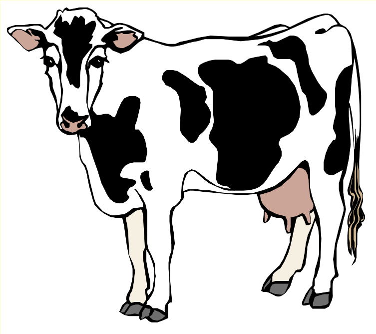
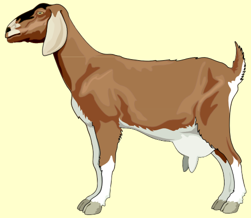
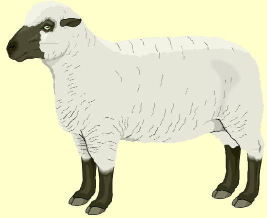






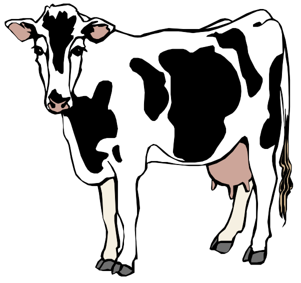
Trans fatty acids and mammary lipogenesis in ruminants

KJ Shingfield

MTT

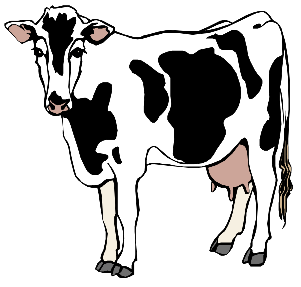
Understanding mammary lipogenesis

- Optimising milk protein and fat content
- Central to improving the nutritional quality of ruminant milk
- Controlled decreases in milk fat has the potential to improve energy balance
- Lipid supplements used to lower greenhouse gas emissions



Nutritional regulation of mammary lipogenesis in the bovine

- In most cases "diet-induced milk fat depression"
- 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) High concentrate/low fibre diets

2) Rations containing marine lipids

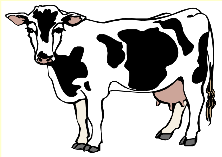
3) Diets containing ionophores

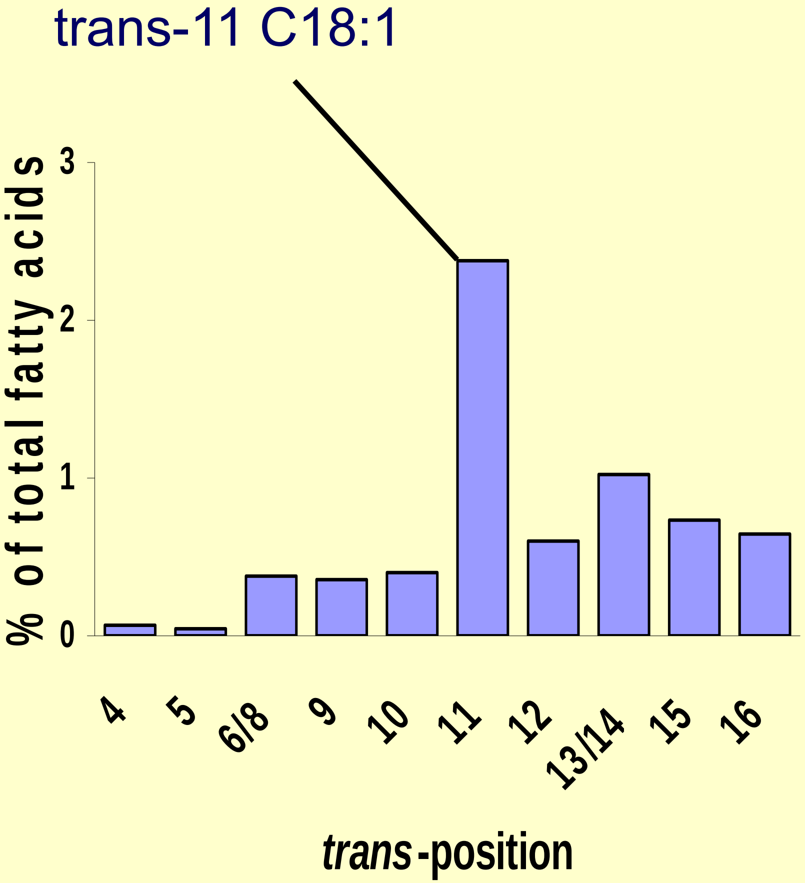
} Addition of polyunsaturated fatty acids

↓

Changes in rumen biohydrogenation

Changes in milk fatty acid composition during dietary induced milk fat depression





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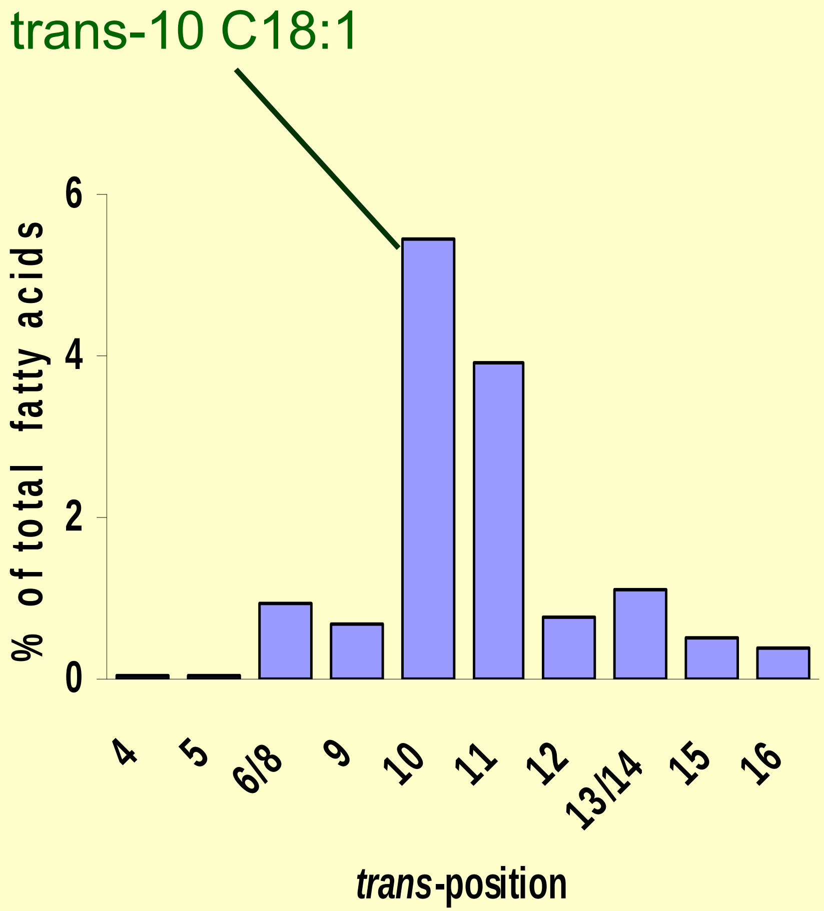


Milk fat depression

Griinari et al., 1998

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 shift

***cis*-9, *cis*-12 $C_{18:2}$ (linoleic acid)** →

***trans*-10, *cis*-12 $C_{18:2}$ (CLA)**

↓

***trans*-10 $C_{18:1}$**

↓ Inhibition

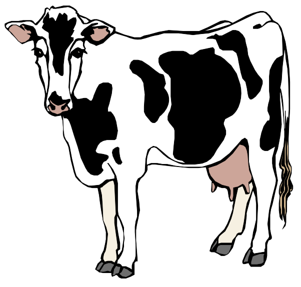
$C_{18:0}$ (stearic acid)

Griinari and Bauman, 1999

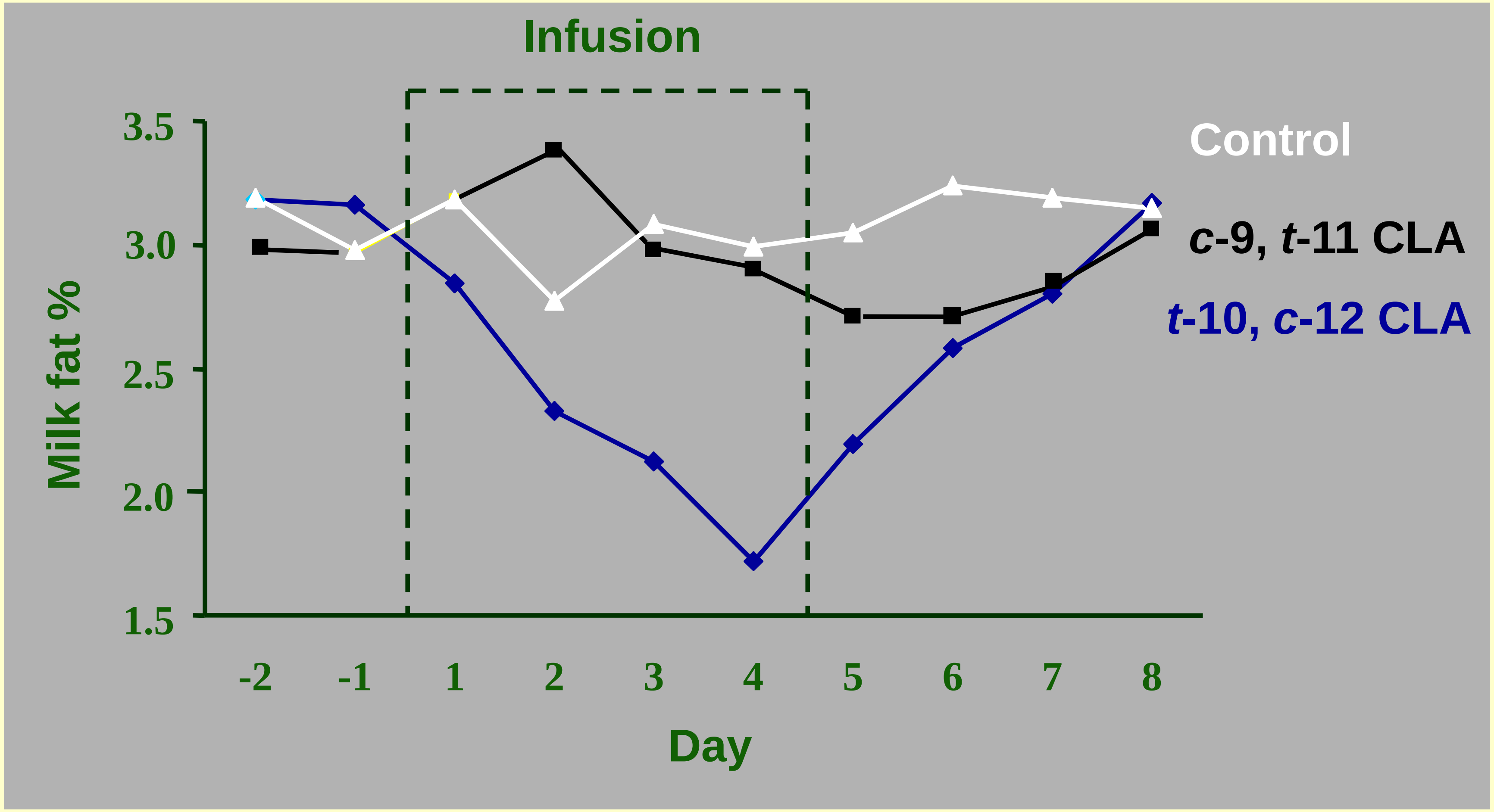
Biohydrogenation theory of milk fat depression

”Mammary synthesis of milk fat is inhibited by unique fatty acids that are produced as a result of the alterations in rumen biohydrogenation.”

Bauman and Griinari, 2001

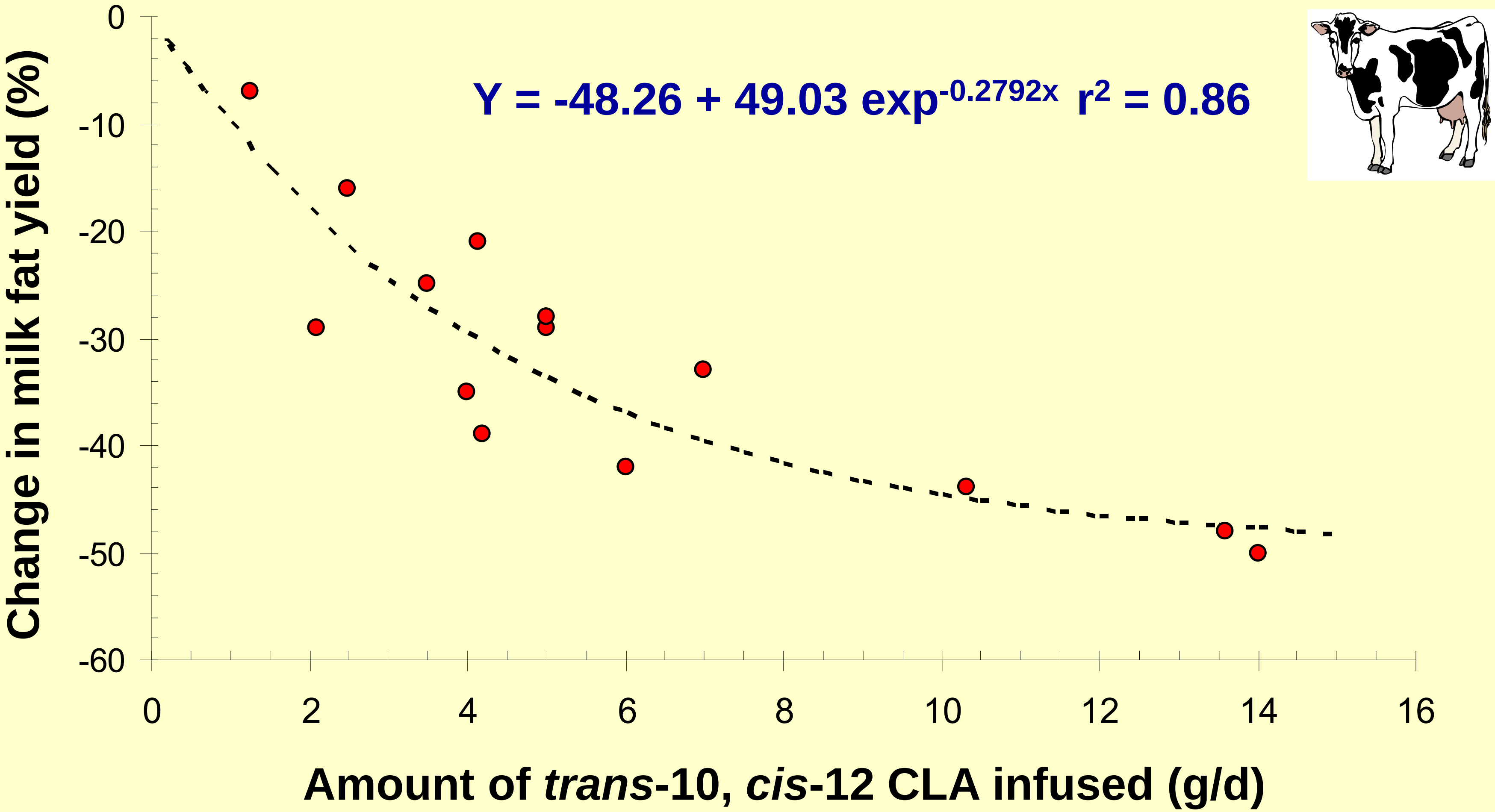


Effect of CLA isomers on milk fat content



Baumgard et al., 2000

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

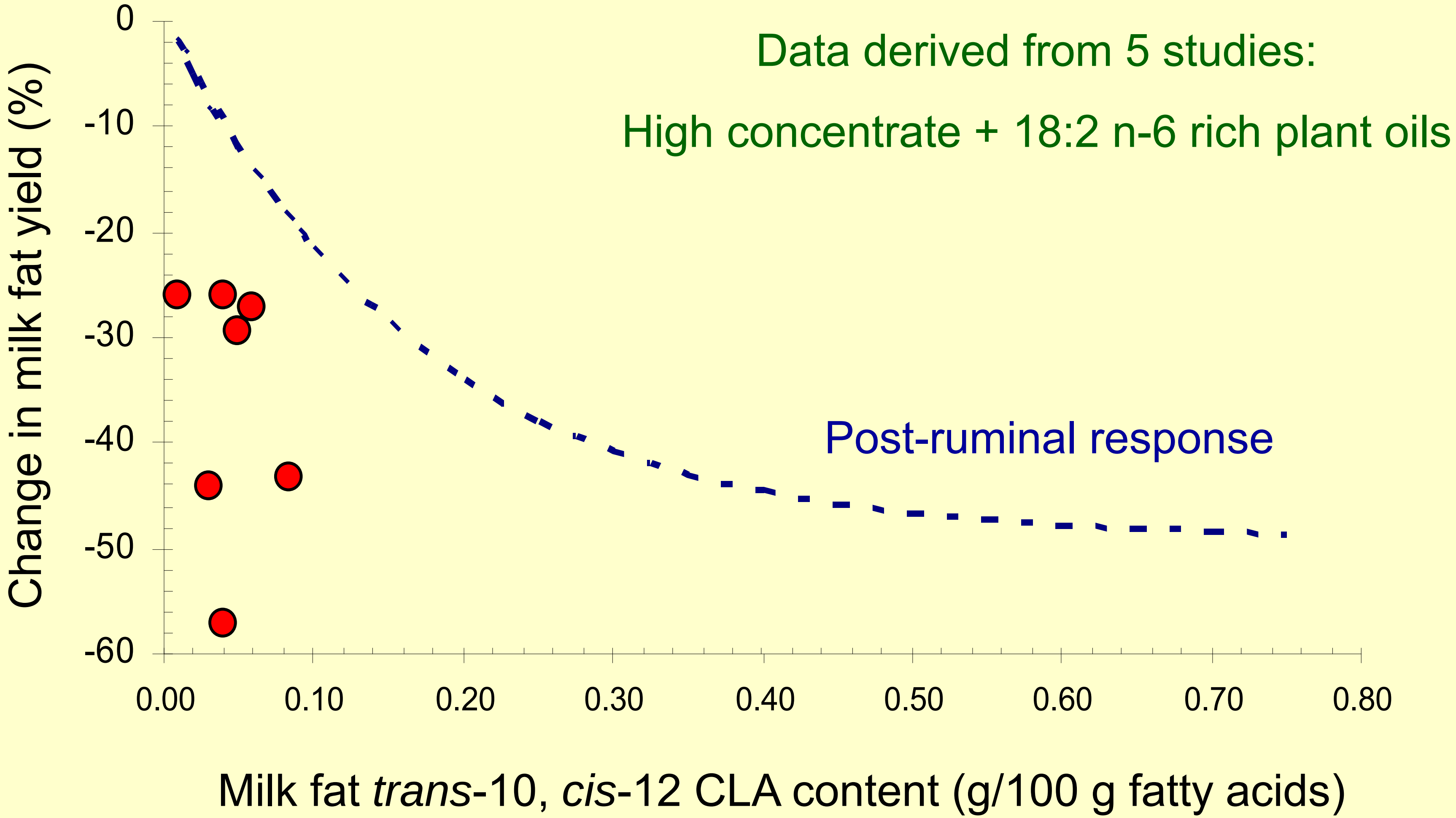


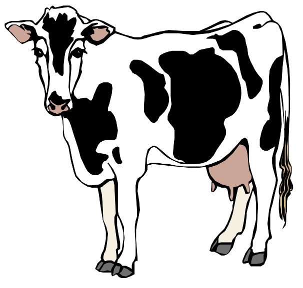
De Veth et al., 2004

Flow of *trans*-10, *cis*-12 CLA leaving the rumen in lactating cows (g/d)

Basal forage	F:C	Lipid supplements	Flow	Reference
Grass silage	60:40	Fish oil	0.02-0.10	Shingfield et al., 2003
Grass silage	60:40	Sunflower oil	0.09-0.36	Shingfield et al., 2008
Grass silage	65:35/ 35:65	None	0.18-0.50	Shingfield et al., unpublished
Grass silage	70:30	Linseed oil	0.04-0.11	Kairenius et al., unpublished
Grass hay	65:35/ 35:65	Linseed oil	0.07-0.10	Loor et al., 2004
Grass hay	35:65	Sunflower/linseed/ fish oil	0.30-1.40	Loor et al., 2005
Maize silage/ Lucerne hay	60:40/ 25:75	None	0.05-0.26	Piperova et al., 2002

Milk fat *trans*-10, *cis*-12 CLA content and milk fat yield during diet induced MFD



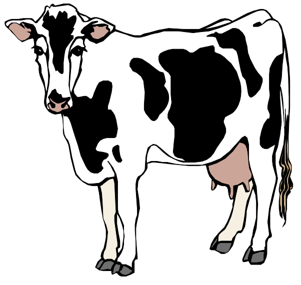


Does *trans*-10, *cis*-12 CLA explain the decreases in milk fat?

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

Role of other biohydrogenation intermediates on milk fat synthesis: the evidence

- Smaller than expected or no increases in milk *trans*-10, *cis*-12 CLA content during diet induced milk fat depression (Griinari and Bauman, 2003)
- Post-ruminal infusions of a mixture of CLA isomers devoid of *trans*-10, *cis*-12 reduced milk fat synthesis (Chouinard et al., 1999)



Range of *trans* fatty acid tested for anti-lipogenic activity

Isomer (s) investigated	Dose (g/d)	Purity (%)	Lipogenic activity	Reference
trans-10, *cis*-12 CLA	10.3	89.5	Inhibitory	Baumgard et al., 2000
trans-9 18:1	25.0	99.0	Neutral	Rindsig and Schultz, 1974
trans-10 18:1	42.6	94.7	Neutral	Lock et al., 2007
trans-11 18:1	12.5	50.0	Neutral	Griinari et al., 2000
trans-12 18:1	12.5	50.0	Neutral	Griinari et al., 2000
cis-9, *trans*-11 CLA	10.1	73.9	Neutral	Baumgard et al., 2000
cis-10, *trans*-12 CLA	1.2	23.2	Tentative	Sæbø et al., 2005
cis-11, *trans*-13 CLA	3.8	39.0	Neutral	Perfield et al., 2004
trans-8, *cis*-10 CLA	3.5	32.0	Neutral	Perfield et al., 2004
trans-9, *cis*-11 CLA	5.0	32.0	Tentative	Perfield et al., 2007
trans-9, *trans*-11 CLA	5.0	>95.0	Neutral	Perfield et al., 2007
trans-10, *trans*-12 CLA	4.6	95.7	Neutral	Sæbø et al., 2005b
cis-6, *trans*-8, *cis*-12 CLNA	4.0	21.8	Neutral	Sæbø et al., 2005a
cis-6, *trans*-10, *cis*-12 CLNA	2.6	14.6	Neutral	Sæbø et al., 2005a
cis-9, *trans*-11, *cis*-15 CLNA	5.0	20.3	Neutral	Gervais and Chouinard, 2008
cis-9, *trans*-13, *cis*-15 CLNA	5.0	20.3	Neutral	Gervais and Chouinard, 2008

Structure of *trans* fatty acids that inhibit lipogenesis

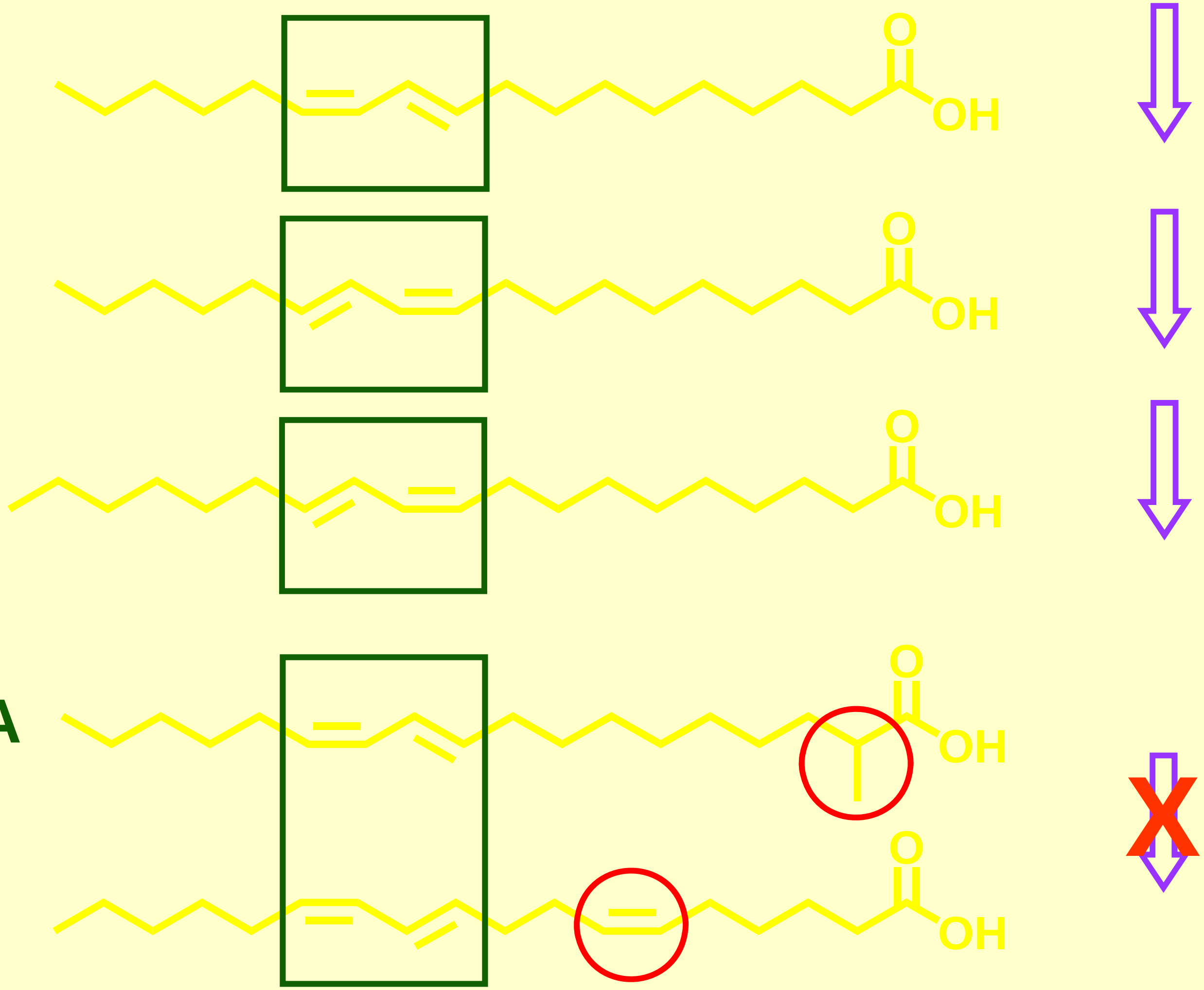
t10, c12 CLA

c10, t12 CLA

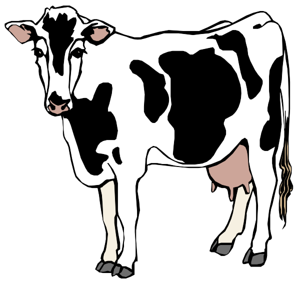
c10, t12 C19:2

α-methyl 10,12 CLA

c6, t10, c12 C18:3



Possible role of *trans*-10 18:1 in the regulation of mammary lipogenesis



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trans-11 18:1	12.5	50.0	Neutral	Griinari et al., 2000
trans-12 18:1	12.5	50.0	Neutral	Griinari et al., 2000
cis-9, *trans*-11 CLA	10.1	73.9	Neutral	Baumgard et al., 2000
cis-10, *trans*-12 CLA	1.2	23.2	Tentative	Sæbø et al., 2005
cis-11, *trans*-13 CLA	3.8	39.0	Neutral	Perfield et al., 2004
trans-8, *cis*-10 CLA	3.5	32.0	Neutral	Perfield et al., 2004
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cis-6, *trans*-8, *cis*-12 CLNA	4.0	21.8	Neutral	Sæbø et al., 2005a
cis-6, *trans*-10, *cis*-12 CLNA	2.6	14.6	Neutral	Sæbø et al., 2005a
cis-9, *trans*-11, *cis*-15 CLNA	5.0	20.3	Neutral	Gervais and Chouinard, 2008
cis-9, *trans*-13, *cis*-15 CLNA	5.0	20.3	Neutral	Gervais and Chouinard, 2008

Post-ruminal infusion study using a mixture of 18:1 isomers enriched with *trans*-10

- 3 x 3 Latin Square
- 14 d experimental periods (5 d infusion + 9 d washout)
- Treatments:

  Control

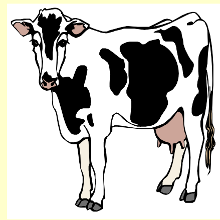
  18:1 preparation to supply 92 g *trans*-10 18:1/d

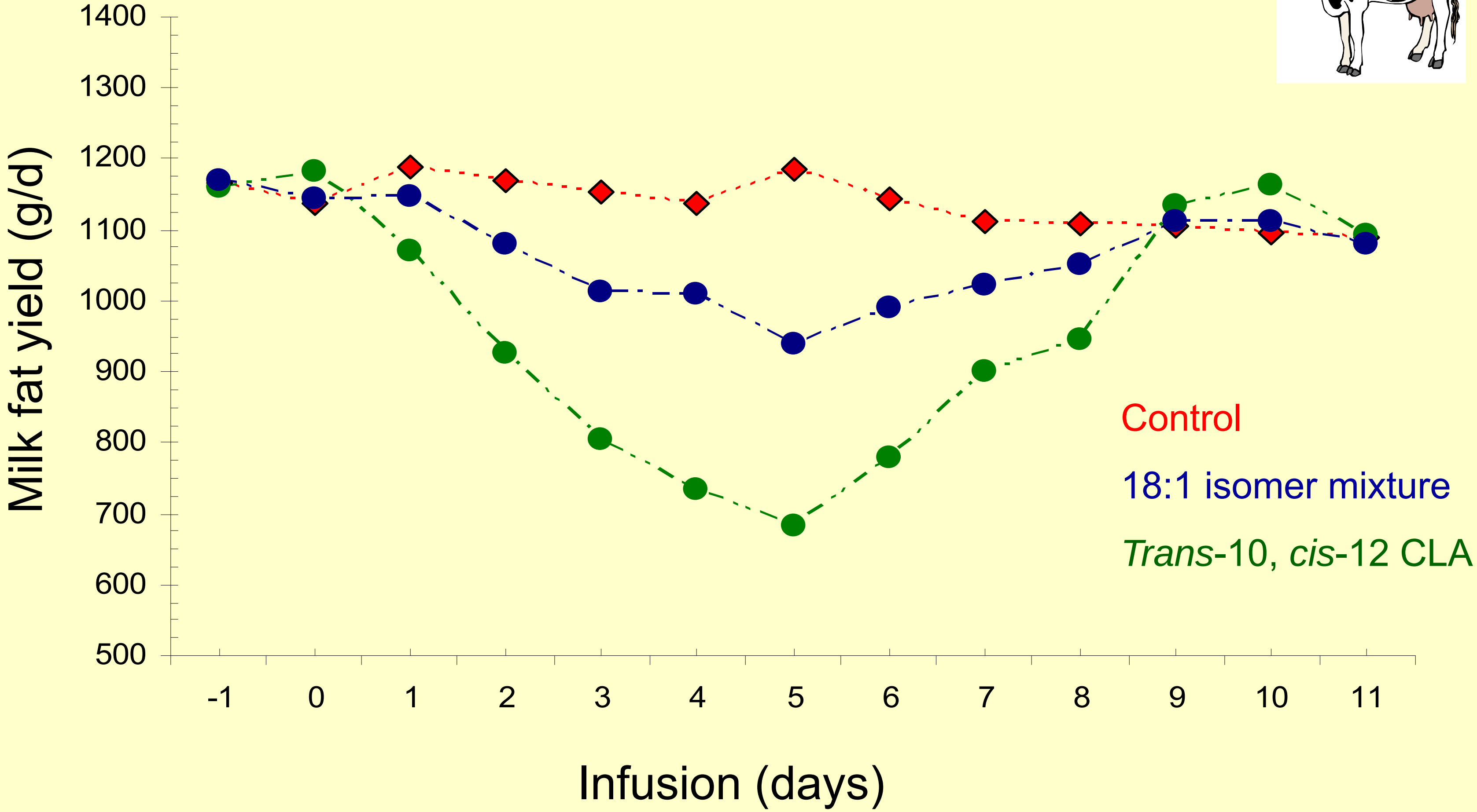
  6.0 g *trans*-10, *cis*-12 CLA/d

Composition of infused 18:1 preparation

Fatty acid	% fatty acids
16:0	0.34
18:0	3.88
cis-9 18:1	8.75
trans-8 18:1	0.33
trans-9 18:1	1.57
trans-10 18:1	38.22
trans-11 18:1	37.48
trans-12 18:1	2.65
trans-13 18:1	0.60
Other 18:1	5.62
CLA	0.00

Effects of infusion treatments on milk fat yield

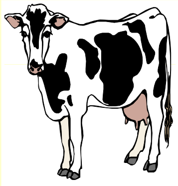




Shingfield et al., 2009

Effect of fish oil in the diet on mammary lipogenesis

Studies of fish oil supplementation and effects on milk fat synthesis



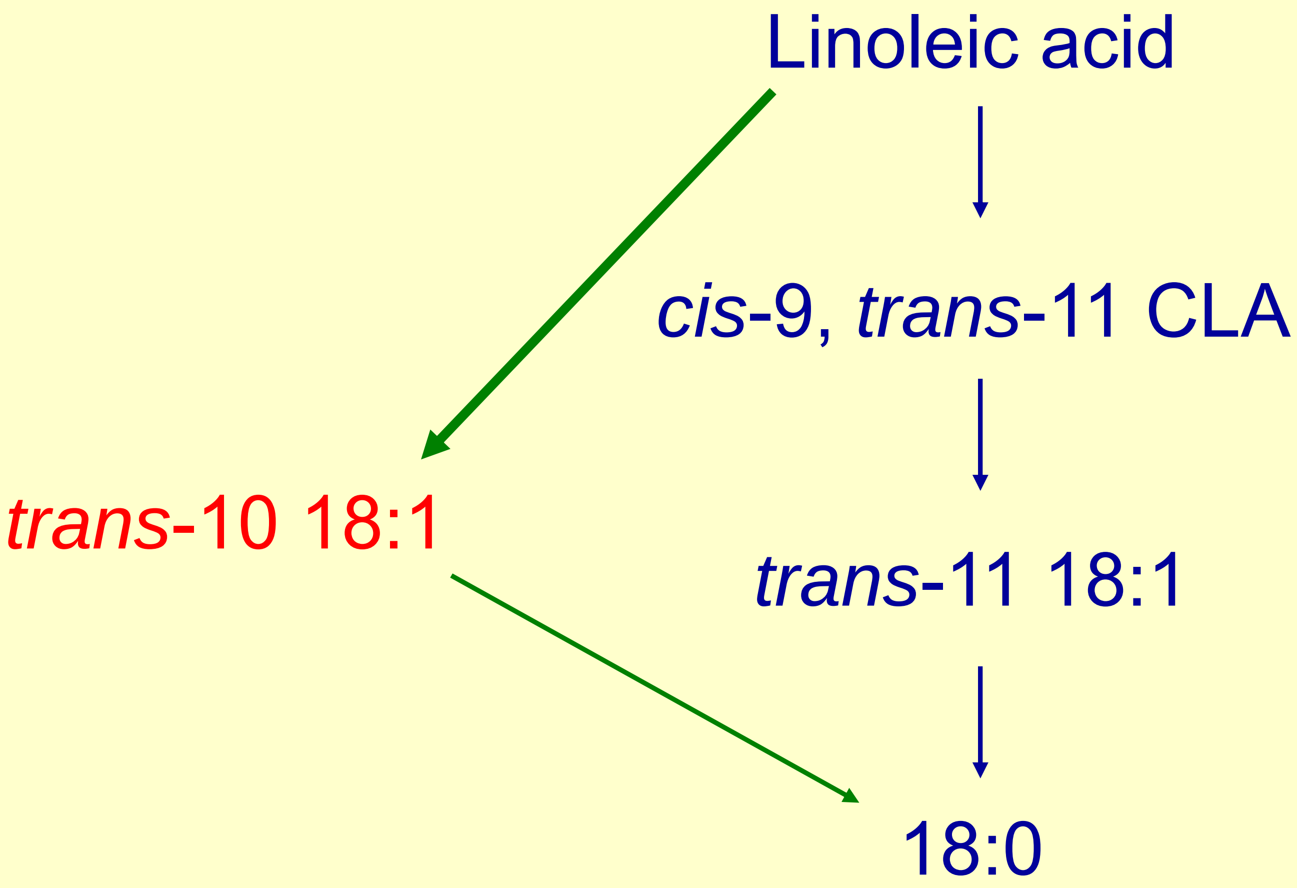
Reference	FO in the diet	% milk fat reduction
Pennington and Davis (1973)	225 g/d	- 16%
Wonsil et al. (1994)	1.5%	-15%
Chilliard et al. (1997)	1.5%	- 34%
Cant et al. (1997)	2.0%	- 30%
Offer et al. (1999)	1.6%	- 24%
Donovan et al. (2000)	1, 2 or 3%	- 6%, - 20%, - 23%
Keady et al. (2000)	1, 2 or 3%	- 4%, - 13%, - 35%
Lacasse et al. (2002)	3.7%	- 38%
Petit et al. (2002)	1.2%	- 25%
AbuGhazaleh et al. (2003)	1%	- 15%
Shingfield et al. (2003)	1.6%	- 7%
Loor et al. (2005)	1.7%	- 29%
Rego et al. (2005)	160/320 g/d	- 14/-33%
Shingfield et al. (2006)	1.5%	- 35%

Effect of fish oil on ruminal biohydrogenation

	Fish oil (g/d)				SEM	*P*	
	0	75	150	300		L	Q
Milk							
Fat (g/kg)	38.0	41.2	32.9	31.5	1.94	*	
Fat yield (g/d)	883	966	783	578	67.9	*	
Flow (g/d)							
18:0	339	314	169	88	19.7	***	NS
18:1 *cis*	23.0	22.0	24.7	33.5	1.85	*	NS
18:1 *trans*	59.9	89.7	157.0	172.9	9.73	***	*
18:1 *trans*-10	4.2	5.6	9.9	56.4	4.52	***	*
18:1 *trans*-11	22.0	32.3	80.8	66.5	4.86	**	**
t-10, *c*-12 CLA	0.104	0.024	0.030	0.049	0.017	NS	*

Shingfield et al., unpublished

Trans-isomer shift induced by fish oil in the diet



Trans 10 shift is indicative of complex changes in ruminal biohydrogenation pathways

	Fish oil (g/d)				SEM	*P*	
	0	75	150	300		L	Q
Flow (g/d)							
18:0-OH	0.85	0.43	1.30	1.41	0.367	NS	NS
18:0-O	1.5	6.0	18.6	15.3	1.94	**	*
18:1 *cis*-11	2.5	2.9	5.2	10.5	0.42	***	*
18:1 *trans*-9	2.0	3.5	6.4	5.7	0.59	**	*
18:2 *trans*-9, *cis*-15	0.16	0.16	0.25	0.65	0.002	***	***
18:2 *trans*-10, *cis*-15	0.27	0.53	0.87	1.83	0.080	***	NS
18:2 *trans*-11, *cis*-15	3.73	5.39	8.96	31.5	1.49	***	**
18:2 *trans*-12, *cis*-15	0.24	0.38	0.40	1.14	0.111	**	NS
18:2 *trans*-10, *trans*-14	1.79	2.95	2.97	6.91	0.734	**	NS
20:2 *trans*-12, *trans*-15	0.27	0.50	0.65	0.95	0.106	*	NS
20:2 *trans*-9, *trans*-15	0.16	0.65	1.05	1.71	0.180	**	NS
20:2 *trans*-11, *trans*-16	0.06	0.21	0.48	1.25	0.048	***	*

Shingfield et al., unpublished

Summary

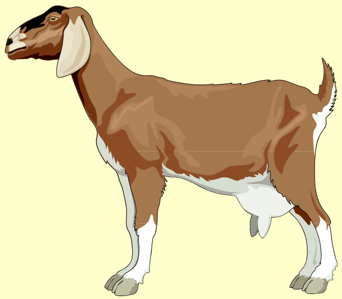
- Reductions in milk fat synthesis on diets containing fish oil is not related to an increase in ruminal *trans*-10, *cis*-12 CLA formation
- The extent of milk fat depression is associated with alterations in biohydrogenation leading to *trans*-10 replacing *trans*-11 as the major 18:1 intermediate leaving the rumen…the so called *trans*-10 shift
- Fish oil results in a dose dependent decrease in the amount of 18:0 leaving the rumen

Role of milk fat fluidity in the regulation of mammary lipogenesis

- Increased content of *trans* fatty acids with a high melting point will impact milk fat fluidity (Chilliard et al., 2000)
- There is a stringent requirement for endogenously synthesized oleic acid (Loor and Herbein, 2003)
- Availability of 18:0 for endogenous synthesis of *cis*-9 18:1 seems to be crucial for milk fat synthesis (Loor et al. 2005)

18:0 ⟶ *trans* 8-10 18:1

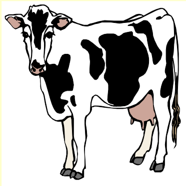
Shingfield and Griinari, 2007; Gama et al., 2008

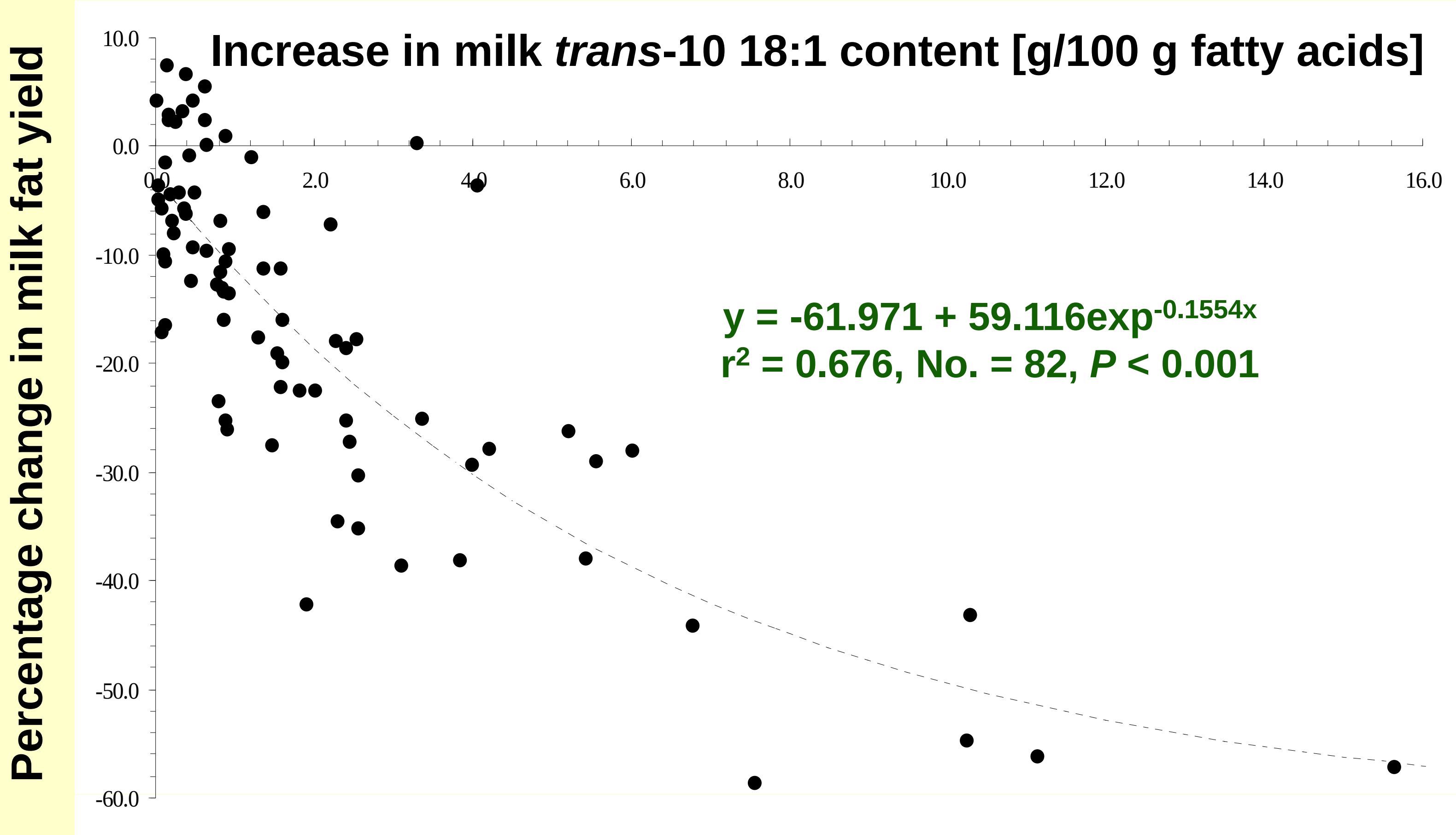


Nutritional regulation of mammary lipogenesis in the ovine and caprine

- 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

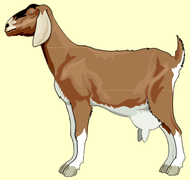
Relationship between mammary lipogenesis and milk *trans*-10 18:1 in the bovine

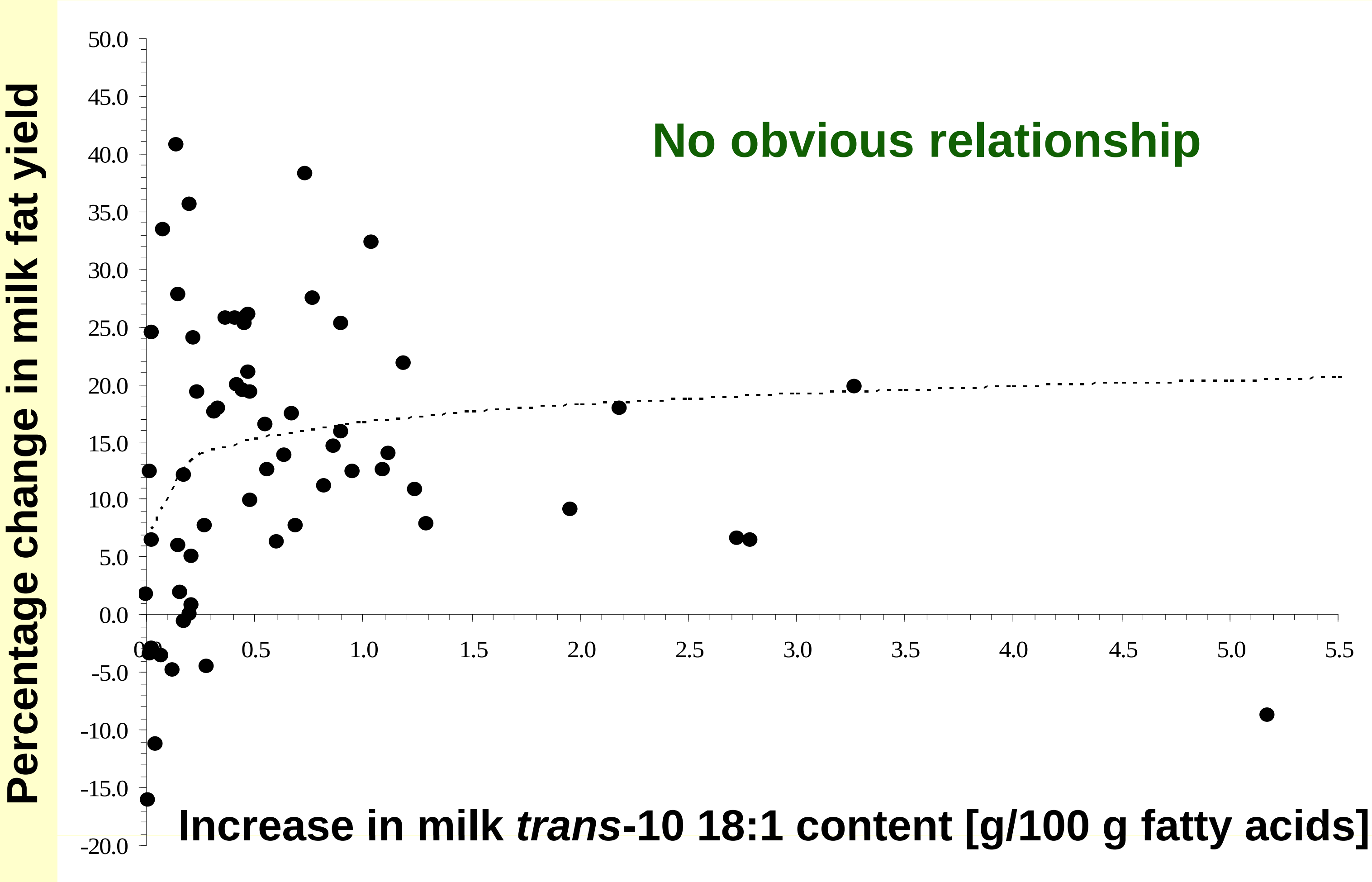




Shingfield et al., unpublished

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



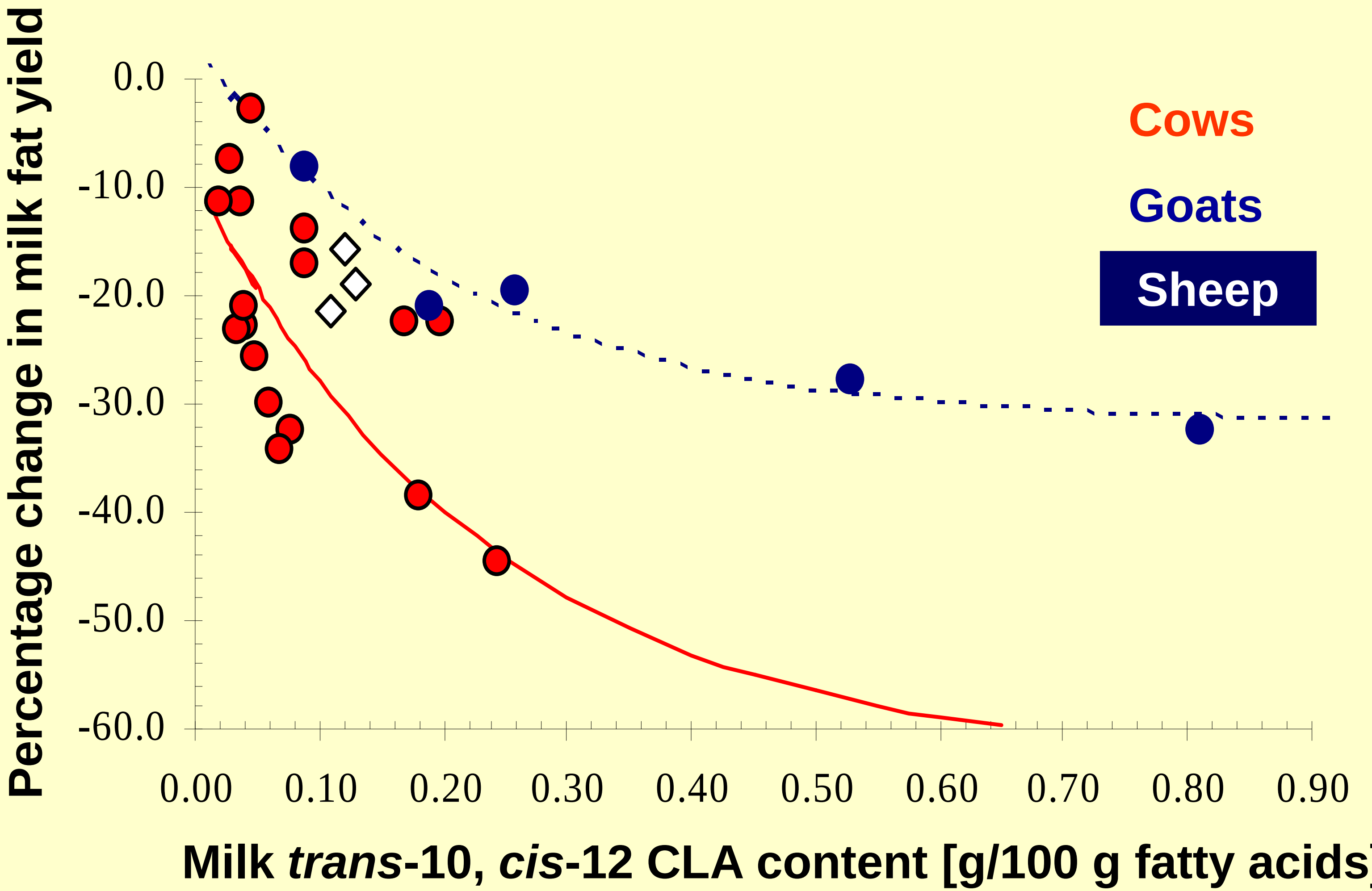


Chilliard et al., unpublished

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

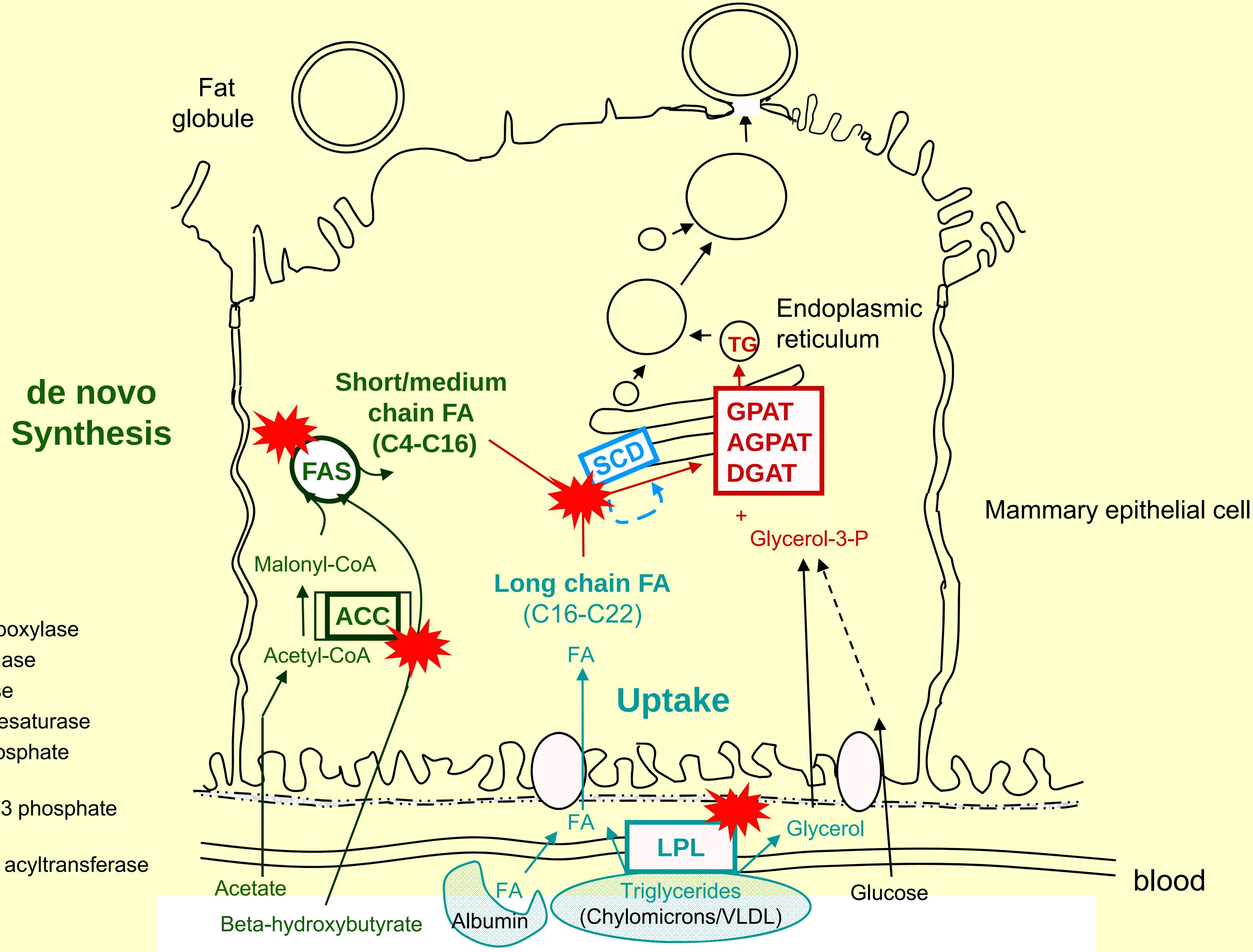
Mammary lipogenic responses in ruminants fed rumen-protected CLA



Shingfield et al., 2009; Shingfield et al., unpublished

Nutritional regulation of mammary lipogenesis in ruminants: molecular dimension

Key genes involved in mammary lipogenesis



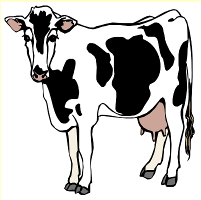
ACC : Acetyl-CoA carboxylase
FAS : Fatty acid Synthase
LPL : Lipoprotein-lipase
SCD : Stearoyl-CoA desaturase
GPAT : Glycerol-3-phosphate acyltransferase
AGPAT : Acylglycerol-3 phosphate acyltransferase
DGAT : Diacylglycerol acyltransferase

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

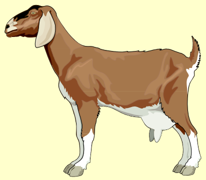


Diets causing MFD or infusion of *trans*-10, *cis*-12 CLA

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



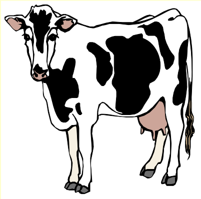
Starch rich diets supplemented with plant oils

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

Nutritional regulation of mammary long chain fatty acid uptake in ruminants

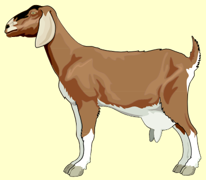


Diets causing MFD or infusion of *trans*-10, *cis*-12 CLA

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

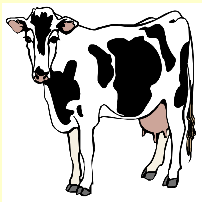
Increase in C18 fatty acid secretion in milk

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

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

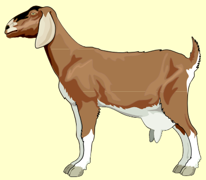


Varies little in response to diet

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

Ahnadi et al., 2002
Baumgard et al., 2002

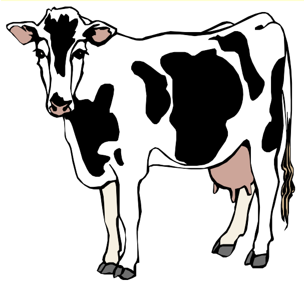
⇨ Lowered SCD1 mRNA abundance



Varies according to basal diet and composition of lipid supplement

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

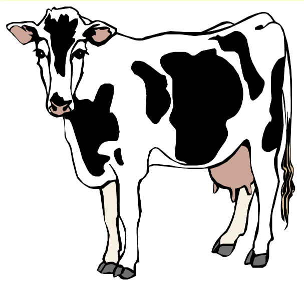
⇨ Formaldehyde-treated linseeds lower SCD1 mRNA abundance
Plant oils often decrease SCD1 activity



Range of *trans* fatty acid tested for effects on milk fat desaturase index

Isomer (s) investigated	Dose (g/d)	Purity (%)	Desaturase Index	Reference
trans-10, *cis*-12 CLA	10.3	89.5	Decrease	Baumgard et al., 2000
trans-9 18:1	41.7	83.0	Neutral	Tyburczy et al., 2008
trans-10 18:1	42.6	94.7	Neutral	Lock et al., 2007
trans-11 18:1	12.5	50.0	Neutral	Griinari et al., 2000
trans-12 18:1	12.5	50.0	Neutral	Griinari et al., 2000
cis-9, *trans*-11 CLA	10.1	73.9	Neutral	Baumgard et al., 2000
cis-10, *trans*-12 CLA	1.2	23.2	ND	Sæbø et al., 2005
cis-11, *trans*-13 CLA	3.8	39.0	Neutral	Perfield et al., 2004
trans-8, *cis*-10 CLA	3.5	32.0	ND	Perfield et al., 2004
trans-9, *cis*-11 CLA	5.0	32.0	ND	Perfield et al., 2007
trans-9, *trans*-11 CLA	5.0	>95.0	Decrease	Perfield et al., 2007
trans-10, *trans*-12 CLA	4.6	95.7	Decrease	Sæbø et al., 2005b
cis-6, *trans*-8, *cis*-12 CLNA	4.0	21.8	Neutral	Sæbø et al., 2005a
cis-6, *trans*-10, *cis*-12 CLNA	2.6	14.6	Neutral	Sæbø et al., 2005a
cis-9, *trans*-11, *cis*-15 CLNA	5.0	20.3	Neutral	Gervais and Chouinard, 2008
cis-9, *trans*-13, *cis*-15 CLNA	5.0	20.3	Neutral	Gervais and Chouinard, 2008

In goats milk fat *trans*-9, *trans*-11 CLA concentrations are higher compared with cows fed comparable diets



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

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**

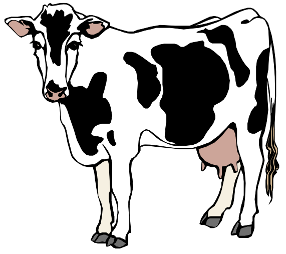
PPAR ?

↓

Downregulation of lipogenic genes

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

⇨ Other regulatory factors e.g. protein kinases may also be involved



Nutritional regulation of the bovine mammary transcriptome

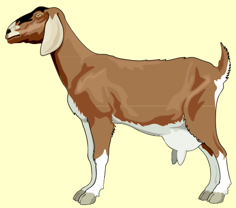
High concentrate diet containing soya bean oil (Loor et al., 2005)

- MFD associated with the (≥ 2 fold) downregulation of 241 genes and (≥ 2 fold) upregulation of 150 genes
- Downregulation of genes associated with fatty acid metabolism e.g. FASN, ACC, SCD, LPL, FABP3, ACSL1
- Downregulation of transcription factors e.g. FASN, ACACA, SCD, LPL, FABP3, ACSL1 but not SREBP1

Fish oil and soya bean oil (Invernizzi et al., 2009)

- MFD associated with the differential expression of 847 genes compared with the control
- Downregulated such as transcription factor YY1
  Upregulated such as HDAC5 and NR3C1

Nutritional regulation of the caprine mammary transcriptome

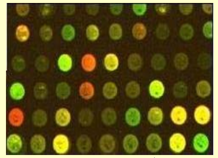


(Ollier et al., *JDS*- in press)

HF-RS *vs* HF → Fat content, Oleic acid (↑)

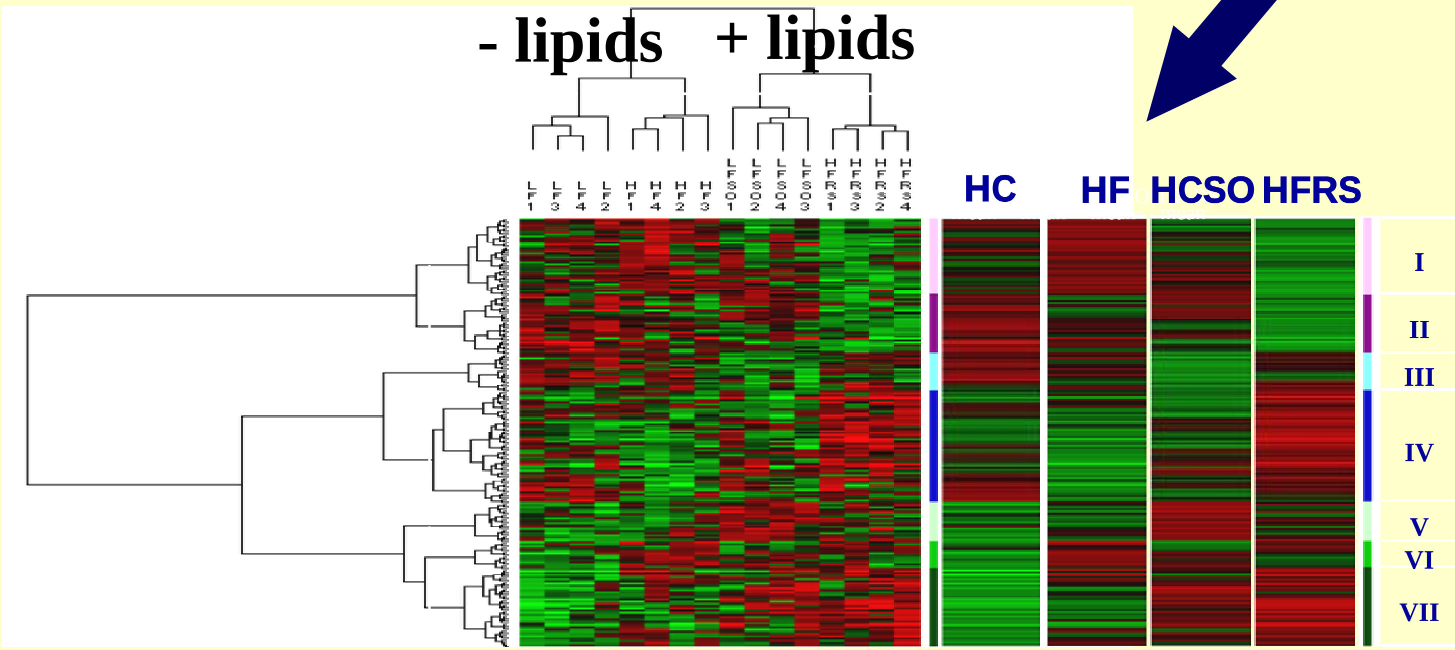
HC-SO *vs* HC → Fat content + yield, Total *trans* FA (↑)

(n=16 lactating goats)



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

Clustering



Gene expression profiles highly dependent on diet

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

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