

Alternative Splicing of Slow Troponin T mRNA in Bovine Skeletal Muscle

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Variation in meat quality, particularly tenderness, is a significant issue for the Australian beef industry. Recent research efforts have been directed towards understanding the underlying genetic factors involved in tenderness. Alternative splicing (AS) is a post-transcriptional mechanism in which a single gene is capable of producing two or more transcripts by joining different 5' and 3' splice sites, often resulting in proteins of distinct functions (Stamm *et al.* 2005). Slow Troponin T (*TNNT1*) mRNA, which encodes a myofibrillar structural protein which undergoes degradation during post-mortem tenderization, occurs as two splice variants in skeletal muscle; however the relationship of these two splice variants to meat quality is unknown. The aim of this study is to investigate the effect of sex and the use of a hormone growth promotant (HGP; Revalor®H; 140mg Trenbolone acetate, 14mg Estradiol) on the mRNA levels of the two splice variants of *TNNT1*.

Brahman steers (M) and heifers (F), of similar live weight (mean \pm SD 311 \pm 31kg), were either administered HGP (M+HGP, n=20; F+HGP, n=21) or not (M-HGP, n=17; F-HGP, n=22) and finished on a similar concentrate diet in a feedlot for 120 days. Muscle samples were collected from the *M. Longissimus lumborum* (LM) at slaughter, and total RNA was isolated (TRIzol reagent), purified and reverse transcribed (Invitrogen). Specific primers were designed (Primer3) to amplify the full and alternate splice variants separately, i.e. one common reverse primer in exon 8, and two forward primers spanning exon 4-6 and 5-6. Quantitative reverse transcriptional (qRT) PCR was performed using SYBR® Green I Dye (Applied Biosystems) and the results were normalised to RPLPO mRNA (using Qgene software) and analysed using PRISM software.

The abundance of the *TNNT1* splice variant was 178 and 203-fold greater than the abundance of the full transcript in the LM muscle of -HGP and +HGP animals, respectively ($P < 0.01$; Figure 1). Further, the ratio of the splice variant to full transcript was greater in M+HGP than F+HGP ($P < 0.01$; Figure 2); there was no difference in this ratio when animals were not treated with the HGP.

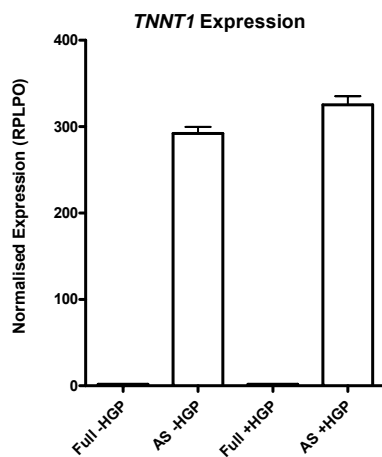


Figure 1. Normalised expression of full and alternatively spliced transcripts of *TNNT1* in -HGP and +HGP LM muscle samples

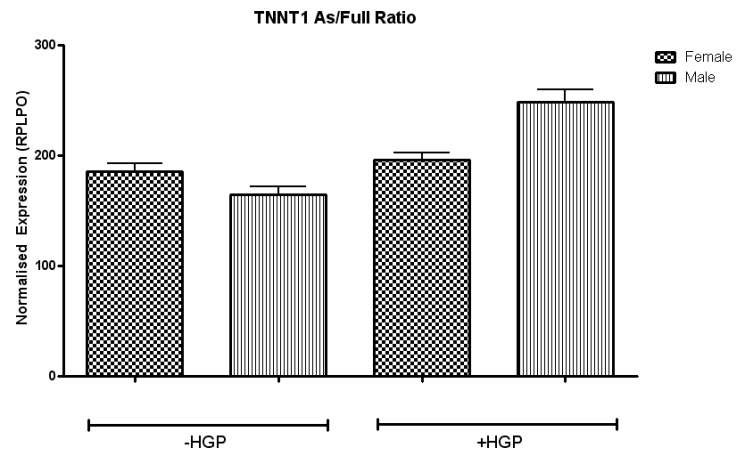


Figure 2. Normalised expression of full and alternatively spliced transcripts of *TNNT1* in male and female +HGP and -HGP LM muscle samples

In conclusion, the *TNNT1* alternate splice variant is more abundant than the *TNNT1* full transcript in the LM muscle of Brahman cattle, possibly suggesting different functional roles. The *TNNT1* splice variant appears to be up-regulated in males compared to females receiving HGP, relative to the full transcript, possibly resulting from the differential response of males and females to growth promotants. The relationship of the *TNNT1* splice variant with objective measurements for tenderness in these cattle will be assessed in the future.

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Stamm, S., Ben-Ari, S., et al. (2005). *Gene* 344: 1.

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