Fat metabolism in mammary gland and body, milk lipolysis and FFA. How to use gene expression as a tool to describe how different diets influence mammary gland lipid metabolism.

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(Milk quality and lactation physiology of dairy goats Seminar 31 May 2010 - UMB, NULS, Norway)

Specificities of lipid metabolism in goats:
- milk FA profile (high C8 & C10, and B-CFA)
- lipolytic system (LPL regulations; flavour)

(e.g. Chilliard et al, 2003; Eknaes, 2009)

Fatty Acids, lipolysis and goat flavour

Fatty Acids, lipolysis and goat flavour

Fat globule triglycerides

- high % C6-C10 FA, mostly on carbon 3
- methyl / ethyl C8 (0.3 g/kg fat)

Lipoprotein lipase
- high % bound to cream
- correlated to lipolysis
- release of FA from carbon 1-3

Free methyl / ethyl C8
Goat flavour


Specificities of lipid metabolism in goats:
- milk FA profile (high C8 & C10, and B-CFA)
- lipolytic system (LPL regulations; flavour)
- genetic polymorphism (aS1-casein/lipids)
- responses to lipid feeding
  (no MFD, high CLA, low lipolysis, ...)

(e.g. Chilliard et al, 2003-2007; Eknaes et al, 2006-2009)
Trans fatty acids and mammary lipogenesis in ruminants

KJ Shingfield, L Bernard, C Leroux and Y Chilliard


1) High concentrate/low fibre diets
2) Rations containing marine lipids
3) Diets containing ionophores

Addition of polyunsaturated fatty acids

Changes in rumen biohydrogenation

Characteristic changes in ruminal biohydrogenation

Diet induced

cis-9, cis-12 C_{18:2} (linoleic acid)
cis-9, trans-11 C_{18:2} (CLA)
trans-11 C_{18:1} (vaccenic acid)
C_{18:0} (stearic acid)

trans-10, cis-12 C_{18:2} (CLA)
trans-10 C_{18:1}
C_{18:0} (stearic acid)

trans-10 shift

Inhibition

Relationship between post-ruminal infusions of trans-10, cis-12 CLA and milk fat yield

\[ Y = -48.26 + 49.03 \exp^{-0.2792x} \quad r^2 = 0.86 \]

(Shingfield ISRP2009, from Griinari and Bauman, 1999)
Does trans-10, cis-12 CLA explain the decreases in milk fat?

<table>
<thead>
<tr>
<th>Reference</th>
<th>Milk 11o,c12 CLA (g/100 g)</th>
<th>Measured</th>
<th>Predicted</th>
<th>Explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperova et al., 2000</td>
<td>0.084</td>
<td>-43.3</td>
<td>-18.8</td>
<td>43.5</td>
</tr>
<tr>
<td>Peterson et al., 2003</td>
<td>0.060</td>
<td>-37.2</td>
<td>-14.2</td>
<td>52.0</td>
</tr>
<tr>
<td>Bell et al., 2006</td>
<td>0.050</td>
<td>-29.5</td>
<td>-12.0</td>
<td>40.7</td>
</tr>
<tr>
<td>Roy et al., 2006</td>
<td>0.040</td>
<td>-26.1</td>
<td>-9.7</td>
<td>37.3</td>
</tr>
<tr>
<td>Mean</td>
<td>0.040</td>
<td>-44.2</td>
<td>-7.3</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Does trans-10, cis-12 CLA explain the decreases in milk fat yield? (Chilliard et al., 2007)

- Diets that cause MFD in cows increase milk fat synthesis in goats (Chilliard et al., 2007)
- Limited data suggest that responses in sheep are more comparable to goats than cows (e.g. Mele et al., 2006; Hervás et al., 2008)
- Species differences may be due to effects on ruminal biohydrogenation or regulation of mammary lipogenesis (Shingfield et al, ISRP 2009)

Relationship between mammary lipogenesis and milk trans-10 18:1 in the bovine

\[ y = -61.971 + 59.116e^{-0.1554x} \]
\[ r^2 = 0.676, \text{ No.} = 82, P < 0.001 \]

(Shingfield et al., unpublished)

Relationship between mammary lipogenesis and milk trans-10 18:1 in the caprine

No obvious relationship

(Chilliard et al., unpublished)
Comparison of mammary lipogenesis and milk trans-10 18:1 between ruminant species

- Increases in milk trans-10 18:1 concentrations are 3-fold higher in cows than goats or sheep.
- Increases in milk trans-10 18:1 are associated with MFD in cows but not in goats or sheep.
- Data suggest that ruminal biohydrogenation is much less susceptible to the trans-10 shift in small ruminants.

(Shingfield et al, ISRP 2009)

Stability of CLA response in goat milk

Mammary lipogenic responses in ruminants fed rumen-protected CLA
Key genes involved in mammary lipogenesis

Nutritional regulation of mammary lipogenesis in ruminants: molecular dimension

Regulation may be mediated via transcription, translation, protein turnover and enzyme activity

- *De novo* fatty acid synthesis (ACC and FASN)
- Fatty acid uptake (LPL)
- Desaturation of fatty acid substrates (SCD)

Nutritional regulation of *de novo* fatty acid synthesis in ruminants

Dietary changes producing MFD or infusion of trans-10, cis-12 CLA may affect ACC and FASN mRNA abundance and activity.

Dietary changes producing MFD or infusion of trans-10, cis-12 CLA may affect LPL mRNA abundance.

Starch rich diets supplemented with plant oils may affect LPL mRNA abundance.

Nutritional regulation of mammary long chain fatty acid uptake in ruminants

Dietary changes producing MFD or infusion of trans-10, cis-12 CLA may affect the secretion of long chain fatty acids in milk.

Starch rich diets supplemented with plant oils may affect the secretion of long chain fatty acids in milk.

LPL activity does not appear to limit mammary long-chain fatty acid uptake in the goat but may be a limiting factor during MFD in cows.
Nutritional regulation of mammary Δ-9 desaturase (SCD1) in ruminants

Varies little in response to diet
- Diets containing fish oil or infusion of trans-10, cis-12 CLA
- Lowered SCD1 mRNA abundance

Varies according to basal diet and composition of lipid supplement
- Formaldehyde-treated linseeds lower SCD1 mRNA abundance
- Plant oils often decrease SCD1 activity

(Y. Chilliard, Norway, 2010)

Concluding remarks #1
1. Ruminal production of trans-10, cis-12 CLA, trans-9, cis-11 CLA and cis-10, trans-12 CLA cannot explain entirely MFD in cows
2. Even in the absence of effects on milk fat synthesis certain trans fatty acids may alter lipogenic gene expression and enzyme activity
3. Effects of trans fatty acids may be mediated at least in part via effects on transcription factors and cellular signalling pathways

(Shingfield et al, ISRP2009)

Concluding remarks #2
4. Mammary lipogenic responses to changes in diet composition differ between ruminant species
5. Some evidence to suggest that differences between ruminant species are related to the effects of diet on ruminal biohydrogenation
6. Indirect comparisons indicate inherent differences in the sensitivity of mammary lipogenic genes to trans fatty acids between ruminant species

(Shingfield et al, ISRP2009)

Nutritional regulation of the caprine mammary transcriptome

Transcriptome analyses (8 500 bovine genes microarray -> statistical analyses)

Cluster analysis of expression profiles highly dependent on diet

(Ollier et al., JDS, 2009)
Impact of food-deprivation on mammary function

Impact of CSN1S1 polymorphism on milk

Altered biological processes

Milk FAs

CSN1S1 genotype

(Chilliard et al., 2008a)

N. goats 33 38
C6:0 2.5 2.2*
C8:0 2.7 3.1** decreases melting point in H Goats
C9:0 0.07 0.08*
C10:0 9.7 11.2**
C11:0 0.09 0.11**
C12:0 4.4 5.2**
C13:0 28.5 28.7**
C14:0 0.74 0.67**
C15:0 0.30 0.27*
C16:0 16.9 15.0** (less desaturated in H goats)
C18:1c9 16.9 15.0**
C18:2n6 2.1 1.9**
CLA 0.33 0.28** (are not related to SCD mRNA levels ?)
Delta 9-desaturation ratios: (n - Chilliard, Norway, 2010)
C10:1/C10 0.025 0.021**
C12:1/C14 0.014 0.012**
C17:1/C17 0.41 0.38*
C18:1c9/C18 2.6 2.1** increases melting point in H Goats
CLA/VA 0.69 0.55**
C913/133 0.71 0.59**
Genotype-Diet (Extr. Linseed) interactions (Chilliard and Rouel, unpubl.)

<table>
<thead>
<tr>
<th>Genotype-Diet (Extr. Linseed) interactions (Chilliard and Rouel, unpubl.)</th>
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</thead>
<tbody>
<tr>
<td>CSN1S1 Genotype</td>
</tr>
<tr>
<td>Diet</td>
</tr>
<tr>
<td>N. goats</td>
</tr>
<tr>
<td>Gen*Det</td>
</tr>
<tr>
<td>Milk fat content (g/kg)</td>
</tr>
<tr>
<td>Lipolysis (g OA/100 g fat)</td>
</tr>
<tr>
<td>C16:0 (% tot. FAs)</td>
</tr>
<tr>
<td>C18:0</td>
</tr>
<tr>
<td>C18:1cis9</td>
</tr>
<tr>
<td>C14:0</td>
</tr>
<tr>
<td>C14:1 / (C14:0+C14:1)</td>
</tr>
<tr>
<td>C18:1cis9 / (C18:0+C18:1)cis9</td>
</tr>
</tbody>
</table>

Response of goat milk fatty acids to oil or oilseeds feeding (Chilliard et al, 2003)

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Linseed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td>Seeds</td>
</tr>
<tr>
<td>Fat content (g/kg)</td>
<td>+3.1</td>
</tr>
<tr>
<td>18:2 +18:3 (%)</td>
<td>+1.3</td>
</tr>
<tr>
<td>VA + RA (%)</td>
<td>+2.7</td>
</tr>
<tr>
<td>Stearic + oleic (%)</td>
<td>+8.3</td>
</tr>
</tbody>
</table>

Goat milk RA & ALA (% total FA) (Chilliard & Ferlay, 2004)

<table>
<thead>
<tr>
<th>RA</th>
<th>ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 control (hay) diets</td>
<td>0.3%</td>
</tr>
<tr>
<td>3 hay diets + linseed oil</td>
<td>3.0-3.5%</td>
</tr>
<tr>
<td>1 hay diet + extr. linseeds</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

Years 2001-2003 (Chilliard et al, 2006 b and 2007)

- 181 Alpine goats in 19 dietary groups
- 5 forages: Maize Silage, Alfafa Hay, Rye-grass Hay, Fress Rye-grass, Natural grassland Hay
- 3 lipid supplements (130g/d) during 3-10 weeks: Oleic sunflower oil (C18:1 n-9), Sunflower oil (C18:2 n-6), Linseed oil (C18:3 n-3)
- Milk from 13 dietary groups used to make cheese by 5 different technologies
**Goat milk saturated FA atherogenic index**

\[ \% \text{C12:0} + 4 \( \% \text{C14:0} \) + \% \text{C16:0} \]

In 7 control diets: 75-85 \% 
In 12 lipid diets: 48-56 \% \( \times 0.65 \)

\[ (Y. \text{ Chilliard, Norway, 2010}) \]

**Goat milk fat oleic acid**

\( \text{C18:1, cis 9} \)

In 7 control diets: 14-18 \% \( \times 1.69 \)
In 12 lipid diets: 25-29 \% 

\[ (Y. \text{ Chilliard, Norway, 2010}) \]

**Goat milk fat linolenic/linoleic ratio**

\( 18:3 \text{n-3}/18:2 \text{n-6} \)

In 4 maize silage diets, without lipids, or with sunflower or oleic sunflower oil:

- 0.05-0.18 \( \times 3.6 \)
- 0.36-0.47 \( \times 6.5 \)

In 4 Hay/Grass diets with linseed oil:

- 0.64-0.85

\[ (Y. \text{ Chilliard, Norway, 2010}) \]

**Goat milk fat rumenic acid equivalent**

\[ \text{rumenic acid + 0.2 vaccenic acid} \]

7 control diets, and 4 oleic sunflower diets: 0.4-1.5 \%
6 Linseed oil diets: 3.2-5.9 \( \times 4.8 \)
2 Sunflower oil diets: 5.7-6.2 \% \( \times 6.3 \)

\[ (Y. \text{ Chilliard, Norway, 2010}) \]
Goat milk fat non-\textit{trans} 11 C18:1 and C18:2 \textit{trans} isomers (\textit{i.e.} except rumenic and vaccenic acids)

- 7 control diets: 0.4-1.6 \%
- 8 Hay/Grass diets + oils: 2.9-6.5
- 4 Maize Silage diets + oils: 6.3-11.3

Flavour defect occurrence (among 7 criteria)

- 5 control diets: 0/7
- 4 oleic sunflower diets: 0.5/7
- 4 linseed oil diets: 1*/7

* small oxidized or fishy flavours

Goat flavour in fresh lactic cheese (score 0-10)

- 5 control diets: 1.54-2.50
- 8 oil supplemented diets: 1.30-2.28

This decrease was probably due to strong decreases in native milk LPL activity and post-milking lipolysis (x 0.54)