Validation of Reference Genes for Gene Expression Profiling in Bovine Tissues

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In the cow-calf segment of beef production within the animal agriculture industry, reproductive success and efficiency are vital to sustainable production of beef. Recent studies have indicated that mRNA transcripts are useful in determining the fertility status of heifers. The aim of this study was to identify reference genes that can be used as endogenously expressed controls in bovine tissues to research the potential of mRNA transcripts to identify heifers that exhibit good or poor reproductive performance.

Primer sets for *Tbp* and *B2m* were designed, and specificity and efficiency tested. *Tbp* and *B2m* were then compared to the *Gapdh* reference gene, which was previously identified, and specificity and efficiency tested. Expression levels of two previously designed and validated target genes, connexin-43 and pannexin-1, were compared following normalization using the reference genes. RNA was isolated from three sets of eight bovine tissues including ovary, heart, muscle, skin, pancreas, kidney, liver, and spleen. The isolated RNA was checked for quality and concentration and used to synthesize cDNA. The cDNA was used to perform quantitative real time PCR, and the results were analyzed using the delta-delta Ct comparison method to determine which reference genes followed the same trend across the tissues (Figure 1). The PCR products from the ovary tissue were run on a gel electrophoresis to confirm the product size and specificity of the generated product for each primer to insure the validity of our results.

Gapdh and *B2m* generally followed the same trend across the tissues, whereas *Tbp* had tissues with differing results. The average cycle threshold for the three reference genes was compared across tissues with *Tbp* being significantly higher than *Gapdh* and *B2m* in muscle (P<0.05). In the heart tissue, there is a significant difference between *Tbp* and *Gapdh* only (P<0.05).

This result indicates that *Tbp* might not be as reliable as *Gapdh* and *B2m* as reference genes. *Tbp* demonstrated significant differences from *Gapdh* and *B2m* in the heart tissue, when comparing the ΔCt normalizations of Connexin-43 to the three reference genes (P<0.05). *Tbp* was also significantly different from *B2m* in the spleen tissue (P<0.05), but not significantly different from *Gapdh* (P>0.05). The general trend of the reference genes was similar across the tissues, when comparing the normalization of Pannexin-1 to the three reference genes. However, *Tbp* again showed a significant difference from *B2m* in the spleen tissue (P<0.05).

As predicted, the results indicated varying expression of the reference genes in the tissues tested, but the expression of the *Gapdh* and *B2m* reference genes followed the same general trend across the tissue types. However, there are significant discrepancies in the expression of *Tbp*, which raises the question of the validity of the use of *Tbp* as a reference gene in certain bovine tissues. *B2m* and *Gapdh* would potentially be suitable reference genes in the tested bovine tissues. The data generated for each potential reference gene in bovine tissues will allow for further study of the specific mRNA transcripts valuable in identifying heifers that will demonstrate either good or poor reproductive performance.

Statment of Research Advisor

Rachel's project goal was to develop markers able to increase the reliability of mRNA data in our lab. Her work resulted in tools that will benefit transcriptional data analysis in studies looking at developing heifer fertility markers.
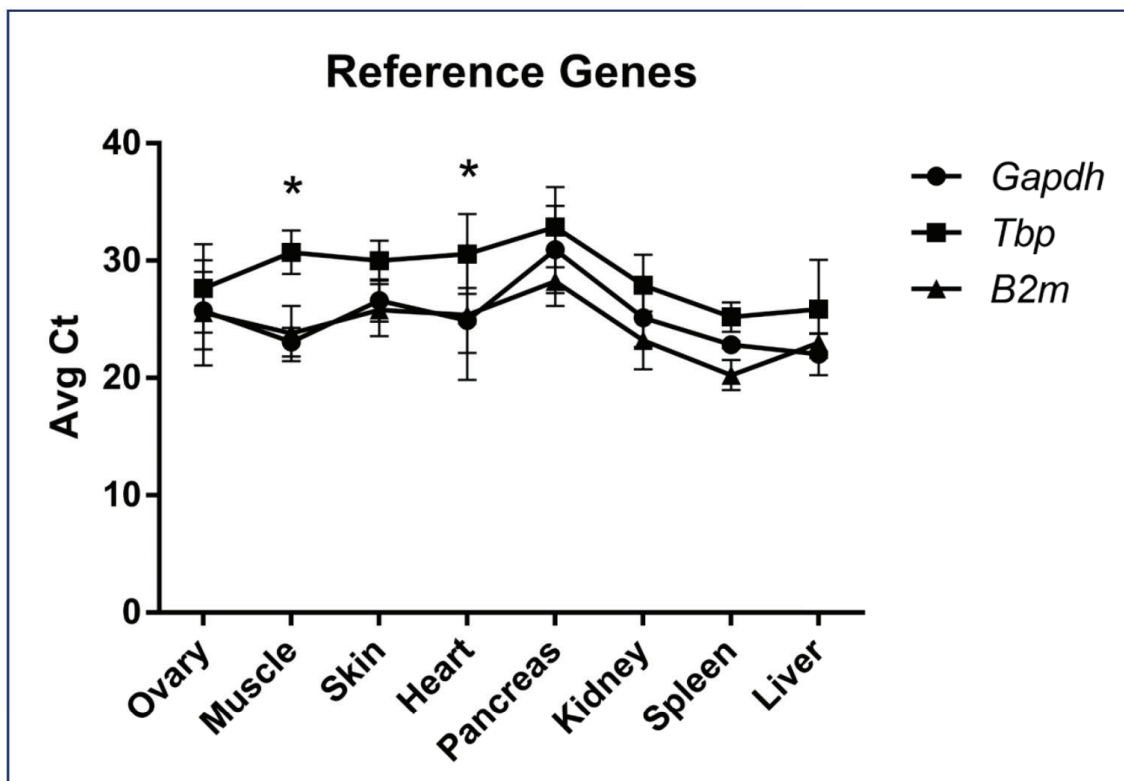
-Paul W. Dyce, Animal Science

Figure 1. Reference Genes: The average Ct (cycle threshold) of the three reference genes across three trials was compared and graphed. *Gapdh* and *B2m* follow the same trend across the various tissues. This is fairly consistent with the trendline of *Tbp*; however, the muscle and heart tissues show statistically significant differences in expression. In muscle, the average Ct of *Tbp* is significantly different from both the *Gapdh* and *B2m* average Ct. However, in the heart tissue, the average Ct of *Tbp* is only significantly different from *Gapdh*. *p<0.05 statistically significant.