Nutrition, Immune Function and Health of Dairy Cattle

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Outline

› Introduction
  › Structural changes in the dairy industry
  › Yield and health and the periparturient animal
  › Sources of variation in immune function
› Energy Balance and Immune Function
› Nutrients and metabolites and immune function
  › Glucose
  › Glutamine
  › BHBA
  › NEFA
› Preventing diseases through nutrition
› Future challenges
Changes in the primary structure in Denmark

From 1984 and onwards these are farms with milk quota

1.5%/year

Consequences of 1,5% increase in milk yield / year?

IMPROVED:
Breeding
Feeding
Management
Environment/Housing

OUTPUT
↑ Milk yield
↑ Efficiency
↓ Health?
↓ Reproduction
Relationship between milk yield and veterinary treatments in Norwegian dairy cows (Solbu, 1983)

Do high yielding cows have increased risk of disease? (Ingvartsen et al. 2003)

- **Based on:**
  - 11 epidemiological studies
  - 14 genetic studies

- **Conclusions:**
  - Contradictory results
  - Phenotypic
    - Unlikely: dystocia, retained placenta, metritis, LDA
    - Probably: mastitis, parturient paresis
  - Genetic Selection
    - Maybe: ketosis, lameness
    - Probably: mastitis

- Reviewing existing literature, even with structured literature selection, is inadequate for elucidating the true relationship between lactational performance and risk of production diseases
### Treatment Rates in Danish Dairy Cows

#### Treatments / 100 cows / year

<table>
<thead>
<tr>
<th></th>
<th>1969-75 (A)</th>
<th>1990 (B)</th>
<th>2007 (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calving assistance</td>
<td>7</td>
<td>7</td>
<td>0.8</td>
</tr>
<tr>
<td>Retained placenta</td>
<td>11</td>
<td>10</td>
<td>9.4</td>
</tr>
<tr>
<td>Reprod. Disorders/Metritis</td>
<td>10</td>
<td>10</td>
<td>7.1</td>
</tr>
<tr>
<td>Parturient paresis</td>
<td>12</td>
<td>5</td>
<td>3.8</td>
</tr>
<tr>
<td>Ketosis</td>
<td>3</td>
<td>4</td>
<td>3.2</td>
</tr>
<tr>
<td>Displaced abomasum</td>
<td>12</td>
<td>12</td>
<td>3.1</td>
</tr>
<tr>
<td>Feet &amp; leg disorders</td>
<td>17</td>
<td>8</td>
<td>12.1</td>
</tr>
<tr>
<td>Mastitis</td>
<td>55</td>
<td>56</td>
<td>43.9</td>
</tr>
<tr>
<td>Other diseases</td>
<td>10</td>
<td>14</td>
<td>18.2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>145</strong></td>
<td><strong>132</strong></td>
<td><strong>103</strong></td>
</tr>
</tbody>
</table>

A: Jørgensen & Nielsen, 1977; B: Andersen, 1991; C: Krog & Trinderup, 2008

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### Disease incidence relative to days from calving

(Ingvartsen et al., 2003)

![Disease incidence graph](image)
Acceleration in milk yield through lactation
(Ingvartsen et al., 2003, Hansen et al. 2006)

> Cause of increased disease risk:
> 1. Probably not yield per se
> 2. Rate of increase in daily milk yield (acceleration) → Adaptational problems. Physiological imbalance?

Sources of variation in immune function – potentially increasing risk of infectious diseases

- Physiological state
- Hormonal status
- Nutrition
- Body condition
- Stress (env., phys., psyc.)
- Exposure to pathogens
- Early life events
- Gender
- Genetics
- Age

Risk of infectious diseases
Physiological – immunological interaction and risk of infections (mod. from Ingvartsen 2003)

Energy Balance and Immune Function: Results from in vivo studies vary and are inconclusive

> Feed restriction (16 yearling Holstein steers)
  – Enhanced PMN gene expression (Perkins et al., 2001)
> Feed restriction/mastitis challenge (Holstein cows)
  – No effect (Perkins et al., 2002)
  – Ketonemic cows = more severe mastitis than non-ketonemic cows (Kremer et al., 1993)
  – Lower PMN phagocytic capability, higher APP in milk (Moyes et al., 2009)
> Epidemiological studies during postpartal NEB
  – ↑ BHBA and NEFA associated with mastitis (Jánosi et al., 2003; Moyes et al., 2009; Nyman et al., 2008)
Dysfunctions observed in periparturient cows

<table>
<thead>
<tr>
<th>Leukocytes</th>
<th>Dysfunction</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neutrophils</strong></td>
<td>↓ Oxidative metabolism <em>in vitro</em></td>
<td>Detilleux <em>et al.</em>, 1995</td>
</tr>
<tr>
<td></td>
<td>↓ Neutrophil chemotaxis <em>in vitro</em></td>
<td>Kehrli <em>et al.</em>, 1989b</td>
</tr>
<tr>
<td></td>
<td>↓ Antibody dependent cell-mediated cytotoxic reaction <em>in vitro</em></td>
<td>Nagahata <em>et al.</em>, 1988</td>
</tr>
<tr>
<td></td>
<td>↓ CD62L and CD18* expression <em>in vivo</em></td>
<td>Cai <em>et al.</em>, 1994</td>
</tr>
<tr>
<td></td>
<td>↓ Phagocytosis</td>
<td>Paape <em>et al.</em>, 1981</td>
</tr>
</tbody>
</table>

| Monocytes/Macrophages | ↑ TNF-α production induced by LPS** | Sordillo *et al.*, 1995 |
|                       | ↓ TNF-α production | Røntved, 2000 |

| **Lymphocytes** | ↓ Number in blood *in vivo* | Kehrli *et al.*, 1989a |
|                 | ↓ Cell division | Park *et al.*, 1992 |
|                 | ↓ IFN-γ production | Kehrli *et al.*, 1989a |
|                 | ↑ CD62L and CD18* expression | Lee & Kehrli, 1998 |

* CD62L and CD18: adhesion molecules involved in the migration from blood into tissue.
** Lipopolysaccharide (LPS) constitutes a part of the cell surface of gram negative bacteria.

Fates of energetic fuels in leukocytes

Adapted from Calder *et al.*, 1990; Newsholme *et al.*, 1986, 2004
Nutrient Supply and Leukocyte Function

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Function</th>
<th>Peri-parturient Supply</th>
<th>Low Levels</th>
<th>Moderate/High Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Proliferation, survival, differentiation; chemotaxis/ phagocytosis energetic fuel</td>
<td>Low</td>
<td>Phagocytic capability</td>
<td>Phagocytosis and chemotaxis</td>
</tr>
<tr>
<td>Glutamine</td>
<td>DNA/RNA synthesis; ROM and NO production; energetic fuel</td>
<td>Low</td>
<td>??</td>
<td>cytokine/ROM production and cell division</td>
</tr>
<tr>
<td>BHBA</td>
<td>Not used as fuel source; no known function</td>
<td>High</td>
<td>No effect</td>
<td>Chemotaxis, respiratory burst, IgM secretion</td>
</tr>
<tr>
<td>NEFA (SFA vs UFA)</td>
<td>Adhesion, phagocytosis, ROM/cytokine production, apoptosis, TLR signaling, T cell activation</td>
<td>High</td>
<td>??</td>
<td>Antigen presentation, cytokine/ROM production; TLR signaling Phagocytosis, necrosis</td>
</tr>
</tbody>
</table>

Glucose and glutamine supply

› The immune cells use glucose and glutamine as energy source, e.g. for their oxidative metabolism (oxidative burst) (Bashan et al., 1993)

› Glutamine constitutes 43% of AA in muscle tissue in dry cows, 24% in early lactation (Meijer et al., 1995)

› Glutamine conc. in plasma can drop by up to 50% at sepsis

› Neutrophils have a lifespan of 7-10 hours

› Monocytes approx. 8 hours in blood, subsequently macrophages in tissue for days or weeks
Glucose and immune function

- Glucose is required by most phagocytic cells (i.e., macrophages and PMN)
  - preferred metabolic fuel during inflammation for activated PMN, macrophages, and lymphocytes (Barghouthi et al., 1995, Gamelli et al., 1996)
- Glucose uptake in murine neutrophils and peritoneal macrophages after stimulation with LPS in vitro (Barghouthi et al., 1995, Schuster et al., 2007)
- Inhibition of glucose uptake phagocytic capabilities and risk of infection in murine macrophages (Barghouthi et al., 1995, Lang and Dobrescu, 1991)
- Cellular uptake of glucose in phagocytic cells is facilitated via the non-insulin dependent glucose transporter (GLUT1) (Fukuzumi et al., 1996)
- Efficient glucose uptake by immune cells is critical for maintaining cellular functions and an optimal host response to invading microbes

But what is the situation in the cows – particularly in early lactation?

Function and target molecule of glutamine in different cells and organs

Adapted from Curi et al., (2007)
Liver metabolic fate of exported muscle glutamine in catabolic states

Liver metabolic fate of exported muscle glutamine in catabolic states

Ketosis and leukocyte function

<table>
<thead>
<tr>
<th>Udder defence mechanisms</th>
<th>Ketotic individuals</th>
<th>Leukocyte functional capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytic defence</td>
<td></td>
<td>In vitro, + ketone bodies</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>Unchanged in human ketosis</td>
<td>Inhibits PMN phagocytosis</td>
</tr>
<tr>
<td>Bact. activity</td>
<td>Prod. of superox. lower in leukocytes from humans in NEB</td>
<td>Inhibited in cultures supplemented with BHB</td>
</tr>
<tr>
<td>Inflammation mediators</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opsonins</td>
<td>Lower IgG tetanus toxin at immunisation</td>
<td></td>
</tr>
<tr>
<td>Chemoattractants</td>
<td>Lower prod. of cytokines from leukocytes from ketotic cows</td>
<td>Lower cytokine prod. in leukocytes from humans in NEB</td>
</tr>
<tr>
<td>Leukocyte chemotaxis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of blood leukoc.</td>
<td>Lower with increasing BHB level in ketotic cows</td>
<td>Ketone bodies inhibit the proliferation of bovine bone marrow cells</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>Lower in leukocytes from spontaneously ketotic cows</td>
<td>Lower chemotaxis if supplemented with ketone bodies</td>
</tr>
</tbody>
</table>

(Suriyasathaporn et al., 2000)
BHBA and AcAc inhibit bovine bone marrow cell proliferation

BHBA inhibits chemiluminescence (CL) of bovine neutrophils at 1.0 and 2.5 mM

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Cellular CL</th>
<th>Cell-free MPO-activity</th>
<th>Superoxide production</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>98.7</td>
<td>95.4</td>
<td>100.1</td>
</tr>
<tr>
<td>0.05</td>
<td>95.9</td>
<td>96.7</td>
<td>96.5</td>
</tr>
<tr>
<td>0.10</td>
<td>95.6</td>
<td>99.2</td>
<td>97.8</td>
</tr>
<tr>
<td>1.00</td>
<td>89.6*</td>
<td>92.2</td>
<td>98.4</td>
</tr>
<tr>
<td>2.50</td>
<td>64.3***</td>
<td>90.9</td>
<td>96.3</td>
</tr>
</tbody>
</table>

Hoeben et al., 1999.
Effect of ketones on immune cells in ruminants

<table>
<thead>
<tr>
<th>Leukocytes</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>↓ cellular chemiluminescence</td>
<td>Hoben et al., 1997</td>
</tr>
<tr>
<td>Monocytes/ macrophages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>↓ mitogen response</td>
<td>Targowski &amp; Klucinski, 1983</td>
</tr>
<tr>
<td></td>
<td>↓ mitogen response at exp. ketosis in calves</td>
<td>Targowski et al., 1985</td>
</tr>
<tr>
<td></td>
<td>↓ mitogen response (milk)</td>
<td>Klucinski et al., 1988</td>
</tr>
<tr>
<td></td>
<td>No effect on mitogen response</td>
<td>Franklin et al., 1991</td>
</tr>
<tr>
<td></td>
<td>No change in the IgM secretion</td>
<td>Nonnecke et al., 1992</td>
</tr>
<tr>
<td></td>
<td>No effect on mitogen response (milk)</td>
<td>Nonnecke &amp; Kehrli, 1985</td>
</tr>
<tr>
<td></td>
<td>No effect on DNA and IgM synt.</td>
<td>Lacetera et al., 2002</td>
</tr>
<tr>
<td></td>
<td>↓ IFN-γ production in ketotic cows</td>
<td>Szuster-Ciesielska et al., 1995</td>
</tr>
</tbody>
</table>

TLRs and inflammatory response

Lee and Hwang, 2006
Shift in plasma NEFA profiles around parturition

Adapted from Douglas et al., 2007

NEFA and TLR signaling

› Parameters italicized always indicates gene expression

› Saturated Fatty Acids:
  › Activate TLR signaling
    › \( \uparrow \text{COX2 expression in murine monocytes} \)
      \( \text{Lee et al., 2001} \)

› Unsaturated Fatty Acids:
  › Fail to activate or inhibit TLR signaling
    › \( \downarrow \text{NFkB expression (Lee et al., 2003)} \)
    › \( \downarrow \text{PPARy (Lee et al., 2004)} \)
Energy balance and TLR signaling in dairy cows: complex - not fully understood

- Dietary-induced NEB in mid-lactation: blood PMN
  - \( \uparrow \) TLR2, TLR4, IL1R2, IL6
  - \( \downarrow \) TNF\( \alpha \) and IRAK1
    Moyes et al., 2010

- Post-partal NEB:
  - \( \downarrow \) ATF3 (feedback inhibitor of TLR4)
  - \( \downarrow \) TRAF6 and IL8
    Stevens et al., 2011

Energy balance and immune function cont’:
Transcription-level

- Mammary: \( \downarrow \) pro-inflammatory response
  - \( \downarrow \) IL-8 Signaling (80% of genes)
  - \( \uparrow \) Anti-inflammatory genes
    (Moyes et al., 2010)

- Other tissues: results vary
  - Spleen: \( \uparrow \) oxidative stress; \( \downarrow \) pro-inflammatory cytokines (Morris et al., 2009)
  - Liver: \( \uparrow \) oxidative stress; \( \uparrow \downarrow \) pro-inflammatory response
    (McCarthy et al., 2010; Douglas et al., 2007)
  - Uterus: \( \uparrow \) pro-inflammatory cytokines and antigen presentation
    (Wathes et al., 2009)
Preventing disease through nutrition

› Avoid overconditioning at calving
› Strategies should be implemented during lactation rather than dry period (Ingvartsen, 2006)
› Limit prepartum energy intake (Dann et al., 2006)
› ↓ NEFA, BHBA and liver TAG postpartum
› ↓ magnitude of change in BCS
› Minimize drops in DMI around calving
› Greater dips associated with ↑ NEFA and BHBA postpartum (Ingvartsen & Andersen, 2000; Ingvartsen, 2006; Drackley et al., 2006)
› Feeding high quality feeds/nutrients to secure optimal nutrient supply and physiological status to secure optimal:
› Production and efficiency, product composition
› immune function and health
› reproduction

Future challenges in prevention in individual cows through precision management

› Have we solved all the problems?

NO!

• Understanding individual differences
• Better in describing cow status and risks
• Animal status oriented strategies based on risk management
Effect parity and TMR energy density on plasma BOHB concentration

Weeks around calving
Parity:
- ∆ = 1.
- □ = 2.
- △ = 3.

Days around calving

Changes in NEFA, BHBA and glucose around calving, example 1 – the expected
Changes in NEFA, BHBA and glucose around calving, example 2 – the mobilizing healthy cow

Changes in NEFA, BHBA and glucose around calving, example 3 – the mobilizing risk cow
Hypothesis & definition regarding Physiological Imbalance (PI)

› Hypothesis: Immune function and health can be improved by reducing the PI in cows, and at the same time it will improve production and reproduction (Ingvartsen et al., 2003, 2006; Ingvartsen and Moyes, 2012)

› Definition of PI: cows whose parameters (e.g. glucose, BHBA, NEFA) deviate from the normal, and who consequently have an increased risk of developing diseases (clinical or subclinical) and reduced reproduction and/or production (Ingvartsen, 2006)

Future focus

› To better understand the immunophysiology of the ruminant animal, particularly in the periparturient period
› New tools in the “age of the –omics”
› To better understand the biological basis of the imbalance measured:
  › quantitative understanding
  › importance for e.g. immune function and risk of disease
  › be able to predict individual animal responses to changes in e.g. nutrient supply or management to overcome the physiological imbalance
› Potentially we lack more specific biomarkers and sensors to take on the challenge of building future automatic and proactive strategies
› New sensors and technology