REALTIME PCR ANALYSIS OF MRNA TRANSCRIPTS IN POST-MORTEM BOVINE MUSCLE

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Introduction

The application of approaches such as transcriptome and proteome analysis has opened up new possibilities for discovering genes, proteins or molecular polymorphisms that control muscle growth and meat quality traits (Hocquette *et al.*, 2001). Transcriptomics enables analysis of the complete set of RNA transcripts produced by the genome at a given time and provides a dynamic link between the genome, the proteome and the cellular phenotype. However to carry out such analysis, high quality undegraded RNA is necessary. The stability of mRNA has emerged as a key step in the regulation of eukaryotic gene expression with half-lives of mRNAs varying from a few minutes to more than 24 hours (Tourrière *et al.*, 2002). In human tissue, extensive RNA stability was observed up to 38-48 h post-mortem (Schramm *et al.*, 1999). Since access to muscle tissue is usually post-mortem, there can be an appreciable time lag in commercial abattoirs between time of slaughter and muscle excision online. Post-mortem stability in fibrous tissue, such as muscle, has not been extensively studied, and is of particular interest in respect of analyzing gene expression in meat-producing animals in relation to meat quality. The aim of this study was to investigate RNA integrity from bovine striploin muscle, collected 0.5 – 48 h post-mortem in an abattoir environment. Subsequently, GAPDH and Calpain II mRNA transcript levels were quantitatively determined using realtime PCR.

Materials and Methods

Heifers were slaughtered under controlled conditions at an abattoir facility at Ashtown Food Research Centre, Dublin. Samples were excised from the *longissimus dorsi* (LD) muscle at intervals of 0.5 – 48 h post-mortem. Samples were stored in RNAlater or flash frozen in liquid nitrogen and stored at –20°C or –80°C as appropriate. Total RNA was isolated using (1) TRIreagent or (2) the commercial Qiagen RNeasy mini-kit and all samples were subsequently DNAsed. The Agilent Bioanalyzer (Agilent Technologies, Germany) was used to calculate the integrity and concentration of total RNA. Samples with RNA Integrity Numbers (RIN) of >7 are considered to be of sufficient quality for downstream gene expression analysis.

Randomly primed reverse transcription was carried out for first strand cDNA synthesis. PCR was performed using primers designed specifically for glyceraldehyde-3 phosphate dehydrogenase (GAPDH 216 bp) (U85042) and calpain II (287 bp) (J05065). RT-PCR products were analysed by agarose gel electrophoresis. Realtime RT-PCR analysis was carried out for mRNA transcript detection using the Faststart SYBR Green 1 master mix kit (Roche Diagnostics, UK). Standard dilutions were used to generate crossing point values which were used to quantify the transcript levels of GAPDH and calpain II of bovine muscle samples collected from 0.5 – 48 h post-mortem.

Results and Discussion

The Agilent Bioanalyzer was used to assess total RNA integrity and concentration and is regarded as a quality control standard for all routine gene expression experiments. Meat samples stored in RNAlater at -20°C, -80°C or flash frozen, from which RNA was isolated using TRIreagent or the commercially available kit all gave high quality total RNA up to 2 days post-mortem (Table 1). Good RNA Integrity Numbers (RIN >7) were obtained for all of these samples. The RIN is a software tool designed to estimate the integrity of total RNA samples. The expert software automatically assigns an integrity number to a eukaryote total RNA sample, with RIN values >7 being considered high quality RNA. Therefore, these extracts are of sufficient quality for gene expression analysis using total RNA.

RT-PCR analysis showed that transcript intensity appeared to remain constant for GAPDH (housekeeping gene) and calpain II (involved in post-mortem meat tenderisation). From initial quantitative realtime RT-PCR analysis, which accurately determines transcript levels, it appears that there is no difference in transcript quantity for GAPDH and calpain II up to 48 h post-mortem, suggesting that transcripts are relatively stable up to 48 h post-mortem (Figure 1). Further samples are being tested over this post-mortem period and the numbers of candidate genes examined will be expanded.

Table 1: RNA Integrity Numbers (RIN) obtained from bovine muscle cellular RNA samples using the Agilent 2100 Bioanlayser. RIN >7 are indicative of high quality RNA, *pm* = time post-mortem, 1 = muscle samples stored in RNAlater at -20°C; 2 = muscle samples stored in RNAlater at 4°C for 24 hours followed by subsequent RNAlater removal and tissue storage at -80°C; 3 = muscle samples flash frozen in liquid nitrogen and stored at -80°C.

Extraction Method	TRIReagent			Qiagen RNeasy kit		
Storage	1	2	3	1	2	3
Sample	RNA Integrity Number (RIN)					
0.5h *pm*	8.5	8.5	8.5	7.4	8.4	7.6
1h *pm*	8.6	8.5	7.9	7.9	7.9	7.5
1.5h *pm*	8.7	8.5	7.9	7.6	7.5	7.5
2h *pm*	8.7	8.6	7.9	8.0	8.0	7.3
3h *pm*	8.3	8.3	8.2	7.7	7.6	7.7
4h *pm*	9.2	8.6	7.7	7.7	7.8	7.5
5h *pm*	8.7	8.2	7.5	7.9	7.9	7.4
24h *pm*	8.1	8.5	7.8	8.1	7.9	7.6
48h *pm*	8.2	8.6	7.6	7.5	7.4	7.5

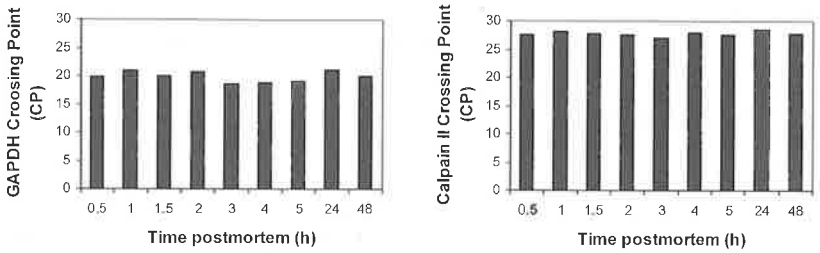

Figure 1: Realtime RT-PCR analysis of GAPDH and calpain II mRNA transcripts. Standard dilutions (of known copy number) generated crossing point values which were used to determine GAPDH and calpain II copy numbers in unknown bovine muscle samples collected from 0.5 – 48 h post-mortem.

Conclusions

The Agilent Bioanalyzer is a fast and reliable method of assessing RNA integrity in addition to making a quantitative measurement. Either flash freezing or storing muscle samples in RNAlater enabled the extraction of high quality total RNA from 0.5 – 48 h post-mortem, indicating high bovine muscle RNA stability post-mortem. High quality total RNA, suitable for gene expression analysis, was isolated from beef up to 2 days post-mortem demonstrating that a time delay between slaughter and sample collection should not adversely affect cellular RNA quality.

References

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