Microanatomical Structure and Physical Characteristics of Thin Tail Hogget with Calpastatin (CAST-1) Genotype Differences

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ABSTRACT

Thin tail sheep has good adaptation in tropics condition, but they have low meat quality. Quality of thin tail hogget can be improved by selection. Calpastatin (CAST) gene is an indigenous inhibitor of calpain that involved in regulation of protein turn over and growth. The objective of this research was to determine the effect of calpastatin-genotype on microanatomical structure and physical characteristics of thin tail hodget. Nine thin tail sheep from Jonggol were used for this research. PCR-RFLP method was carried out to identify genetic variation of calpastatin gene, based on the identification of CAST variation genotype. It was found that MM and MN genotypes for calpastatin gene with TT as a single Calpain genotype variation. The sheep was clustered based on the variation of calpastatin gene, 5 sheep had MM genotype and 4 sheep had MN genotype. Physical and microanatomical characteristics were analyzed from their meats. Sheep with MN genotype showed tougher meat, it was characterized with a greater of muscle fiber surface area, the number of muscle per muscle bundle and muscle bundle area and harder meat tenderness than in MM genotypes. Hypertrophy and hyperplasia of MN were greater than MM.

Key words: calpastatin, microanatomical characteristics, physical characteristics, thin tail sheep

ABSTRAK

Domba ekor tipis (DET) merupakan ternak lokal yang memiliki kemampuan adaptasi lingkungan yang bagus, namun dari segi kualitas dan kuantitas daging masih perlu ditingkatkan. Peningkatan produksi pada DET salah satunya melalui seleksi. Gen kalpastatin (CAST) diduga tidak hanya memberikan pengaruh terhadap keempukan daging postmortem tetapi juga terhadap pertumbuhan otot. Tujuan penelitian ini adalah mempelajari sifat fisik dan struktur mikroanatomi daging DET pada variasi gen kalpastatin yang berbeda. Penelitian ini menggunakan sembilan ekor DET. Sampel diambil dari domba yang memiliki genotipe kalpastatin MM (5 ekor) dan MN (4 ekor) dengan genotipe kalpain yang sama, yaitu TT. Domba yang dipilih adalah DET jantan dengan umur siap potong berkisar 1-1,5 tahun (I1), dengan kisaran bobot badan 17-20 kg, sampel daging domba dianalisa sifat fisik dan mikroanatomi ototnya. Hasil deteksi keragaman menunjukkan bahwa hanya diperoleh 2 variasi genotipe, yaitu MM dan MN untuk gen kalpastatin, dan tidak ditemukan genotipe NN. M menunjukkan alel kalpastatin normal, sedangkan N menunjukkan alel kalpastatin yang mengalami mutasi. DET genotipe MN memiliki luas penampang serabut otot, jumlah otot per fasikulus dan luasan fasikulus yang lebih besar tetapi memiliki daging yang lebih keras dibandingkan dengan yang bergenotipe MM. Hipertrofi dan hiperplasia pada MN lebih besar daripada MM.

Kata kunci: kalpastatin, mikroanatomi, sifat fisik, domba ekor tipis

INTRODUCTION

Thin tail sheep is one of potential indigenous livestock for meat production. Although the body weight is relatively small, thin tail sheep are adaptable to the limited availability of food and high temperature condition, also the mortality rate is relatively low (Sumaryadi & Manalu, 1999). Indonesian thin tail sheep is also have higher resistantability of nematode parasite, especially fasciolosis (Pleasance *et al.*, 2011). Productivity and quality of Indonesian thin tail sheep need to be improved in order to enhance sheep farmer prosperity (Priyanto *et al.*, 2000).

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Improvement in molecular biology techniques allows livestock selection can be done up to gene level by determining diversity of genes that affects the productivity of livestock. Cast-1 as gene marker associated with growth trait in the local sheep regulates the synthesis of calpain and calpastatin (Sumantri *et al*., 2008). Calpain and calpastatin are included in the calpain system (Camou *et al*., 2007). Calpain system is a proteolytic enzyme that contributes to the tenderness of post-slaughter meat (Allais *et al*., 2011). Calpain system has three members, those are μ-calpain, m-calpain, and calpastatin. The μ-calpain and m-calpain are a protease whose enzyme activity and influenced by Ca^{2+} ions. In live animals, calpain enzyme has a function of protein degradation in myofibrillar structure (Scanes, 2003).

 Calpastatin is an enzyme to inhibit protein degradation by μ-calpain, m-calpain. Increasing of calpastatin activity leads to increase muscle mass (hypertrophy) (Kemp *et al*., 2009). Calpastatin together with myostatin has a role in regulating muscle growth rate. Variation in calpain system genes is expected to influence not only on the rate of postmortem meat tenderness, but also affecting on muscle growth. This research was aimed to determine the relationship between physical characteristics and microanatomical structure of male thin tail sheep muscle with variation genotype calpastatin (CAST-1) differences.

MATERIALS AND METHODS

Materials

This experiment used nine heads of male thin tail sheep with average weight 17-20 kg. Those sheep have changed a couple of milkteeth to permanent teeth, belong to the Jonggol Animal Science Teaching and Research Unit (JASTRU). Five sheeps were used for MM samples and four sheep were for MN samples. The only TT as a single genotype of calpain gene variations was found in early CAST-1determination which is as a wild type. Sheep were reconditioned for 3 mo in the Small Ruminant Field Laboratory, Faculty of Animal Science, Bogor Agricultural University. The sheep was represatated thin tail sheep maintained in extensive pasture systems.

The equipments used in this study consisted of instrument for analysis of muscle physical characteristics and microanatomical structure. Meat physical characteristics analysis tools were pH meter, carper press, planimeter, Warner-Bratzler, and bimetal thermometer. Analysis tool of for muscle microanatomy were a set of surgical instruments, glass cup, measuring cups, glass objects, glass cover, microtomes, incubators and a light microscope equipped with a camera.

Methods

Sheep calpastatin gene was extracted, identified and detected with refers to Sambrook *et al*. (1989). The variation of calpastatin gene was caused by mutation at 261st nitrogenous base position. Calpastatin gene as long as 622 pb of PCR product was cleaved by MspI restric-

tion enzyme that produce M and N allele (Sumantri *et al*., 2008). The M allele is a normal allel of calpastatin gene, but N allele is a mutated gene from G nitrogenous bases to A nitrogenous base at 261st nitrogenous base position. Determination of CAST-1 gene variations was only found MM and MN.

The sheeps were slaughtered with refers to **Baihaqi** & Herman (2012). Carcasses were divided according to the eight commercial pieces by Borton *et al*. (2005). Physical characteristic analysis of the meat was done on day 3 after slaughtered that stored in chilling room. The meat portion for physical analysis was taken from loin section. Observation of physical characteristics included pH lamb meat, water holding capacity (WHC), tenderness and cooking loss. pH value was measured by a pH meter by AOAC (1995), WHC was analyzed based on the percentage of water that comes out (mgH_2O) with Hamm method as described by Marino *et al*. (2011). Tenderness was evaluated by Warner-Bratzler shear force (Santos *et al*., 2008). Cooking loss was calculated to determine the percentage of shrinkage during the cooking process refered to Everts *et al*. (2010).

Preparation of microanatomical structure observation refered to Gorocica-Buenfil *et al*. (2007). Muscles used as samples for microanatomical structure observation were *M. sternocephalicus* and *M. gluteus medius*. Slide staining was performed by Haematoxylin-eosin staining (HE) while collagen tissue staining was using Masson Trichrome (Morgan *et al*., 2004). Samples were then observed with a microscope and digital camera for digitally imaging of microanatomy muscle. Digital image was processed by a modified muscle measurement methods according to Albrecht *et al*. (2006) using the Corel Draw X3 programe. Parameters measured were muscle fibre surface area, muscle bundle area, amount of the muscle per muscle bundle, percentage of the muscle area per muscle bundle, percentage of the connective tissue per muscle bundle, distance between muscle bundles and the percentage of connective tissue within perimysium.

Data Analysis

The collected data were analyzed using hypothesis testing two-tailed Student's test (multiple samples) to compare between calpastatin gene variations of MM and MN (Steel & Torrie, 1991).

RESULTS AND DISCUSSION

Physical Characteristics of Meat

The physical characteritics of meat are the meat quality important parameters. The result showed that MM genotype had a lower value of tenderness (P<0.05) compared with MN (Table 1). Tenderness point of MM was 2.24 kg/cm² which was belong to the category of very soft, whereas the MN was at 3.45 kg/cm² belongs to the category of soft. This finding was differed with Casas *et al*. (2006), who stated that the heterozygote (CT) of CAST between normal and mutated (transition from cytosine to tymine) had lower value of Warner-Bratzler shear force than normal CAST (CC) in *Bos taurus, Bos*

Table 1. The average value of physical characteristics of thin tail hogget with different genotype calpastatin

Parameters	Genotipe of calpastatin			
	MM (n= 5)	CV (%)	MN (n= 4)	CV (%)
Tenderness (kg/cm²)	2.24±0.32[a]	14.37	3.45±0.63[b]	18.34
pH	5.44±0.15	2.81	5.47±0.15	2.65
Cooking loss (%)	46.10±3.49	7.57	45.49±3.87	8.50
Water Holding Capacity (%H₂O)	40.00±5.28	13.20	37.00±2.23	6.03

Note: Means in the same rows with different superscript differs significantly (P<0.05). CV= coefficient of variation.

indicus and cross breed of those. These conditions indicated that the CAST-1 gene mutations contributed a significant effect on meat tenderness. The results of physical characteristics measurements indicated that the identification of calpastatin genetic can be used as a reference of marker in determining meat tenderness. The MN had the calpain enzyme inhibitory activity lower than MM. These data showed a contradiction with the results of Dagong *et al*. (2011) which stated that the difference of genotype CAST-1 at thin tail sheep did not give a significant effect on meat tenderness. This differences might be affected by differences of gene region intron 5-exon 6 (Dagong *et al*., 2011) and intron 1. In this study, sheep used had different weight and original place so that resulted in a higher coefficient of variation. This study used sheep derived from a single location with the same maintenance system, grazing on the similar age and body weight, so the coefficient of variation was less than Dagong *et al*. (2011). Moreover, time for withering was also influencing on CAST-1 enzyme activity which looked after withering for 36 hours (Camou *et al*., 2007).

pH value, WHC, and cooking loss between MM and MN were no significant difference. The MM pH value was 5.44, whereas the MN was 5.47. These value were in accordance with the limits isoelectric point of pH rigormortis, it indicates that the process was completed. Water holding capacity of MM consisted of 40.00% H₂O,

whereas the MN was at 37.00% H₂O. This finding demonstrated that the ability of the protein to bind meat-free water was higher in MN than MM. Cooking loss of MM was 46.10%, while the MN was 45.49%. Cooking loss difference between MM and MN was found 0.61% greater in MM than MN. This means that the mass loss during cooking of MM was higher than in MN. Cooking loss and WHC was quite high in both treatments. This indicates that myofibril proteins were degraded due to proteolytic enzymes. Water holding in MM was greater than MN. It is associated with meat tenderness values that MM was more tender than MN. This tenderness condition might be an indication that proteolytic enzymes in MM meat had a higher activity than in MN genotypes.

Microanatomical Structure of Meat

Observations on the axis of the body's muscles microanatomy representation were performed on *M. sternocephalicus* while for representing the locomotor muscles were used *M. gluteus medius*. The MN had a muscle fibre surface area, muscle bundle area, amount of the muscle per muscle bundle, percentage of the muscle area per muscle bundle, distance between muscle bundle, percentage of the connective tissue within muscle bundle were greater (P<0.05) than MM both in the axis of the body and locomotor muscle (Table 2 and 3).

In *M. sternocephalicus*, muscle fibre surface area of MM was 591.18 μm², whereas the MN was 638.48 μm². MN had muscle fibre surface area 47.3 μm² greater than in MM. Muscle bundle area of MM was 50,880 μm², whereas the MN at 82,648 μm². MN had muscle bundle area 31,768 μm² greater than in MM. This value indicated that the MN had higher muscle bundle development. Amount of the muscle per muscle bundle of MM was 79.40 whereas MN was at 124.20. Number of the muscle per muscle bundle MN 44.80 was greater than MM. This value showed that the growth hyperplasia in MN was higher than in MM. This was consistent with Kerth *et al*. (2003), which stated that the higher activity of calpastatin in callipyge sheep gave greater muscle fibre area in *semitendinosus*, *longissimus* and *supraspinosus* muscle of Hampshire x Rambouillet cross sheep.

Table 2. Microanatomy analysis of *M. sternocephalicus* of the thin tail hogget with different genotype calpastatin

Parameters	Genotipe of calpastatin					
	MM (n=5)		CV (%)	MN (n=4)		CV (%)
Muscle fibre surface (μm²)	591.18±	74.39[a]	12.58	638.48±	52.85[b]	8.28
Muscle bundle area (μm²)	50,880.00±10,486.30[A]		20.61	82,648.00±8,282.50[B]		10.02
Sum of muscle/ muscle bundle	79.40±	11.24[A]	14.15	124.20±	6.61[B]	5.32
Muscle area/muscle bundle (%)	92.63±	0.23[A]	0.24	95.97±	0.58[B]	0.61
Connective tissue/muscle bundle (%)	7.37±	0.23[A]	3.05	4.03±	0.58[B]	14.43
Distance between muscle bundle (μm)	30.80±	2.95[A]	9.58	56.80±	14.04[B]	24.72
Connective tissue within perimicium (%)	43.54±	0.59[A]	1.37	55.48±	12.19[B]	21.98

Note: [ab]Means in the same rows with different superscript differs significantly (P<0.05); [AB]Means in the same rows with different superscript differs significantly (P<0.01). CV= coefficient of variation.

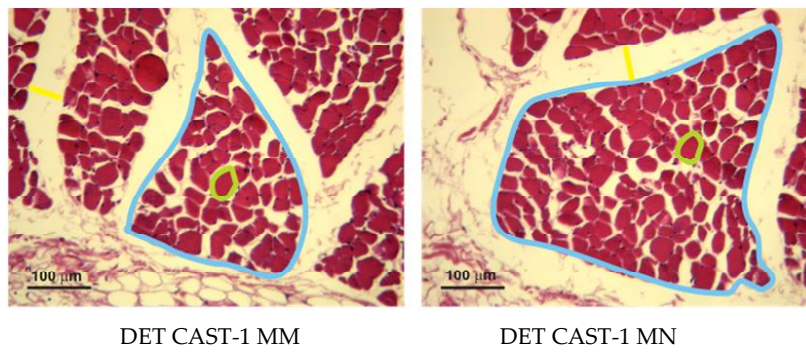DET CAST-1 MM   DET CAST-1 MN

Figure 1. Cross-section of *M. sternocephalicus* the thin tail hogget with different genotype calpastatin.
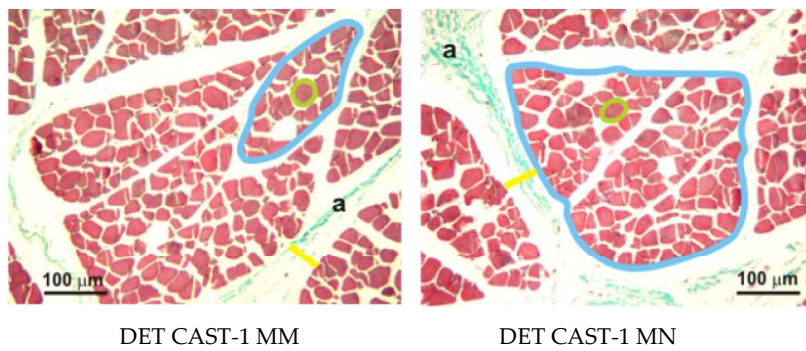(——): Muscle fibre; (——): Muscle bundle; (——): distance between muscle bundle.

DET CAST-1 MM   DET CAST-1 MN

Figure 2. Collagen (a) condition of *M. sternocephalicus* in thin tail hogget with different genotype calpastatin.
(——)

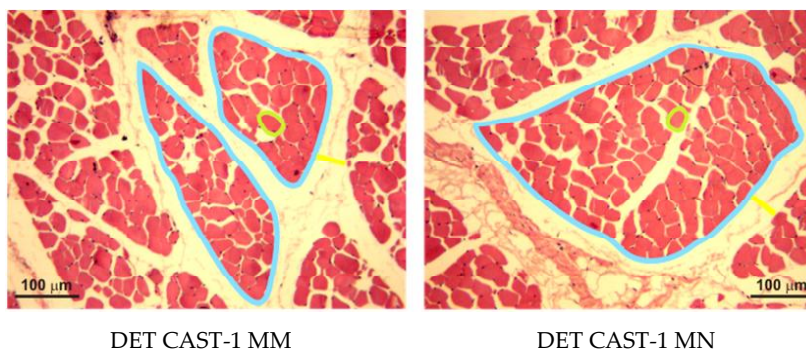DET CAST-1 MM   DET CAST-1 MN

Figure 3. Cross-section of *M. gluteus medius* the thin tail hogget with different genotype calpastatin.
(——): Muscle fibre; (——): Muscle bundle; (——): distance between muscle bundle.

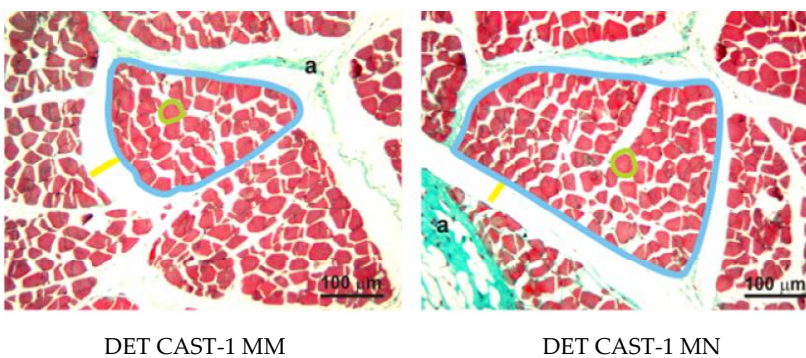DET CAST-1 MM   DET CAST-1 MN

Figure 4. Collagen (a) condition of *M. gluteus medius* in thin tail hogget with different genotype calpastatin.
(——): Muscle fibre; (——): Muscle bundle; (——): distance between muscle bundle.

Table 3. Microanatomy analysis of *M. gluteus medius* of the thin tail hogget with different genotype calpastatin

Parameters	Genotipe calpastatin			
	MM (n= 5)	CV (%)	MN (n= 4)	CV (%)
Muscle fibre surface (μm²)	585.26± 24.37[a]	4.16	633.50± 33.21[b]	5.24
Muscle bundle area (μm²)	80,774.00±12,950.60[A]	16.03	98,812.00±8,895.80[B]	9.00
Sum of muscle/ muscle bundle	125.60± 18.41[A]	14.65	151.20± 16.41[B]	10.85
Muscle area/muscle bundle (%)	93.16± 0.40[A]	0.43	95.90± 0.93[B]	0.97
Connective tissue/muscle bundle (%)	6.84± 0.40[A]	5.87	4.10± 0.93[B]	22.74
Distance between muscle bundle (μm)	31.60± 5.98[A]	18.93	55.60± 7.83[B]	14.08
Connective tissue within perimicium (%)	42.52± 1.07[A]	2.52	59.81± 5.79[B]	9.69

Note: [ab]Means in the same rows with different superscript differs significantly (P<0.05); [AB]Means in the same rows with different superscript differs significantly (P<0.01). CV= coefficient of variation.

Percentage of the muscle area per muscle bundle and percentage of connective tissue per muscle bundle indicated the proportion of connective tissue between the muscles in muscle bundle (Sharafi & Blamker, 2011). The percentage of muscle area per muscle bundle of MM was 92.63%, while the MN was 95.97%. The proportion of muscle in MN was greater (P<0.05) than in MM, the percentage of connective tissue per muscle bundle of MM was 7.37%, while the MN was 4.03%. Therefore the proportion of connective tissue in the MM was larger (P<0.05) than in MN. Distance between muscle bundle and the higher percentage of perimysium connective tissue in meat caused the higher weighness of meat perimysium (Sharafi & Blamker, 2010). Connective tissue in Masson Trichrome staining was indicated by the presence of a bluish green color. Distance between muscle bundle DET-1 MN CAST was greater (P<0.05) compared with DET CAST-1 MM by a margin of 26 μm. The percentage of collagen in the DET perimysium CAST-1 MN was greater (P<0.05) compared with DET CAST-1 MM with a difference of 11.94%.

The conditions of *M. sternocephalicus* was also found in *M. gluteus medius*. Muscle fibre surface area of MN had a larger size than in MM with a difference of 48.24 μm². The development of muscle hypertrophy MN was higher (P<0.05) than in MM. The muscle bundle area of MN was also broader than in MM with a difference of 18.038 μm². Muscle bundle vast amount is also affected by the amount of muscle per muscle bundle. The amount of muscle per muscle bundle in MN greater than MM by a margin of 25.60 muscles. The proportion of muscle in muscle bundle on MN had greater (P<0.05) than in MM by a margin of 2.74%. Muscle fibre surface area could be an indication of the growth of muscle hypertrophy, the increase in muscle size during postnatal growth, whereas the amount of muscle per muscle bundle could be an indication of the growth of muscle hyperplasia, which is an increase in the number of muscle cells during prenatal growth (Albrecht *et al.*, 2006). This indicated that the level of hyperplasia and hypertrophy of MN was higher than in MM. The connective tissue proportion in muscle bundle of MM was even greater (P<0.05) than in MN with a difference of 2.38%.

Muscle bundle sectional comparison of muscle and connective tissue between treatment conditions on *M. sternocephalicus* was presented in Figure 1 and 2. Muscle bundle sectional comparison of muscle and connective tissue between treatment conditions in *M. gluteus medius* was presented in Figure 3 and 4.

Distance between muscle bundle and perimysium percentage of collagen can be used as an indicator in meat tenderness, that the greater the distance between fasiculus and percentage of perimisum connective tissue in the meat will produce the increase of hard tenderness (Brewer *et al.*, 2007). Distance between muscle bundle of MN greater (P<0.05) than MM by a margin of 24 μm. The percentage of collagen in the perimysium of MN was greater (P<0.05) than MM by a margin of 11.94%. This explains the MM meat was softer than MN as the smaller percentage of collagen in the muscle perimysium.

CONCLUSION

Sheep with MN genotype showed tougher meat, it is characterized by greater of muscle fiber surface area, number of muscle per muscle bundle and muscle bundle area and harder meat tenderness than in MM genotypes. Hypertrophy and hyperplasia of MN are greater than MM genotypes.

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