Effects of rumen protected choline during transition phase on haematology of dairy cows

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S12 Nutrition of the high yielding cow
Introduction

Previous findings on choline in dairy cows

• Milk production and quality ⇒ generally, increased milk fat production
• Liver metabolism ⇒ improved prevention of fatty liver and ketosis
• Reproduction ⇒ (?)
• Haematology ⇒ (?)

2 types of role for choline:
  • Role of choline *per se*
  • Role as a methyl donor

Choline, metabolism, and haematology
Introduction

Choline *per se*

PtdCho component
60 % of milk phospholipids contain choline → phospholipids are 0.5-1.0 % of total milk lipids → 105-210 mg/L of choline-containing phospholipids ⇒ choline is a limiting metabolite in lactating mammary tissue (Pinotti et al., 2002)

PtdCho essential component of VLDL and cannot be substituted by other phospholipids (Pinotti et al., 2002)

Increased synthesis of PtdCho → building of membranes during phagocytosis (García Gil et al., 1982; Biochem J)
Introduction

Choline as methyl donor

2 principal methyl donors in animal metabolism
- choline → betaine
- methionine → S-adenosyl-methionine

Methyl groups may be derived from
- exogenous sources: Met, betaine, choline
- *de novo*: tetrahydrofolate (THF) system → folic acid and vitamin B12
Introduction
Choline as methyl donor

Labile methyl groups: choline and Met are interrelated.
High producing dairy cow: Met is the 1st limiting a.a.
Elevated requirement for Met for transmethylation reactions and milk protein synthesis ⇒ altered methyl group metabolism (Lobley et al., 1996)
Ruminants → conservative methyl group metabolism
  • elevated rate of de novo methyl group synthesis from the one-carbon pool
  • low rate of methyl catabolism (low activity of choline oxidase)

Interchangeability → 28 % of Met is used for choline synthesis (Emmanuel and Kennelly, 1984)
Why haematology in nutrition?

**Leukocyte**

- progenitor cells (bone marrow): ketone bodies inhibit growth and differentiation → ↓ circulating PMN (Hoeben et al., 1999)

- adipocytes in bone marrow? (Mikhail et al., 1997)

- development and proliferation: pH, BHBA (Donovan et al., 2003)

- functions: hormones (insulin, Okouchi et al., 2002), metabolites, vitamins, and trace minerals (Lacetera et al., 2002; Politis et al., 2004; Weiss and Hogan, 2007)

- BHBA → ↓ NEU extracellular traps and bactericidal activity (Grinberg et al., 2008)
Why haematology in nutrition?

**Leukocyte**

- Phagocytosis of apoptotic circulating PMNs: liver and Kupffer cells status *(Shi et al., 2001)*

**Erythrocyte**

- Nutritional status for vitamins and trace minerals
- Membrane fatty acids

**Aim of this work**

To examine the effects of supplementation of rumen protected choline (RPC) to transition cows on haematological features
Material and Methods

Animals

- 22 Italian Friesian cows (parity ≥ 2) randomly assigned by
  - expected calving date
  - parity
  - previous milk yield
- to either be supplemented with rumen-protected choline (RPC) from d –21 relative to expected parturition until 35 DIM, or to consume basal diet only (CON)

Herd management and data recording

- cows milked with an automatic milking system
- milk yield was recorded at each milking
## Material and Methods

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit</th>
<th>Prepartum</th>
<th>Lactation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>% as fed</td>
<td>67.8</td>
<td>54.7</td>
</tr>
<tr>
<td>Estimated DMI</td>
<td>kg/d</td>
<td>11.3</td>
<td>20.1</td>
</tr>
<tr>
<td>Nutrients (on DM basis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{NE}_L$</td>
<td>Mcal/kg</td>
<td>1.38</td>
<td>1.64</td>
</tr>
<tr>
<td>ME</td>
<td>Mcal/d</td>
<td>24.3</td>
<td>49.2</td>
</tr>
<tr>
<td>CP</td>
<td>%</td>
<td>13.6</td>
<td>16.2</td>
</tr>
<tr>
<td>NDF</td>
<td>%</td>
<td>51.0</td>
<td>35.5</td>
</tr>
<tr>
<td>Met (estimated)</td>
<td>g/d</td>
<td>18</td>
<td>43</td>
</tr>
</tbody>
</table>
Material and Methods

Treatments

• CON: TMR basal dry-cow diet until calving, followed by TMR basal lactation diet
• RPC: TMR basal dry-cow diet + 50 g of RPC per cow top dressed product (Sta-Chol®, Ascor Chimici, Italy) until calving, followed by TMR basal lactation diet + 50 g of RPC per cow top dressed product just after TMR distribution
• RPC with 50% choline as choline chloride

Blood sampling

• before TMR distribution
• jugular blood samples: once just before the trial start, and then weekly until the 10th wk of lactation
• 5 mL evacuated sampling tubes, with K₃-EDTA as anticoagulant
Material and Methods

**Blood analysis**

- automatic hematological analyzer (Cell Dyn 3700, Abbott Diagnostici, Italy)

Determined features:

- WBC; NEU; LYM; MONO; EOS; BASO
- RBC; HGB; HCT; MCV; MCH; MCHC
- PLT; MPV; PDW
Material and Methods

Statistical analysis

- pre- and post-partum data separately analyzed
- randomized block design, mixed model
- main effects: choline supplementation (RPC vs. CON), week of trial (-3, -2, -1 and 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10), and their interactions
- cow repeated in time
Results and Discussion

- WBC count

<table>
<thead>
<tr>
<th>wk from calving</th>
<th>log10 (K mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-4</td>
<td>3.00</td>
</tr>
<tr>
<td>-2</td>
<td>3.10</td>
</tr>
<tr>
<td>0</td>
<td>3.20</td>
</tr>
<tr>
<td>2</td>
<td>3.30</td>
</tr>
<tr>
<td>4</td>
<td>3.40</td>
</tr>
<tr>
<td>6</td>
<td>3.50</td>
</tr>
<tr>
<td>8</td>
<td>3.60</td>
</tr>
<tr>
<td>10</td>
<td>3.70</td>
</tr>
<tr>
<td>12</td>
<td>3.80</td>
</tr>
</tbody>
</table>

* indicates significant difference between groups.

RPC and CON groups are compared.
Results and Discussion

**Neutrophils count**

![Graph showing neutrophils count over wk from calving]

- **RPC**
- **CON**

Log$_{10}$ (K mL$^{-1}$) vs wk from calving.
Results and Discussion

Neutrophils percentage

wk from calving

% WBC

RPC
CON

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Results and Discussion

Eosinophils count

![Graph showing eosinophils count over weeks from calving]

- **RPC**
- **CON**

* indicates a significant difference.
Results and Discussion

Eosinophils percentage

wk from calving

% WBC

RPC
CON

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Results and Discussion

Choline and neutrophils

- regulating n° of PMNs in the circulation and prompt removal of senescent PMNs → maintaining normal immune function and preventing tissue injury
- active phagocytosis of apoptotic circulating PMNs was essentially limited to the liver
- PMNs phagocytosed by Kupffer cells (Shi et al., 2001) ⇒ a role for choline in this activity?

- PtdCho to building PMN membrane during phagocytosis (García Gil et al., 1982) ⇒ a choline effect to improve PMN function in phagocytosis?
Results and Discussion

Milk yield

- **RPC**
- **CON**

wk from calving

kg/d

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Abeni et al., 2007
Results and Discussion

Plasma insulin concentration

log10 (ng L⁻¹)

wk from calving

RPC
CON

* Abeni et al., 2007
Results and Discussion

Plasma NEFA

log_{10} (\text{mol L}^{-1})

RPC
CON

* Abeni et al., 2007
Hypothesis

Cause of liver injury

Mediators

Inflammatory phase

Metabolic syndrome

Fat accumulation

Impaired lipid metabolism

Adipocytokines

NEFA

Kupffer cells

Adapted from Bataller and Brenner, 2005
Results and Discussion

Choline and eosinophils

• Difficult to explain:
  - Data variance
  - Few available papers on this topic

• Regulation of eosinophil adhesion by lysophosphatidylcholine (Zhu et al., 2007) ⇒ improved adhesion and, as consequence, transmigration of EOS in tissues?

• Choline treatment (in mouse) significantly inhibited eosinophilic airway inflammation (Mehta et al., 2007) ⇒ a choline effect in reducing allergen-induced inflammation?
Results and Discussion

### Lymphocytes count

The graph illustrates the lymphocytes count over time from calving. The y-axis represents the log10 (K mL⁻¹) scale, and the x-axis represents weeks from calving. The graph compares two groups: RPC and CON.

- **RPC** (Red Circle): Indicates the lymphocytes count for the reduced protein concentration group.
- **CON** (Blue Circle): Indicates the lymphocytes count for the control group.

Key observations:
- A significant difference (*) is noted between the two groups at certain weeks.
- The lymphocytes count appears to increase gradually over the weeks post-calving.
Conclusion

- Cows fed RPC had lower total leukocyte count ($P<0.001$), neutrophil count ($P<0.05$), lymphocyte count ($P<0.001$), and eosinophil count ($P<0.001$) than CON cows throughout their first 10 weeks of lactation.

- Small differences between treatments on erythrocyte-related variables, both before and after calving.

- Supplementation of transition cow diet with RPC affected the number of circulating leukocyte in early lactation, but further research is necessary to better understand the implications of these effects on transition cow health.
Thank you for your attention