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Regulation of Milk Fat Synthesis

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Milk Fat Affected by Many Factors

Nutritional Factors

- fiber in the diet
- specific feeds
- feeding strategy
- ionophores

→ **Milk fat** ←

Non-nutritional Factors

- genetics
- stage of lactation
- season
- parity
- ambient temperature

History of Milk Fat Depression

- **Recognized by Boussingault in 1845**
- **Naturally occurs with certain diets**
  - **high concentrate, low fiber**
  - **low in effective fiber**
  - **plant and fish oil supplements**
  - **unsaturated fatty acids**

Boussingault

- **Milk fat reduced but milk yield and other milk components unaffected**

Diet Induced Milk Fat Depression

- **Occurs under two circumstance**

1) Altered ruminal fermentation

2) Dietary polyunsaturated fatty acids

Milk Fat Depression Theories

- Shortage of precursors for milk fat synthesis
- Direct inhibition of milk fat synthesis

Shortage of Precursors

- Acetate deficiency (↓ rumen production)
- β-hydroxybutyrate deficiency (anti-ketogenic effects of propionate)
- Glucogenic-insulin theory (tissue competition)

Overall studies provide little or no support for theories involving a shortage of precursors for milk fat synthesis

Glucogenic-insulin Theory

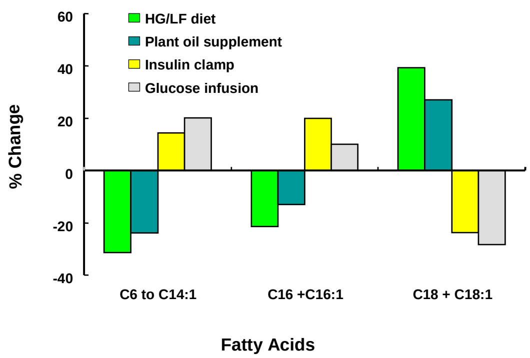
High grain:forage diet
↓
Increased propionate
↓
Increased glucose
↓
Increased insulin
↓
Milk fat depression

Acetate Shortage Theory and MFD

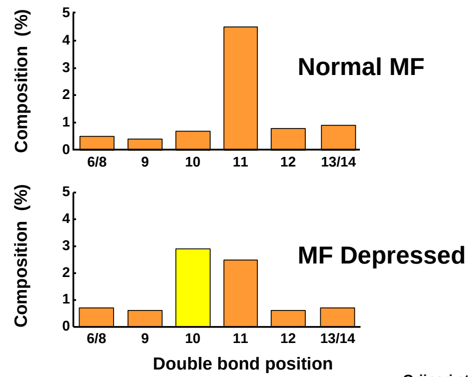
	Normal diet	HG/LF Diet
Rumen Production, moles/d		
Acetate	29.4	28.1[a]
Propionate	13.3	31.0[b]
B-hydroxybutyrate	7.0	9.1[c]

[a]Davis et al. 1967
[b]Bauman et al. 1971
[c]Palmquist et al. 1969

Changes in Milk Fat Composition



trans-18:1 Profile in Milk Fat



Griinari et al., 1998

Trans Theory of Milk Fat Depression

Altered rumen microbes and fermentation

↓

Formation of *trans* fatty acids in the rumen

↓

Inhibition of mammary lipogenic enzymes

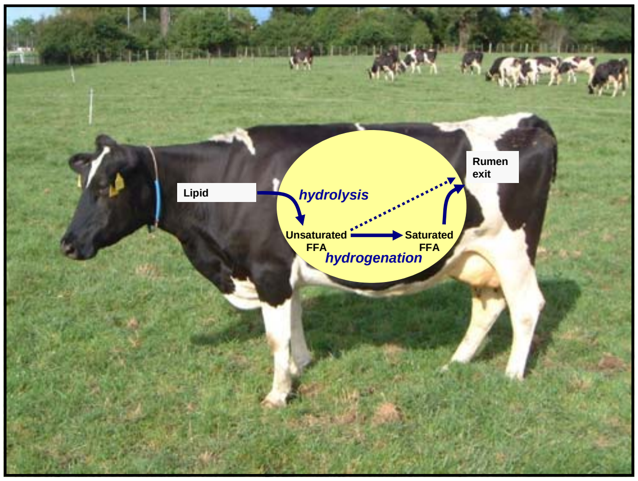
↓

Milk fat depression

- Davis and Brown (1970)
- Selner & Schultz (1980)

Lipid Nutrition in Ruminants

The Rumen Complicates Things



Rumen Biohydrogenation

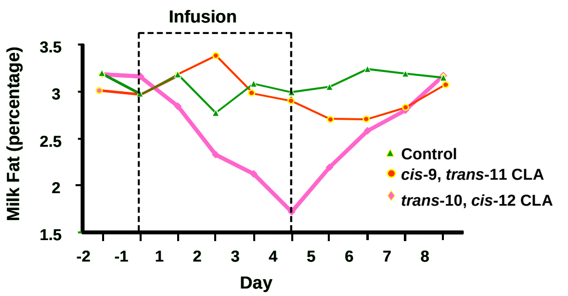
linoleic acid
(*cis*-9, *cis*-12 $C_{18:2}$)
↓
rumenic acid
(*cis*-9, *trans*-11 CLA)
↓
vaccenic acid
(*trans* -11 $C_{18:1}$)
↓
stearic acid ($C_{18:0}$)

Altered pathways

Alternate CLA isomers
trans-10, *cis*-12 CLA
↓
trans- $C_{18:1}$ isomers
trans-10 $C_{18:1}$
↓
stearic acid ($C_{18:0}$)

Griinari and Bauman, 1999

Effect of CLA Isomers on Milk Fat



Baumgard et al., 2000

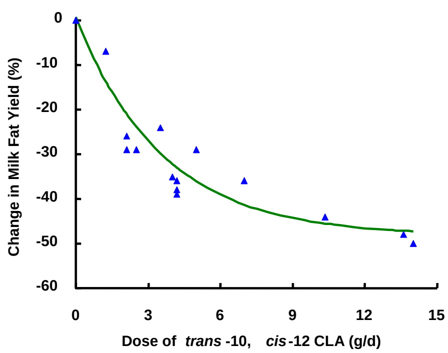
“BIOHYDROGENATION THEORY” of milk fat depression

Under certain conditions rumen biohydrogenation results in unique fatty acids that are potent inhibitors of milk fat synthesis –

Milk fat depression is an interaction between ruminal metabolism and mammary synthesis of milk fat

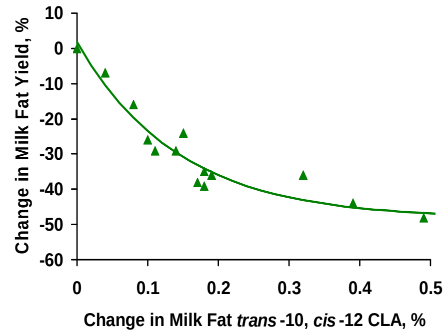
Bauman and Griinari, 2001

trans-10, *cis*-12 CLA Reduces Milk Fat



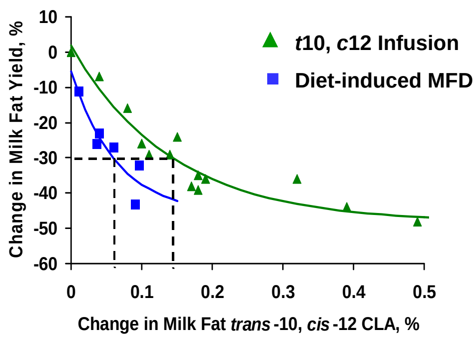
de Veth et al., 2004

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

Range of CLA Isomers in Milk Fat

Conjugated 18:2

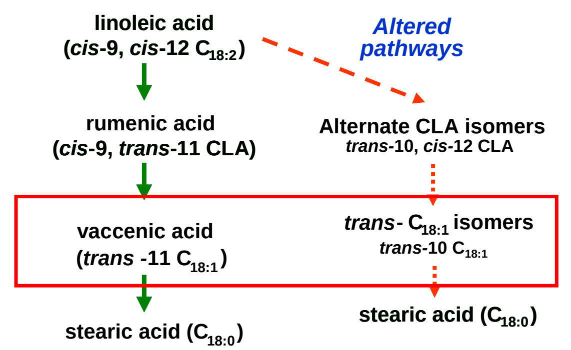
Isomer	% of total CLA isomers
trans-7, *cis*-9	1.2-8.9
trans-7, *trans*-9	0.02-2.4
trans-8, *cis*-10	0.06-1.5
trans-8, *trans*-10	0.2-0.4
trans-9, *trans*-11	0.8-2.9
cis-9, *trans*-11	72.6-91.2
trans-10, *cis*-12	0.03-1.5
trans-10, *trans*-12	0.28-1.3
cis-11, *trans*-13	0.18-4.7
trans-11, *cis*-13	0.07-8.0
trans-11, *trans*-13	0.28-4.2
cis-12, *trans*-14	0.04-0.80
trans-12, *trans*-14	0.33-2.8
cis-*cis* isomers	0.06-4.8

Lock and Bauman, 2004

CLA Isomers Tested in the Dairy Cow

- *trans*-8, *cis*-10 CLA
- *cis*-9, *trans*-11 CLA
- *trans*-9, *trans*-11 CLA
- *trans*-9, *cis*-11 CLA
- *trans*-10, *cis*-12 CLA
- *cis*-10, *trans*-12 CLA
- *trans*-10, *trans*-12 CLA
- *cis*-11, *trans*-13 CLA

Rumen Biohydrogenation



Griinari and Bauman, 1999

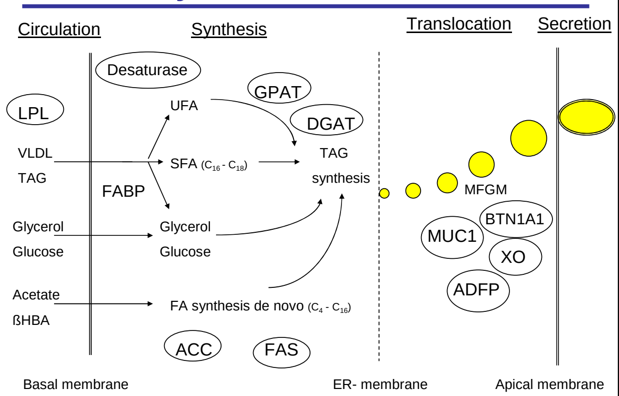
trans-18:1 Isomers Tested in the Dairy Cow

	Previous	Recent
trans-9	25 g/d[1]	46 g/d[3]
trans-10		43 g/d[4]
trans-11	12.5 g/d[2]	7.5-47.5 g/d[3,5]
trans-12	12.5 g/d[2]	

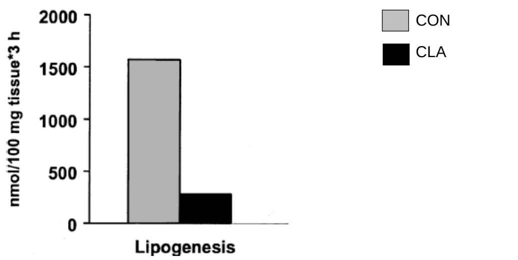
None affected milk fat synthesis, although all were incorporated into milk fat.

[1]Rindsig and Schultz (1974)
[2]Griinari et al. (2000)
[3]Tyburczy et al. (2007)
[4]Lock et al. (2006)
[5]Shingfield et al. (2007)

Milk Fat Synthesis



Direct Action in Mammary Tissue



Baumgard et al., 2002

- Specific ⬇ lipogenesis in mammary tissue

Levels Regulation

DNA

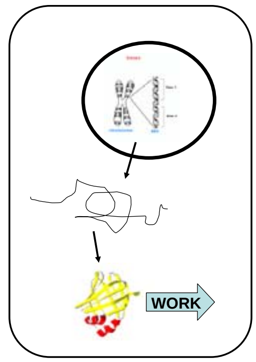
Transcription

mRNA

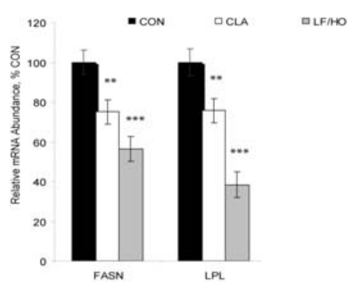
Translation and stability

Protein

Activity and stability

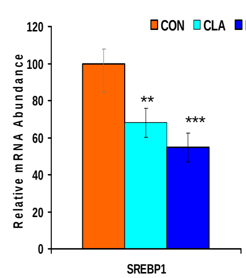


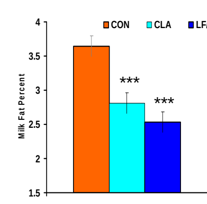
Mammary mRNA During MFD



MFD results in a coordinated decrease in expression of lipogenic enzymes

SREBP1 During MFD





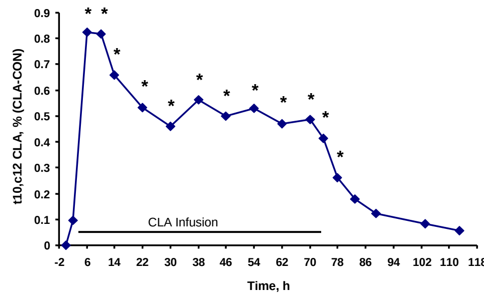
- ⬇ Expression of SREBP1
- Central regulator of lipid synthesis
- Highly expressed in lactating mammary tissue

Harvatine and Bauman, 2006

Temporal Analysis of MFD

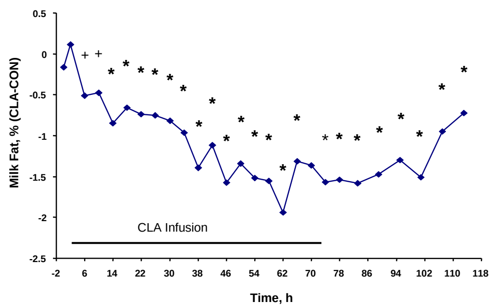
- Question- How fast can we induce MFD

*t*10,*c*12 CLA in Milk Fat



- Quickly reached steady state CLA in milk

Change in Milk Fat Percent



- Milk fat percent progressively decreased after 2 h

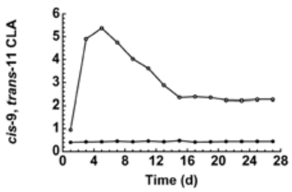
Temporal Response to Diet

- Observed temporal milk fat yield and milk FA profile of cows fed a high oil diet (high PUFA)

  Shingfield et al. 2006

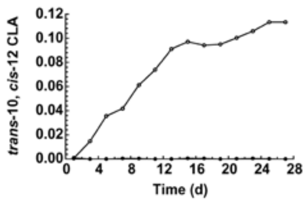
- Milk fat yield progressively decreased after 6-8 d and maximal reached at ~18 d

Dynamic Milk FA Profile

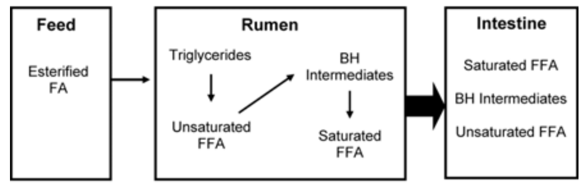


cis-9, trans-11 CLA
Rumenic Acid
Does not Induce MFD

trans-10, cis-12 CLA
Induces MFD

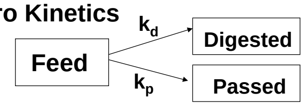


Model of Ruminal Fat Metabolism

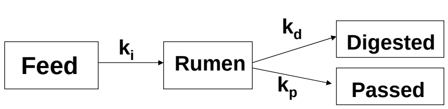


Modeling Rumen Kinetics

- **In Vitro Kinetics**



- **In Vivo: Pool and Flux**



– **Fractional rate = Flux / Pool Size (g/h / g = /h)**

(Mertens, 1993; Firkins et al. 1998)

Kinetics of Ruminal Fat Metabolism

- **Ruminally and duodenally cannulated cows**
  - Duodenal flow
  - Ruminal pool size
- **Treatments**
  – Saturated FA- prilled, hydrogenated FA
  – Intermediate FA saturation
  – Unsaturated FA- calcium salts of FA

* Cottonseed in basal ration

Harvatine and Allen, 2006

Ruminal Passage Rate, %/h

	Sat	Int	Uns	SE	*P* L	*P* Q
cis-C18:1	4.7	4.9	4.8	0.31	NS	NS
C18:2	2.6	2.5	2.6	0.19	NS	NS
C18:3	6.9	6.9	7.1	0.65	NS	NS

- **Slow ruminal passage rate of FA**

Harvatine and Allen, 2006

Demonstrating Ruminal Adaptation

- **Ruminal contents switched between cows fed high and low forage diet**

Satter and Bringe, 1969

- 70 % of maximal reduction in milk fat by 3 days
- Complete milk fat depression within 5-6 d

Dietary Risk Factors

- **Associative Effects**
  - **Synergistic/Antagonistic**
    - **Dietary carbohydrate profile**
    - **Rate and extent of fermentation**
    - **Effective fiber**
    - **Feeding strategies/management**
    - **Silage fermentation/quality**
    - **Dietary fat level**
    - **Profile and availability of dietary fat**
    - **Rumen modifiers- ionophore**
    - **Cow effect (level of intake etc)**
    - **Ruminal N balance**

Can fatty acids be used to troubleshoot milk fat problems?

Mechanism for Diet-Induced MFD

- Increased duodenal flow of specific intermediates

  (ie. *trans*-10, *cis*-12 CLA)

  - **Increased passage rate**
    - Intermediates flow out of the rumen before complete biohydrogenation
  - **Changes in rumen environment**
    - Change in microbial populations
      - Shift in biohydrogenation pathways
      - Lower rate of biohydrogenation

Trends/Similarities

- Alteration of rumen environment cause changes in fatty acid biohydrogenation
- Increased duodenal flow/milk fat content of *trans*-18:1 fatty acids but more specifically *trans*-10 18:1 correlated with MFD
- Increased duodenal flow/milk fat content of *trans*-10, *cis*-12 CLA and other CLA isomers

Individual isomers are important

- Decreasing dietary forage *(Peterson et al. 2003)*
  - Increased milk total *trans*-18:1 by 1.7 fold
  - Increased milk *trans*-10 C18:1 by 4.5 fold
  - Decreased milk fat yield 27%
- Addition of full fat ESB *(Peterson et al. 2002)*
  - Increased milk total *trans*-18:1 1.7 fold
  - Increased milk *trans*-10 C18:1 1.5 fold
  - No effect on milk fat yield

Interpretation of Results

- Duodenal flow of total *trans*-18:1 is not the best indicator for milk fat depression
- Rumen environment is critical and associative effects have large impact

Can the absorbed FA profile be modeled and applied on farm to predict MFD??

CPM dairy has a fatty acid sub-model

Overview of CPM Dairy FA Model

- **Feed ingredients are assigned**
  - FA profile
  - Rate of hydrolysis
- **Free fatty acids can undergo BH**
- **Fatty acids are assigned constant BH rates**
- **Passage rate from the rumen**
  - Same as the feed they originate from
- **Model then predicts**
  - Duodenal flow and absorption of individual FA

Overview of CPM Dairy FA Model

- **Uses simplified biohydrogenation pathways**
- **Limitations**
  - Biohydrogenation rates remain constant regardless of rumen environment
  - Does not account for other 18:2 fatty acids (CLA isomers)
  - *trans*-18:1 fatty acids are grouped
  - *cis*-18:1 is directly biohydrogenated to 18:0 although this fatty acid is known to flow through the *trans*-18:1 pool (Mosley et al., 2002)

Testing the CPM sub-model

- **Decreased dietary forage** *(Peterson et al., 2003)*
  - 53% forage diet vs 16% forage diet
  - Decreased milk fat yield 27%
- **Model predicts:**
  - *trans*-18:1 duodenal flow increases 34 g/d
  - Total *trans*-18:1 flow of 91 g/d
- **Observed:** Milk Fat
  - Total *trans*-18:1 increased 74%
  - *trans*-10 18:1 increased 450%.
  - Increased *trans*-10, *cis*-12 CLA

Testing the CPM sub-model

- **Fish oil supplementation** *(Whitlock et al., 2002)*
  - Addition of 2% fish oil
  - Decreased milk fat yield 29%
- **Model predicts:**
  - A 5 g/d increase in *trans*-18:1 duodenal flow for a total *trans*-18:1 value of 55 g/d
- **Observed:** Milk Fat
  - Total *trans*-18:1 increased 500%
  - *trans*-10 18:1 increased 800%.
  - Increased *trans*-10, *cis*-12 CLA

Testing the CPM sub-model

- **Increased dietary PUFA** *(Harvatine and Allen, 2004)*
  - Ca Salts of LCFA (UNS) vs no fat (CON) with cottonseed in the basal ration
  - Decreased milk fat yield 20%
- **Model predicts:**
  - 84 g/d increase in 18:2 and a 15 g/d increase in *trans*-18:1 duodenal flow with addition of Ca salts
- **Observed:** Duodenal Flow
  - 18:2 unchanged
  - *trans*-18:1 increased 100 g/d
  - Increased *trans*-10, *cis*-12 CLA

Interpretation of Results

- Associative effects of starch, fiber, and oil level on biohydrogenation are not accounted for by the CPM model
- Model incorrectly predicts PUFA as Ca salts will bypass rumen biohydrogenation

Conclusion

- **MFD caused by unique fatty acids originating from ruminal biohydrogenation**
- **These FA isomers are very potent**
- **Mechanism involves regulation of lipogenic gene expression**
- **Rumen environment is critical**
  - Biohydrogenation is not constant
  - Associative effects have large impact

Implications

- **Some FA also good for human health**
  - Future goal may be to increase functional food properties of milk by increasing specific *trans* FA
- **Reducing and rescuing MFD**
  - Know the risk factors
  - Sometimes additive, sometimes explosive
  - Associative effects hinder providing rules
    - Polyunsaturated FA load
    - Diet fermentability
    - Stability of ruminal fermentation

Nutritional Genomics in Action

- **Physiologically relevant**
- **Biologically significant**

Acknowledgements

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Thank You. Questions???