Transcriptional regulations of milk protein and lactose synthesis by the diet in dairy cows

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Overall bovine milk composition

Proteins
(30-40 g/ 100 g)

Carbohydrates
(50 g/ 100 g)

Ash (0.8 g/ 100 g)

Lipids
(35 g/ 100 g)

80 % casein
20% whey protein
(mainly α-lactalbumin)

Mainly lactose

Mainly calcium

Mainly Triacylglycerols

They account for the osmotic pressure in milk

=> Milk Yield

The biosynthesis of casein and lactose in the mammary epithelial cell

DNA

↓ transcription ?

mRNA

↓ translation

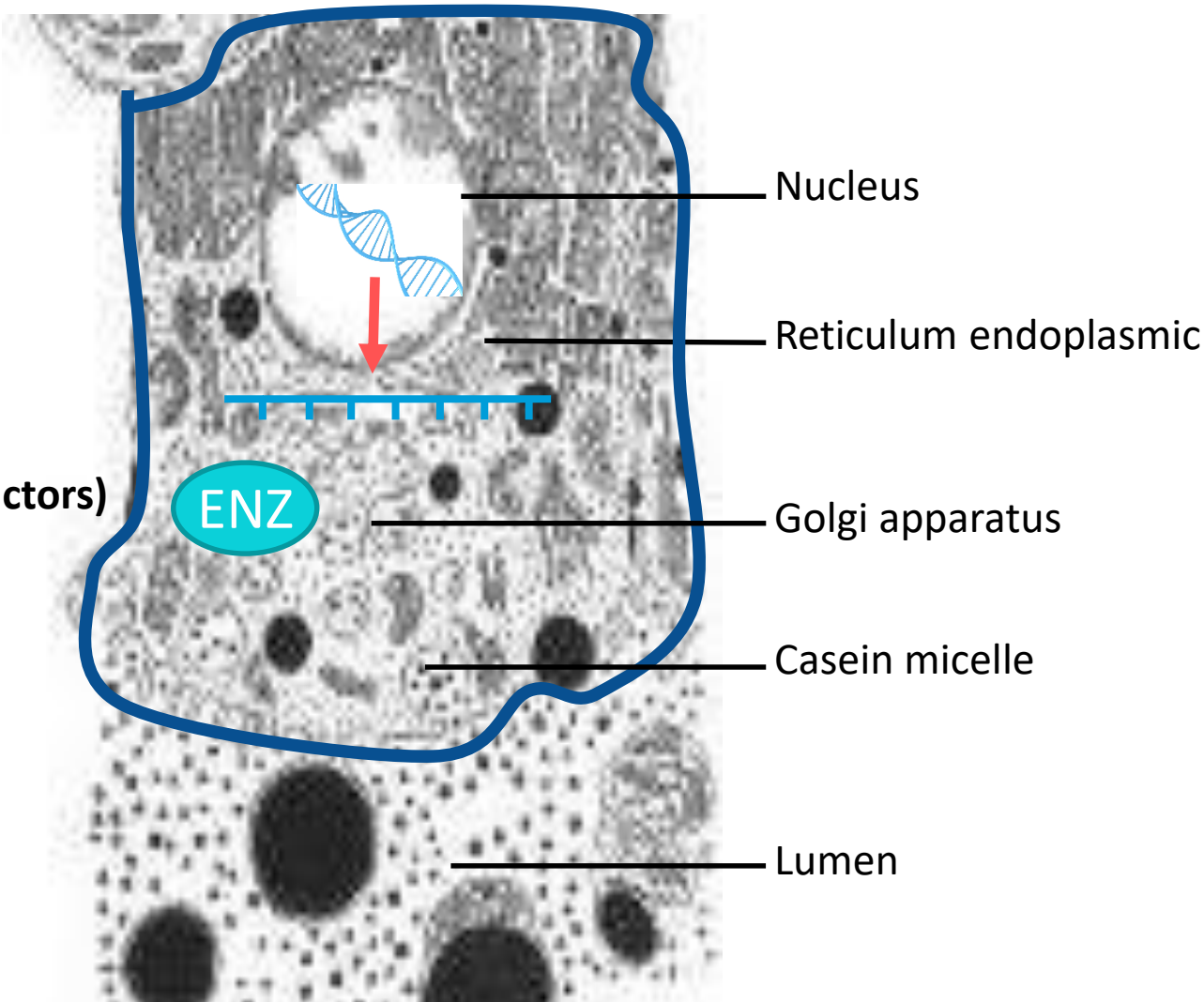
Proteins

(milk proteins, enzymes, co-factors)

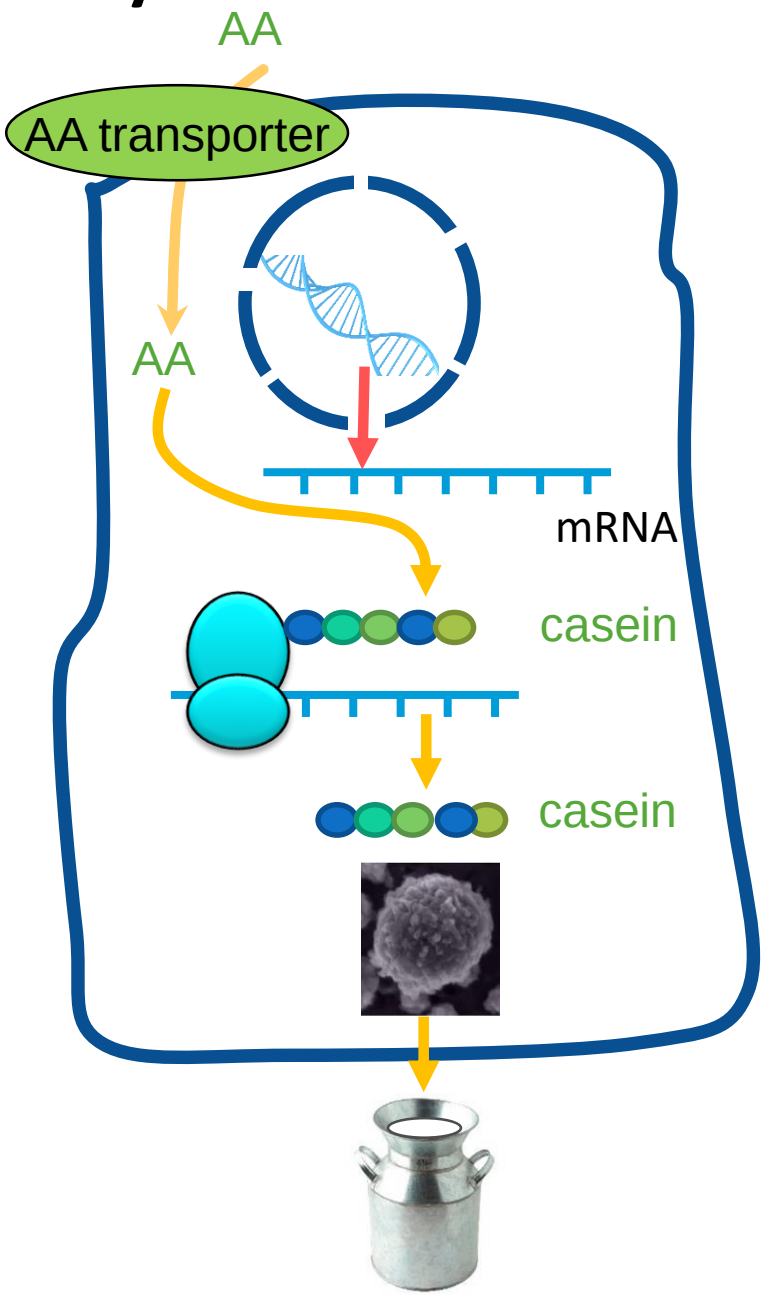
↓

Milk constituents

(lactose, caseins…)



Casein biosynthesis in the mammary epithelial cell



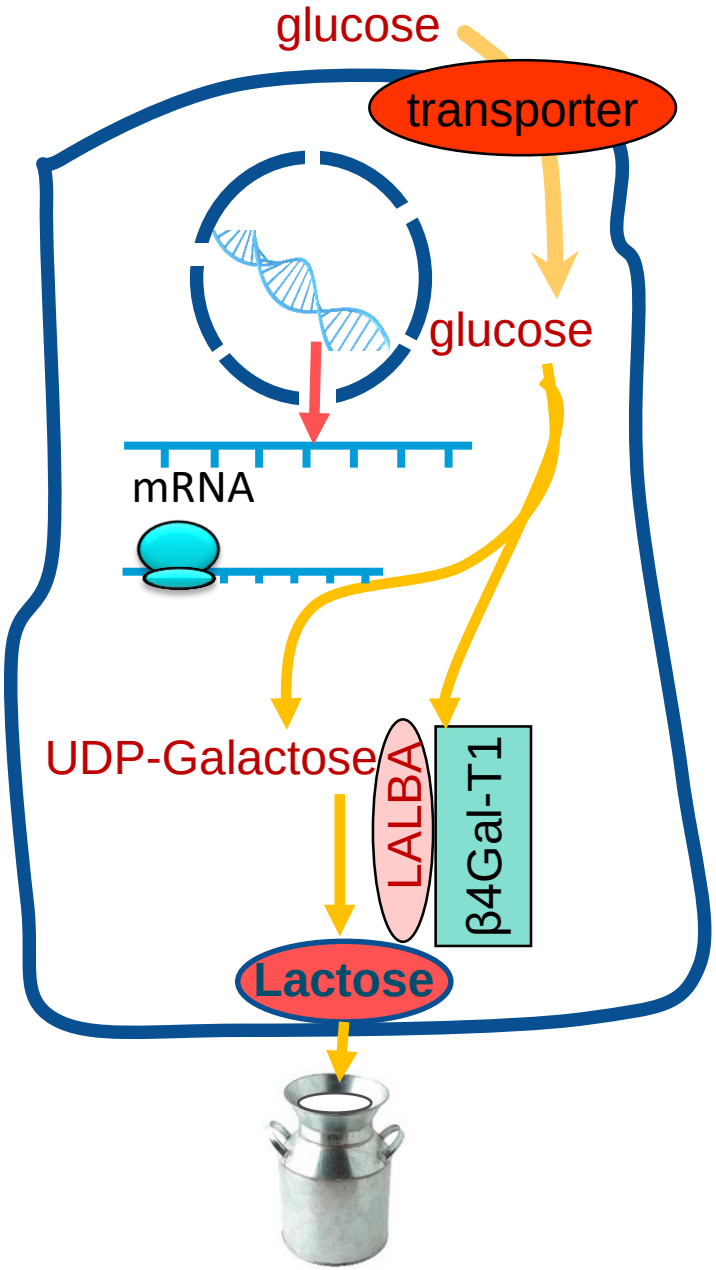
AA transporters
(SLC7A1, SLC7A5, SLC7A7,
SLC6A14, SLC1A2, SLC38A2, SLC1A5)

Casein mRNA
(CSN1S1, CSN1S2, CSN2, CSN3)

Casein proteins

Casein micelles

Lactose biosynthesis in the mammary epithelial cell



Glucose transporters

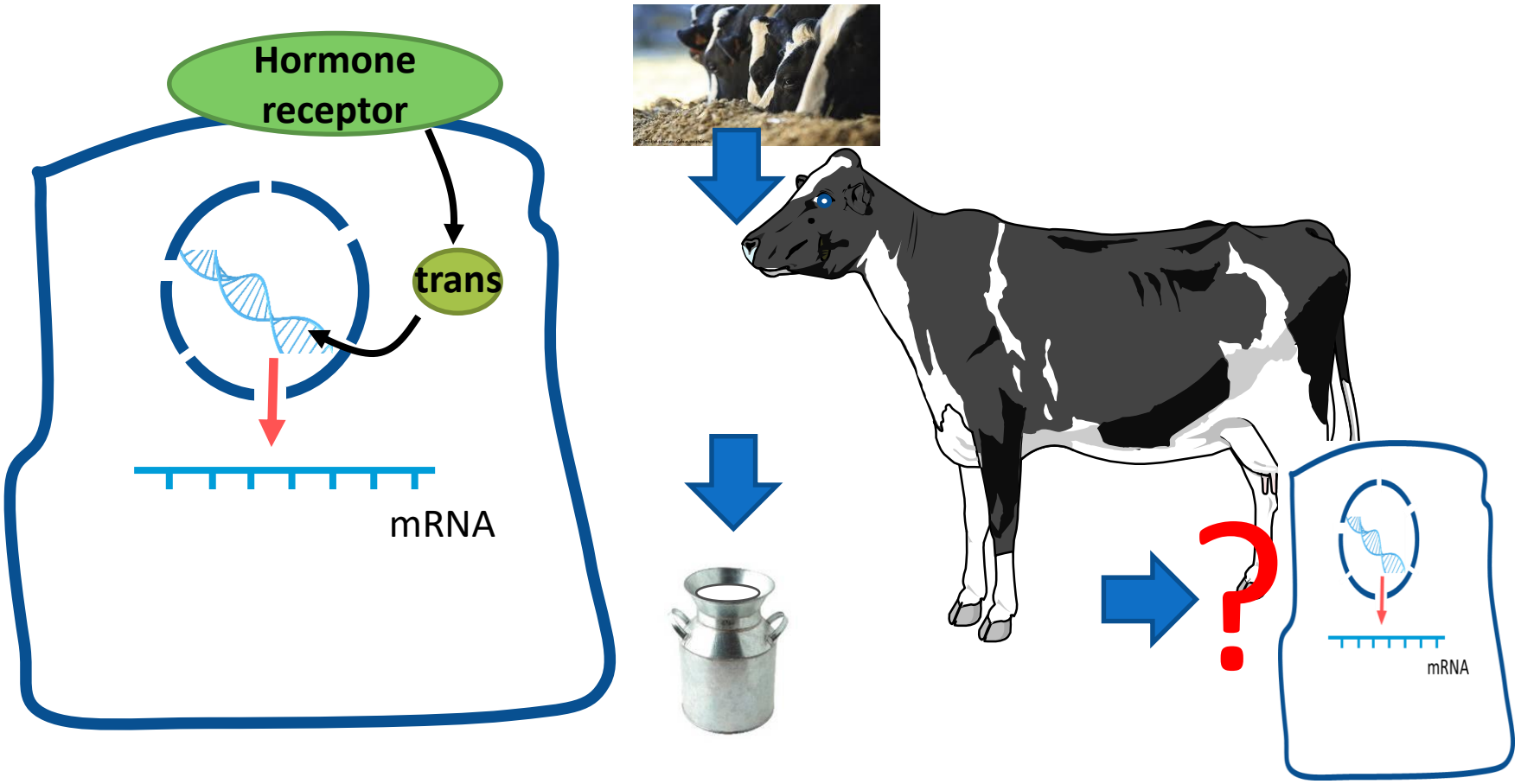
GLUT-1 (SLC2A1), -3, -5, -8, -12
SGLT-1 (SLC5-A1) and -2

Lactose synthase

Enzyme: Galactosyltransferase (β4Gal-T1)
Co-facteur: **α–lactalbumin** (LALBA)

Lactose

Objectives of the review



While the effects of mammary development and galactopoietic hormones on the expression of the genes involved in milk lactose are known, the impact of nutrition has been less investigated. We and other carried out studies to investigate the effect of feed restriction (4 trials) and protein restriction (2 trials) on the expression of genes involved in milk protein and lactose synthesis in dairy cows.

OUTLINE

Transcriptional regulations of milk protein and lactose synthesis by the diet in dairy cows

Part 1: Material and methods to analyse gene expression in bovine mammary tissue extracts

Part 2: Effects of feed restriction

Part 3: Effects of protein restriction

Conclusions

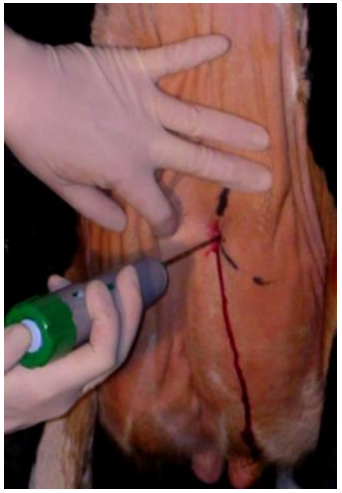
Material and methods 1/2

Mammary cell or tissue sampling

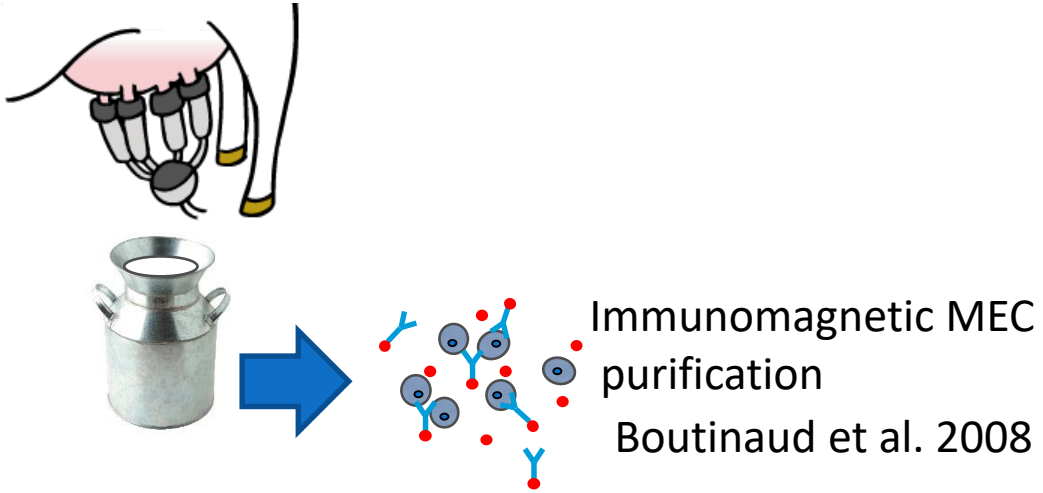
Studying transcriptomic regulations entails the collection of RNA.
Sampling of mammary tissues can be performed after slaughter or biopsy.
However theses techniques are invasive.

An alternative from milk: the purification of Mammary Epithelial Cells (MEC)

Slaughter



Biopsie

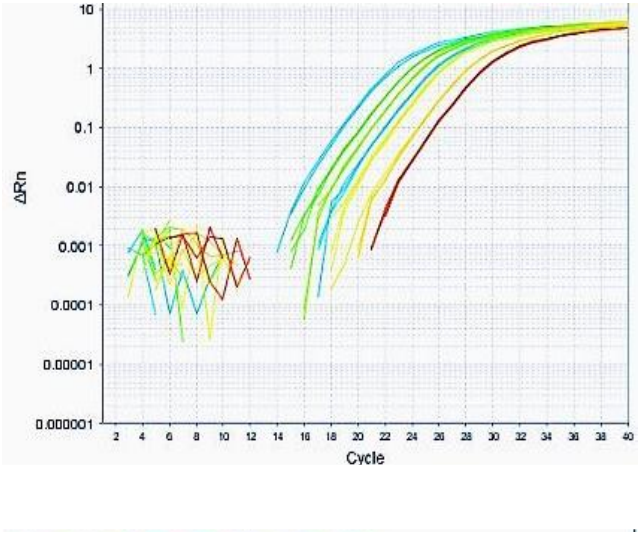


Milk

Material and methods 2/2

Analyses of RNA expression

The gene expression was evaluated by real time qPCR
in mammary tissue samples or MEC purified from milk.



Mammary epithelial cells isolated from milk are a valuable, non-invasive source of mammary transcripts (Boutinaud et al. 2015).

OUTLINE

Transcriptional regulations of milk protein and lactose synthesis by the diet in dairy cows

Part 1: Material and methods to analyse gene expression in bovine mammary tissue

Part 2: Effects of feed restriction

Part 3: Effects of protein restriction

Conclusions

Feed restriction treatments

	Dessauge et al. 2011	Sigl et al. 2014	Boutinaud et al. 2008	Herve et al. submitted
Lactation stage	early	early and mid	mid	mid
Duration	11 weeks	3 days	2 weeks	4 weeks
Level of feed restriction	50%	30%	30%	20%
Samples	mammary tissue	milk purified MEC	milk purified MEC	milk purified MEC + mammary tissue

RESULTS

Effects of FR on protein and lactose yields

	Dessauge et al. 2011	Sigl et al. 2014	Boutinaud et al. 2008	Herve et al. submitted
	delta	delta	delta	delta
Milk yield (kg/d)	- 13 kg (- 39 %)	- 2 kg and - 2 kg	- 3.8 kg (- 14 %)	- 3.2 kg (- 8 %)
Protein yield (g/d)	- 454 g (- 45%)	- 76 g and - 96 g	- 180 g (- 21 %)	- 143 g (- 13 %)
Lactose yield (g/d)	- 674 g (- 41%)		- 192 g (- 15 %)	- 251 g (- 13 %)
Protein content (g/kg)	- 3.1		- 2.3	- 1.4
Lactose content (g/kg)	- 1.4		NS	- 1
Casein content (g/kg)			-1.8	- 1.4

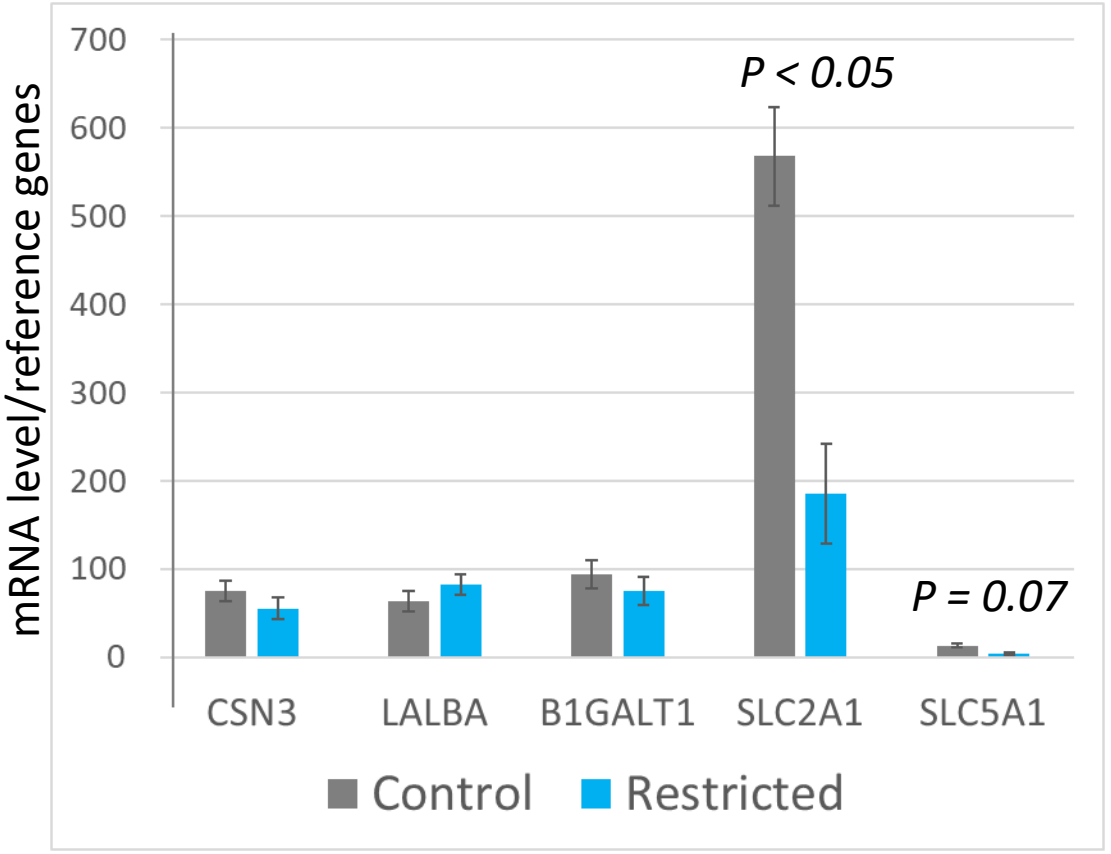
Feed restriction induced reductions in milk lactose and protein synthesis in all trials with sometimes but not always changes in milk protein and lactose composition.

Effects of a moderate feed restriction at mid-lactation 1/2

A 30% FR at mid-lactation for 2 weeks.

	Boutinaud et al. 2008
	delta
Milk yield (kg/d)	- 3.8 kg (-14 %)
Protein yield (g/d)	- 180 g (-21 %)
Lactose yield (g/d)	- 192 g (-15 %)
Protein content (g/kg)	- 2.3
Lactose content (g/kg)	NS

Milk purified MEC

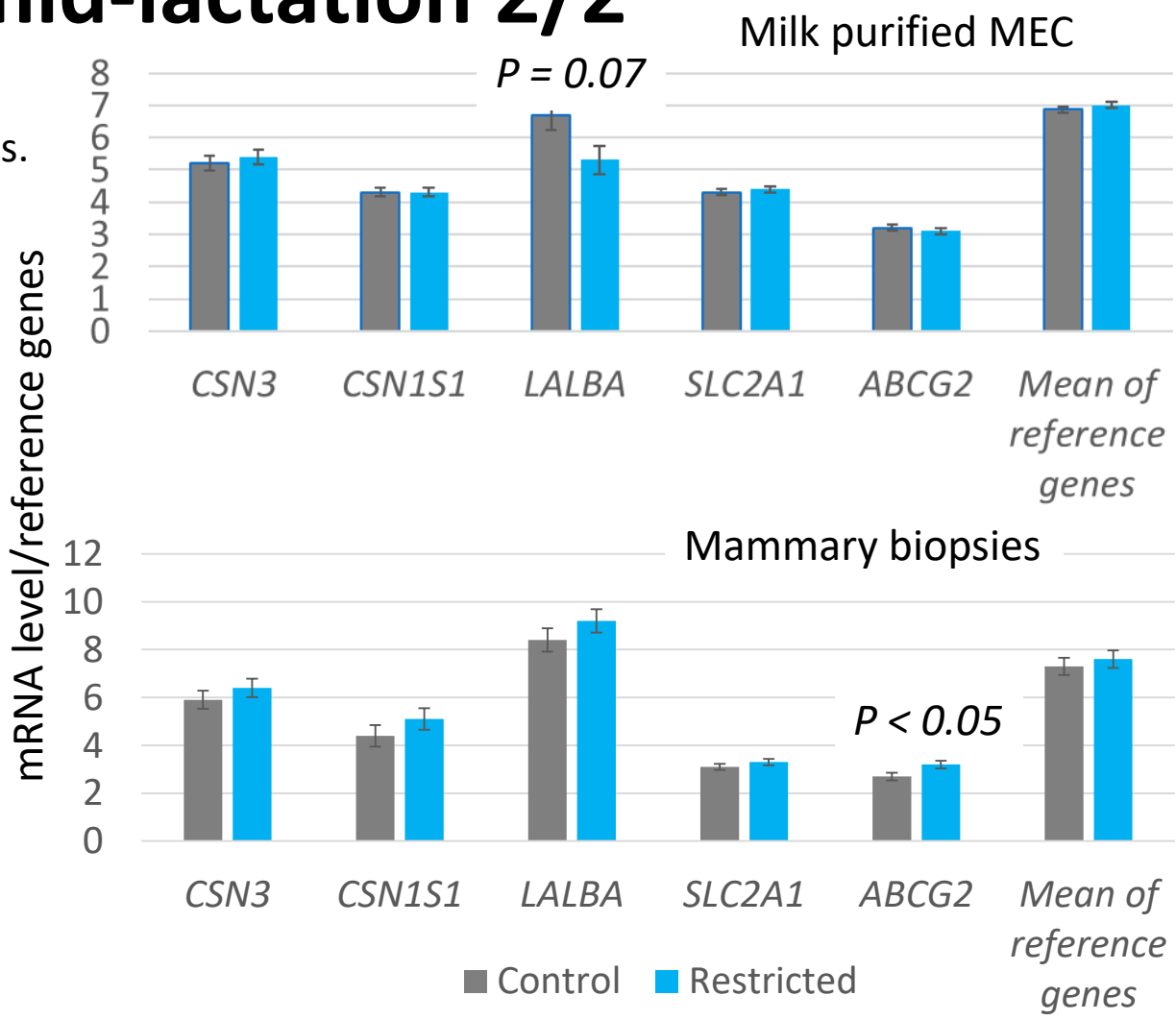


A moderate feed restriction at mid-lactation induced reductions in the mRNA level of the two main glucose transporters without affecting milk protein gene expression.

Effects of a moderate feed restriction at mid-lactation 2/2

A 20% FR at mid-lactation for 4 weeks.

	Herve et al. submitted
	delta
Milk yield (kg/d)	- 3.2 kg (-8 %)
Protein yield (g/d)	- 143 g (-13 %)
Lactose yield (g/d)	- 251 g (-13 %)
Protein content (g/kg)	- 1.4
Lactose content (g/kg)	- 1
Casein content (g/kg)	- 1.4



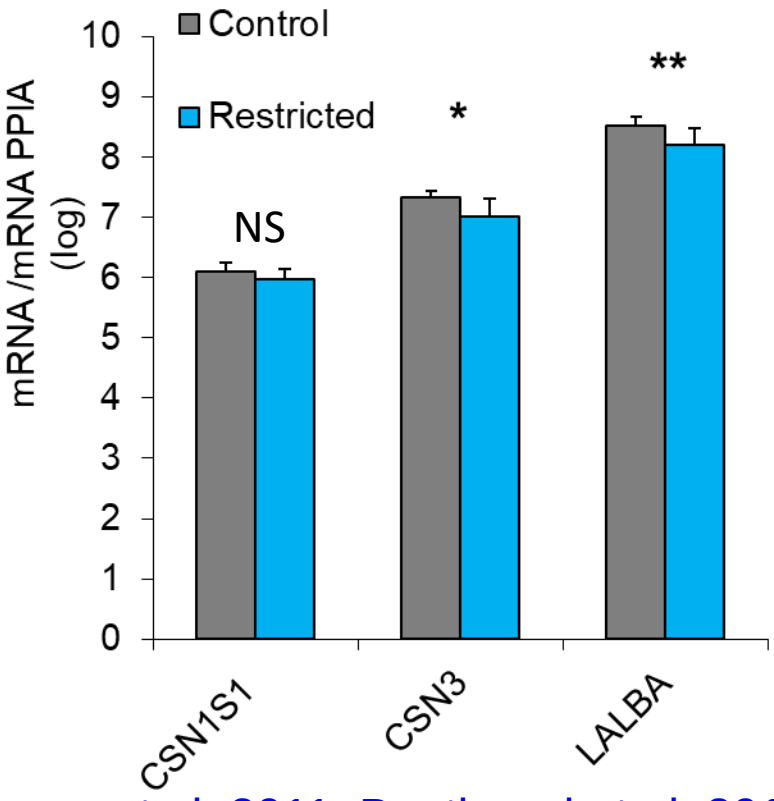
A moderate feed restriction at mid-lactation induced only minor changes in mammary transcripts.

Effects of a severe feed restriction at early-lactation

A 50% FR at early lactation for 11 weeks.

Dessauge et al. 2011

	delta
Milk yield (kg/d)	- 13 kg (- 39 %)
Protein yield (g/d)	- 454 g (- 45%)
Lactose yield (g/d)	- 674 g (- 41%)
Protein content (g/kg)	- 3.1
Lactose content (g/kg)	- 1.4



Dessauge et al. 2011, Boutinaud et al. 2016

A severe feed restriction (- 50%) in early lactation inducing a reduction of - 38% milk, - 45% protein and – 41% lactose yield was associated with decreases in LALBA and K-casein gene expression without change in alphaS1 casein gene expression.

Effects of a moderate feed restriction at early and mid-lactation

A 30% FR at early and mid-lactation for 3 days.

	Sigl et al. 2014
	delta
Milk yield (kg/d)	- 2 kg and - 2 kg
Protein yield (g/d)	-76 g and - 96 g

Sigl et al. 2014

	Early Lactation	Mid Lactation
CNS3	↗	=
CNS1S1	↗	=
LALBA	↗	=
SLC2A1	↘	↘

A moderate feed restriction (30% of allowance) induced a decrease in the transcript levels of SLC2A1 at both stages of lactation. It also induced an unexpected increase in milk protein mRNA levels at early lactation without affecting it on a later stage of lactation.

Summary of results about the effects of feed restriction

	Dessauge et al 2011	Sigl et al. 2014		Boutinaud et al. 2008	Herve et al submitted
	Early/severe	Early/moderate	Mid/moderate	Mid/moderate	Mid/moderate
CNS3	↘	↗	=	=	=
CNS1S1	=	↗	=		=
LALBA	↘	↗	=	=	↘
SLC2A1		↘	↘	↘	=

- In most studies, moderate feed restriction (20 or 30% of allowance) induced a decrease in the transcript levels of SLC2A1, coding for the main transmembrane transporters of glucose in MEC.

- Other results are less consistent:

✓ Moderate feed restriction induced an increase in casein and LALBA transcripts in early lactation (in 1 trial) but not on a later stage (in 3 trials) and a downregulation for *LALBA* at mid lactation.

✓ Severe restriction at early lactation induced a downregulation of milk protein transcripts (except for CNS1S1).

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Protein restriction treatments

	Haque et al. 2015	Omphalius et al. 2018
Animals	4 Holstein cows at mid lactation	4 Holstein cows at mid lactation
Experimental design 2x2 factorial	LP vs HP 2 AA profiles	LP vs HP LE vs HE
Level of protein restriction	**102 vs 108** g of metabolizable protein / kg of DM*	**92 vs 170** at 14 UFL/d **69 vs 125** at 18 UFL /d g of metabolizable protein / kg of DM*
Samples	milk purified MEC	milk purified MEC

HP, high protein
LP, low protein
HE, high energy
LE, low energy

*INRA, 2007

RESULTS / Effects of Protein restriction on protein and lactose yields

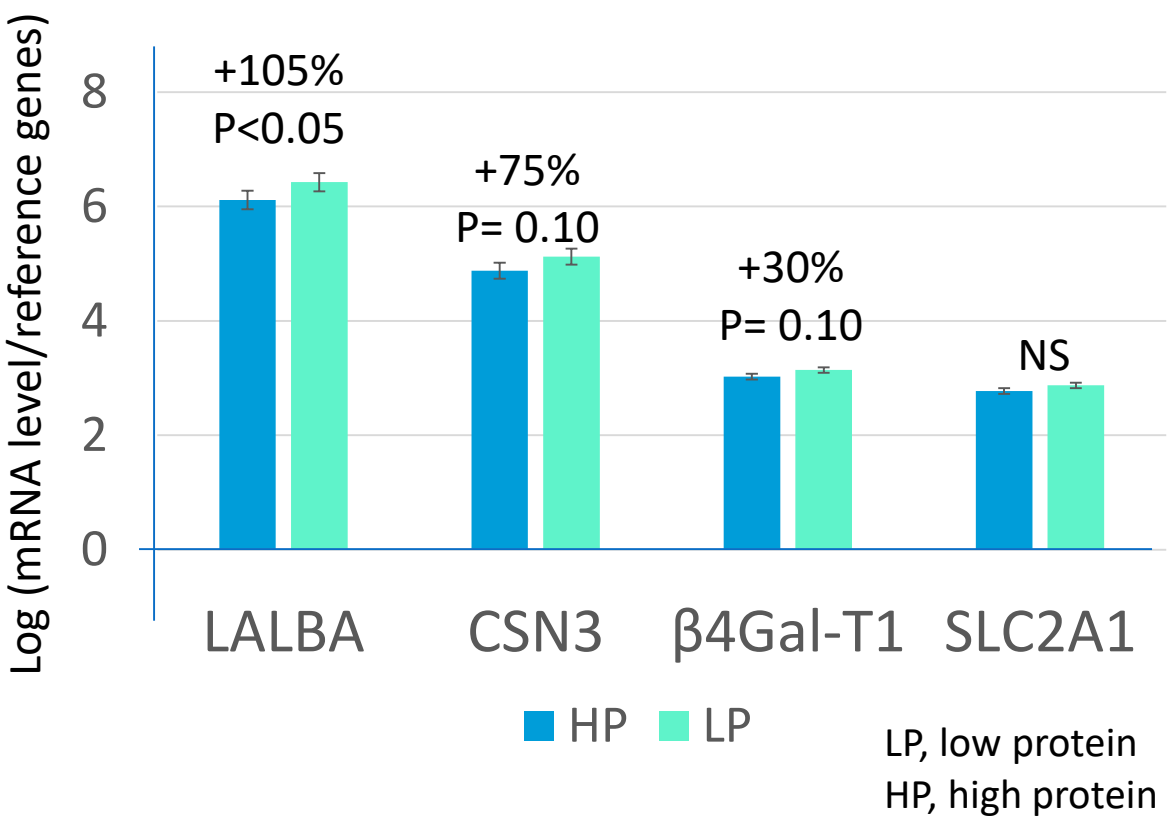
	Haque et al. 2015	Omphalius et al. 2018	
	Delta LP vs HP	Delta LP vs HP at LE	Delta LP vs HP At HE
Milk yield (kg/d)	- 1.3 kg (- 4 %)	- 3.6 kg (- 16 %)	- 4 kg (- 16 %)
Protein yield (g/d)	- 50 g (- 5 %)	- 80 g (- 13 %)	- 108 g (- 16 %)
Lactose yield (g/d)	- 64 g (- 4 %)	- 143 g (- 14 %)	- 250 g (- 21 %)
Protein content (g/kg)	NS	NS	NS
Lactose content (g/kg)	NS	NS	NS

LP, low protein
HP, high protein
LE, low energy
HE, high energy

Protein restrictions induced reductions in milk lactose and protein synthesis in all trials.

Effects of Protein restriction (1/2)

	Haque et al. 2015 Delta LP vs HP
Milk yield (kg/d)	- 1.3 kg (- 4 %)
Protein yield (g/d)	- 50 g (- 5 %)
Lactose yield (g/d)	- 64 g (- 4 %)



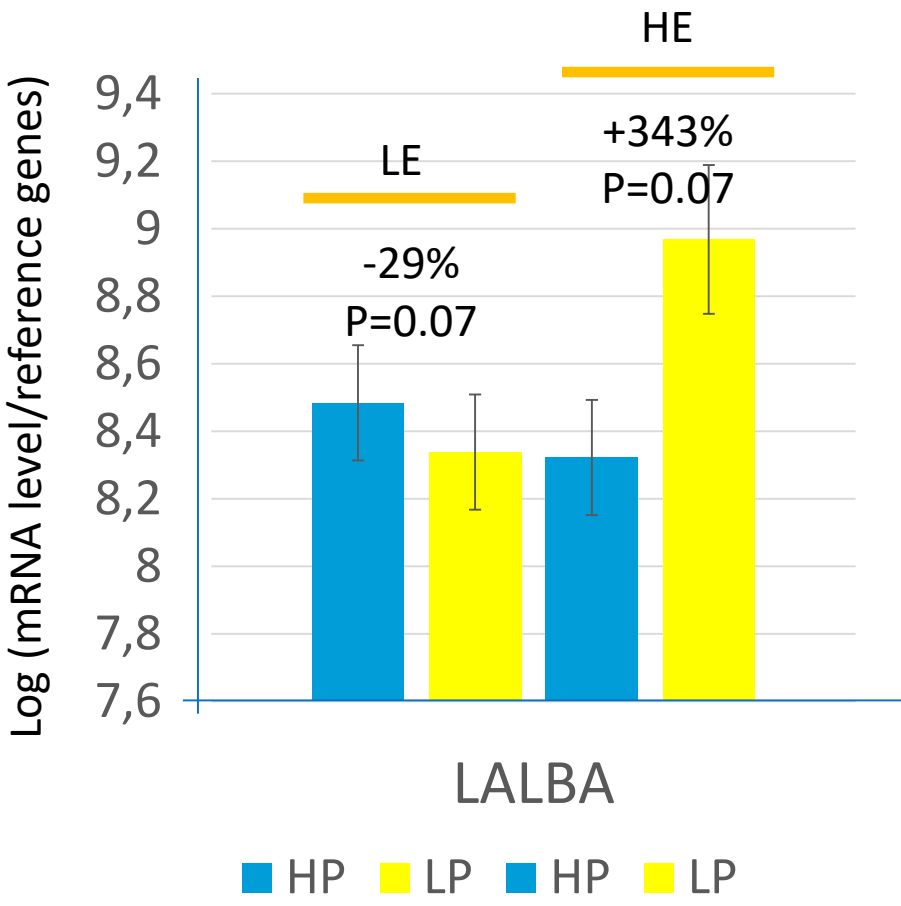
LP, low protein
HP, high protein

A moderate protein restriction induced an unexpected increase in LALBA transcript.

Effects of Protein restriction 2/2

LP, low protein
HP, high protein
LE, low energy
HE, high energy

	Omphalius et al. 2018	
	Delta LP vs HP at LE	Delta LP vs HP at HE
Milk Yield (kg/d)	- 3.6 kg (- 16 %)	- 4 kg (- 16 %)
Protein Yield (g/d)	- 80 g (- 13 %)	- 108 g (- 16 %)
Lactose Yield (g/d)	- 143 g (- 14 %)	- 250 g (- 21 %)



The effect of the protein restriction on LALBA transcripts seems to vary according to the level of energy.

Conclusions 1/2

DNA

transcription

mRNA

translation

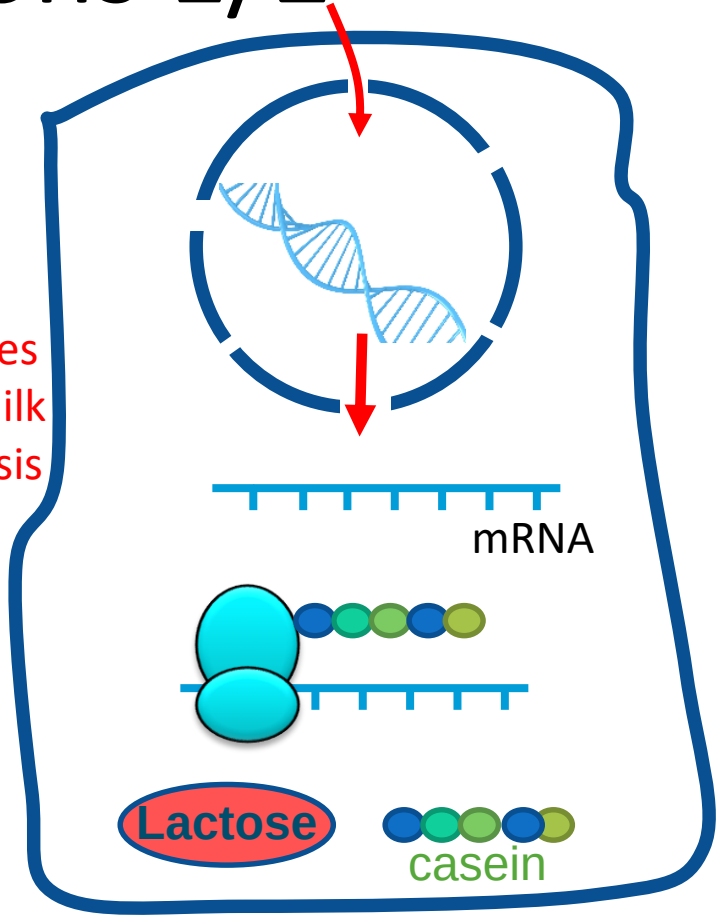
Proteins

(milk protein, enzyme, co-factors)

?

Milk constituents

Discrepancies in the changes in transcripts involved in milk protein and lactose synthesis according to studies.



- The effects of feed or protein restriction on mammary transcripts involved in milk lactose and protein synthesis likely depend on the severity and the duration of the restriction, the stage of lactation, and the level of energy in the diet.
- In some cases, manipulating the diet induced variations in transcript expression opposite to that observed for milk protein and lactose yields suggesting that other mechanisms than modification of gene expression may modulate protein and lactose synthesis (enzyme activity? as suggested by Norgaard et al. 2008.)

Conclusions 2/2

DNA

transcription

mRNA

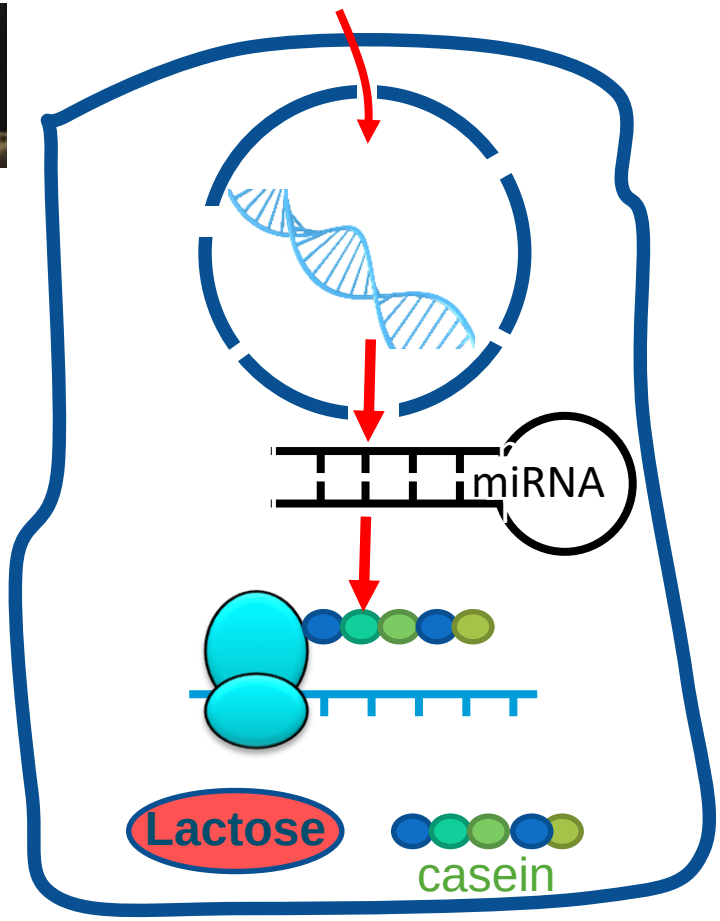
translation ?

Proteins

(milk protein, enzyme, co-factors)

Milk constituents

(lactose, caseins…)



- RNA seq? Other genes involved in the secretion of milk constituents such as genes involved in endoplasmic reticulum biogenesis as suggested by Nichols et al.2017.
- Regulation of translation via miRNA regulations? As suggested by Mobuchon et al., 2015.

Thank you for your attention and great thanks to:

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Feed restriction treatments

	Dessauge et al. 2011	Sigl et al. 2014	Boutinaud et al. 2008	Herve et al. submitted
Lactation stage	early	early and mid	mid	mid
Duration	11 weeks	3 days	2 weeks	4 weeks
Level of feed restriction	50%	30%	70%	80%
Restriction mode	Diet with 60% grass silage and 40 % hay instead of 55% Maize and 15 % and alfafa and 30% concentrate	Diet without the 5 or 6 kg of concentrate	Same diet wit a reduction of the offers	Same diet wit a reduction of the offers
Samples	mammary tissue	milk purified MEC	milk purified MEC	milk purified MEC + mammary tissue

Protein restriction treatments

	Haque et al. 2015	Omphalius et al. 2018
Animals	4 Holstein cows at mid lactation	4 Holstein cows at mid lactation
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Level of protein restriction	**102 vs 108** g of digestible protein in the intestin / kg of DM*	**92 vs 170** at 14 UFL/d **69 vs 125** at 18 UFL /d g of digestible protein in the intestin / kg of DM*
Restriction mode	Diet Corn silage (63%) Soybean meal (+/- 6%) ajusted with ground dehydrated wheat meal, ground corn and potato pulp.	Diet Corn silage (from 83 to 60 %) Energy concentrate (from 2 to 30%) Soybean meal (from 0.8 to 33%)
Samples	milk purified MEC	milk purified MEC

HP, high protein
LP, low protein

HE, high energy
LE, low energy

*INRA, 2007