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Proteomics Of Colour Stability In New Zealand Lamb Meat (#302)

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Introduction

Beef and lamb which is a bright cherry-red colour is regarded as superior quality and freshness, but it is short lived [1]. Extending this shelf-life is critical for the New Zealand meat industry which relies on high-value chilled products that are shipped and sold overseas.

In earlier work [2] we have compared meat from lamb sires selected for different colour stability and identified 5 colour stable and 5 colour-labile lines. In this experiment we have vacuum packed loins from these lines, aged them for eight weeks and then displayed them under simuylated retail display conditions (4-6C air temperature, >1000 Lux daylight) for six days. The objective was to to find proteins that differed between stable and labile lines using differential fluorescent staining (DIGE) on 2-dimensional gels (2D-PAGE).

Methods

Pooled samples of 100mg from 8 animals of each sire within the 2 colour groups (stable and labile) were prepared. Proteins were extracted by homogenising these samples in 5ml of lysis buffer (7 M urea, 2 M thiourea, 1% dithiothreitol (DTT), pH 8.5 for 1 min), vortexing and centrifuging. The protein concentrations of the supernatant was determined using the 2D-Quant kit (GE Healthcare, USA). The pH of each sample was adjusted to 8.5, using sodium hydroxide. An internal sample was prepared from a mixture of equal amount of both samples being analysed. The protein samples were labelled with either Cy3 or Cy5 cyanine dye and the internal standard with Cy2 dye. Each sample contained a total of 150 µg protein. The labelled samples underwent isoelectric focussing on IPG strips (pH 3-11 NL, 24cm) with passive rehydration overnight and then subsequently focused until 70,000Vh was reached. The IPG strips were reduced before proceeding to second dimension separation using 12.5% Tris-glycine polyacrylamide gels. The gels were scanned using a Typhoon FLA 9,500 scanner (GE Healthcare) at 100 µm resolution. Gel analysis was carried out using Delta2D v4.5 software (Decondon, Greifswald, Germany). The spot matching and protein quantification was performed automatically using the Cy2 images as internal standards.

In-gel digestion was performed based on (Deb-Choudhury et al., 2010) [3] with minor modifications. LC–MS/MS was carried out on a Bruker nano-Advance liquid chromatography (BrukerDaltonics, Bremen, Germany) coupled to a Bruker amazon Speed ETD ion trap mass spectrometer. Protein identification procedure used ProteinScape v3.1.0 and Mascot Percolator (Clerens et al., 2010) [4].

Table 1: Gel-labelling scheme

Gel	Cy2	Cy3	Cy5
1	Internal Standard	Labile Day 0	Labile Day 6
2	Internal Standard	Labile Day 6	Labile Day 0
3	Internal Standard	Labile Day 0	Stable Day 0
4	Internal Standard	Stable Day 0	Stable Day 6
5	Internal Standard	Stable Day 6	Stable Day 0
6	Internal Standard	Stable Day 6	Labile Day 6

Results

Table 2 Proteins that were significantly higher in labile samples.

Protein ID	Spot	kDa	Scores (ProteinScape)	IAlign match	MASCOT coverage	UniPROT ID
Serum albumin precursor	1	69.1	944.4	100%	32%	P14639
Cytochrome b-ca complex	2	52.9	382.4	59%	28%	P24959
actin, alpha skeletal muscle	3	42	588.1	100%	34%	P68133
Triosephosphate isomerase 1	4	26.7	1,164.10	100%	84%	W5P5W9

Six proteins were found in higher amounts in labile samples than stable samples. Four of these were identified by mass spectrometry: *actin*,a major constituent of the contractile apparatus of skeletal muscle; *serum albumin*, the most abundant protein in serum; *cytochrome b-ca complex*, part of the mitochondrial respiratory chain and *triose phosphate isomerase,* part of the glycolysis pathway and important in the production of pyruvate, amino acids and glycerol.

Conclusion

This study has found consistent with the hypothesis that there is a genetic basis to colour stability. The sire effect has been linked to increased levels of particular proteins. We are now using metabolomics to investigate whether this leads to changes in other molecules.

Notes

Acknowledgements

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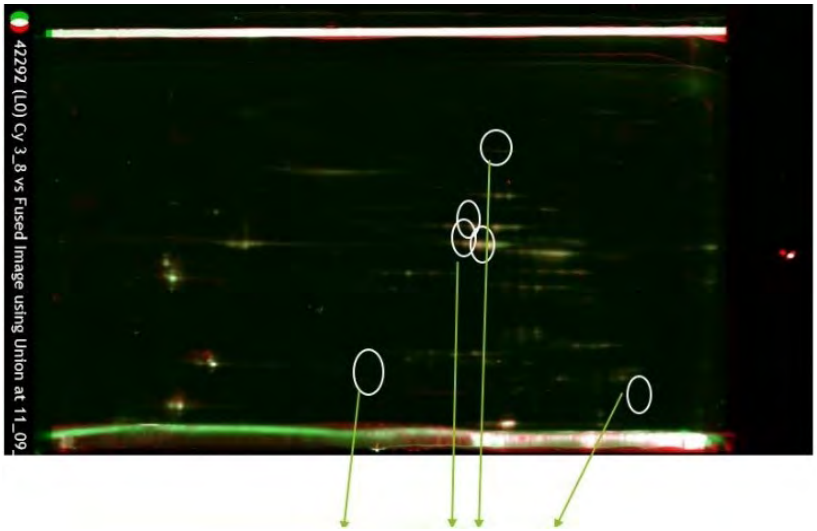

Figure 2
The position of the four proteins identified as different using DIGE

Notes

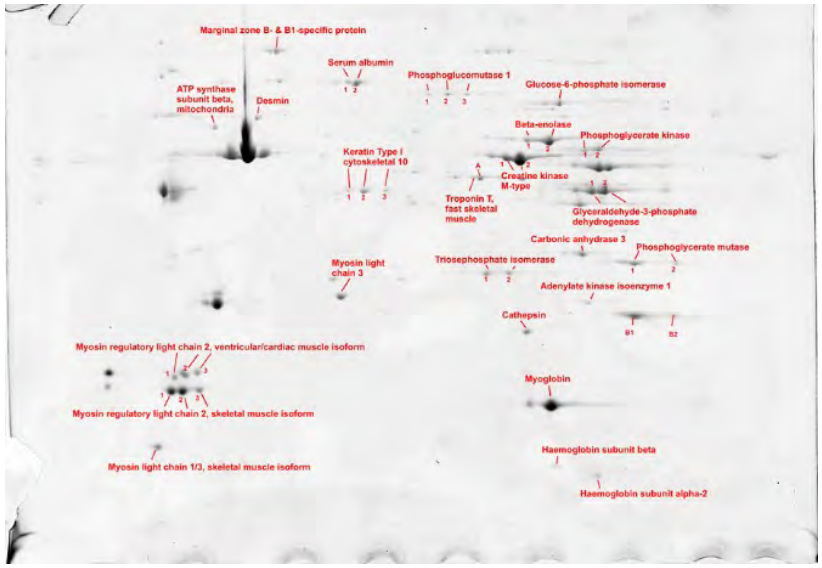

Figure 1.
Proteins identified on Coomassie blue stained 2D-PAGE

Notes