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An independent validation association study of carcass quality, shear force, intramuscular fat percentage and omega-3 polyunsaturated fatty acid content with gene markers in Australian lamb ☆

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ABSTRACT

Previous association studies revealed several single nucleotide polymorphisms (SNPs) that explained the observed phenotypic variation for meat tenderness and long-chain omega-3 polyunsaturated fatty acid (PUFA) content of Australian lamb. To confirm the validity of these associated SNPs at predicting meat tenderness and omega-3 PUFA content, an independent validation study was designed. The OvineSNP50 genotypes of these animals were used to impute the 192 SNP Meat Quality Research (MQR) panel genotypes on nearly 6200 animals from the Cooperative Research Centre for Sheep Industry Innovation Information Nucleus Flock and Sheep Genomics Falkiner Memorial Field Station flock. Association analysis revealed numerous SNP from the 192 SNP MQR panel that were associated with carcass quality – fat depth at the C-site and eye muscle depth; shear force at day 1 and day 5 after slaughter (SF1 and SF5); and omega-3 PUFA content at $P < 0.01$. However, 1 SNP was independently validated for SF5 (i.e. CAST_101781475). The magnitude of the effect of each significant SNP and the relative allele frequencies across Merino-, Maternal- and Terminal-sired progeny was determined. The independently validated SNP for SF5 and the associated SNP with omega-3 PUFA content will accelerate efforts to improve these phenotypic traits in Australian lamb.

1. Introduction

The efficient production of meat that is of consistent quality and value is necessary for the sheep meat industry to remain competitive (Pethick, Warner, & Banks, 2006). The Australian sheep meat industry is focussed on appropriate management strategies (pre- and post-slaughter) incorporated with genome assisted breeding programs (i.e. genomic selection) to meet these expectations (Rowe, 2010). Thus, identifying gene markers that can predict carcass quality, shear force at day 1 and day 5 after slaughter (SF1 and SF5), intramuscular fat (IMF) percentage and omega-3 polyunsaturated fatty acid (PUFA) content in lamb are of great interest to the Sheep Meat Industry.

Carcass quality traits can assist in predicting the overall saleable meat yield or proportions of fat, lean and bone on carcass (Gardner et al., 2010; Hopkins, 1994; Hopkins & Fogarty, 1998; Stanford, Jones, & Price, 1998). Saleable meat yield is an important financial determinant of the overall value of the carcass (Hopkins, Wotton, Gamble, Atkinson, Slack-Smith, & Hal, 1995). Carcass quality traits targeted by genetic breeding programs include: post-weaning weight (PWWT), fat depth at the C-site (PFAT) and eye muscle depth (PEMD). Studies investigating the potential of Australian Sire Breeding Values (ASBVs) for high PWWT showed that available nutrition significantly affected the sire's genetic potential for growth (Hegarty et al., 2006). Gardner et al. (2010) showed that progeny from Merino-, Maternal- and Terminal-sires with high ASBVs for PWWT had increased weights at slaughter and hot carcass weights (HCWT). Further studies revealed that sires with high ASBVs for PEMD (i.e. 1 mm) showed increases in loin depth on carcass of 0.6 mm (Hegarty et al., 2006). This increase

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in loin depth was not affected by available nutrition (Hegarty et al., 2006).

Meat tenderness is the principal desirable attribute associated with meat eating quality and can be measured objectively by shear force (Hopkins, Toohey, Warner, Kerr, & van de Ven, 2010), trained panellists (Safari, Fogarty, Ferrier, Hopkins, & Gilmour, 2001) or consumers (Hopkins, Walker, Thompson & Pethick, 2005). Numerous studies have shown that the greatest biological factor which contributes to the meat tenderisation process, post slaughter are calcium-activated proteases (i.e. the calpains) and their activity. To date, 14 different calpain gene family members have been identified (Goll, Thompson, Li, Wei, & Cong, 2003). Three members of the calpain family are ubiquitously expressed in skeletal muscle, μ-calpain (CAPN1), *m*-calpain (CAPN2) and calpain 3 (CAPN3). Associated with the calcium-activated proteases is the calpain specific inhibitor, calpastatin (CAST).

IMF percentage has also been shown to affect meat tenderness and the overall sheep meat eating quality of lamb (i.e. flavour, juiciness, tenderness and overall likeability). In beef cattle, there is a genetic correlation between IMF percentage and meat tenderness (Reverter, Johnston, Perry, Goddard, & Burrow, 2003). For Australian lamb to achieve consumer satisfaction – "good every day" score (MSA grade – 3 out of 5); lamb meat must contain an IMF percentage between 4 – 5% (Hopkins, Hegarty, Walker, & Pethick, 2006a).

In human diets, red meat is an important source of omega-3 PUFAs (Howe, Meyer, Record, & Baghurst, 2006). Numerous studies have identified the health benefits of omega-3 PUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Psota, Gebauer, & Kris-Etherton, 2006). In humans, both EPA and DHA have cardiovascular and anti-inflammatory health benefits (Marik & Varon, 2009; Psota et al., 2006; Swanson, Block, & Mousa, 2012). Docosapentaenoic acid (DPA) is another omega-3 PUFA, however the nutritional content of DPA cannot currently be claimed as an Omega-3 under the Food Standards Code (Howe, Buckley, & Meyer, 2007). Thus, achieving levels of omega-3 PUFAs that comply with recommended dietary guidelines (i.e. "good source) has been proposed as a goal for lamb production systems (Pethick et al., 2006). In mammals, the omega-3 PUFA biosynthetic pathway is controlled by fatty acid desaturases (i.e. FADS1, FADS2, FADS3) and elongases (for example, ELOVL2) (Sprecher, Luthria, Mohammed, & Baykousheva, 1995).

The Sheep Industries OvineSNP50 chip (Illumina, CA, USA) which includes 54,977 genome wide SNP includes 6 SNP in close proximity to genes that influence shear force: CAPN1 (OAR21:47225725), CAPN2 (OAR12:28694194) and CAPN3 (OAR7:39000331) and CAST (OAR5:101742566, OAR5:101792466 and OAR5:101853472) and four SNP in close proximity to genes that potentially influence omega-3 PUFA content; FADS1 (OAR21:43637798 and OAR21:43646542) and FADS3 (OAR21:43754091and OAR21:43748475). Preliminary genome wide association studies (GWAS) have shown that these SNP in close proximity are not capable of predicting shear force in lamb. However, 10 SNP on the OvineSNP50 chip (Illumina, CA, USA) were associated with omega-3 PUFA levels in lamb (B.J. Hayes, pers. comm.).

Thus, identifying SNP in closer proximity to or within these genes could improve genomic predictions for shear force and omega-3 PUFA content in sheep. Recent findings reported by Knight et al. (2012) identified 182 SNP is close proximity to or within the genes previously associated with SF1, SF5 and omega-3 PUFA content in lamb. Association studies revealed 3 SNP in CAST and CAPN2 genes that were associated with SF5 (i.e. meat tenderness) and no SNP within the FADS1/2/3, ELOVL2 and SLC26A10 gene regions associated with long-chain omega-3 fatty acid content of Australian lamb (Knight et al., 2012). Thus, a 192 SNP Meat Quality Research (MQR) panel was designed containing the 182 new SNP identified by Knight et al. (2012) and the 10 SNP on the OvineSNP50 chip (Illumina, CA, USA) that were associated with omega-3 PUFA levels in lamb.

The aim of this study was to validate the associated SNPs at predicting SF5 and long-chain omega-3 PUFA content in lamb in an independent sheep population. The OvineSNP50 genotypes of the independent sheep population were used to impute the 192 SNP MQR genotypes on nearly 6200 animals from the Sheep CRC INF and Sheep Genomics Falkiner Memorial Field Station (FMFS) flock. Using the imputed genotypes of nearly 6200 animals, this paper reports the associations of each SNP on the MQR panel with carcass quality, SF1, SF5, IMF percentage and omega-3 PUFA content. Linear mixed model analysis was then performed to estimate the magnitude of effect of all significant SNP. The relative allele frequencies of all significant SNP is reported across Merino-, Maternal- and Terminal-sired progeny.

2. Materials and methods

2.1. Phenotypic data

Phenotypic data used in the association study was collected from the Sheep CRC INF flock (van der Werf, Kinghorn, & Banks, 2010) and Sheep Genomics FMFS flock (White et al., 2012). The INF animals were located at eight geographically different sites across Australia and the FMFS animals were raised in Deniliquin, NSW, Australia. The eight geographical sites for the INF included: Kirby Research Station, University of New England, Armidale, NSW; Trangie Agricultural Research Centre, NSW; Cowra Agricultural Research and Advisory Station, NSW; DPI Hamilton Centre, Vic.; DPI Rutherglen Centre, Vic., Struan Research Station, SA; Turretfield Research Station, SA and Great Southern Agricultural Research Institute, Katanning, WA. All dams used in the INF and FMFS flocks had a Merino background that ranged from fine wool to strong wool. The sires were either from Terminal, Maternal (i.e. Border Leicester, Coopworth and East Friesian), or Merino breeds. Whilst the Merino sheep were mostly purebred, the remaining breeds this study represented were mainly crossbred animals because of their crosses with Merino ewes. Therefore both research flocks (i.e. the INF and FMFS) represented the majority of flock structures seen in the Australian Sheep Industry. Table 1 represents all the breeds used in this study.

Table 1
Number of progeny by breed of sire and dam for the Cooperative Research Centre for Sheep Industry Innovation Information Nucleus flock (before backslash) and the Sheep Genomics Falkiner Memorial Research Station flock (after backslash) (BL – Border Leicester, BL x EF – Border Leicester x East Friesian, MER – Merino, PD – Poll Dorset, PD x WS – Poll Dorset x White Suffolk, PM – Poll Merino, EF – East Friesian, WD – White Dorper, WS – White Suffolk, XB – cross breed and BL x MER – Border Leicester x Merino).

SIRE Breed	DAM Breed						
	MER	PD	PM	WS	XB	BL X MER	Totals
BOND	6/0	0/0	0/0	0/0	0/0	0/0	6/0
BOOROOLA	44/0	0/0	0/0	0/0	0/0	0/0	44/0
BL	405/124	0/10	5/0	0/7	0/0	0/49	587/190
BL X EF	0/66	0/2	0/0	0/2	0/0	0/25	0/95
MER	415/1274	0/0	10/0	0/2	0/0	0/0	491/1276
PD	518/103	0/9	13/0	0/7	638/0	0/44	1511/163
PD X WS	0/143	0/12	0/0	0/13	0/0	0/45	0/213
PM	247/0	0/0	6/0	0/0	0/0	0/0	297/0
COOPWORTH	64/66	0/5	5/0	0/5	0/0	0/22	69/98
CORRIEDALE	61/0	0/0	6/0	0/0	0/0	0/0	67/0
DOHNE MERINOS	69/0	0/0	8/0	0/0	0/0	0/0	77/0
DORPER	27/0	0/0	0/0	0/0	0/0	0/0	27/0
EF	3/0	0/0	0/0	0/0	0/0	0/0	3/0
HAMPSHIRE DOWN	5/0	0/0	0/0	0/0	0/0	0/0	5/0
ILE DE FRANCE	12/0	0/0	0/0	0/0	2/0	0/0	14/0
PRIME SAMM	83/0	0/0	9/0	0/0	0/0	0/0	92/0
RESEARCH	2/0	0/0	0/0	0/0	0/0	0/0	2/0
SOUTHDOWN	16/0	0/0	0/0	0/0	9/0	0/0	25/0
SUFFOLK	92/0	0/0	2/0	0/0	129/0	0/0	293/0
TEXEL	91/0	0/0	1/0	0/0	130/0	0/0	328/0
WD	53/0	0/0	0/0	0/0	0/0	0/0	53/0
WS	337/49	0/5	17/0	0/8	526/0	0/28	1179/90
Totals	2550/1825	0/43	82/0	0/44	1434/0	0/213	4066/2125