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## Characterization of isoforms of equine $\alpha_{s1}$ -casein: post-transcriptional variants and phosphorylation post-translational variants

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Unlike bovine caseins, equine and human caseins have variable degrees of phosphorylation. Before initiating research on the influence of the degree of phosphorylation on interactions with minerals of nutritional interest, equine caseins have to be precisely characterised. Equine  $\alpha_{s1}$ -casein exhibits complex electrophoretic patterns in bidimensional electrophoresis and in urea-electrophoresis which might be explained by the presence of several post-transcriptional variants (alternative splicing of exons and use of cryptic site) and post-translational variants (variable degree of phosphorylation). The purpose of this study was to elucidate the microheterogeneity of  $\alpha_{s1}$ -casein of Haflinger mare's milk. The  $\alpha_{s1}$ -casein was purified from whole sodium caseinate by hydrophobic interaction chromatography (HIC). Two fractions containing  $\alpha_{s1}$ -casein were obtained and were submitted to enzymatic dephosphorylation to determine the masses of the different isoforms present in milk. An original method of dephosphorylation associating alkaline phosphatase and acid phosphatase was developed to reach a complete dephosphorylation prior to LC/ESI-MS analysis of the apoforms. The full-length protein and three alternative splicing variants involving exon-skipping of exon 7 ( $\Delta 7$ ) or exon 14 ( $\Delta 14$ ) or both ( $\Delta 7-14$ ) were characterized by mass spectrometry. Furthermore, four variants resulting from the use of a cryptic splice site leading to the loss of the residue Gln-91 were identified for the full-length protein ( $\Delta Q$ ) and for each splicing variant ( $\Delta 7-Q$ ,  $\Delta 14-Q$ ,  $\Delta 7-14-Q$ ). The two fractions obtained by HIC were pooled and subfractionated by anion-exchange chromatography and analysed by LC/ESI-MS. Thanking to the determination of the masses of the different apoforms, the degree of phosphorylation of the full-length  $\alpha_{s1}$ -casein and of each of the splicing variants was determined. The  $\Delta 7$ ,  $\Delta 7-14$ ,  $\Delta 7-Q$  and  $\Delta 7-14-Q$  variants carried two to six phosphate groups whereas the full-length  $\alpha_{s1}$ -casein, and the  $\Delta 14$  and  $\Delta 14-Q$  variants had from two to eight phosphate groups. In conclusion, 36 different isoforms of  $\alpha_{s1}$ -casein were identified in Haflinger mare's milk explaining the complexity of its electrophoretic patterns.