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Evaluation of stable reference genes and expression analysis of ATP1A1 gene in various tissues of riverine buffaloes (*Bubalus bubalis*)

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Riverine buffaloes are major livestock species contributing greatly to the dairy sector of our country. Till date, no suitable reference genes are available to normalize the transcriptional data across tissues of riverine buffaloes. Also the expression profiling of alpha1 sodium-potassium adenosine triphosphatase (*ATP1A1*) gene, an important candidate for its key role in adaptation and heat tolerance is not known across various tissues of riverine buffaloes. In the present study, a panel of 10 reference genes (*GAPDH, ACTB, UXT, RPS15, RPL4, RPS9, RPS23, HMBS, β2M and EEF1A1*) were evaluated using three different algorithms, geNorm, NormFinder and BestKeeper to identify most stable reference in tissue samples (mammary gland, kidney, spleen, liver, heart, intestine, ovary, lung, muscle, brain and fat). The M-value given by geNorm ranged from 0.9797 (RPS9 and UXT) to 1.7362 (RPS15). From the most stable to the least stable, genes were ranked as: UXT/RPS9>RPL4>RPS23>EEF1A1>B-ACTIN>HMBS>GAPDH>B2M>RPS15. NormFinder analysis ranked the reference genes according to the stability value as: UXT>RPS23>RPL4>RPS9>EEF1A1>HMBS>β-ACTIN>β2M>GAPDH>RPS15. Based on the crossing point SD value and range of fold change expression, BestKeeper analysis classified the genes from most to least stable as: RPS9> RPS23/UXT>RPL4>GAPDH>EEF1A1>β-ACTIN>HMBS>β2M>RPS15. Our data identified RPS23, RPS9, RPL4 and UXT gene to be the most stable and appropriate reference genes that could be utilized for normalization of transcriptional data in various tissues of buffalo. Our data showed expression of ATP1A1 in all the tissues though expression level varied in the following order: Kidney>heart>brain>lung>ovary>liver>fat>mammary glands>intestine>spleen> muscle. Relative expression of mRNA ATP1A1 in kidney was 4 folds higher expression thanin muscles. Hence the present study provides panel of stably expressed reference genes that can be utilized for functional studies in riverine buffaloes. In future such study will pave way to study functional aspects of buffalo specific genes including ATP1A1 that will enrich the bubaline genome.

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Polymorphism in prolactin promoter region and egg production performance in Kadaknath hens

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Present study was carried out to study egg production performance and polymorphism of Prolactin gene at 24 bp indel locus at promoter region (PRL24). Egg production performances were recorded as age at first laying (AFE), Body Weight at First Egg (WFE), Mean Egg Weight (MEW) and Total No. of Eggs at 90 days of laying (TEN). DNA was isolated from 2-3 of blood of 20 birds collected from wing vein. PRL24 locus for indel polymorphism was amplified by PCR and the product was resolved on 6% native PAGE for genotyping. The AFE (d), WFE (Kg), MEW (g) and TEN of Kadaknath hens in the present study were found to be 188.00±0.71, 1.26±0.03, 42.83±0.21 and 37.75±0.59 respectively. The prolactin gene locus PRL24 showed two alleles I & D and three genotypes: II, ID & DD. The frequencies of I and D alleles at this locus were 0.55 & 0.45 respectively. The birds of D allele had a significantly ($P<0.05$) better TEN than birds of I allele.

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