Serum Haptoglobin as an Indicator for Calving Difficulties and Postpartal Diseases in Transition Dairy Cows

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**TRANSITION PERIOD**

- 3 weeks from expected calving date to 3 weeks postpartum
  - Elevated incidence of metabolic and infectious diseases
  - Increased exposure and susceptibility of the mammary gland and uterine tract to bacteria
Transition Period

20% older cows die  90% become ill

Milk Loss and Treatment Costs

Cow Quality of Life decreased
Profit and Milk Quality decreased
**Haptoglobin**

- Acute Phase Protein
- Primary synthesis in the liver
  - Secondary synthesis in various body tissues
    - Mammary gland
    - White blood cells
    - Adipose tissue
    - Ovaries
ACUTE PHASE RESPONSE

- Body’s response to infectious agents that can cause stress, trauma, and inflammation
  - Innate immune system

- Haptoglobin primarily serves to prevent further tissue damage and promote repair
  - Proportional to severity of challenge
Antioxidant

Anti-Inflammatory Agent

Immunomodulator

Bacteriostat

Tissue-Regeneration Agent

Functions of Haptoglobin
Prevents future tissue damage and promotes tissue generation
HAPTOGLOBIN IN BOVINE

- Proposed as an indicator of acute and chronic diseases
- Limited sensitivity (percent of animals detected as sick)
  - Delayed reaction (24 hr) to tissue damage or infection
  - Decreases after an acute infection
  - Does not always go up during disease
Hp Increase
2-3 g/L

Baseline Concentration
25-35 mg/L

Challenge

≤ 24 hours, >10-fold

Peaks between 60 & 80 hrs Up to 14 d
Objective 1

Evaluate whether peripartal [Hp] were associated with:
• Health status and severity
• Type and number of diseases

Objective 2

• Examine whether prepartal [Hp] indicate birth complications
• Examine whether [Hp] were elevated prior to clinical signs of diseases
**Hypothesis 1**

Haptoglobin concentrations will increase in the peripartal period:
- In relation to health status, severity, type and number of diseases

**Hypothesis 2**

Haptoglobin concentrations will increase in the peripartal period:
- Prepartum in cows that had birth complications
- Prior to the onset of clinical signs of diseases
METHODS

- Van Beek Dairy in Monroe, Oregon, in Spring and Summer of 2010
- 161 multiparous Holstein cows
- 4 weeks prior to expected calving date to 4 weeks post-calving
METHODS: ANIMAL MANAGEMENT

- Between days -28 and 100 postpartum, cows were monitored daily for signs of diseases.
- Medical treatment was provided and recorded by herd manager and recorded in Dairy Comp (Valley Ag. Software, Inc., Tulare, CA).
**Blood Collection**

- Blood samples were taken according to the figure below (0 = day of calving)
**Blood Collection**

- 5-8 mL of blood was taken from the coccygeal vein or artery in a 10 mL serum vacutainer tube
- Samples were placed on ice and transported to lab
  - Serum was separated by centrifugation at room temperature for 20 minutes at 1600 x g
  - Stored at -20°C until chemical analysis
**Blood Analysis**

- Samples were analyzed using a bovine haptoglobin enzyme-linked immunosorbent assay (ELISA)
  - Life Diagnostics, Inc., Catalog number: 2410-7
- Procedure was conducted according to manufacturer's instructions
**Classification of Groups**

- **Disease Status and Severity**

**Healthy (n=19)**
- No medical treatment
- SCC < 1,000,000 cells/mL and
- BHBA < 1.3 mmol/L

**Mild Disease (n=49)**
- Treated but no glucose precursors or antibiotics,
- SCC > 1,000,000 cells/mL or
- BHBA > 1.3 mmol/L

**Severe Disease (n=63)**
- Treated with antibiotics with withdrawal period
- Oral or I.V. glucose precursors

**Died/Sold (n=30)**
- Died or sold in the first 100 days postpartum
<table>
<thead>
<tr>
<th>Disease Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (n=20)</td>
<td>No medical treatment</td>
</tr>
<tr>
<td>Mild Disease (n=17)</td>
<td>Treated without glucose precursors</td>
</tr>
<tr>
<td>Other Diseases (n=19)</td>
<td>Diseases other than ketosis, metritis, or mastitis</td>
</tr>
<tr>
<td>Ketosis (n=20)</td>
<td>BHBA &gt; 1.3 mMol/L</td>
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<tr>
<td>Metritis (n=21)</td>
<td>Placental retention or purulent/putrid vaginal or cervical discharge</td>
</tr>
<tr>
<td>Mastitis (n=17)</td>
<td>Milk flakes, swelling, or SCC &gt; 1,000,000 cells/mL</td>
</tr>
<tr>
<td>2+ Diseases (n=47)</td>
<td>Cows with more than 1 disease</td>
</tr>
</tbody>
</table>
# Classification of Groups

- **Healthy (n=63)**
  - Healthy cows or cows with mild diseases

- **Other Severe Disease (n=70)**
  - Severe disease without birth complications

- **Birth Complications (n=28)**
  - Twinning (n=16)
  - Hard pull or C-Section (n=8)
  - Both (n=4)
CLASSIFICATION OF GROUPS

- First Treatment Time

- No Treatment (n=39)
- Treated D-21 to -1 (n=14)
- Treated D0 to 3 (n=50)
- Treated D4 to 7 (n=25)
- Treated D8 to 28 (n=28)
SUMMARY OF CLASSIFICATION GROUPS

- Disease Status and Severity
- Disease Number and Type
- Birth Complications
- First Treatment Time
Figure 1: Elevated serum haptoglobin concentrations during the first week postpartum indicate disease status and severity of dairy cows during the peripartal period. Cows in the two severe groups had greater [Hp] than mild diseases ($P < 0.001$).
Figure 2: Compared to healthy cows, sick cows had greater peak [Hp] in the first wk after calving ($P < 0.001$). Cows with severe diseases had greater peak [Hp] than the mild/healthy groups in the first wk after calving ($P < 0.001$). Cows that were sold or died had greater peak [Hp] than cows with severe disease in wk 2 to 4 postpartum ($P = 0.04$).
Figure 3: Compared to healthy cows, sick cows had greater [Hp] AUC values in the first wk postpartum ($P < 0.001$). Cows with severe diseases had greater [Hp] AUC values than the mild/healthy groups in the first wk postpartum ($P < 0.001$). Cows that were sold or died had greater [Hp] AUC values than cows with severe disease in wk 2 to 4 postpartum ($P = 0.02$).
Figure 4: Disease number and type affect [Hp] AUC values. Cows with ketosis, metritis, and 2 or more diseases had the greatest [Hp] AUC values in wk 1 postpartum. Cows with mastitis and 2 or more disease had the greatest [Hp] AUC values in wk 2 to 4 postpartum.
Figure 5: Disease number and type affect peak [Hp]. Cows with ketosis, metritis, and 2 or more diseases had the greatest peak [Hp] in wk 1 postpartum. Cows with mastitis and 2 or more disease had the greatest peak [Hp] in wk 2 to 4 postpartum.
Figure 6: Cows with versus without birth complications had greater [Hp] at days -14 prepartum ($P < 0.001$)
Figure 7: Compared to cows without birth complications, cows with birth complications had greater [Hp] AUC values ($P < 0.001$) and peak concentrations ($P = 0.004$) in the last 3 wks prepartum.
Figure 8: Cows that were treated first within day 0 to 3 postpartum had greater [Hp] at days -7 ($P = 0.05$) and -3 ($P = 0.01$) postpartum.
Figure 9: Cows that were treated first between 4 and 7 days postpartum had greater [Hp] at days 1 ($P = 0.04$) and 3 postpartum ($P < 0.001$).
**Figure 10:** Cows that were treated first between 8 and 28 days postpartum had greater [Hp] at days 3 ($P = 0.002$) and 7 ($P < 0.001$) postpartum.
Elevated serum haptoglobin concentrations during first week postpartum indicate disease:

- Incidence
- Severity
- Number
- Type
SUMMARY

- Elevated serum haptoglobin concentrations precede birth complications and clinical diagnosis and treatment of peripartal diseases
CONCLUSION

- Serum haptoglobin may assist in early detection and treatment of diseases in early lactation
IMPACT

- Increased profit
- Shorter time period between parturition and resumption of estrus cycle
- Consistent dairy products for consumers
- Happy cows! 😊
Future Research

- Repeat the study on a larger scale and at various farms that differ in management protocols
  - Include heifers
  - Diseases to be diagnosed by a veterinarian
Thank you:

- Dr. Gerd Bobe and my fellow laboratory peers
- Diamond V, ER Jackman Internship Support Program, and USDA’s Multicultural Scholars Program
- Van Beek Dairy
- Family and friends!
QUESTIONS?
FUNCTIONS OF HAPTOGLOBIN

- Antioxidant
  - Binds to free-floating hemoglobin to prevent unwanted oxidation
  - Transports complex to CD163 receptor on monocytes, then degraded in the lysosomes
FUNCTIONS OF HAPTOGLOBIN

- Anti-Inflammatory Agent
  - Prevents oxidation damage of cells, hence, the release of pro-inflammatory cytokines
  - Inhibits the activity of cyclooxygenase (COX) and lipoxygenase (LOX) in platelet cells
    - COX and LOX promote inflammation and oxidation of LDL
FUNCTIONS OF HAPTOGLOBIN

- Tissue-Regeneration Agent
  - Promotes the migration of fibroblasts needed for tissue regeneration
  - Inhibits the activities of matrix metalloproteinases, which promote tissue breakdown
FUNCTIONS OF HAPTOGLOBIN

- Bacteriostat
  - Prevents the growth of pathogenic bacteria that require the iron from hemoglobin
FUNCTIONS OF HAPTOGLOBIN

- Immunomodulator
  - Attracts monocytes and macrophages to site of infection
    - Binds to decrease their production of pro-inflammatory cytokines