Abstract

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Biochemical Sciences

Candidate: Michaela Růžičková

Supervisor: Ing. Petra Matoušková, Ph.D.

Title of diploma thesis: Reference gene selection for mRNA quantification in *Haemonchus contortus*

*Haemonchus contortus* is a parasitic nematode, whose multiresistance to antihelmintics means global problem, threatening mostly small ruminants farming. Genom and transcriptom have been published in 2013, allowing gene expression studies to be conducted. Reference genes for this studies have not been validated yet. Use of suitable reference genes is essential for accurate normalization of gene expression levels. Aim of this work was to identify and validate potential reference genes for gene expression studies in *H. contortus* adults. Eleven genes were chosen, stability of their expression was assessed in males and females of two *H. contortus* strains, one drug-susceptible (ISE) and one multi-drug-resistant (WR). Total RNA was extracted and reverse transcribed to cDNA. cDNA was diluted and analyzed using quantitative real-time PCR with SYBR Green I detection. Expression stability was evaluated by computer programs BestKeeper, geNorm, NormFinder and the comparative C_{T} method. Ncbp, ama, sodc, gapdh and farb were found to be most stable genes. By this study we demonstrated, that the combination of commonly used gapdh gene and at least one of the other best ranked genes would be appropriate for gene expression studies in *H. contortus* adults.