Changes in the mammary gland transcriptome due to diet-induced milk fat depression in dairy cows

Background

• Increased incidence of metabolic and reproductive disorders in dairy cattle

• Negative energy balance is considered to be important determinant of metabolic health and fertility

• One strategy to manage negative energy balance during early lactation is to decrease milk energy secretion through nutritionally controlled reductions in milk fat content (milk fat depression, MFD).
Milk fat depression

Altered biohydrogenation

Diets:
- Easily fermented compounds
- Polyunsaturated fatty acids

Rumen

Atypical Metabolites

Omasum

Reduced milk fat production
Altered fatty acid composition

Dale E. Bauman and Mikko J. Griinari, Livest Prod Sci. 70:15-29, 2001
Objective

• To unravel the molecular mechanism of MFD

→ To develop nutritional solutions to improve energy balance of cows in critical periods of lactation
Experimental design

4X4 Latin square

- 4 cows
- 4 diets (HF, HFO, LF, LFO)
- 4 x 35 d experimental periods

Sampling

- Intake
- Rumen and omasal digesta
- Rumen fluid
- Milk
- Tissue biopsies
- Urine & feces

Analysis
## The effect of diets on milk

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF</td>
<td>HFO</td>
<td>LF</td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk, kg/d</td>
<td>26.7</td>
<td>25.7</td>
<td>29.7</td>
</tr>
<tr>
<td>Fat, g/d</td>
<td>1050</td>
<td>1076</td>
<td>1195</td>
</tr>
<tr>
<td>Protein, g/d</td>
<td>901</td>
<td>823</td>
<td>1013</td>
</tr>
<tr>
<td>Lactose, g/d</td>
<td>1161</td>
<td>1122</td>
<td>1254</td>
</tr>
<tr>
<td><strong>Concentration, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>3.94</td>
<td>4.19</td>
<td>4.11</td>
</tr>
<tr>
<td>Protein</td>
<td>3.38</td>
<td>3.23</td>
<td>3.48</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.34</td>
<td>4.34</td>
<td>4.21</td>
</tr>
</tbody>
</table>
Temporal changes in milk fat yield

Fat yield g/d

Day of study

Biopsies
Mammary gene expression by RNAseq

Sample preparation
- RNA extraction
- TrueSeq library construction
- Flow cell preparation

Illumina HiSeq
- 100 bp pair-end
- 50M reads/sample

Data Analysis
- Read mapping against reference genome
- Transcript annotation
- Differential expression
- Functional annotations
Transcriptional landscape of mammary gland
Functional analysis

- Highest expressing genes are coding for:
  - milk proteins
  - lactosesynthetase complex
  - proteins involved in ubiquitination (post-translational modification of proteins)

- The functions of other highly expressed genes are (top 10%):
  - Cholesterol synthesis
  - ATP synthesis
  - mRNA translation
  - Endoplasmic reticulum
Mammary lipogenic gene expression

Gene networks driving bovine milk fat synthesis during the lactation cycle.
Differential gene expression (HF vs LFO)

- 35 genes were differentially expressed on day 8, FDR < 0.05
- 87 genes were differentially expressed on day 15, FDR < 0.05
Differential gene expression (HF vs LFO)

- 35 genes were differentially expressed on day 8, FDR < 0.05
- 87 genes were differentially expressed on day 15, FDR < 0.05

Mammary lipogenic genes were not among these significant genes
Functional annotation of DE genes

Day 8 (KEGG pathways, 0 hits; GO-terms, 2 hits, (FDR < 0.05))

- Organic acid binding
- Cellular traffic
- Glucose/insulin metabolism
- Endocytosis
- Cell signalling
- Regulation of transcription

Day 15 (KEGG pathways, 7 hits; GO-terms, 12 hits, (FDR < 0.05))

- Extracellular matrix
- Regulation of actin cytoskeleton
- Endocytosis
- Cell signalling
- Regulation of transcription
Conclusions

• The major effect of diet induced milk fat depression in the mammary gland seems to be on adaptation of other mechanisms than lipogenesis as recently suggested for CLA mediated MFD (Kramer et al. 2013)

• Indicated mechanisms suggest remodelling of cellular structures similarly as in CLA induced MFD in mice (Kadegowda et al. 2013, Journal of Lipids)

• The results are preliminary and require further analysis and verification
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