Development of a PCR-RFLP Test to Discriminate between Beta-Casein B and $\text{C}^{\text{BT}}$ Allele in Romanian Buffalo

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SUMMARY

The only genetic variant known in the $\beta$-casein locus in Italian Buffalo and other European buffalo breeds, namely B, is currently used as a genetic marker to identify adulteration with cattle milk of buffalo cheeses, after a procedure of plasmin digestion and IEF migration of digestion products (gamma caseins) (Addeo et al., 1990). In our previous work we identified with a 14% frequency and confirmed by molecular analysis the hypothesis of the existence of two new additional genetic variants in Romanian Buffalo, not observed in other European or Asian breeds (Balteanu et al., 2008), renamed $\alpha$S1-casein B$^{\text{BT}}$ and $\beta$-casein $^{\text{BT}}$ ($\text{BT} =$ Transylvanian Buffalo) (Balteanu, 2010). The substitution of a G (found in B allele) with a C (found in C$^{\text{BT}}$ allele) from exon 7, leads to a Lys to Asn substitution in the position 68 of mature protein. This changes plasmin digestion pattern of the two protein variants and as a consequence leads to a misidentifying of buffalo cheese authenticity using the European official detection method. Therefore the objective of this study was to set up a PCR-RFLP test as an alternative test for the oficial method. Based on the G-C substitution (exon 7) a PCR-RFLP test was set up, with specific primers designed from intron 6-exon 7, covering a 723 bp fragment. The digestion was performed with DdeI enzyme (Fermentas, Vilnius, Lithuania). As long as this new $\beta$-casein variant appears only in Romanian buffalo it could be used as genetic marker for Romanian Buffalo cheeses traceability.

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REFERENCES