DIETARY MANIPULATION OF PORCINE INTRAMUSCULAR FAT LEVELS AND SUBSEQUENT PROTEOMIC ANALYSIS

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Keywords: pork, intramuscular fat, *longissimus dorsi*, protein profile, bioanalyzer

Introduction

The quality and nutritive value of meat are critical traits for today's consumers with fat content becoming increasingly important to the consumer. Although pork meat with low fat content is desirable for reducing human caloric intake, an intramuscular fat level (IMF) below 2.5% is related to lower sensory quality traits (Fernandez *et al.* 1999). Studies suggest that dietary conditions can modulate IMF content at slaughter. Restriction of energy intake results in a decreased IMF content (Candek-Potokar *et al.*, 1998; Gondret and Lebret 2002; Wood *et al.*, 1996) and a low-protein diet results in an increased IMF content when compared to adequate diets (Adeola and Young 1989; Karlsson *et al.*, 1993). In the current study we were interested in exploiting this fact to produce pigs divergent for IMF content and to investigate if changes induced at the cellular level could be monitored through examination of protein profiles early post slaughter. Parallel studies will consider other factors such as differential gene expression. Therefore, the objective of this study was to administer dietary treatments to alter the IMF status in pork *M. longissimus dorsi* and to examine if differences are reflected at a proteomic level.

Materials and Methods

Forty pigs were offered one of 5 diets ad-libitum containing 13.8 MJ digestible energy and varying lysine levels: **Treatment 1**; 12.5 g/kg lysine from 40 kg to 65 kg and 10.5 g/kg lysine from 65 kg to slaughter, **Treatment 2**:10.5 g/kg lysine from 40 kg to slaughter, **Treatment 3**: 8.5 g/kg lysine from 40 kg to 65 kg and 10.5 g/kg lysine from 65 kg to slaughter, **Treatment 4**: 8.5 g/kg lysine from 40 kg to slaughter, **Treatment 5**:12.5 g/kg lysine from 40 kg to slaughter. Animals were then slaughtered according to standard commercial procedures in an Irish abattoir and samples of *M. longissimus dorsi* were taken for protein analysis at 3 hours post mortem. Compositional analysis was performed (Bostian *et al.*, 1985) to determine IMF content. Soluble protein extracts were prepared using 5.0% TCA following a modification of the method of Stoeva *et al.*, (2000). Extracts, from treatment groups which were significantly different for IMF, were analysed on an Agilent 2100 Bioanalyzer using a Protein 200 Plus Assay (Agilent Technologies, 76337 Waldbronn, Germany). These extracts were also run on a one dimensional sodium dodecyl sulphate-polyacrylamide gel (1-DE SDS-PAGE gel; 12% acrylamide).

Results and Discussion

Animals produced on diets with varying lysine levels showed altered IMF levels at slaughter. Those on treatment 5 showed a significantly lower level of intramuscular fat than those on treatment 3 and 4 (0.39% Vs 1.49%, $P<0.05$; Table 1). The greatest divergence in IMF level 5 was observed between treatment groups 3 and 5, therefore these groups were chosen for further protein analysis as these samples were most likely to have markedly different protein profiles. Protein extracts from Treatment 3 and 5 were investigated using a 'lab on a chip' technology (Bioanalyzer, Agilent Technologies). This novel approach enables a reduction in labour and time for protein analysis and possesses the added advantage of having a minute sample requirement. However this novel approach did not conclusively identify protein differences between IMF extremes as the protein profiles of both treatment 3 and 5 were similar. The only variation of note occurred in the lower molecular weight region of the samples (6-21 kDa), however the existence of inter group variation within this region precluded meaningful analysis (Figure 1). Comparable results were obtained by analyzing the protein samples from each treatment electrophoretically using a 1-DE gel (Figure 2). As both 1-DE electrophoresis and the Bioanalyzer act on the same principle of separating proteins according to their molecular weight, 2-DE electrophoresis may prove a more comprehensive method for identifying subtle deviations in protein profiles.

Table 1: Intramuscular fat levels (IMF) in pigs *post mortem*.

Treatment	Mean IMF (%)	Std Dev	Sample size(n)
1	0.64^{ab}	0.341	7
2	1.22^{ab}	0.744	6
3	1.54^{ac}	0.906	7
4	1.49^{a}	0.727	7
5	0.39^{b}	0.219	8

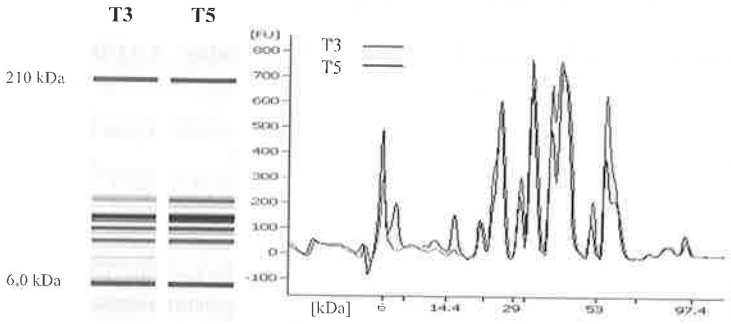

Figure 1: Bioanalyzer gel and electropherogram images of TCA extracts from samples with extremes of IMF levels.

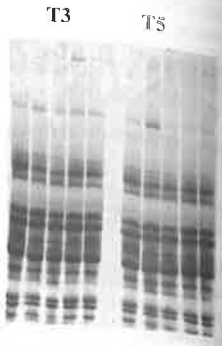

Figure 2: SDS-PAGE analysis of proteins from treatments 3 & 5.

Conclusion

Dietary treatments with varying lysine levels resulted in variation in IMF content in pork *longissimus dorsi* muscle. Protein profiles were investigated using both Bioanalyzer protein chip and 1-DE SDS-PAGE techniques. We have shown that protein deviations which may occur between pork samples of different IMF levels are not detected in proteins isolated by TCA extraction. It is possible that differences in protein profiles are being masked by co migration of the proteins of interest. The use of 2-DE electrophoresis would overcome this problem. Also more pronounced proteomic differences earlier in the live animal may be evident in myofibrillar and/or connective tissue. While results proved inconclusive in this instance, the Bioanalyzer did prove a useful tool for analysis of muscle protein species.

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