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Marker-assisted selection for meat quality and the ovine calpastatin gene

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ABSTRACT

The calpain-calpastatin system (CCS) comprises a family of calcium-dependent neutral proteases, the calpains, and calpastatin, a specific inhibitor of the calpains that regulates their activity *in vivo*. The CCS is found in most animal tissues and influences many important processes including muscle development and degeneration, meat tenderisation postmortem, cataract formation and fertility. The calpains have been shown to play the major role in postmortem tenderisation in beef, lamb and pork by degrading specific muscle structural proteins. The level of postmortem calpastatin appears critical in determining the ultimate tenderness of aging muscles. Tenderness has been shown in consumer surveys and tasting panel tests to be the most important characteristic determining the overall eating quality of meat.

In using a molecular genetic approach to studying meat quality in sheep we have chosen the ovine calpastatin gene (CAST) as a candidate gene for meat quality. We have described a three allele system of polymorphic variants (CAST *a*, *b* and *c*) in a region of the ovine CAST. Since 1995 we have carried out slaughter trials on small groups of Dorset Down hoggets and Dorset Down x Coopworth lambs to ascertain any association between meat quality traits and the molecular markers in CAST. Sheep with the genotypes of CAST *aa*, *ab* and *ac* were compared. These trials indicate an association of the CAST genotype *ac* with increased liveweight gain (+12-17%, $p < 0.05$), increased age-corrected carcass weight (+15-18%, $p < 0.05$), but also increased longissimus dorsi shear force (+4-12%, nonsignificant) compared to sheep with the CAST genotype *aa*.

Keywords: sheep; calpastatin; weight gain; marker-assisted selection.

INTRODUCTION

The calpain-calpastatin system (CCS) comprises a family of calcium-dependent neutral proteases, the calpains, and calpastatin, a specific inhibitor of the calpains that regulates their activity *in vivo* (Sorimachi *et al.*, 1997). The CCS is found in all animal tissues studied and influences many important processes including muscle development and degeneration, meat tenderisation postmortem, cataract formation and fertility. The calpains have been shown to play the major role in postmortem tenderisation in beef, lamb and pork by degrading specific muscle structural proteins (Huff-Lonergan *et al.*, 1996). The degradation of muscle myofibril proteins such as titin and nebulin by the calpains during carcass aging is the main biochemical change responsible for postmortem meat tenderisation. The level of calpastatin at 24 hours postmortem appears critical in determining the ultimate tenderness of aging muscles (Koochmaraie *et al.*, 1995). A better understanding of the mechanism by which the CCS influences the tenderisation process will enable meat producers to provide higher quality meat to consumers. Tenderness has been shown in consumer surveys and tasting panel tests to be the most important characteristic determining the overall eating quality of meat (Miller *et al.*, 1995).

Characterisation of molecular genetic variation in the ovine calpastatin gene has revealed the presence of three allelic variants, *a*, *b* and *c*, detectable by PCR-SSCP (Roberts *et al.*, 1996). All three CAST alleles are present in most, if not all, breeds with the *a* allele being predominant in all

flocks so far studied with alleles *b* and *c* usually below a frequency of 10% (Palmer *et al.*, 1997). As a result sheep homozygous for alleles *b* and *c* are very rare and as yet no attempt has been made to include these *bb* and *cc* animals in trials studying meat quality. If these variants of the ovine calpastatin gene are to be used commercially it must be shown that there is an association between the presence of particular CAST alleles and differences in meat quality. Secondly the cost of genotyping must be reduced to allow lamb producers to increase their returns without heavy set up costs.

The objective of this work was to study the association of meat quality in sheep with the ovine calpastatin gene (CAST) as a candidate gene for this attribute. Here we describe the results of three slaughter trials with two breeds of sheep of known CAST genotype to examine the association between CAST genotype and sheep meat quality characteristics.

MATERIALS AND METHODS

Animals

Slaughter trials used either yearling Dorset Down hoggets, being culled from the Lincoln University Lean Tissue Selection flock, or Dorset Down x Coopworth lambs (4 - 5 months old), breeding and age details of these groups are shown in Table 1. Trials A and B used Coopworth ewes mated to single Dorset Down rams and all the lambs that survived to slaughter were analysed. Trial C used yearling Dorset Down hoggets culled from the Lincoln University

Lean Tissue Selection flock. The ewes and lambs were grazed on pasture at Lincoln University and slaughtered and processed by PPCS at either the Fairton or Canterbury plants.

CAST gene genotyping

The calpastatin genotype was determined by the method of Roberts *et al.*, (1996).

Tenderness Assessment

Lamb LD samples were taken as bone-in chops (40 mm thick) at 24 h postmortem and frozen at -20°C prior to tenderness determination. Before shear force determinations the samples were thawed to 2°C and cooked in “Tuflex” plastic bags (Transpak Industries, Auckland, New Zealand) by immersing in a water bath at 80°C until they reached an internal temperature of 75°C. Internal meat temperatures were measured with Fluke Type K temperature probes attached to Fluke 52 meters. At least 10 samples (10 x 10 x 25 mm) were placed separately in the MIRINZ tenderometer (Devine and Graafhuis, 1995) and the shear force (kgF) required to cut the fibres determined. The mean kgF for each sample was calculated according to MIRINZ bulletin 872 (1991).

Data

Sheep were weighed at weaning and immediately prior to transport to slaughter and hot carcass weight, carcass grade and *longissimus dorsi* (LD) tenderness were determined for each carcass. Data was analysed using general linear model ANOVA with Minitab version 11.

RESULTS

Slaughter trials have been performed on groups of yearling Dorset Down hoggets and Dorset Down x Coopworth lambs to assess whether variation in the CAST locus is associated with meat quality traits (Table 1). The results of three slaughter trials are shown in Table 2. Preliminary trials (data not shown) had suggested little difference in meat quality between sheep with CAST genotype of *aa* and *ab*. This finding was confirmed in Trial C, but the first two trials concentrated on examining differences between *aa* and *ac* animals. Trial A involved lambs sired by a single sire and sex matching between the CAST genotype groups allowed valid comparisons of growth and meat quality parameters. This trial provided evidence that lambs with CAST genotype *ac* had faster weight gains (+12%) than the *aa* lambs from the same sire. However as the *c* allele was inherited from the lambs’ dams this was a source of variation that was eliminated by using a sire with CAST genotype *ac* in Trial B.

Trial B showed a trend for faster growth rate for lambs with CAST genotype *ac*. However in Trial B none of the measured parameters showed a significant difference between lambs with genotypes *aa* and *ac* and the amount of the difference between the two groups was greatly reduced compared to Trial B, although the trends in most other parameters remained the same. The most likely explanation for the differences between Trials A and B is the ad-

Table 1: Design of slaughter trials.

Trial A			Trial B			Trial C					
Dorset Down x Coopworth [#]			Dorset Down x Coopworth [§]			Purebred ... Dorset Down					
1 sire (aa)*			1 sire (ac)*			12 sires (7 aa, 3 ab, 2 ac)*					
Progeny			Progeny			Progeny					
Genotype	Ewes	Rams	Total	Genotype	Ewes	Rams	Total	Genotype	Ewes	Rams	Total
aa	3	9	12	aa	24	18	42	aa	0	24	24
								ab	0	8	8
ac	3	4	7	ac	11	18	29	ac	0	5	5

[#]mean age 141 days [§]mean age 172 days ... mean age 407 days

*The CAST genotype of the sires is shown in brackets.

Table 2: Lamb production and meat quality parameters of lambs of CAST *aa*, *ab* and *ac* genotypes.

Trial A						
1996-97						
1996-born Dorset Down x Coopworth lambs						
Slaughter	Liveweight gain	Carcass weight	Carcass weight gain	Weaning weight	LD Tenderness	
(kg)	(kg/day)	(kg)	(kg/day)	(kg)	(kgF)	
Genotype						
<i>aa</i> (n = 12)	35.3±1.3 ^a	0.247±0.009 ^a	14.8±0.69 ^a	0.104±0.005	27.6 ^a	6.03±0.33 ^a
<i>ac</i> (n = 7)	39.9±1.5 ^b	0.276±0.008 ^b	17.8±0.67 ^b	0.123±0.004	30.0 ^a	5.94±0.37 ^a
	(+13%)	(+12%)	(+20%)	(+18%)	(+8.7%)	(-2.2%)
Trial B						
1997-98						
1997-born Dorset Down x Coopworth lambs						
Slaughter	Liveweight gain	Carcass weight	Carcass weight gain	Weaning weight	LD Tenderness	
(kg)	(kg/day)	(kg)	(kg/day)	(kg)	(kgF)	
Genotype						
<i>aa</i> (n = 42)	31.6±0.54 ^a	0.185±0.004 ^a	13.1±0.27 ^a	0.077±0.002 ^a	30.1±0.75 ^a	6.07±0.42 ^a
<i>ac</i> (n = 29)	33.1±0.98 ^a	0.194±0.005 ^a	13.4±0.51 ^a	0.078±0.003 ^a	30.2±1.1 ^a	6.29±0.34 ^a
	(+4.7%)	(+4.9%)	(+2.3%)	(+1.3%)	(+0.5%)	(+3.6%)
Trial C						
1997-98						
1997-born Dorset Down rams						
Slaughter	Liveweight gain	Carcass weight	Carcass weight gain	Weaning weight	LD Tenderness	
(kg)	(kg/day)	(kg)	(kg/day)	(kg)	(kgF)	
Genotype						
<i>aa</i> (n=24)	44.4±1.3 ^a	0.109±0.003 ^a	18.6±0.63 ^a	0.046±0.002 ^a	30.5±1.2 ^a	8.01±0.25 ^a
<i>ab</i> (n=8)	45.1±1.7 ^a	0.111±0.005 ^a	19.6±0.94 ^a	0.048±0.002 ^{ab}	30.9±2.0 ^a	8.32±0.53 ^a
<i>ac</i> (n=5)	52.1±2.0 ^b	0.127±0.004 ^b	21.7±0.88 ^b	0.053±0.002 ^b	28.3±3.1 ^a	8.97±0.65 ^a
	(+17%)	(+17%)	(+17%)	(+15%)	(-8%)	(+12%)

^{a,b}Values in the same column lacking a common superscript differ (*P* < 0.05)

verse effect of the severe 1997-98 summer drought in Canterbury. The lambs in Trial B gained very little weight after weaning compared to the lambs in Trial A. Trial C made use of a group of Dorset Down ram hoggets being culled from the Lincoln University Lean Tissue Selection flock. Data from this trial shows that animals with CAST genotype *ac* have a significant growth advantage over those with genotype *aa* even at one year of age and that LD shear force is greater in *ac* animals even though this difference is not significant.

DISCUSSION

The data presented here is suggestive that the ovine CAST gene is a major gene which can affect weight gain in sheep. A major gene effect occurs when there is evidence that variation within a single gene strongly affects a quantitative trait, in contrast to the additive effects of multiple genes. We have screened individuals from a number of sheep breeds for variation in the CAST locus and found a three allele system to be widely distributed amongst the flocks studied (Palmer *et al.*, 1997). Data from the slaughter trials presented here shows that with favourable grazing conditions lambs with CAST genotype *ac* can achieve up to 17% increase in weight gain compared to *aa* lambs. This can translate to up to 18% increased carcass weight gain. This effect appears to occur after lambs are weaned and seems to be maintained at least until one year of age. There appears to be little difference in growth or quality traits between *aa* and *ab* lambs.

The growth effect has been observed with both paternal and maternal inheritance of the CAST *c* allele and occurred in both purebred Dorset Down lambs and Dorset Down x Coopworth lambs suggesting that this is not a breed or flock dependent phenomenon. Such an increase in liveweight gain and LD shear force is consistent with an increase in the level of calpastatin expression in muscle as has been observed following the administration of b-adrenergic agonist (Speck *et al.*, 1993). However we have not yet shown whether there is a CAST genotype dependent change in muscle calpastatin expression in sheep. The increase in LD shear force that appears to be associated with the CAST *ac* genotype, is not a desirable characteristic. However the increase is small and in all cases nonsignificant and also appears to be associated with increasing age. In *ac* lambs slaughtered at less than six months of age meat tenderness seems likely to be well within acceptable levels.

It is not clear whether the increase in weight shown by *ac* lambs is due to an increase in any particular tissue type, measurements in forthcoming trials will attempt to elucidate this. It appears that ovine CAST gene has potential as a target marker-assisted selection to improve slaughter lamb production. Animals carrying the CAST *c* allele appear to be present in most flocks, although often at low frequency. This approach is therefore one that sheep producers could profitably use to increase the carcass weights of slaughter lambs with relatively little extra expenditure or prolonged breeding strategies to introduce the desirable allele.

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