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RNA secondary structure and alternative splicing in the 3'-UTR of DGAT1 gene in the indicine Guzerat breed

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The DGAT1 gene encodes diacylglycerol O-acyltransferase (EC 2.3.1.2), the rate limiting enzyme for triglyceride synthesis. This gene is the most important known component of the genetic variance in parameters of milk production and composition discovered so far. In several Bos taurus breeds, the SNP DGAT1 K232A explains 2 to 50% of the variance in such parameters. DGAT1 232K allele was correlated with less milk protein and higher fat contents. To the other hand, the DGAT1 232A allele was correlated with lower saturated fat, leading to a healthier milk for human consumption. We found low frequencies of DGAT1 232A in Zebu breeds in Brazil (between 0 and 2%). However, the fact that the allele of interest is rare does not mean that there are no other breed specific gene variations that could be used in association studies. Genetic variations leading to alternative splicing or changing RNA secondary structures that may impact gene regulation have not yet been described for this gene. Therefore, we sequenced the DGAT1 3'-untranslated region in a group of eight individuals of the indicine Guzerat breed and performed a search for regulatory elements. Six new SNPs one indel were identified. The allele DGAT1 indel (+) is involved in a significant gain in milk production. Significant associations ($p = 0.05$) of the indel polymorphism were found for BVs of lactation in 305 days, total fat and protein. In silico analysis suggested the existence of possible ORFs in the 3´region of this gene. Besides, six haplotypes were identified with Phase software, which were used in the ascertain of the effects of these genetic variations upon RNA secondary structure and upon alternative splicing in the 3´region. Alternative splicing was ascertained with ASSP and ASPICDB softwares and the prediction of RNA secondary structure was developed with Vienna package. Using the ASPICDB it was possible to identify eight alternative splicing

sites that were already described for this gene. Variations between haplotypes were identified with he ASSP software. When compared to the reference sequence, haplotype 5 presented a new acceptor splicing site and the absence of an alternative isoform. Haplotype 6 presented a displacement of an acceptor splicing site and the absence of two alternative isoforms. For the analysis of secondary structure, we considered the structures generated from the minimum free energy (MFE). Each one of the six haplotypes presented some differences in the configurations of the handles, but haplotype 5 was the most divergent. This initial analysis allowed us to conclude that all polymorphisms founded may play a different role in the functional regulation granting subsequent in vitro analysis.