BOVINE GAPDH EXPRESSION USING REAL-TIME RT-PCR

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Summary. Gene expression analysis is increasingly important in biological research, while real-time reverse transcription PCR (RT-PCR) is becoming the method of choice for high-throughput and accurate expression profiling of selected genes. The aim of this study is measuring GAPDH expression levels for future researche as housekeeping gene for IGF-1. For compensating of variations in input RNA amounts and efficiency of reverse transcription, differnt endogenous housekeeping genes have been quantified, and results were normalized to these values. However, the normalization using housekeeping genes, in many cases of IGF-1 studies, was unsuccessful. We measured the GAPDH expression in different cattle tissues, blood, liver and skeletal muscle, with ready-to-use kits from Roche. For the experiments the real-time RT-PCR LightCycler technology was used. The GAPDH expression determination with SYBR Green I was performed with high linearity (R=1.0) and with small mean squared error (Error=0.098) over three orders of magnitude of molecules. The highest gene expression was observed in cattle muscle. The recommendation for changes in quantification protocol has been given. The most effective temperature for quantification is 84 - 85°C.

Abbreviations used: GAPDH - glyceraldehyde-3-phosphate-dehydrogenase, IGF-1 – insulin like growth factor 1, RT-PCR – reverse transcription polymerase chain reaction.

Keywords: glyceraldehyde-3-phosphate dehydrogenase, housekeeping gene, IGF-1, real-time RT-PCR, LightCycler.