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POSTMORTEM STABILITY PROFILE OF BOVINE MUSCLE RNA

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Introduction

The production of consistently tender, well-flavoured and juicy meat products remains elusive in the meat production sector, with the complex *postmortem* tenderisation process still not fully understood. Meat quality may be affected by differential expression of muscle genes and their protein products in response to factors such as nutrition, handling, environment or growth rate. The new scientific orientation towards transcriptome and proteome analysis has opened up new possibilities for discovering molecular predictors (genes, proteins or molecular polymorphisms) that control muscle growth and meat quality traits (Hocquette *et al*., 2001). The identification of such genes expressed in skeletal muscle tissue is a necessary step toward a better understanding of the genetic basis of meat production (Davoli *et al*., 1999) and may have a revolutionary impact on understanding the biology of meat quality. However to carry out such analysis, high quality undegraded RNA is necessary. The half-lives of mRNAs may vary from a few minutes to more than 24 hours and changes in mRNA stability are ultimately reflected in the amount of protein produced, the stability of mRNA has emerged as a key step in the regulation of eukaryotic gene expression (Tourrière *et al*., 2002). As there can be a considerable time lag in commercial abattoirs between time of slaughter and muscle excision online, it is important to determine whether RNA stability in muscle tissue, particularly in M. *longissimus dorsi* (striploin), is influenced by time *postmortem*.

Postmortem 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.

Objectives

The objective of this research was to determine the RNA integrity and quantity from bovine muscle tissue, collected *postmortem* in an abattoir environment. Total RNA stability was measured over a 14-day *postmortem* period, by assessing cellular RNA integrity using electrophoresis, the Agilent Bioanalyzer system, RT-PCR and real time RT-PCR.

Methodology

Heifers were slaughtered under controlled conditions at the abattoir facility at the National Food Centre, Dublin, Ireland. Samples were excised from the *longissimus dorsi* (LD) muscle at intervals of 1 hour to 14 days for the first animal (14 days is the normal length of the *postmortem* ageing process) and 0.5 hour to 2 days for the second animal (thereby focusing on the early *postmortem* period). Samples were stored in RNAlater or flash frozen in liquid nitrogen and stored at –20°C or –80°C as appropriate. RNAlater (Ambion, Inc.) is a tissue storage reagent that rapidly permeates tissue to stabilise and protect cellular RNA without jeopardising the quality or quantity of RNA (Reisz-Porszasz *et al*., 2003). Total RNA was isolated from 100mg of frozen tissue by (1) homogenisation in TRIreagent and precipitation with chloroform and isopropanol or (2) with the commercial Qiagen RNeasy mini-kit. All samples were DNAsed to remove contaminating genomic DNA.

The Agilent Bioanalyzer (Agilent Technologies, Germany) was used to calculate the integrity and concentration of total RNA, as it offers the advantage of assessing RNA integrity in addition to making a quantitative measurement and is regarded as a quality control standard for all routine gene expression experiments, i.e. microarray and realtime PCR analysis. Samples with rRNA ratios (28S/18S) of 1 or greater and values of greater than 13 for '% of Total Area of the 28S peak' 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 the housekeeping genes glyceraldehyde-3 phosphate dehydrogenase (GAPDH) (Accession number U85042) and beta-actin (Accession number AF191490). RT-PCR products were analysed on agarose gels using electrophoresis. Realtime RT-PCR analysis was carried out for DNA detection using SYBR Green 1 (Roche Diagnostics, Ltd., UK).

Results & Discussion

The *postmortem* stability profile of bovine muscle is of particular interest as regards gene expression analysis since *postmortem* tissues have traditionally been regarded as unsuitable sources of RNA for gene expression studies due to the rapid degradation of RNA.

Using gel electrophoresis, 28S and 18S rRNA bands were detectable up to 8 days *postmortem*, with visible degradation being observed as the *postmortem* interval increased up to 14 days (Figure 1).

The Agilent Bioanalyzer was used for a more accurate and reproducible assessment of total RNA integriy and concentration. Samples analysed initially with the Bioanalyzer showed that total RNA integrity decreases dramatically from 2 day to 14 days *postmortem* so further analysis focussed on the 0.5 hour to 2 day *postmortem* interval. Meat samples stored in RNAlater from which RNA was isolated using TRIreagent or the commercially available kit gave high quality total RNA up to 2 days *postmortem* (Figure 2). Good rRNA ratios (>1) and values for '% of Total Area of 28S peak' (>13) were obtained for these samples. These extracts are of sufficient quality for gene expression analysis using total RNA. Total RNA isolation from meat samples flash frozen in liquid

nitrogen gave a lower RNA integrity than those stored in RNAlater (Figure 2). Poly(A) RNA (mRNA) makes up between 1-5% of total cellular RNA and is most frequently used for detection and quantitation of extremely rare mRNAs, and while high quality total RNA indicates good overall RNA stability, mRNA integrity will have to be assessed for generating an accurate gene expression profile.

RT-PCR transcripts of varying intensity were evident up to 10 days *postmortem* for GAPDH and 9 days *postmortem* for beta-actin (Figure 3). Band intensity decreased between day 2 and day 3 *postmortem*. Despite the overall stability of RNA specific for GAPDH and beta-actin, individual RNAs specific for other genes may undergo more rapid decay and is currently being investigated. Realtime RT-PCR analysis, which is a more sensitive technique to determine transcript levels, showed that GAPDH transcripts were evident up to 6 hours *postmortem* after which RNA integrity and transcripts begin to decrease (Figure 4).

Conclusions

Determining RNA integrity and quantity is an essential step in generating an accurate gene expression profile. Storing muscle samples in RNAlater, stabilised and protected RNA samples for extraction of high quality total RNA. The Agilent Bioanalyzer is a fast and reliable method of assessing RNA integrity in addition to making a quantitative measurement. High quality total RNA, suitable for gene expression analysis, was isolated from beef up to 2 days *postmortem* demonstrating that a time delay between slaughter and sample collection should not adversely affect cellular RNA quality.

References

Davoli, R., Zambonelli, P., Bigi, D., Fontanesi, L. & Russo, V. 1999. Analysis of expressed sequence tags of porcine skeletal muscle. Gene 233:181–188.

Hocquette, J.F., Cassar-Malek, I., Listrat, A., Jurie, C., Jailler, R. & Picard, B. 2001. Some recent advances in muscle biology and its regulation by nutrition: consequences for bovine meat quality. Recent Advances in Animal Nutrition in Australia 13:135–143.

Reisz-Porszasz, S., Bhasin, S., Artaza, J.N., Shen, R., Sinha-Hikim, I., Hogue, A., Fielder, T.J. & Gonzalez-Cadavid, N.F. 2003. Lower skeletal muscle mass in male transgenic mice with muscle-specific overexpression of myostatin. Am J Physiol Endocrinol Metab, 285(4):876–888.

Tourrière, H., Chebli, K. & Tazi, J. 2002. mRNA degradation machines in eukaryotic cells. Biochimie 84:821–837.

Tables and Figures

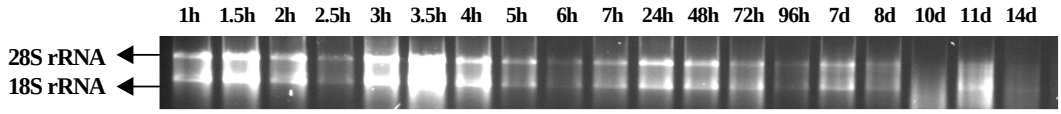
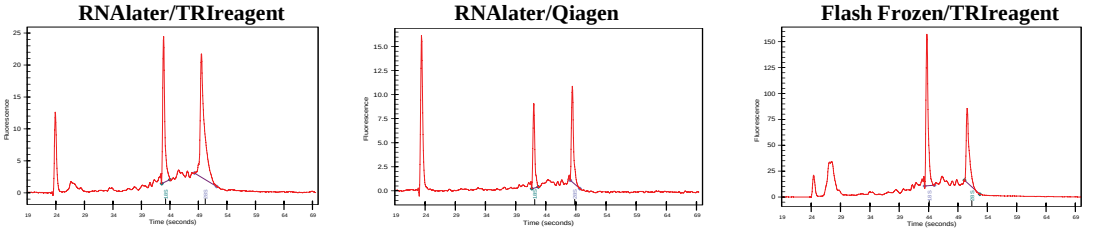


Figure 1. Total RNA samples isolated over a *postmortem* ageing period of 1 hour to 14 days and analysed using gel electrophoresis. RNA degradation was observed as the *postmortem* interval increased up to 14 days.



RNAlater/TRIreagent	
RNA Area	136.55
RNA Conc	82.92 ng/µl
rRNA ratio [28S/18S]	1.41

Fragment	Name	% of Total Area
1	18S	17.31
2	28S	24.41

RNAlater/Qiagen	
RNA area	44.02
RNA conc	18.77 ng/µl
rRNA ratio [28S/18S]	1.1

Fragment	Name	% of Total Area
1	18S	17.81
2	28S	19.66

Flash Frozen/TRIreagent	
RNA area	862.23
RNA conc	468.57 ng/µl
rRNA ratio [28S/18S]	0.57

Fragment	Name	% of Total Area
1	18S	16.81
2	28S	9.51

Figure 2. Examples of Bioanalyzer results of RNA samples stored in RNAlater or flash frozen in liquid nitrogen and total RNA isolation using TRIreagent or Qiagen RNeasy mini-kit. Samples with rRNA ratios [28S/18S] of >1 and '% of Total Area of 28S peak' values of > 13 are considered to be indicative of high quality RNA.

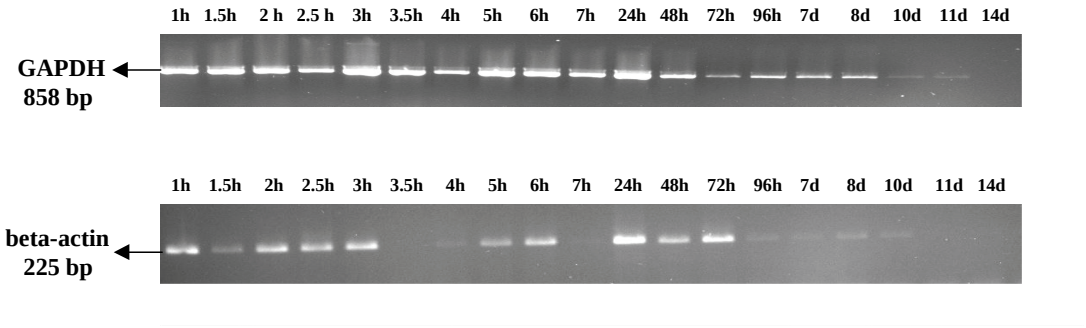


Figure 3. RT-PCR was carried out on RNA samples (1h – 14d) using GAPDH (858 bp) and β-actin (225 bp) specific primers and PCR products were analysed using agarose gel electrophoresis. Band intensity decreased as the *postmortem* interval increased up to 14 days.

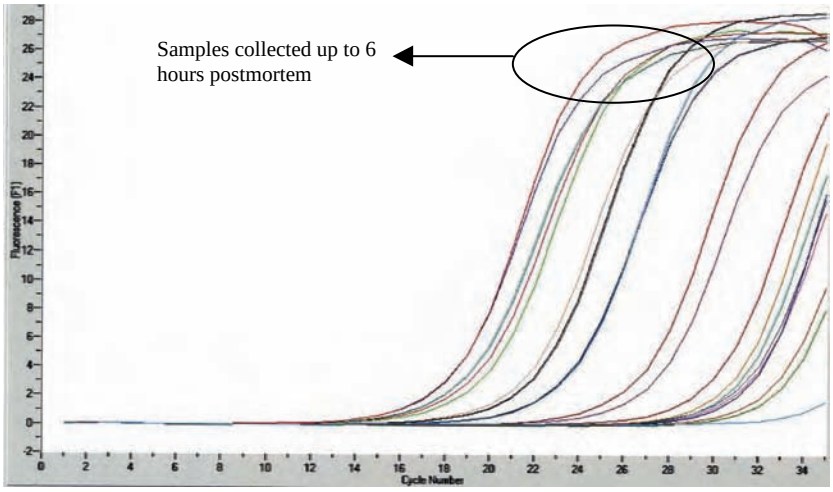


Figure 4. Realtime RT-PCR analysis was carried out using GAPDH specific primers on RNA samples (1h – 14d). PCR products were detected using SYBR Green 1. Transcript integrity decreased as the *postmortem* interval increased up to 14 days.