Impact of nutrition on the immune system of cattle

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Possible implications of nutrition and other environmental factors on the functionality of the immune system

Diet composition

- Feed intake
  - Intake of energy and nutrients
  - Intake of unwanted compounds

Metabolism and nutritional status

Immune system

Environment
Possible implications of nutrition and other environmental factors on the functionality of the immune system

Diet composition

Feed intake

Intake of energy and nutrients

Intake of unwanted compounds

Ruminal metabolism

Metabolism and nutritional status

Immune system

Environment
Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle (Zebeli & Metzler-Zebeli, 2012)

- Disrupted tight junctions
- Systemic pathway - portal vein
- Lymphatic pathway - lymphatic ducts
- Fever
- Lower feed intake
- Lipolysis
- Metabolic changes stress
- High concentrate, low fibre diets
- LPS
- SAA
- CRP
- Haptoglobin
- Interplay between rumen digestive disorders and diet-induced inflammation in dairy cattle (Zebeli & Metzler-Zebeli, 2012)
**Haptoglobin**
- Binding hemoglobin
- Bacteriostatic effect
- Stimulation of angiogenesis
- Role in lipid metabolism/development of fatty liver in cattle
- Immunomodulatory effect
- Inhibition of neutrophil respiratory burst activity

**Serum amyloid A**
- Transport of cholesterol from dying cells to hepatocytes
- Inhibitory effect on fever
- Inhibitory effect on the oxidative burst of neutrophilic granulocytes
- Inhibitory effect on in vitro immune response
- Chemotaxic effect on monocytes, polymorphonuclear leucocytes and T-cells
- Induction of calcium mobilisation by monocytes
- Inhibition of platelet activation

**C-reactive protein**
- Complement activation and opsonisation
- Modulation of monocytes and macrophages, cytokine production
- Binding of chromatin
- Prevention of tissue migration of neutrophiles

*Fold-increase in cattle:*
- **Haptoglobin**: > 10
- **Serum amyloid A**: 1 - 10
- **C-reactive protein**: 0
Associations between rumen endotoxin and plasma acute phase protein concentrations in cows

**Lactating cow**

- Ru. LPS/SAA [ng/mL] vs. Barley [%]
- CRP [µg/mL] vs. Barley [%]

**Dry, non-gravid cow**

- Ru. LPS [EU*10000/mL] vs. Week
- Haptoglobin [µg/mL] vs. Week

P-values:
- p_{lin/qu.}<0.01

References:
- Zebeli et al. (2010)
- Dänicke et al. (2014)
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Intake of energy and nutrients

Intake of unwanted compounds

Metabolic status

Immune system

Environment
Physiological events in the transition period with relevance for metabolism and immune system

Non-esterified fatty acids

Milk energy

Beta-OH-butyrate

Energy intake

Energy balance

Changes (relative to initial values)

Weeks relative to calving

-3 -2 -1 0 1 2 3 4 5 6 7 8 9
Non esterified fatty acids (NEFA) and beta-OH-butyrates (BHB) inhibit the \textit{in vitro} proliferative response of bovine peripheral mononuclear cells dose-dependently.

**Fatty acid**  | **IC$_{50}$ [µM]**
--- | ---
cis-9, trans-11 conjugated linoleic acid | 56
Linoleic acid | 105
NEFA [C16:0 (29.8%), C16:1 (6.1%), C18:0 (15.6%), C18:1 (41.5%)] | 81

Renner et al., 2013

Schulz et al., 2015
Relationship between non-esterified fatty acids (NEFA), 3-β-hydroxybutyrate (BHB) and stimulation index (SI) of cows *ex vivo* (Dänicke et al., 2012)

\[
SI = 6.1 - 0.002 \cdot \text{NEFA} - 6.7 \cdot \text{BHB} + 6.3 \cdot \text{BHB}^2 \quad (r^2=0.562, \ RSD=1.7)
\]

○ CON: day 0; ● CON: day 21, □ CLA50: day 0; ■ CLA50: day 21; △ CLA100- day 0; ▲ CLA100: day 21
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Diet composition

- Feed intake
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Metabolic status

- Immune system

Environment
Designs for studying the role body reserves on metabolism and immune system in cows differing in physiological state

Transition cow

- High BCS
- Low BCS

Dry, non-gravid cow

- High BCS

Energy requirement for:

- Maintenance
- Gravidity (Fetus, body accretion)
- Maintenance
- Milk energy
- Maintenance
- Body accretion (mainly fat)
Body condition of cows differing in physiological state

**Transition cow**

**Dry, non-gravid cow**

Energy requirement for:

- Maintenance
- Gravidity (Fetus, body accretion)

Drong et al., 2016

- Maintenance
- Milk energy

Locher et al., 2015

- Maintenance
- Body accretion (mainly fat)
Energy balance of cows differing in physiological state

Transition cow

- High BCS
- Low BCS

Dry, non-gravid cow

- High BCS

Energy requirement for:
- Maintenance
- Gravidity (Fetus, body accretion)
- Maintenance
- Milk energy
- Maintenance
- Body accretion (mainly fat)

Drong et al., 2016
Dänicke et al., 2016
NEFA and BHB of cows differing in physiological state

**Transition cow**

- **NEFA**
  - High BCS: Red circles
  - Low BCS: Blue circles
  - **p**\(_{\text{week} \times \text{group}} < 0.001\)

- **BHB**
  - High BCS: Red circles
  - Low BCS: Blue circles
  - **p**\(_{\text{week} \times \text{group}} < 0.001\)

**Dry, non-gravid cow**

- **NEFA**
  - Low BCS: Red circles
  - **p**\(_{\text{week}} < 0.001\)

- **BHB**
  - Low BCS: Red circles
  - **p**\(_{\text{week}} < 0.001\)

Drong et al., 2016

Dänicke et al., 2016
**Ex vivo proliferative response of bovine peripheral mononuclear cells in dependence on physiological state**

**Transition cow**
- **Low BCS**
- **High BCS**

- $p_{\text{week} \times \text{group}} < 0.001$

**Dry, non-gravid cow**
- **Low BCS**
- **High BCS**

Energy requirement for:
- **Maintenance**
- **Gravidity (Fetus, body accretion)**
- **Maintenance**
- **Milk energy**
- **Maintenance**
- **Body accretion (mainly fat)**

Drong et al., 2016

Dänicke et al., 2016
Lymphocyte proportion and CD4+ to CD8+ ratio in dependence on physiological state

*Transition cow*

*Dry, non-gravid cow*

Energy requirement for:

- Maintenance
- Gravidity (Fetus, body accretion)
- Milk energy
- Maintenance
- Body accretion (mainly fat)

Drong et al., 2016

Dänicke et al., 2016
Activation of indolamine-2, 3-dioxygenase (IDO) and its consequences (Schröcksnadel et al., 2006)

Cattle:
Induction of endometrial IDO-mRNA-expression together with a locally increased kynurenine/tryptophan ratio (=IDO-activity) was suggested as an immunological mechanism to establish embryo tolerance (Groebner et al., 2011).
Institute of Animal Nutrition

ACAT2

ACMSD

AOX1

CYP1A1

DDC

Tryptophan

Protein turnover

Dietary & microbial Trp

Tryptophan-2,3-dioxygenase (TDO)

Indolamine-2,3-dioxygenase (IDO)

Kynurenine

LPS

IF-Gamma

00303 S0996
(c) Kaesman Laboratories
**Interferon-γ (IFN-γ) secretion in ConA-stimulated PBMC in dependence on BCS** (Lacetera et al., 2005)

![Graph showing IFN-γ secretion over time relative to parturition](image)

- Thin
- Medium
- Overconditioned

**Tryptophan catabolism in dependence on BCS** (Hüther et al., 2016)

![Graph showing kynurenine to tryptophan ratio over time relative to parturition](image)

- Low BCS
- High BCS

A higher kynurenine to tryptophan ratio indicates a higher IDO/TDO activity and suggests a lower immuno-reactivity.
Granulocyte numbers, unstimulated (basal) and stimulated ROS formation of granulocytes in dependence on physiological state

**Transition cow**

**Dry, non-gravid cow**

**Energy requirement for:**
- Maintenance
- Gravidity (Fetus, body accretion)

- Maintenance
- Milk energy
- Maintenance
- Body accretion (mainly fat)

*Drong et al., 2016*

*Dänicke et al., 2016*
Monocyte numbers in dependence on body condition and physiological state (Eger et al., 2015)

**Significant differences:**
A,B,C: BCS high  
A,b,c: BCS low  
*: between groups

**Monocytes**

- **BCS high**
- **BCS low**

**cM**

- high phagocytic activity, most abundant

**intM**

- high ROS formation, pro-inflammatory

**ncM**

- high phagocytic activity, patrolling?
Flow cytometric assessment of glucose uptake of monocytes from cows differing in body condition (Eger et al., 2016)

Glucose uptake of all monocytes decreased after parturition
Possible implications of nutrition and other environmental factors on the functionality of the immune system

- Essential oils (EO)
- Diet composition
  - Feed intake
    - Intake of energy and nutrients
    - Intake of unwanted compounds
- Monensin (Mo)
- Metabolism and nutritional status
  - Immune system
- Environment
Substances with potential effects on ruminal microbiota

**Essential oils**
- Eugenol
  - ![Eugenol structure](image)
- Thymol
  - ![Thymol structure](image)

**Antibiotic**
- Monensin A
  - ![Monensin A structure](image)

Design for investigation of the effects of essential oils (EO) as additive and Monensin (Mo) as slow release intraruminal bolus for ketosis prevention in pre-disposed cows (Drong et al., 2015)

BCS=4*
BCS>3,5 (n=48)
Mo (n=16) EO (n=16)

BCS=2*
BCS<3,0 (n=16)

* Mansfeld et al., 2000
Rumen microbiome analysis by using the 16S Ilumina MiSeq-technique (Schären et al., 2017)

**Similarity among samples**

→ Significant difference between microbiome of HC/MO animals compared to others. \( P < 0.001 \)
→ No difference between HC/EO and control (LC, HC).

**Sample diversity / alpha-diversity variables**

Significantly lower species diversity in HC/MO samples:
→ only 162 vs. 170 species detected!

### Table

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>( \text{Mean} / \text{SE} )</th>
<th>( \text{Significance} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chao1</td>
<td>HC</td>
<td>171.3 / 2.2</td>
<td>0.323</td>
</tr>
<tr>
<td></td>
<td>LC</td>
<td>172.4 / 4.3</td>
<td></td>
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<tr>
<td></td>
<td>MO</td>
<td>166.8 / 5.7</td>
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</tr>
<tr>
<td></td>
<td>EO</td>
<td>171.0 / 7.5</td>
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<tr>
<td><strong>Observed species</strong></td>
<td>HC</td>
<td>171.0 / 4.5</td>
<td>0.015</td>
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<tr>
<td></td>
<td>LC</td>
<td>170.5 / 4.0</td>
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<tr>
<td></td>
<td>MO</td>
<td>162.5 / 4.0</td>
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<tr>
<td></td>
<td>EO</td>
<td>170.5 / 8.5</td>
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<tr>
<td><strong>Shannon index</strong></td>
<td>HC</td>
<td>4.41 / 0.23</td>
<td>0.020</td>
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<tr>
<td></td>
<td>LC</td>
<td>4.43 / 0.16</td>
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<tr>
<td></td>
<td>MO</td>
<td>4.20 / 0.13</td>
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</tr>
<tr>
<td></td>
<td>EO</td>
<td>4.39 / 0.37</td>
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</tr>
</tbody>
</table>

*Blue = LC; Green = HC/MO; Red = HC/EO; Orange = HC

Oral rumen liquid samples collected at 56 DIM.
**Differences between groups in rumen microbiota on family level (Schären et al., 2017)**

<table>
<thead>
<tr>
<th>Taxonomy</th>
<th>Global</th>
<th>EO:LC</th>
<th>EO:HC</th>
<th>EO:MO</th>
<th>LC:HC</th>
<th>MO:LC</th>
<th>MO:HC</th>
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<tbody>
<tr>
<td>Archaea - Euryarchaeota - Methanobacteria - Methanobacteriales - Methanobacteriaceae</td>
<td>0.846</td>
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<td>Bacteria - Actinobacteria - Bifidobacteriales - Bifidobacteria - Bifidobacterium</td>
<td>0.141</td>
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<tr>
<td>Bacteria - Actinobacteria - Bifidobacteriales - Bifidobacteriaceae - uncultured and other</td>
<td>0.229</td>
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<tr>
<td>Bacteria - Actinobacteria - Coriobacteriia - Coriobacteriales - Coriobacteriaceae</td>
<td>0.025</td>
<td>0.610</td>
<td>0.913</td>
<td>0.425</td>
<td>0.937</td>
<td>0.024</td>
<td>0.108</td>
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<tr>
<td>Bacteria - Bacteroidetes - Bacteroidia - Bacteroidales - BS11 gut group</td>
<td>0.001</td>
<td>0.839</td>
<td>0.998</td>
<td>0.029</td>
<td>0.915</td>
<td>0.001</td>
<td>0.012</td>
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<tr>
<td>Bacteria - Bacteroidetes - Bacteroidia - Bacteroidales - Prevotellaceae</td>
<td>0.050</td>
<td>1.000</td>
<td>0.316</td>
<td>0.049</td>
<td>0.316</td>
<td>0.049</td>
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<td>Bacteria - Bacteroidetes - Bacteroidia - Bacteroidales - RF16</td>
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<td>Bacteria - Bacteroidetes - Bacteroidia - Bacteroidales - Rikenellaceae</td>
<td>0.000</td>
<td>0.807</td>
<td>1.000</td>
<td>0.001</td>
<td>0.778</td>
<td>0.026</td>
<td>0.000</td>
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<tr>
<td>Bacteria - Bacteroidetes - Bacteroidia - Bacteroidales - S24-7</td>
<td>0.064</td>
<td>0.948</td>
<td>0.978</td>
<td>0.227</td>
<td>0.999</td>
<td>0.063</td>
<td>0.082</td>
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<td>Bacteria - Candidate division TM7</td>
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<td>Bacteria - Cyanobacteria - SHA-109</td>
<td>0.001</td>
<td>0.970</td>
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<td>0.982</td>
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<td>Bacteria - Firmicutes - Clostridia - Clostridiales - Family XIII Incertae Sedis</td>
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<tr>
<td>Bacteria - Firmicutes - Clostridia - Clostridiales - Lachnospiraceae</td>
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<td>0.948</td>
<td>0.825</td>
<td>0.006</td>
<td>0.991</td>
<td>0.043</td>
<td>0.085</td>
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<td>Bacteria - Firmicutes - Clostridia - Clostridiales - Lachnospiraceae</td>
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<td>Bacteria - Firmicutes - Clostridia - Clostridiales - Veillonellaceae</td>
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<tr>
<td>Bacteria - Firmicutes - Erysipelotrichi - Erysipelotrichales - Erysipelotrichiaceae</td>
<td>0.151</td>
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<tr>
<td>Bacteria - Firmicutes - Gammaproteobacteria - Aeromonadales - Succinivibrionaceae</td>
<td>0.183</td>
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<tr>
<td>Bacteria - Spirochaetes - Spirochaetales - Spirochaetaceae - Treponema</td>
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<tr>
<td>Bacteria - Tenericutes - Mollicutes - Anaeroplasmatales - Anaeroplasmataceae</td>
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<tr>
<td>Bacteria - Tenericutes - Mollicutes - RF9</td>
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<tr>
<td>Unassigned</td>
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<td>0.908</td>
<td>1.000</td>
<td>0.001</td>
<td>0.911</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Clear alterations due to MO observed on family level

Differences between groups in rumen microbiota on family level (Schären et al., 2017)
LPS concentration in rumen fluid in dependence on BCS (LC, HC), Monensin (HC/MO) oder essential oils (HC/EO) (Drong et al., 2016)

\[ p_{\text{group}} = 0.001 \]
\[ p_{\text{group} \times \text{time}} = 0.454 \]
Haptoglobin in blood in dependence on BCS, Monensin (Mo) or essential oils (EO) (Drong et al., 2016)

\[ p_{\text{group}} = 0.489 \]
\[ p_{\text{time}} < 0.001 \]
\[ p_{\text{group \times time}} = 0.182 \]
Tryptophan metabolism in dependence on BCS (LC, HC), Monensin (HC/MO) or essential oils (HC/EO) (Drong et al., 2016)

- **Kynurenine [µmol/L]**
  - \( p_{\text{group}} = 0.072 \)
  - \( p_{\text{time}} < 0.001 \)
  - \( p_{\text{group} \times \text{time}} = 0.229 \)

- **Tryptophan [µmol/L]**
  - \( p_{\text{group}} = 0.317 \)
  - \( p_{\text{time}} < 0.001 \)
  - \( p_{\text{group} \times \text{time}} = 0.479 \)

- **Kynurenine to tryptophan ratio**
  - \( p_{\text{group}} = 0.022 \)
  - \( p_{\text{time}} < 0.001 \)
  - \( p_{\text{group} \times \text{time}} = 0.040 \)
Non-esterified fatty acids (NEFA) in blood in dependence on BCS, Monensin (Mo) or essential oils (EO) (Drong et al., 2015)

- $p_{\text{group}} = 0.001$
- $p_{\text{time}} < 0.001$
- $p_{\text{group} \times \text{time}} = 0.013$
β-hydroxybutyrate (BHB) in blood in dependence on BCS, Monensin (Mo) or essential oils (EO) (Drong et al., 2015)

\[ p_{\text{group}} = 0.003 \]
\[ p_{\text{time}} < 0.001 \]
\[ p_{\text{group} \times \text{time}} = 0.008 \]

Propionate metabolism in Mo-group:
- Propionate
- Glucose
- mRNA-expression of acetyl-CoA acetyltransferase 2
- mRNA-expression of lactate dehydrogenase A
Possible implications of nutrition and other environmental factors on the functionality of the immune system

Essential oils (EO) ➔ Diet composition

- Feed intake
- Intake of energy and nutrients
  ➔ Metabolism and nutritional status
  ➔ Immune system

Monensin (Mo) ➔

- Intake of unwanted compounds

Environment
Possible implications of nutrition and other environmental factors on the functionality of the immune system

- Essential oils (EO) → Diet composition → Feed intake
- Feed intake → Intake of energy and nutrients → Metabolism and nutritional status
- Metabolism and nutritional status → Immune system
- Immune system → BVDV

- Diets high in energy and nutrients:
  - Intake of unwanted compounds
- Monensin (Mo)

- Monensin (Mo) → Intake of energy and nutrients → Metabolism and nutritional status → Immune system → BVDV
BVDV-antibody detection after BVDV-vaccination prior to (a.p.) or after (p.p.) calving in dependence on BCS, Monensin (Mo) or essential oils (EO) (Drong et al., 2016)

Percent sample value [PP, %]

Cut off

Days relative to calving

-42 -35 -28 -21 -14 -7 0 7 14 21 28 35 42 49 56
Physiological events in the transition period with relevance for metabolism and immune system

Non-esterified fatty acids

Milk energy

Beta-OH-butyrate

Energy intake

Energy balance

Weeks relative to calving
Effects of a decreasing milking frequency from 2 x daily (□) to 1 x daily (■) during the first 7 days post partum on milk yield and energy status (Loiselle et al. 2009).
Possible implications of nutrition and other environmental factors on the functionality of the immune system

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Intake of unwanted compounds

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Environment
Many thanks for your attention!