Association of polymorphic variations in calpastatin with meat tenderness and yield of retail meat cuts in lambs

R. Bickerstaffe*, J.G.H. Hickford, K. Gately & H. Zhou
Agriculture & Life Sciences Division, Lincoln University, Canterbury, New Zealand
E-mail: bickerst@lincoln.ac.nz

Abstract

Calpains, particularly u-calpain, have been implicated in myofibrillar protein degradation. Calpastatin is a natural occurring inhibitor of calpains and consequently the balance of calpain –calpastatin activity in muscles is believed to dictate the rate of tenderization in post-mortem meat. Research in beef cattle has shown that there is a link between the presence of specific calpastatin variants and post-mortem meat tenderization, whilst other researchers have found there are no linkages.

This research investigates whether polymorphic variants in ovine calpastatin in lambs (n = 152) are associated with any post-mortem differences in the tenderness of the LD or in the yield of retail meat cuts. There were no associations between the presence of any one of the eight allelic variations of calpastatin in the lambs with the tenderness of lamb loins.

There was a significant association (p=0.05) between the presence of three calpastatin variants and the yield of short-loins. One calpastatin variant resulted in an 15% increase in the weight of the short-loins.

The research illustrates that in lambs there is a lack of association between the measured allelic variants of calpastatin and meat tenderness, but the yield of short-loins is influenced by specific calpastatin allelic variants. The relationship between the allelic variants and muscle calpastatin activity has yet to be determined.

Introduction

The goal of meat retailers is to provide consistent tender meat to match the requirement of the increasing numbers of discerning consumers, whilst the objective of stock producers is to select animals that grow fast and produce muscles yielding desirable cuts of meat commanding a premium price in the market place.

The common molecular and biochemical factor that links meat tenderness and muscle yield is the skeletal calpain isomers and the calpain inhibitor, calpastatin. The balance of enzymic activity between calpains and calpastatin determines the extent of proteolytic activity and, consequently, the breakdown of myofibrillar proteins.

In live animals, high muscle yields as promoted by high muscle calpastatin activity as exemplified in the callipyge sheep, whilst under post-mortem conditions, low calpastatin activity is required for high quality tender meat.

In the bovine and ovine, a calpastatin gene has been identified on chromosome 7 and 5 respectively. Polymorphic variations in the gene have been identified but the linkages between the polymorphic variants and meat tenderness or yield have been inconsistent.

This research investigates whether there are any predictive linkages between eight variants of the ovine calpastatin gene with the tenderness of the longissimuss dorsi (loin) or the yield of meat cuts from 152 lambs raised in different environments.

Materials and Methods

Lambs, aged between 10-12 months, sourced from 70 farms and representing all major sheep breeds with a known history and environmental background eg. lowland versus alpine were slaughtered at the same export processing plant. The lambs (3-5 per farm) were selected from uniform lines of lambs (n=100 or higher) post-slaughter on the chilling floor with 16 to 19kg hot carcass weights. After a standard export market chilling regime, all the carcases 30h post-slaughter were boned out by the same skilled personnel to yield the standard cuts of retail meat. All the retail cuts, fat trimmings and waste were weighed to determine the yields of marketable product from each carcass.

A 40mm 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 80^0C until they reached an internal temperature of 75^0C as measured by a temperature probe inserted into the chop. After cooling, 10 rectangular samples (10 x 10 x 25mm) of meat were cut along the fibres and the shear force (KgF) to cut across the fibres determined using a MIRINZ tenderometer.

A meat sample (5 g) was removed from the rib loin for DNA extraction and genotyping. DNA was isolated from the meat sample using a phenol/chloroform method. The calpastatin (CAST) genotypes were typed by two separate methods using the PCR-SSCP technique. Method 1 is described by Zhou et al (2007) and

method 2 by Palmer et al (1998). Amplicons representative of the known CAST alleles were included in each polyacrylamide gel and their banding patterns were used as standards for allele identification. Method 1 yielded four genotypes labelled a,b,c and d. Method 2 yielded four separate genotypes labelled A, B, C & D.

Results and Discussion

Carcasses (n=152) were initially cut into three primals: leg, full loin and forequarter. From the three primals seven retail sub-primal cuts were obtained: Foreshank, Bone-in-Neck, Square cut shoulder, Flaps, Short-loin (backstrap), French Rack and Part boned (PB) leg. All cuts were trimmed to the same specifications. The mean carcass weight and the yield of the three primals and seven retail cuts are in Table 1.

Table 1. Mean carcass weight (kg) and yield of meat cuts (kg) from the carcass (n=152)

CW	Leg	Full Loin	FQ	FS	Bone in neck	SCS	Flaps	SL	FR	PB Leg
17.15	5.73	4.89	6.53	0.60	0.53	4.72	1.61	1.56	0.81	4.72
±	±	±	±	±	±	±	±	±	±	±
1.88	0.61	0.69	0.71	0.07	0.79	5.56	0.28	0.24	0.12	0.55

CW= Carcass weight; FQ = Forequarter; FS = Foreshank; SCS = Square cut shoulder; SL = Shortloin; FR = French rack.

The mean tenderness of the LD from all the carcasses was 6.65 ± 2.55 KgF. The results, however, do not show whether the genetic background of the stock had any affect on any of the measured parameters. As stated, the research was to investigate whether the presence or absence of specific alleles of calpastatin could be linked to variations in the tenderness of the LD or the yield of the retail cuts. For this purpose, statistical analysis on the data was performed 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 calpastatin alleles and meat tenderness or yield. One method tested allelic effects (presence or absence of a given allele) and the other tested genotypic effects. Table 2 shows the linkage between the eight variants of calpastatin and loin tenderness.

Table 2. Allelic effects of the eight variants of calpastatin gene loin tenderness (KgF) in lambs.

	Tenderness mean ± SE (KgF)		
Allele	Without Allele	With Allele	P-value
a	6.63 ± 2.62 (n=53)	6.61 ± 2.52 (n=99)	0.95
b	6.35 ± 2.45 (n=36)	6.70 ± 2.59 (n=116)	0.48
c	6.54 ± 2.58 (n=116)	6.85 ± 2.46 (n=36)	0.53
d	6.59 ± 2.52 (n=150)	8.30 ± 5.60 (n=2)	0.35
A	7.14 ± 6.72 N=5)	6.72 ± 0.72 (n=139)	0.72
B	6.71 ± 2.44 (n=123)	6.93 ± 3.02 (n=16)	0.71
C	6.78 ± 2.58 (n=128)	6.39 ± 2.04 (n=16)	0.56
D	6.74 ± 2.47 (n=135)	6.75 ± 3.3 (n=9)	0.99

Table 2 shows there was no association between the presence of any one of the eight allelic variations of ovine calpastatin with the tenderness of lamb loins. In beef cattle some researchers have demonstrated there is a link between the presence of specific calpastatin variants and post-mortem tenderisation whilst other researchers have found there are no linkages. These lamb results support there is no linkage between the calpastatin variants and loin tenderness.

Table 3 shows the linkages between the eight calpastatin variants and the yield of retail cuts. There were no associations between the yield of any retail cut with the presence or absence of variants b,c, B or D. There were, however, some linkages between the yield of flaps, shortloin, French rack and PB-leg with the variants a,d,D,B and C. Three different variants (a,A,B) of calpastatin had a significant affect on the yield of the shortloin (LD). For example, allele A produced a 15% increase in the retail weight of the shortloins.

Table 3. Allelic effects of eight variants of calpastatin gene on the yield of retail cuts in lambs

Allele	Flaps		Shortloin		French Rack		PB Leg	
	Without Allele	With Allele	Without Allele	With Allele	Without Allele	With Allele	Without Allele	With Allele
a	1.56±0.30 (n=53) p = 0.08	1.64±0.26 (n=99)	1.52±0.24 (n=53) p = 0.05	1.59±0.23 (n=99)	-	-	-	-
d	-	-	-	-	0.81± 0.11 (n=150) p = 0.07	0.95± 0.17 (n=2)	-	-
A	1.34±0.15 (n=5) p = 0.03	1.61±0.27 (n=139)	1.35±0.15 (n=5) p = 0.04	1.56±0.23 (n=139)	-	-	4.27±0.36 (n=5) p = 0.05	4.74±0.53 (n=139)
B	1.62±0.2 (n=128) p = 0.09	1.51±0.27 (n=21)	1.57±0.22 (n=123) p = 0.05	1.47±0.26 (n=21)				
C	1.62±0.27 (n=128)	1.49±0.24 (n=16)	-	-	-	-	-	-

The reason why particular calpastatin variants influence the calpain-calpastatin activity balance and, as a consequence, meat yields is not known. Specific experiments are required on a large group of animals to verify the above results and to address the questions as to whether specific calpastatin variants influence calpastatin mRNA levels, calpastatin activity or specific functional domains which influence calpastatin expression.

Conclusion

There has been considerable interest in identifying DNA markers to assist producers to select stock that produce increased amounts of quality tender meat for the market place. To-date, DNA markers which have increased the yield of meat cuts have been associated with adverse effects on meat tenderness: e.g. Callipyge and Carwell genes. The results reported here show that the gene marker selection of lambs based on calpastatin variants can increase the yield of premium priced cuts of meat without any adverse effects on the eating quality of the meat. This is s positive benefit for the described calpastatin markers. Discovering the underlying mechanism creating these results and why the Longissimus dorsi muscle, in particular, responds to the allelic variations in calpastatin and increases its muscle size without affecting its tenderness remains a scientific challenge.

Acknowledgements

The authors thank PPCS for access to animals and facilities and to Dr Chris Frampton for providing statistical advice. This research was funded by the Foundation of New Zealand Research Science & Technology (FRST) and Lincoln University Research Fund.

References

Casas, E. et al. 2006. Effects of calpastatin and μ-calpain markers in beef cattle on tenderness traits. J. Anim. Sci. **84**, 520-525.

Corva, P. et al. 2007. Association of CAPN1 and CAST gene polymorphisms with meat tenderness in Bos Taurus beef cattle from Argentina. Genetics and Molecular Biology **30**, 1064-1069.

Geesink, G.H. & Koohmaraie, M. 1999. Postmortem proteolysis and calpain/calpastatin activity in callipyge and normal lamb biceps femoris during extended post-mortem storage. J. Anim. Sci. **77**, 1490-1501.

Goll, D.E., Neti, G., Mares, S.W. & Thompson, V.F. 2008. Myofibrillar protein turnover: The proteasome and the calpains. J. Anim. Sci. 86. E19-35.

Koohmaraie, M. & Geesink, G.H. 2006. Contribution of post-mortem biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Science **74**, 34-43.

Palmer, B.R., Roberts, N., Hickford, J.G.H. & Bickerstaffe, R. 1998. PCR-RFLP for MspI and NcoI in the ovine calpastatin gene. J. Anim. Sci. **76**, 1499-1500.

Schenkel, F.S. et al. 2006. Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. J. Anim. Sci. **84**, 291-299.

Zhou, H., Hickford, J.G. & Gong, H. 2007. Polymorphism of the ovine calpastatin gene. Mol. Cell Probes **21**, 242-244.