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 metabolim 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(1)

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

(1) *Chilliard et al (2003)*

Specificities of lipid metabolim 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

(published in ANIMAL, 2010, vol 4, pages 1140-1166)

Characteristics of diets that cause milk fat depression

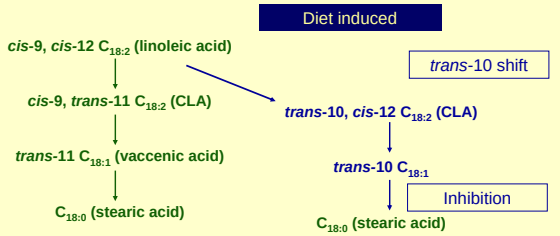
1) High concentrate/low fibre diets
2) Rations containing marine lipids
3) Diets containing ionophores

} Addition of polyunsaturated fatty acids

↓

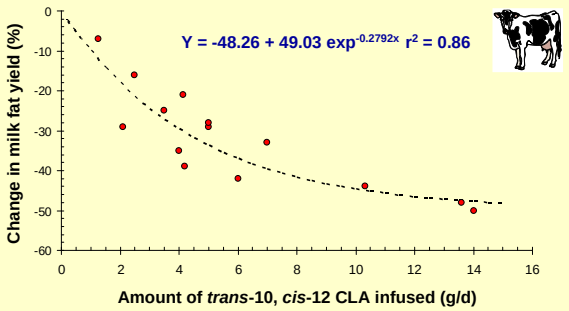
Changes in rumen biohydrogenation

Characteristic changes in ruminal biohydrogenation



(Shingfield ISRP2009, from Griinari and Bauman, 1999)

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



(Shingfield ISRP2009, from De Veth et al., 2004)

Does *trans*-10, *cis*-12 CLA explain the decreases in milk fat?

Reference	Milk t10,c12 CLA (g/100 g)	Change in milk fat yield (%)		
		Measured	Predicted	Explained
Piperova et al., 2000	0.084	-43.3	-18.8	43.5
Peterson et al., 2003	0.060	-27.2	-14.2	52.0
Bell et al., 2006	0.050	-29.5	-12.0	40.7
	0.040	-26.1	-9.7	37.3
Roy et al., 2006	0.040	-57.3	-9.7	17.0
	0.030	-44.2	-7.3	16.5
Mean				34.5

(Shingfield et al, ISRP 2009)

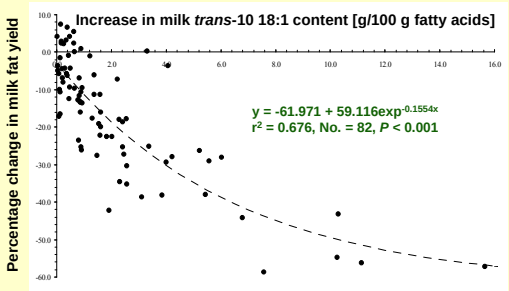
(Y. Chilliard, Norway, 2010)

Nutritional regulation of mammary lipogenesis in the ovine and caprine

- **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)

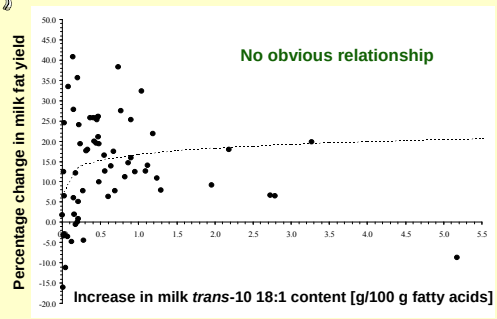
(Y. Chilliard, Norway, 2010)

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



(Y. Chilliard, Norway, 2010) *Shingfield et al., unpublished*

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



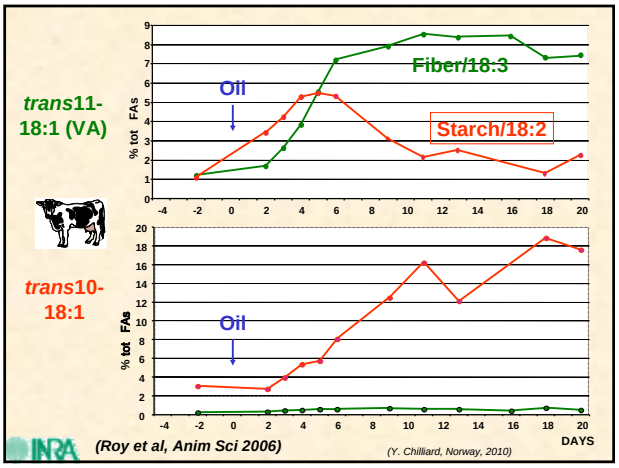
(Y. Chilliard, Norway, 2010) *Chilliard et al., unpublished*

Comparison of mammary lipogenesis and milk *trans*-10 18:1 between ruminant species

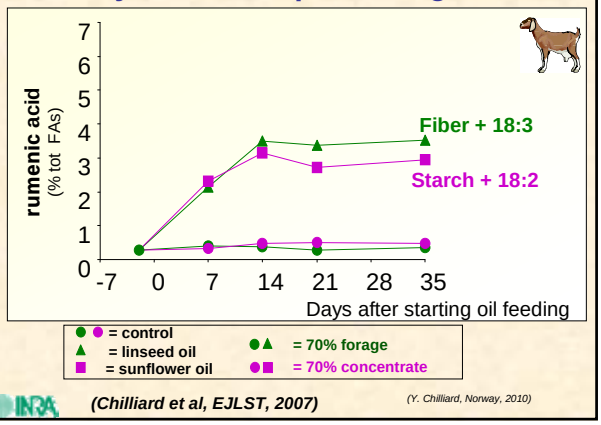
- **Increases in milk *trans*-10 18:1 concentrations are 3-fold higher in cows than goats** or sheep
- Increases in milk *trans*-10 18:1 are associated with MFD in cows but not in goats or sheep
- Data suggest that ruminal biohydrogenation is much less susceptible to the *trans*-10 shift in small ruminants

(Shingfield et al, ISRP 2009)

(Y. Chilliard, Norway, 2010)



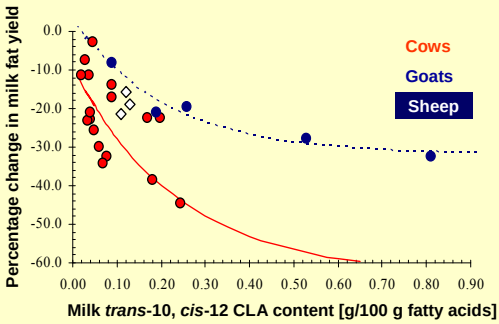
Stability of CLA response in goat milk



(Chilliard et al, EJLST, 2007)

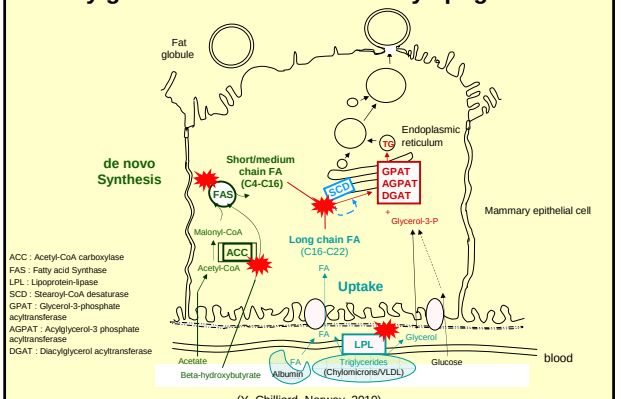
(Y. Chilliard, Norway, 2010)

Mammary lipogenic responses in ruminants fed rumen-protected CLA



(Y. Chilliard, Norway, 2010) **Shingfield et al., 2009 & unpubl.**

Key genes involved in mammary lipogenesis



(Y. Chilliard, Norway, 2010)

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)

(Y. Chilliard, Norway, 2010)

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

Diets causing MFD or infusion of *trans*-10, *cis*-12 CLA

30-59% decrease in C10-C16 secretion in milk

Piperova et al., 2000
Ahnadi et al., 2002
Baumgard et al., 2002
Peterson et al., 2003
Harvatine and Bauman, 2006

⇨ Lowered ACC and FASN mRNA abundance and activity

Starch rich diets supplemented with plant oils

Chilliard et al., 2007
Bernard et al., 2009

5-32% decrease in C10-C16 secretion in milk

⇨ No change in ACC and FASN mRNA abundance and activity

(Y. Chilliard, Norway, 2010)

Nutritional regulation of mammary long chain fatty acid uptake in ruminants

Diets causing MFD or infusion of *trans*-10, *cis*-12 CLA

Decrease in C18 fatty acid secretion in milk

Ahnadi et al., 2002
Harvatine and Bauman, 2006

⇨ Lowered LPL mRNA abundance

Starch rich diets supplemented with plant oils

Increase in C18 fatty acid secretion in milk

Bernard et al., 2005a,b
Bernard et al., 2009a,b

⇨ No change or increase in LPL mRNA abundance/activity

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

(Y. Chilliard, Norway, 2010)

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

Ahnadi et al., 2002
Baumgard et al., 2002 ⇨ Lowered SCD1 mRNA abundance

Varies according to basal diet and composition of lipid supplement

Bernard et al., 2005a,b
Bernard et al., 2009a,b ⇨ 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)

(Y. Chilliard, Norway, 2010)

Concluding remarks #2

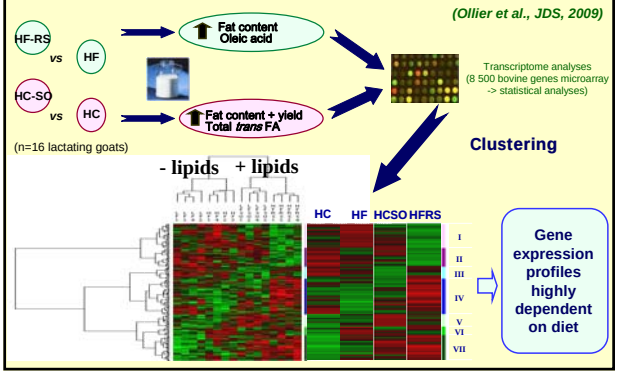
4. Mammary lipogenic responses to changes in diet composition **differ between ruminant species**
5. Some evidence to suggest that differences between ruminant species are related to the effects of diet on **ruminal biohydrogenation**
6. Indirect comparisons indicate inherent differences in the **sensitivity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

(Shingfield et al, ISRP2009)

(Y. Chilliard, Norway, 2010)

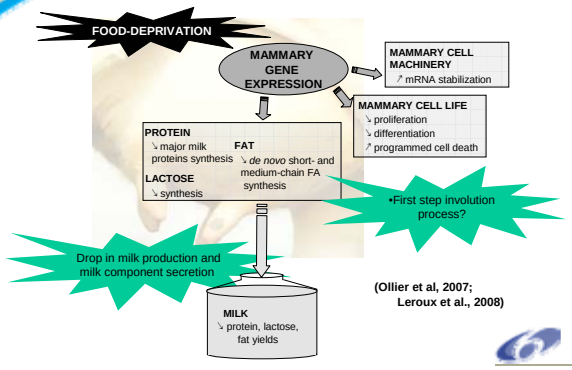
Nutritional regulation of the caprine mammary transcriptome

(Ollier et al., JDS, 2009)



Impact of food-deprivation on mammary function

LIPGENE

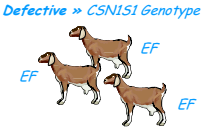


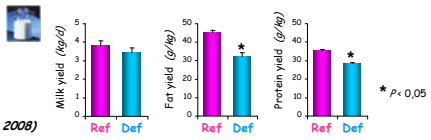
(Ollier et al, 2007; Leroux et al., 2008)

Impact of CSN1S1 polymorphism on milk

« Reference » CSN1S1 Genotype

« Defective » CSN1S1 Genotype





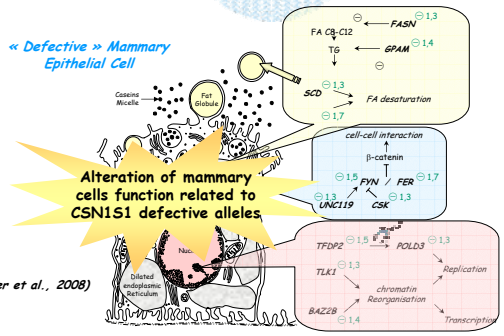
(Ollier et al., 2008)

C. Leroux et al., 24th PPS 2008

DIET AGRICULTURE ENVIRONMENT

Altered biological processes

« Defective » Mammary Epithelial Cell



Alteration of mammary cells function related to CSN1S1 defective alleles

(Ollier et al., 2008)

C. Leroux et al., 24th PPS 2008

DIET AGRICULTURE ENVIRONMENT

Milk FAs	Low	High	CSN1S1 genotype
N. goats	33	38	*(Chilliard et al, 2006a)*
C6:0	**2.5**	**2.7***	
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****	
C16:0	**30.5**	**28.7****	
C16:1c9	0.74	0.67**	
C17:1	0.30	0.27*	
C18:0	**6.6**	**7.4****	**(less desaturated in H goats)**
C18:1c9	**16.9**	**15.0****	
C18:2c9t13	0.12	0.10**	
C18:2n6	**2.1**	**1.9****	
CLAc9t11	0.33	0.28**	
Delta 9-desaturation ratios:			(are not related to SCD mRNA levels ?)
C10:1/C10	**0.025**	**0.021****	
C14:1/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****	
c9t13/t13	**0.71**	**0.59***	

(Y. Chilliard, Norway, 2010)

Genotype-Diet (Extr. Linseed) interactions *(Chilliard and Rouel, unpubl.)*

CSN1S1 Genotype	High	High	Low	Low	
Diet	Control	ELS	Control	ELS	
(N. goats)	(23)	(23)	(24)	(24)	Gen*Diet P<
Milk fat content (g/kg)					0,09
Lipolysis (g OA/100 g fat)					0,0004
C10:0 (% tot. FAs)					0,0003
C16:0					0,02
C18:0					0,002
C18:1cis9					0,10
C14:1 / (C14:0+C14:1)					0,02
C18:1cis9 / (C18:0+C18:1 cis9)					NS

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

Supplement	Linseed	
	Oil	Seeds
Fat content (g/kg)	+3.1	+6.0
18:2 +18:3 (%)	+1.3	+0.5
VA + RA (%)	+2.7	+0.3
Stearic + oleic (%)	+8.3	+11.8

INRA

(Y. Chilliard, Norway, 2010)

Goat milk RA & ALA *(% total FA)*

(Chilliard & Ferlay, 2004)

		RA	ALA
2 control (hay) diets	:	0.3%	0.5-0.8%
3 hay diets + linseed oil	:	3.0-3.5%	1.3-1.7%
1 hay diet + extr. linseeds	:	2.1%	2.7%

INRA

(Y. Chilliard, Norway, 2010)

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

(Y. Chilliard, Norway, 2010)

Goat milk saturated FA atherogenic index

[% C12:0 +4 (% C14:0) + % C16:0]

In 7 control diets : 75-85 %

In 12 lipid diets : 48-56 %

X 0.65

(Y. Chilliard, Norway, 2010)

Goat milk fat oleic acid

(C18:1, *cis* 9)

In 7 control diets : 14-18 %

In 4 oleic sunflower oil diets : 25-29 %

X 1.69

(Y. Chilliard, Norway, 2010)

Goat milk fat linolenic/linoleic ratio

(18:3 n-3/18:2 n-6)

In 4 maize silage diets, without lipids, or with sunflower or oleic sunflower oil : 0.05-0.18

In 2 maize silage diets with linseed oil : 0.36-0.47

In 4 Hay/Grass diets with linseed oil : 0.64-0.85

X 3.6

X 6.5

(Y. Chilliard, Norway, 2010)

Goat milk fat rumenic acid equivalent

[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

2 Sunflower oil diets : 5.7-6.2 %

X 4.8

X 6.3

(Y. Chilliard, Norway, 2010)

Goat milk fat non-*trans* 11 C18:1 and C18:2 *trans* isomers

(i.e. except rumenic and vaccenic acids)

7 control diets : 0.4-1.6 %

X 4.7

8 Hay/Grass diets + oils : 2.9-6.5

X 8.8

4 Maize Silage diets + oils : 6.3-11.3

(Y. Chilliard, Norway, 2010)

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

(Y. Chilliard, Norway, 2010)

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

5 control diets : 1.54-2.50

X 0.89

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)

(Y. Chilliard, Norway, 2010)