Understanding Milk Fat Synthesis

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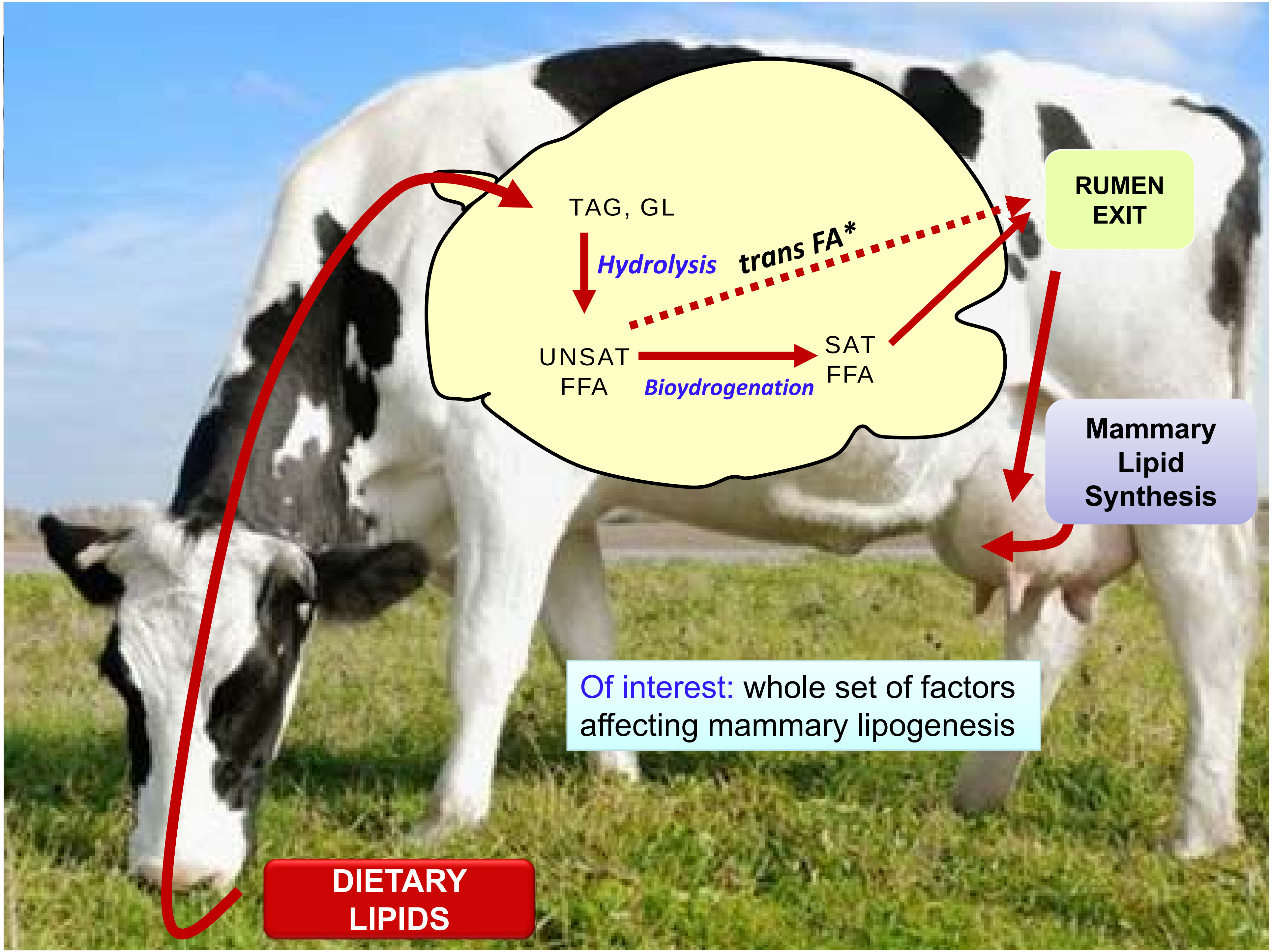
UNIVERSITÉ LAVAL

GRIPHA

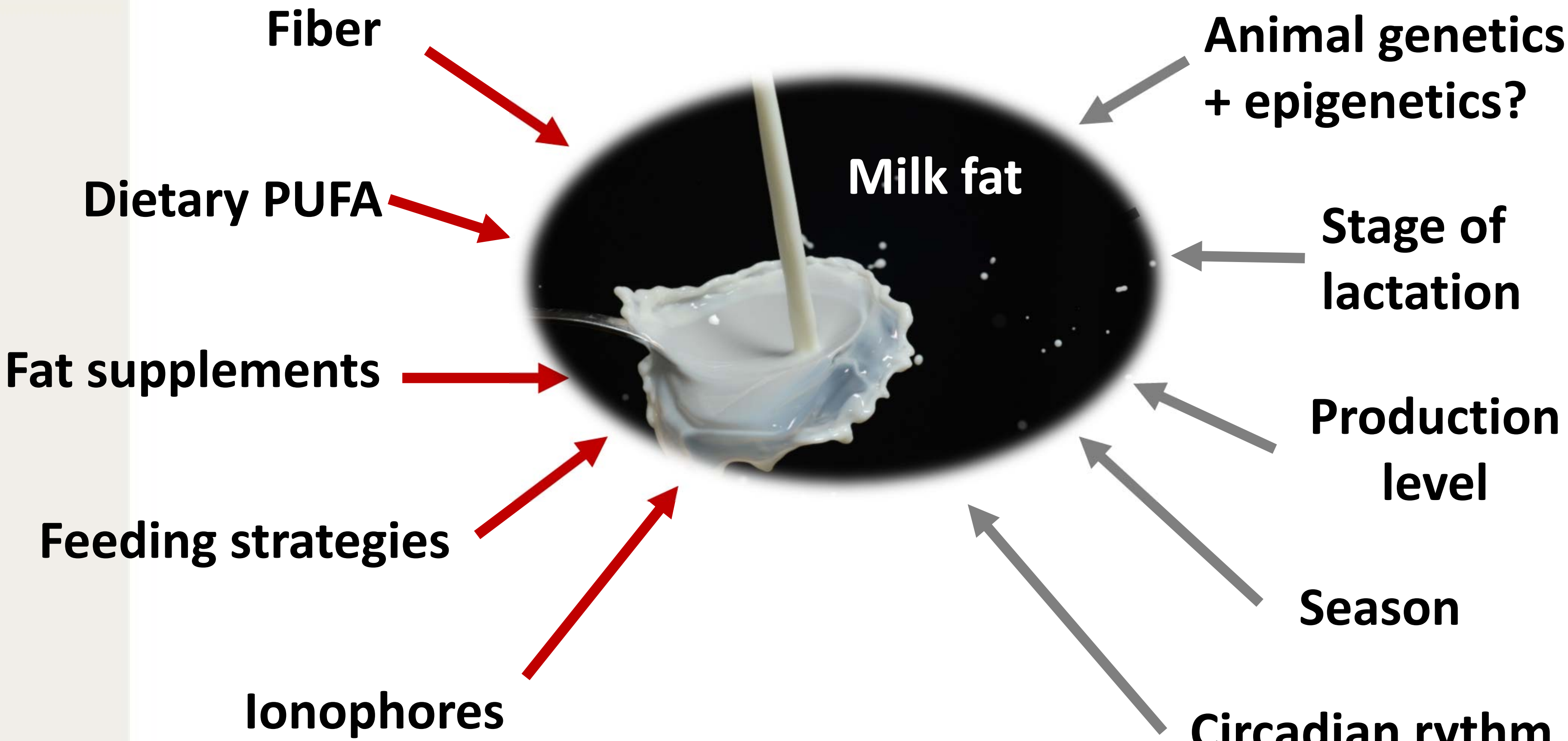
STELA



TAG, GL
Hydrolysis
trans FA*
UNSAT FFA
Bioydrogenation
SAT FFA
RUMEN EXIT
Mammary Lipid Synthesis
DIETARY LIPIDS
Of interest: whole set of factors affecting mammary lipogenesis

Numerous factors affect milk fat

Nutritional factors | **Non-nutritional factors**



Milk fat depression, an old problem

i) Recognized by Boussingault in 1845

i) Occurs under certain dietary conditions

- High concentrate/low fiber
- Plant and fish oil supplements (PUFA)

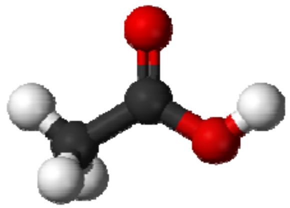
Boussingault

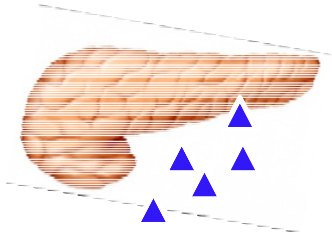
iii) Particular phenotype:

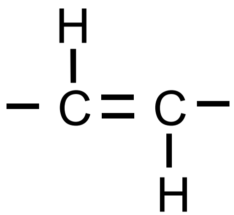
- Specific decrease in fat yield (up to 50%)
- Decrease in fatty acid yield
    - *de novo* > preformed
    - increase in specific *trans* fatty acids (FA)

Time course of MFD theories

1845	1951	1962	1970	2001
Precursor shortage (low fat in high beet diets)	Precursor shortage (C2 deficit in LF* diets)	Glucogenic-insulin theory (decreased NEFA)	*trans* FA theory (in LF/HO* diets)	Specific bioactive FA (*t*10*c*12 CLA) (in LF/HO diets)





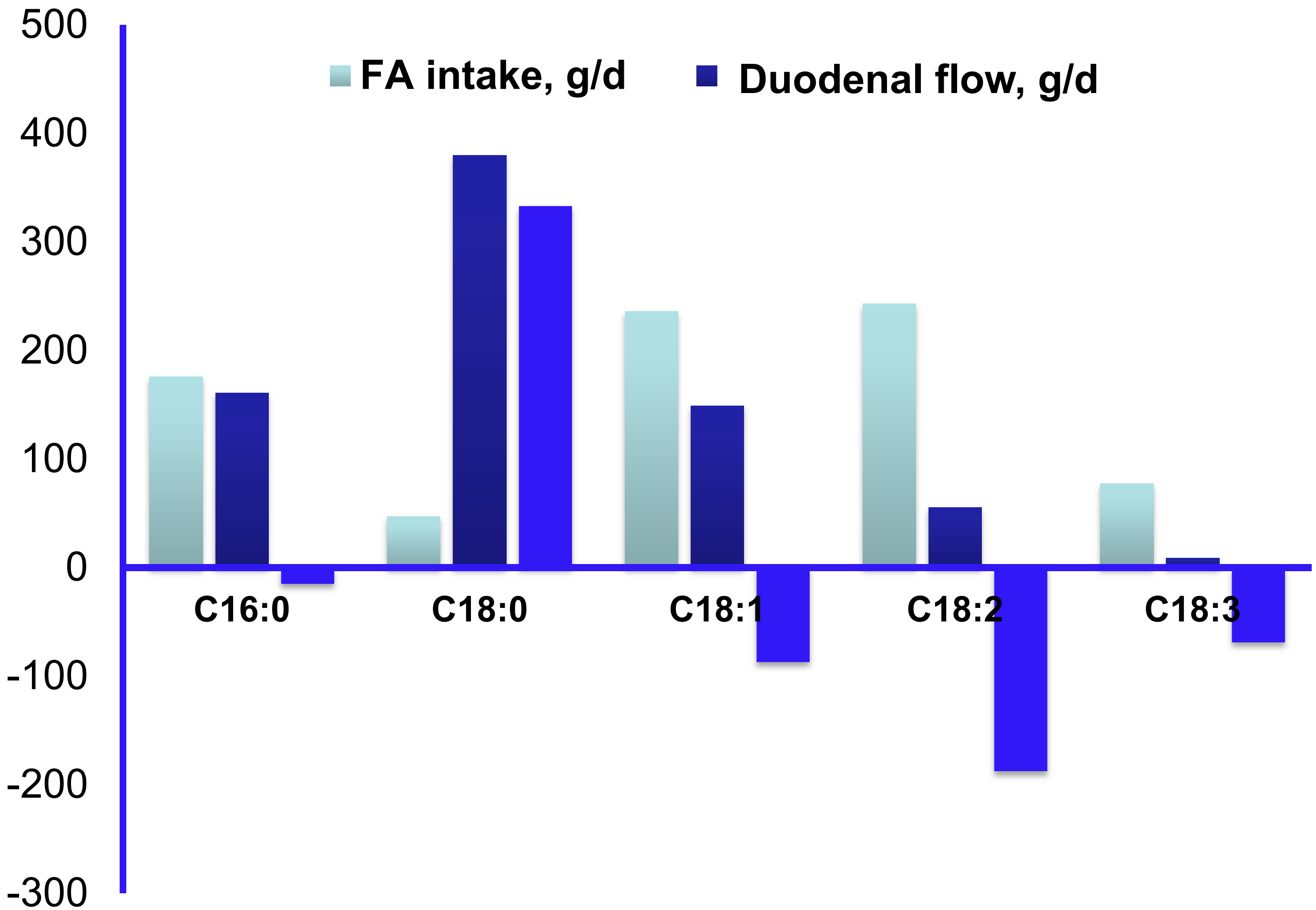


Boussingault — **Tyznik and Allen** — **McClymont and Vallance** — **Davis and Brown** — **Bauman and Griinari**

*LF=low forage
*HO=high oil

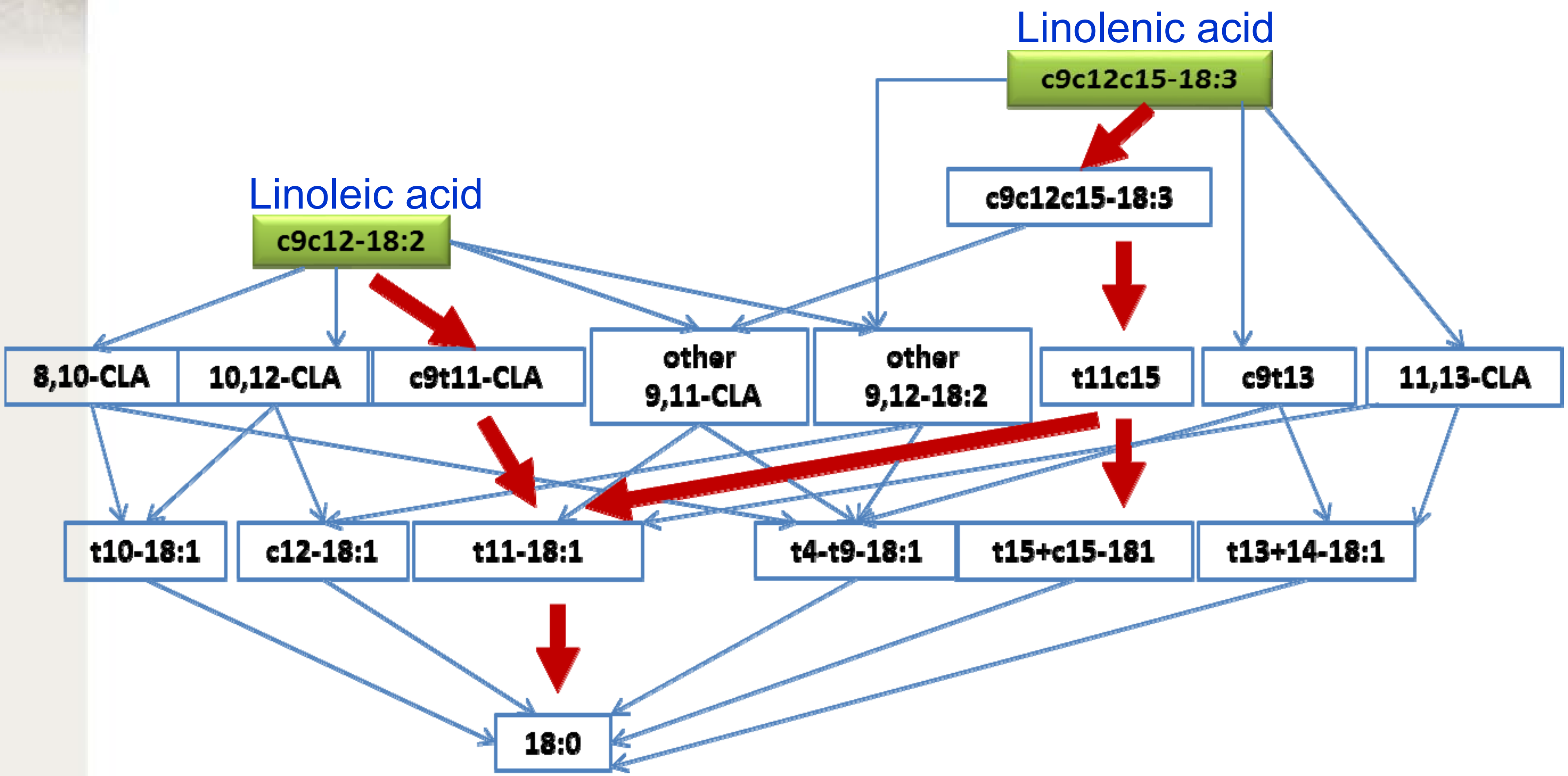
CLA = Conjugated linoleic acid

Evidence of biohydrogenation



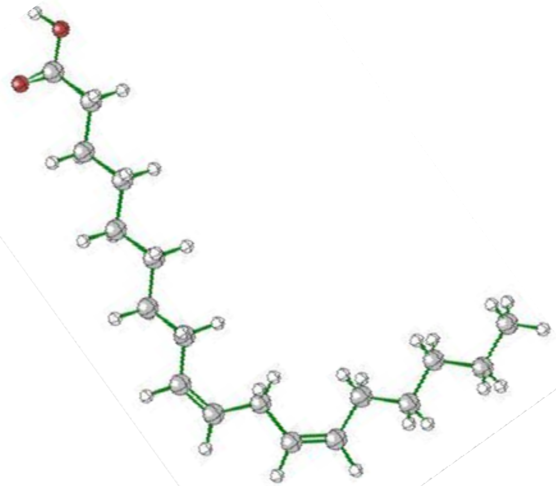
Adapted from Boerman et al. 2015

Biohydrogenation is complex!



Lourenço et al., 2010

Biohydrogenation pathways can be altered



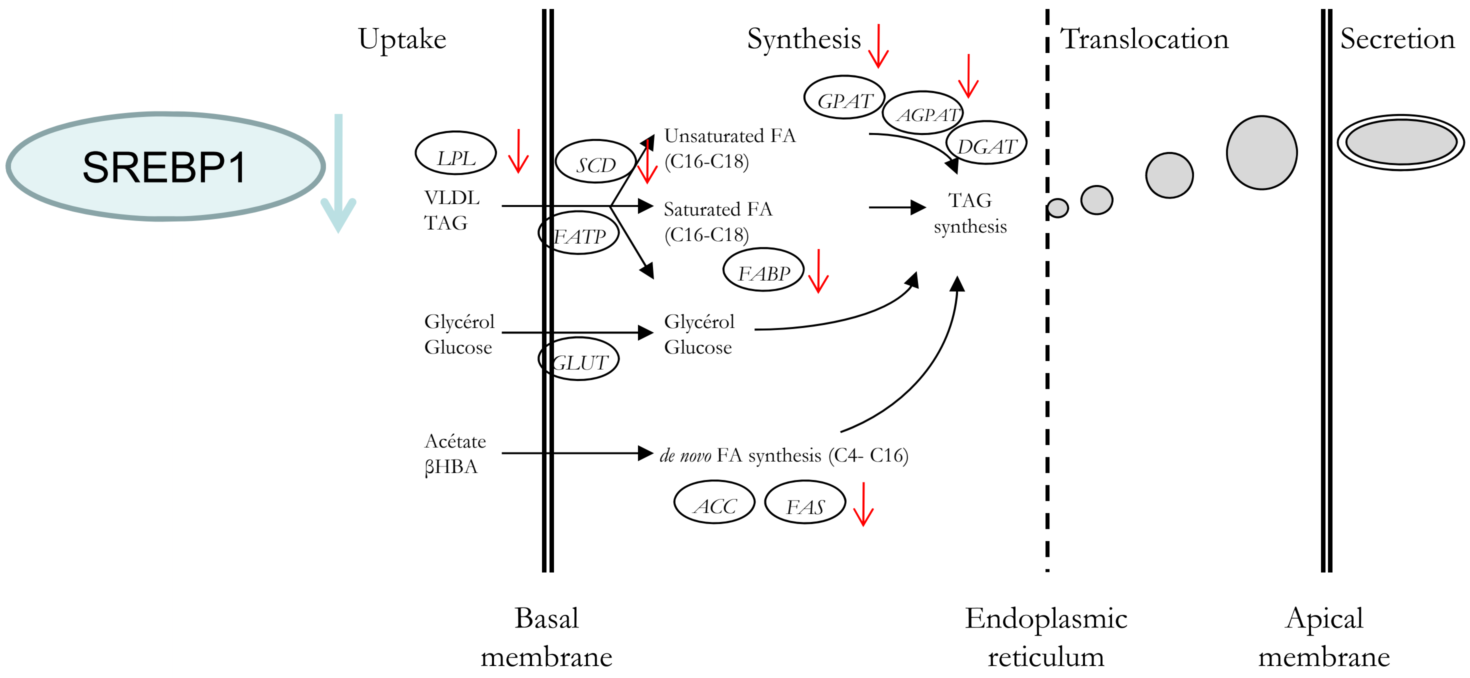
Biohydrogenation of linoleic acid

Linoleic acid
18:2 *cis*-9, *cis*-12

Conjugated linoleic acid 18:2 *cis*-9, *trans*-11	Conjugated linoleic acid 18:2 *trans*-10, *cis*-12
↓	↓
trans vaccenic acid 18:1 *trans*-11	18:1 *trans*-10
↓	↓
stearic acid 18:0	stearic acid 18:0

Coordinated gene downregulation

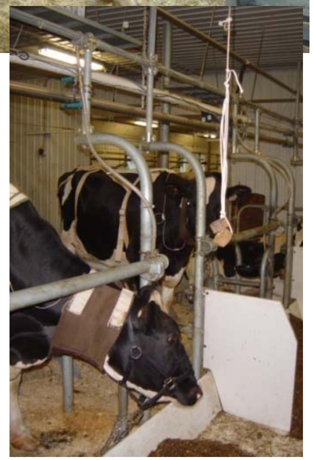
- Milk fat synthesis and secretion



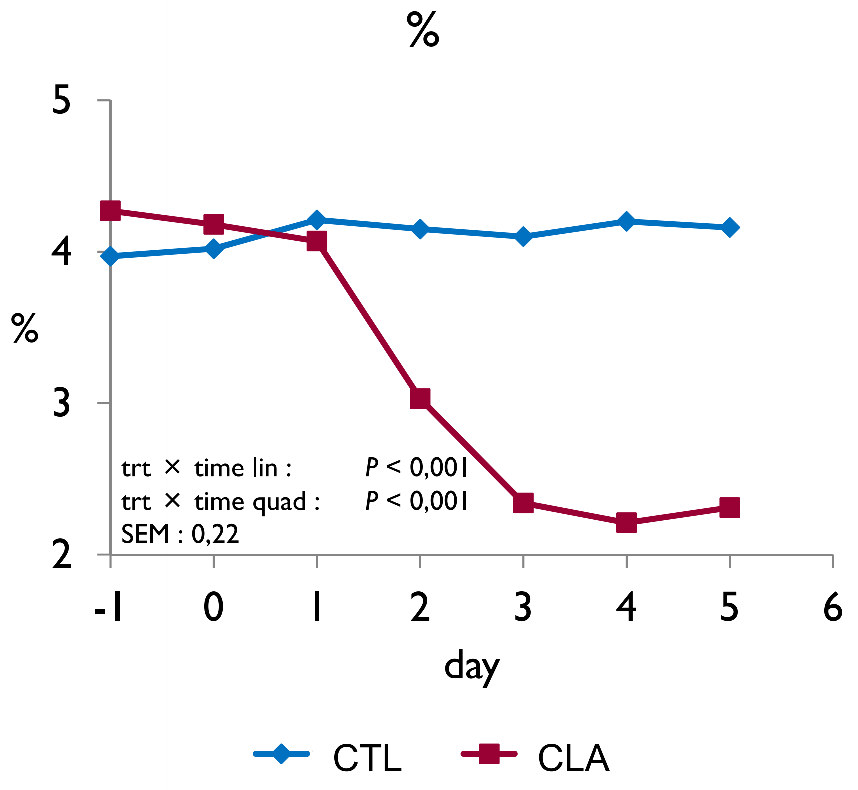
- *trans*-10, *cis*-12 CLA inhibits lipogenesis in adipose tissue via the synthesis of proinflammatory proteins.

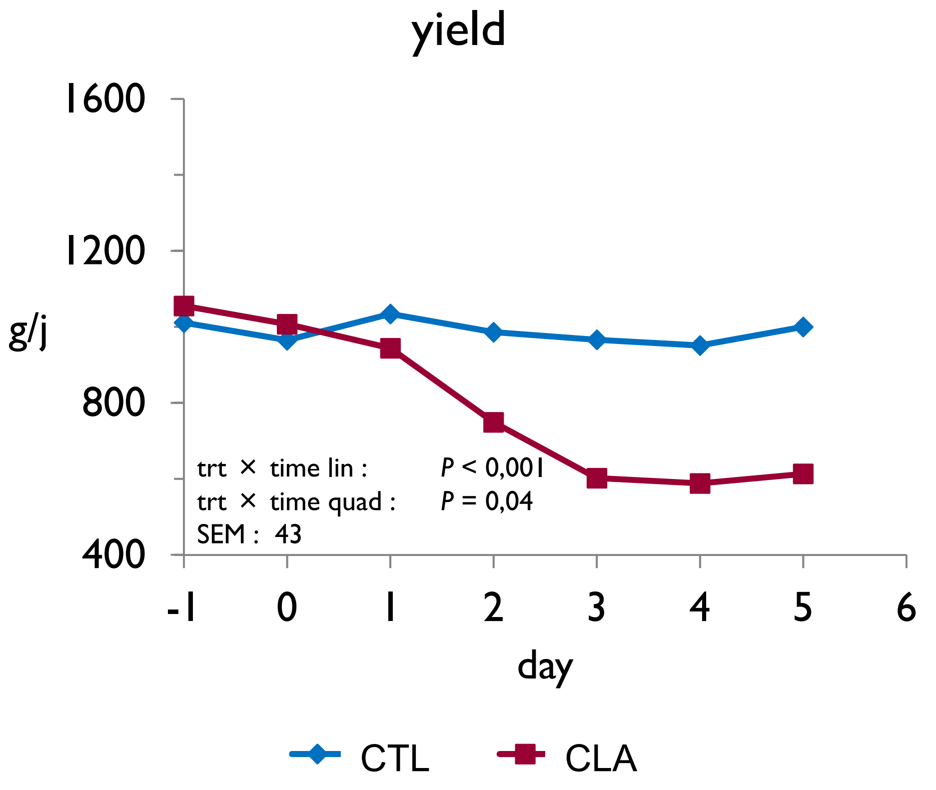
- Cross-over design
  - 4 cows (195 ± 6 DIM)
  - 2 treatments:
    - Intravenous infusions of 5 d
    - Lipid emulsion (15 % w/w) :

      10 g/d of 18:2 *cis*-9, *cis*-12 (CTL)
      10 g/d of 18:2 *trans*-10, *cis*-12 (CLA)
    - 23-d washout between periods



Milk fat


%
5
4
%
3
2
trt × time lin : P < 0,001
trt × time quad : P < 0,001
SEM : 0,22
-1 0 1 2 3 4 5 6
day
CTL CLA

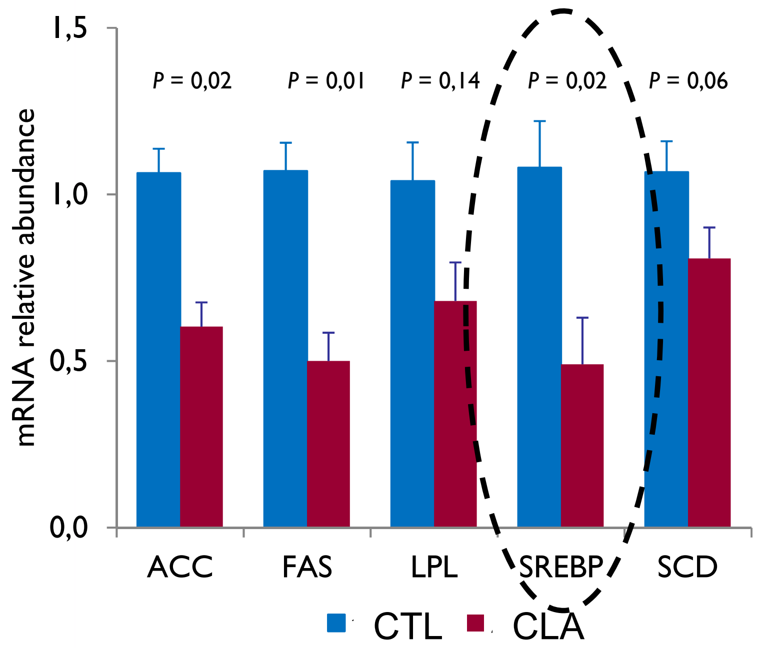

yield
1600
1200
g/j
800
400
trt × time lin : P < 0,001
trt × time quad : P = 0,04
SEM : 43
-1 0 1 2 3 4 5 6
day
CTL CLA

Effects of CLA on lipid metabolism of the mammary tissue in lactating dairy cows

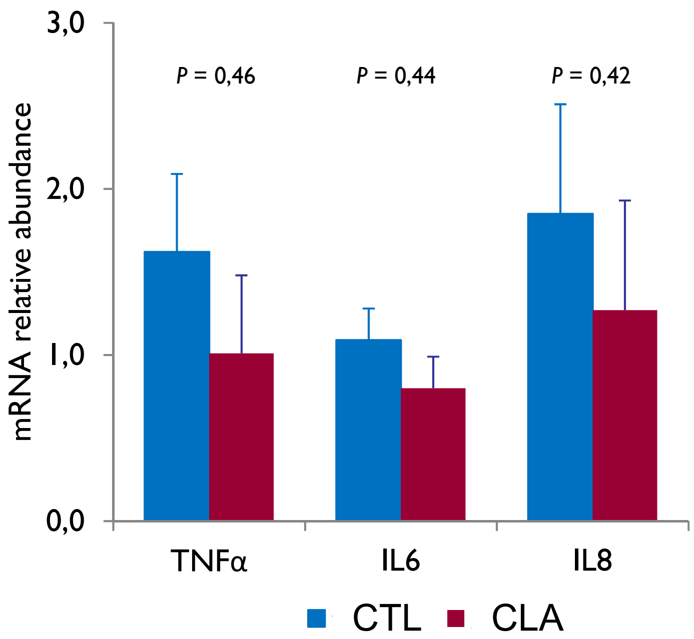
Gervais et al., 2009

Gene expression

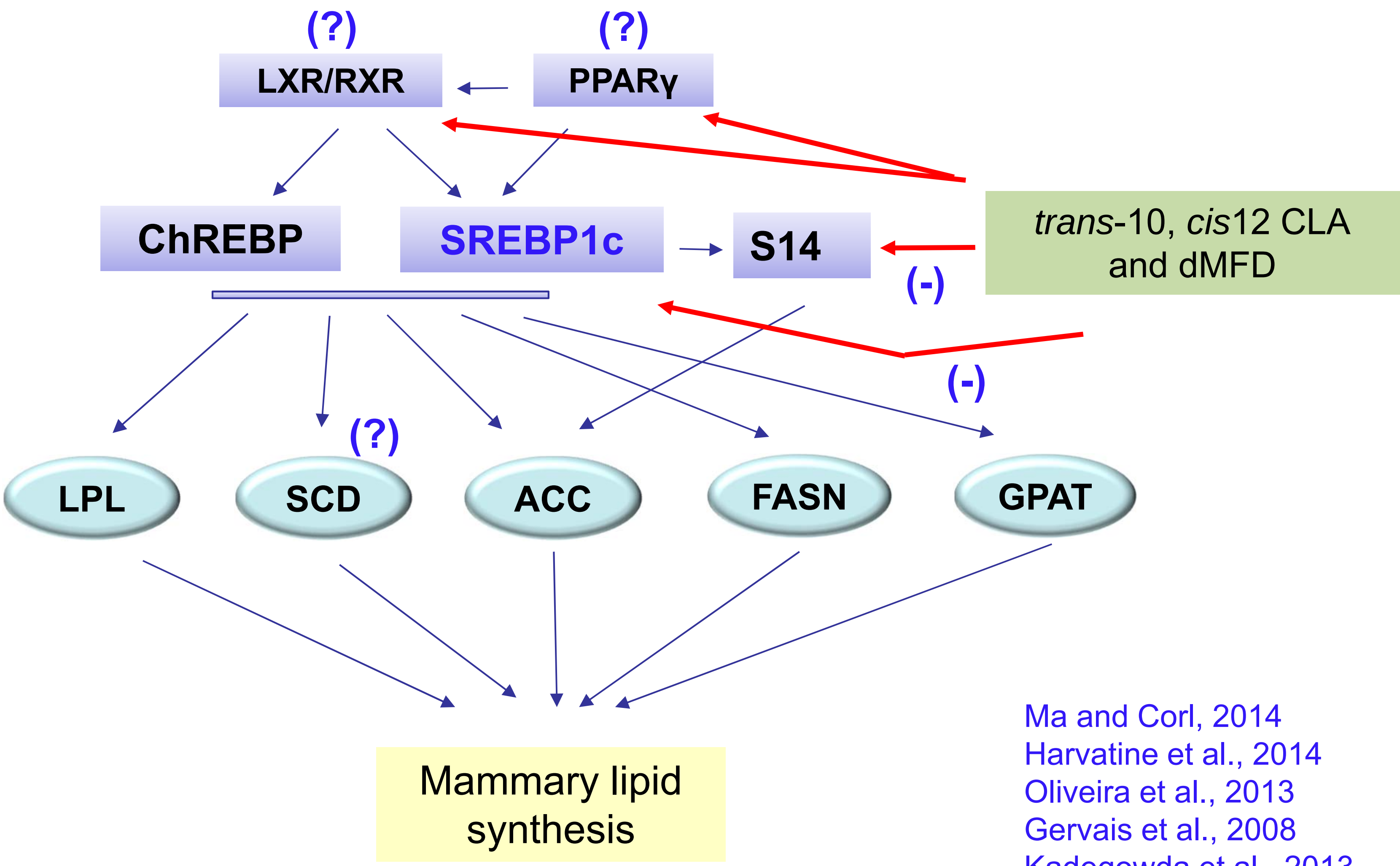
Lipogenic activity



Proinflammatory activity

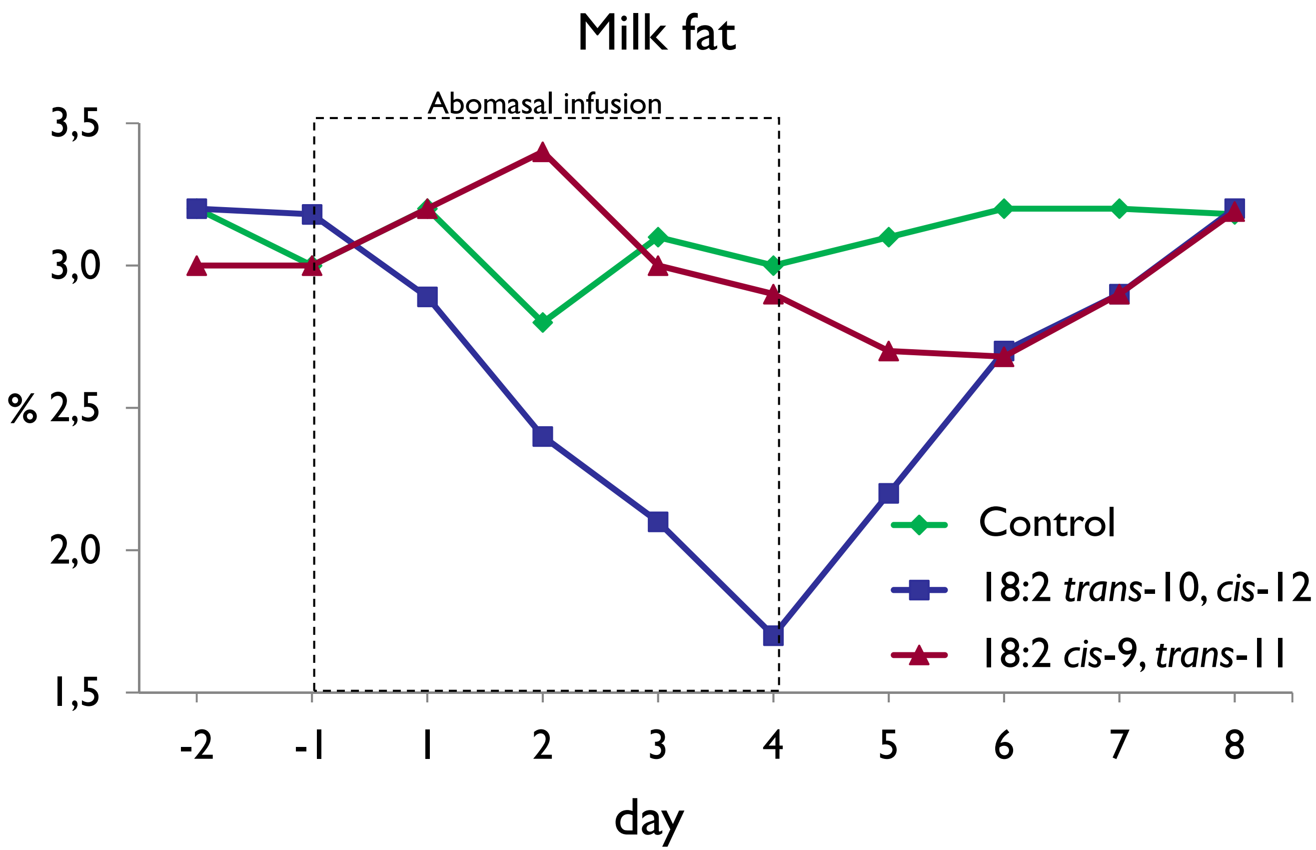


Working model of lipogenesis inhibition during MFD



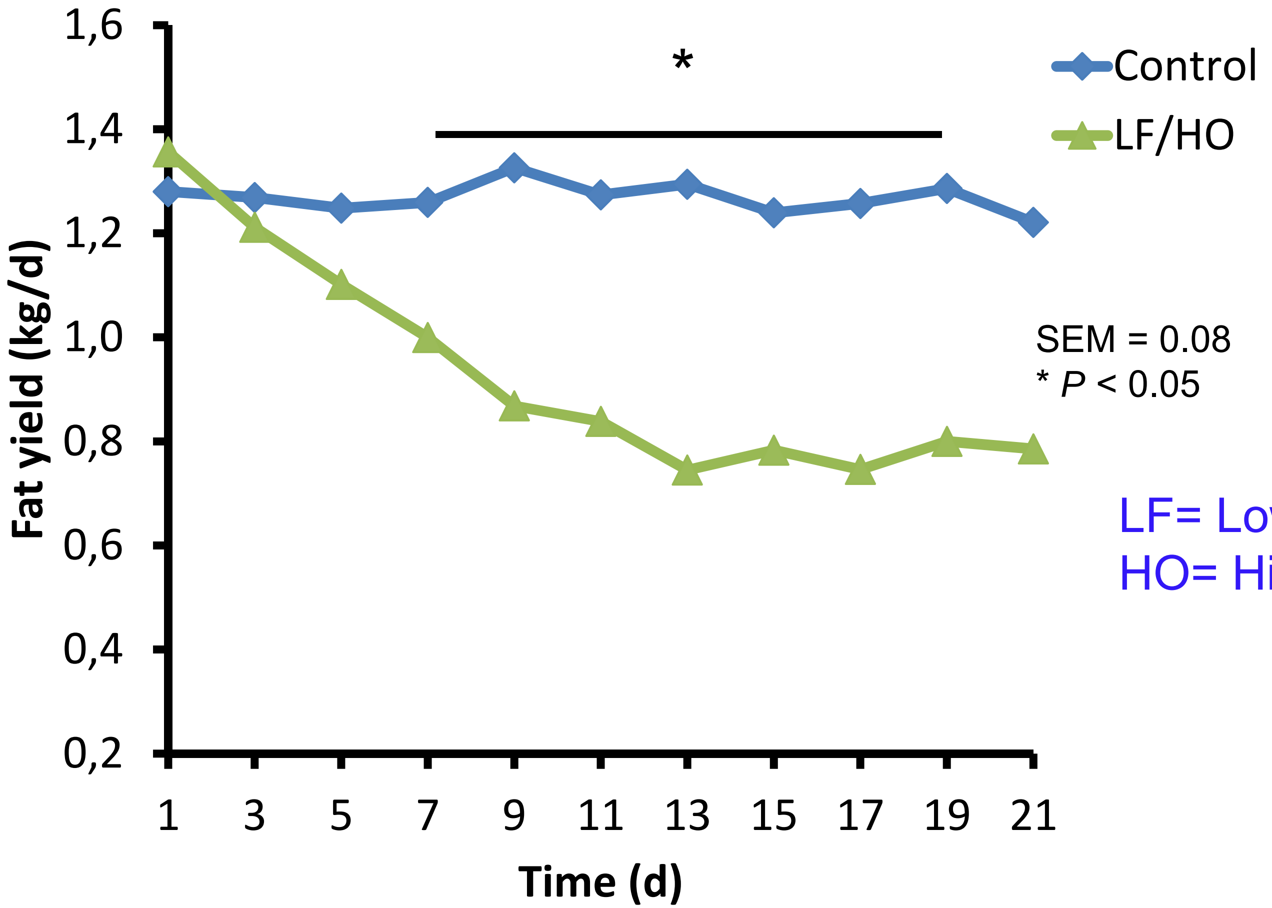
Ma and Corl, 2014
Harvatine et al., 2014
Oliveira et al., 2013
Gervais et al., 2008
Kadegowda et al., 2013

Mammary response to CLA is rapid!



Baumgard et al. (2000)

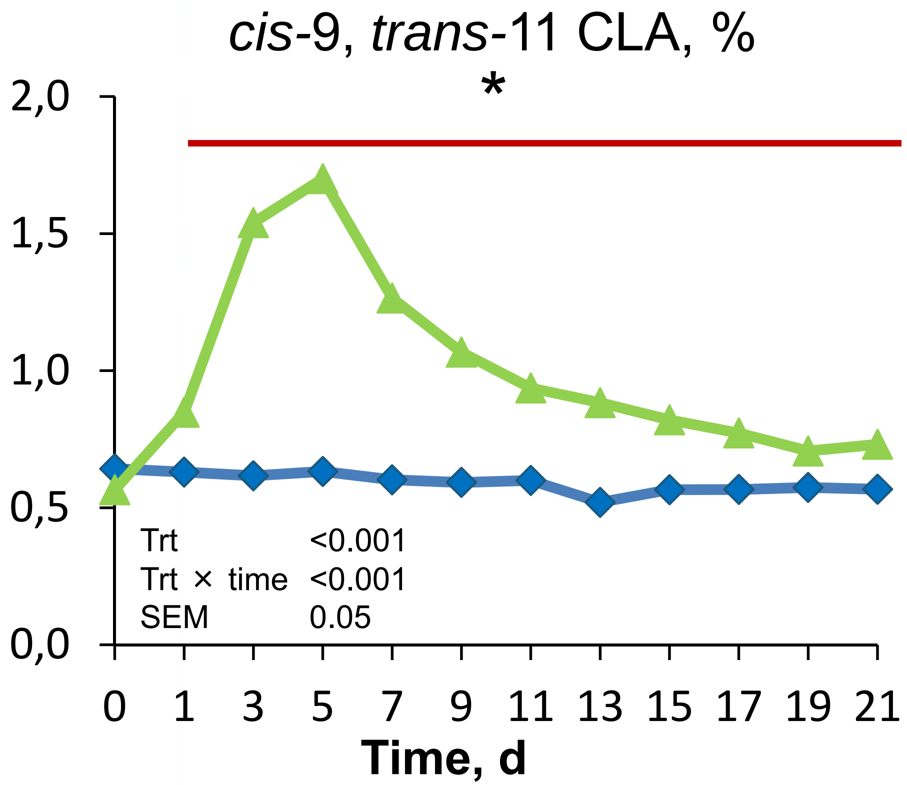
Diet induced milk fat depression is progressive

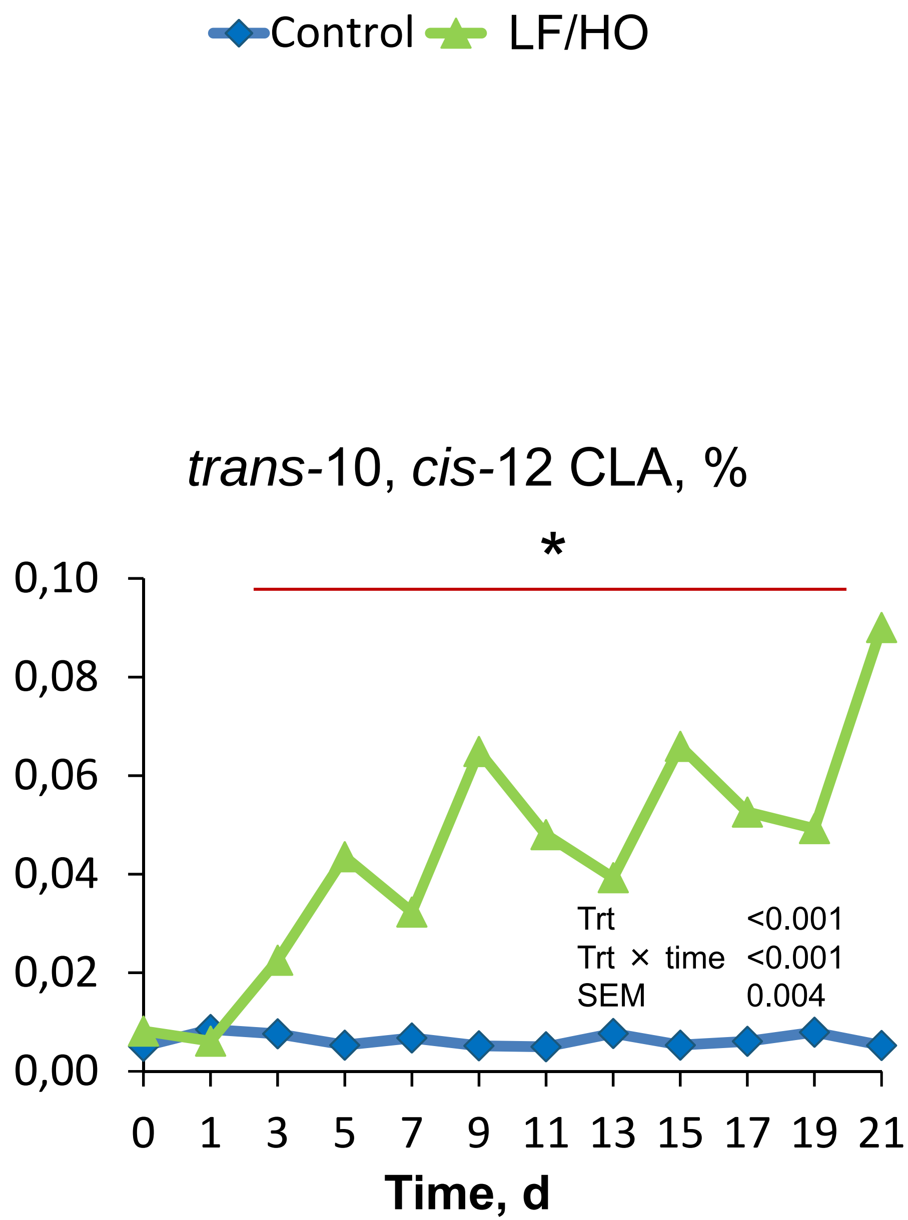


LF= Low forage
HO= High oil

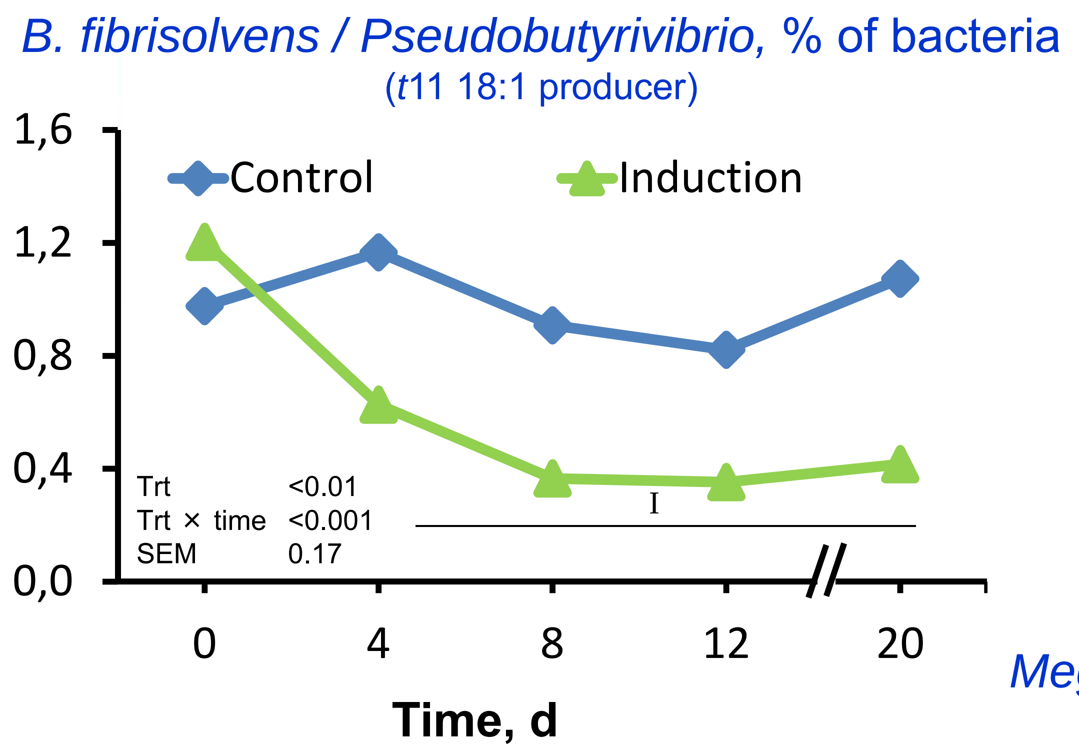
Rico and Harvatine, 2013

Induction of MFD

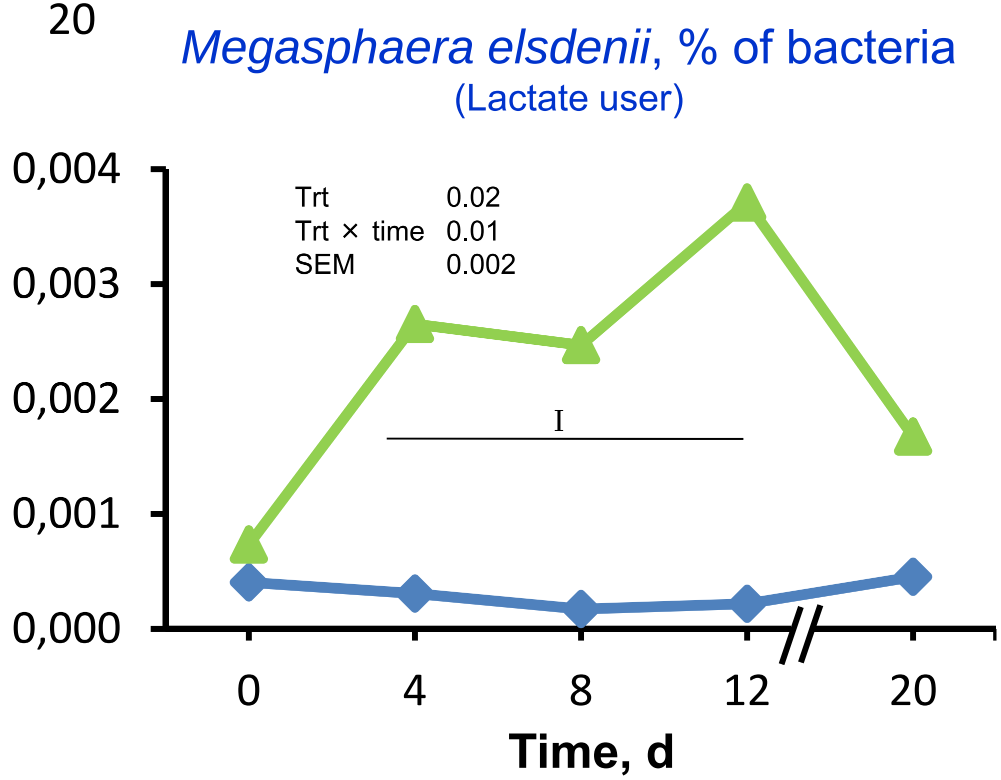


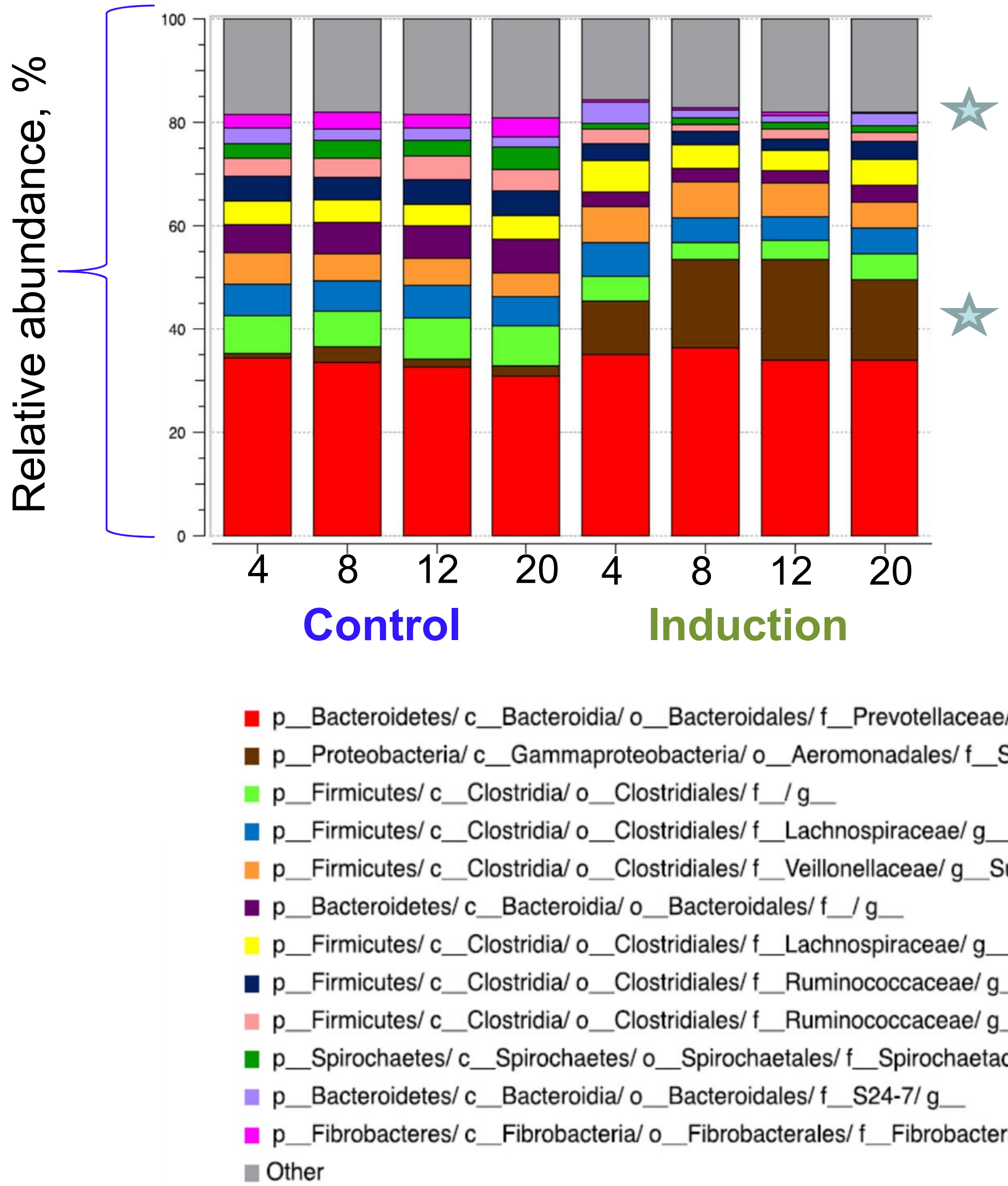


* $P < 0.05$


B. fibrisolvens / Pseudobutyrivibrio, % of bacteria
(t11 18:1 producer)
1,6
1,2
0,8
0,4
0,0
Control
Induction
Trt <0.01
Trt × time <0.001
SEM 0.17
I
0
4
8
12
20
Time, d

I = $P < 0.05$

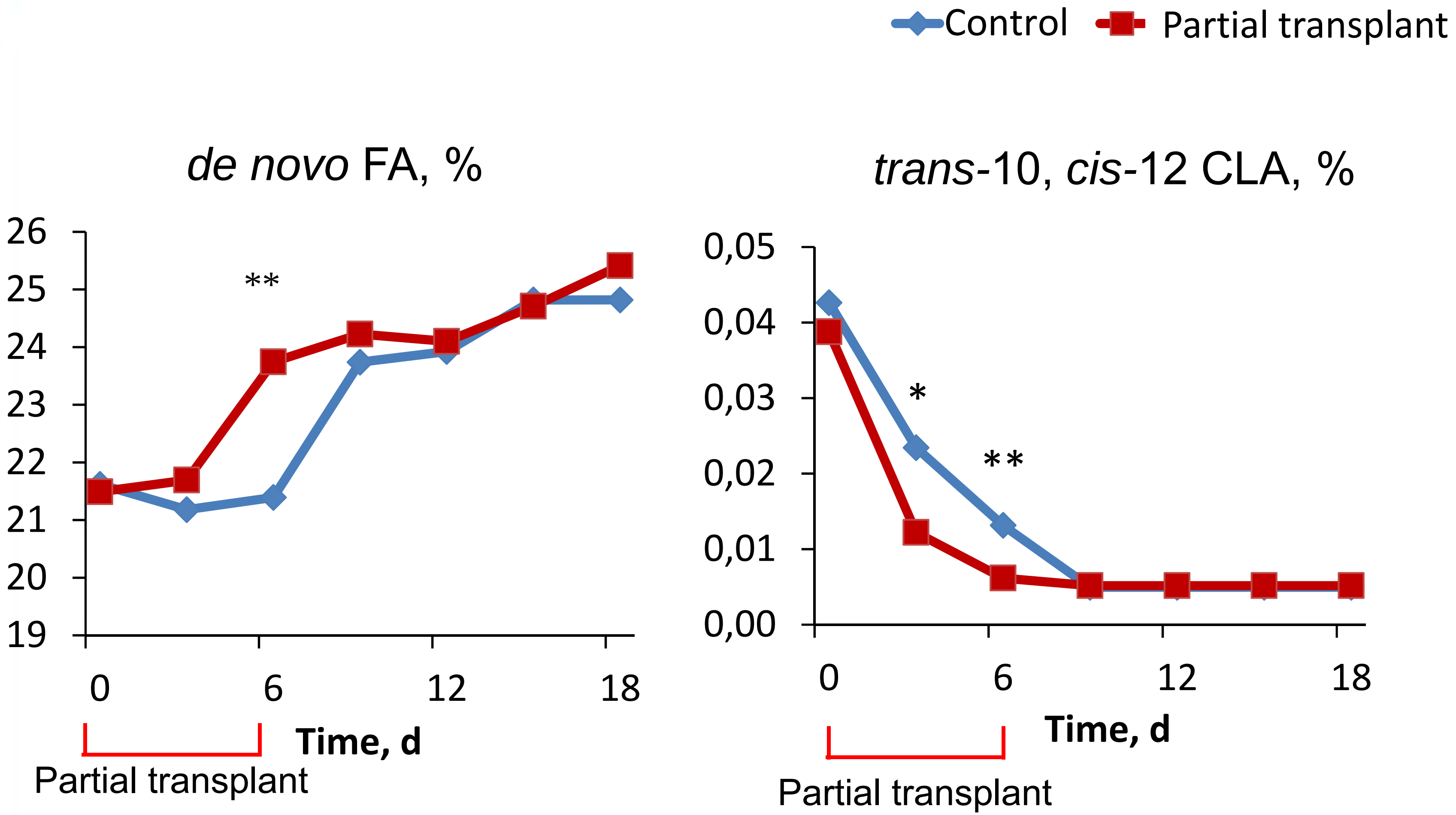

Megasphaera elsdenii, % of bacteria
(Lactate user)
0,004
0,003
0,002
0,001
0,000
Trt 0.02
Trt × time 0.01
SEM 0.002
I
0
4
8
12
20
Time, d



Ilumina sequencing of genomic DNA (16S rRNA)

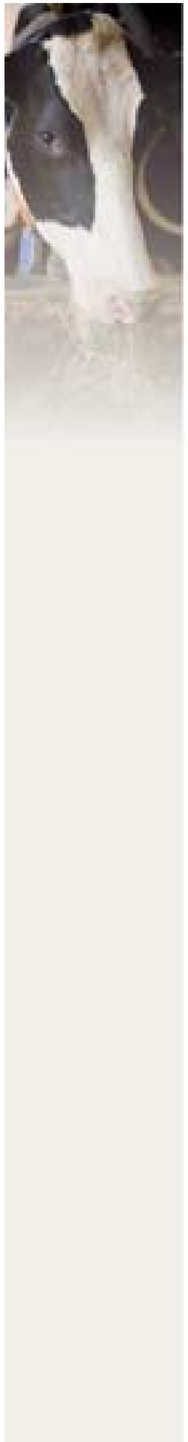
Azad et al., 2015

Microbial adaptation may limit speed of recovery from MFD

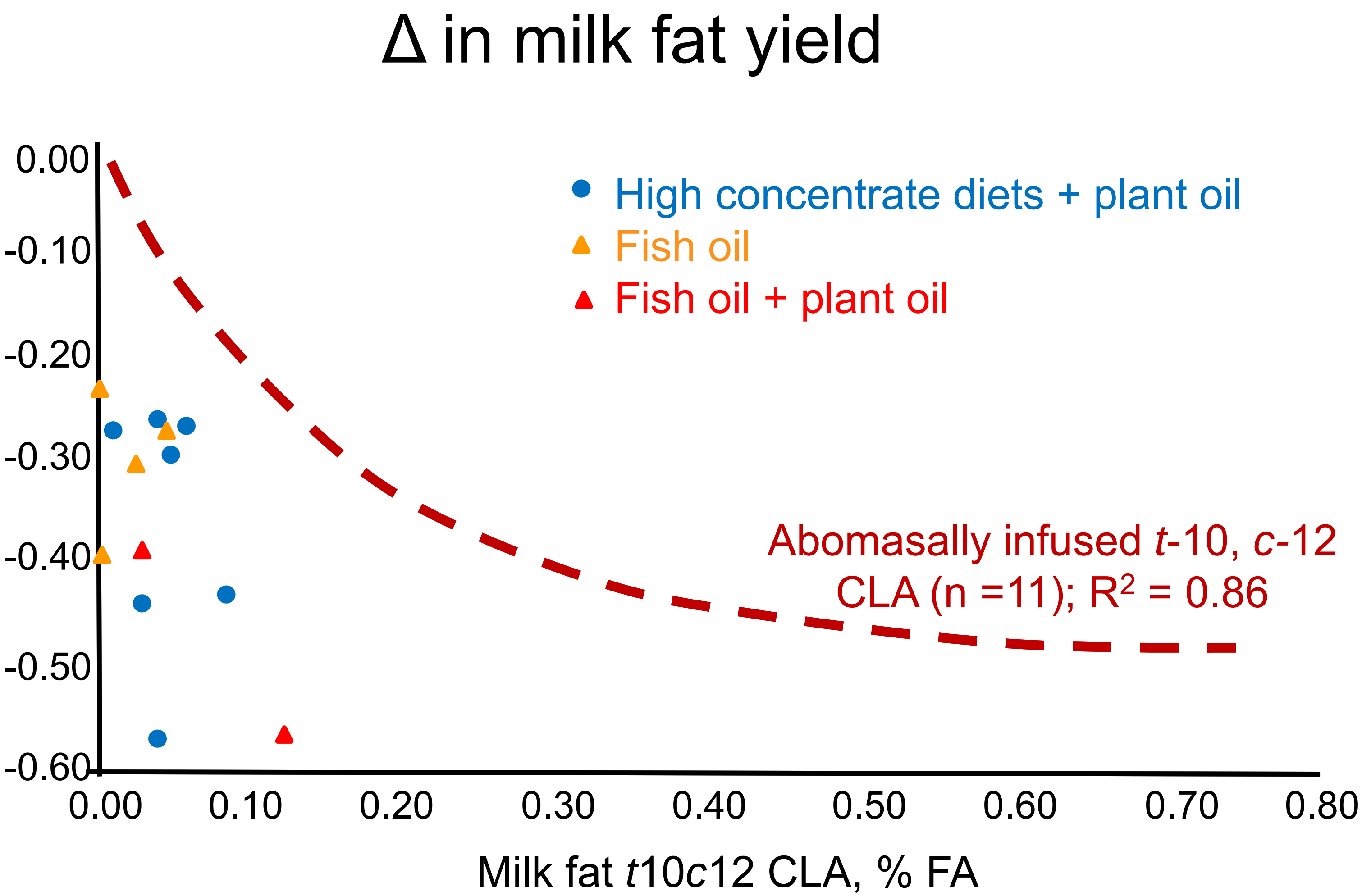


* = $P < 0.05$
** = $P < 0.01$

Rico et al., 2014



Reduction in milk fat = *f*(*t*10*c*12 CLA) ?



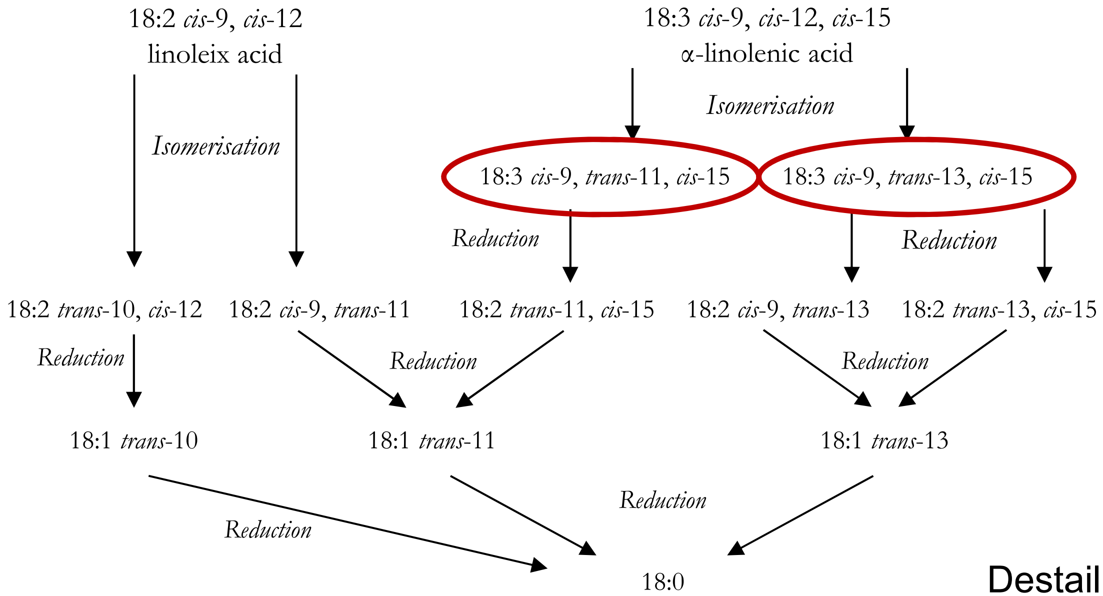
Adapted from Shingfield and Griinari, 2007

Effects of Intravenous Infusion of Conjugated Diene 18:3 Isomers on Milk Fat Synthesis in Lactating Dairy Cows

Conjugated FA isomer	Effect on mammary lipogenesis	Reference	Conjugated FA isomer	Effect on mammary lipogenesis	Reference
18:2			18:3		
trans-8, cis-10	∅	Perfield et al. 2004	cis-6, trans-10, cis-12	∅	Sæbø et al. 2005b
trans-9, cis-11	↓	**Perfield et al. 2007**	cis-6, trans-8, cis-12	∅	Sæbø et al. 2005b
cis-9, trans-11	∅	Baumgard et al. 2000 Baumgard et al. 2002 Loor et Herbein 2003			
trans-9, trans-11	∅	Perfield et al. 2007			
trans-10, cis-12	↓	**Baumgard et al 2000**			
cis-10, trans-12	↓	**Sæbø et al. 2005a**			
trans-10, trans-12	∅	Sæbø et al. 2005a			
cis-11, trans-13	∅	Perfield et al. 2004			

Effects of Intravenous Infusion of Conjugated Diene 18:3 Isomers on Milk Fat Synthesis in Lactating Dairy Cows

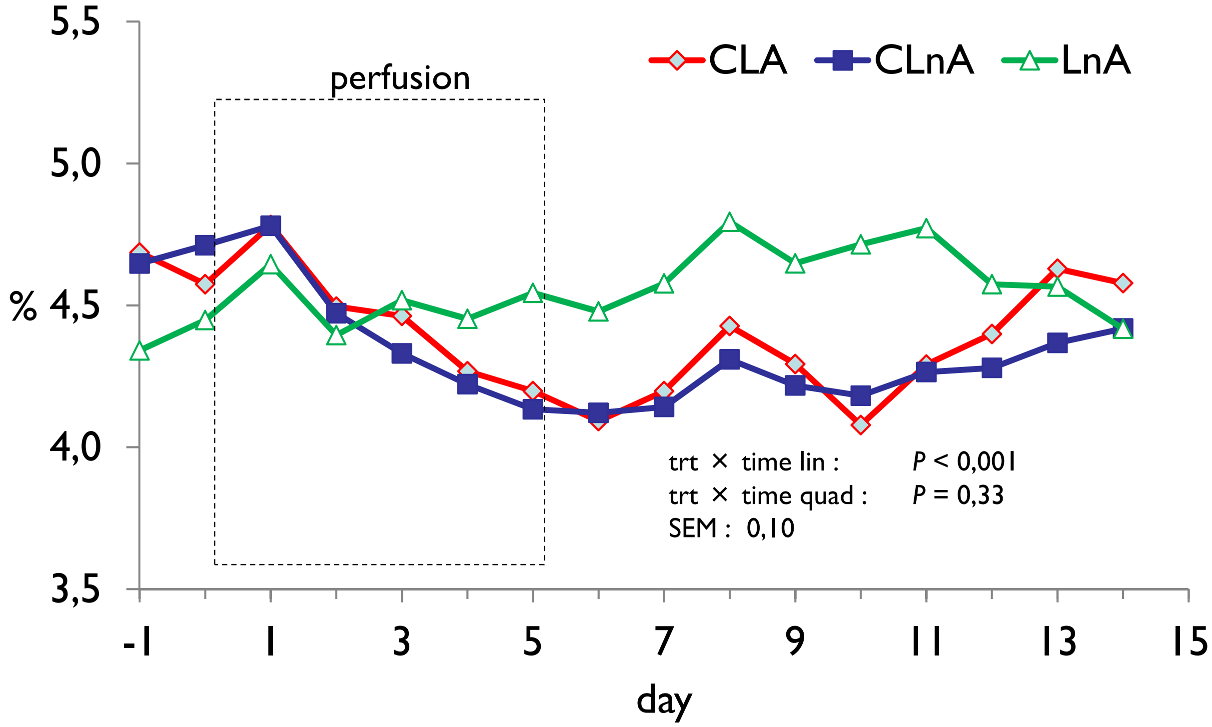
- It is possible that other biohydrogenation intermediates have an impact on mammary tissue lipogenesis



Destaillat et al., 2005

- In rats, conjugated linolenic acids (CLnA) induce a more important decrease in adipose tissue than *trans*-10, *cis*-12 CLA. (Koba et al., 2002)

Milk fat



CLA
CLnA
LnA
perfusion
5,5
5,0
% 4,5
4,0
3,5
-1
1
3
5
7
9
11
13
15
day
trt × time lin : P < 0,001
trt × time quad : P = 0,33
SEM : 0,10

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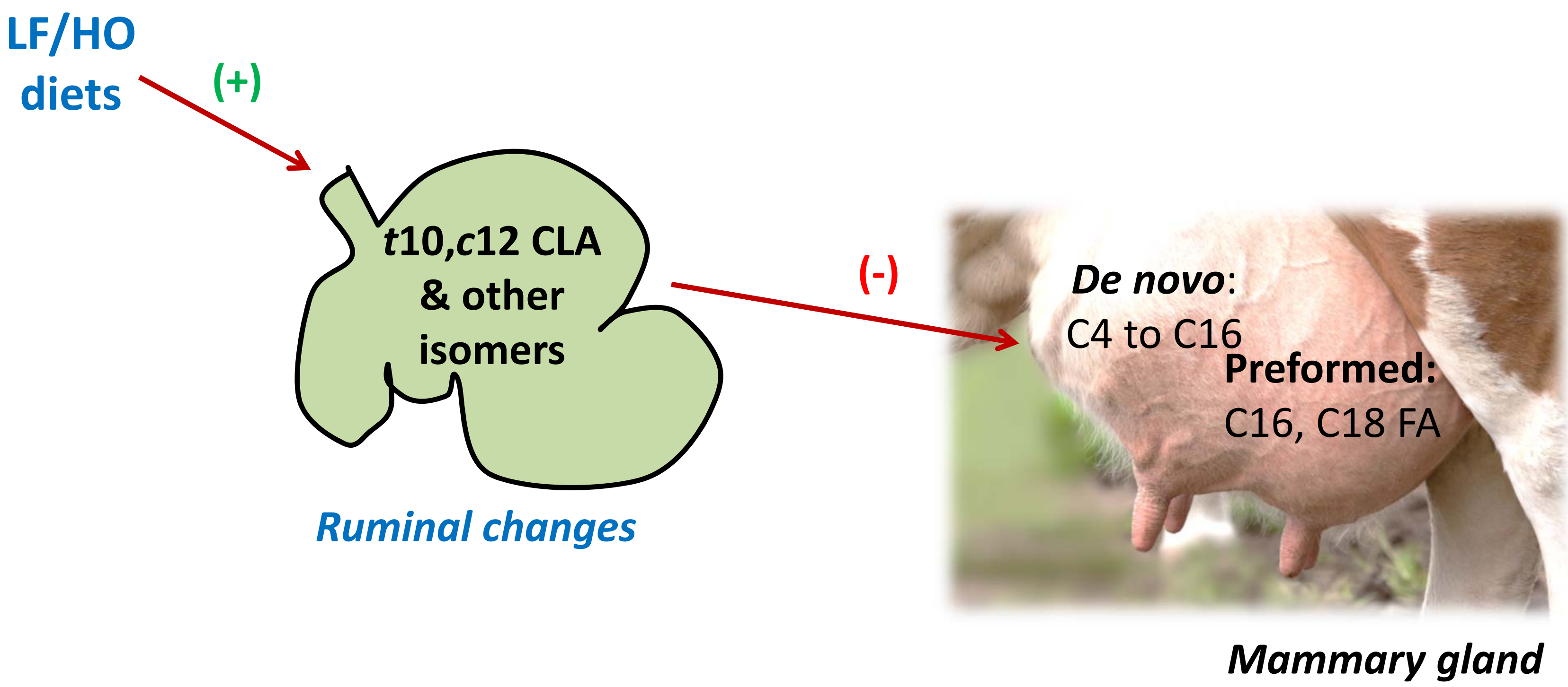
trans-9, cis-11	↓	**Perfield et al. 2007**	cis-6, trans-8, cis-12	Ø	Sæbø et al. 2005b
cis-9, trans-11	Ø	Baumgard et al. 2000 Baumgard et al. 2002 Loor et Herbein 2003	cis-9, trans-11, cis-15	Ø	Gervais and Chouinard, 2008
trans-9, trans-11	Ø	Perfield et al. 2007	cis-9, trans-13, cis-15	Ø	Gervais and Chouinard, 2008
trans-10, cis-12	↓	**Baumgard et al 2000**			
cis-10, trans-12	↓	**Sæbø et al. 2005a**			
trans-10, trans-12	Ø	Sæbø et al. 2005a			
cis-11, trans-13	Ø	Perfield et al. 2004			

Current model of milk fat depression



However, rumen outflow known intermediates is insufficient to fully explain observed milk fat reductions

Biohydrogenation theory of MFD

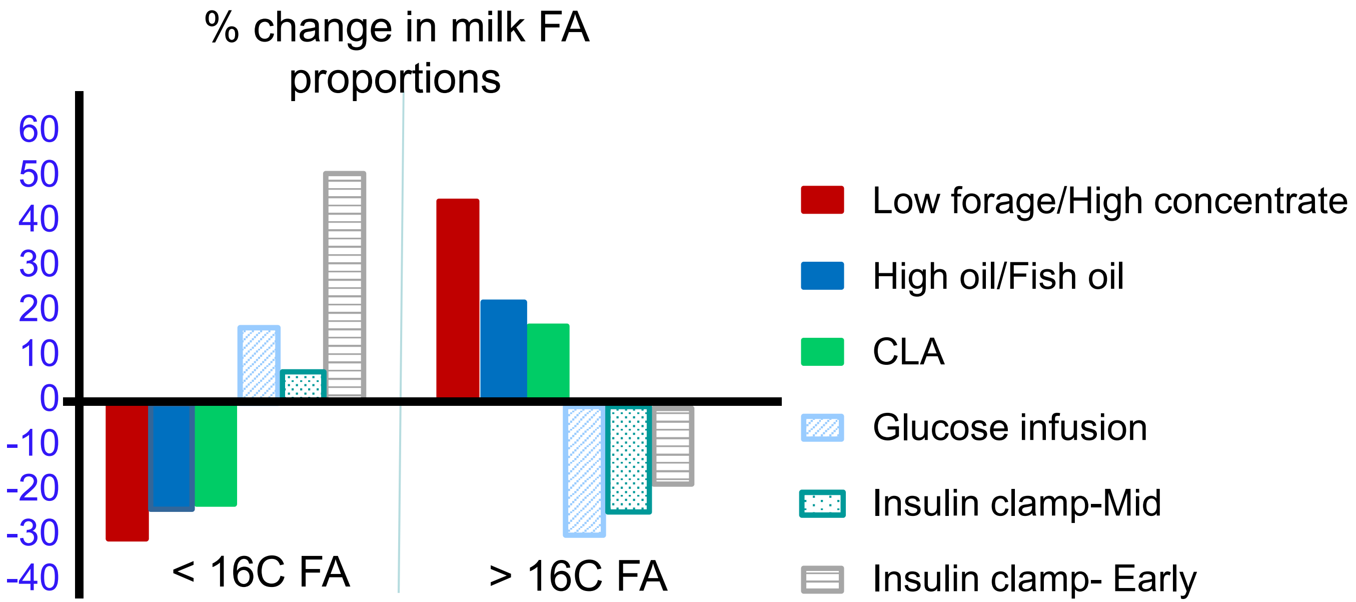
"Under certain conditions, rumen biohydrogenation results in unique fatty acids that are potent inhibitors of milk fat synthesis"

Bauman and Griinari, 2001

Insulin secretagogues & milk fat

Abomasal infusions of glucose or propionate decreased milk fat % and yield in a dose dependent manner.

Maxin et al., 2010 (meta-analysis)



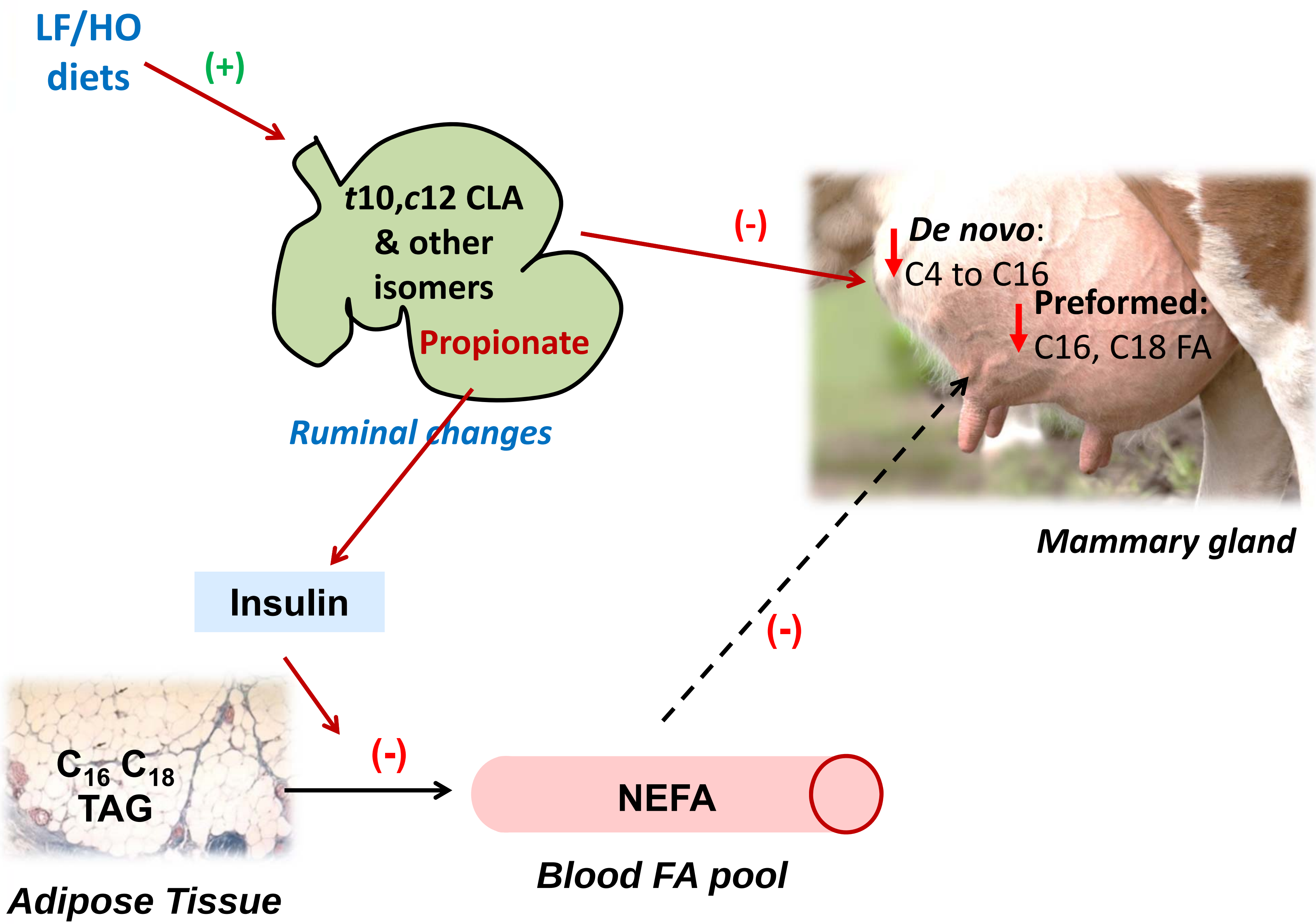
Adapted from Harvatine et al., 2009

Biohydrogenation theory of MFD

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

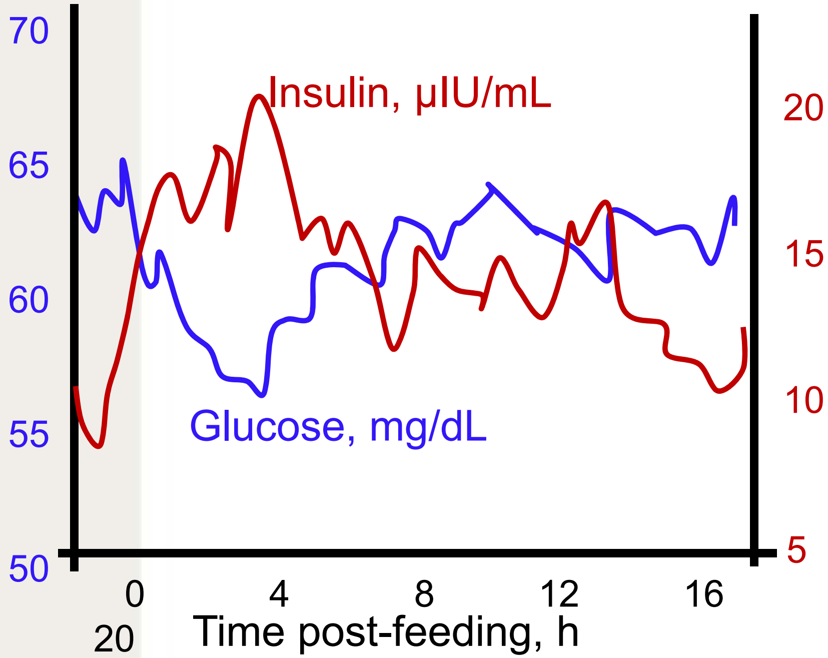
Bauman and Griinari, 2001

Other factors affecting milk fat synthesis

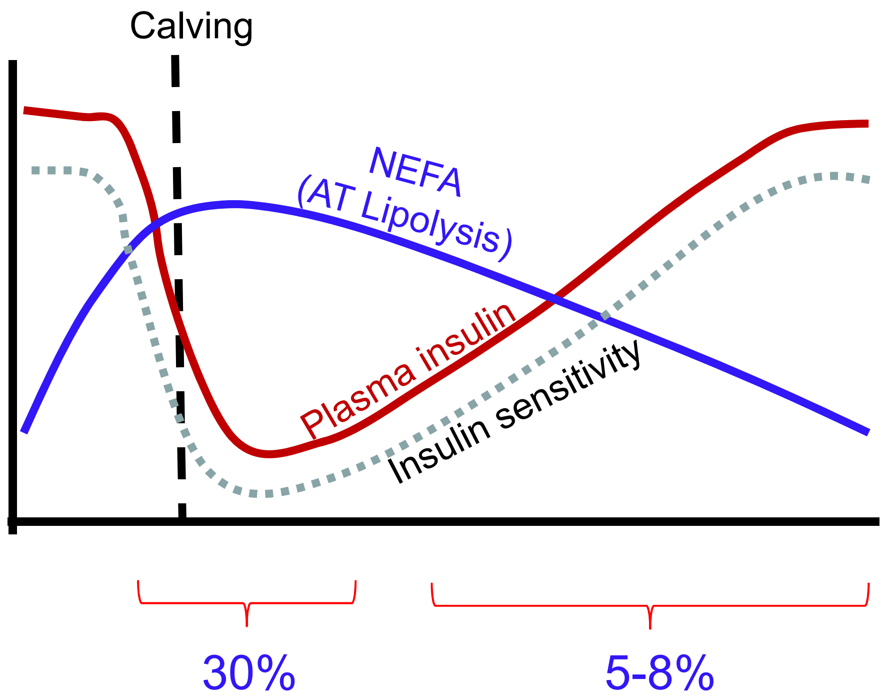


Insulin changes

Daily pattern,
Homeostasis



During lactation,
Homeorhesis

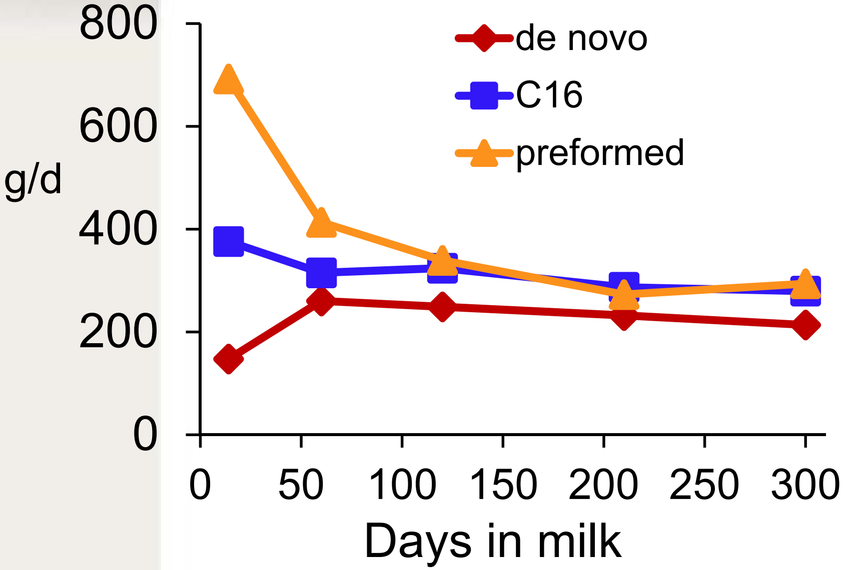


Reduction in milk fat yield during insulin clamp

Oba and Allen, 2003

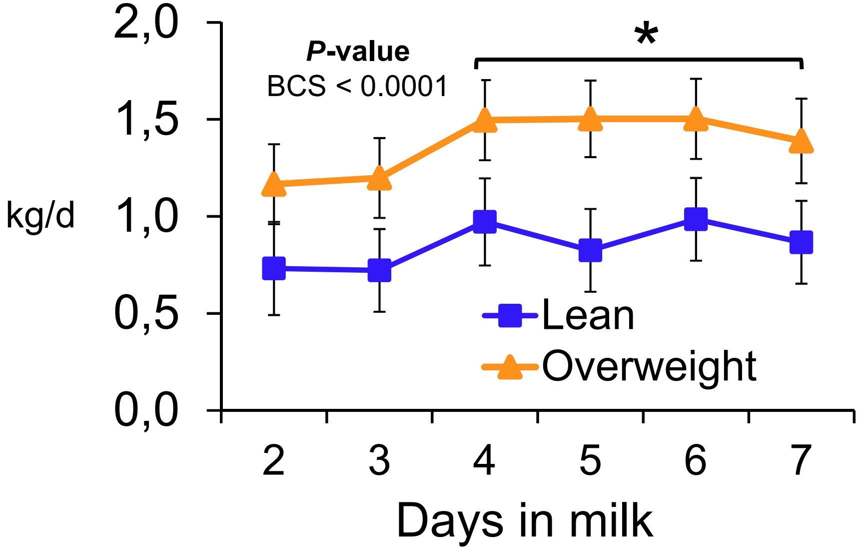
NEFA for milk fat during lactation

Milk fat yield



Baumann et al., 2015

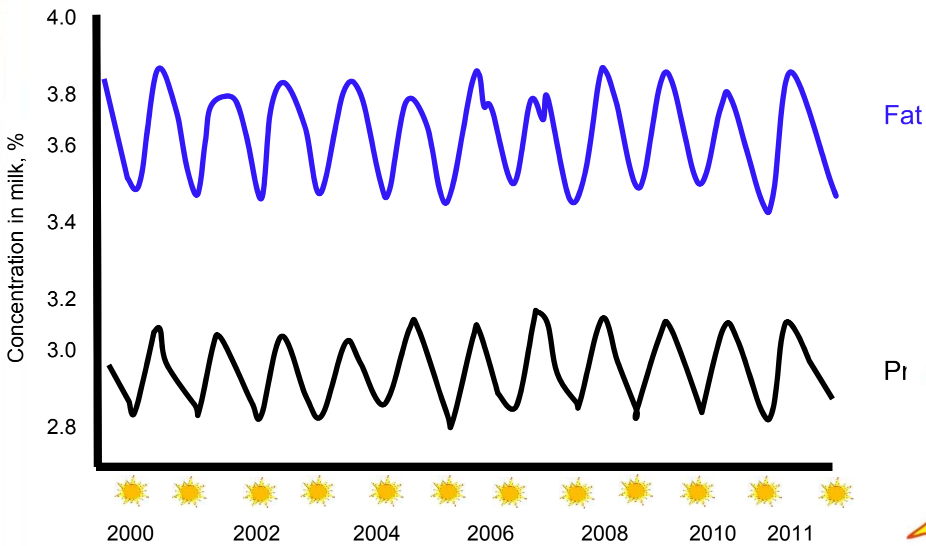
Milk fat yield



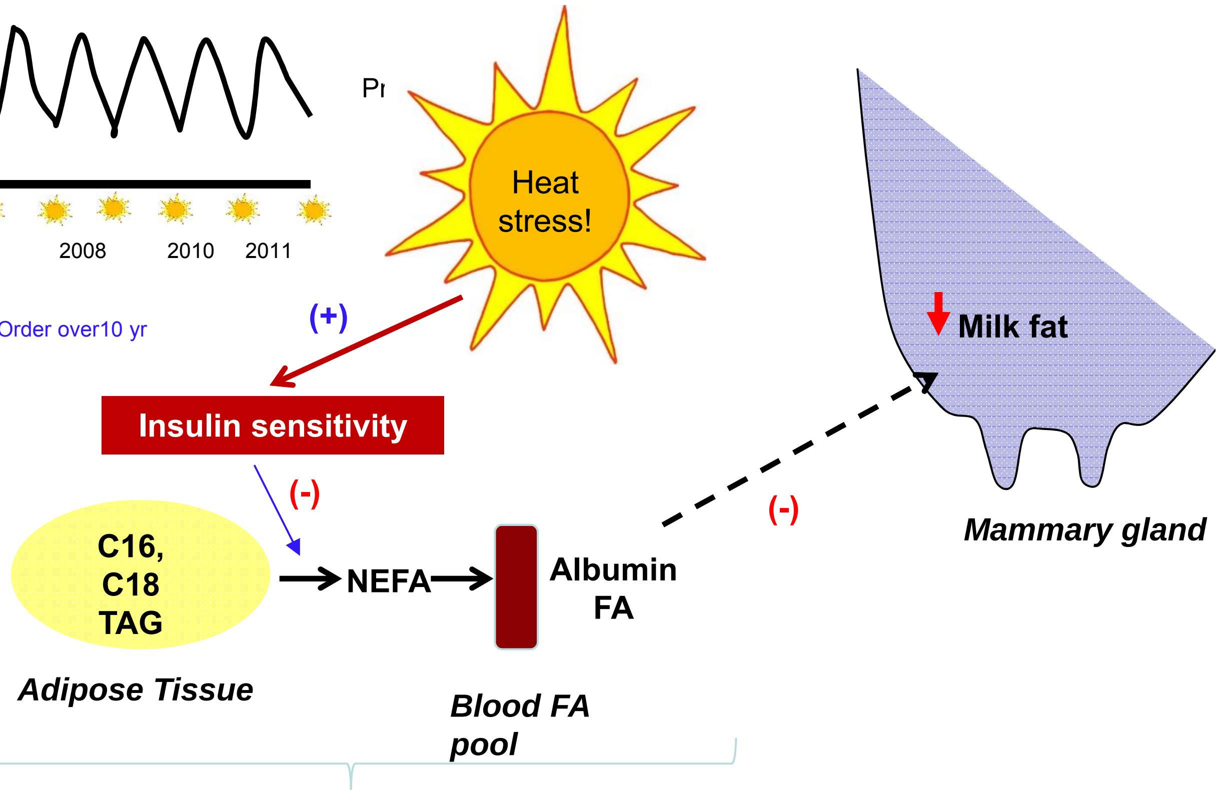
(NEFA increase 2-3x 1st week postpartum)

Rico et al., 2015

Milk fat is reduced during heat stress



Southwest Milk Order over10 yr



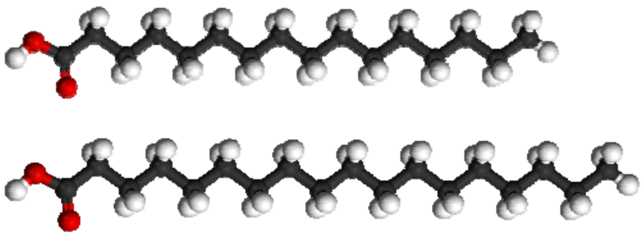
Adapted from Wheelock et al., 2010

Summary

- Milk fat synthesis is affected by bioactive BH intermediates & ruminal adaptation is a key rate limiting step (changes are reversible).
- NEFA are used as a substrate for milk fat synthesis, thus, decreased adipose mobilization results in lower milk fat.
- Homeorhetic effects of insulin may explain some cases of MFD (Heat stress, high concentrate diets, ionophores).

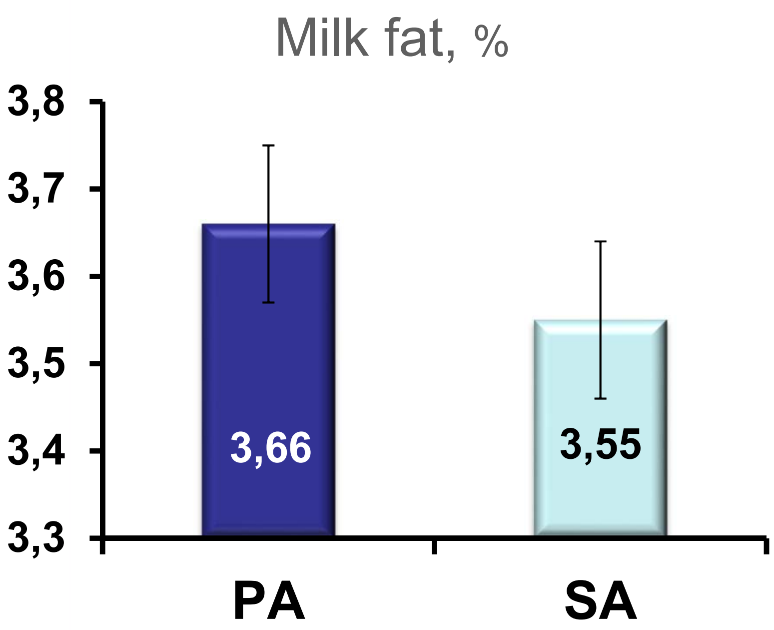
PA increases milk yield

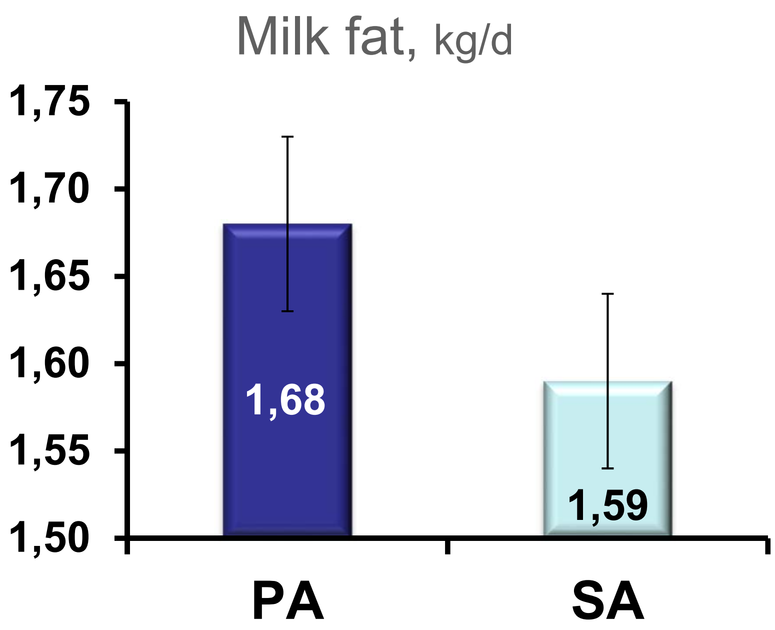
MICHIGAN STATE UNIVERSITY



Palmitic acid, C16:0

Stearic acid, C18:0





Rico et al. 2014

Differences between saturated FA supplements

- 16:0 enriched supplement increased milk fat quadratically with dietary inclusion level (Mosley et al. 2007)
- Higher potential of 16:0 to increase milk fat relative to 18:0 (Rico et al. 2014)
- Shorter chains (8:0 – 10:0) perturb ruminal microflora and thus fermentation, but…..are metabolized differently when absorbed at the intestine

Hypothesis

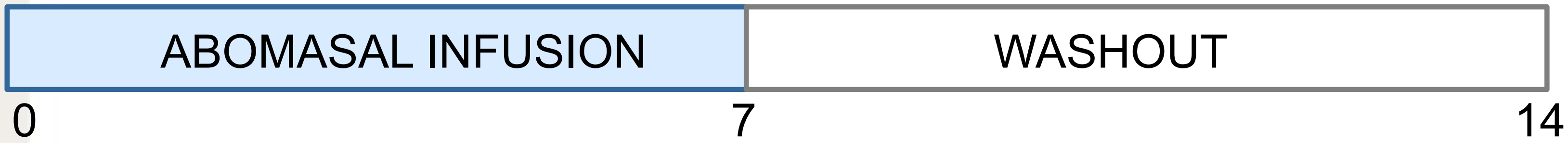
- Abomasally-infused fat supplements with varying chain length will differently affect performance and milk composition in dairy cows

Objective

- To test the effect of FA chain length of saturated fat supplements on milk production and composition in lactating dairy cows

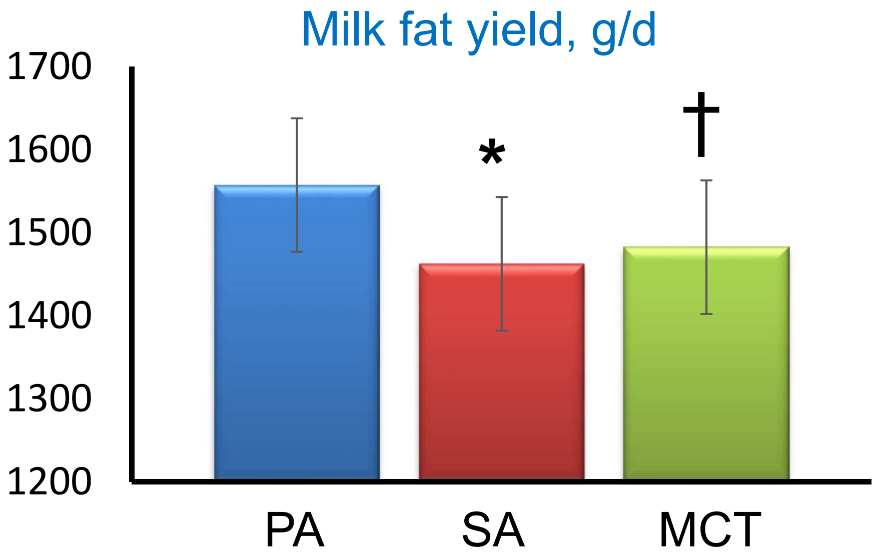
Design and treatments

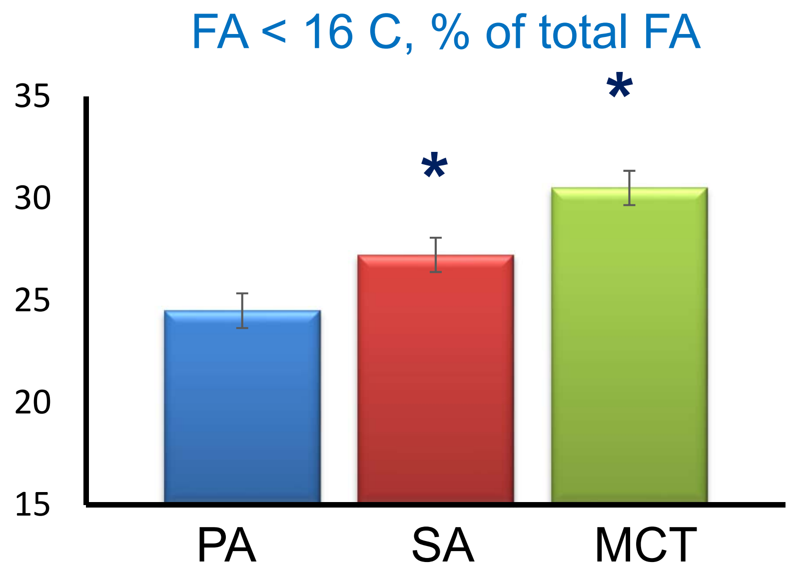
- 11 cannulated Holstein dairy cows (150 ± 52 DIM) in replicated Latin square design (3 periods of 14 d)



- Emulsions providing 280 g/d of FA:
  - Free FA enriched in palmitic acid (PA; >85% 16:0)
  - Free FA enriched in stearic acid (SA; 98% 18:0)
  - Medium-chain triglycerides (MCT; 8:0 and 10:0)

Saturated FA chain length altered milk fat during mid-lactation





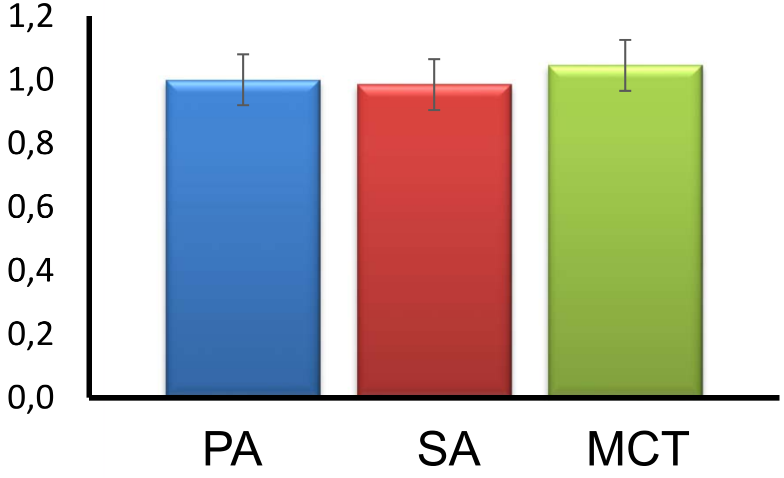
PA = C16:0
SA = C18:0
MCT = C8:0 + C10:0

* vs. PA $P < 0.05$
† vs. PA $P < 0.10$

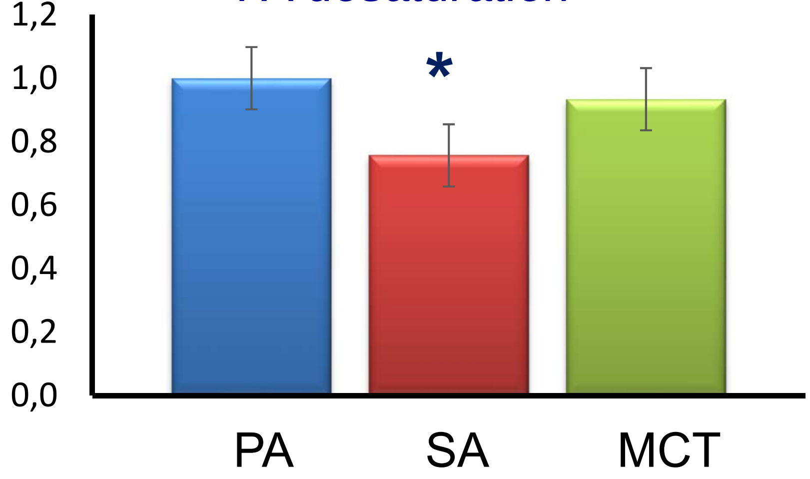
Rico et al., 2015

Mammary lipid metabolism genes fold changes ($2^{-\Delta\Delta Ct}$)

LPL
FA uptake

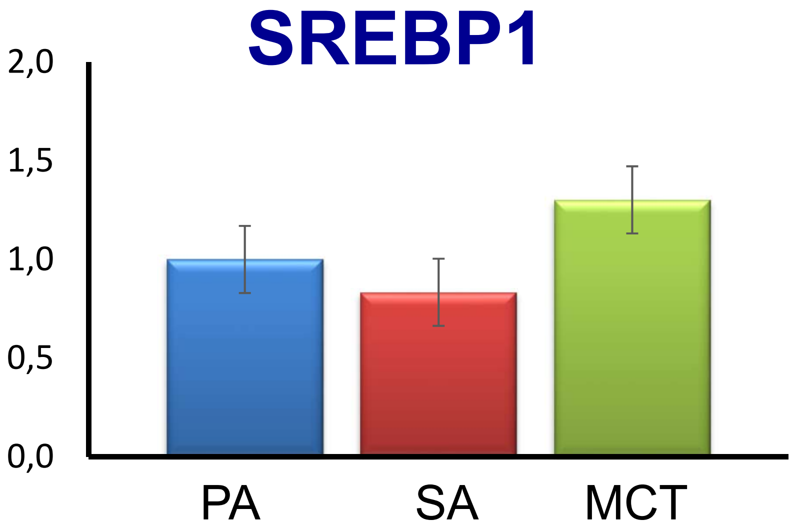


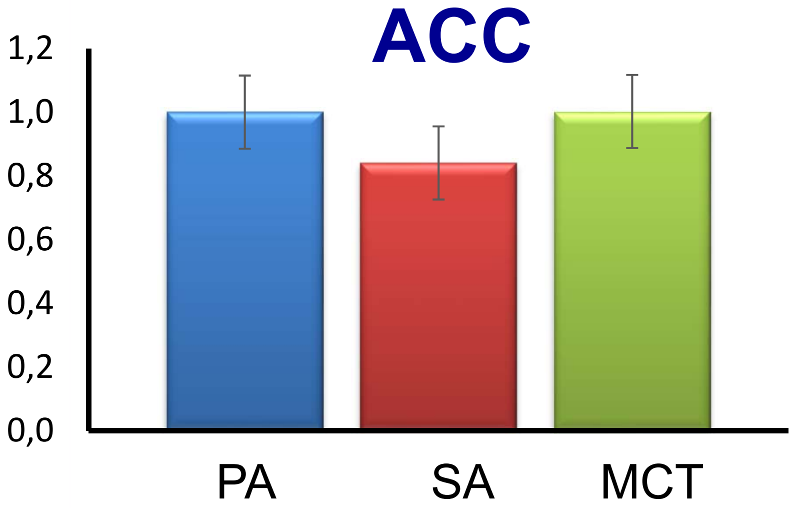
SCD1
FA desaturation

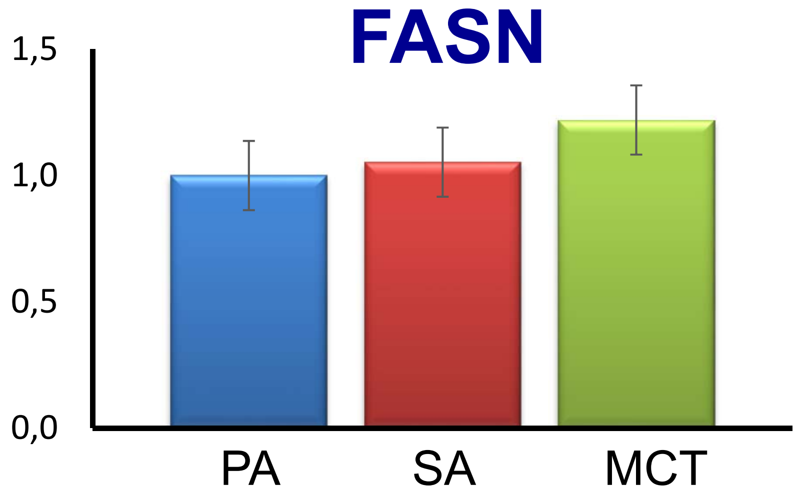


* vs. PA $P < 0.05$

Mammary lipid metabolism genes fold changes ($2^{-\Delta\Delta Ct}$)







Summary of results

- PA increased milk fat synthesis and efficiency relative to SA
- Higher 10:0 and 12:0 with MCT

  …….known to be oxidized in the liver?
- Transcription of key mammary lipogenic genes was not affected
- Liver pyruvate kinase was reduced by PA

Effects of dietary fatty acid chain length on performance of early lactation dairy cows

- Twenty one multiparous Holstein dairy cows (6 ± 2 DIM) in randomized complete block design

- Fat supplements fed for 28 d at **2%** of ration DM were:

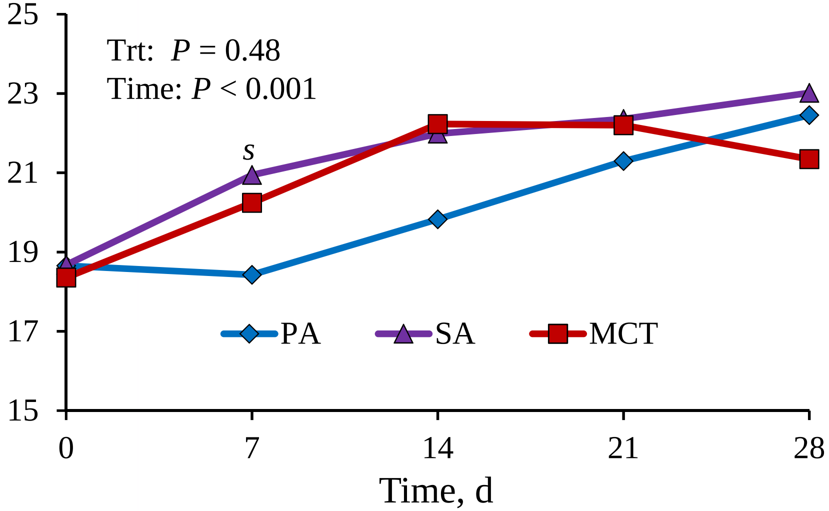
  1) free FA enriched in palmitic acid (>85% 16:0; PA)

  2) free FA enriched in stearic acid (35% 16:0; 45% 18:0; SA)

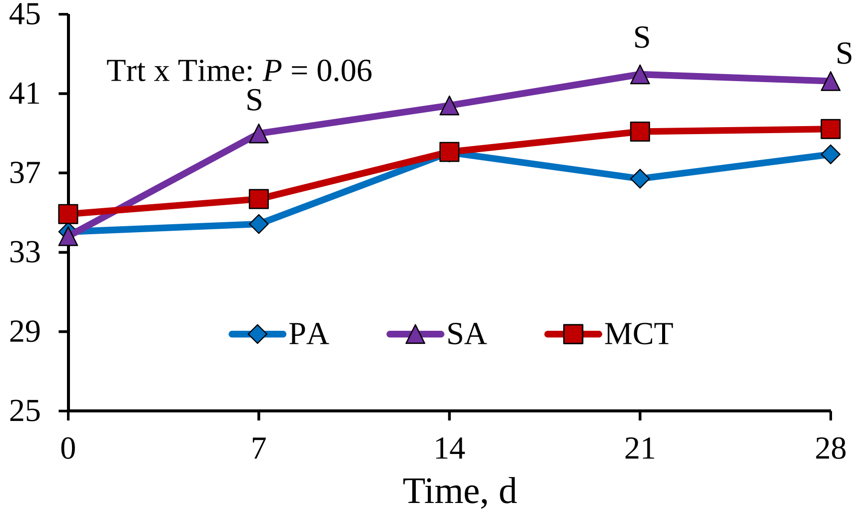
  3) medium-chain triglycerides (25% 8:0 and 10:0 mix; MCT) protected in a saturated FA matrix (57% 16:0 and 43% 18:0)

Milk yield reduced by PA, minor DMI effects

DMI, kg/d



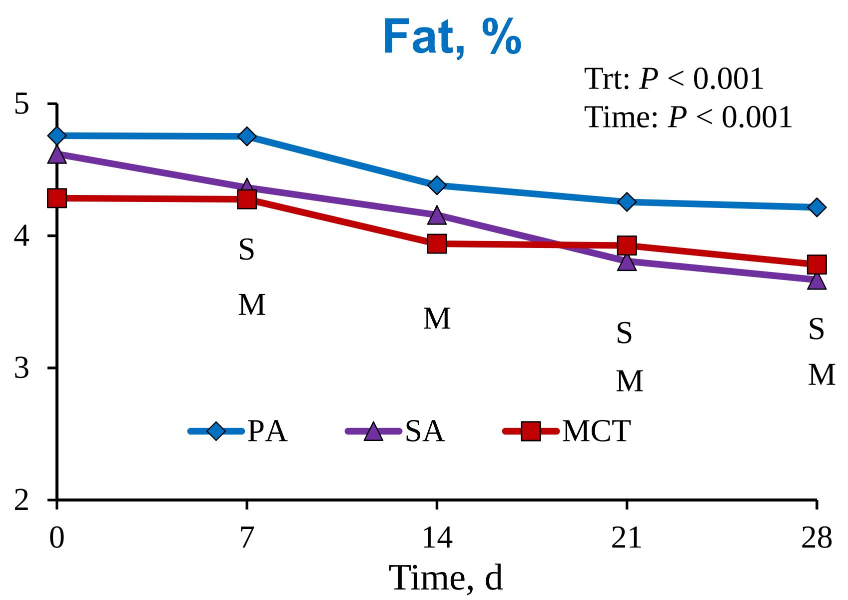
MY, kg/d

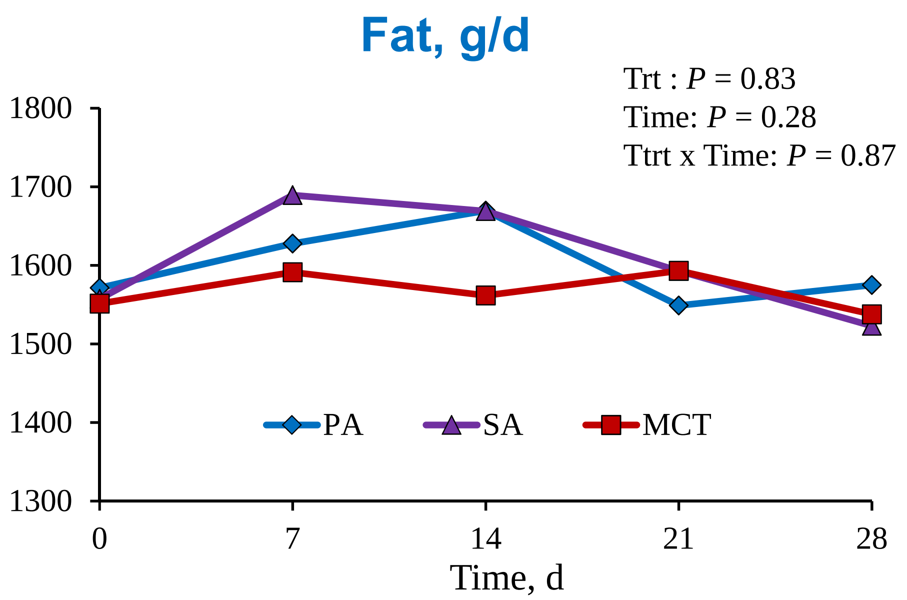


PA vs. SA
S: *P* < 0.01; ***s***: *P* < 0.10

PA vs. MCT
M: *P* < 0.01; ***m***: *P* < 0.10

Milk fat concentration and yield effects





PA vs. SA
S: *P* < 0.01; ***s****:* *P* < 0.10

PA vs. MCT
M: *P* < 0.01; ***m***: *P* < 0.10

Conclusions

- Dietary FA chain length affected milk yield with minor effects on DMI and no effect on ECM
- PA failed to increase milk fat yield in contrast to previous studies
- Moderate effects on other milk components

Questions