Gut Health and the Microbiome is a Big Deal

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General Synopsis

- Stress
- Reduced Feed Intake
- “Leaky Gut”
- Activated Immune System
- Nutrient Reprioritization to non-productive functions
- Reduced Performance
- $$ Reduced Income $$
Gastrointestinal Epithelial: aka Gut Barrier

- **Reticulo-rumen and omasum**
  - Stratified squamous epithelium
    - 4 distinct strata
    - Multiple layers (maybe > 10 layers thick)
      - 85 um separating “outside” from self
    - No mucus

- **Rest of GIT**
  - Columnar epithelium
    - Single layer epithelium
      - 20 um separating “outside” from self
    - Mucus lined

Steele et al., 2016 JDS
Human Intestinal Disorders

- Diseases associated with increased intestinal permeability (leaky gut)
  - 1/3 of adults have a barrier issue
    - Crohn’s disease
    - Irritable bowel syndrome
    - Celiac disease
    - Colitis
    - Ulcer colitis
    - Alcoholism

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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
- Rumen Acidosis
- Distant Inflammation
- Weaning
- Large Intestine PTN Fermentation
- Small Intestine Bacteria Overgrowth
Glucose is primarily made from propionate
Lactose is made from glucose
72 g of glucose/ 1 kg of milk
Milk yield is primarily determined by the amount of synthesized lactose
Heat Stress: Economics and Food Security

- **Cost:** (lost productivity, mortality, product quality, health care etc.)
  - American Agriculture: > $4 billion/year
    - $1.7 billion in dairy industry
  - Global Agriculture: > $150 billion/year

- It will get worse in the future if:
  - Climate change continues as predicted
  - Genetic selection continues to emphasize milk synthesis, lean tissue accretion, piglets/sow etc..
    - Heat producing processes

St. Pierre et al., 2003; Baumgard and Rhoads, 2013
Results of Heat Stress

- Decrease in production (milk and growth)
- Reduced body condition
- Acute health problems
- Rumen acidosis (hidden costs)
- Significant drop in pregnancy rate
  - See Albert DeVries webinar
- High incidence of abortions
- High death loss

Added all up … costly!
Heat Stress Questions??

- Does the decrease in feed intake explain the reduced milk yield during heat stress?

**Indirect vs. direct effects of heat**

- If we have a better understanding of the biological reasons *WHY* heat stress reduces production, we’ll have a better idea of how to alleviate it.
Lactation:
Effects of Heat Stress on Feed Intake

Heat stress ↓ feed intake by ~30 %

Rhoads et al., 2009
Effects of Heat Stress on Milk Yield

Heat stress \( \downarrow \) yield \( \sim 45\% \)
Pair-feeding \( \downarrow \) yield by \( \sim 19\% \)

Thus, \( \downarrow \) feed intake only accounts for \( \sim 50\% \) of the reductions in milk yield

Wheelock et al., 2010
Baumgard et al., 2011
Body Weight Loss

Rhoads et al., 2009
Heat Stress Reduces Basal and Stimulated Adipocyte Lipolysis

Rhoads et al., 2009
Wheelock et al., 2010
Baumgard and Rhoads, 2013
Heat Stress Increases Basal and Stimulated Insulin Secretion

Wheelock et al., 2010
Glucose Tolerance Test

Wheelock et al., 2008

P < 0.05
Heat Stress Cows Secrete ~400 g less lactose/day than Pair-Fed Thermal Neutral Controls.

Is the liver producing ~ 400 g less glucose/day? Or is extra-mammary tissues utilizing ~400 g more/day? 

Rhoads et al., 2009
Wheelock et al., 2010
Extra mammary tissues utilize ~ 400 g more glucose/day during heat stress.

Indicates glucose is preferentially being utilized for processes other than milk synthesis (maybe the immune system?) during heat stress.

Period: $P < 0.05$

Baumgard et al., 2011
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
Biology of Heat Stress Symptoms
Heat Stress and Gut Health

- Diversion of blood flow to skin and extremities

- Coordinated vasoconstriction in intestinal tissues
  - Reduced nutrient and oxygen delivery to enterocytes
  - Hypoxia increases reactive oxygen species (ROS)

- Reduced nutrient uptake increases rumen and intestinal osmolarity in the intestinal lumen
  - Multiple reasons for increased osmotic stress

Baumgard and Rhoads, 2013
Intestinal Morphology

Thermal Neutral

Heat Stress

Pair-fed

Pearce et al., 2011
Heat Stress and Gut Health

- When bacteria (or parts of bacteria) enter the body they cause an immune response

- Lipopolysaccharide (LPS) stimulates the immune system

- LPS promotes inflammation production….catabolic condition
  - Inflammation….($\text{TNF}_\alpha$, IL-1 etc..)
    - Reduced appetite
    - Stimulates fever
    - Causes muscle breakdown
    - Induces lethargy
    - ....reduces productivity
Blood stream

Submucosa

Healthy TJs

Comromised TJs

Hypoxia

HIF-1α

TJs

Actin

Myosin

MLCK

NFκB

PGE2

TNFα

IL-1β

IL-16

INFγ

APP

TJs

Lumen

Blood stream
The effects are rapid!
Plasma LPS & LBP

Pearce et al., 2015
Heat Stress Summary

- Direct and indirect effects
  - ↓DMI only accounts for 50% of reduced milk yield
- Hyperinsulinemia
- Blunted adipose mobilization
- Liver remains sensitive to catabolic signals
- Leaky gut
  - Inflammation and acute phase protein response
- Unknown whereabouts of 400 g of glucose
Leaky Gut and Ketosis?
### Transition Period Disorders: Mediated Largely by NEFA

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

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

Only 50% of cows in North America 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?
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
<table>
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<tr>
<th>SARA Inducer</th>
<th>pH &lt; 5.6 (min/d)</th>
<th>Δ LPS (EU/mL)</th>
<th>Δ LPS (EU/mL)</th>
<th>Δ SAA (µg/mL)</th>
<th>Δ Haptoglobin (µg/mL)</th>
<th>Δ LBP (µg/mL)</th>
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<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>
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<tr>
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<td>279</td>
<td>+ 47,579</td>
<td>+ 0.52</td>
<td>+ 269.2</td>
<td>+ 476</td>
<td>+ 34.9</td>
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</table>

Adapted from Khafipour et al., 2009a,b
Grain Acidosis

Alfalfa Pellet Acidosis

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?

Yuan 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
Abuajamieh et al., 2015

- **Trt**: $P = 0.02$
- **Day**: $P = 0.26$

Days relative to calving

**LPS**

EU/ml

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 gut permeability reduces productivity and alters energetic and inflammatory indices in otherwise healthy dairy cows.

Are the results similar to heat stress and ketotic cows???
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: $P = 0.05$

BHBA

Day: $P = 0.08$

Trt x Day: $P = 0.09$

Stoakes et al., 2014
Summary

- Intentionally induced leaky gut markedly affected production and metabolism: responses similar to ketosis and heat stress.

- Feed restriction (by itself) negatively affected intestinal barrier function.
  - Does this have implications to on farm “out of feed” scenarios??
12 hours of feed restriction (40% of ad libitum intake) causes leaky gut in the pig

Pearce et al., 2015

12 TN: 12 hours of thermal neutral ad libitum fed conditions
12 PF: 12 hours of pair-feeding in thermal neutral conditions
12 HS: 12 hours of heat stress and ad libitum feed intake
12 HS-Zn: 12 hours of heat stress and fed Availa-Zn
Effects of Feed Withdrawal (100%) on Milk Yield

How often are cows without feed for ≥ 6 hours on your farm?

Kvidera al., 2015
Could 12 h of FR causes leaky gut in cattle?

- Don’t know….depends upon mechanism
  - Decreased luminal nutrient delivery….then likely not
    - Continues out-flow of ruminal nutrients

- Psychological stress
  - Hunger-induced ACTH-Cortisol action…..then possible
    - Cortisol causes leaky gut in multiple models
    - Intestinal Mast cells are responsive to nerves
    - Upon CRF stimulation they release proteases and TNFα
      - Both proteases and TNFα cause tight junction breakdown
        - Dr. Adam Moeser’s papers
The GIT is enervated by both the CNS and ENS

https://www.lumennatura.com/2013/01/30/study-craniosacral-therapy-and-the-digestive-system/
So the Gut Becomes Leaky....the Immune System is Activated.......who Cares?

Mmmm, Tastes like a combination of Who Cares?
&
So What?

Tell someone who cares
Evolution of the Immunometabolic Field
First recognized the unique metabolism of cancer cells (1927)
- Large glucose consumers
- Switch from oxidative phosphorylation → aerobic glycolysis
- 1931 Noble Prize

Also observed activated lymphocytes become highly glycolytic (1958)

Mentored Sir Hans Krebs

Drinking buddy with Albert Einstein
Glucose and the Immune System

- At rest, immune cells can burn multiple fuels
- Once activated, immune cells only want glucose
- How much glucose does the immune system use?
- Milk synthesis is regulated by lactose synthesis….glucose is precursor to lactose
Can we quantify this amount of glucose?
### Cow # 8341

<table>
<thead>
<tr>
<th>Min</th>
<th>Blood Sample</th>
<th>Glucose [mg/dL]</th>
<th>Glucose ROI [mL/hr]</th>
<th>Tr (€)</th>
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<tr>
<td>60 (1 hr)</td>
<td>✓</td>
<td>96</td>
<td>0</td>
<td>101.3</td>
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<td>70</td>
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<td>84</td>
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<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>
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<tr>
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<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>
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<td>150</td>
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<td>180 (3 hr)</td>
<td>✓</td>
<td>55</td>
<td>75</td>
<td>100.7</td>
</tr>
</tbody>
</table>

Target [Glu] Range: 61-67

Stoakes et al., 2015
Total Glucose Deficit (g)

Control: 483 g
LPS: 1259 g
LPS-Eu: 1553 g

$1553 \text{ g} - 483 \text{ g} = 1070 \text{ g glucose/12 h}$

$2.4 \text{ lb/12 h} \ldots \text{or} \ 4.7 \text{ lb/day}$

Stoakes et al., 2015
8.4 Mcal of energy!....$$$$$
Heat Stress $\rightarrow$ Leaky Gut $\rightarrow$ ↑ LPS $\rightarrow$ ↑ Insulin

↓ Feed Intake $\rightarrow$ Immuno-activation

↓ Performance $\rightarrow$ Immune System Nutrient Utilization
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
Target Mitigation Strategies

- Prevent infection (obvious)
- Encourage feed intake
  - Ensure 100% feed availability
- Minimize psychological stress
- Maximize digestion prior to large intestine
  - Dietary strategies
- Prevent rumen acidosis
  - Dietary Strategies
- Enhance intestinal permeability
  - Dietary strategies
- Immunomodulation

Dairy Producer’s Responsibility
Nutritionist and Producer’s Responsibility
Nutritionist’s Responsibility
Lactating Dairy Cow Metabolic Adaptation to Heat Stress and Maladaptation to Lactation

Summary
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**
Metabolic Inflexibility: Decreased Insulin Sensitivity
Glucose redirected to immune system

Unsuccessful Transition
Fatty Liver, Excessive Ketone Synthesis
Reduced DMI

Baumgard and Rhoads, 2013
Metabolic Inflexibility Remains Insulin Sensitive

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
Summary

- Heat stress, ketotic and feed-restricted cows and emotionally stressed cows have a similar metabolic and endocrine fingerprint
  - Leaky gut is a common denominator

- The activated immune system utilizes an enormous amount of glucose.

- Dietary and management strategies
Conclusions

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

- 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.
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