Fatty acid metabolism and milk fat depression

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Milk Fat

• Fat is the major energy component of milk
  – Economic value
  – Physical and manufacturing properties

• Milk fat content is markedly affected by diet
  – High grain
  – Vegetable oils
  – Marine oils
Fatty Acids

• Long carbon chains that contain a methyl group (CH$_3$) at one end and a carboxyl group (COOH) at the other

• Fatty acids make lipids energy rich

• Characterized by:
  – Number of carbons
    • Chain length
  – Number of double bonds
    • Degree of unsaturation
  – Location and orientation of these bonds
    • Non-conjugated, conjugated, cis, trans
Nomenclature and Structure

Saturated – single bonds

\[
\text{C} \quad \text{C} \quad \text{C} \quad \text{C} \quad \text{C} \quad \text{C} \quad \text{C} \quad \text{C} \quad \text{C}
\]

Unsaturated – double bonds

\[
\text{C} \quad \text{C} \quad =\text{C} \quad \text{C} \quad \text{C} \quad =\text{C} \quad \text{C} \quad \text{C} \quad \text{C}
\]
Nomenclature and Structure

**cis**

C

H

C

H

C

H

C

H

**trans**

C

H

C

H

C

H

C

H
Nomenclature and Structure

non-conjugated

conjugated
Hydrolysis

Lipids → Free fatty acids

Bacterial lipases + H₂O
Biohydrogenation

- **Linoleic acid**
  - (cis-9, cis-12 18:2)
  - **Rumenic acid**
    - (cis-9, trans-11 CLA)
    - Vaccenic acid
      - (trans-11 18:1)
      - Stearic acid
        - (18:0)
  - **trans-10, cis-12 CLA**
    - **trans-10 18:1**
    - Stearic acid
      - (18:0)
Biohydrogenation

- Linolenic acid
  
  \((\text{cis}-9, \text{cis}-12, \text{cis}-15 \ 18:3)\)

- \(\text{cis}-9, \text{trans}-11, \text{cis}-15 \ 18:3\)

- \(\text{trans}-11, \text{cis}-15 \ 18:2\)

- \(\text{trans}-15 \text{ or } \text{cis}-15 \ 18:1\)

- Vaccenic acid
  
  \((\text{trans}-11 \ 18:1)\)

  - Stearic acid
    
    \((18:0)\)
Rumen Biohydrogenation

Linolenic Acid
\( \text{cis-9, cis-12, cis-15 C}_{18:3} \)

\( \text{cis-9, trans-11, cis-15 C}_{18:3} \)

\( \text{trans-11, cis-15 C}_{18:2} \)

\( \text{trans-15 or cis-15 C}_{18:1} \)

Linoleic Acid
\( \text{cis-9, cis-12 C}_{18:2} \)

\( \text{cis-9, trans-11 CLA} \)

\( \text{trans-11 C}_{18:1} \)

Stearic Acid \( \text{C}_{18:0} \)

Unique conjugated linoleic acid (CLA) isomers

Numerous \( \text{trans-18:1} \) isomers

altered fermentation
Fat Digestion in the Rumen

- **TG**
- **Glycolipids**
- **TG or FFA**

**Hydrolysis**

- **Glycerol**
- **VFAs**

**Biohydrogenation**

- **trans FFAs**
- **PL**

**Microbes**

- **FFAs**
- **Inert fats**
- **TG or FFA**
Rumen Biohydrogenation Pathways

Unsaturated FA

Trans intermediates

Saturated FA
Polyunsaturated fatty acid losses

Loor et al. (2004)
# Major Fatty Acids in Milk Fat

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>% (weight basis)</th>
<th>Common Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0</td>
<td>4</td>
<td>Butyric</td>
</tr>
<tr>
<td>6:0</td>
<td>3</td>
<td>Caproic</td>
</tr>
<tr>
<td>8:0</td>
<td>2</td>
<td>Caprylic</td>
</tr>
<tr>
<td>10:0</td>
<td>3</td>
<td>Capric</td>
</tr>
<tr>
<td>12:0</td>
<td>4</td>
<td>Lauric</td>
</tr>
<tr>
<td>14:0</td>
<td>11</td>
<td>Myristic</td>
</tr>
<tr>
<td>16:0</td>
<td>29</td>
<td>Palmitic</td>
</tr>
<tr>
<td>16:1</td>
<td>2</td>
<td>Palmitoleic</td>
</tr>
<tr>
<td>18:0</td>
<td>12</td>
<td>Stearic</td>
</tr>
<tr>
<td>18:1</td>
<td>25</td>
<td>Oleic</td>
</tr>
<tr>
<td>18:2</td>
<td>2</td>
<td>Linoleic</td>
</tr>
<tr>
<td>18:3</td>
<td>1</td>
<td>Linolenic</td>
</tr>
</tbody>
</table>
Triglyceride Synthesis

- 98% of milk fat is triglyceride
- Glycerol backbone with 3 ester-linked FA
Milk Fat Synthesis

**Circulation**
- LPL
- VLDL
- TAG
- Glycerol
- Glucose
- Acetate
- βHBA

**Synthesis**
- Desaturase
- UFA
- SFA \((c_{16} - c_{18})\)
- Glycerol
- Glucose
- FA synthesis de novo \((c_4 - c_{16})\)

**Translocation**
- TAG synthesis

**Secretion**
- TAG
- VLDL
- TAG
- Milk
- Fat

**Basal membrane**
- ER- membrane
- Apical membrane
Major Fatty Acids in Milk Fat

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>% (weight basis)</th>
<th>Fatty Acid Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6:0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>8:0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td>3</td>
<td><strong>De novo synthesis</strong></td>
</tr>
<tr>
<td>12:0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>29</td>
<td><strong>De novo and preformed</strong></td>
</tr>
<tr>
<td>16:1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>18:1</td>
<td>25</td>
<td><strong>Uptake of preformed</strong></td>
</tr>
<tr>
<td>18:2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>18:3</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
## Factors Influencing Milk Fat

<table>
<thead>
<tr>
<th>Nutritional Factors</th>
<th>Non-nutritional Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Dietary fiber</td>
<td>• Genetics</td>
</tr>
<tr>
<td>• Specific feeds</td>
<td>• Stage of lactation</td>
</tr>
<tr>
<td>• Feeding strategy</td>
<td>• Season</td>
</tr>
<tr>
<td>• Ionophores</td>
<td>• Parity</td>
</tr>
<tr>
<td></td>
<td>• Ambient temperature</td>
</tr>
</tbody>
</table>
Milk Fat Depression in the Dairy Cow

- Recognized by Boussingault in 1845
- Naturally occurs with certain diets
- Milk fat reduced but milk yield and other components unaffected
Milk Fat Depression Characteristics

• Diet-induced
  – High concentrate, low fiber
  – Low in effective fiber
  – Plant and marine oil supplements
  – Unsaturated fatty acids

• Specific for milk fat, up to 50% decrease

• Decreased yield of all fatty acids, but greatest for de novo synthesized fatty acids
MFD Changes Milk Fat Composition

Bauman and Griinari (2003)
Biohydrogenation Theory

• MFD is the result of direct inhibition of milk fat synthesis at the mammary gland by unique fatty acid intermediates formed during rumen biohydrogenation of PUFA.

Bauman and Griinari (2003)
trans-10, cis-12 CLA in Milk Fat

Adapted from de Veth et al. (2004)
Post-Ruminal Infusion vs. Diet-Induced MFD

Adapted from Peterson et al. (2003) and Griinari and Bauman (2005)
$C_{18:1}$ trans Fatty Acids

<table>
<thead>
<tr>
<th>Double Bond Position</th>
<th>Normal Milk Fat</th>
<th>MF Depressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/8</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>11</td>
<td>4.0</td>
<td>3.0</td>
</tr>
<tr>
<td>12</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>13/14</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Griinari et al. (1997)
C_{18:1} trans Fatty Acids

Normal Milk Fat

MF Depressed

Linoleic acid

trans-10, cis-12 CLA

Rumenic acid

Vaccenic acid

trans-10 18:1

Stearic acid (18:0)

Stearic acid (18:0)

Griinari et al. (1997)
Milk fat content (%) vs. Rumen outflow of trans-10 18:1 (g/d)

- Piperova et al. (20)
- Loor et al. (22)
- Shingfield et al. (21)
- Lundy et al. (23)

Equation: $y = 4.59x^{-0.14}$

$R^2 = 0.52$
Role for *trans*-10 18:1

- *trans*-10, *cis*-12 CLA is the most extensively studied potent inhibitor of milk fat synthesis
- MFD is correlated with *trans*-10 rumen outflow
  - Does not directly inhibit milk fat synthesis
  - Marker of altered biohydrogenation
- Other *trans* and conjugated PUFA may decrease milk fat

Lock et al. (2007)
Monensin and Milk Fat Depression

- Ionophores disrupt biohydrogenation
- Monensin targets gram positive bacteria
  - Includes group “B” bacteria that hydrogenate monenes
  - Potentially results in build-up of intermediates
  - Not a problem in normal biohydrogenation pathways

Fellner et al. (1997)
Biohydrogenation

1) Isomerization
Linoleic acid (cis-9, cis-12 18:2)

2) Hydrogenation
Vaccenic acid (trans-11 18:1)

3) Hydrogenation
Stearic acid (18:0)

Group “A”
Rumenic acid (cis-9, trans-11 CLA)

trans-10, cis-12 CLA

trans-10 18:1

Monensin-targeted

Group “B”
Monensin and Milk Fat Depression

- No single risk factor for MFD
- Monensin has potential to amplify the effect of other risk factors
- Monensin decreased milk fat content 0.13%
- Decreases in fat yield seem related to increased presence of dietary unsaturated FA

Duffield et al. (2008)
Rumen Unsaturated FA Load

• RUFAL reflects total dietary unsaturated FA supply entering the rumen daily
  – Total feed ingredient fatty acid composition
    • Oleic (C18:1)
    • Linoleic (C18:2)
    • Linolenic (C18:3)
  – Marker for altered biohydrogenation

Lock and Jenkins (2009)
Unsaturated FA × Monensin

- Lack of available data to support interaction of monensin and unsaturated FA

- Heterogeneity of data in meta-analysis
Dietary C18:1 and C18:2 Concentrations

- Total dietary EE% in parentheses
- 6 fat blend trts test C18:1, C18:1^2, C18:2, C18:2^2, C18:1 × C18:2 interaction

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No monensin $\times$ C18:1 or C18:2 effect on milk fat yield
Monensin and Milk Fat Depression

• Monensin changes the rumen environment

• Diets with the capacity to lead to MFD are further influenced by monensin

• With most diets, an effect is not observed and reductions are only apparent for content, not yield
“Butter is just a little stick of smiles and happiness”
– Paula Deen

http://inventorspot.com/articles/food_sculpture_16966
Increasing Milk Fat Yield

• Milk fat composition is easily manipulated
• Increases with saturated LCFA infusion
  – Decreases proportion of de novo FA
  – Unsaturated FA can reduce DMI
• LCFA are derived from dietary sources
• Short- and medium-chain FA are essential for milk triacylglycerol synthesis

Kadegowda et al. (2008)
Abomasal Butterfat Infusion

- Treatments:
  - 400 g/d butterfat
  - 245 g/d LCFA mixture matching LCFA in butterfat

- Compare effects of short- and medium-chain FA

- Limited infusions to avoid DMI reduction

Kadegowda et al. (2008)
Kadegowda et al. (2008)
Abomasal Infusion of Butterfat Increases Milk Fat Yield

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Butterfat</th>
<th>LCFA</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk, kg/d</td>
<td>31.8</td>
<td>33.7</td>
<td>33.1</td>
<td>2.5</td>
</tr>
<tr>
<td>FCM, 3.5%</td>
<td>32.87</td>
<td>37.72</td>
<td>35.04</td>
<td>2.71</td>
</tr>
<tr>
<td>Fat, %</td>
<td>3.74</td>
<td>4.26</td>
<td>3.79</td>
<td>0.19</td>
</tr>
<tr>
<td>Fat yield, g/d</td>
<td>1,178</td>
<td>1,421</td>
<td>1,279</td>
<td>107.7</td>
</tr>
</tbody>
</table>

Kadegowda et al. (2008)
Abomasal Infusion of Butterfat Increases Milk Fatty Acid Yields

Kadegowda et al. (2008)
Increasing Milk Fat Yield

• Infusion of LCFA did not alter milk fat yield
  – FA <16:0 were not reduced, but maintained
• Butterfat and LCFA similarly increased FA >16:0
  – Changes in palmitate to stearate ratios may have influenced LCFA treatment fat yields
• Medium-chain FA transfer efficiency increased with chain length
  – Yield of 14:0 was greater for cows infused with butterfat
• Short- and medium-chain FA may be limiting factors for increasing milk fat synthesis
Take Home Message

• Milk fat yield can be increased with fat supplementation
Take Home Message

• Milk Fat Depression requires:
  – Altered rumen environment
  – Supply of PUFA
Take Home Message

• No single factor is responsible for MFD – interactions of potential risk factors can change the rumen environment and increase MFD risk.
Questions?