TRANSCRIPTONAL ANALYSIS OF OVINE *M. LONGISSMUS THORACIS ET LUMBORUM* FOR GENES INVOLVED IN CARCASS AND MEAT QUALITY TRAITS.

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Abstract –The identification of regulatory genetic polymorphisms robustly associated with carcass and meat quality traits has the potential to enhance the rate of genetic gain in lamb quality through genomic selection programmes. The objective of this experiment was to identify differentially expressed genes (DEGs) in the *M. longissimus thoracis et lumborum* (LTL) **from twin lambs that had divergent fatness phenotypes. Carcass and meat quality traits were measured on 64 pairs of lambs that were sired by rams from terminal-sire breeds. An index of fatness, based on subcutaneous fat depth, carcass fat score, and the concentrations of fat and moisture in samples of LTL, was used to identify the 10 twin pairs exhibiting the greatest within-pair divergence in fatness. The expression profile of 34 genes known to be involved in fat metabolism was evaluated for this study. Five genes (***ADIPOQ, MMP1, MSTN, LDLR, LPL)* **with within-litter differential expression profiles** ($P < 0.05$) were identified**. The promoter region of each of these genes is currently being sequenced to identify functional SNPs for use in future breeding programmes.**

Key Words – Differentially expressed genes, qPCR, lamb eating quality, carcass fat, functional SNPs

I. INTRODUCTION

Lamb is a good source of dietary protein and has low cholesterol, balanced amino acids, good tenderness and juiciness, which are positive attributes from a consumer perspective (1). One of the main factors affecting lamb eating quality is fat content, which is positively correlated with juiciness, taste and tenderness (2, 3). There is evidence that breeding for increased muscling (higher lean meat yield) and faster growth rate is associated with increased toughness of meat and reduced intramuscular fat levels with subsequent detrimental effects on eating quality (4). The aim of this study was to identify differentially expressed genes associated with fatness index in lamb *M. longissmus thoracis et lumborum* (LTL), using a divergent full-sibling model, with a view to future identification of regulatory polymorphisms associated with gene expression and eating quality.

II. MATERIALS AND METHODS

All procedures were carried out under license in accordance with the European Community Directive, 86-609-EC. Lambs born and raised as twins (64 pairs) were used; they were sired by rams from 1 of 3 terminal terminal-sire breeds (Suffolk, Texel and Charollais). All animals were slaughtered (at commercial slaughter weights) by electrical stunning followed by exsanguination, and 2 g of *M. longissmus thoracis et lumborum* were collected and stored in RNAlater (Ambion Inc., Austin, TX) within one hour postmortem. Carcasses were classified for fatness and conformation, and subcutaneous fat depth over the 12th rib was measured on the cold carcass after cutting when samples of LTL were retained for proximate analysis. A panel of 63 gene-specific primers (Table 1, only 5 significant genes) were designed using the web-based software program 'primer3' (http:// frodo.wi.mit.edu/primer3/) and obtained from a commercial supplier (MWG). Total RNA extraction from the LTL (50 mg of tissue), cDNA synthesis, and quantitative real-time PCR were performed according to the methods described by Alam et al. (5). The software package qbaseplus (Biogazelle, Belgium) was used for geNorm analysis and calculation of calibrated normalized relative quantities (CNRQ) of DNA. Data on carcass fat score, fat depth, and the composition of the fat (IMF) and moisture concentrations of LTL were combined (Proc PRINCOMP; SAS 2012) to yield an index of within-litter divergence for fatness. The 10 litters with the most divergent members were used, thus yielding 10 pairs with divergent fatness phenotypes (High or Low). Average gene expression values for the Low and High groups were compared (Proc GLM; SAS 2012) for genes identified as exhibiting significantly increased within-litter variation in expression level based on the coefficient of variation and an experiment wise error rate of 0.05.

III. RESULTS AND DISCUSSION

The expression level of 3 genes (*ADIPOQ, MMP1, MSTN)* was elevated ($P < 0.05$) in the high-fat animals, while the expression level of 2 genes *(LDLR, LPL*) was lower ($P < 0.05$) in the high-fat animals (Figure 1).

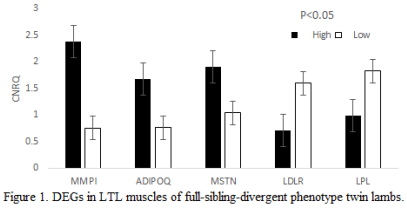

Figure 1. DEGs in LTL muscles of full-sibling-divergent phenotype twin lambs.

Table 1. An overview of genes and their functions

Gene Name (Gene symbol)	Accession No.	Function
Matrix metallopeptidase 1 *(MMP1)*	XM_012095308.2	Positive correlation with water holding capacity
Adiponectin, (*ADIPOQ*)	XM_012097437.2	Marbling
Myostatin *(MSTN)*	NM_001009428.1	Skeletal muscle and fat development
Low density lipoprotein receptor*(LDLR)*	XM_004008531.3	Endocytosis of low density lipoprotein
Lipoprotein lipase *(LPL)*	NM_001009394.1	Triglycerides metabolism

All of the genes that differed significantly between the High and Low fatness index animals have a known role in fat metabolism. For example, *MSTN* is involved in regulation of skeletal muscle growth and adipose development (6, 7). Tempfli (8) reported that *ADIPOQ* expression was not significantly different between groups in back fat level, however muscle expression was remarkably higher ($P < 0.05$) in fat-type animals. *MMP1* plays a key role in adipogenesis, stimulating tissue remodelling during adipose tissue expansion in obesity. The *LDLR* is a major determinant of plasma cholesterol levels and involved in endocytosis of low density lipoprotein.

IV. CONCLUSION

Of the thirty four genes known to be involved in fat metabolism that were examined, five genes with differential expression profiles between animals that had different fatness phenotypes were identified. SNPs in the promoter regions of these genes are currently being identified and will be tested for associations with fatness in a larger population of lambs. Such SNPs can be incorporated into the national breeding programme for sheep.

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