Altering rumen biohydrogenation to improve milk fatty acid profile of dairy cows

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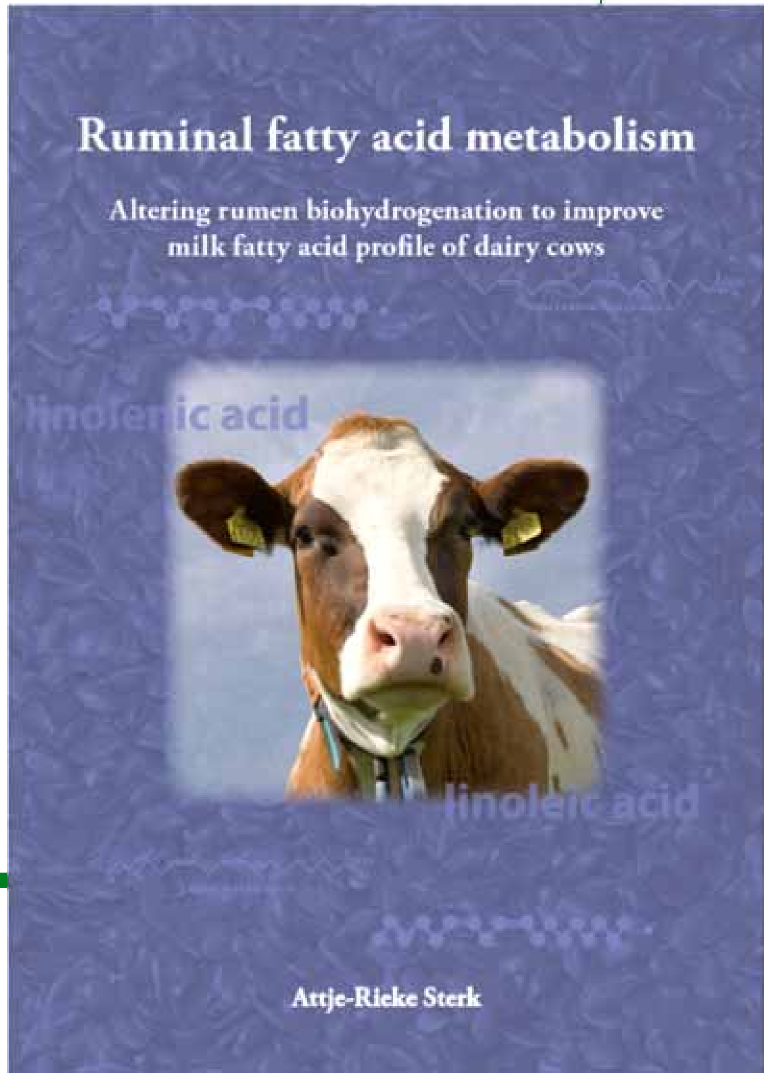
Thesis: Ruminal fatty acid metabolism

- Introduction
- Meta-analysis
- In vitro research
- In vivo research
- Conclusions



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Milk fatty acid composition

- Milk fatty acids
  - De novo synthesis of fatty acids
  - Uptake of preformed fatty acids

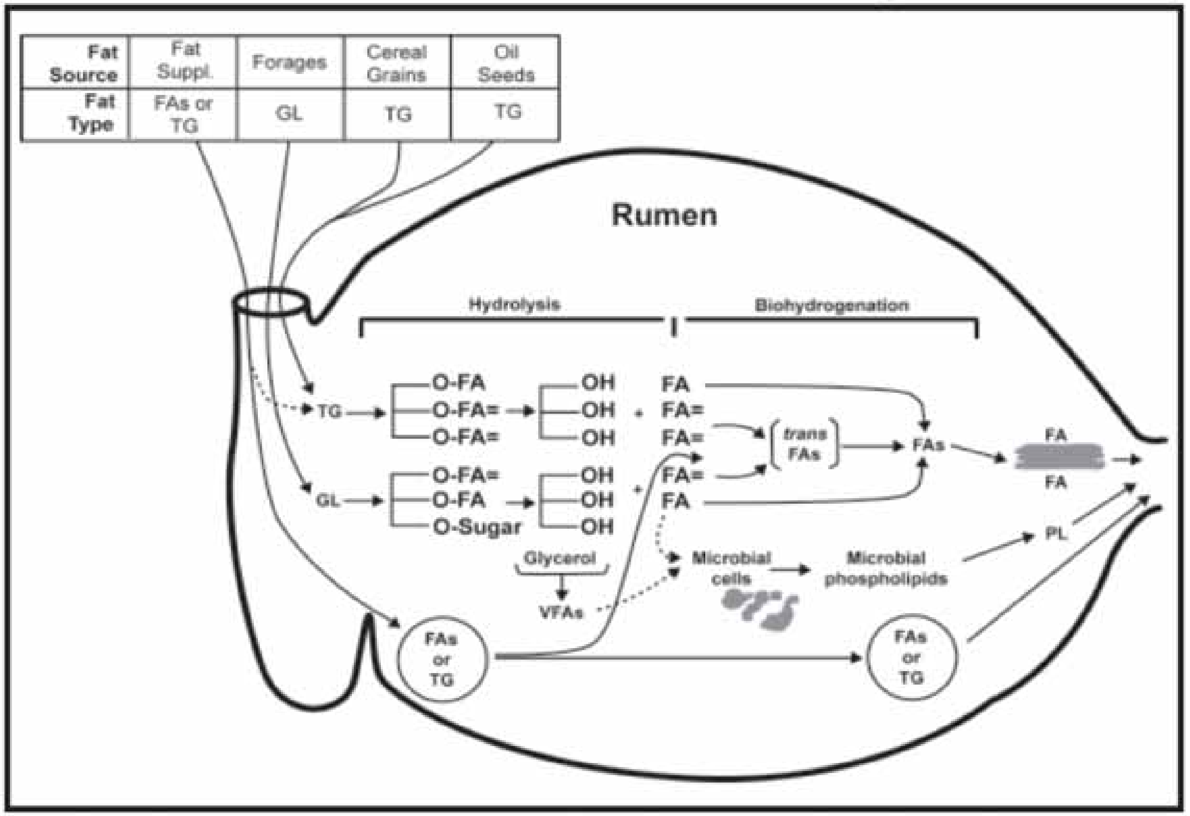
- Milk fatty acid composition
  - Saturated fatty acids (SFA)
  - Mono-unsaturated fatty acids (MUFA)
  - Poly-unsaturated fatty acids (PUFA)

  } Unsaturated fatty acids (UFA)

- Human health
  - ↓ SFA
  - ↑ UFA
  - ↑ Specific health promoting PUFA (e.g. omega-3, CLA)

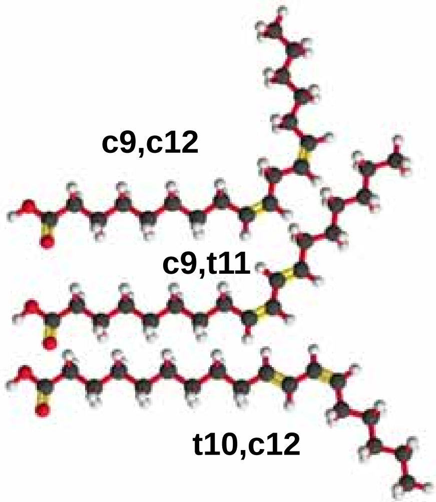
Fat metabolism in the rumen

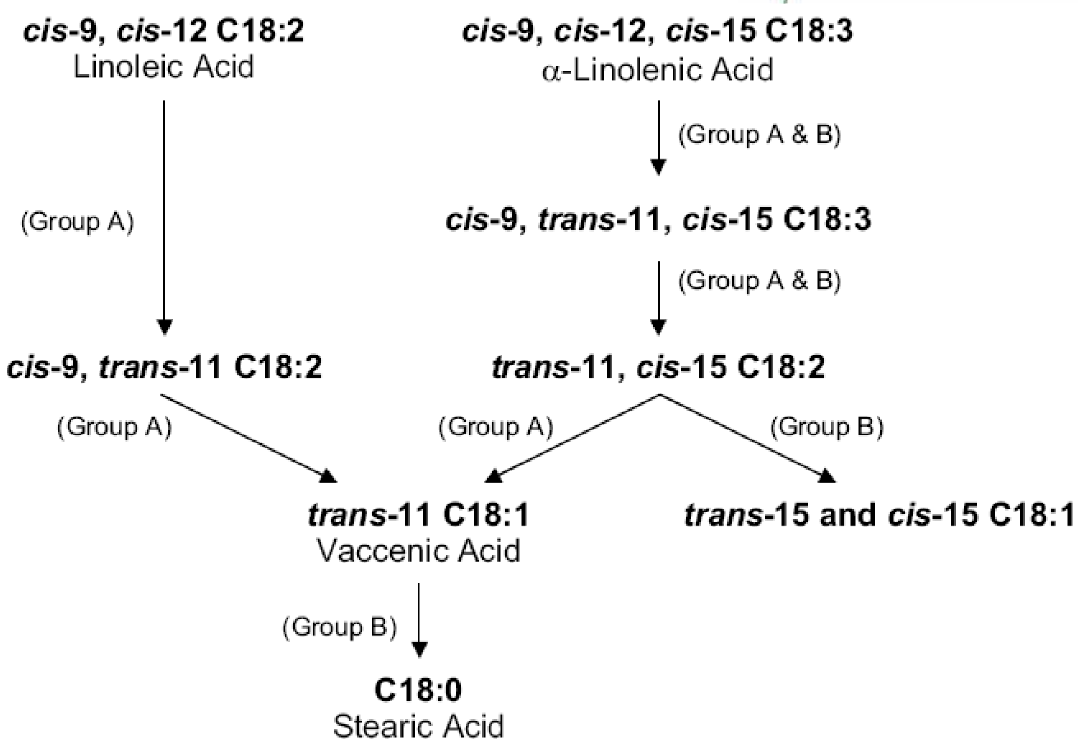
- Intake of fat
  - Forages
  - Concentrates
- Hydrolysis
- Biohydrogenation
  - Isomerization
  - Hydrogenation



Biohydrogenation

- Classical pathways



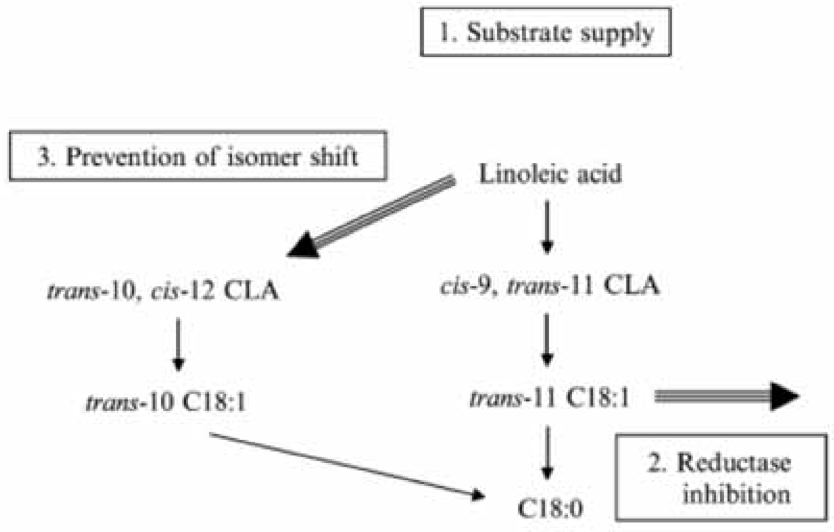


(Adapted from Harfoot and Hazlewood, 1997)

Changes in rumen environment

- Substrate supply
  - Fatty acid composition => more UFA
  - Protection of UFA

(Adapted from
Griinari and Shingfield, 2002)



- Influencing bacterial population
  - Inhibition of complete biohydrogation to stearic acid
- Prevention of isomer shift
  - Prevent milk fat depression

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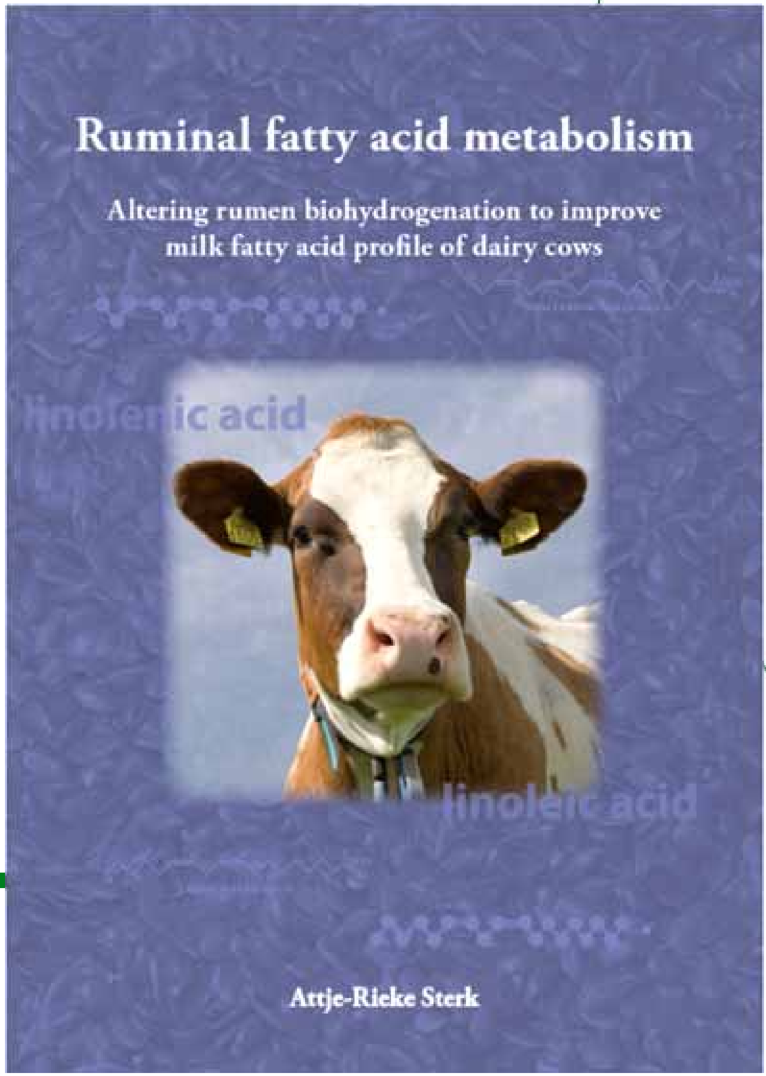

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Meta-analysis – Introduction

- A large number of studies on responses of milk fatty acid profile to fat supplementation is published
- Effects of interfering factors is difficult to assess
- Form of fat supplementation and addition of fish oil can affect biohydrogenation

- Objective was to study the effect of different fat sources, their technological form and/or addition of fish oil, and characteristics of the basal diet (forage type, NDF content, FA content) on milk fatty acid profile in lactating dairy cows

Meta-analysis – Materials and methods

- Database containing 50 experiments with 151 treatments
- Different fat sources
  - Unsupplemented, rapeseed, soybean or sunflower, linseed, fish
- Different technological forms of the fat source
  - Oil, seed, protected, added fish oil
- Different main forage types in the rations
  - Alfalfa silage, barley silage, maize silage, maize silage/haylage, grass silage, haylage
- Mixed model analysis to model milk fatty acid profile
  - Continuous variables (FA and NDF content of the ration)
  - Class variables (fat source, technological form and forage type)

Meta-analysis – Results and conclusions

- The relationship between the dietary nutrient composition and milk fatty acid profile is affected by the form of fat supply and the main forage type in the ration

- This results in significant differences in several milk fatty acids:
  - Within fat sources supplied as oil, seed, protected or with added fish oil
  - Within fat sources supplied to diets containing alfalfa silage, barley silage, maize silage, maize silage/haylage, grass silage or haylage

- Full paper available in thesis and soon in Journal of Agricultural Science

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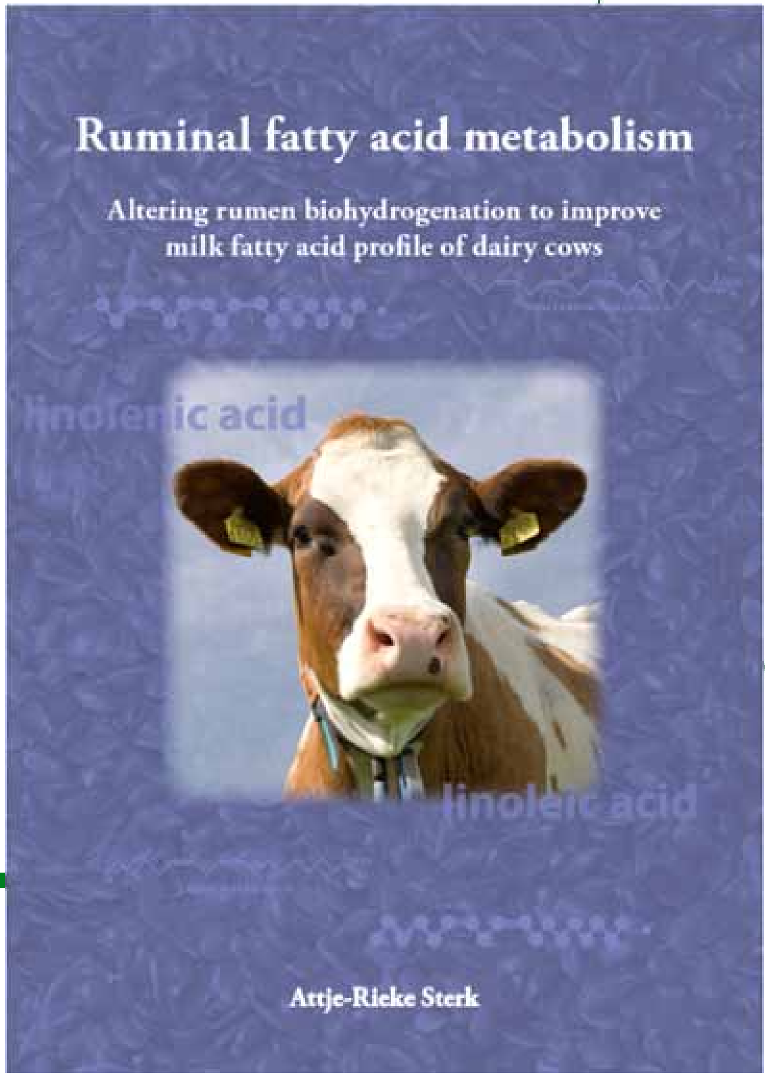

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In vitro research - Introduction

- To improve milk fatty acid profile there is a need to increase postruminal flow of unsaturated fatty acids (UFA)
- Development of UFA protection technologies
    - Chemical protection (e.g. formaldehyde)
    - Alterations of fatty acid structure (e.g. Ca-salts)
    - Technological protection (e.g. extrusion)
    - Inhibition of complete biohydrogenation by the addition of DHA
- In vitro evaluation of effectiveness of protection
    - Estimation of kinetic parameters (e.g. kh and lag time)
    - Calculation of effective biohydrogenation
    - Estimation of effect on rumen fermentation

In vitro research - Materials and methods

- 8 Treatments:
  - Pure linseed oil (LO)
  - Crushed linseed (CL)
  - Extruded whole linseed (70 % linseed/30 % wheatbran; EL)
  - Extruded crushed linseed (70 % CL/30 % wheatbran; ECL)
  - Formaldehyde treated linseed (FCL)
  - Micronized crushed linseed (MCL)
  - DHA in combination with linseed oil (DL)
  - Commercial extruded linseed product (CEL)

- Incubation with mixed rumen fluid + buffer (0, 0.5, 1, 2, 4, 6, 12, 24 h)
- Fatty acid analysis using gas chromatography
- Disappearance of C18:3n3

In vitro research – Results and conclusions

- Exponential model (Orskov and McDonald, 1979; Enjalbert et al., 2003)
- $Y = \exp^{(-kh * (\text{incubation time} - \text{Lag time}))}$
- Effective biohydrogenation (kp = 6 %/h):
  $Kh * \exp^{(-kp * \text{Lagtime})} / (kh + kp)$ (Dhanoa et al., 1999)

Treatment	LO	CL	EL	ECL	MCL	FCL	DL	CEL
C18:3n3, %	0.37	0.40	0.66	0.30	0.35	0.59	0.44	0.22
Kh, %/h	6.65	4.13	1.79	5.93	4.53	2.47	4.42	8.04
Lag time	1.49	2.21	0.25	2.00	2.01	2.99	1.37	2.00
Eff. bh, %	43.5	35.6	22.7	43.7	38.1	24.3	38.3	50.8

- Full paper available in thesis and Journal of Dairy Science 93: 5286-5299

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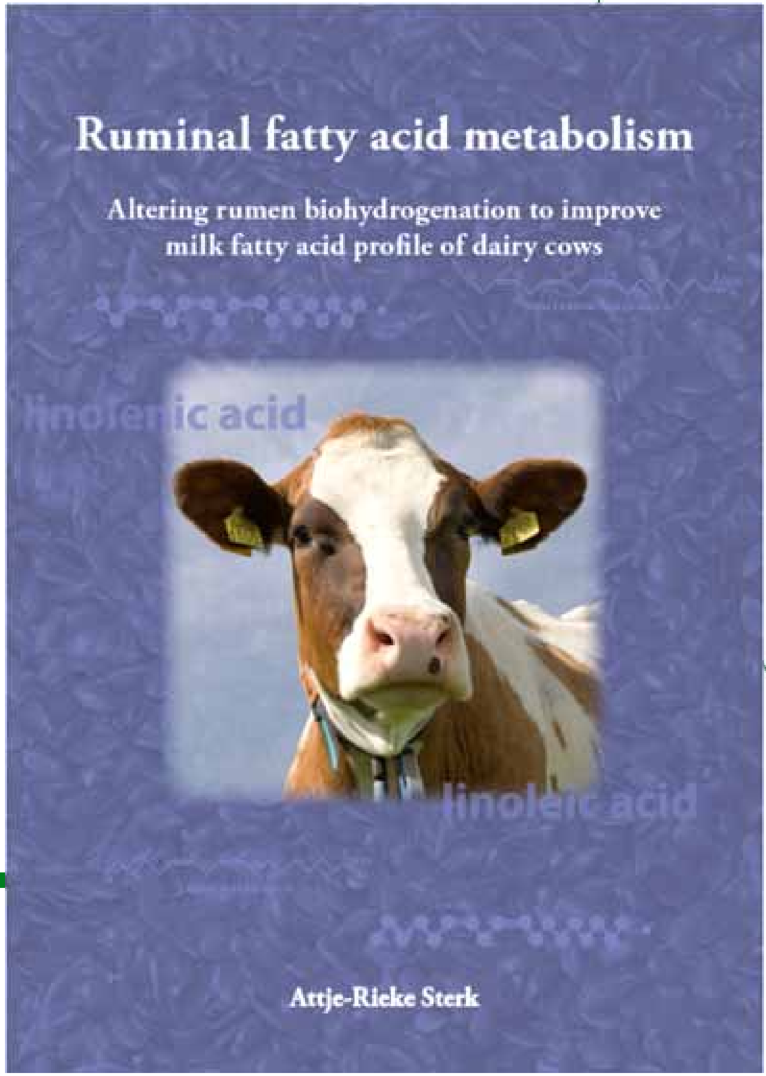

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In vivo research - Introduction

- Two experiments
  - Latin square experiment in which fatty acid intake, omasal fatty acid flows and plasma and milk fatty acid profiles were measured from cows fed different linseed treatments
  - Multivariate design experiment to estimate milk fatty acid profile from cows fed an increasing proportion of crushed linseed in combination with varying forage type and forage to concentrate ratio

Experiment 1 - Introduction

- Very low transfer efficiency of C18:3n3 from feed to milk fat
- Marked differences between lipids entering versus lipids leaving the rumen
- Development of different rumen protection technologies
- In vitro research showed lower biohydrogenation of C18:3n3 for EL and FCL
- DHA addition to LO can influence the accumulation of different biohydrogenation intermediates

- Objective was to determine effects of supplementing different linseed treatments on nutrient intake, nutrient flows into the omasal canal, production performance, and FA profile in plasma and milk fat of lactating dairy cows

Experiment 1 - Materials and methods

- 4 x 4 Latin square design with 4 cows receiving 4 treatments in 4 periods
- Treatments were:
  - Crushed linseed (CL)
  - Extruded whole linseed (EL)
  - Formaldehyde treated linseed oil (FL)
  - DHA in combination with linseed oil (DL)

- Measurements:
  - DMI, milk yield, milk composition
  - Nutrient and FA flows to omasal canal
  - Nutrient digestibilities (rumen, faecal) and FA biohydrogenation
  - FA profile in plasma triglycerides and FA profile in milk fat

Experiment 1 - Results
DMI, milk yield and milk composition

	Dietary treatment				SEM	P-value
	CL	EL	FL	DL		
DMI, kg/d	20.4	20.8	21.6	19.8	1.30	0.502
Milk yield, kg/d	33.1	31.4	33.7	29.7	2.41	0.402
Milk fat, g/kg	43.0[a]	47.5[a]	46.7[a]	32.7[b]	5.70	0.002
Milk protein, g/kg	31.8	32.7	32.6	30.9	1.17	0.552

Experiment 1 - Results
Nutrient and fatty acid flows to omasal canal

	Dietary treatment				SEM	P-value
	CL	EL	FL	DL		
DM, kg/day	13.4	13.2	13.1	12.5	1.28	0.667
NDF, kg/day	3.0	3.0	2.8	2.8	0.43	0.659
Cfat, kg/day	1.3	1.3	1.1	1.2	0.94	0.491
C18:0, g/day	368.5[a]	342.6[a]	331.6[a]	148.0[b]	32.20	0.007
C18:1t10, g/d	7.6[b]	6.2[b]	5.4[b]	149.6[a]	20.76	0.005
C18:1t11, g/d	35.6[ab]	26.0[b]	32.6[ab]	92.2[a]	14.72	0.034
C18:2t11c15	11.6	8.5	17.3	45.4	9.28	0.100
C18:3n3	21.8[b]	33.8[a]	15.5[b]	4.6[c]	2.59	<0.001

Experiment 1 - Results
Nutrient and fatty acid flows to omasal canal

	Dietary treatment				SEM	P-value
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C18:1t10, g/d	7.6[b]	6.2[b]	5.4[b]	149.6[a]	20.76	0.005
C18:1t11, g/d	35.6[ab]	26.0[b]	32.6[ab]	92.2[a]	14.72	0.034
C18:2t11c15	11.6	8.5	17.3	45.4	9.28	0.100
C18:3n3	21.8[b]	33.8[a]	15.5[b]	4.6[c]	2.59	<0.001

Experiment 1 - Results
Nutrient digestibility and fatty acid biohydrogenation

	Dietary treatment				SEM	P-value
	CL	EL	FL	DL		
Rumen DC						
DM	34.9	36.6	39.2	37.2	2.75	0.370
NDF	60.1	61.0	63.6	60.6	3.78	0.439
Whole tract DC						
DM	73.3[ab]	72.7[b]	74.5[ab]	74.7[a]	0.93	0.025
Cfat	71.3[b]	64.8[c]	78.5[a]	80.4[a]	1.06	<0.001
FA biohydrogenation						
C18:3n3	94.0[b]	90.9[c]	95.4[b]	98.5[a]	0.49	<0.001

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Experiment 1 - Results
Fatty acid profile in plasma triglycerides

	Dietary treatment				SEM	P-value
	CL	EL	FL	DL		
Plasma FA, g/100 g FA						
C18:0	43.06[a]	42.04[a]	37.11[a]	17.88[b]	3.157	0.001
C18:1t10	0.65[b]	0.63[b]	0.58[b]	11.50[a]	1.673	0.007
C18:1t11	1.84[b]	1.78[b]	2.11[b]	6.72[a]	0.640	0.001
C18:2t11c15	0.51[b]	0.54[b]	0.66[b]	2.02[a]	0.278	0.015
C18:3n3	1.22[z]	1.35[z]	3.60[y]	1.12[z]	0.526	0.043

Experiment 1 - Results
Fatty acid profile in plasma triglycerides

	Dietary treatment				SEM	P-value
	CL	EL	FL	DL		
Plasma FA, g/100 g FA						
C18:0	43.06[a]	42.04[a]	37.11[a]	17.88[b]	3.157	0.001
C18:1t10	0.65[b]	0.63[b]	0.58[b]	11.50[a]	1.673	0.007
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C18:3n3	1.22[z]	1.35[z]	3.60[y]	1.12[z]	0.526	0.043

Experiment 1 - Results
Fatty acid profile in milk fat

	Dietary treatment				SEM	P-value
	CL	EL	FL	DL		
Milk FA, g/100 g FA						
C18:0	14.25[a]	14.94[a]	13.49[a]	6.57[b]	1.312	0.002
C18:1t10	0.43[b]	0.57[b]	0.33[b]	7.47[a]	1.095	0.006
C18:1t11	1.31[b]	0.63[b]	1.06[b]	3.20[a]	0.323	0.006
C18:2t11c15	0.31[b]	0.23[b]	0.27[b]	0.98[a]	0.135	0.015
C18:3n3	0.87[b]	0.83[b]	3.19[a]	0.46[b]	0.253	<0.001

Experiment 1 - Results

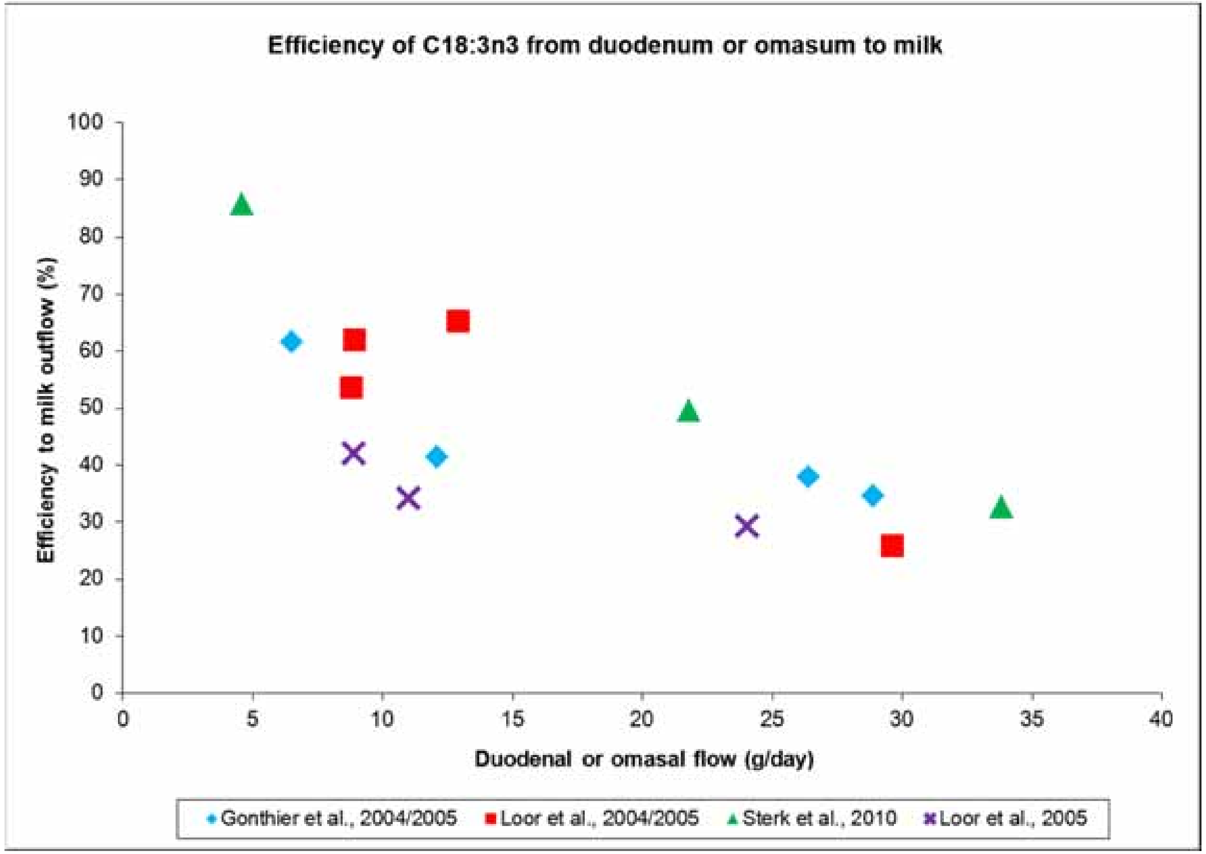
Fatty acid profile in milk fat

	Dietary treatment				SEM	P-value
	CL	EL	FL	DL		
Milk FA, g/100 g FA						
C18:0	14.25[a]	14.94[a]	13.49[a]	6.57[b]	1.312	0.002
C18:1t10	0.43[b]	0.57[b]	0.33[b]	7.47[a]	1.095	0.006
C18:1t11	1.31[b]	0.63[b]	1.06[b]	3.20[a]	0.323	0.006
C18:2t11c15	0.31[b]	0.23[b]	0.27[b]	0.98[a]	0.135	0.015
C18:3n3	0.87[b]	0.83[b]	3.19[a]	0.46[b]	0.253	<0.001

Experiment 1 - Results
Transfer efficiency

	Dietary treatment				SEM	P-value
	CL	EL	FL	DL		
Intake to milk	3.21[b]	3.04[b]	13.06[a]	1.30[b]	0.628	<0.001
Omasum to milk	59.2[b]	33.5[b]	287.8[a]	89.1[b]	16.33	<0.001

Experiment 1 - Results
Transfer efficiency



- Full papers available in thesis and submitted to Journal of Dairy Science

Experiment 2 - Introduction

- Fatty acid profile in milk largely dependent on fatty acid intake and rumen fatty acid metabolism
- The composition of the basal diet affects fatty acid intake and rumen fatty acid metabolism
- Few direct comparisons exist between the basal diet (type of forages, forage to concentrate ratio) and lipid supplements
- Varying multiple factors simultaneously allows quantification of the curvature in relationships as well as interactions among factors
- Objective was to evaluate the effects of forage type and F/C ratio in combination with supplementation of different levels of crushed linseed on intake, milk yield and composition, and milk fatty acid profile

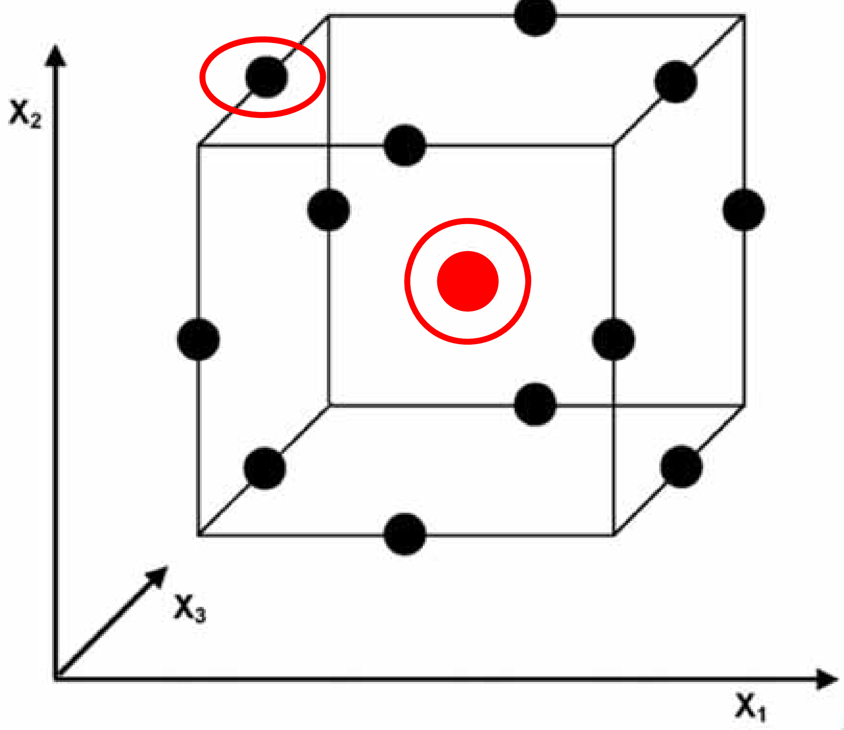
Experiment 2 – Materials and methods

- Multivariate design (Box-Behnken design)
- 3 factors
  - Forage type : 20%, 50%, 80% grass silage vs maize silage
  - F/C ratio : 65:35, 50:50, 35:65
  - Crushed linseed : 1%, 3%, 5% of DM

- 13 treatments (including center point treatment)
- 4 periods of 3 weeks (last 3 days measurements)
- 4 treatments per period (including repetition of center point)
- 4 groups of 9 cows

Experiment 2 – Materials and methods

Treatment	Forage type	F/C ratio	Crushed linseed
1	20% GS	65:35	3% of dm
2	80% GS	65:35	3% of dm
3	20% GS	35:65	3% of dm
4	80% GS	35:65	3% of dm
5	20% GS	50:50	1% of dm
6	80% GS	50:50	1% of dm
7	20% GS	50:50	5% of dm
8	80% GS	50:50	5% of dm
9	50% GS	65:35	1% of dm
10	50% GS	35:65	1% of dm
11	50% GS	65:35	5% of dm
12	50% GS	35:65	5% of dm
CP	50% GS	50:50	3% of dm



Experiment 2 - Materials and methods

- Individual measurements:
  - Feed samples for chemical analysis and FA profile
  - DMI
  - Milk yield and composition
  - Milk FA profile

- Statistical analysis:
  - Measurements averaged per cow per period
  - Response surface analysis according to St.Pierre and Weiss (2009)
  - Response surface plots

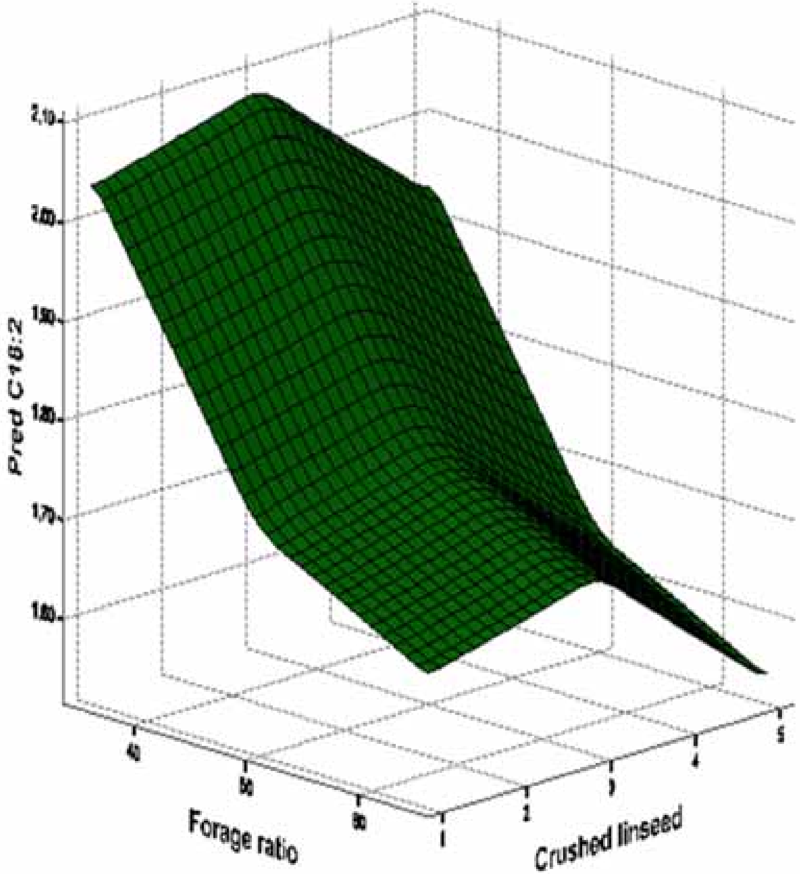
Experiment 2 - Results

Simple statistics

Variable	N	Mean	Std. Deviation	Minimum	Maximum
Intake					
DMI, kg/d	116	23.0	3.6	15.2	31.2
C18:2*n*-6, g/d	116	222	51	119	345
C18:3*n*-3 , g/d	116	213	73	69	408
Milk yield and composition					
Milk production, kg/d	143	41.2	7.31	25.3	61.9
Fat, %	144	3.81	0.55	2.25	4.94
Protein, %	144	3.14	0.24	2.58	3.84
Lactose, %	144	4.82	0.29	3.73	5.58
Milk FA profile, g/100 g FA					
C18:0	144	10.88	1.76	5.58	16.12
Cis-9 C18:1	144	16.69	2.50	12.94	26.29
Trans-10 C18:1	144	0.71	0.66	0.24	3.43
Trans-11 C18:1	144	1.26	0.35	0.42	3.34
C18:2*n*-6	144	1.78	0.29	1.19	2.82
C18:3*n*-3	144	0.79	0.17	0.41	1.22

Experiment 2 - Results
Response surface for C18:2*n*-6

- C18:2*n*-6 % = 3.68 – 0.0036 x Grass % – 0.0608 x Forage % + 0.1209 x CL % + 0.00048 x Forage $\%^2$ – 0.0247 x CL $\%^2$



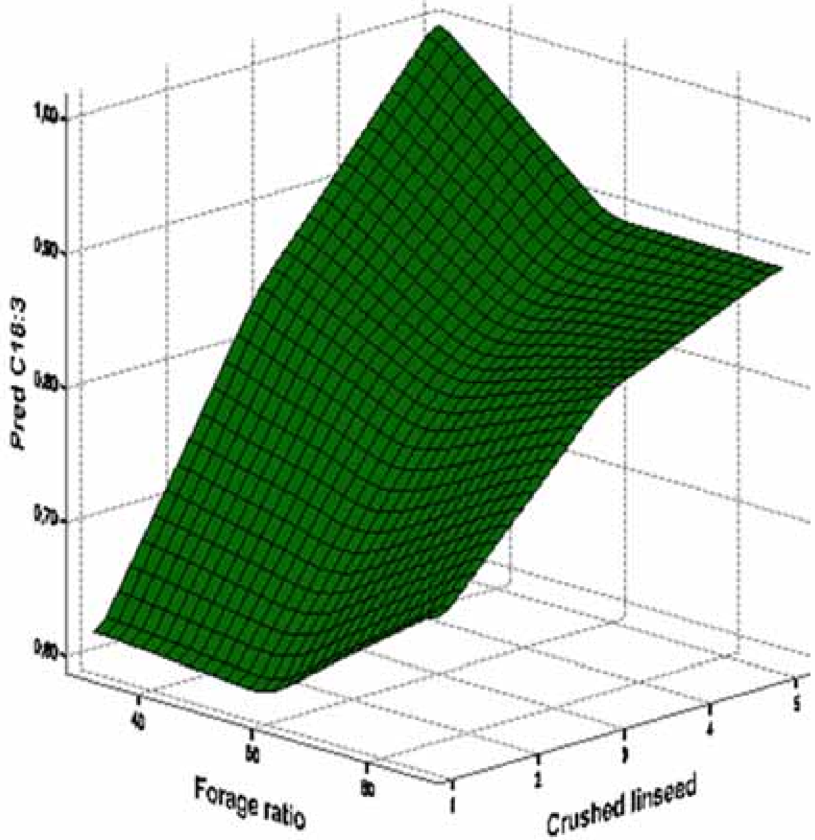
P-values model:

Intercept	:	< 0.001
Grass %	:	0.002
Forage %	:	0.004
CL %	:	0.048
F $\%^2$	:	0.014
CL $\%^2$	:	0.017

link to success

Experiment 2 - Results
Response surface for C18:3*n*-3

- C18:3*n*-3 % = 0.71 + 0.0025 x Grass % – 0.0191 x Forage % + 0.2196 x CL % + 0.00024 x Forage %^{2} – 0.0102 x CL %^{2} – 0.0017 x Forage % x CL



P-values model:

Intercept	:	0.018
Grass %	:	< 0.001
Forage %	:	0.074
CL %	:	< 0.001
F %^{2}	:	0.027
CL %^{2}	:	0.073
F % x CL %	:	0.034

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Experiment 2 - Conclusions

- There were interactions between crushed linseed supplementation and F/C ratio for the proportions of C18:3n3 and several biohydrogenation intermediates
  - Highest levels were achieved when the diet contained 5% crushed linseed and a 35:65 F/C ratio
- There were linear effects of shifting from 80% grass silage to 80% maize silage on milk fatty acid profile
- Overall, the effect of adding crushed linseed on the milk fatty acid profile is significantly dependent on the F/C ratio and the forage type in the basal diet
- Full paper available in thesis and soon in Journal of Dairy Science

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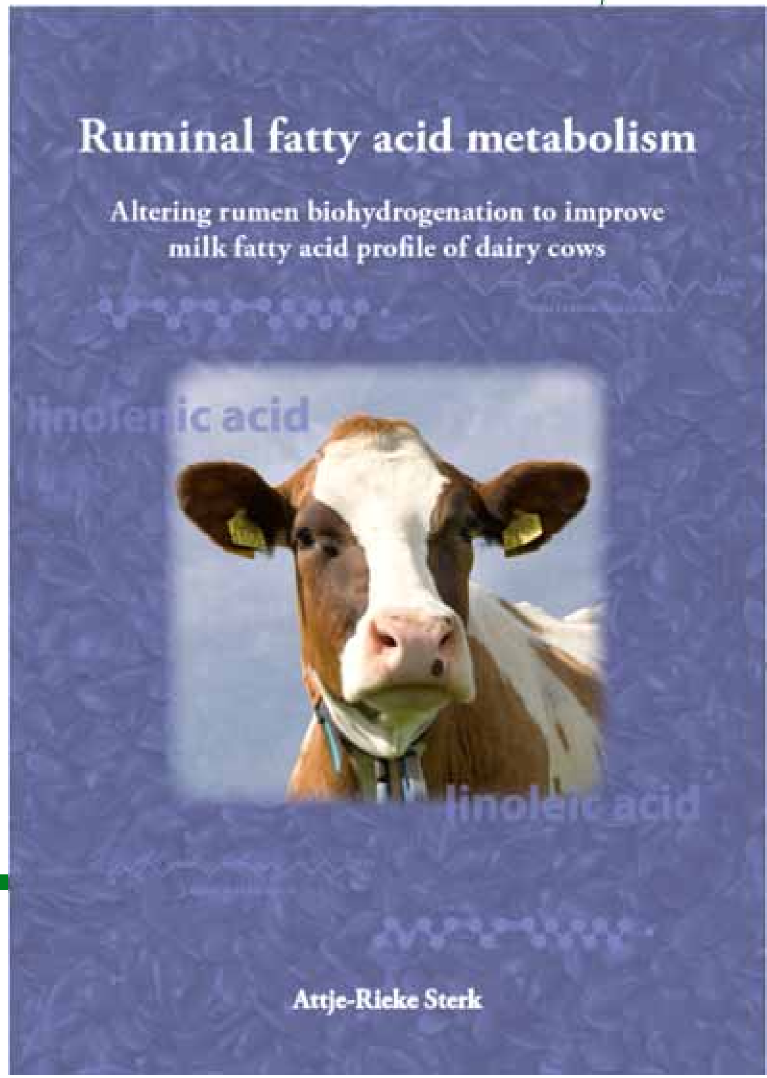

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Conclusions

- Fatty acid profile in milk fat is largely influenced by
  - Fatty acid intake
  - Ruminal fatty acid metabolism
  - Lipid mobilisation
  - Mammary gland metabolism

- Beneficial changes in milk fatty acid profile can be achieved when the ration of dairy cows is altered

Thank you for your attention!

- a.sterk@agrifirm.com

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