Genetic association of delta-six fatty acid desaturase single nucleotide polymorphic molecular marker and muscle long chain omega-3 fatty acids in Australian lamb

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Abstract— **For sustainable prime lamb production in the Australian sheep industry and a better understanding of the relationships between fat metabolism-related genes and sheep muscle long chain omega-3 polyunsaturated fatty acids, we investigated the genetic association between polyunsaturated fatty acids (PUFAs), delta-6 desaturase (FADS2) and fatty acid binding protein(FABP) gene clusters in crossbred sheep. Thirty-one single nucleotide polymorphisms (SNPs) were genotyped in *Longissimus dorsi* muscle samples from 362 crossbred prime lambs sired by five genetically divergent rams. Total intramuscular lipid long chain fatty acid levels were analysed using gas chromatography. Genetic association was tested for significance using mixed model analyses in SAS fitting genotype, sire breed and sex as fixed effects and sire as a random variable. FAPB SNP was highly significantly associated (P<0.05) with 18:4n-3 (stearidonic acid), while FADS2 SNP was significantly associated (P<0.05) with intramuscular levels of eicosapentaenoic (C20:5n-3) and docosahexaenoic (C22:6n-3) acids. The results suggest that this SNP is in linkage disequilibrium with functional lipid synthesis pathway associated with higher delta-six desaturase activity. For the first time, this study provides evidence for an association between genetic variants of FADS2 and omega-3 PUFA in sheep muscle. This SNP could potentially be a novel marker of choice for prime lamb producers to effectively select for enhanced muscle omega-3 fatty acid content in their breeding flock.**

Keywords— **Omega-3 fatty acids, FADS2, FABP4.**

I. INTRODUCTION

Prime lamb production is a significant component of the Australian sheep meat industry whose farm gate value has increased from $0.5 to $2.2 billion[1]. The fatty acid composition of muscle tissues of ruminants is an important meat eating quality trait because of its relationship with flavour and tenderness and published results suggest it could be controlled by genetic factors such as lipid synthesis and fatty acid metabolism-related genes[2]. Long chain omega-3 polyunsaturated fatty acids (PUFA) are beneficial in lowering cholesterol levels, hence reducing the risk of artherosclerosis and other heart-related complications. Delta-5 and delta-6 desaturases are key enzymes in PUFA metabolism and several factors like fatty acids in the diet and biological tissues may influence desaturase activity[3].

Fatty acid binding proteins (FABPs) are proteins that reversibly bind fatty acids and other lipids and, nine tissue-specific cytoplasmic FABPs have been identified[4]. Fatty acid binding protein 4 (FABP4), which is expressed in adipose tissue, interacts with peroxisome proliferator-activated receptors and binds to hormone-sensitive lipase and therefore, plays an important role in lipid metabolism and homeostasis in adipocytes[5].

Genetic variability at the FABP4 locus has been shown to be associated with plasma lipid levels, type-2 diabetes, and coronary heart disease risk[4]. Therefore, FABP4 is a candidate gene affecting fatness traits of mammals, but Barendse et al. (2009)[6] reported that its association with fatness traits in cattle and other livestock species is not consistent from one study to another. For instance, genetic polymorphisms of the FABP4 gene were significantly associated with marbling and carcass weight [7] and backfat thickness[8] in Korean Hanwoo cattle, but only with palmitoleic acid in Japanese Black cattle[9]. In sheep muscle, information on the genetic association between FABP4 and FADS2 and long-chain PUFA is scanty, hence our main aim in this paper was to investigate these relationships in Australian prime lambs.

II. MATERIALS AND METHODS

Animals and experimental design: A half-sib experimental design was utilised in this study. Five top-EBV rams acquired from Tasmanian Sheep Stud Breeders comprising Dorset, Texel, White Suffolk, East Friesian and Coopworth were mated to purebred Merino ewes at a ratio of 1:120 ewes in separate paddocks in a commercial farming operation in the Coal River Valley, Tasmania, to generate 500 first cross prime lambs.

Animal management: Lambs were run in sire groups under similar management conditions to minimise environmental variation. The lambs were marked, vaccinated and electronically tagged at 6 weeks and run as one mob within a large scale commercial farming operation. The flock was raised on forage oats and fescue as basal diet with ad-lib barley grain until the attainment of 44kg when they were slaughtered. Blood sampling was by jugular venipuncture into vacutainers containing EDTA. *Longissimus dorsi* muscle tissue samples from 369 of the prime lambs were collected and transported to the laboratory and stored at -20°C until ready for genomic DNA and lipid extraction.

Fatty acid analysis: Longissimus dorsi muscle samples of approximately 1g size, were used for fatty acid analysis. Lipid was extracted using a modified Bligh and Dyer protocol[10] (Bligh and Dyer, 1959). This involved a single phase extraction, $CHCl_3$/MeOH/H2O (1:2:0.8, by vol.), followed by phase separation to yield a total lipid extract (TLE). An aliquot of the TLE was trans-methylated in methanol:chloroform:hydrochloric acid (10:1:1, v/v/v) for 2 hours at 80°C. After addition of water, the mixture was extracted three times with hexane: chloroform (4:1, v/v) to obtain fatty acid methyl esters (FAME) which were concentrated under a stream of nitrogen gas. An internal injection standard (19:0 FAME) was added and analysed by gas chromatography using an Agilent Technologies 7890A GC (Palo Alto, California, USA) equipped with a Supelco Equity-1 fused silica capillary column (15 m×0.1 mm). Helium was used as the carrier gas. Samples were injected, by a split/splitless injector auto-sampler operated in splitless mode, at an oven temperature of 120°C. After 1 min, the oven temperature was raised to 270°C at 10°C per min and finally to 300°C at 5°C and held for 5 min. Individual component peak identification was confirmed by mass spectral data and by comparing retention time with those obtained for authentic and laboratory standards. GC peak areas were converted to mg / 100g using the 19:0 FAME internal injection standard prior to statistical analysis.

Genomic DNA extraction: Genomic DNA from blood samples was extracted using the UltraClean® 96 Well BloodSpin® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA). Muscle samples were extracted using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). DNA concentration of all samples was assessed using the NanoDrop 8000 UV/VIS spectrometer (Thermo Scientific, Wilmington, DE, USA) along with purity by determination of the 260/280 nm ratio.

Analysis of FABP4 and FADS2 polymorphisms: To identify SNP, the International Sheep Genomics Consortium 454 read archive (https://isgcdata.agresearch.co.nz/) was BLAST-searched using bovine mRNA sequences of FABP4 (NM001114667) and FADS2 (NM001083444). A total of 19 (FABP4) and 50 (FADS2) 454 reads were retrieved and aligned using Sequencer v4.7 before putative SNP were identified as base changes between overlapping reads. Three variant positions were identified and used to develop fluorogenic 5' nuclease assays formatted for analysis using the Applied Biosystems (AB) 7900HT "TaqMan' sequence detection system. FABP4 SNP1 and FABP4 SNP2 are in intron 2 of the FABP4 gene located on sheep chromosome 9 at Mb position 60.52 as defined by sheep genome v1.0 (https://www.biolives.csiro.au/cgi-bin/gbrowse/oar1.0/). FADS2 SNP1 is located within the 3' UTR of the FADS2 gene located on sheep chromosome 21 at Mb position 43.71. The primers and probes used for genotyping animals at each of the SNP are provided in Table 1. Reactions were conducted in 10µl containing 5ng of genomic DNA and standard conditions. End point allele discrimination analysis to assign the genotype of each animal was performed using the Sequence Detection System (SDS) software version 2.2 (Applied Biosystems).

Statistical analysis: A mixed linear model in SAS fitting the fixed effects of SNP locus, breed, and sex with sire as a random effect was run.

III. RESULTS AND DISCUSSION

It was evident from Table 2 that there were significant associations between FAPB4 SNP with C18:4n-3 and EPA, while the FADS2 SNP was associated with EPA and DHA. This finding is in agreement with the report of Malerba et al 2008[11] who genotyped 13 SNPs located on the FADS1-FADS2-FADS3 gene cluster in serum phospholipids and reported strong associations with arachidonic acid (C20:4n-6), linoleic (C18:2n-6), alpha-linolenic (C18:3n-3) and eicosadienoic (C20:2n-6) acids, but not stearidonic (C18:4n-3), eicosapentaenoic (C20:5n-3) and docosahexaenoic (C22:6n-3) acids levels[12]. More recently, it was confirmed that polymorphisms of the Delta-5 (FADS1) and Delta-6 (FADS2) desaturase genes were associated with the levels of several long-chain n-3 and n-6 PUFAs in serum phospholipids[13]. In the plasma also, genetic variability in the FADS1-FADS2 gene cluster revealed that several SNPs were associated with higher delta-6 (FADS2) desaturase activity and lower delta-5 (FADS1) desaturase activity [14].

For the first time, this study provides evidence for an association between genetic variants of FADS2 and omega-3 PUFA in sheep muscle. This SNP could potentially be a novel marker of choice for prime lamb producers to effectively select for enhanced muscle omega-3 fatty acid content in their breeding flock. However, more data are needed with larger flocks because the estimates were based on 369 sheep only with a tendency for the contribution to the observed phenotypic variance to be overestimated in smaller data sets.

Table 1 SNP primer sequences for FAPB4 and FADS2

Gene	SNP	Oligonucleotide Name	Oligonucleotide Sequence
FABP4	FABP4_SNP1	forward primer	GACAGGAAAGTCAAGGTGAGGAATA
	FABP4_SNP1	reverse primer	CCTCCTTCTACAAAATGGCTTGCTA
	FABP4_SNP1	vic-probe	AGAGTAAAAGCCTGATTTATA
	FABP4_SNP1	fam-probe	AGTAAAAGCCTGGTTTATA
	FABP4_SNP2	forward primer	GAGGAATAAAGAACTGGAGCAGAGT
	FABP4_SNP2	reverse primer	CCTCCTTCTACAAAATGGCTTGCTA
	FABP4_SNP2	vic-probe	ATAGGCAGCAGTCGTTTA
	FABP4_SNP2	fam-probe	TAGGCAGCAGTTGTTTA
FADS2	FADS2_SNP1	forward primer	CCCCTGACCTGGCCATT
	FADS2_SNP2	reverse primer	CCAAGTCCAGAGCCTGTGA
	FADS2_SNP3	vic-probe	AGAGCTCAGCAGAAGC
	FADS2_SNP4	fam-probe	AAGAGCTCAACAGAAGC

Table 2 SNP associations with LC-PUFA (p-values)

	FABP4	FADS2
C18:4n-3	0.015*	0.497
EPA (20:5n3)	0.045*	0.049*
DHA (C22:6n3)	0.438	0.053*
EPA+DHA	0.302	0.959
EPA+DPA+DHA	0.892	0.114

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