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EFFECT OF STRESS ON KEY PROTEINS IN ENERGY METABOLISM OF LAMB

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I. INTRODUCTION

A major goal in meat science is to determine the pre- and post-mortem conditions that will provide consistent high-quality meat. This is particularly important for New Zealand where most of the meat is exported to countries with stringent criteria. Earlier studies have shown that acute exercise stress causes reproducible changes in lamb meat quality associated with high pH [1]. It has also been shown that muscle type influences the nature and extent of these changes. A potential mechanism for these changes is differences in energy metabolism caused by changes in key enzymes associated with glycolysis. Glycolysis is a key pathway in post-mortem as oxygen supply to the muscle ceases after slaughter. The current study determines the levels of five of these proteins in four muscles and how exercise stress changes their abundance. Three of these enzymes, PGK1 (Phosphoglycerate kinase), ENO3 (Beta enolase) and PKM2 (Pyruvate kinase), occur within the glycolysis pathway and other two, glycogen phosphorylase and PGM1 (Phosphoglucomutase), allow the mobilisation of the muscle's glycogen stores. Four of the enzymes were chosen from literature reports [2] while glycogen phosphorylase was identified by the change in abundance at its molecular mass in SDS-PAGE gels. The longer-term purpose of this work is to find markers of meat quality.

II. MATERIALS AND METHODS

The samples were taken from 9-month-old ewe lambs who were part of an acute exercise stress trial [1] where the quality of meat from 10 lambs that were exercised just before slaughter were compared to meat from a control group that was kept quietly in their pens. Samples from four different muscles, were removed within 30 min of slaughter, *Longissimus lumborum* (LL, fast/glycolytic); *Gracilis* (G, intermediate) *Semimembranosus* (SM, intermediate); and *Supraspinatus* (SS, slow/oxidative), snap frozen in liquid nitrogen and stored at -80C until analysis. The 10ug of sarcoplasmic protein samples were separated at 200 V for 60 min on denaturing NuPAGE 4-12% acrylamide gels following the manufacturer's instructions and stained with GelCode blue. Specific proteins were analysed by western blotting. Proteins separated on NuPAGE gels were transferred to PVDF membrane. All primary antibodies were incubated with membrane for 16 hours, the secondary antibody (Goat anti-rabbit IgG H&L HRP (Horseradish Peroxide)) and Precision Protein Strep Tactin HRP were incubated with the membrane for 6 hours. Membranes were exposed to Clarity Western ECL Substrate (Bio-Rad) and images were taken with ChemiDoc MP (Bio-Rad). The effect of treatment on protein bands was analysed with a General Linear Model in Mintab21 (Minitab LLC) to account for samples being split across two runs. Differences between muscle groups were analysed using control-only data with One-Way ANOVA in Mintab21 (Minitab LLC).

III. RESULTS AND DISCUSSION

Protein gel electrophoresis revealed several protein bands that were altered by stress. These changes varied with muscles and the most consistent was an increased abundance at 17kDa and 98kDa with stress which was significant in G, SM and SS muscles (Figure 1A). Western blotting confirmed the presence of myoglobin at 17kDa and glycogen phosphorylase at 98kDa.

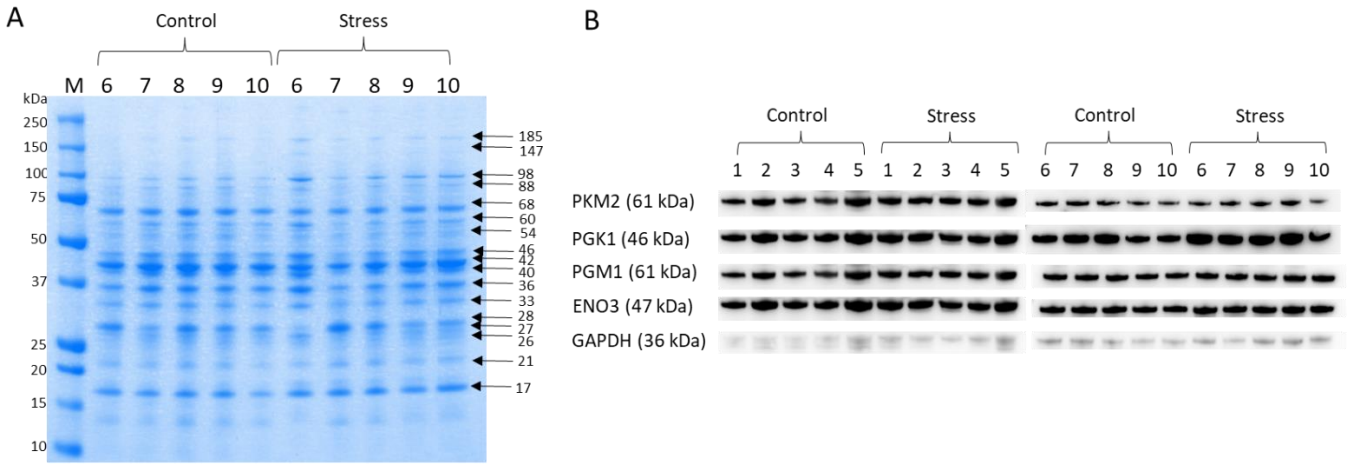


Figure 1. A) Image of stained gel in Image Lab 6.1 (Bio-Rad) of protein profiles with 17 identified bands from Supraspinatus proteins from control and exercise stress (stress) lambs. B) Antibody detection of PKM2, PGK1, PGM1, ENO3 and GAPDH in the Semimembanosus of 1-10 control and acute exercise stress (stress) animals. GAPDH was used as a house protein for loading differences.

Analysis of the specific enzymes in glycolysis showed significant differences between muscles (Table 1). The largest difference was between LL (fast/glycolytic) and SS (slow/oxidative) with ENO3 and PGM1 higher in SS and PGK1 higher in LL . The muscles also varied in response to exercise stress. The SM muscle was the most responsive, with 3 out of the 4 enzymes changing relative quantity in stressed animals. In contrast there were no significant changes in G.

Table 1 - Western Blot analysis of PGK1, PGM1, ENO3, PKM2 relative to GAPDH for LL, G, SM, SS muscles. Relative quantity generated in Image Lab 6.1 (Bio-Rad). The results are given as mean plus standard deviation (n=10). Control groups of muscles (row-wise) that do not share letter notation are statistically different ($p<0.05$). Enzymes where stress caused a significant change in abundance are in bold.

	LL		G		SM		SS	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress
PGM1	4.1 ± 1.1^{b}	5.5 ± 2.1	5.6 ± 2.5^{ab}	4.2 ± 0.7	6.2 ± 2.6^{ab}	4.6 ± 1.4	8.1 ± 3.4^{a}	6.2 ± 2.6
PGK1	9.7 ± 7.0^{a}	7.9 ± 3.9	3.6 ± 2.1^{b}	3.8 ± 0.8	**4.3 ± 1.6^{b}**	**6.6 ±1.9**	**1.8 ± 1.9^{b}**	**5.2 ± 4.1**
ENO3	4.8 ± 1.2^{b}	5.9 ± 2.5	7.0 ± 3.5^{b}	4.9 ± 0.9	**9.8 ± 4.8^{ab}**	**6.6 ± 2.2**	**14.1 ± 6.1^{a}**	**6.9 ± 3.2**
PKM2	**6.0 ± 1.9^{a}**	**3.1 ± 0.9**	1.4 ± 0.7^{c}	1.3 ± 0.6	**3.2 ± 1.6^{bc}**	**2.4 ± 0.9**	5.0 ± 3.4^{ab}	3.1 ± 2.2

IV. CONCLUSION

There has been a muscle specific response of a number of proteins to stress. Work is continuing to correlate these changes to other markers of meat quality.

ACKNOWLEDGEMENTS

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