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NZ3m
Milks Mean More

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Effect of protein polymorphisms on milk composition, coagulation properties, and protein profile in dairy sheep

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Introduction

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Sheep milk: Water (~80-83%)

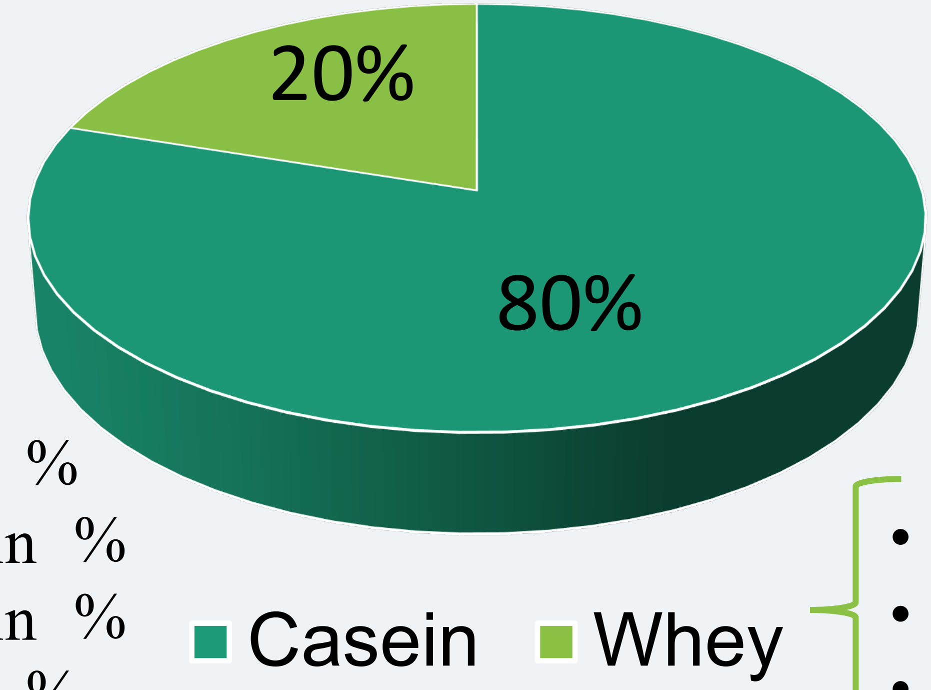
17% Total Solids

- Fat, 6.5%
- Protein, 5.25%
  - → Casein proteins, in micelles, rennet sensitive
  - → Whey, in water-phase of milk, heat sensitive
- Lactose, 4.90%

(Wendorff & Haenlein, 2017)

Introduction

Sheep milk- True Protein



Affected by:
ewe breed,
genetics,
stage of
lactation,
age, feeding

- κ-casein %
- α_{s1}-casein %
- α_{s2}-casein %
- β-casein %

- α-lactalbumin %
- β-lactoglobulin %
- other minor proteins

Introduction

Casein and whey proteins exist in various phenotypic forms!

Phenotypes= here referred to as "protein polymorphisms"

Consequence of:

1. genetic variations (SNPs)→ AA sequence modifications

and/ or

2. post-translational modifications (PTMs)→ glycosylation/phosphorylation

Introduction

Protein profile could be affecting processability due to:

- *Quantitative* differences: protein composition

  (% of κ-CN, αs1-CN, αs2-CN, β-CN, α-LA, and β-LG in TP)

- *Qualitative* differences: direct effect of <u>protein polymorphisms</u>

Objective

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To investigate:

The effects of protein polymorphisms on sheep milk production, composition and processability

Materials and Methods

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Flock

- 470 test-day records from 147 ewes
- Milked in mid-late lactation
  (from 50 to 182 days of lactation)
- East-Friesian + Coopworth genetics
- White x Black
- Extensive pasture-based farming

Figure 1. Dairy ewes from a New Zealand flock

Materials and Methods

Milk composition

- Traditional composition analyses from MilkoScan (Foss Analytics):

  Fat %

  Protein %

  Casein %

  Lactose %

- Total calcium content (Arsenazo III method, RX Daytona Plus clinical analyser)

Materials and Methods

Determination of proteins

- RP-HPLC by comparing the elution times with those in the literature (Trujillo, et al., 2000)
- Agilent 1260 Infinity II LC system
- Sample preparation (Bobe et al., 1998)
- Separation of milk protein fractions (Bonfatti et al., 2008)

Materials and Methods

Milk processability traits:

1. Milk Coagulation Properties (MCP) (McMahon & Brown, 1982)
2. Individual Laboratory Cheese Yield (ILCY) (Othmane et al. 2002)
3. Heat coagulation time (HCT) in an oil bath at 140 °C (Cole & Tarassuk 1946)

Materials and Methods

RCT= Rennet coagulation time, minutes

K20= Time to reach curd firmness of 20 mm, minutes

A30= Curd firmness at 30 minutes, millimeters

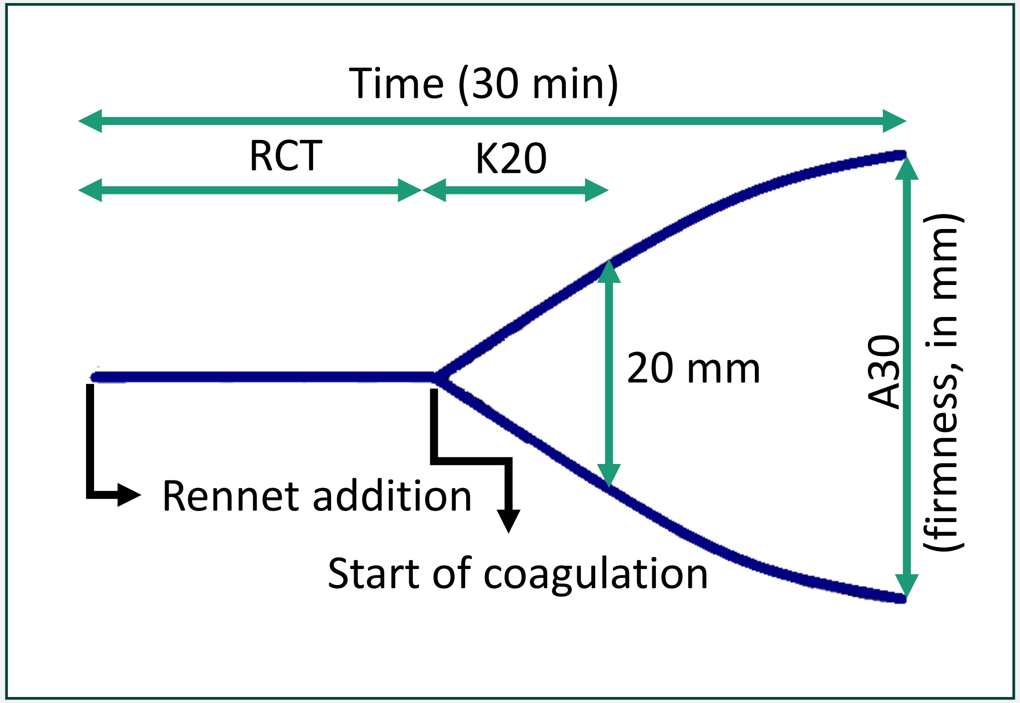


Figure 2. Diagram of coagulation and curd firmness as a function of time, recorded with the Formagraph. Adapted from McMahon & Brown (1982).

Materials and Methods

Figure 2. Formagraph analyses

Figure 3. Individual laboratory cheese yield

Materials and Methods

Statistical analyses

- SAS version 9.4 software (SAS Institute Inc., Cary, NC, USA)
- Mixed linear model including fixed and random effects:

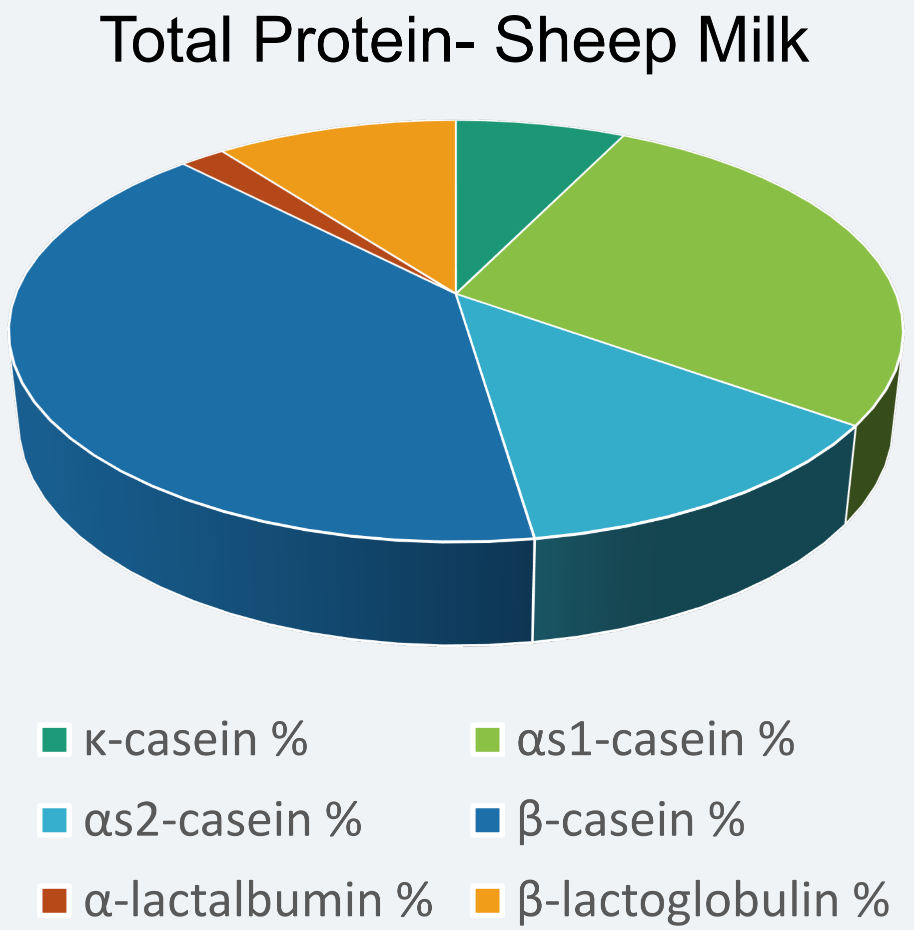
y= ewe coat colour + litter size + parity number + stage of lactation + polymorphism (one at a time)

+ deviation median lambing date + ewe + random error

Results

Table 1. Average protein composition.

Protein fraction	N	Mean	SD
κ-casein %	470	7.28	1.3
α_{s1}-casein%	470	27.58	1.78
α_{s2}-casein %	470	12.78	1.58
β-casein %	470	40.06	1.96
α-lactalbumin %	470	1.94	0.61
β-lactoglobulin%	470	10.36	1.25



Results

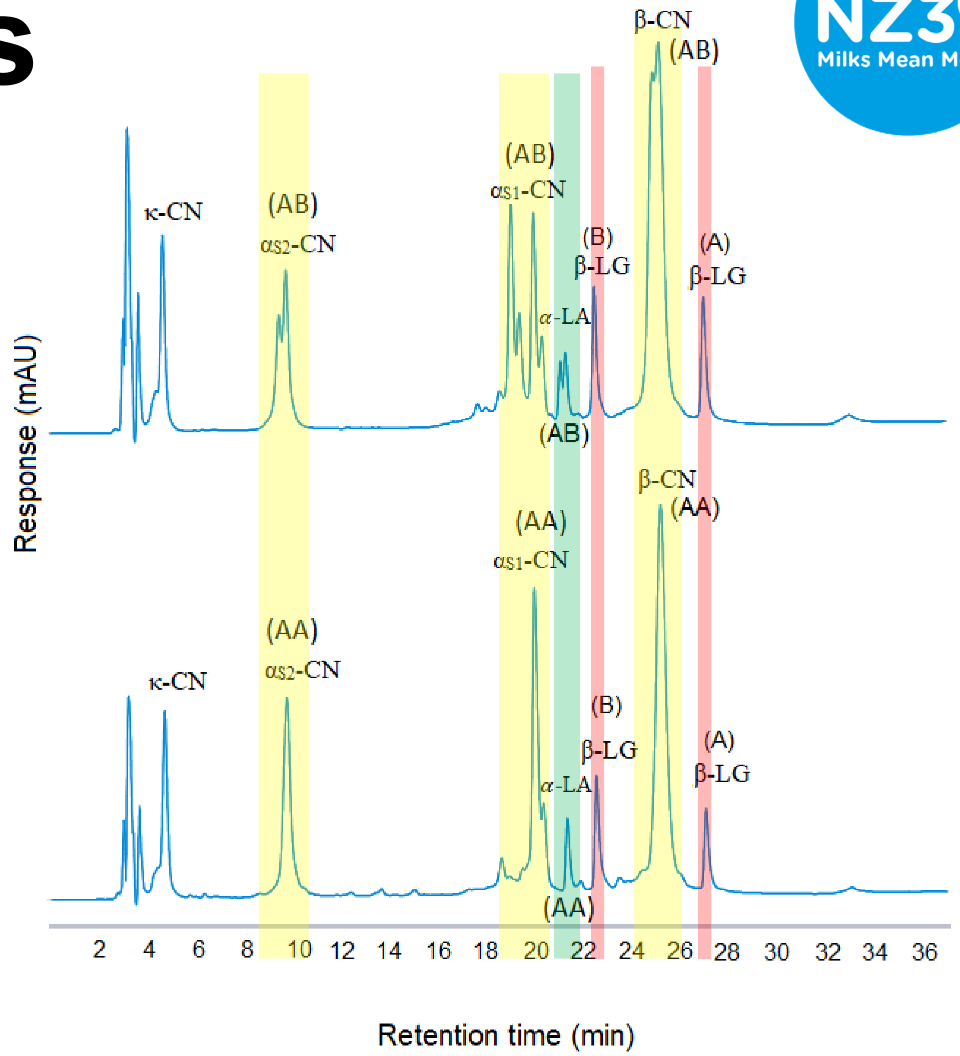


Figure 4. Main milk protein polymorphisms (self-named) observed through RP-HPLC, in a New Zealand dairy sheep flock.

Results

Genome-wide Association Study for β-lactoglobulin%, with 50K SNPs

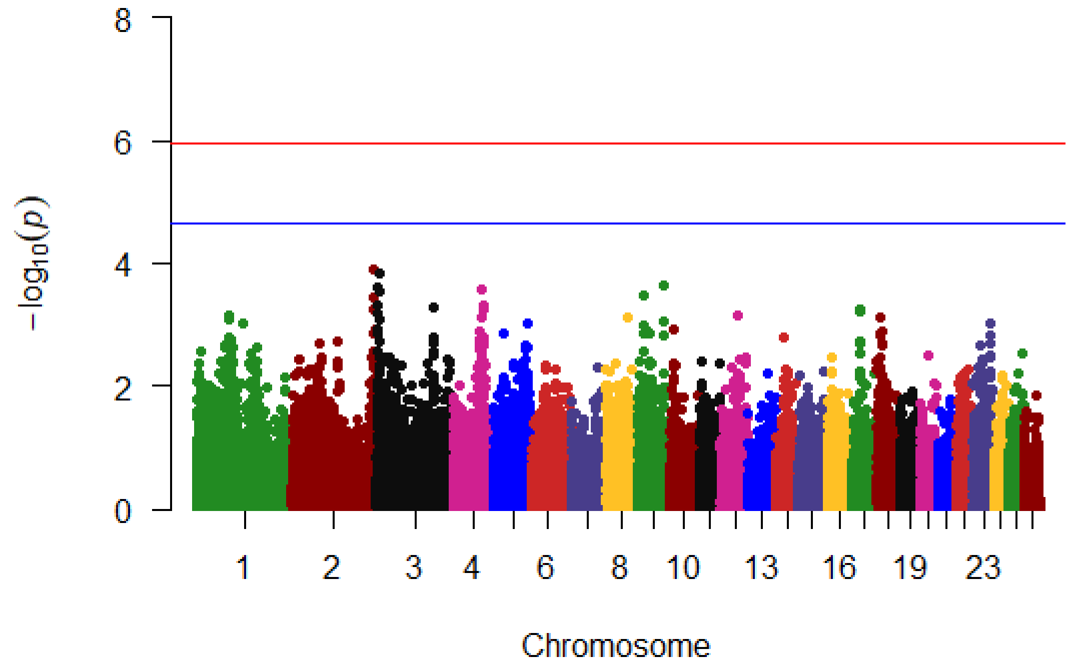


Figure 5. Manhattan plot for −log10 (p-values) of marker effects for β-lactoglobulin%. The genome-wide significance threshold of Bonferroni correction is represented by the red line at −log10 (p-value) = 5.96.

Results

Genome-wide Association Study for β-lactoglobulin%, with 50K SNPs

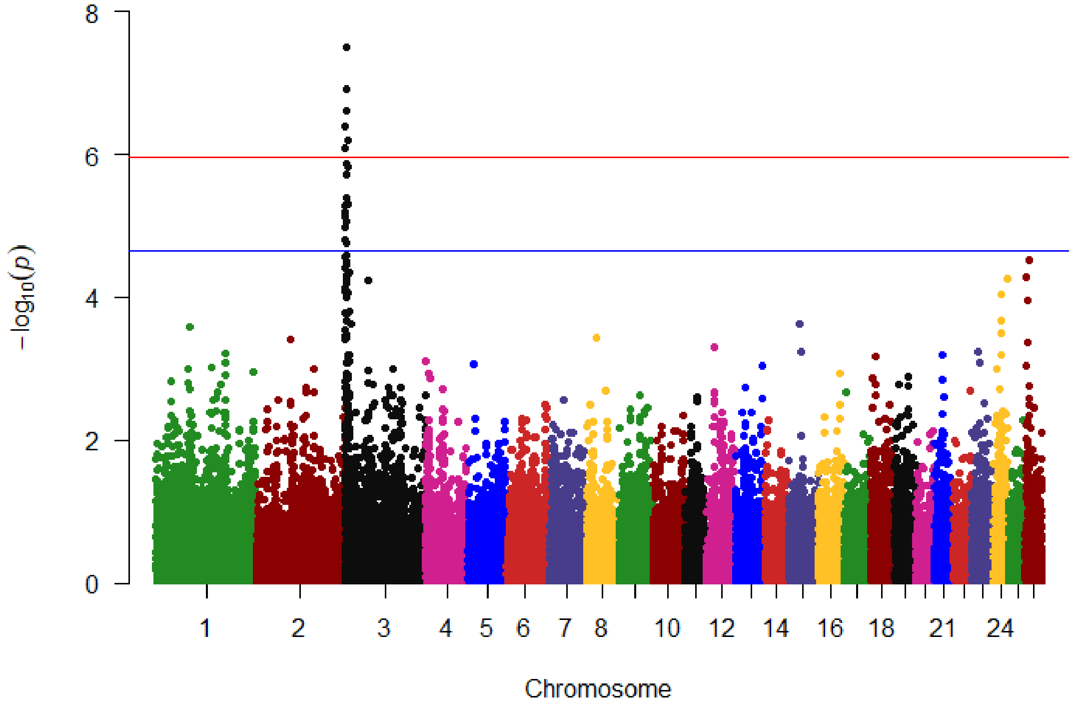


Figure 5. Manhattan plot for −log10 (p-values) of marker effects for β-lactoglobulin B%. The genome-wide significance threshold of Bonferroni correction is represented by the red line at −log10 (p-value) = 5.96.

Results

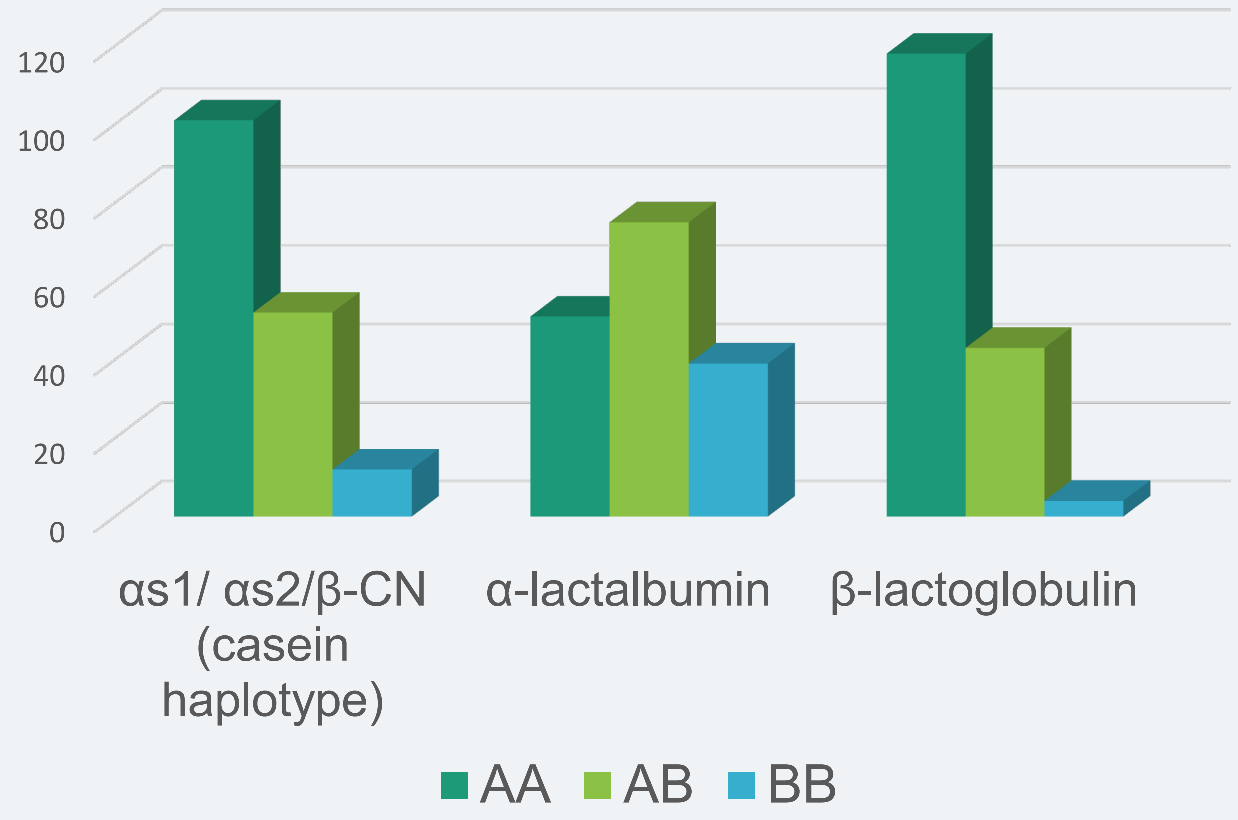


Figure 6. Frequencies of <u>self-named</u> milk protein polymorphisms, as aggregate casein proteins and as separate whey protein fractions, in a New Zealand dairy sheep flock.

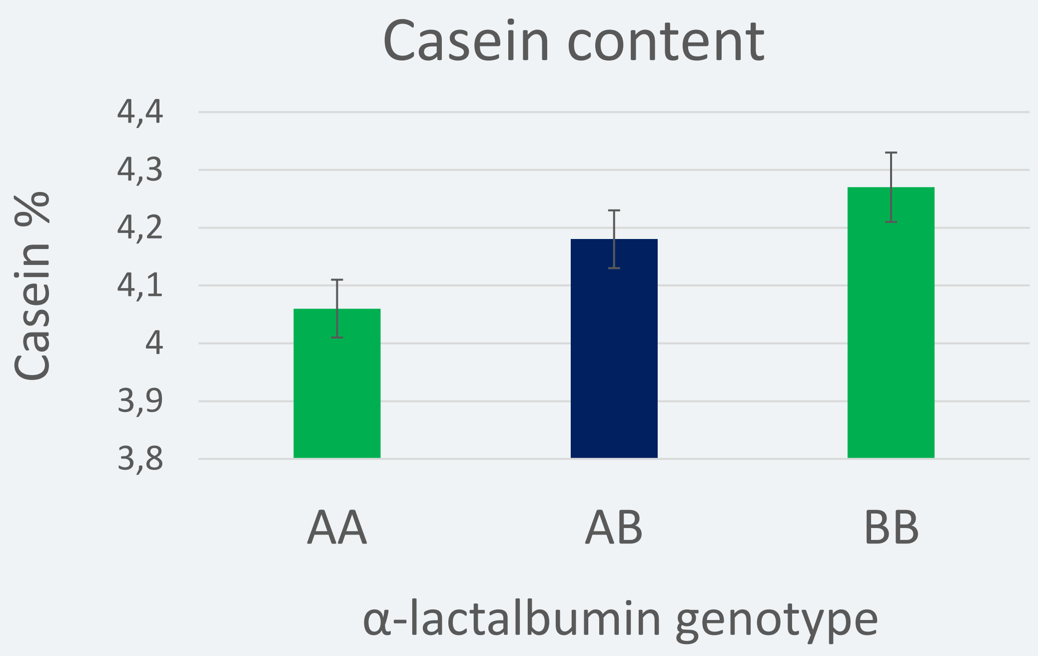
Results

Table 2. F values of effects of protein polymorphisms on milk production and composition of dairy sheep in a New Zealand flock.

Trait	Casein polymorphism (αs1/αs2/β-CN)	α-lactalbumin polymorphism	β-lactoglobulin polymorphism
Milk yield (L/day)	0.79	0.94	0.18
Fat (%)	2.53	2.63	2.62
Protein (%)	2.43	3.38*	0.46
Lactose (%)	2.6	2.89	0.21
Casein (%)	1.35	4.58**	0.3

Results

Figure. Least squares means (± standard errors) of casein % for the different α-lactalbumin genotypes.



Results

Table 3. F values of effects of protein polymorphisms on protein composition of milk from dairy sheep in a New Zealand flock.

Trait	Casein polymorphism (αs1/αs2/β-CN)	α-lactalbumin polymorphism	β-lactoglobulin polymorphism
κ-casein %	1.07	0.09	0.32
αs1-casein %	41.50***	1.22	1.45
αs2-casein %	60.40***	1.2	0.01
β-casein %	4.00*	1.08	0.44
α-lactalbumin %	0.02	15.60***	1.08
β-lactoglobulin %	8.00**	0.67	0.44

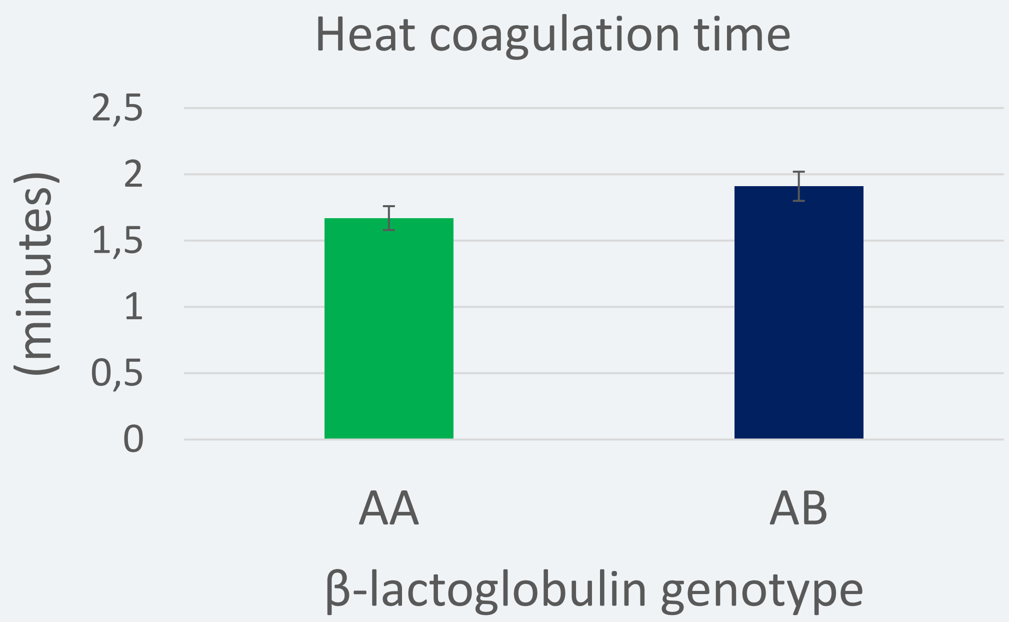
Results

Table 4. F values of effects of protein polymorphisms on processability of milk from dairy sheep in a New Zealand flock.

Trait	Casein polymorphism (αs1/αs2/β-CN)	α-lactalbumin polymorphism	β-lactoglobulin polymorphism
Milk coagulation			
RCT (minutes)	0.26	0.43	1.7
K20 (minutes)	0.01	1.62	0.12
A30 (millimetres)	0.19	1.16	0.43
ILCY (%)	0.01	0.65	0.6
HCT (minutes)	3.26	0.08	5.02*
pH	2.96	0.7	3.5

Results

Figure. Least squares means (± standard errors) of heat coagulation time for the different β-lactoglobulin genotypes.



Results

β-LG AB milk coagulated 14 seconds later than AA milk (at 140°C)

- Tyrosine of β-LG A has a role in the hydrophobic interactions, affecting the stability of micelles, and therefore B should show a higher denaturation onset temperature than ovine A (Amigo, et al. 2000)

- For bovine milk, β-LG variant B was also considered more thermostable than variant A (Keppler, Sönnichsen, Lorenzen, & Schwarz, 2014)

Conclusion

- Quantitative and qualitative variability of milk proteins were evidenced in the small flock of dairy sheep
- There was a significant effect of β-lactoglobulin polymorphism on milk heat stability, this could be of interest to the manufacturers of milk powder and beverages

Future

Selectively breed dairy sheep for desirable milk protein profile:

- Large genomic studies to understand correlations between protein polymorphisms and processability traits
- Need commercial sheep milk protein standards
- Include of relevant SNPs in BeadChips of lower density or whole genomic imputation

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