Insights into Immunology for the Transition Dairy Cow

NMC Industry-Sponsored Seminar

3:30pm to 5:30pm

January 31, 2016

Glendale, AZ
Insights into Immunology for the Transition Dairy Cow

1. Understanding Periparturient Immune Suppression
   • Paul Rapnicki, DVM MBA
   • Elanco Animal Health

2. Improving transition cow health by preventing metabolic stress
   • Lorraine Sordillo, MS PhD
   • Michigan State University
Understanding Periparturient Immune Suppression

Paul Rapnicki, DVM MBA
Dairy Technical Consultant
Elanco Animal Health

NMC 55th Annual Meeting
Industry Sponsored Seminar
Periparturient Immune Suppression

Periparturient Immune Suppression

• Evidence & Understanding

• Management Implications
What is Immune Suppression?

• Literally means “*Diminished immune responsiveness*”

• Immune system is a highly diverse part of our bodies that fights disease

• Involves cellular and non-cellular mechanisms that are often interdependent

• Periparturient immune suppression is rather significant
  
  – Typically, this is in the area of a 25 to 40% decline in both neutrophil function (innate immunity) and lymphocyte function (acquired immunity)*

Immunity is a whole-body system that protects the cow from infections.

Two Major Branches of the Immune System

- **Acquired Immunity**
  This is what most producers think about for their herd — things like vaccines and antibodies.

- **Innate Immunity**
  This is the first cellular line of defense against bacterial invasion, where macrophages and neutrophils respond to quickly kill bacteria.
Neutrophils are key in mastitis protection

• Work from early mastitis researchers demonstrated the importance of Neutrophils

• Neutrophils predominant (97%) of cells in mastitic milk

Founding father of modern immunology
Elle Metchnikoff (Russian zoologist)

• Neutrophil role in mastitis has been recognized a long time. . . .
• In his 1908 Nobel Prize Acceptance Lecture he described disease as
• “a battle between a morbid agent, the external microorganism, and the mobile cells of the organism itself. A cure would represent the victory of the cells, and immunity would be the sign of an activity on their part sufficiently great to prevent an invasion of microorganisms.”
Mastitis origins to immunology

• Metchnikoff cited the Swiss veterinary expert, Zschokke:

  • Zschokke observed phagocytosis of streptococci in the battle against bovine mastitis was a good sign.

  • If phagocytosis was insignificant or not present, cows were written off as no longer capable of producing good milk.

  • Not only must phagocytes engulf the microorganisms, but that the devouring cells must utterly destroy the microorganisms.

  • In some cases, streptococci of mastitis were found to "destroy the phagocytes after being engulfed by them thus liberating themselves to carry on their deadly work."
Effective Neutrophil Activity Includes:

- Attachment to vascular wall, diapedesis and chemotaxis to site of infection
- Ingestion of invading bacteria
- Destruction of ingested bacteria
Neutrophils: “The Professional Phagocyte”
Neutrophil death and removal

Pathogen Clearance

Early Apoptosis

Late Apoptosis

Secondary Necrosis

Proteases ROIs

Tissue damage

Inflammation

Macrophage Phagocytosis

↓ Inflammation

↑ Tissue repair

http://medicine.cf.ac.uk/person/eamon-mcgreal/research/.
Immunity is a whole-body system that protects the cow from infections.
Evidence for Periparturient Immune Suppression
Is this cow immune suppressed?
Retained Placenta (RP)

• During the third and final stage of labor, the placenta is released and the process of uterine involution begins

• Beagley et al. Review (2010) reported:
  – RP definition is varied in the literature, ranging from retention of the placenta for 8 to 48 hours postpartum
  – Most published studies define RP in cattle at 12 to 24 hours

Retained Placenta – Role of Immune Function

• Beagley et al. Review (2010) reported:
  – Decreases in immune function play an important role in the mechanism of placental retention

• Gunnink (1984)
  – Demonstrated differences in neutrophil function in cows that would further develop retained placenta

Retained Placenta – Role of Immune Function

• Kimura et al. (2002)
  - RP is caused by immune dysfunction at calving
    • Unifying theory that helps explain epidemiological evidence that deficiency or excess of a variety of nutrients or hormones can affect the incidence of RP
    - Cows developing retained placenta have impaired neutrophil function, as assessed by chemotaxis toward cotyledon supernatant preparations and myeloperoxidase activity

Boyden Chamber Assay

White blood cells

Homogeniz. Placenta

Membrane with Microscopic pores

Chemotactic activity of neutrophils toward cotyledon supernatant in cows with retained placenta was significantly lower ($P < 0.01$) than in cows without retained placenta before parturition.

In cows that had RP post-calving, pre-calving myeloperoxidase activity was less compared to cows that did not have RP.

Is this cow immune suppressed?
Clinical Metritis

- Kelton et al. (1998)
  - A cow is considered to have clinical metritis if she had a postpartum condition characterized by an abnormal (i.e., not including lochia or clear oestral mucus) cervical discharge, vaginal discharge, or both or abnormal uterine content.
  - These characteristics are not an exhaustive list of clinical signs associated with this condition but rather represent the minimum criteria on which the diagnosis of the condition was based.

# Clinical Illness Scoring for Metritis

<table>
<thead>
<tr>
<th>Category</th>
<th>CIS Score</th>
<th>Uterine Discharge Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skipped</td>
<td>Null</td>
<td>No examination</td>
</tr>
<tr>
<td>No Metritis Detected</td>
<td>0</td>
<td>Examined – visual inspection of vaginal vault</td>
</tr>
<tr>
<td>Mild Metritis</td>
<td>1</td>
<td>Examined, Odor Present AND Non-Watery Discharge on Metriclecheck or Palpation if Not Visible</td>
</tr>
<tr>
<td>Moderate Metritis</td>
<td>2</td>
<td>Examined, Odor Present AND Watery Discharge on Metriclecheck or Palpation if Not Visible</td>
</tr>
<tr>
<td>Acute Puerperal Metritis</td>
<td>3</td>
<td>Examined, Odor Present AND Watery Discharge on Metriclecheck or Palpation if Not Visible</td>
</tr>
</tbody>
</table>

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*adapted from Dohmen et al. (1995), Overton et al. (2003), Urton et al. (2005), Sheldon (2006), Benzaquen et al. (2007), and Huzzey et al (2007).

*Signs of systemic illness include, but are not limited to, body temperature $\geq 39.5^\circ\text{C}$, decreased milk yield, dullness or other signs of toxemia, decreased dry matter intake, elevated heart rate, and dehydration (Sheldon et al., 2008; Haimerl and Heuweis, 2014)
Score = 2 or 3 (odor and watery)
Clinical Metritis – Incidence

Figure 1. Frequency distribution of metritis incidence by days postpartum in a sample of 753 metritis cases that occurred over a one-year period in dairies in Ohio, New York, and California.

Clinical Metritis – Role of Immune Function

• Nearly all dairy cattle experience bacterial contamination of the uterus for 2 to 3 weeks after calving

• Therefore. . . . in the immediate postpartum period, it is logical to assume that a prompt and effective response of the innate immune system is critical for bacterial clearance and prevention of clinical metritis


Clinical Metritis – Role of Immune Function

• Leukocytes from cows diagnosed with endometritis (cloudy discharge and enlarged uterus at 4 weeks post-calving) exhibited poor phagocytic activity at -1, 1, 2, 3, and 4 wks around calving

Clinical Metritis – Role of Immune Function

- In cows with metritis or endometritis, blood neutrophil functions were significantly impaired compared to unaffected cows.

- Significantly lower neutrophil myeloperoxidase (MPO) activity than cows with normal uterine health activity.

Effective Neutrophil Activity Includes:

- Attachment to vascular wall, diapedesis and chemotaxis to site of infection
- Ingestion of invading bacteria
- Destruction of ingested bacteria
  - Oxidative burst (ROI)
  - MPO-H₂O₂-Halide
  - Defensins
  - Lysozyme
  - Lactoferrin
Clinical Metritis – Role of Immune Function

• Impairment of the immune status during the periparturient period in cattle increases the risk of uterine infection

• Infection of the uterus is largely influenced by the balance between bacterial contamination and the immune status around parturition

Is this cow immune suppressed?
CHECK HER OUT FOR CLINICAL MASTITIS
REvisa SI TIENE MASTITIS CLINICA

Three easy questions help check cows for clinical mastitis.
Tres preguntas sencillas ayudan a comprobar si las vacas tienen mastitis clínica.

1. Is the milk abnormal in appearance (watery, flakes, clots)?
¿La leche se ve anormal (grumos, coágulos, se ve aguada)?

2. Does the udder have signs of inflammation (pain, swelling, redness, heat, firmness)?
¿La ubre tiene signos de inflamación (dolor, hinchazón, enrojecimiento, calor)?

3. Is she acting sick (fever, not eating, depressed)?
¿La vaca actúa enferma ( fiebre, no está comiendo, deprimida)?

RECORD THE CLINICAL MASTITIS EVENT IN THE DAILY FARM RECORDS:
- ID of the cow and date of the event
- Note which quarter is affected
- Note the severity (mild, moderate, or severe) of the case

ANOTA EL EVENTO DE MASTITIS CLÍNICA EN EL SISTEMA DE INFORMACIÓN DIARIA DE LA LECHERÍA.
- Número de identificación de la vaca y fecha del evento
- Anota cuál es el cuarto afectado
- Anota el grado de severidad del caso (leva, moderado 0 severo)

NOTIFY THE LEAD HERDSPERSON THAT A NEW CASE OF CLINICAL MASTITIS HAS BEEN FOUND SO THAT A TREATMENT DECISION CAN BE MADE.
NOTIFÍQUE A LA PERSONA ENCARGADA QUE SE HA ENCONTRADO UN NUEVO CASO DE MASTITIS CLÍNICA PARA TOMAR UNA DECISIÓN SOBRE EL TRATAMIENTO.

NUMBER OF "YES" ANSWERS DETERMINES SEVERITY:
0: no clinical mastitis 1: mild 2: moderate 3: severe

EL NÚMERO DE RESPUESTAS CON "SÍ" DETERMINA EL GRADO DE SEVERIDAD:
0: no tiene mastitis clínica 1: leve 2: moderado 3: severo

(800) 420-4441
www.elanco.us
Evidence for periparturient immune suppression


Epidemiology: Mastitis on Well-Managed Dairies

• > 80% of cows with DHI linear SCS <5
• Still have unacceptable frequencies of IMI and clinical mastitis
• Clinical mastitis by coliforms, bacteriologically negative (mostly coliforms) and environmental streptococci account for >80% of cases.
• 20% of clinical cases during first 7 d after calving

Immune dysfunction linked to mastitis

- Incidence and severity of mastitis is greatest during the periparturient period in both humans and dairy cows.
- During this time, the mammary gland experiences increased exposure to a plethora of mastitis-causing pathogens.
- At the same time, important immunological defenses of the host are compromised.

Neutrophil Anatomy Key structures

- Nucleus
- Cytoplasm
  - Granules
    - Primary
    - Secondary
- Membrane

Neutrophil Anatomy Key structures

- **Nucleus**
- **Cytoplasm**
  - Granules
    - Primary
    - Secondary
- **Membrane**
  - Loss of pseudopodia due to phagocytosis of milk fat globules and casein
  - Milk neutrophil less functional than blood PMN

Periparturient Immune Suppression

Parturition impact on neutrophil function

Neutrophil Phagocytosis-associated Oxidative Metabolism

Periparturient Immune Suppression

Evidence for Periparturient Immune Suppression
Periparturient Immune Suppression Causes

- Cumulative effect of a multitude of factors
- Superimposed ↑ estrogen and progesterone levels
- Endorphins ↑
- Cortisol ↑ - not the sole contributor
- Negative energy & protein balance of the cow
- Genetic susceptibility
- Mother nature deliberately but transiently suppressing the immune system
Periparturient Immune Suppression

• Evidence & Understanding

• Management Implications
Management Implications

All transition dairy cows go through a period of Negative Energy Balance and Immune Suppression

The issues are:

- the degree (how much)
- the success of adaptation (how long)

Management Implications

• Taking action during the 60 days before calving and 30 days after helps protect the health and production potential of the entire herd

• “Taking Action” = Making Investment Decisions = $
Total Cost Per Calving Analysis

INVESTMENT
Investment decisions

CONSEQUENCE
Direct costs + Indirect costs + Emotional costs

TOTAL COST PER CALVING

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Total cost per incident case of clinical mastitis occurring in the first 30 days in milk

Preventive Veterinary Medicine 122 (2015) 257–264

The cost of clinical mastitis in the first 30 days of lactation: An economic modeling tool

E. Rollin a, K.C. Dhuyvetter b, M.W. Overton a,b,*

 a Department of Population Health, University of Georgia, Athens, GA 30602, United States
 b Elanco Animal Health, 2500 Innovation Way, Greenfield, IN 46140, United States
Objective:

Estimate the cost of clinical mastitis (CM) that occurs during the first 30 days in milk in a typical North American dairy

Method:

A deterministic partial budget model
“All models are wrong, but some are useful”

-George Box
References


## Model Assumptions: General Herd Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit 1</th>
<th>Unit 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk price</td>
<td>$0.46/kg</td>
<td>$0.21/lb</td>
</tr>
<tr>
<td>Marginal milk value calculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feed price (Lactating cow TMR)</td>
<td>$0.28/kg DM</td>
<td>$0.13/lb DM</td>
</tr>
<tr>
<td>Energy density of lactating ration (Mcal NeL)</td>
<td>1.70/kg DM</td>
<td>0.77/lb DM</td>
</tr>
<tr>
<td>Energy required to produce marginal milk</td>
<td>0.71 Mcal NEI/kg</td>
<td>0.32 Mcal NEI/lb</td>
</tr>
<tr>
<td>Marginal milk value</td>
<td>$0.35/kg</td>
<td>$0.16/lb</td>
</tr>
<tr>
<td>Non-saleable milk value (feed calves)</td>
<td>$0.36/kg</td>
<td>$0.16/lb</td>
</tr>
<tr>
<td>Non-saleable milk value (discard)</td>
<td>$0.00/kg</td>
<td>$0.00/lb</td>
</tr>
<tr>
<td>Market cow price (slaughter)</td>
<td>$1.84/kg</td>
<td>$0.84/lb</td>
</tr>
<tr>
<td>Reproductive efficiency (21 day Pregnancy Rate)</td>
<td></td>
<td>22%</td>
</tr>
</tbody>
</table>

1USDA  2Milk Replacer mfg
<table>
<thead>
<tr>
<th>Model Assumptions: Animal Parameters</th>
<th>Overall</th>
<th>Lact = 1</th>
<th>Lact &gt; 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of calvings</td>
<td></td>
<td>38%</td>
<td>62%</td>
</tr>
<tr>
<td>Animals calving</td>
<td>1000</td>
<td>380</td>
<td>620</td>
</tr>
<tr>
<td>Average value of animal(^1,(^2)</td>
<td></td>
<td>$2094(^1)</td>
<td>$1973(^2)</td>
</tr>
<tr>
<td>Culling risk over lactation</td>
<td>37%</td>
<td>25%</td>
<td>46%</td>
</tr>
<tr>
<td>Body weight</td>
<td>662 kg</td>
<td>544 kg</td>
<td>735 kg</td>
</tr>
<tr>
<td></td>
<td>1460 lbs</td>
<td>1200 lbs</td>
<td>1600 lbs</td>
</tr>
<tr>
<td>Market value</td>
<td>$1173</td>
<td>$964</td>
<td>$1301</td>
</tr>
<tr>
<td>Milk production average (1-30 DIM)</td>
<td>28 kg</td>
<td>23 kg</td>
<td>32 kg</td>
</tr>
<tr>
<td></td>
<td>62 lbs</td>
<td>50 lbs</td>
<td>70 lbs</td>
</tr>
</tbody>
</table>

\(^1\)LMIC \(^2\) Estimated based on curvilinear depreciation model (unpublished)
## Model Assumptions: Mastitis Parameters

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>Lact = 1</th>
<th>Lact &gt; 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Mastitis incidence (1-30 DIM)(^1)</td>
<td>6%</td>
<td>6%</td>
<td>6%</td>
</tr>
<tr>
<td>Recurrence risk (% of 1(^{st}) cases with a 2(^{nd}) case)(^2,3)</td>
<td>34%</td>
<td>26%</td>
<td>38%</td>
</tr>
<tr>
<td>Recurrence risk (% of 1(^{st}) cases with a 3(^{rd}) case)(^2,3)</td>
<td>13%</td>
<td>8%</td>
<td>16%</td>
</tr>
<tr>
<td><strong>Severity of CM cases(^4)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild mastitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate mastitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe mastitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Causative agent(^5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of first cases Gram positive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of first cases Gram negative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of first cases “Other”</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Itemization & Estimation of Costs:

Direct Costs
- Incurred closer to the mastitis event
  - Diagnostics
  - Therapeutics
  - Non-saleable milk
  - Veterinary service
  - Labor
  - Death

Indirect Costs
- Tend to be more delayed
- “Lost opportunity”
  - Milk production loss
  - Premature culling and replacement
  - Reproductive loss
**Direct costs: $128**

<table>
<thead>
<tr>
<th>Cost</th>
<th>Economic Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostics</td>
<td>$10</td>
</tr>
<tr>
<td>Therapeutics</td>
<td>$36</td>
</tr>
<tr>
<td>Non-saleable milk</td>
<td>$25</td>
</tr>
<tr>
<td>Veterinary Service</td>
<td>$4</td>
</tr>
<tr>
<td>Labor</td>
<td>$21</td>
</tr>
<tr>
<td>Death Loss</td>
<td>$32</td>
</tr>
</tbody>
</table>
## Indirect costs: $316

<table>
<thead>
<tr>
<th>Cost</th>
<th>Economic Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Future milk production loss</td>
<td>$125</td>
</tr>
<tr>
<td>Premature culling and replacement loss</td>
<td>$182</td>
</tr>
<tr>
<td>Future reproductive loss</td>
<td>$9</td>
</tr>
</tbody>
</table>

These are only counted for the incident case in the first 30 DIM
Total cost per incident case of clinical mastitis occurring in the first 30 days in milk

<table>
<thead>
<tr>
<th>Cost</th>
<th>Economic Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Costs</td>
<td>$128</td>
</tr>
<tr>
<td>Indirect Costs</td>
<td>$316</td>
</tr>
<tr>
<td>Total Cost</td>
<td>$444</td>
</tr>
</tbody>
</table>
DEDICATED to the DEDICATED

Thanks!
Improving Transition Cow Health by Preventing Metabolic Stress

Lorraine M. Sordillo
College of Veterinary Medicine
Michigan State University
Presentation Overview

• Metabolic Stress (nutrient metabolism, inflammation, oxidative stress)
• Biomarkers of metabolic stress
• Monitoring and intervention strategies
Metabolic and Infectious Diseases in Transition Cows

Metabolic Stress is the Common Link

The nexus between nutrient metabolism, oxidative stress and inflammation in transition cow

L. M. Sordillo\textsuperscript{A,B} and V. Mavangira\textsuperscript{A}

Destructive feedback loops that enhance disease susceptibility.

Altered Nutrient Metabolism

Dysfunctional Inflammation

Metabolic Stress Triad

Oxidative Stress
Altered Nutrient Metabolism

- Transition from late lactation to dry period
- Transition from late gestation to early lactation
- Fetal demands
- Onset of copious milk production
- Linked to changes in nutrient requirements

Increased Need:
- Energy
- Proteins
- Glucose
- Minerals
- Vitamins
Altered Nutrient Metabolism

Nutrient Requirements

Dry Matter Intake
Altered Nutrient Metabolism

Negative Energy Balance

Energy Ingested  
Energy Required

Energy (Meal NEI/day)

Days from Calving

Babcock Institute
USDBUNON01679
Altered Nutrient Metabolism

Metabolic Adaptations to NEB

- Lipid and protein mobilization
- Alterations in blood lipids
- Increased nonesterified fatty acids (NEFA)
- Increased beta-hydroxybutyrate (BHB)

Suriyasathaporn et al., 2000
Lipid Mobilization and Immunity

Impact of Concentration

**Plasma NEFA**

- **Low Concentrations** (<0.3 mEq/L)
  - Normal immune cell functions
  - Regulated inflammation
  - Decreased risk of metabolic and infectious diseases

- **High Concentrations** (>0.7 mEq/L)
  - Abnormal immune cell functions
  - Increased inflammation
  - Increased risk of metabolic and infectious diseases
## Consequences of Altered Nutrient Metabolism

*Compromised immunity increases disease susceptibility*

<table>
<thead>
<tr>
<th>Transition Period</th>
<th>Observed Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative Energy Balance</strong></td>
<td>• Reduces antibody formation (<a href="#">van Knegsel, 2007</a>) and neutrophil functions (<a href="#">Hammon et al., 2006</a>)</td>
</tr>
<tr>
<td><strong>Elevated NEFA</strong></td>
<td>• Compromised lymphocyte functions (<a href="#">Lacetera et al. 2004</a>), impaired cytokine production (<a href="#">Scalia et al 2006</a>), reduced neutrophil function (<a href="#">Ster et al 2012</a>) and altered endothelial cell adhesion (<a href="#">Contreras et al 2012</a>)</td>
</tr>
<tr>
<td><strong>Ketosis (BHB levels)</strong></td>
<td>• Impairs neutrophil function (<a href="#">Grinberg et al., 2008</a>)</td>
</tr>
<tr>
<td><strong>Body Condition Score</strong></td>
<td>• Alters cytokine production (<a href="#">O’Boyle et al., 2006</a>) and reduces lymphocyte functions (<a href="#">Lacetera et al., 2005</a>)</td>
</tr>
</tbody>
</table>
Amplification of Lipid Mobilization

Clinical Coliform Mastitis

Plasma NEFA (n=4)

Metabolic Adaptations During the Vital 90 days

*Balance is Essential*

![Diagram showing metabolic homeostasis and inflammation](diagram.png)

Inflammation
(Essential Innate Immune Response)

- Purposes of inflammation:
  - eliminate or neutralize source of injury
  - repairs damaged tissues
  - restores normal tissue function
  - complex network of factors
  - tightly regulated response

“Balance is Essential”
Inflammatory Dysfunction in Periparturient Cows

Immunopathogenesis

- Inflammatory responses help disease progression
  - Inability of local defenses to adequately detect and eliminate pathogen (immune evasion)
  - Uncontrolled leukocyte recruitment and activation of inflammatory response
  - Delicate balance of robust response and inflammatory resolution mechanisms is lost
  - Bystander damage to host tissues
  - **MASTITIS**
Cytokines in Milk During Mastitis

Robust Initial Response

Shuster et al, 1995
Cytokine Response in Periparturient Cows

Coliform Mastitis Challenge

Proinflammatory Phenotype in Periparturient Cows

Blood Monocytes Produce More Inflammatory Cytokines

Lipid Mediators and Inflammation

**Oxylipid Biosynthesis**

- **Regulates onset and resolution of inflammation**
  - Fatty acids are substrates for oxygenation
  - Catabolism by enzymes (Cyclooxygenase, COX and Lipoxygenase, LOX)
  - Prostaglandins, thromboxanes & leukotrienes
  - Targets of NSAID therapy
  - Complex network of oxidized lipid mediators
Pro-Inflammatory Oxylipid Biosynthesis
(Streptococcus uberis infected mammary glands)

**Changes with S. uberis mastitis**
- Targeted analyses of 25 oxylipids using LC/MS
- Identified novel oxylipids not targeted by NSAIDS
- Correlations between the HODEs and inflammatory markers (cytokines & adhesion molecules)

**Figure:**
- Bar graph showing the concentration of 9-HODE and 13-HODE in uninfected and S. uberis-infected tissue samples.
- Significantly different (P ≤ 0.05) from uninfected mammary tissue samples.

Hydroxyoctadecadienoic acid (HODE)

*Ryman et al., 2015 Prostaglandins & Lipid Mediat. 121:207-217*
Systemic Inflammation in Periparturient Cows

Pro-inflammatory Oxylipids in Plasma

HODE = pro-inflammatory class
oxo-ODE = anti-inflammatory class

(Hydroxy-octadecadienoic acid)

Systemic Inflammation in Periparturient Cows

Biomarkers of Inflammation

Plasma Acute Phase Proteins

Clinically Healthy

- **↓ Positive Acute Phase Proteins**
  (haptoglobin; C-reactive protein; ceruloplasmin; serum amyloid A)

- **↑ Negative Acute Phase Proteins**
  (albumin; retinol binding protein)

J.J. Loor et al., 2005 Physiol. Genomics 23:217
Inflammation and Disease Susceptibility

More Inflammation is Associated with Higher Disease Risk

Bertoni et al., 2008. J Dairy Sci. 91:3300

Based on acute phase protein expression (+/-)
Periparturient Metabolic Stress

Role of Oxidative Stress

Oxidative Stress

Inflammation

Causes a low-grade inflammation in the absence of disease
**What is Oxidative Stress?**

*Definition:* Disturbance in the prooxidant-antioxidant balance resulting in damage to cells and tissues, Sies, 1985

- **Lipids**
  - peroxidation
  - membrane damage

- **Protein**
  - enzymes
  - receptors

- **Nucleic Acid**
  - mutations
  - carcinogenesis
What are Pro-oxidants?
Reactive Oxygen Species (ROS)

**Free Radicals**
- Super oxide anion ($O_2^*$)
- Hydroxyl radical (OH•)
- Peroxyl (RO$_2^*$)
- Alkoxyl (RO•)
- Hydroperoxyl (HO$_2$•)

**Non-Radical**
- Hydrogen peroxide ($H_2O_2$)
- Hypochlorous acid (HOCl$^-$)
- Singlet oxygen ($^{1}O_2$)
- Peroxynitrate (ONO$_2$•)
Oxidative Stress in Periparturient Cows

Sources of Reactive Oxygen Species (ROS)

• High energy demand for milk synthesis and secretion
• Electron transport chain produces ROS
• Phagocytic cells produce bactericidal ROS
• Peroxisomal fatty acid metabolism
• Moderate amounts of ROS are essential

Sordillo et. al., 2007, J. Dairy Sci. 90:1186.
Oxidative Stress in Clinical Coliform Mastitis

Evidence of Lipid Peroxidation

Anti-Oxidant Defense

• Anti-oxidant defenses reduce harmful ROS
• Molecules capable of slowing or preventing the oxidation of other molecules
• **Radical scavengers**: ascorbic acid, α-tocopherol, β-carotene
• **Enzymes**: SOD, catalase, glutathione peroxidase, thioredoxin reductase
### Micronutrients and Immunity

*Associated with controlling oxidative stress*

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Observed Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Selenium</strong></td>
<td>● improves neutrophil function; decreases severity of mastitis</td>
</tr>
<tr>
<td><strong>Vitamin E</strong></td>
<td>● increase neutrophil killing; decrease clinical mastitis</td>
</tr>
<tr>
<td><strong>β-carotene</strong></td>
<td>● increases killing by phagocytes; increase lymphocyte proliferation</td>
</tr>
<tr>
<td><strong>Chromium</strong></td>
<td>● improves lymphocyte functions; increased antibody responses</td>
</tr>
<tr>
<td><strong>Copper &amp; Zinc</strong></td>
<td>● deficiency decreases neutrophil functions &amp; increases susceptibility to disease</td>
</tr>
</tbody>
</table>
Decline in Antioxidant Micronutrients During the Periparturient Period

Plasma Vitamin E

Plasma Vitamin A

Goff and Stabel. 1990 J. Dairy Sci. 73:3195
Oxidative Stress During the Periparturient Period

Antioxidant Potential of Whole Blood
Reduction of Cu^{++} to Cu^{+}

Lipid Hydroperoxide Levels in Plasma
Indicator of Free Radical Damage

**Obesity and Oxidative Stress in Dairy Cows**

*Lipid Mobilization & Pro-oxidant Production*

---

**Body Condition Score**

<table>
<thead>
<tr>
<th></th>
<th>Low (&lt;2.5)</th>
<th>Medium</th>
<th>High (&gt;3.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS</td>
<td>142.2±3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>129.3±5.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.4±3.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>TBARS, nmol/mL</td>
<td>1.77±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.69±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.10±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

---

**NEFA**

- **High BCS**

![NEFA Graph](chart)

**BHBA**

- **High BCS**

![BHBA Graph](chart)

---

Obesity, Oxidative Stress, and Inflammation

Enhances Metabolic Stress

Increased TNF-α expression in high BCS cows (>3.5)

<table>
<thead>
<tr>
<th>TNF-α source</th>
<th>High BCS</th>
<th>Normal BCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma (ng/ml)</td>
<td>.53 + .05*</td>
<td>.41 + .05</td>
</tr>
<tr>
<td>LPS Whole blood (ng/ml)</td>
<td>6.73 ± 1.33</td>
<td>4.70 ± .46</td>
</tr>
</tbody>
</table>

*Significant difference with respect to BCS (P<0.05).

Reduced Antioxidant Potential

Reduction of Cu++ to Cu+

Oxidative Stress During the Periparturient Period

*Imbalance causes tissue damage and health disorders*

- Metabolism
- Inflammation

- Vitamin E
- Selenium

\[
\begin{align*}
\text{Oxidants} & : \quad O_2^- \quad H_2O_2 \quad \text{hydroperoxides} \\
\text{Antioxidants} & : \quad \text{catalase} \quad \text{superoxide dismutase} \\
& \quad \text{glutathione peroxidase} \quad \text{thioredoxin reductase}
\end{align*}
\]
Controlling Metabolic Stress Starts at Dry-off

*Diseases at 30 days after calving can be a result of cow management in the 60 days before calving*
Controlling Metabolic Stress Starts at Dry-off

A need for effective monitoring systems and benchmarking

Prospective Monitoring

Proactive Interventions

Disease Incidence

Retrospective Monitoring

Reactive Interventions
Adoption of Best Management Practices to Reduce Production Losses in Transition Dairy Cattle

L.M. Sordillo (PD), B. Norby, A. Contreras, R. Malinowski, & R. Martinez
Michigan State University, East Lansing, MI
S. Shaik, North Dakota State University, Fargo, ND

Objectives:
1. Identify biomarkers of metabolic stress during the dry period that can be used as indicators of disease risk during early lactation.

1. Develop a practical decision-making tool that monitors biomarkers of metabolic stress and suggest improvements in managing dry cows.
What Are Biomarkers?

- **Objective measurements used as an indicator of:**
  - Normal physiological processes
  - Health disorders
  - Response to intervention (management, drugs, nutritional supplements)
Methods of Assessing Metabolic Stress

Biomarkers for Individual Cow Diagnostics

Lipid Mobilization
- NEFA
- Beta-hydroxybutyrate
- Calcium
- Dry matter intake
- Body condition score

Inflammation
- Albumin
- Cholesterol
- Retinol
- Serum amyloid A
- Haptoglobin
- Blood cell counts

Oxidative Stress
- Anti-oxidant potential
- Reactive oxygen species
- F₂ Isoprostanes
- TBARS

*Only NEFA, BHB, and hypocalcemia are validated as disease predictors at the herd level.*
Controlling Metabolic Stress Starts at Dry-off

*What Biomarkers Can Predict Early Lactation Disease Risk?*

**Prospective Monitoring**

**Proactive Interventions**

**Monitoring Metabolic Stress Risk**

(minimum 15 cows/cohort)
Risk Assessment Tool

Reduce transition cow disease risk

Dairy Consultant Enrollment

Download iPad app from Apple App Store

Consultation and Decision Making with Farm Management Team

Report output includes farm scores, recommended actions, and user content

Use app as a guided walk-through of farm conditions in The Vital 90 Days

The Vital 90 Days
Vital 90™ Days Risk Assessment Tool

- **Facilitated Physical Exam Tool**
  - Facility/housing system
    - Cow comfort
  - Management
    - Cow handling & movement
    - Environment
  - Nutrition
    - Specific focus on
      - Water
      - Energy
      - Metabolizable protein
      - Cow - BCS
Controlling Metabolic Stress

Requires coordinated adaptations across multiple physiological systems

- Altered Nutrient Metabolism
- Dysfunctional Inflammation
- Metabolic Stress
- Oxidative Stress
Practical Considerations

- **Reduce intense lipid mobilization during the transition period**
  - Minimize reductions in dry matter intake (DMI)
  - Design diets to increase energy without affecting DMI
  - Prevent over-conditioning in the dry period

- **Optimize inflammatory responses and oxidant balance**
  - Reduces sources of stress (heat stress, exposure to pathogens)
  - Micronutrient supplementation (Se, Vitamin E, etc.)
  - Immunomodulators (vaccines, supplements, etc.)

- **Comprehensive Approach to Reduce Metabolic Stress**
  - Monitoring Programs
  - Inclusive of entire dry period and early lactation
  - Early intervention
Global Dairy Immunity Awareness Survey
January 31, 2016

Paul Rapnicki, DVM, MBA
Dairy Technical Consultant
Elanco Animal Health

Among dairy producers in the U.S., Brazil, France, Germany, UK, Spain and New Zealand
Elanco recently conducted a global survey among dairy farmers to better understand their views about the consequences of immune suppression around calving and the impact on successful lactation cycles.
Worldwide Survey Among Dairy Farmers

More than **1,200** dairy farmers surveyed

Seven countries, including U.S., Brazil, France, Germany, UK, Spain and New Zealand

U.S.

- **187** dairy farmers; **91%** owners
- **850** avg. herd size
- **33** avg. years of experience
Key Findings

Unprompted, U.S. dairy farmers identified three top healthcare priorities:

- 28% Repro.
- 32% Mastitis
- 40% Nutrition

96% of U.S. dairy farmers indicate the Vital 90 Days are “very important”

- More than 90% of farmers worldwide rank The Vital 90 Days as “very important”
- #1 reason for this importance across all seven countries...

Achieving Full Lactation Potential
Understanding of Immune Suppression in Dairy Cows

Producers are knowledgeable that immune suppression leads to risk of disease

- On scale of 1 to 7, U.S. farmers rank their knowledge of immune suppression as 5.4
- Yet, only 6% of U.S. farmers listed immune suppression as a concern

U.S. ranking of top five immune suppression consequences:

- Mastitis: 59%
- Pneumonia: 48%
- Ketosis: 30%
- Metritis: 27%
- Milk Fever: 18%
Dairy farmers across the globe are largely unfamiliar with the term “neutrophils”

- Between 9% and 36% - depending on country - express unfamiliarity

- Neutrophils are the primary type of white blood cell that recognize and kill harmful bacteria

- Farmers are missing the link between immune suppression and the decline in level and function of neutrophils
Producers Are Talking to Their Veterinarians About Protecting Their Cows’ Immune Systems

Have you ever initiated a conversation with your veterinarian regarding protecting your cows’ immune systems around the time of calving?

- Yes 83%
- No 17%

(n = 187)
Opportunities for Managing Cow Health

Veterinarians fill a vital role in helping producers understand immune suppression

- Producers are looking for solutions and are open to talking with their veterinarians about immune suppression
- According to the survey, producers feel empowered to address immune suppression and they want their veterinarians to be proactive with the conversation and ways to address

Why?

- Producers want to take the best care of their cows
- Minimize disruption to their routine
- Accomplish work faster/easier

End Goal: Achieving Full Lactation Potential
Questions?
Thank you for all that you do!