The association between polymorphic variations in calpain 3 with the yield and tenderness of retail lamb meat cuts

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Abstract

Calpain 3 is a muscle specific protease whose exact metabolic role has yet to be determined. In humans, allelic variations in calpain 3 have been associated with muscle loss. In livestock, the impact of myofibrillar calpain 3 protein changes on muscle mass changes and meat tenderization has been controversial. This research investigates the association between three allelic variants of calpain 3 with the tenderness of the LD and the yield of retail meat cuts from 152 lamb carcasses boned out according to market specifications. There were no correlations between any of the allelic variants with the tenderness of the LD. There were increases in the weights of fat trimmed leg and foreshank retail cuts which are ascribed to significant genotypic differences in calpain 3a and calpain 3c. The yield of the leg and fore shank cuts increased 2.45 to 3.25% with the presence of the specific alleles. This research illustrates that a phenotypic variation in the yield of meat cuts is associated with genetic variations in calpain 3 in livestock. This suggests that calpain 3 may have a role in determining muscle mass.

Introduction

Calpain 3 is a muscle specific protease (Sorimachi *et al*, 1989) unlike the ubiquitous occurring calpains 1 and 2. Interest in calpain 3 increased dramatically when an association was made between allelic variations in calpain 3 and muscle wasting which is expressed clinically as Limb Girdle Muscular Dystrophy 2A (LGMD) in humans (Richard *et al*, 1995). To-date, 280 pathogenic mutations in calpain 3 have been identified (Kramerova *et al*, 2007). It is now accepted that a loss in calpain 3 results in muscle wasting. However, it is still an open question as to whether calpain 3 has a significant role in regulating the metabolic turnover of cytoskeletal proteins and, consequently, muscle mass or whether calpain 3 acts as a key signalling protease directing metabolic events (Goll *et al*, 2008). Interesting, in induced muscle atrophy in humans, calpain 3 was the only calpain to respond significantly to a reduction in muscle mass after a 2 week period of immobilisation (Greenhaff, 2006).

In livestock, the role of myofibrillar calpain 3 protein has been controversial. In lambs, changes in myofibrillar calpain 3 protein has been associated with muscle mass changes and with the rates of post-mortem meat tenderisation and a role for calpain 3 in myofibril protein degradation has been suggested (Ilian *et al*, 2001, 2004). An opposite view is held by Geesink *et al* (2005) based on calpain 3 changes in knock out mice. Immunofluorescence microscopy has shown that calpain 3 is associated with the Z-disc and M-lines of ovine sarcomeres (Ilian *et al*, 2003).

Polymorphic variations in calpain 3 have been identified in ovine exon 10 (Zhou *et al*, 2006), 12 and 13 (Chung *et al*, 2007). This research investigates whether there are any predictive linkages between the three variants of the calpain 3 gene at the exon 10 position with the tenderness of the Longissimus dorsi (loin) or the yield of three primals and seven sub-primal retail cuts from 152 lambs raised in different environments.

Materials and Methods

Uniform lines of lambs (n=100 or higher), aged 10-12 months, were sourced from 70 farms and slaughtered at the same export processing plant. 3 to 5 lamb carcasses from each farm, in the weight range 16 to 19 kg were selected 2h post-slaughter. After exposing the carcasses to a standard chilling regime, carcasses at 30h post-slaughter were boned out to yield the standard three primal cuts: Leg, Full Loin and Forequarter. From these primals, seven sub-primal market cuts were obtained: Foreshank, Bone-in-Neck, Square cut shoulder, Flaps, Shortloin (Backstrap), French rack and Part boned (PB) leg. All cuts were trimmed to the same retail market specifications. Primal cuts, sub-primal retail cuts, fat trimmings and waste were weighed to determine the product yield from each carcass.

A 40 mm long Longissimus dorsi (LD) sample was removed from the 7-rib rack to provide a bone-in rib loin chop for tenderness determinations. The bone-in chops were cooked in 'Tuflex' plastic bags by immersion in water at 80^{o}C until they reached an internal temperature of 75^{o}C. After cooling, rectangular samples (10 x 10 x 25 mm) were cut along the fibres and the shear force (KgF) to cut across the fibres determined using a MIRINZ tenderometer.

A meat sample (5g) was removed from the ribloin for DNA extraction and genotyping. DNA was isolated using a phenol/chloroform method. The genomic DNA was PCR amplified using the PCR primers 5'-

CTCTCAGGATGTCCTACG-3' and 5'-CTGGGGAAGTTGGGCAG-3'. The PCR amplicons were separated on 14% polyacrylamide and from the banding patterns three calpain 3 variants were visually identified: CAPN3a, CAPN3b and CAPN3c.

Results and Discussion

The mean cold carcass weight for the lambs (n=152) was 17.15 ± 1.88 kg of which 27.5%, 27.5%, 9.4%, 9.1%, 4.7%, 3.5% and 3.1% consisted of the PB Leg, Shoulder, Flaps, Shortloin, French rack and Bone-in neck. LD tenderness from all carcasses was 6.65 ± 2.55 KgF.

To investigate whether the presence or absence of the three alleles of calpain 3 could be linked to variations in LD tenderness or the yield of the three primals and seven sub-primal retail cuts, the data was statistically analysed using SPSS version 15, with the presence or absence of an allele in each lamb genotype coded with a 1 or 0 respectively. Two models were used to test the association between the calpain 3 alleles and meat tenderness or meat yield. One method tested allele effects, the other genotype effects. Table 1 shows the linkages between the three calpain 3 variants and loin tenderness.

Table 1. Allelic effects of three calpain 3 variants on loin tenderness (KgF) in lambs (n=152).

Allele	Tenderness means ± SE (KgF)		P-value
	Without Allele	With Allele	
Calpain 3a	6.45 ± 2.13 (n=27)	6.70 ± 2.64 (n = 120)	0.64
Calpain 3b	6.60 ± 2.50 (n=88)	6.74 ± 2.64 (n=59)	0.75
Calpain 3c	6.73 ± 2.54 (n=97)	6.51 ± 2.58 (n=20)	0.62

Table 1 shows there was no association between the presence of any one of the three calpain 3 allelic variants and loin tenderness. There have been no previous observations on this relationship. Table 2 shows the statistical relationship (p-value) between the three calpain 3 genotypes and the yield of the three primal cuts and seven sub-primal retail cuts.

Table 2. Statistical relationship (p-value) between calpain 3 genotypes (calpain 3a, 3b and 3c) with the yield of specific meat cuts.

Allelic Variant	Measured Parameter			
	Primal Leg	PB Leg	Foreshank	French rack
Calpain 3a	0.05	0.03	0.05	NS
Calpain 3b	NS	0.07	NS	NS
Calpain 3c	0.05	0.02	0.03	0.09

Note (i) Calpain 3a, 3b, 3c showed no relationships with the Full Loin, Forequarter, Bone-in Neck, Square cut shoulder, Flaps or Shortloin.

(ii) NS signifies the p value was above 0.10

Table 2 illustrates that calpain 3a and 3b genoytpes were associated with significant difference in the primal and PB leg weights. The same two genotypes, calpain 3a and 3b, were also associated with significant differences in foreshank weights. There was a trend for calpain 3a to be associated with differences in French rack weights and calpain 3b with differences in PB-leg weights. Table 3 shows the mean yields of the various cuts of meat relative to a 17 kg carcass.

Table 3. Allelic effects of calpain 3 variants on the yield of retail cuts from lamb carcasses(mean = 17 Kg)

Calpain3 Variant	Primal Leg Weight (kg)		PB Leg Weight (kg)		Foreshank Weight (kg)		French Rack Weight (kg)	
Allele	Without	With	Without	With	Without	With	Without	With
3a	5.62±0.58 n=27	5.76*±0.62 n=120	4.61±0.51 n=27	4.74*±0.56 n=120	0.58±0.06 n=27	0.60*±0.07 n=120	0.79±0.12 n=27	0.82±0.11 n=120
3b	5.71±0.61 n=88	5.76±0.61 n=59	4.69±0.56 n=88	4.74†±0.54 n=59	0.60±0.07 n=88	0.59±0.06 n=59	0.82±0.11 n=88	0.81±0.12 n=59
3c	5.70±0.63 n=97	5.80*±0.57 n=50	4.68±0.56 n=97	4.79*±0.52 n=50	0.59±0.06 n=97	0.61*±0.07 n=50	0.81±0.1 N=97	0.83†±0.1 n=50

Note * Signifies a significant phenotypic difference with the genotype. † Signifies a trend.

Table 3 shows that the presence of calpain 3a or 3b genotypes produce a 1.8 to 2.4% increase in primal leg weights and a 2.4 to 2.8% increase in PB-leg weights. The same two genotypes (3a, and 3b) were associated with a 2.0 to 3.4% increase in foreshank weights. These are not dramatic changes but they are consistent. That is lambs possessing genotypes calpain 3a and 3c show consistent increases in leg weights.

Lamb legs are valuable retail products commanding premium prices in some markets. Any selection tool to identify lambs that yield high leg weights would be beneficial to the industry.

There are no previous reports linking calpain 3 genotypes to market retail cuts but Chung *et al* (2007) reported that two calpain variants were associated with increased (11.8 to 13.3%) lamb birth weights. The results reported by Chung and this report are consistent. Both pieces of research indicate that calpain 3 variants might be associated with differences in muscle development and/ or growth.

Conclusion

There was no correlation between any of the three calpain 3 allelic variants with the tenderness of the LD muscle. This is the first report that two calpain 3 genotypes are linked to increases in the weight of meat cuts. The leg weights were increased by 1.8 to 2.4% and Foreshanks by 2.0 to 3.4%. The mechanism by which calpain 3 variants have an affect on muscle mass is unknown and needs investigation. A benefit to the industry is that two variants have the potential to be genetic markers that promote muscle mass without any adverse effects on meat tenderness.

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