Changes in the mammary gland transcriptome due to diet-induced milk fat depression in dairy cows

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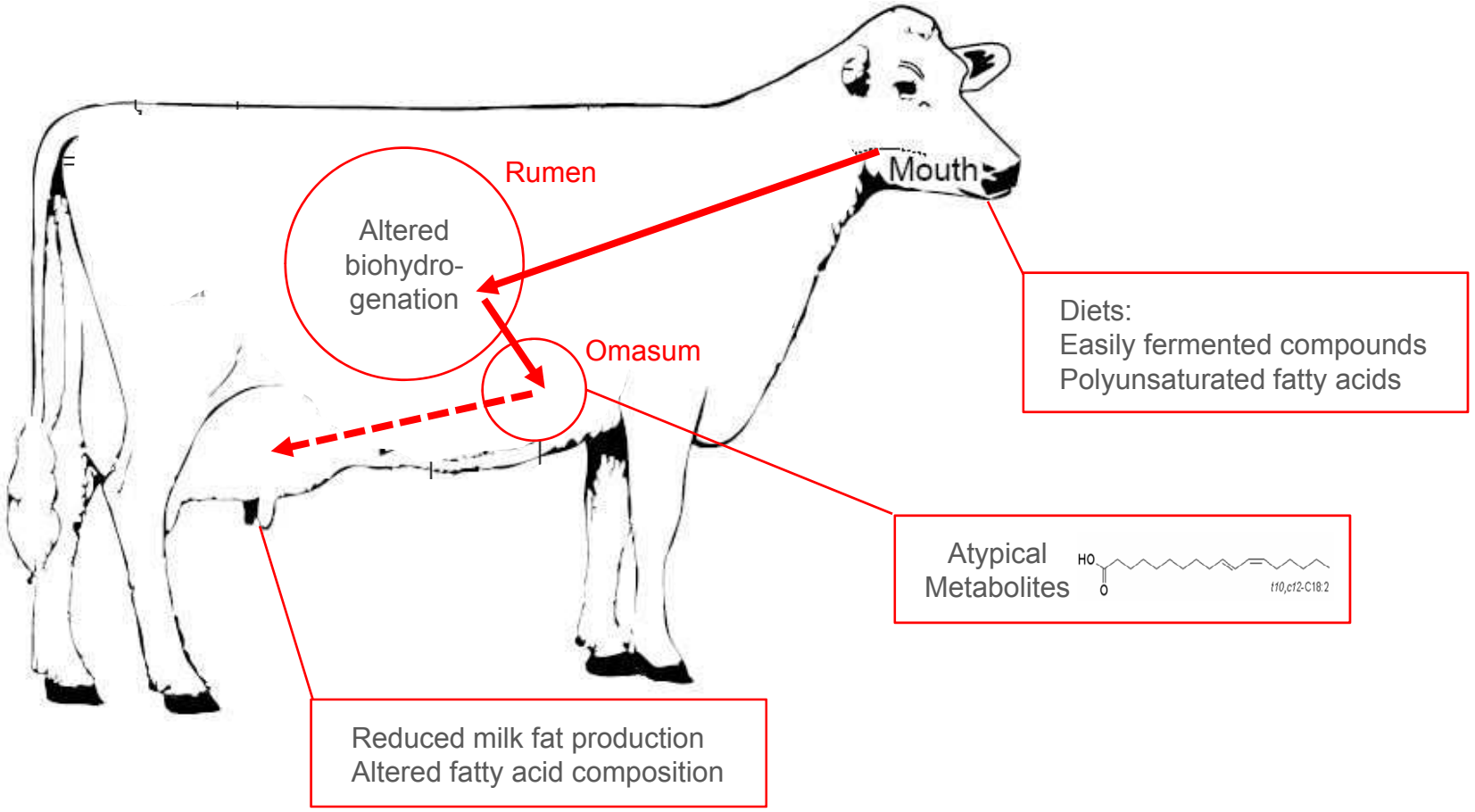
Background

- Increased incidence of metabolic and reproductive disorders in dairy cattle
- Negative energy balance is considered to be important determinant of metabolic health and fertility
- One strategy to manage negative energy balance during early lactation is to decrease milk energy secretion through nutritionally controlled reductions in milk fat content (milk fat depression, MFD).

Milk fat depression



Dale E. Bauman and Mikko J. Griinari, Livest Prod Sci. 70:15-29, 2001

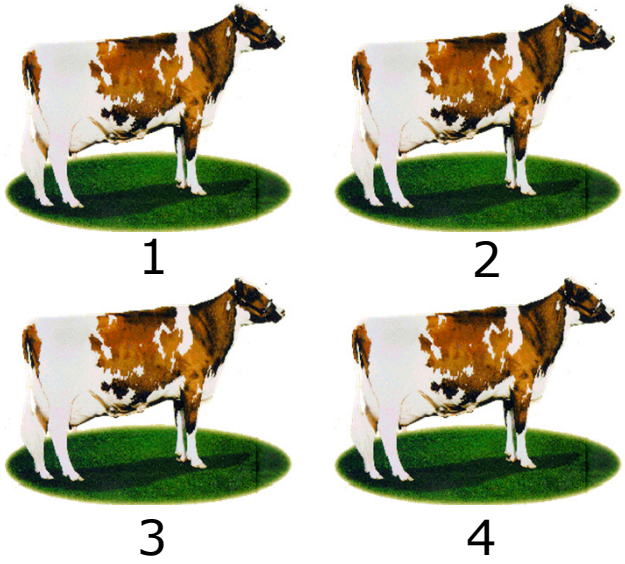
Objective

- To unravel the molecular mechanism of MFD

→ To develop nutritional solutions to improve energy balance of cows in critical periods of lactation

Experimental design

4X4 Latin square



- 4 cows
- 4 diets (HF, HFO, LF, LFO)
- 4 x 35 d experimental periods

Sampling

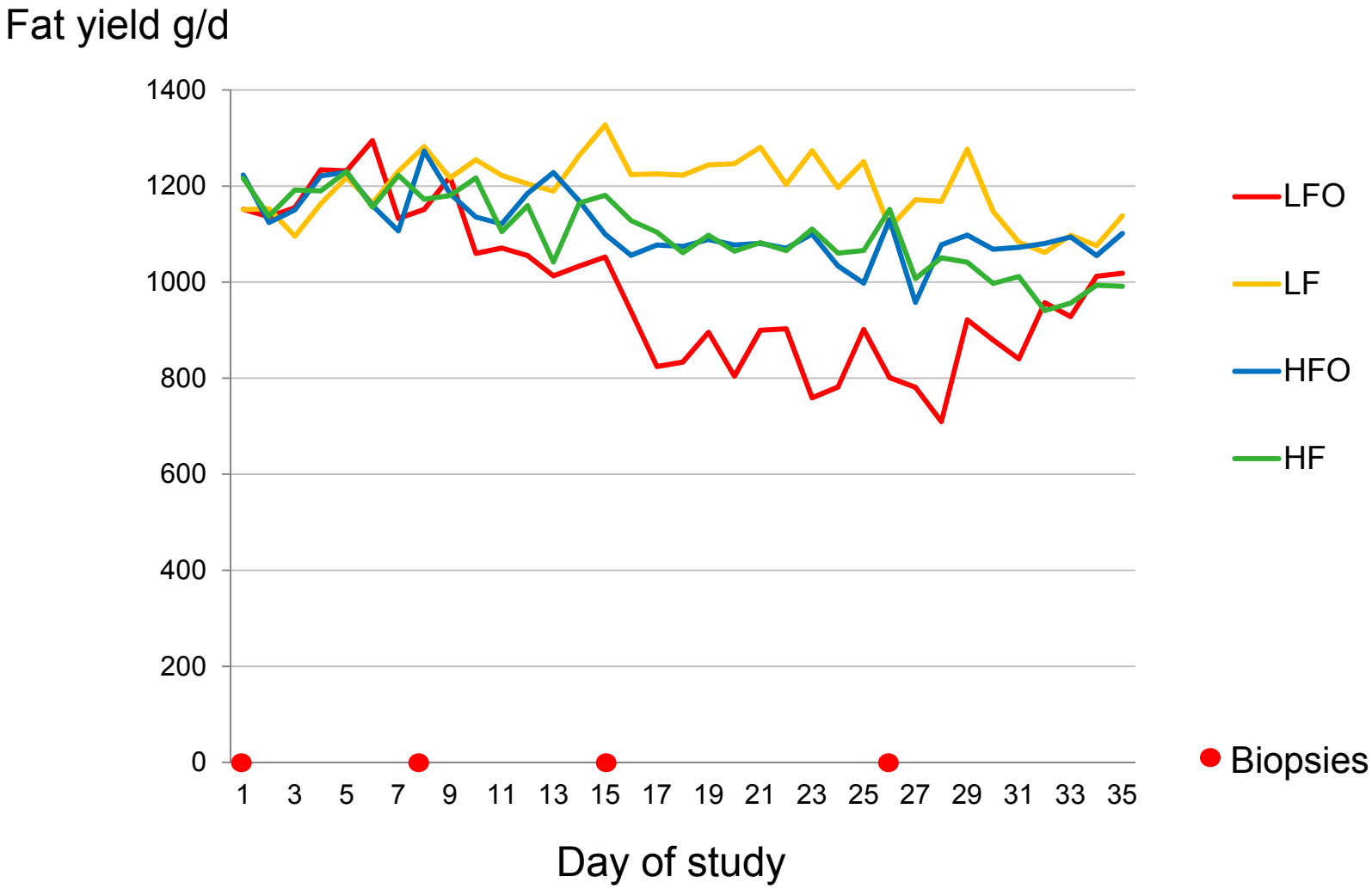
- Intake
- Rumen and omasal digesta
- Rumen fluid
- Milk
- Tissue biopsies
- Urine & feces

Analysis

The effect of diets on milk

	Treatment				SEM	*P*-value		
	HF	HFO	LF	LFO		F	O	FxO
Yield								
Milk, *kg/d*	26.7	25.7	29.7	28.9	2.50	0.12	0.60	0.97
Fat, *g/d*	1050	1076	1195	821	94.6	0.38	<0.05	<0.05
Protein, *g/d*	901	823	1013	1012	60.9	<0.05	0.42	0.43
Lactose, *g/d*	1161	1122	1254	1215	120.8	0.29	0.64	1.00
Concentration, *%*								
Fat	3.94	4.19	4.11	2.88	0.224	<0.05	0.07	<0.05
Protein	3.38	3.23	3.48	3.67	0.152	0.11	0.89	0.29
Lactose	4.34	4.34	4.21	4.18	0.094	0.16	0.88	0.88

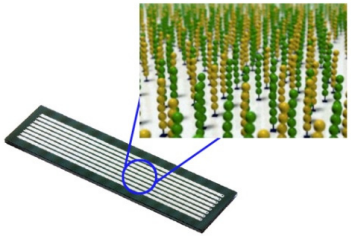
Temporal changes in milk fat yield



Mammary gene expression by RNAseq

Sample preparation

- RNA extraction
- TrueSeq library construction
- Flow cell preparation



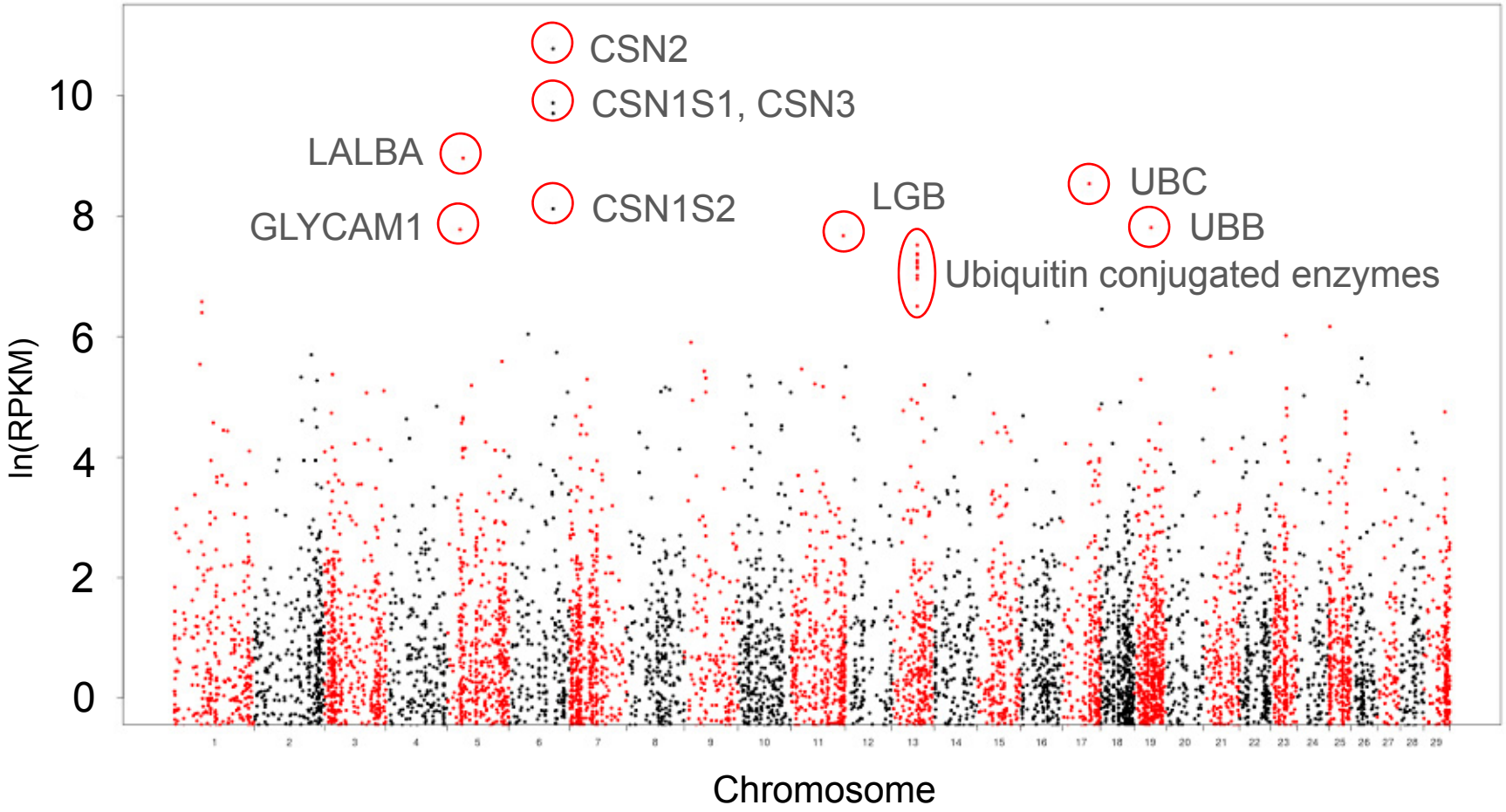
Illumina HiSeq

- 100 bp pair-end
- 50M reads/sample

Data Analysis

- Read mapping againts reference genome
- Transcript annotation
- Differential expression
- Functional annotations

Transcriptional landscape of mammary gland

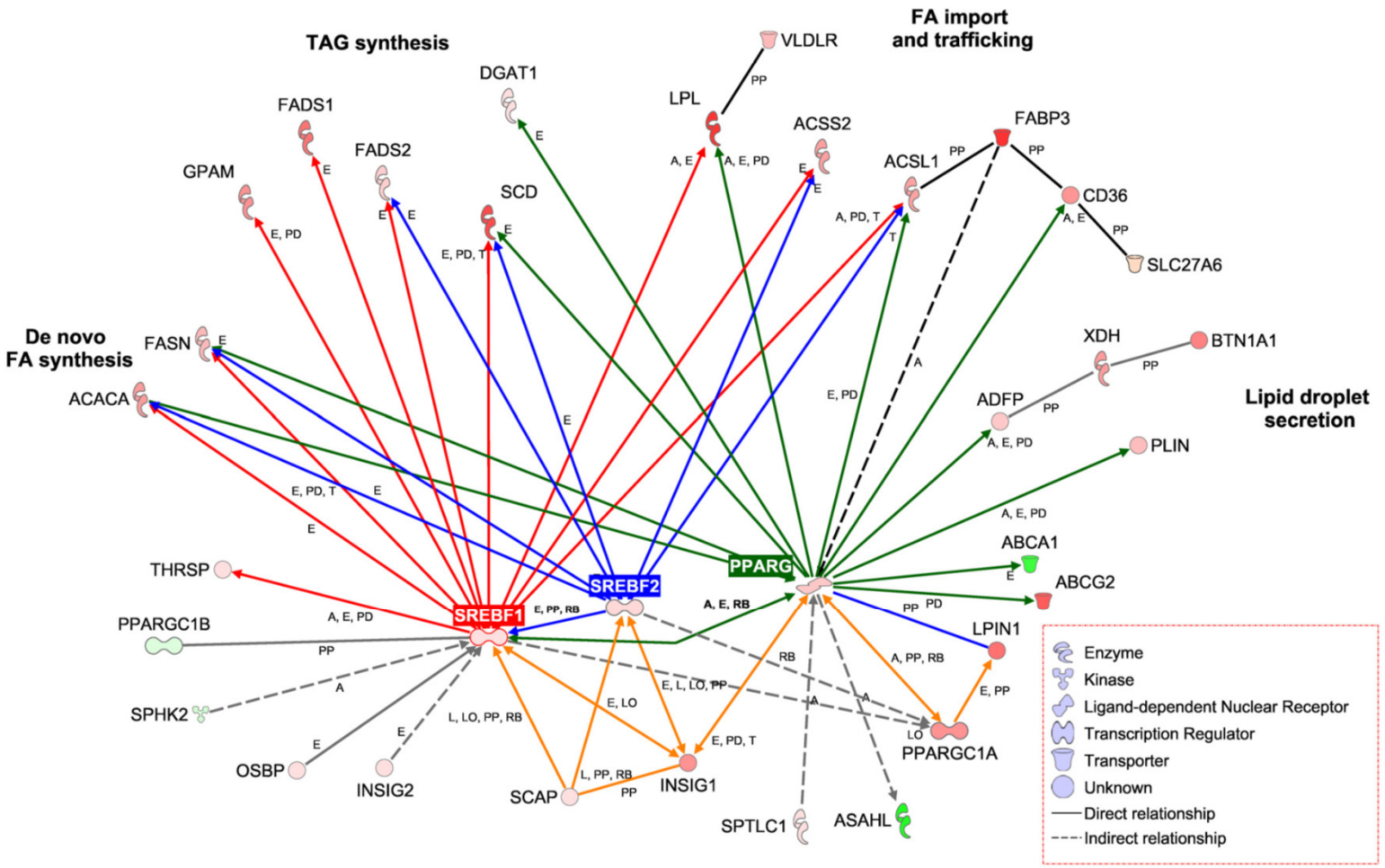


Functional analysis

- Highest expressing genes are coding for:
  - milk proteins
  - lactosesynthetase complex
  - proteins involved in ubiquitination (post-translational modification of proteins)

- The functions of other highly expressed genes are (top10%):
  - Cholesterol synthesis
  - ATP synthesis
  - mRNA translation
  - Endoplasmic reticulum

Mammary lipogenic gene expression



Gene networks driving bovine milk fat synthesis during the lactation cycle.
Bionaz & Loor (2008) BMC Genomics, 9:366

Differential gene expression (HF vs LFO)

- 35 genes were differentially expressed on day 8, FDR < 0.05
- 87 genes were differentially expressed on day 15, FDR < 0.05

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Mammary lipogenic genes were not
among these significant genes

Functional annotation of DE genes

Day 8 (KEGG pathways, 0 hits;GO-terms, 2 hits, (FDR < 0.05))

- Organic acid binding
- Cellular traffic
- Glucose/insulin metabolism
- Endocytosis
- Cell signalling
- Regulation of transcription

Day 15 (KEGG pathways, 7 hits;GO-terms,12 hits, (FDR < 0.05))

- Extracellular matrix
- Regulation of actin cytoskeleton
- Endocytosis
- Cell signalling
- Regulation of transcription

Conclusions

- The major effect of diet induced milk fat depression in the mammary gland seems to be on adaptation of other mechanisms than lipogenesis as recently suggested for CLA mediated MFD (Kramer *et al*. 2013)

- Indicated mechanisms suggest remodelling of cellular structures similarly as in CLA induced MFD in mice (Kadegowda *et al*. 2013, Journal of Lipids)

- The results are preliminary and require further analysis and verification

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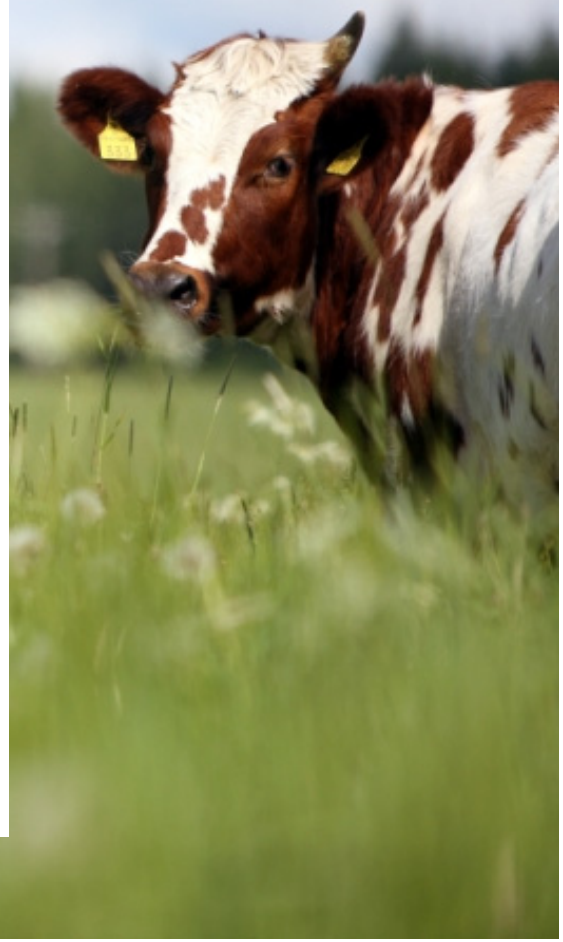

Thank you

MTT Agrifood Research Finland, the Finnish Forest Research Institute (Metla), the Finnish Game and Fisheries Research Institute (RKTL) and the statistical services of the Information Centre of the Ministry of Agriculture and Forestry (Tike) are to be merged under a new entity called *Natural Resources Institute Finland (Luke)* as of 1 January 2015.

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