Variation in phosphorylation degree of bovine α_{s2}-casein

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Caseins are highly phosphorylated milk proteins interacting with calcium phosphate to form large colloidal structures called casein micelles. Phosphorylation of casein is an important post-translational modification occurring in the mammary gland under the action of kinases. However, the molecular mechanism behind is not fully understood yet. These kinases recognize the consensus motif as a glutamic acid, phosphor-serine residue, or aspartate at the position n+2 relative to the target serine or threonine residue (**Ser**/Thr-x-**Glu/SerP**/Asp; Mercier, 1981). This modification is essential for the construction and the stabilization of the micelle structure which is based on interactions between phospho-serine or phospho-threonine residues of casein and calcium phosphate. α_{s2}-casein (α_{s2}-CN) is of particular interest since it is the most phosphorylated casein and exhibits various phosphorylation states. In addition, it has been the least investigated since its phosphorylated isoforms are difficult to purify and quantify. The objective of this study was to investigate the variation in the phosphorylation degree of α_{s2}-CN among individual cows.

Milk samples from 500 French Montbéliarde cows were collected as part of a R&D project (FROM'MIR)*, and were analyzed by liquid chromatography coupled with electrospray ionization mass spectrometry (LC/ESI-MS) to determine milk protein composition. Relative protein concentration of each α_{s2}-CN phosphorylated isoform was quantified by mass signal intensity (Miranda *et al.*, 2013). Method reproducibility was assessed by calculating the coefficient of variation (CV) of relative protein concentration for all α_{s2}-CN phosphorylated isoforms from a reference milk sample that was analyzed after every 10 milk samples. In total, 50 reference milk samples were analyzed.

α_{s2}-CN was characterized with multiple phosphorylation states containing 10 to 14 phosphate groups (10P-14P). This is the first study that shows the presence of α_{s2}-CN-14P, and the first method to determine the relative concentration for each α_{s2}-CN phosphorylated isoform. CV values for reproducibility of the relative protein concentration were below 6% for all the α_{s2}-CN phosphorylated isoforms. Furthermore, CV values for relative protein concentration regarding all the α_{s2}-CN phosphorylated isoforms (> 10%) were larger than CV values for reproducibility, which implies a significant variation in phosphorylation degree of α_{s2}-CN among individuals. Regarding to the correlation between the relative concentration of the different phosphorylation states of α_{s1}-CN and α_{s2}-CN, α_{s1}-CN-9P had negative correlation with α_{s2}-CN-10P and α_{s2}-CN-11P (-0.74 and -0.72, respectively), but positive correlation with α_{s2}-CN-13P and α_{s2}-CN-14P (0.69 and 0.67, respectively). This observation supports the previous finding on the relationship between α_{s1}-CN-8P and α_{s1}-CN-9P, which suggests that two different sets of genes are involved in regulation of phosphorylation isoforms (Bijl *et al*., 2014).

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