

## Selection of Suitable Reference Genes for Assessing Endurance Related Traits in a Native Pony Breed of Zanskar at High Altitude

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**Abstract :** High performance of endurance in equid requires adaptive changes involving physio-biochemical, and molecular responses in an attempt to regain homeostasis. We hypothesized that the identification of the suitable reference genes might be considered for assessing of endurance related traits in pony at high altitude and may ensure for individuals struggling to potent endurance trait in ponies at high altitude. A total of 12 mares of ponies, Zanskar breed, were divided into three groups, group-A (without load), group-B, (60 Kg) and group-C (80 Kg) on backpack loads were subjected to a load carry protocol, on a steep climb of 4 km uphill, and of gravel, uneven rocky surface track at an altitude of 3292 m to 3500 m (endpoint). Blood was collected before and immediately after the load carry on sodium heparin anticoagulant, and the peripheral blood mononuclear cell was separated for total RNA isolation and thereafter cDNA synthesis. Real time-PCR reactions were carried out to evaluate the mRNAs expression profile of a panel of putative internal control genes (ICGs), related to different functional classes, namely glyceraldehyde 3-phosphate dehydrogenase (GAPDH),  $\beta_2$  microglobulin ( $\beta_2M$ ),  $\beta$ -actin (ACTB), ribosomal protein 18 (RS18), hypoxanthine-guanine phosphoribosyltransferase (HPRT), ubiquitin B (UBB), ribosomal protein L32 (RPL32), transferrin receptor protein (TFRC), succinate dehydrogenase complex subunit A (SDHA) for normalizing the real-time quantitative polymerase chain reaction (qPCR) data of native pony's. Three different algorithms, geNorm, NormFinder, and BestKeeper software, were used to evaluate the stability of reference genes. The result showed that GAPDH was best stable gene and stability value for the best combination of two genes was observed TFRC and  $\beta_2M$ . In conclusion, the geometric mean of GAPDH, TFRC and  $\beta_2M$  might be used for accurate normalization of transcriptional data for assessing endurance related traits in Zanskar ponies during load carrying.

**Keywords :** endurance exercise, ubiquitin B (UBB),  $\beta_2$  microglobulin ( $\beta_2M$ ), high altitude, Zanskar ponies, reference gene

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