still not know how much glucose the immune system needs *in vivo*?

Prerequisite for developing mitigation strategies

What’s the Problem?:

- Dynamic and ubiquitous distribution of the immune system throughout tissues
  - Allows for quasi tissue/organ quantification but….
  - Complicates whole-body quantification
Etiology of Our Hypothesis

- All leukocytes become obligate glucose utilizers
- Adipocytes, myocytes and hepatocytes reduce glucose utilization
- Liver glucose output increases
- .......but hypoglycemia still occurs

Hmmmmmm?????????
LPS Challenge & Blood Glucose

Time

Glucose

Hepatic Glucose output & Peripheral Insulin Resistance

Immune system glucose utilization >

Hepatic Glucose output & Peripheral Insulin Resistance

Immune system utilization
Can we quantify this amount of glucose?
### Cow # 8341

<table>
<thead>
<tr>
<th>Min</th>
<th>Blood Sample</th>
<th>[Glucone] (mg/dL)</th>
<th>Glucose ROI (mL/hr)</th>
<th>Tr (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 (1 hr)</td>
<td>✓</td>
<td>96</td>
<td>0</td>
<td>101.3</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>84</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td>79</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>✓</td>
<td>91</td>
<td>0</td>
<td>100.8</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>98</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td></td>
<td>116</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>120 (2 hr)</td>
<td>✓</td>
<td>115</td>
<td>0</td>
<td>101.2</td>
</tr>
<tr>
<td>130</td>
<td></td>
<td>102</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>140</td>
<td></td>
<td>87</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>✓</td>
<td>68</td>
<td>0</td>
<td>100.9</td>
</tr>
<tr>
<td>160</td>
<td></td>
<td>49</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>170</td>
<td></td>
<td>54</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>180 (3 hr)</td>
<td>³³³</td>
<td>55</td>
<td>75</td>
<td>100.7</td>
</tr>
<tr>
<td>190</td>
<td></td>
<td>56</td>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

Target [Glu] Range: 61-67

Stoakes et al., 2015
Milk Yield (kg)

Time Relative to Bolus (hr)

Pre-Trt 6 12

Control LPS LPS-Eu

Trt: $P<0.01$

Stoakes et al., 2015
Total Glucose Deficit (g) = 1553 g – 483 g = 1070 g glucose/12 h

Stoakes et al., 2015
Study Limitations

- Glucose uptake by other tissues
  - ↓ insulin sensitivity in adipose
    - (Song et al., 2006, Shi et al., 2006, Poggi et al., 2007)
  - ↓ insulin sensitivity in muscle

Conclusion: 1 kg/12 h is underestimated!

- Glucose output by liver
  - Increased
    - (Lang et al., 1993, McGuinness et al., 1993, Ling et al., 1994)
8.4 Mcal of energy!
Practical Implications: Growth

- Immune System Glucose: ~1000 g/d
  - 1000 g of CHO x 4.1 kcal/g = 4,100 kcal

- Protein synthesis: 10 kcal/g (Patience, 2012)

- 4,100 kcal ÷ 10 kcal/g = 410 g of protein

- 410 g PTN ÷ ~30% dm = ~1,366 g of lean tissue
### Conserved Response

<table>
<thead>
<tr>
<th>Species:</th>
<th>Immune glucose utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steers:</td>
<td>1.0 g/kg ( BW^{0.75}/h ) (Kvidera et al., 2016)</td>
</tr>
<tr>
<td>Pigs:</td>
<td>1.1 g/kg ( BW^{0.75}/h ) (Kvidera et al., 2015)</td>
</tr>
<tr>
<td>Cows:</td>
<td>0.7 g/kg ( BW^{0.75}/h ) (Kvidera et al., 2017)</td>
</tr>
<tr>
<td>Cows:</td>
<td>1.0 g/kg ( BW^{0.75}/h ) (Horst et al, unpublished)</td>
</tr>
</tbody>
</table>
Calcium and Immune System

- Inflammation plays a key role in hypocalcemia (Zebeli et al., 2013; Hendy and Canaff, 2016)

- Ca is involved in immune system activation (Hendy and Canaff, 2016)

- Immunoactivation/inflammation can be induced experimentally by infusing lipopolysaccharide (LPS)
  - LPS is a cell wall component of gram-negative bacteria which elicits a robust immune response (Waldron et al., 2003; Kvidera et al., 2017)
Ca and Immune System

- I.V. LPS decreases circulating Ca in late lactation (Kvidera et al., 2017; Horst et al., 2017)

![Graph showing ionized calcium levels over time](image1)

Kvidera et al., 2017

![Graph showing ionized calcium levels over time](image2)

Horst et al., 2017
Immuno-activation and Hypocalcemia?

- Maybe pathogen/antigen exposure during the periparturient period also contributes to milk fever.

- LPS is toxic to developing follicles (Hansen et al., U of FL)

- ..??? maybe the reason why negative outcomes during the transition period are all correlated with each other is because most of them can be causal connected with LPS.
Can “leaky gut” explain suboptimal production frequently observed in animal agriculture?

- Heat Stress
- Inadequate feed intake
  - “off-feed event”
    - The negative effects on growth and milk yield are bioenergetically unexplainable by reduced feed intake
  - Transition period
    - Cause of ketosis?
- Weaning
- Shipping
- Overcrowding
- Unpalatable feed
- Drought
Can the Feed or Animal Health Industry do anything about leaky gut????

Targets:
- Direct action at intestine
- Indirect via:
  - Increased feed intake
  - Rumen acidosis prevention
    - Hind gut acidosis prevention
  - Improved immune function
Target Mitigation Strategies

- Prevent infection (obvious)
- Encourage feed intake
- Maximize digestion prior to large intestine
  - Dietary strategies
- Prevent rumen acidosis
  - Dietary Strategies
- Enhance intestinal permeability
  - Dietary strategies
- Immunomodulation
- Minimize psychological stress
### Potential nutritional strategies to ameliorate intestinal permeability

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Presumed Mechanism of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate</td>
<td>Acidosis prevention</td>
</tr>
<tr>
<td>Glutamine</td>
<td>↑ intestine integrity</td>
</tr>
<tr>
<td>Zinc</td>
<td>↑ intestine integrity, antioxidant</td>
</tr>
<tr>
<td>Dairy Products</td>
<td>↑ intestine integrity</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Selenium</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>↑ intestine integrity</td>
</tr>
<tr>
<td>Betaine</td>
<td></td>
</tr>
<tr>
<td>Conjugated Linoleic Acid</td>
<td>↑ Energy balance</td>
</tr>
<tr>
<td>Chromium</td>
<td>↑ Feed Intake, enhance immune cell function</td>
</tr>
<tr>
<td>Yeast, yeast extract/DFM</td>
<td>Acidosis prevention &amp; ↑ Feed Intake</td>
</tr>
<tr>
<td>Ionophores</td>
<td>Acidosis prevention</td>
</tr>
<tr>
<td>β-glucan</td>
<td>Immune modulation</td>
</tr>
<tr>
<td>Mannanoligosaccharide</td>
<td>↑ intestine integrity</td>
</tr>
<tr>
<td>Rehydration therapy</td>
<td>↑ intestine integrity &amp; ↑ Feed Intake</td>
</tr>
<tr>
<td>Butyrate</td>
<td>↑ intestine integrity</td>
</tr>
<tr>
<td>Mycotoxin binders</td>
<td>↑ intestine integrity</td>
</tr>
<tr>
<td>OmniGen-AF</td>
<td>Immune modulation</td>
</tr>
<tr>
<td>Organic acids</td>
<td>↑ intestine integrity</td>
</tr>
<tr>
<td>Plant botanicals</td>
<td>↑ intestine integrity</td>
</tr>
</tbody>
</table>

Baumgard et al., 2014
Dairy Cow

Maladaptation to Lactation

Summary
Metabolic Flexibility: Decreased Insulin Sensitivity

Baumgard and Rhoads, 2013
Unsuccessful Transition

Metabolic Flexibility: Decreased Insulin Sensitivity

Glucose redirected to immune system

Baumgard and Rhoads, 2013
Ketosis: When to intervene?

- **Treat:**
  - High ketones
  - Not coming into milk
  - Not aggressively eating
  - Looks lethargic and melancholic
  - Has a mild fever

- **Don’t mess with**
  - High ketones….but she’s
  - Eating like a champ
  - Milking like a world-record holder
  - Looks great
  - No fever
High NEFA and ketones are needed to support maximum milk production.

In a “crashing cow”, NEFA and ketones are a “reflection” of the underlying insult.

Ketosis appears to be an immuno-metabolic event.

- Leaky gut…and other sources of inflammation could be etiological origin.
Conclusions

- Leaky gut and endotoxin infiltration may play important roles (if not the origin) in suboptimal productivity commonly observed in animal agriculture, and ketosis in particular.

- Strategies that can improve intestinal integrity need to be researched...in a “stressed model”

- If leaky gut is the fundamental cause of many typical on-farm problems....then it is a financial problem that dwarfs all others combined.
Acknowledgments

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- Diamond V
- ASCUS
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