GENETIC PARAMETER ESTIMATION OF HIGH VALUE MILK PROTEINS AND WHEY TO CASEIN RATIO IN DANISH HOLSTEIN

N. A. POULSEN, T. T. LE, V. R. GREGERSEN, B. CHRISTENSEN, E.S. SØRENSEN, L.B. LARSEN & A.J. BUITENHUIS

AARHUS UNIVERSITY
DEPARTMENT OF FOOD SCIENCE

12 FEBRUARY 2018

NINA AAGAARD POULSEN
ASSISTANT PROFESSOR

Milk Genomics Initiative

Minerals and vitamins

Fatty acids

Coagulation properties

Metabolites

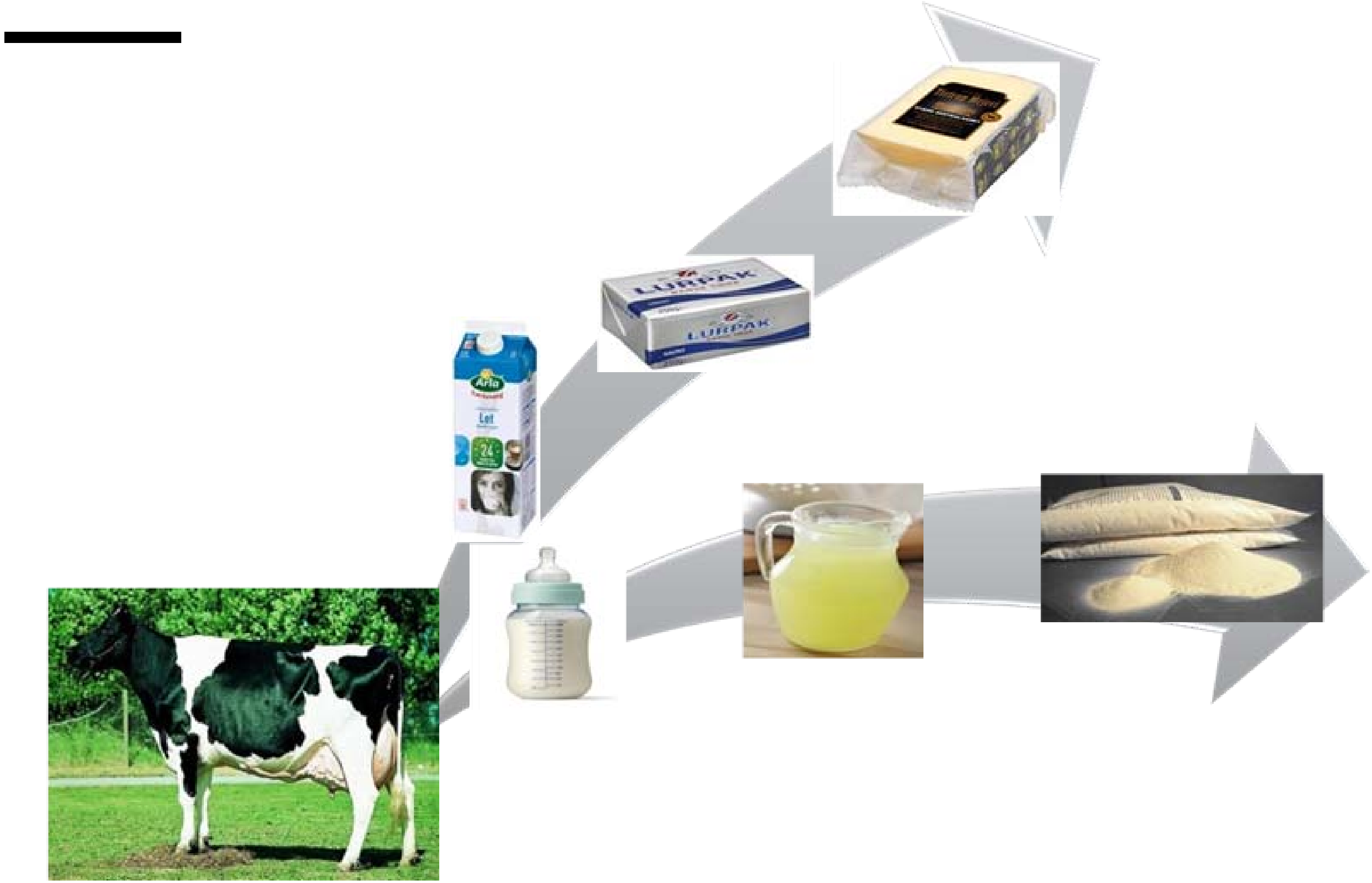
Protein composition

Oligo-saccharides

Environmental effect

Genetic effect

Increasing focus on whey components



Breeding for high value protein (Big Milk):
OPN, α-LA, β-CN and B12-binding protein, whey to casein ratio

Human milk versus bovine milk

Protein	Human milk	Bovine milk
Total protein	9-11 g/L	35 g/L
α-lactalbumin	2.4 g/L (24 %)	0.5 g/L (1.5%)
β-casein	1.3 g/L (13%)	10 g/L (28%)
Osteopontin	138 mg/L	18 mg/L
Transcobalamin	22-180 nmol/L	?
Whey/casein ratio	~60:40	20:80

Human milk gold standard for production of infant formula

Aim: Improved infant formula with optimal functionalities

Milk proteins:

•source of amino acids,

•facilitate digestion and uptake of other nutrients (β-casein - calcium, transcobalamin – vitamin B12)

•Physiological effects – enhance immune function, defence against e.g. pathogenic bacteria, development of the gut

Aim of study

Identify QTLs using a GWAS approach (BF < 0.05) :

$Y_{ijklm} = u + parity_i + herd_j + b_1*DIM_k + b_2*e^{-0.05DIMk} + b3* SNP_m + animal_l + e_{ijklm}$

Estimate heritabilities:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_E^2}$$

Conventional farms - indoor feeding,

Study design: maximized genetic variation, minimized environmental variation

POP1
Danish Holstein
N = 322
Bovine HD

Partiy 1-3
Average: 1.77

DIM: 138-227
Average: 181

#Herds: 19

POP2
Danish Holstein
N= 341
Bovine SNP50

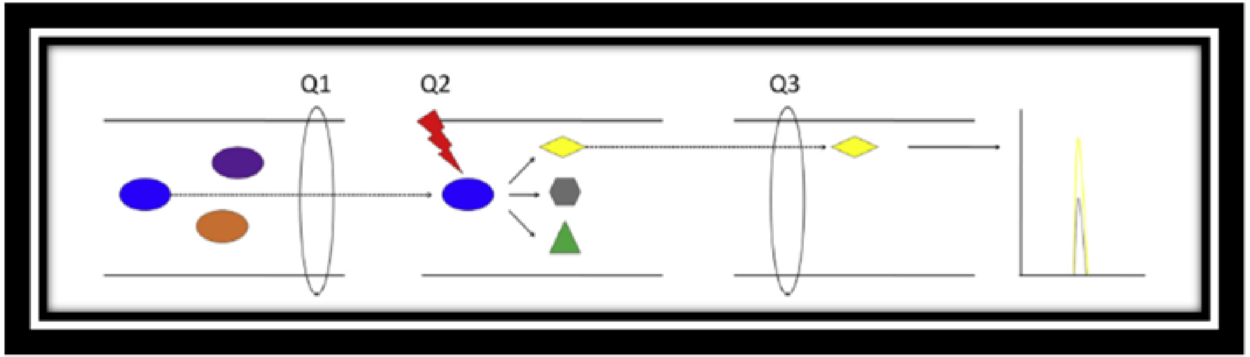
Partiy 1-6
Average: 1.98

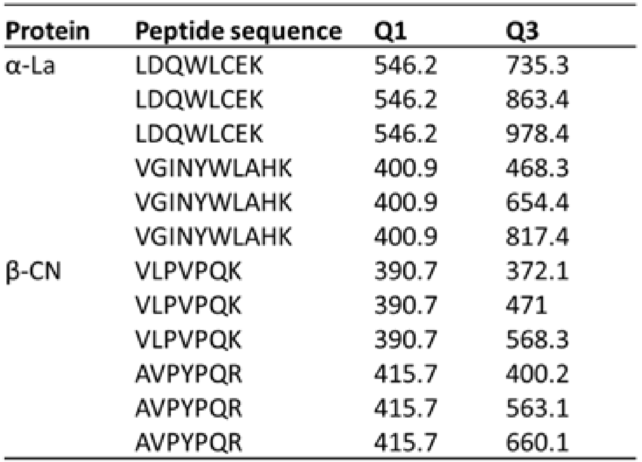
DIM: 4-877
Average: 180

#Herds: 3

Absolute quantification of α-LA and β-CN

Quantification by multiple reaction monitoring (MRM)

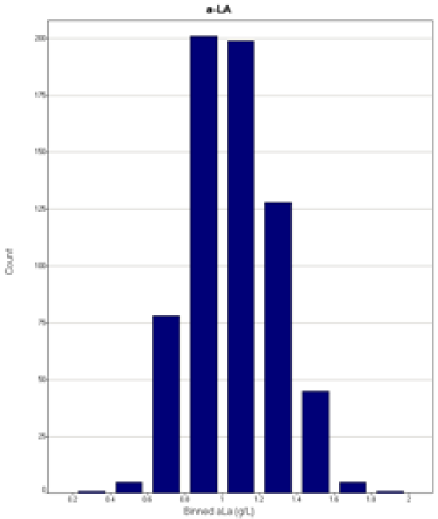


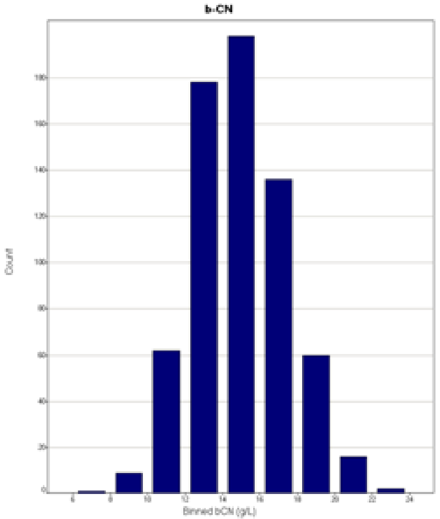


Protein	Peptide sequence	Q1	Q3
α-La	LDQWLCEK	546.2	735.3
	LDQWLCEK	546.2	863.4
	LDQWLCEK	546.2	978.4
	VGINYWLAHK	400.9	468.3
	VGINYWLAHK	400.9	654.4
	VGINYWLAHK	400.9	817.4
β-CN	VLPVPQK	390.7	372.1
	VLPVPQK	390.7	471
	VLPVPQK	390.7	568.3
	AVPYPQR	415.7	400.2
	AVPYPQR	415.7	563.1
	AVPYPQR	415.7	660.1

- Using a triple quadrupole mass spectrometry instrument (Le et al., 2017, IDJ 67, 2-15)
- Quantification based on determination of the amount of the specific peptides from α-LA and β-CN generated by tryptic cleavage, representing unique parts of the protein sequence

Results for α-LA and β-CN





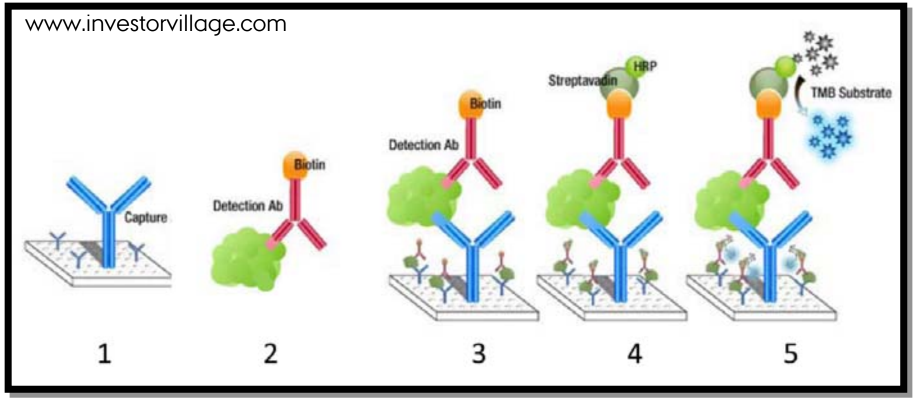
α-LA mean: **1.1 g/L** (0.4-1.9 g/L)
h^2**: 0.12** (0.09)

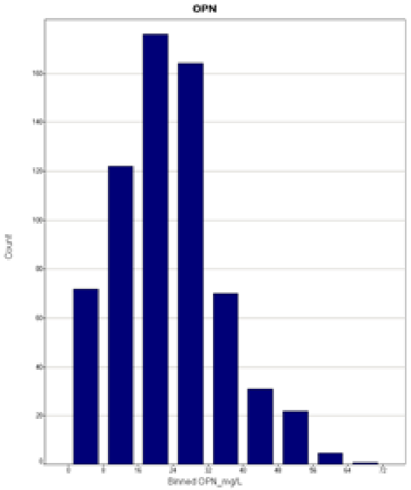
β-CN mean: **14.9 g/L** (7.5-23.4 g/L)
h^2: **0.05** (0.07)

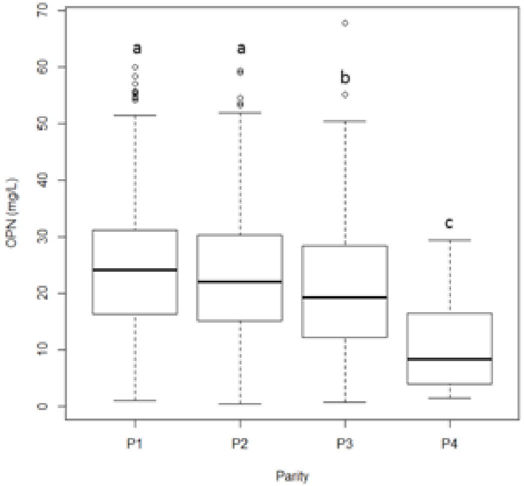
- **Large variation of specific proteins in bovine milk**
- **Low h^2**
- **GWAS: No significant SNPs found for α-LA or β-CN**

Results for OPN

Sandwich ELISA for absolute quantification of OPN


www.investorvillage.com

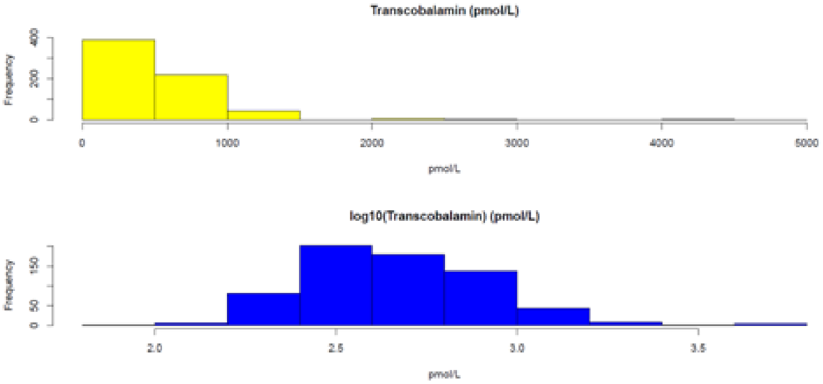


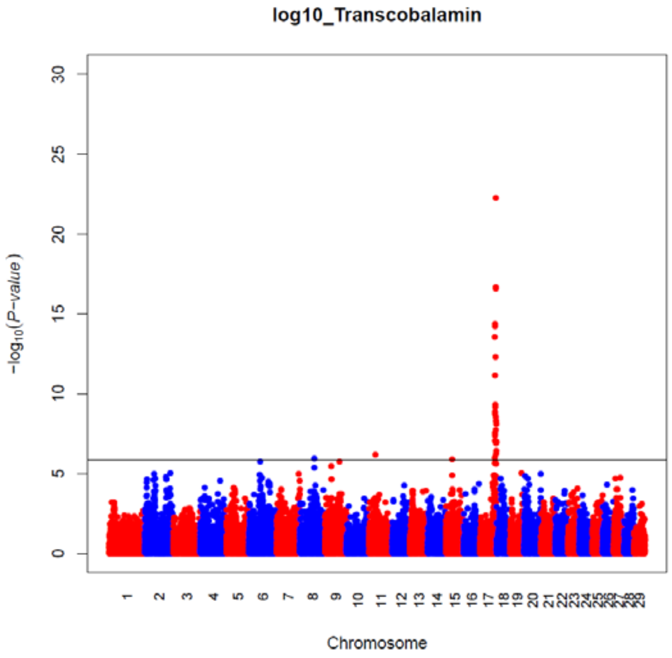


OPN mean: **23 mg/L** (0.4-68 mg/L)
h^2: **0.15** (0.09)

- **Large variation and higher levels of OPN in bovine milk than reported earlier**
- **Significant effect of parity observed**
- **Low h^2**
- **GWAS: No significant SNPs detected**

Results for transcobalamin



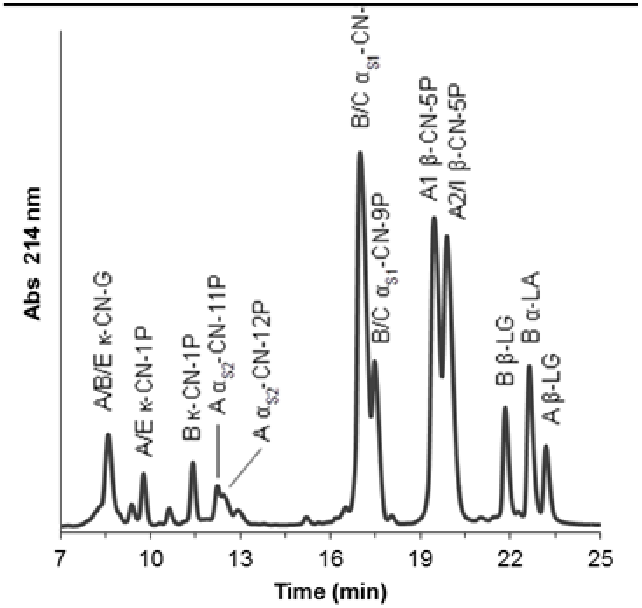


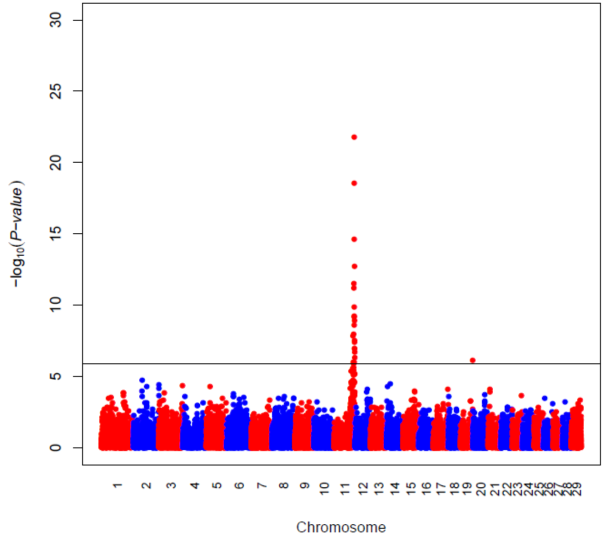
Mean: 557 pmol/L (96-4672 pmol/L)
h^2: 0.44 (0.12), Log_{10}Transcobalamin 0.61 (0.14)

GWAS on transformed data reveal major QTL on BTA17
No obvious candidate genes for transcobalamin found within this region, but need to be explored more

Results for whey:casein ratio

LC/ESI-MS for relative quantification





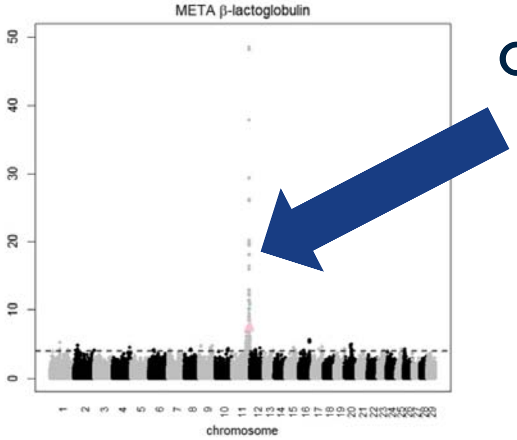
Whey:casein ratio
Mean: **0.15** (0.08-0.26)
h^2: **0.40** (0.11)

- **Moderate heritability**
- **Significant QTL, BTA 11**
- **Most significant SNP was located within PAEP, which is the gene encoding β-lactoglobulin (β-LG)**

Association to relative protein contents

	Danish Holstein		
Trait[1]	Mean	SD	h^2 (SE)
Protein%	3.43^a	0.26	0.47 (0.19)
Casein%	2.66^a	0.12	0.43 (0.18)
α_{s1}-CN%	0.26^a	0.03	0 (0.12)
α_{s2}-CN%	0.05^a	0.01	0.14 (0.15)
β-CN%	0.36^a	0.03	0.05 (0.13)
κ-CN%	0.06^a	0.01	0.77 (0.21)
α-LA%	0.03^a	0.01	0.40 (0.19)
β-LG%	0.08^a	0.02	0.58 (0.20)

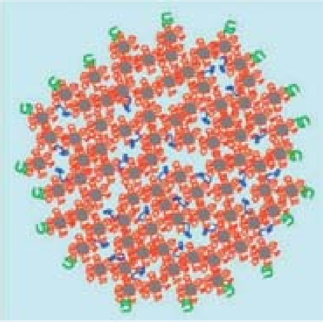
Buitenhuis et al., 2016, BMC Genetics 17:114



QTL on BTA11 including PAEP for β-LG%

Neither absolute (g/L) nor relative (%) β-CN seem to be heritable
Relative contents of α_{s1}-CN%, α_{s2}-CN%, and β-CN% have low heritabilities

κ-CN%, α-LA%, and β-LG% moderate to high heritabilities
Variation in PAEP most likely control variation in β-LG%



Dalgleish et al. 2011

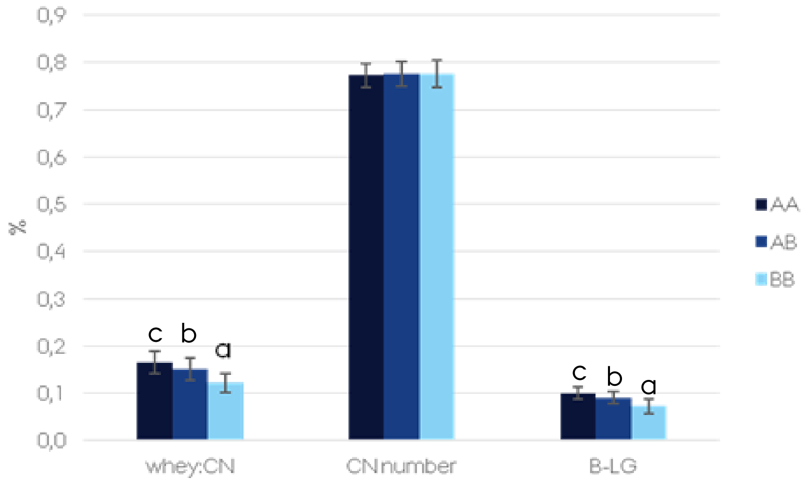
Association to β-LG genetic variants

β-LG variant A and B identified by LC/ESI-MS

Well-known to affect β-LG%, but probably also explains most of the variation in whey:casein ratio

Thus relative β-LG rather than variations in the caseins (>80% of the proteins) seem to determine the variation in whey:casein ratio

This is also however not seen for the casein number measured by IR (Milkoscan)



Conclusions

- Robust methods for quantification of high value proteins in milk developed
- Low heritabilities and no significant SNPs detected for α-LA, β-CN and OPN
- Parity seems to effect OPN composition
- Moderate heritabilities for whey:casein ratio, significant QTL on BTA 11, most likely related to variation in PAEP controling relative β-LG variation
- Moderate heritability for transcobalamin

- Good potential to increase the content of specific proteins and whey:casein ratio through selective breeding

Acknowledgements

Danish Milk Levy Fund

Food Science, Aarhus University: Lotte Bach Larsen, and Thao T. Le

Molecular Biology and Genetics, Aarhus University: Bart Buitenhuis, Vivi Gregersen, Brian Christensen, Esben Skipper Sørensen, Grum Gebreyesus, and Christian W. Heegaard

Aarhus University Hospital: Ebba Nexø and Eva Greibe

Arla

AARHUS UNIVERSITET