

Expression and characterization of functional low-phenylalanine kappa-casein and its isolation from the milk of transgenic rabbits

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To work towards meeting the nutritional requirements of patients suffering certain metabolic diseases (e.g. phenylketonuria, needing a low-phenylalanine diet throughout their lives) transgenic rabbits were created to express low-phenylalanine (Phe) κ -casein in their milk. The feasibility of producing a mutated milk protein in parallel with the wild type milk constituents was demonstrated. Rabbit was chosen as model animal because its superior qualities compared to smaller laboratory as well as larger livestock animals. A chimeric gene construct containing the mammary gland specific, rabbit whey acidic protein promoter and the coding region of the rabbit κ -casein gene was modified by site specific oligonucleotide directed mutagenesis. Through modifying the corresponding codons all except one Phe amino acid residues present in the mature protein were mutated (Phe-39-Tyr, Phe-76-Tyr, Phe-88-Val, and Phe-125-Ser) to amino acids present in the human or mouse/rat sequences at the same positions to minimize alterations in the secondary structure. Phe at position 126 was preserved to maintain digestibility of the protein with chymosin/rennin, which is the first step in milk clotting. Via microinjection this mutated gene construct was used to create two transgenic rabbit lines with transgene copy numbers of 6 (line 82) and 3 (line 63). Transgenic rabbits produced the recombinant κ -casein at high concentration through the whole lactation period in their milk. Altered milk composition did not influence the lactation performance or overall health of the transgenic females. The incorporation of the mutated rabbit κ -casein into the casein micelles resulted in a significant (Student t test $P < 0.01$) reduction of the average micelle size. An increase in the viscosity of transgenic rabbit milk compared to the control was also observed. The low-Phe κ -casein was digestible with chymosin and the rennet clotting time was reduced compared to control milk. The recombinant κ -casein was separated from its native counterpart and from the other milk proteins by a one-step HPLC method on a reversed-phase C4 column. In the future low-Phe caseins produced in transgenic animals could be used as dietary replacements to help meet the special requirements of certain consumer groups.