Nutritional regulation of mammary lipogenesis and milk fat in ruminant: contribution to sustainable milk production

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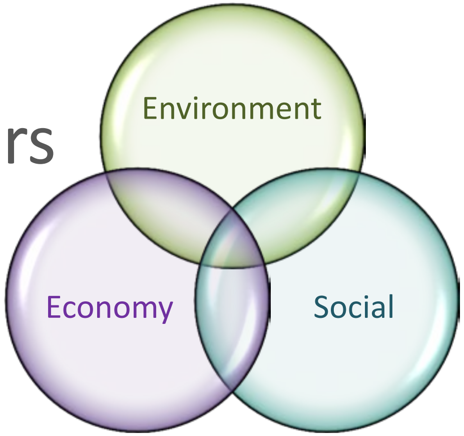
- **Introduction**
- Nutritional regulation of milk fat synthesis and composition
- Rumen biohydrogenation of dietary unsaturated fatty acids
- Mammary lipogenesis
  - Nutritional regulation of mammary lipogenic gene expression
  - Mammary bioconversion of long-chain fatty acids and milk quality
- Conclusions

Context : developement of animal science to sustainable production systems

Sustainability: a concept based on 3 pillars



- Societal expectations
  - Product quality and safety
  - Animal welfare
- Socio-economic viability
  - Animal performances/Profit
- Environment protection

Contribution of dairy science to sustainable production systems: focus on milk fat synthesis

Performances of dairy ruminants

- Nutrient partitioning

⇨ **Control of milk fat synthesis**

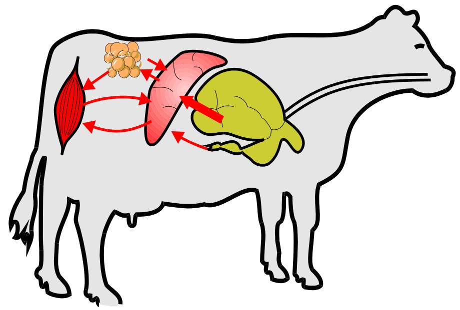
- **Quality of dairy products**
  - Fat: processing, organoleptic quality
  - Fatty acid composition: nutritional quality

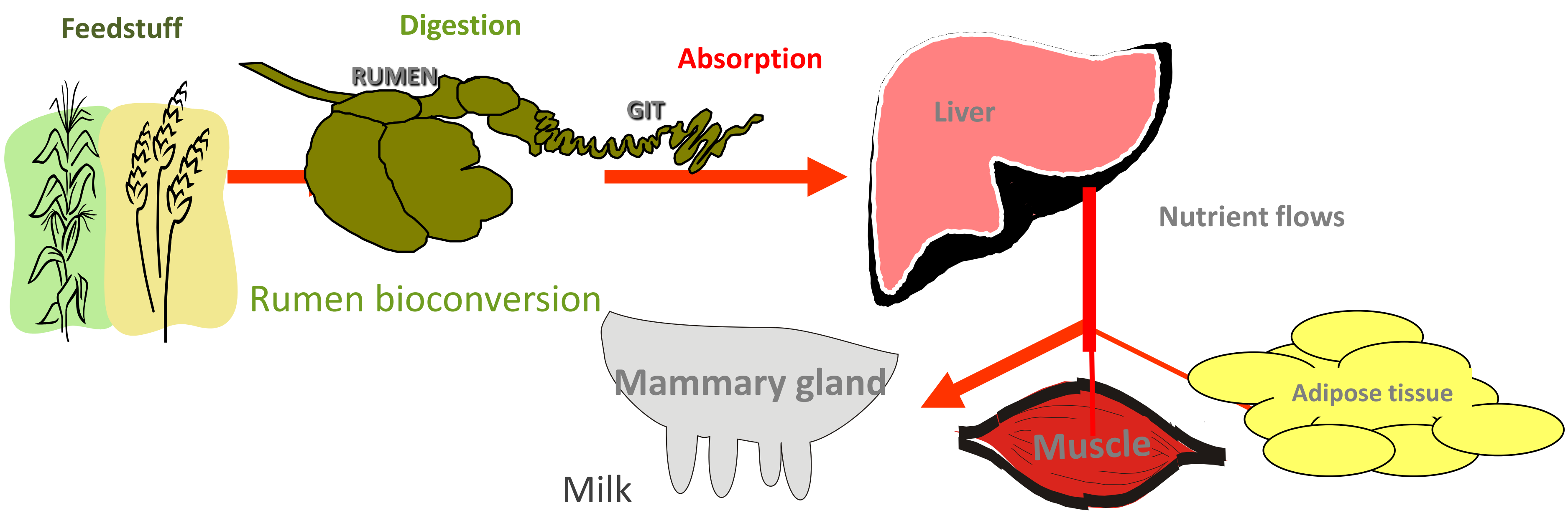
⇨ **Mammary metabolism: gene expression**

Analysis of the animal lactation function

Physiology: nutrient partitioning in the lactating ruminant

whole animal metabolism





⇨ **modulation of metabolism to priorise MG during lactation (teleophoresis*)***

Nutrition: in addition to physiological state, the diet influences mammary metabolism and gene expression in ruminants

- The quantity and composition of diet modulate metabolic pathways and (maybe) the expression of many genes acting in concert
- The mechanisms by which nutrients turn specific genes on or off are still poorly understood in ruminants

Understanding mammary lipogenesis

- Optimising milk fat content:
  Central to improving the nutritional quality of ruminant milk for human
- Controlled decrease in milk fat yield has the potential to improve energy balance during early lactation
- Lipid supplements used to lower greenhouse gas emissions *(Morgavi et al., 2010; Martin et al., 2010; Chilliard et al., 2009)*

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Variation factors of milk composition

		Fat	Protein
Animal	Species	+ + +	+ + +
	Breed	+	+
	Lactation stage	+ +	+ +
Environment (season, diet)			
	Temperature	(+)	(+)
	Photoperiod	(+)	(+)
	Feeding factors	+ + + *Quantity*	+
Interactions	Energy balance	+ +	+ +

Nutritional regulation of mammary lipogenesis in the bovine

- In most cases “diet-induced milk fat depression” (MFD): Situations where feeding a specific diet or dietary ingredient results in a reduction in milk fat synthesis
- Decreases in milk fat yield typically 10-30%, but in more extreme cases can result in more than 50% reduction in milk fat output

Characteristics of diets that cause milk fat depression

1) Rich-starch diets

2) High concentrate/low fibre diets + PUFA

3) Rations containing marine lipids

Presence of PUFA

Addition of PUFA

↓

Changes in rumen biohydrogenation of FA ***(following section)***

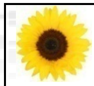
Use of MFD diet as a nutritional management tool

- Positive effects on **Animal performance**
  - Increase milk production
  - Improve energy balance during early lactation and limit body fat mobilisation
  - Enhance reproductive efficiency and next lactation

- **Negative effects**
  - Animal health: acidosis
  - Ressources: high grains and oilseed diets
    - in competition with human nutrition
    - high cost
  - Product: high level of trans-FA and *n*-6 FA in milk

Response of dairy ruminants to 2 types of MFD diets

2 types of MFD diet




Sunflower oil (18:2n-6) + starch

Marine oil (20:5n-3, 22:6n-3)

Fat content (%)

	Sunflower oil + starch	Marine oil
Cows	↓↓	↓↓

(Boeckaert et al 2008; Loor et al 2005; Rego et al 2009; Angulo et al., 2012)

	Sunflower oil + starch	Marine oil
Ewes	= ↑	↓

(Hervás et al 2008 ; Toral et al 2010)

	Sunflower oil + starch	Marine oil
Goats	= ↑	=

(Chilliard et al 2007, Bernard et al 2009, 2010)

Extreme response of goats vs cows

Fatty acids involved in the MFD in bovine

- Remind the biohydrogenation theory of MFD: "Mammary synthesis of milk fat is inhibited by unique fatty acids that are produced as a result of the alterations in rumen biohydrogenation." *(Bauman & Griinari, 2001)*

- trans fatty acid tested and outlined for their anti-lipogenic activity:
  - trans-10, cis-12 CLA *(Baumgard et al., 2000)*
  - trans-9, cis-11 CLA *(Perfield et al., 2007)*
  - cis-10, trans-12 CLA *(Sæbø et al., 2005)*

  But cannot explain entirely MFD in cows

- Increases in milk trans-10 18:1 are associated with MFD in cows *(Shingfield et al., 2010)*

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SCIENCE & IMPACT

Overall targets for altering milk fat composition to improve long-term human health

Cow milk FA composition
- Saturated FA (~68% TFA)
- Monounsaturated FA (~29% TFA)
- *Trans*-FA (2-6% TFA)
- Polyunsaturated FA (~3% TFA)

Nutritional quality
negative effects (in excess)
positive effects

(Jensen, 2002; Chilliard, 2006)

- Decreasing medium chain saturated fatty acids* and increasing *cis*-9 18:1* concentrations…without substantial increases in *trans* fatty acids

- Enhancing polyunsaturated* fatty acid content

- Increasing bioactive lipid* components e.g. 4:0, 15:0 *iso*, *cis*-9, *trans*-11 conjugated linoleic acid (CLA)

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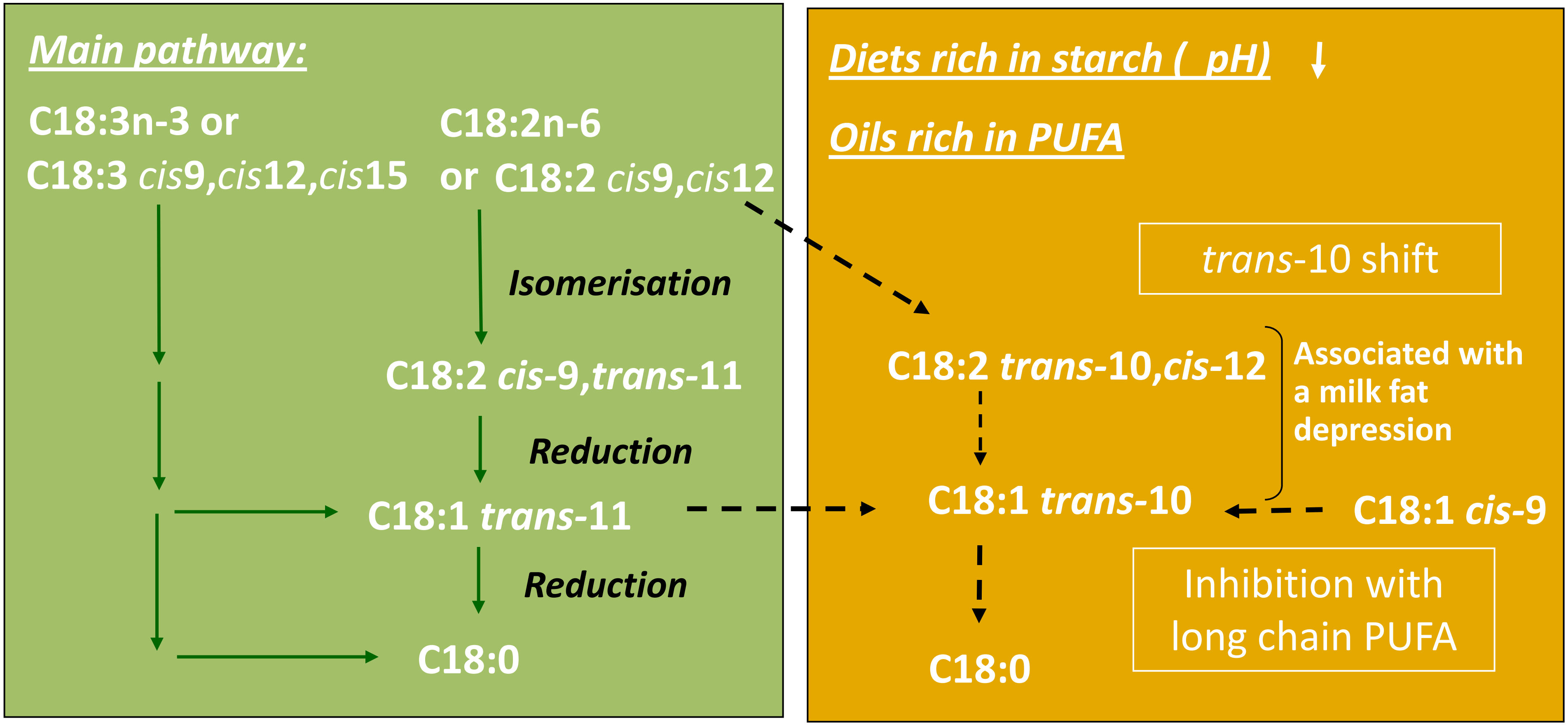
Biohydrogenation of unsaturated fatty acids in the rumen

- 58-87% of oleic acid is hydrogenated
- 70-95% of linoleic acid is hydrogenated
- 85-100% of linoleic acid is hydrogenated

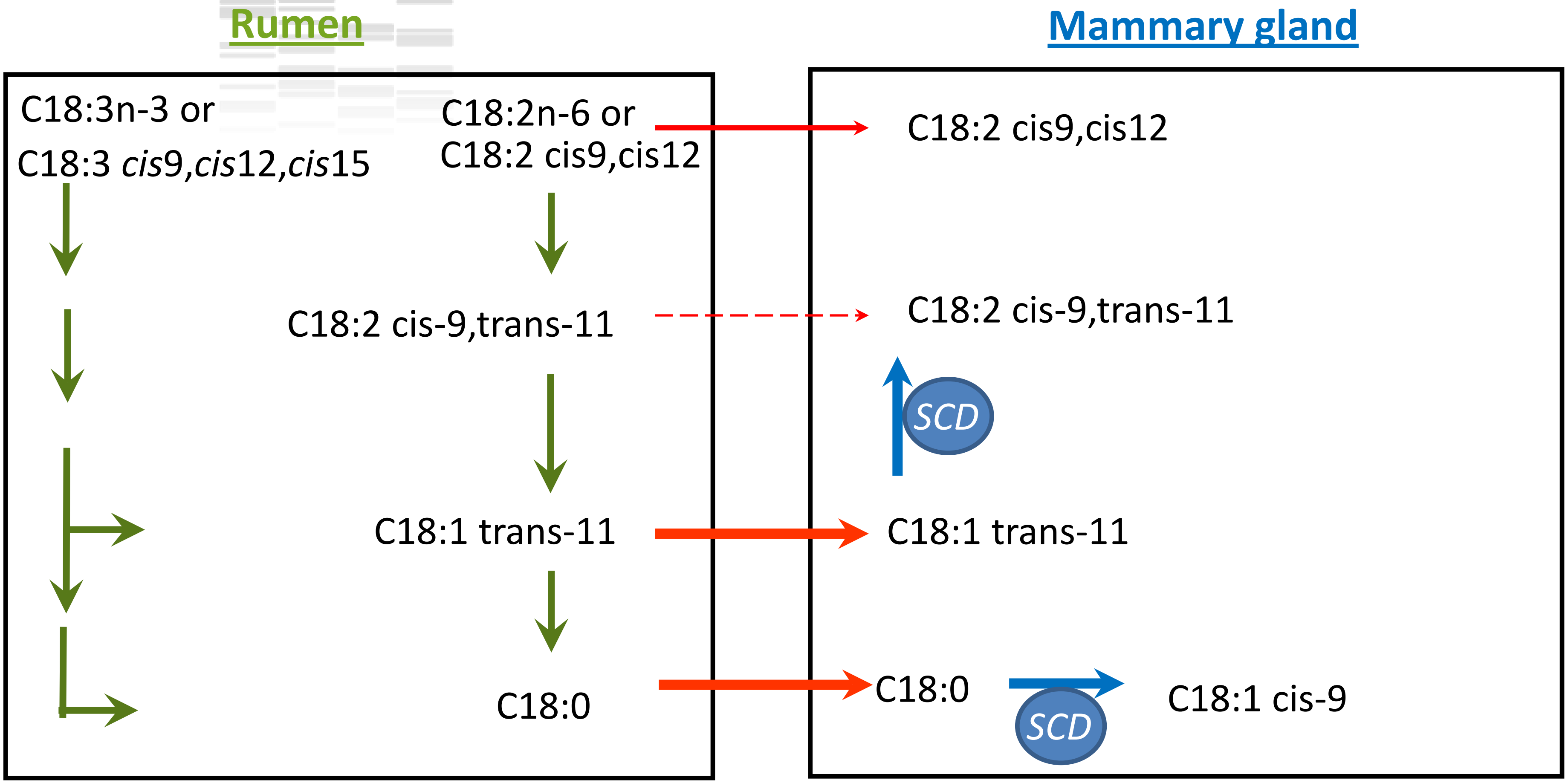
- Conversion to 18:0 is incomplete and biohydrogenation intermediates containing a trans double accumulate in the rumen

Synthesis of trans-10 fatty acids in the rumen

(from Griinari and Bauman, 1999;)



Role of SCD protein in the ruminant mammary gland



- **Milk fat fluidity** : FA saturated in the rumen and mammary desaturated in MG
- Improve milk **nutritional quality**

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Tools to study mammary lipogenesis

Level of study

- Animal
- Tissue
- Cells
(from tissue, milk)
- Molecular

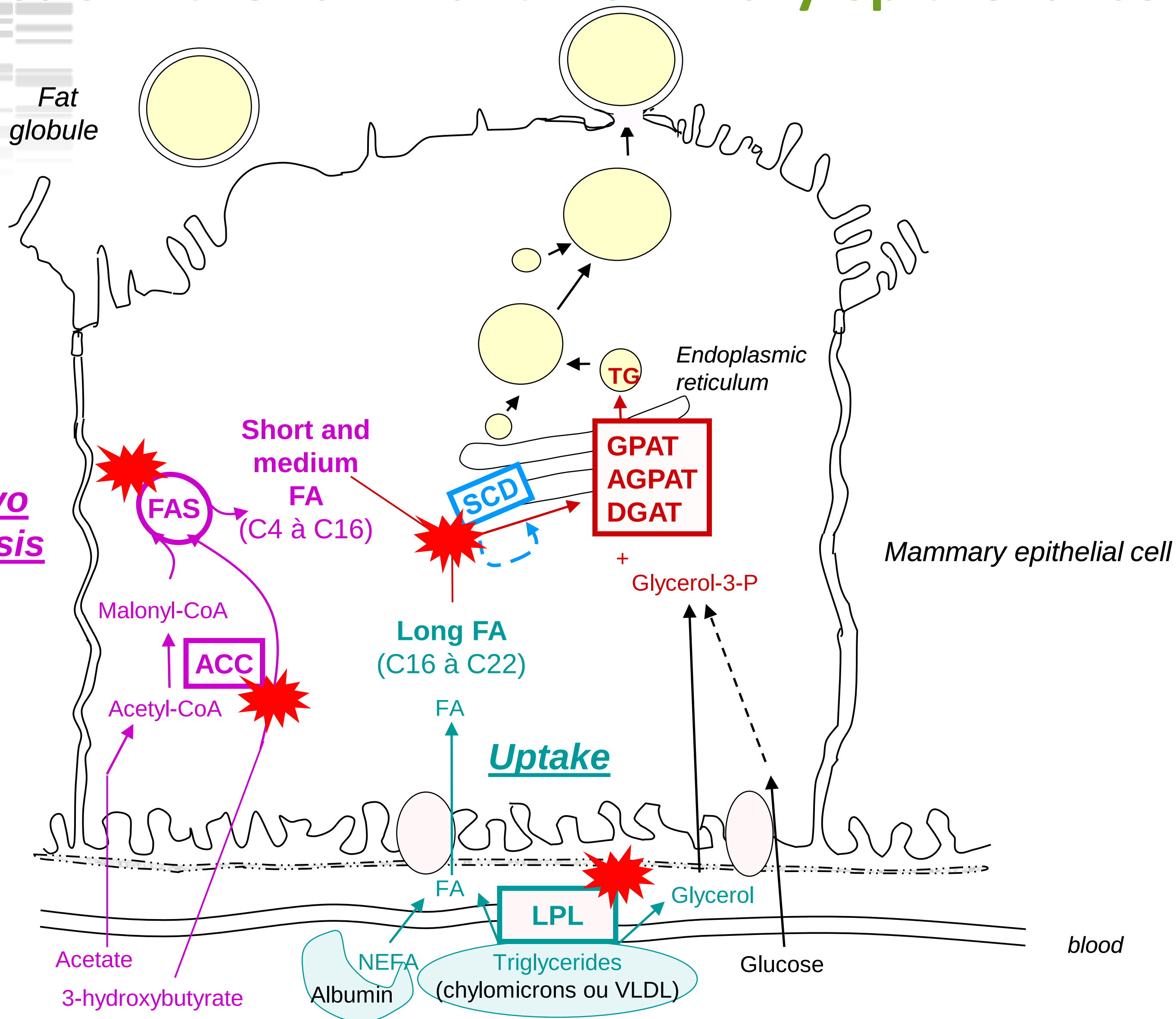
Measures

- Gene expression
- Protein (activity)
- Metabolic pathway
- Substrate flow

Methods

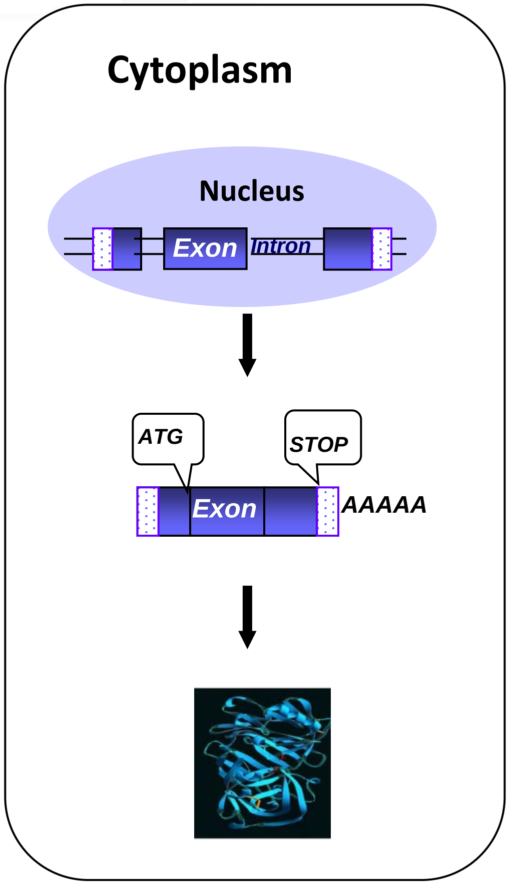
- Multi-catheterised animals/blood sampling MV (Fick principle)
- Biopsies
- Mammary epithelial cell culture/mammary explants
- RT-PCR, NGS, transcriptome
- Western Blot, immunochemistry, RIA/Elisa, proteome
- Metabolic tracers (13C-FA), metabolome

Milk fat synthesis in the ruminant mammary epithelial cell



ACC : Acetyl-CoA carboxylase
FAS : Fatty acid Synthase
LPL : Lipoproteine-lipase
SCD : Stearoyl-CoA desaturase
GPAT : Glycerol-3-phosphate acyltransferase
AGPAT : Acylglycerol-3 phosphate acyltransferase
DGAT : Diacylglycerol acyltransferase

Regulation of gene expression studies



Level of regulation

Gene → Genomic

↓ ***Transcription***

mRNA (s) → **Transcriptomic (microarray) and /or candidate gene expression (RT-PCR)** ★

↓ ***Traduction***

Protein(s) → Proteomic

Activation Enzyme activity ★

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    - ***De novo* lipid synthesis**
    - **Uptake of FA**
    - **Desaturation of FA**
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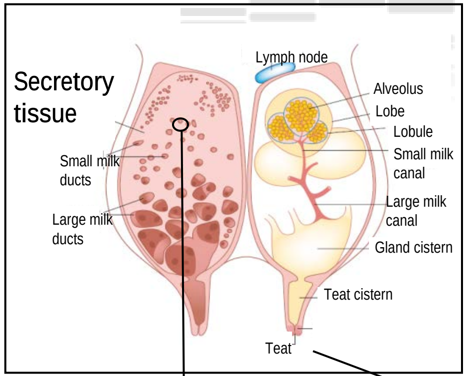
Relationships between nutrition, genes expression and milk fatty acids composition



Tissue *(biopsy)*

Milk

Level of approach

Measures *(methods)*

mRNA of candidate genes *(RT-PCR)*

Protein : *Enzyme activity*

- *De novo* lipogenesis: ACC et FAS ↔ **C4-C16**
- Uptake: LPL ↔ **≥ C18**
- Desaturation: SCD ↔ **cis-9 FA: desaturation ratios**

Fatty acid composition:

- **C4-C16**
- **≥ C18**
- **cis-9 FA: desaturation ratios**

Effect of nutrition on mammary lipogenic gene expression

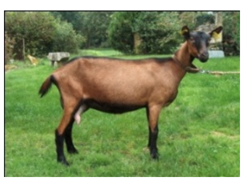
Peterson et al., 2003*
Piperova et al., 2000*
Ahnadi et al., 2002*
Invernizzi et al., 2010*
Harvatine and Bauman., 2006*
Angulo et al., 2012*
Delbecchi et al., 2001
Mach et al., 2011
Jacobs et al., 2011

Few data available *in vivo* and *in vitro*

In vivo studies :

- **on cows:** essentially with **extreme dietary conditions*** that induce MFD and with post-ruminal infusion studies of **t10,c12-CLA**

Baumgard et al., 2002; Harvatine and Bauman, 2006; Gervais et al., 2009

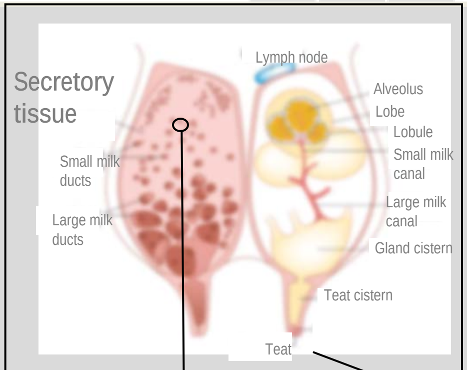


- **on goats:** with more usual dietary conditions **(lipid supplements)**

Bernard et al., 2005a,b; 2009a,b; 2012; Ollier et al., 2009

→ **Database to study the effect of nutrition on mammary gene expression**

Relationships between nutrition, genes expression and milk fatty acids composition



Tissue *(biopsy)*

Milk

<u>Level of approach</u>

<u>Measures *(methods)*</u>

mRNA of candidate genes *(RT-PCR)*

Protein : *Enzyme activity*

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Fatty acid composition:

- **C4-C16**
- **≥ C18**
- **cis-9 FA: desaturation ratios**

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

Piperova et al., 2000
Ahnadi et al., 2002
Baumgard et al., 2002
Peterson et al., 2003
Harvatine and Bauman, 2006
Gervais et al., 2009
Angulo et al., 2012

Diets that induce MFD or *trans*-10, *cis*-12 CLA infusion

30-59% decreases of milk C10-C16 secretion

⇒ Decreases:

(i) mRNA abundance of *ACACA* and/or *FASN*
(ii) enzymes activities of ACC and/or FAS

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

Diets rich in starch supplemented with plant oils

5-32% decreases of milk C10-C16 secretion

⇒ Absence of variation of ACC and FAS (mRNA and enzyme activity)

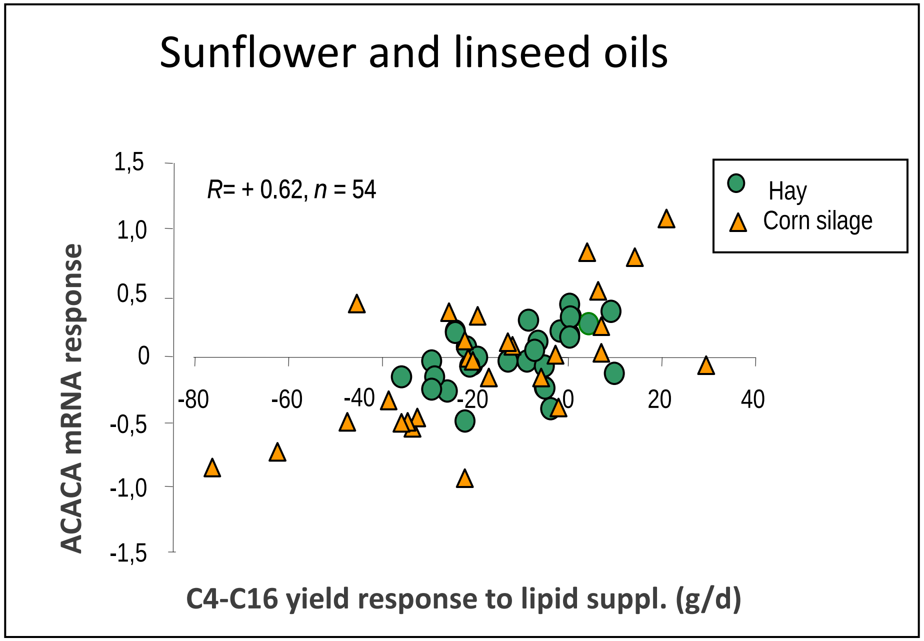
Relationships between genes expression and milk fatty acids composition

• de novo lipogenesis : *ACACA* and *FASN* ↔ Milk C4-C16

Diet supplemented with lipids :

Individual data:

(n=54, from 91 biopsies in 2 LS 3X3)



- *ACACA* mRNA response related to milk C4-C16 yield
- Mean/diet: ↓18-32% C4-C16 yield and small variation of m RNA and enzymes activities (ACC and FAS)

(Bernard et al 2008)

Nutritional regulation of uptake of long-chain FA : LPL

• Uptake : LPL ↔ ≥ C18

Ahnadi et al., 2002
Harvatine and Bauman, 2006

Diets that induce MFD or *trans*-10, *cis*-12 CLA infusion

Decrease in secretion of milk C18 FA

⇨ Decrease LPL mRNA

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

Starch rich diets supplemented with plant oils

Increase in C18 FA secretion in milk (>100%)

⇨ 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

⇨ **Substrate availability? Other key genes : CD36, FABP… ?**

Nutritional regulation of mammary Δ-9 desaturase (SCD1) in ruminants

Ahnadi et al., 2002
Baumgard et al., 2002
Angulo et al., 2012
(Gervais et al., 2009)

Varies little in response to diet

Diets containing fish oil or infusion of *trans*-10, *cis*-12 CLA

⇨ ↓ SCD1 mRNA ***(SCD5 =)***

(Jacobs et al., 2011 : ↓ SCD1 mRNA with SoyO vs LO and RO)

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

Varies according to basal diet and composition of lipid supplement

⇨
- Formaldehyde-treated linseeds lower SCD1 mRNA abundance
- Plant oils (rich in 18:1n-9, 18:2n-6, 18:3n-3): often decrease SCD1 activity

⇒ Transcriptional and/or post-transcriptional regulation of SCD1 according to FA

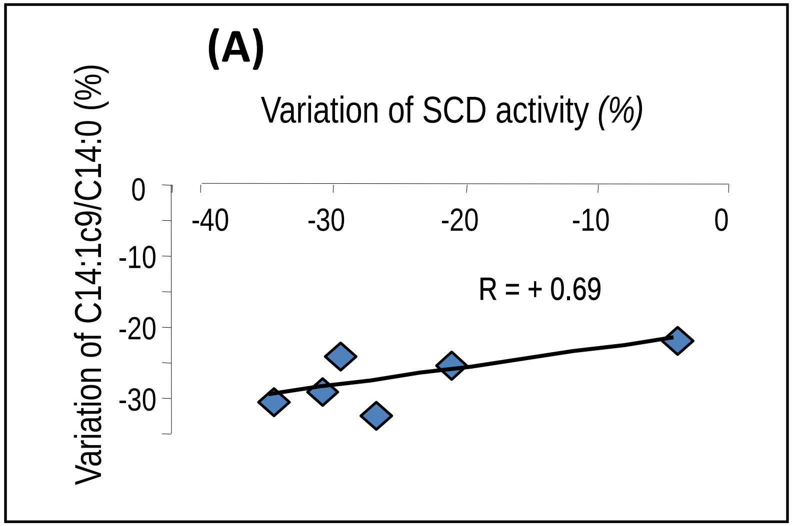
⇒ Balance between uptake and synthesis of MUFA: SCD implication in the regulation of the milk fat fuidity

Relationship between SCD activity and milk FA

- **Desaturation: SCD ↔ cis-9 FA : desaturation ratios**

c9-14:1/14:0
c9-16:1/16:0
c918:1/18:0
c9,t11-18:2/t11-18:1

Hay based diets supplemented with lipids (oils and seeds)



(Bernard et al 2009a, 2009b)

- **Relationship between milk desaturation ratio and enzyme activity**
- **↓ enzyme activity**

Rich-starch diets supplemented with lipids

- **↓ of milk desaturation ratios**
- **No effect on enzyme activity (No effect on mRNA)**

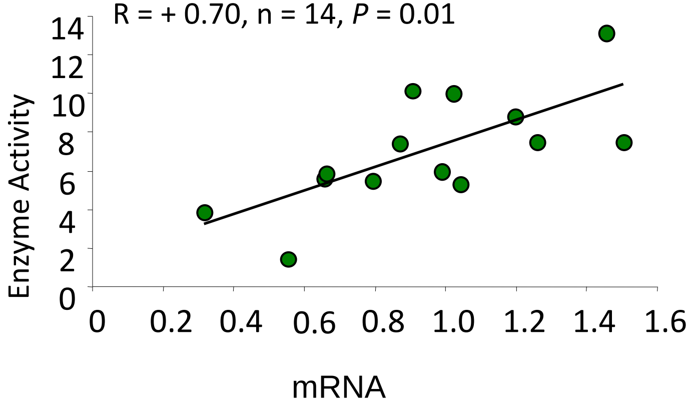
⇓

- Representativeness of milk desaturation ratios ?
- Limits of the ex-vivo enzyme assay?

Differents levels of gene regulation

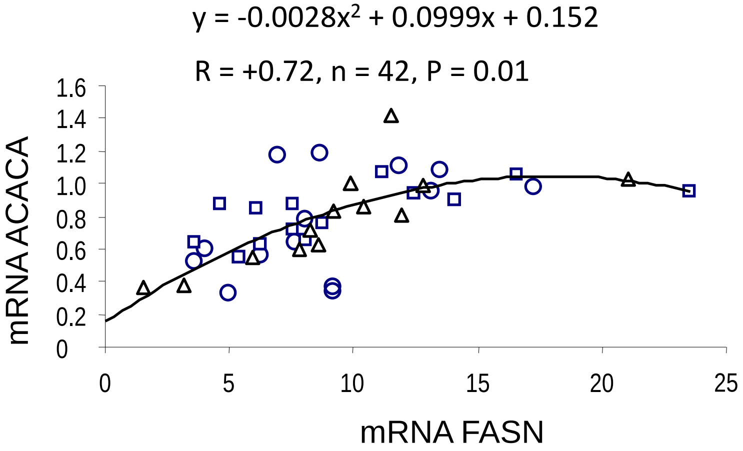
With rich-starch diet supplemented with lipids :

(1) ex. ACACA



1. Transcriptional regulation of ACACA and FASN

(2) : Relationship between mRNAs



2. Common regulatory elements?

→ Transcription factors?

3. Transcriptional and/or post-transcriptional regulation of SCD1 depending on lipid supplements and nature of based diet

(Bernard et al 2008, 2009b, 2012)

Fatty acids and transcription factors

Major transcription factors involved in lipid metabolism:
SREBP1 and PPAR (α, β, γ)

Effects of FA on transcription factors may be :

- **direct** by labelling on transcription factors : PPAR/RXR/LXR.
- or **indirect** via transcription and/or regulation activity of transcription factor : SREBP1.

Nutritional regulation of mammary lipogenesis: role of transcription factors

In-vivo
MFD diet
trans-10, *cis*-12 CLA

Harvatine and Bauman, 2006
Harvatine et al., 2008
Gervais et al., 2009
Angulo et al., 2012

In-vitro
trans-10, *cis*-12 CLA/*trans*-10 18:1
MAC-T (BME-UV) cells

Peterson et al., 2003
McFadden et al., 2008
Kadegowda et al., 2009

↓INSIG 1,2
↓S14

↓ **SREBP-1 mRNA and/or activated protein**

PPARγ?
LXR

Lengi and Corl, 2010

SREBP1-siRNA (MACT)
Ma and Corl, 2012

↓

Downregulation of lipogenic genes

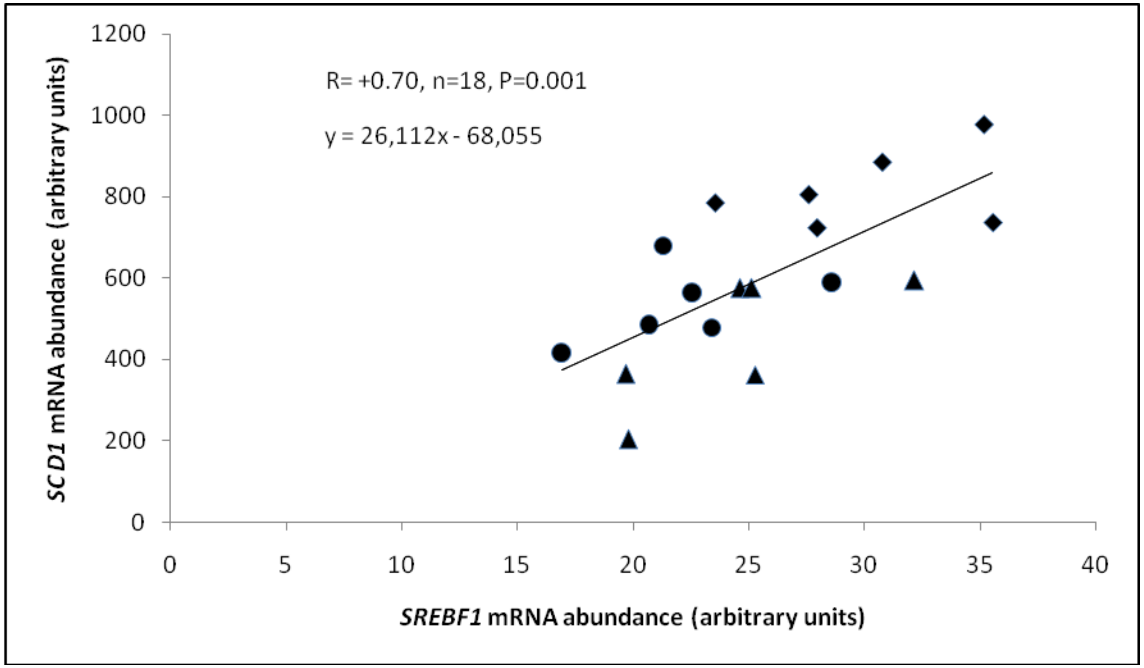
LXR in MEC goat
Wang et al, 2012

⇨ **Role of SREBP1 and Spot 14 as central regulators of MFD in the cow**

⇨ **Other regulatory factors eg. protein kinase (AMP-K, PKB/Akt, Erk, PERK…), miRNA to explore…**

Ex: relationship between mRNA abundances of stearoyl-CoA desaturase (*SCD1*) and sterol regulatory element binding transcription factor 1 (*SREBF1*) in mammary tissue

Cows were fed diets supplemented with rumen-protected saturated fat (SAT, ■), or a combination of linseed oil and algae (LINA, ▲), or a combination of sunflower oil and algae (SUNA, ●). The mRNA abundances are expressed as abundance relative to the geometric mean of 3 reference genes (*PPIA, EIF3K* and *UXT*).



From Angulo et al., 2013

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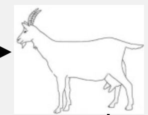
Ex: Measure of the in vivo metabolic activity of the SCD

- Direct methods: tracer methodology
  - 13C-18:0, -16:0,-14:0, 18:1trans-11 in cows *(Mosley et al., 2006)*
  - 13C-18:1trans-11 in goats *(Bernard et al., 2010)*
- Indirect methods :
  - Duodenal and milk FA flows in cows *(Shingfield et al., 2007; Glasser et al., 2008)*
  - Inhibition of SCD using sterculic acid in cows *(Griinari et al., 2000; Corl et al., 2001; Kay et al., 2004)* and in ewes *(Bichi et al., 2012)*

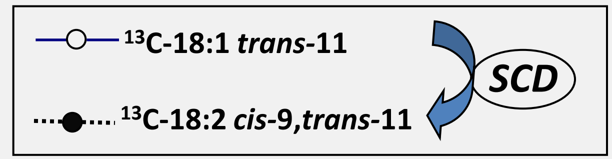
Use of 13C-labelled fatty acids : example of 13C-18:1trans-11 to study Δ9-desaturation pathway in goats

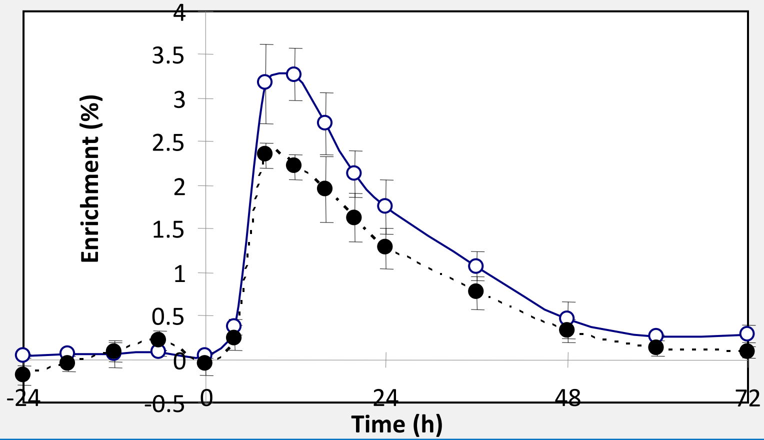
(Bernard et al 2010)

^{13}C-18:1 trans-11 (<u>iv injection</u>)



Total <u>milk sampling</u>





<u>Enrichment kinetics</u> of milk fat in ^{13}C-18:1*trans*-11 and ^{13}C-18:2*cis*-9,*trans*-11

- **~ 32% of C18:1trans-11 uptaken is Δ9-desaturated in the mammary gland**
- **63 to 73% of milk C18:2cis-9,trans-11 comes from delta-9 desaturation of C18:1trans-11**

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Conclusions

1. Characterisation of effect of nutrition on milk fat
2. Application of molecular tools : mechanisms of milk fat synthesis
3. MFD diets act via alteration of rumen metabolism and production of specific trans-FA in cows
4. Production of specific trans-FA in cows alters mammary lipogenic gene expression
5. Indirect comparisons indicate a lower sensitivity of goats mammary lipogenic genes to trans-FA compared to cows
6. Still little data on transcription factors

Thank you for your attention !