Polymorphisms in the Pyruvate Kinase Gene and Milk Production Traits in Dairy Cattle

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Abstract:

Milk production is a critical trait in dairy cattle, and identifying genetic factors that influence this trait is paramount in modern livestock breeding programs. Pyruvate kinase liver and RBC (PKLR) is a candidate gene that has been implicated in milk production traits in cows due to its role in glycolysis, a key metabolic pathway. This study aims to explore the potential impact of non-synonymous single nucleotide polymorphisms (nsSNPs) in the *PKLR* gene on protein stability and function using a suite of computational tools. First, we identified 170 nsSNPs within the *PKLR* gene and subjected them to a rigorous computational analysis. Four widely used *in silico* tools, SIFT, Polyphen-2, SNAP2, and Panther, were employed to predict the deleteriousness of these nsSNPs. Surprisingly, these tools classified only 18 out of the 170 nsSNPs as potentially deleterious. This initial screening suggested that most nsSNPs in PKLR may not have a significant functional impact. To delve deeper into the potential impact of these nsSNPs, we assessed their effect on protein stability. We utilized multiple computational methods, including I-mutant, MUpro, CUPSTAT, SDM, and Dynamut, to predict the change in protein stability resulting from amino acid substitutions. Our analysis revealed that 9 out of the 18 nsSNPs were associated with decreased protein stability, indicating a potential functional consequence. The conservation of these nsSNPs across evolution was also evaluated using ConSurf analysis. Strikingly, all 18 nsSNPs were found to be located in evolutionarily conserved regions of the PKLR protein. This conservation suggests that alterations in these positions could have significant functional consequences. Further InterPro investigation identified two distinct domains within the PKLR protein: the Pyruvate Kinase barrel domain and the Pyruvate Kinase C Terminal domain. Out of the 18 identified nsSNPs, 12 were in the Pyruvate Kinase barrel domain, while the remaining 6 were in the Pyruvate Kinase C Terminal domain. This domain-specific distribution may provide insights into the functional significance of these nsSNPs. We generated a 3D model of the PKLR protein to gain structural insights using MODELLER software. This model was rigorously validated through Ramachandran plot analysis and Prosa, which collectively indicated a high-quality structure. Subsequently, we performed energy minimization calculations for both the native and mutated structures using SWISS PDB Viewer with GROMOS 96 program. Notably, three structural and four functional residues exhibited total energy values higher than the native model, suggesting that these mutant structures were less stable. To confirm the impact of these nsSNPs on PKLR protein

structure and function, we conducted Molecular Dynamics simulations. These simulations provide dynamic insights into how these genetic variants might affect the protein's behaviour over time. This study provides valuable insights into functional genetic variations in PKLR that may influence milk production traits in cattle, offering a foundation for further experimental investigations and potential applications in selective breeding programs.

Keywords: protein stability; evolutionary conservation; structural analysis; computational tools; genetic variations.

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