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Expression levels of 14 genes found as RNA in cells extracted from milk

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Background

What determines the shape of the lactation curve?

Can we use new knowledge to improve selection for dairy traits?

Biology of lactation

Milk yield is the result of three major biological processes

- Mammary cell production and differentiation
- Milk secretion rate per cell
- Cell death rate

Background

- What determines the shape of the lactation curve?
- Three processes
  - Mammary cell production and differentiation
  - Milk secretion rate per cell
  - Cell death rate

New question: Can we use gene expression levels as an indicator of secretion rate throughout lactation?

Supplementary question: Can we do this with samples from a commercial herd?

14 genes studied

- Lactose – *B4GALT1, LALBA, UGDH, UGP2*
- Fat – *ACACA, DGAT1, FASN, SCD*
- Prolactin – *JAK2*
- Protein – *CSN1S1, LALBA*
- Cell cycle etc. – *ARNTL2, CYSLTR2, FOXH1, UTRN*

Milk samples

- Milk samples collected from 20 cows over a 5 month period from the RVC commercial herd
- 120 whole milk samples were transferred to the laboratory for analysis – each sample analysed twice
- RNA extracted from mammary epithelial cells in the milk
- Expression levels of 14 genes measured in each sample using qRT-PCR and capillary-gel electrophoresis
- Farms records on milk production were taken from routine monthly recording
- Daily milk yield (DMY; kg/d), fat and protein % (FP, PP), somatic cell count (an indicator of mastitis; SCC) and day of lactation (DIM)

Data and analysis

- 14 sets of gene expression ratios (GER), relative to housekeeper gene
- SCC, all GER skewed – $\log_{10}$ transformed for analysis
- Fixed model fitted to each GER using ASReml
  - $GER_{ijk} = \mu + DIM + C_i + T_j + SCC + e_{ijk}$
  - μ - mean log expression ratio
  - DIM – fitted as increasing polynomial
  - C_i - cow effect (i = 1 to 20)
  - T_j - sampling time (j = 1 to 6)
  - SCC - somatic cell count
  - e_{ijk} - random error term.

Data and analysis - 2

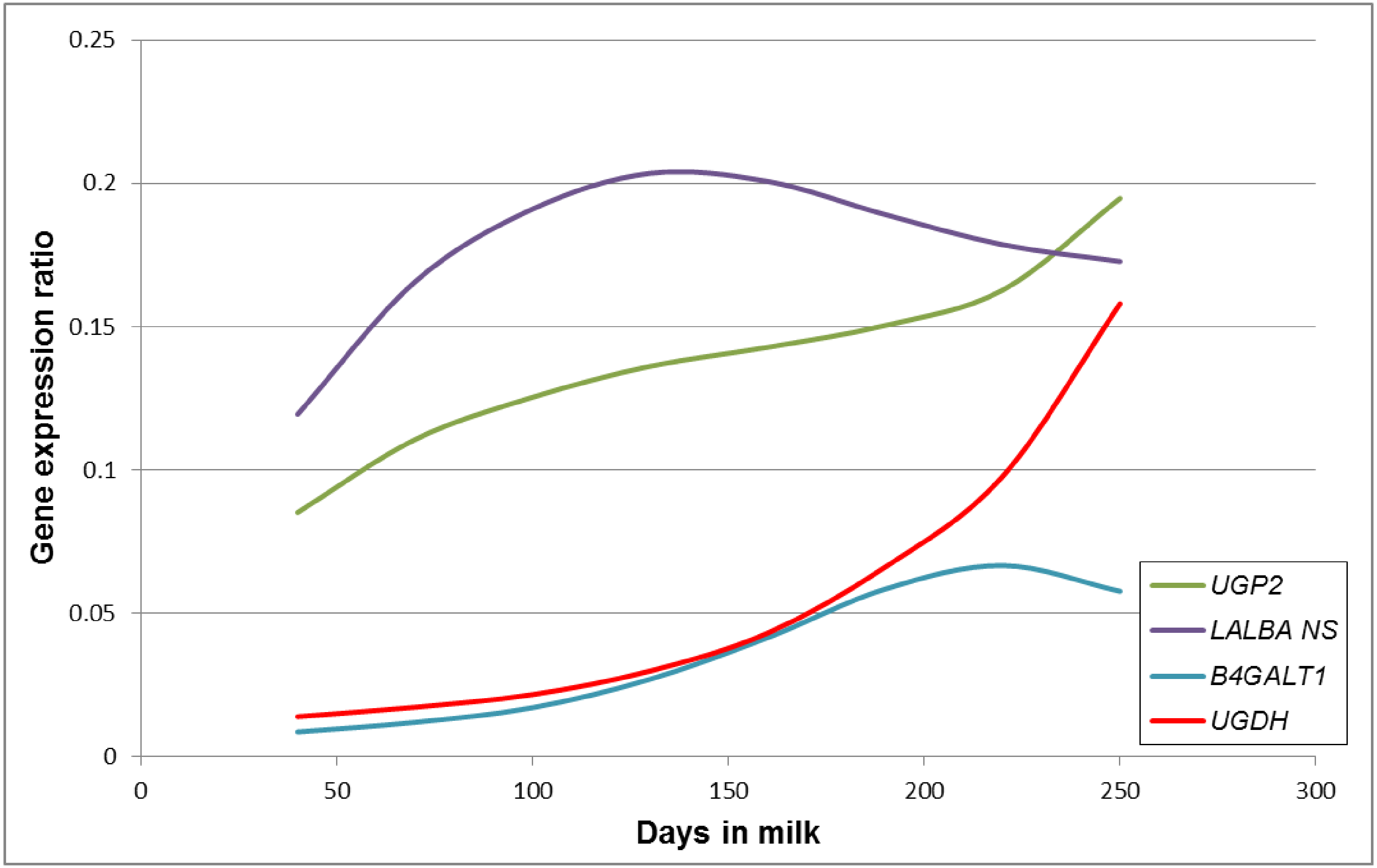
- GER across 50-250 DIM recovered from ASReml analyses
- Correlation coefficients calculated between all GER traits – bivariate fixed model in ASReml
- Correlations considered significantly different from 0 when greater than twice the SE
- Correlations between GER and milk traits also calculated

RESULTS

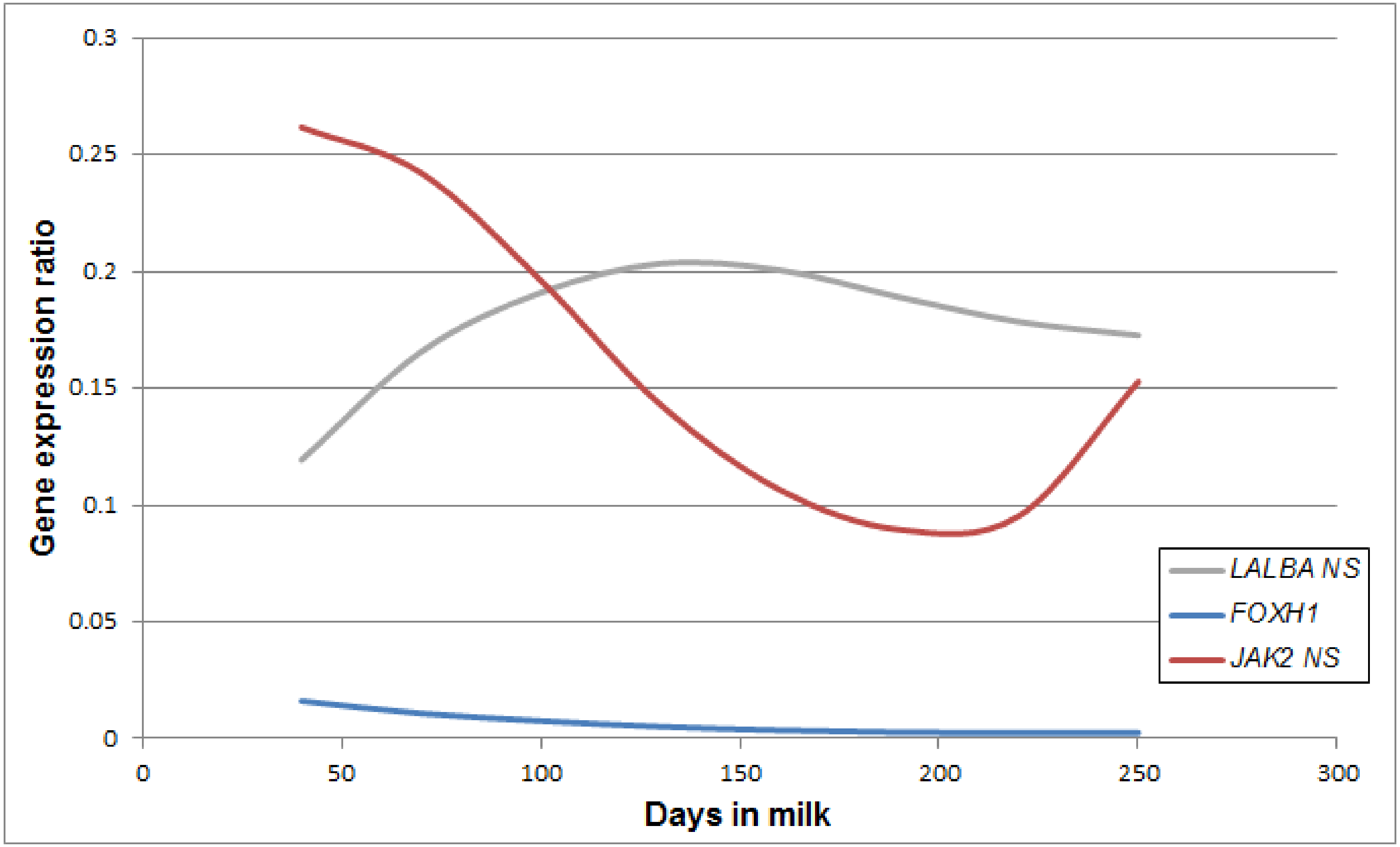
ANOVA summary

Gene	DIM	Order	Cow	Sample	SCC
ACACA	NS	-	***	NS	NS
ARNTL2	NS	-	***	**	**
B4GALT1	***	1	***	***	NS
CSN1S1	**	2	***	NS	NS
CYSLTR2	***	3	*	NS	NS
DGAT1	NS	-	NS	***	NS
FASN	***	3	*	***	NS
FOXH1	***	1	*	***	NS
JAK2	NS	-	***	***	**
LALBA	NS	-	***	NS	NS
SCD	***	1	***	***	NS
UGDH	***	1	***	**	NS
UGP2	***	1	***	***	*
UTRN	NS	-	***	*	NS

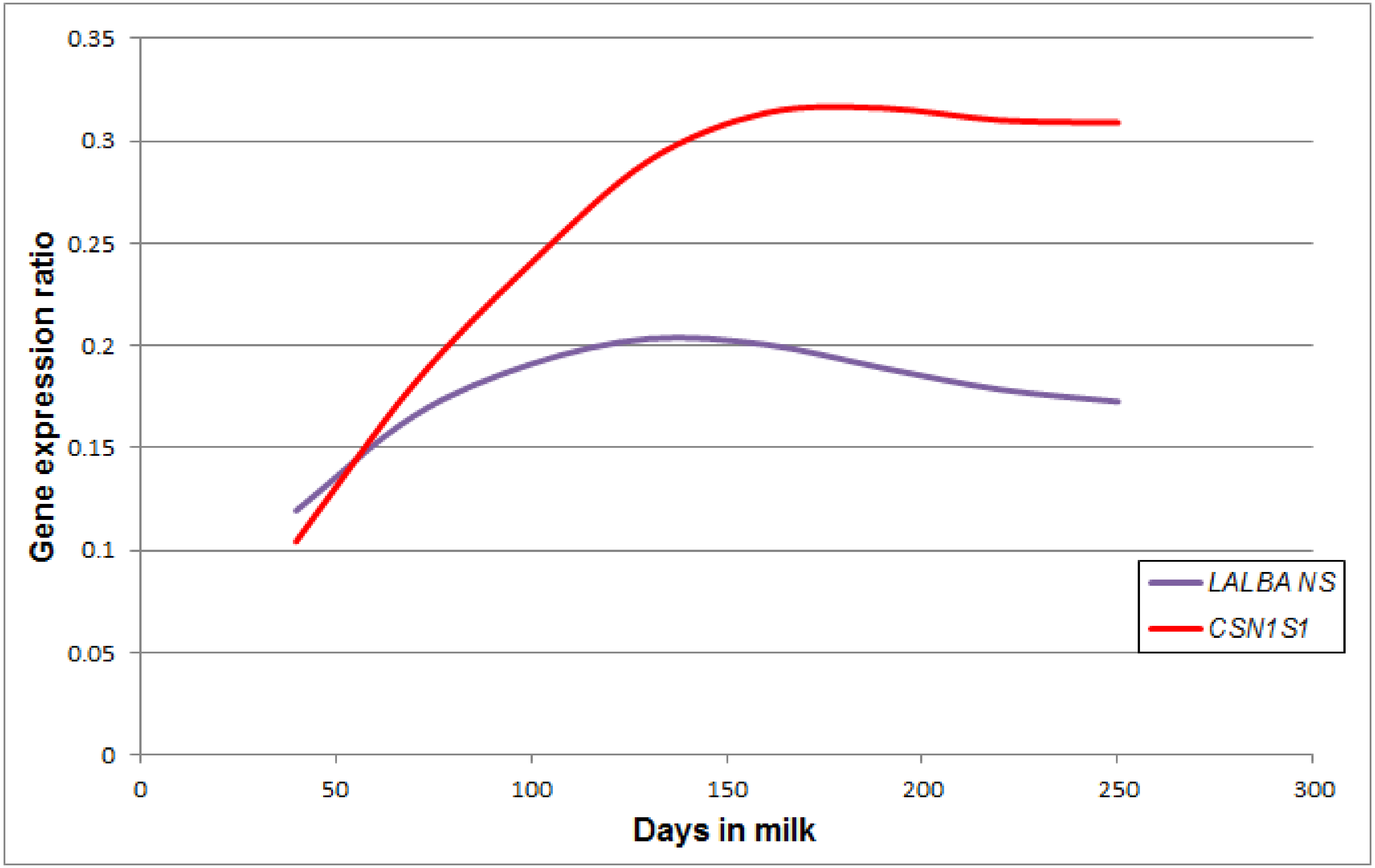
Lactose genes



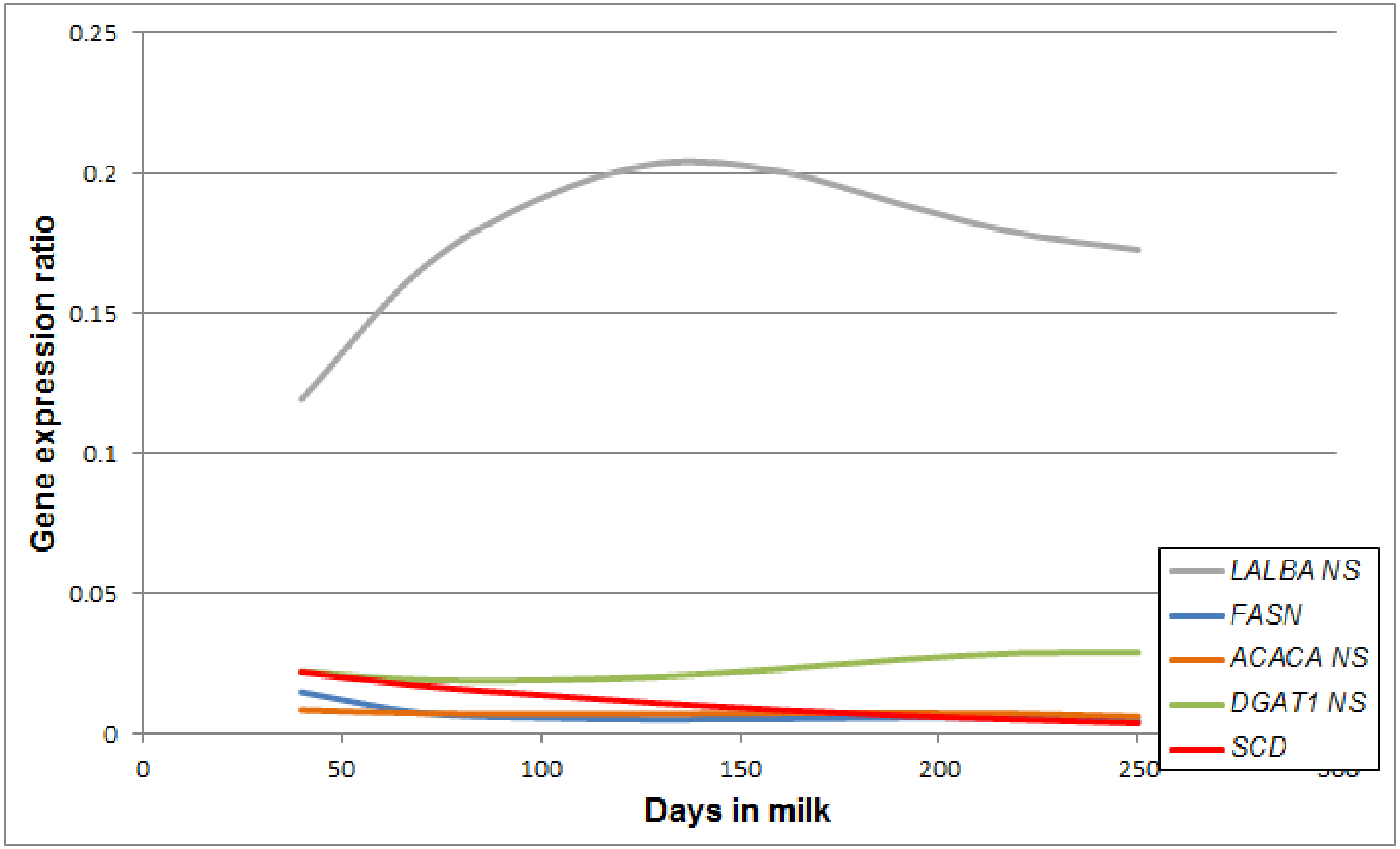
Prolactin genes



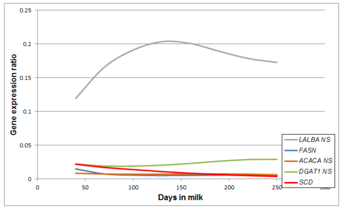
Protein genes

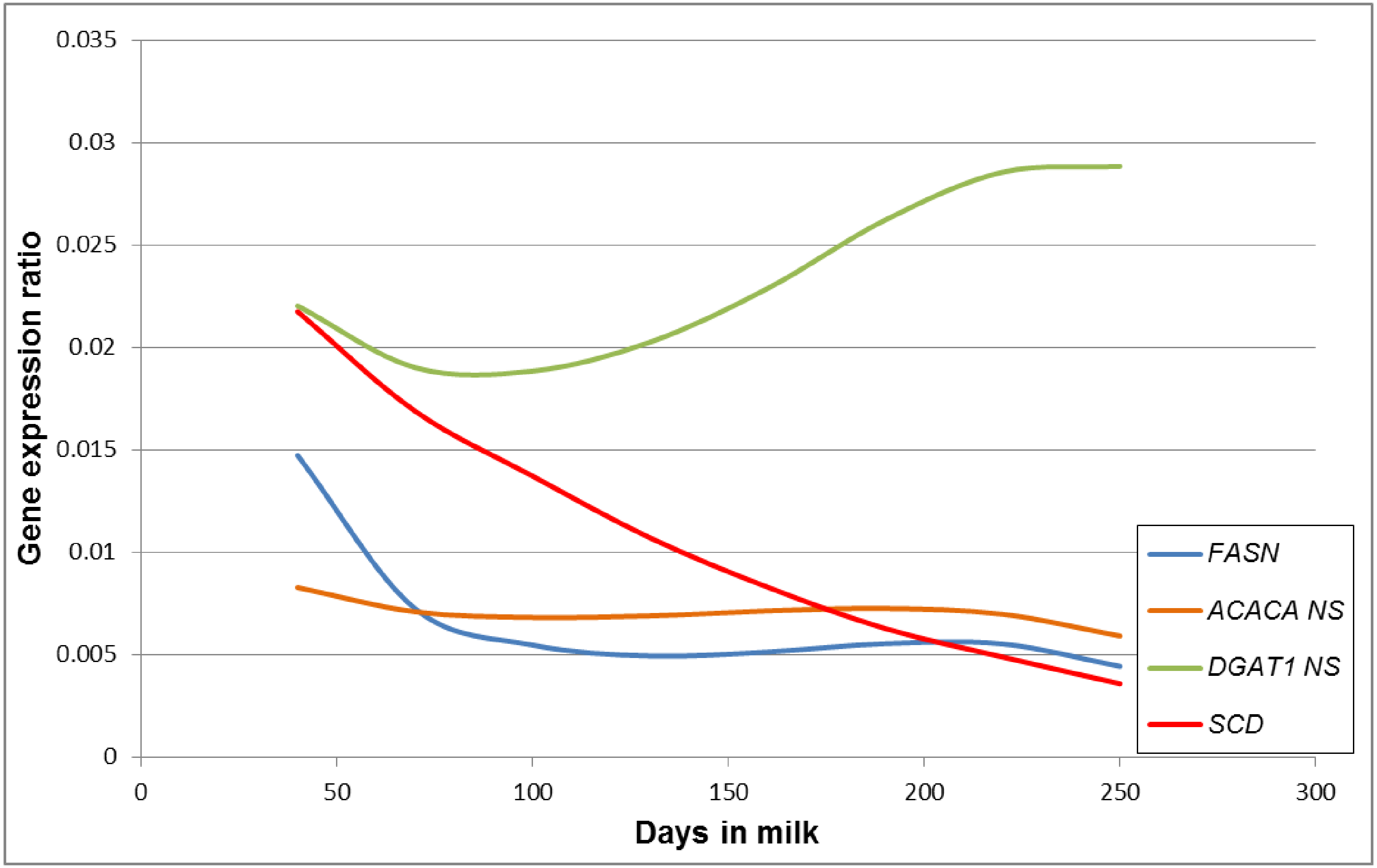


Fat genes

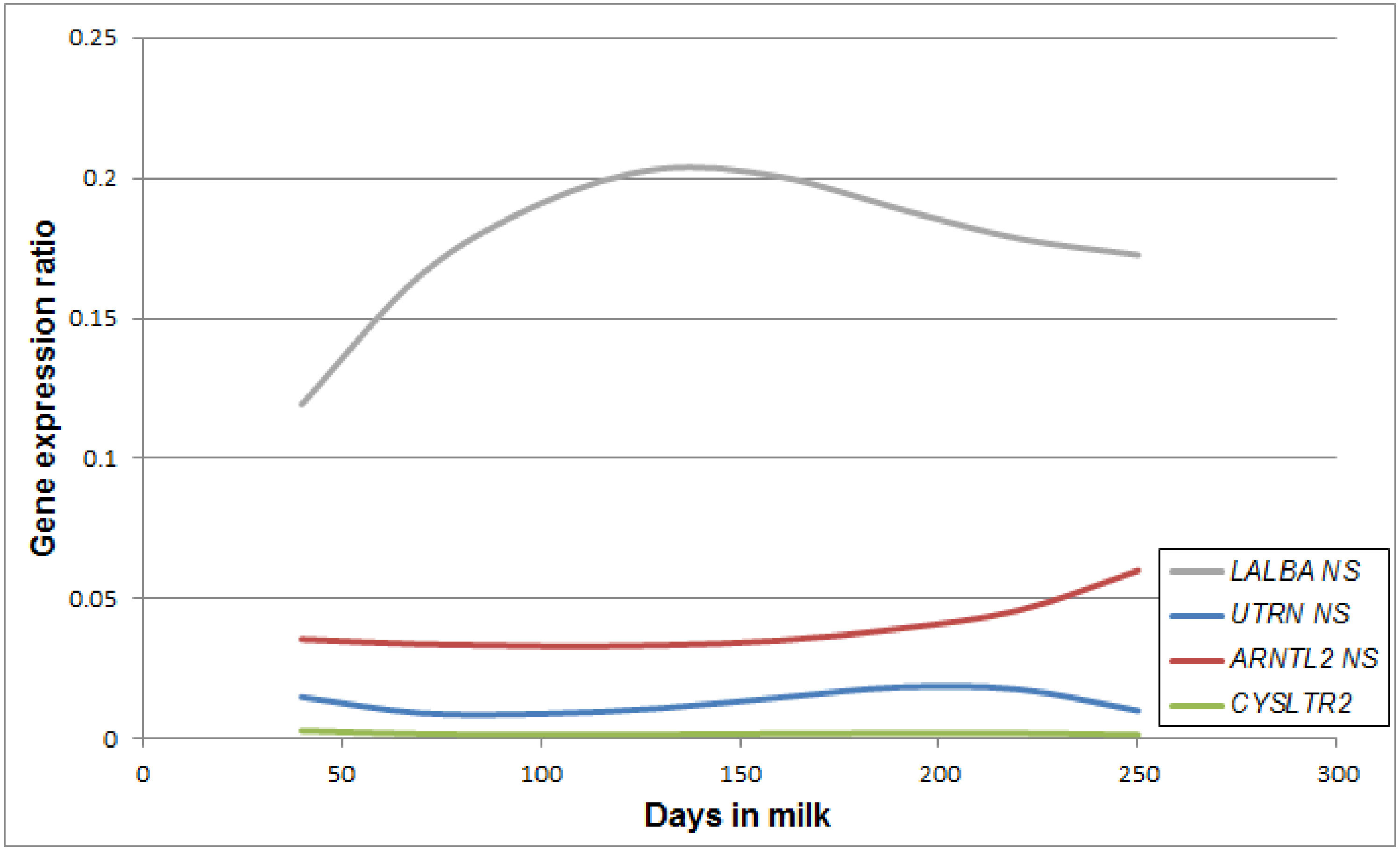

Gene expression ratio
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
250
Days in milk
LALBA NS
FASN
ACACA NS
DGAT1 NS
SCD

Fat genes


0.25
0.2
0.15
0.1
0.05
0
Gene expression ratio
0
50
100
150
200
250
300
Days in milk
LALBA NS
FASN
ACACA NS
DGAT1 NS
SCD


Gene expression ratio
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
50
100
150
200
250
300
Days in milk
FASN
ACACA NS
DGAT1 NS
SCD

Cell cycle etc. genes



Correlations – Lactose genes

	B4GALT1	*LALBA*	*UGDH*
LALBA	-0.05		
UGDH	**0.53**	**-0.27**	
UGP2	**0.61**	0.08	**0.46**

Values in bold significantly different from 0

Correlations – Fat genes

	ACACA	*DGAT1*	*FASN*
DGAT1	**0.22**		
FASN	0.07	**0.23**	
SCD	**0.50**	**0.22**	**0.27**

Values in bold significantly different from 0

Correlations – Cell cycle etc. genes

	ARNTL2	*CYSLTR2*	*FOXH1*
CYSLTR2	0.05		
FOXH1	-0.02	**0.31**	
UTRN	**0.42**	0.05	**0.22**

Values in bold significantly different from 0

Correlations – Other genes

	CSN1S1	*JAK2*	*LALBA*
JAK2	-0.13		
LALBA	**0.83**	-0.14	
SCD	**0.39**	0.06	**0.40**

Values in bold significantly different from 0

Genes and production

	Milk	Fat%	Protein %	Fat weight	Protein weight
ACACA			**0.15**	**-0.20**	
B4GALT1				**-0.17**	
CSN1S1			**-0.14**		
CYSLTR2	**-0.21**		**0.28**	**-0.21**	
FASN	**-0.25**		**0.18**	**-0.19**	**-0.17**
LALBA			**-0.16**		
SCD		**-0.18**	**0.16**		
UGDH	**-0.16**		**0.15**		
UTRN	**-0.20**		**0.18**	**-0.18**	

Implications

- RNA can be extracted from milk with biologically credible results
- Gene expression of several key genes varies across lactation
- Low level relationship between GER and production traits
- Further analyses required to determine if GER can be used to estimate secretion rate in lactation

Acknowledgements

- Boltons Park Farm for collaboration with milk samples
- BBSRC for funding equipment

Gene	Mean	SD	Skewness	Mean log$_{10}$ values	SD log$_{10}$ values
ACACA	0.012	0.04	13.6	-2.15	0.37
ARNTL2	0.119	0.17	3.2	-1.43	0.86
B4GALT1	0.069	0.11	5.0	-1.55	0.68
CSN1S1	0.449	0.32	1.0	-0.62	0.75
CYSLTR2	0.009	0.04	11.3	-2.71	0.54
DGAT1	0.076	0.15	6.0	-1.63	0.80
FASN	0.032	0.18	9.7	-2.19	0.53
FOXH1	0.027	0.11	12.2	-2.29	0.76
JAK2	0.270	0.28	2.8	-0.83	0.60
LALBA	0.331	0.27	1.4	-0.75	0.68
SCD	0.024	0.07	7.9	-2.03	0.53
UGDH	0.123	0.20	3.2	-1.42	0.75
UGP2	0.206	0.21	2.8	-0.88	0.45