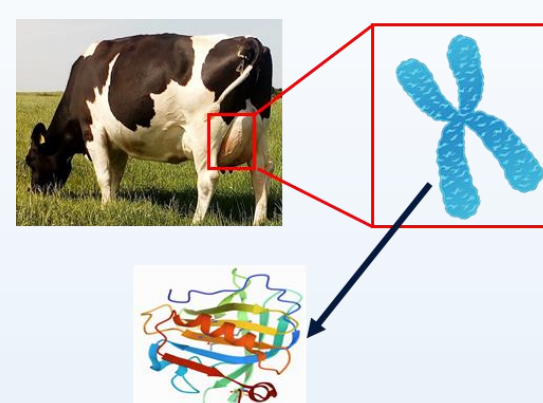


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Introduction:



- The nitrogen content of milk is distributed among the caseins, whey proteins and non-protein nitrogen, each of which has an effect on the nutritional value of milk.
- Genetic factors have a major influence on the protein profiles of milk, which in turn are reported to influence milk yield, composition, and milk processability.

Objectives:

- Investigate natural variations in the nitrogen composition and protein profile of individual cows.
- Determine potential correlations between specific protein variants and milk nitrogen fractions and determine the potential for tailoring milk for specific protein dominant product classes.

Methods:

- Samples were fractionated and total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) of all individual milk samples were determined using the Kjeldahl method.



Kjeldahl Titration

- The identification, separation, and quantification of constituent casein and whey proteins was carried out by Reverse Phase High Performance Liquid Chromatography (RP-HPLC).



HPLC

- Heat stability was measured using the oil bath method.
- Casein micelle size and zeta-potential was measured by Malvern Zeta sizer.

Results:

Table 1. Descriptive analysis of 95 individual milk samples

Variable	Mean
Milk yield (kg/milking)	8.78 ± 3.71
SCC	175775 ± 353076
Lactose (%)	4.75 ± 0.16
Total solids (%)	14.30 ± 0.82
Crude protein (%)	3.77 ± 0.38
True protein (%)	3.59 ± 0.45
NCN (%)	0.11 ± 0.02
NPN (%)	0.03 ± 0.004
% Casein	3.08 ± 0.47
% Whey protein	0.53 ± 0.13
Casein number	80.89 ± 3.54

Analysis:

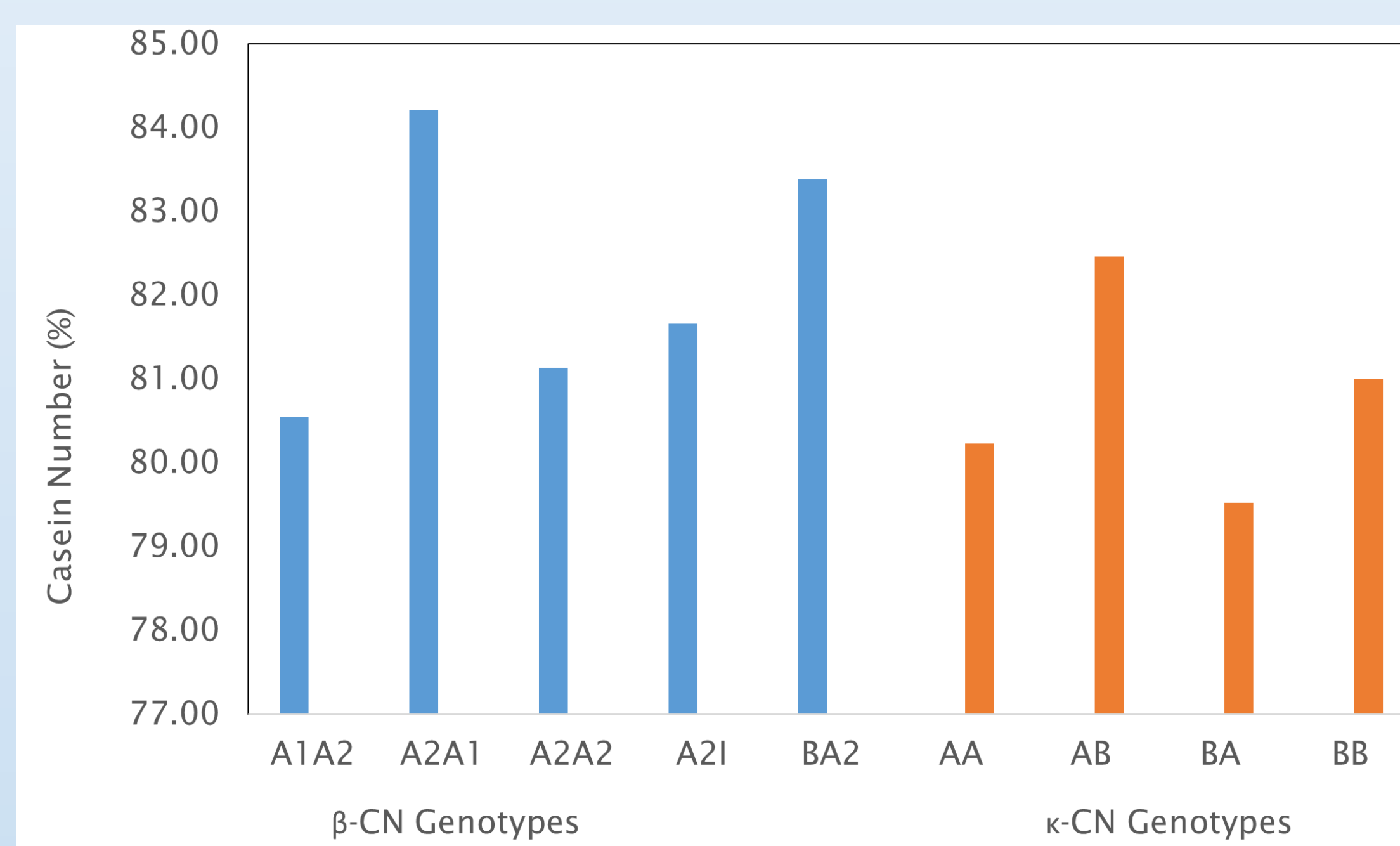


Figure 1. Analysis of different genotypes with casein number, showing moderate differences between β -CN and κ -CN genotypes and milk casein number.

- The presence of κ -CN AB genotype resulted in positive observations in casein number.
- β -CN A2A1 genotype resulted in an observable increase in casein number.

Table 2. Average heat stability, casein micelle size, zeta potential and casein number of high CN:WP ratio and low CN:WP ratio groups of cows.

Casein Number Category	Average heat stability (min)	Micelle Size (nm)	Zeta Potential (mV)	Casein Number
High casein number	12.02 ± 2.82	151.45 ± 17.94	- 24.4 ± 1.93	86.02 ± 5.59
Low casein number	10.65 ± 4.32	161.87 ± 9.37	- 25.7 ± 2.51	75.9 ± 2.83

- **Observations:** that high casein number milk had smaller average micelle size.
- Insubstantial variation in zeta-potential between both categories.
- A slight increase in heat stability was apparent for high casein milk samples.

Discussion and conclusions:

- Correlation analysis revealed several relationships between the genotypes and protein fractions. However, only the β -LG genotype shows a statistically significant effect on the κ -CN concentration and β -CN genotype shows a statistically significant effect on milk characteristics.
- A better understanding of the functional properties of milk protein genetic variants is key to improving the value of dairy products and the processing behaviour of these protein variants.
- Preliminary results investigating the genotypes, protein profiles and nitrogen fractions, from a random selection of cows, demonstrates that different protein variants present in the milk of individual cows impacts milk composition and has potential to be utilised for different product streams.

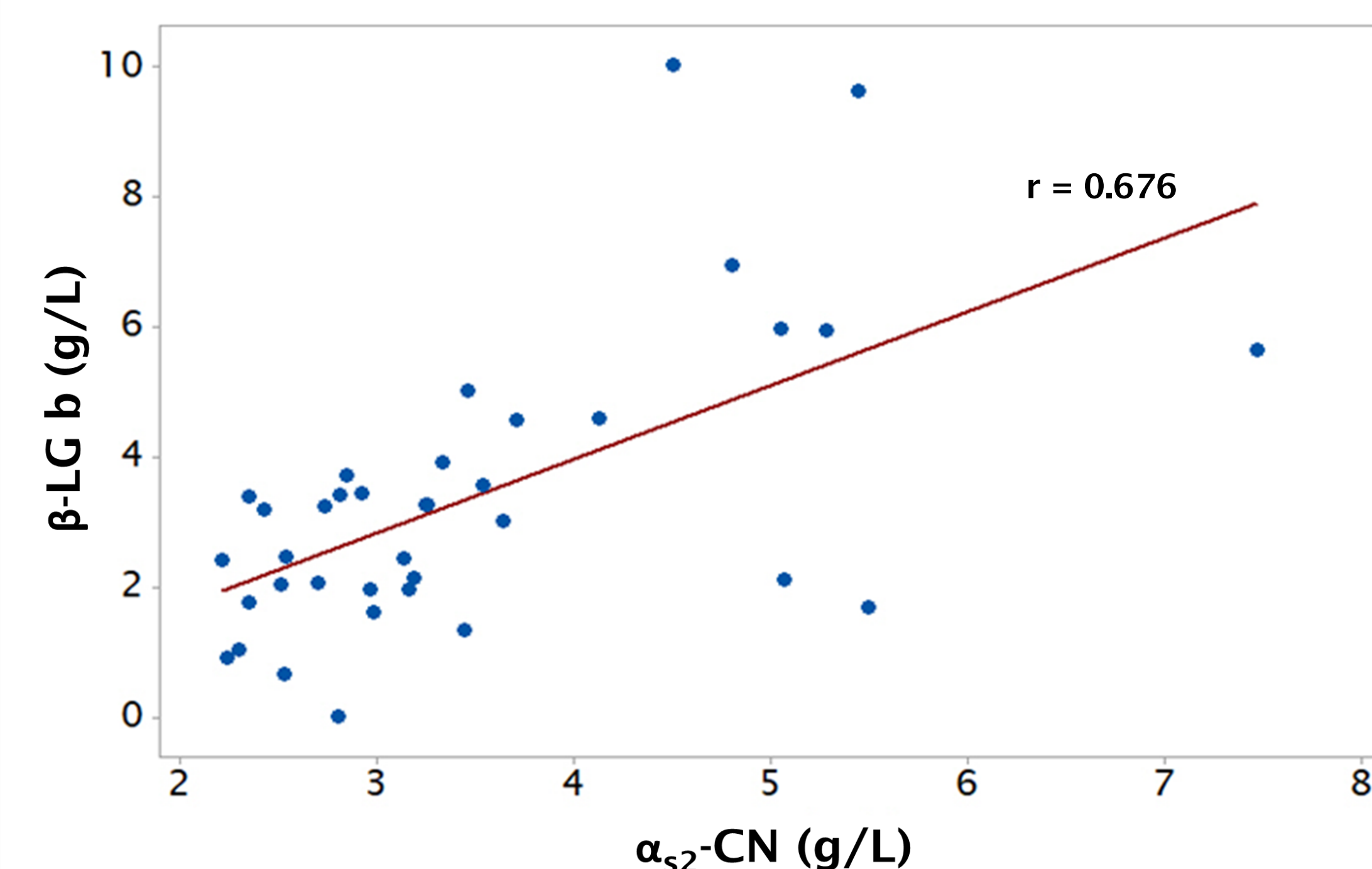


Figure 2. Scatterplot indicating the relationship between individual protein fractions (n = 37).

- α_{s2} -CN (g/L) was positively correlated with β -LG level (g/L; $p < 0.05$).

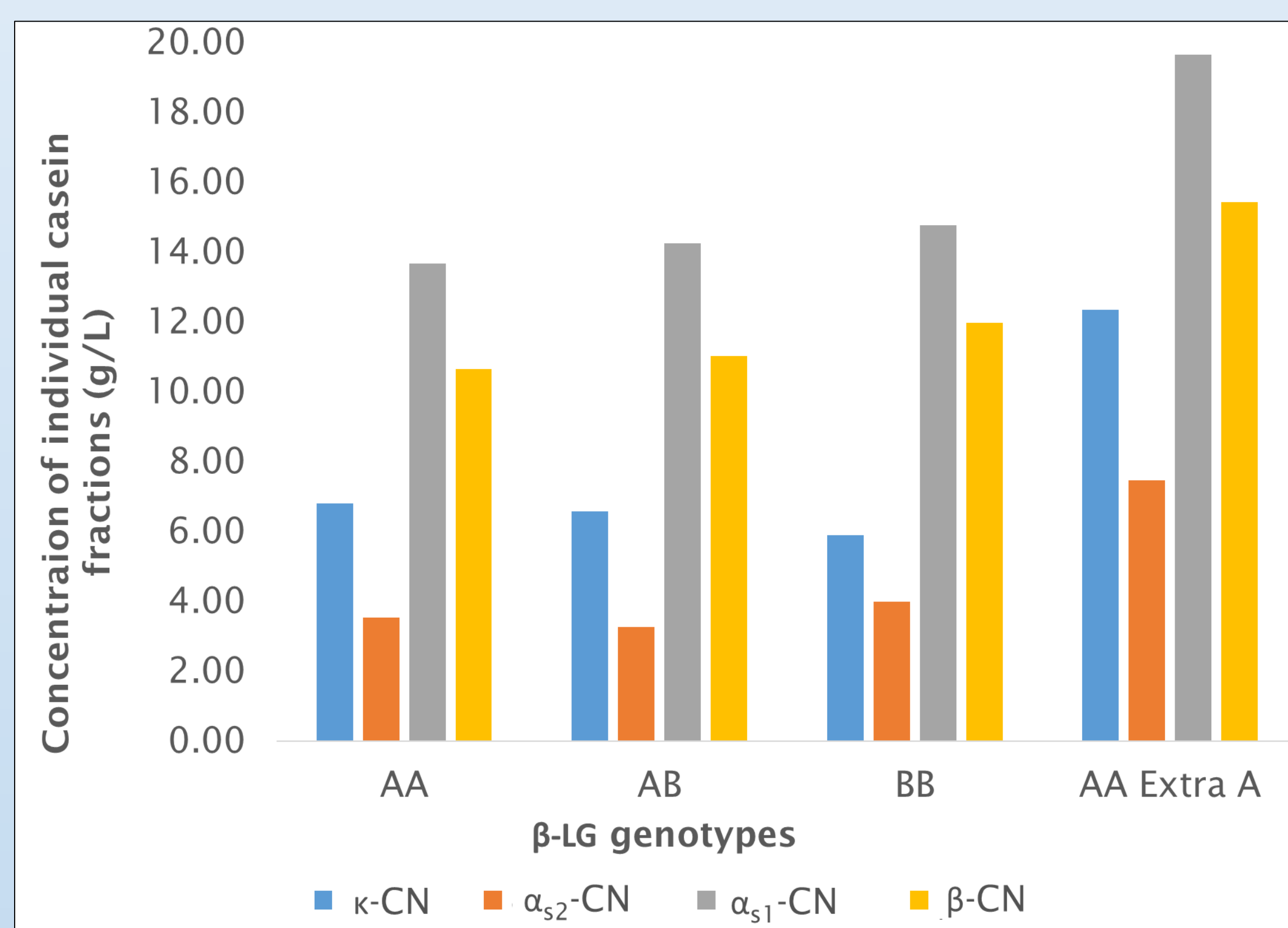


Figure 3. Relative concentration of casein fractions for milk of cows expressing different β -LG genotypes

- The κ -CN AA extra A genotype significantly influences α_{s2} -CN levels ($p < 0.05$).
- The significance of the result for κ -CN AA extra A should be interpreted with caution as only 2 individual samples had this genotype.

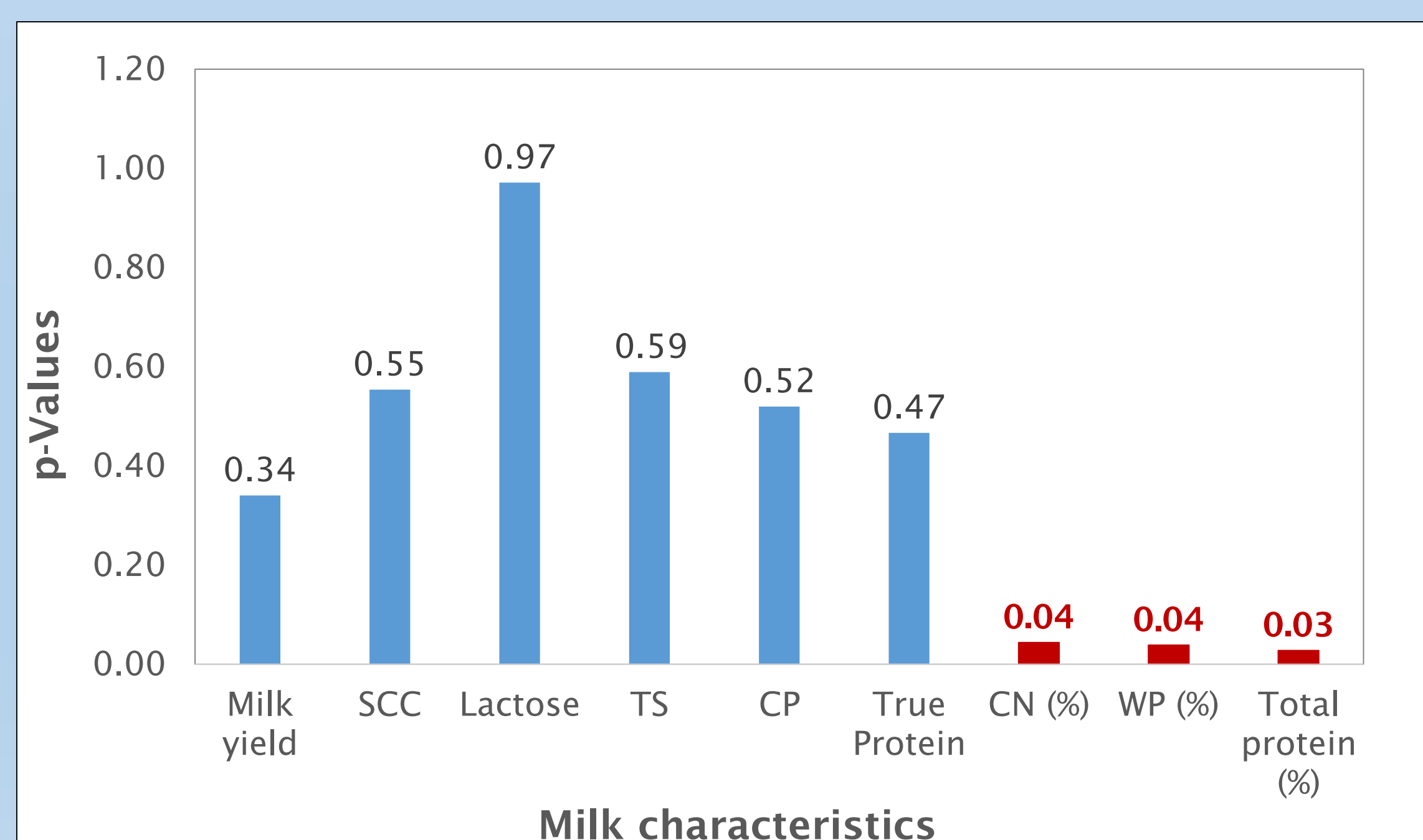


Figure 4. One-way ANOVA analysis of the effect of β -CN genotypes on milk characteristics (red effects are statistically significant).

- Results indicate that β -CN genotype has a statistically significant influence on:
 - Total casein % ($p = 0.04$)
 - Total whey protein % ($p = 0.04$)
 - Total protein % ($p = 0.03$)