Physical Meat Characteristics of Local Thin Tail Sheep based on Calpastatin (CAST) Genotype variation

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Abstract

Calpastatin (CAST) is an indigenous inhibitor of calpain that involved in regulation of protein turn over and growth. The objective of this research was to identify genetic polymorphisms in the part of intron 5 - entire exon 6 of CAST gene in local sheep and their association with meat quality and muscle composition. A PCR-SSCP method was carried out to identify genetic variation of CAST gene. In total 401 heads of sheep from 8 subpopulations were investigated, three groups of samples were thin tail sheep from Sukabumi, Jonggol and Kissar. The rest samples were Priangan sheep from Margawati and Wanaraja and fat tail sheep from Donggala, Sumbawa and Rote islands. Twenty one heads from Jonggol were used for meat and muscle identification. SSCP analysis revealed that three different SSCP patterns corresponded to three different alleles in the CAST locus (CAST-1, 2 and 3 allele) with six different genotypes. Genetic variation between local sheep populations were calculated based on genotypic and allelic frequencies. Most populations studied were polymorphic, with genotype frequencies of CAST-11, CAST-12, CAST-22, CAST-13, CAST-23, and CAST-33 were 29.7%, 38.2%, 24.2%, 2.5%, 4.5% and 0.7% respectively. CAST-1 and 2 alleles were most commonly found in all populations with total frequency 95.8%, while rare allele was CAST-3 (4.2%) and only found in thin tail population. Based on sequence analysis identified a non-synonymous amino acid variation in exon 6 induced Gln/Leu substitusion. There was no association between CAST alleles and genotypes with meat quality.

Key words: calpastatin, local sheep, meat quality, PCR-SSCP

Introduction

Local sheep is one of the genetic resource potential to be developed. This is due to their several advantages which are prolific, good adaptability to the harsh

environments, disease resistance, shorter production cycles and relatively requires small capital. In addition, in some densely populated areas like Java, sheep are able to substitute some beef that have to be imported each year. Based on 2008 data, special needs of sheep in West Java alone reach the range of 3.343.365 heads (Ditjennak 2009). The amount of the request indicates that the prospects for sheep farming is still wide open.

In relation to sheep development efforts, some weaknesses of the local sheep is that their slaughter weights and daily body weight gain are low vaired between 54 - 174 g/head/day (Yamin *et al.* 2009), in addition, the carcass quality and meat is also highly variable and do not meet international market standards. In order to solve the problem the genetic quality improvement efforts are needed to increase productivity, carcass and meat quality so the impact on lamb production will increase the contribution of lamb to total meat production in the country that currently only around 5% (Ditjennak 2009).

Molecular biotechnology advances allow the selection can be done at the DNA level through the use of marker genes that have an association with the highly ecomomic traits. One of marker genes associated with body weight in local sheep was calpastatin (Sumantri *et al*. 2008). Numerous other studies have also shown a relationship of calpastatin gene with carcass quality (Schenkel *et al*. 2006), and meat quality, especially tenderness (Casas *et al*. 2006; Curi *et al*. 2009).

Calpastatin (CAST) is a member of the calpain-calpastatin system involving three molecules of the enzyme μ-calpain, m-calpain and calpastatin that serves as both calpain inhibitor. This system plays a important role in diverse physiological processes such as regulation of protein turn over and growth (Goll *et al*. 1992), and myoblast migration (Dedieu *et al*. 2003), therefore CAST is believed as a good candidate gene for growth, carcass and meat quality.

Information on meat quality based on various calpastatin gene genotype in the local sheep currently is not yet available, so the information is needed in order to describe the effect of this gene on meat quality.

Materials and Methods

Sample and Genotyping

This study used 21 heads of Thin Tail Sheep (TTS) from UP3J Jonggol that reared intensively. Sheep then grouped based on their genotype variation based on Dagong et. al. (2011) methods. A pair of PCR primer, forward: 5'-GTTATGAATTGCTTTCTACTC-3' and reverse: 5'-ATACGATTGAGAGACTTCAC-3' was designed to amplify part of intron 5 and whole exon 6 of CAST gene, as described by Zhou et al. (2007). PCR amplification was carried out in 25 μl reaction containing 50-100 ng genomic DNA, 0.25 μM of each primer, 200 μM dNTPs (Fermentas), 4.0 μM Mg^{2+}, 0.5 U of Toptaq DNA polymerase (Qiagen, Hilden, Germany), and 1x

the reaction buffer. The condition of thermal cycling consisted of pradenaturation at 95 °C for 5 min, followed by 35 cycles of denaturation 95 °C for 30 s, annealing 56 °C for 45 s, and extension 72 °C for 45 s. The final extension step was at 72 °C for 5 min. Amplification was carried out in a thermal cycler (Mastercycler Personal 22331, Eppendorf, Germany). The PCR amplicon were checked on 1.5% agarose gels in 0.5 x TBE buffer containing 10% of ethidium bromide at 100 volt for 45 min and visualized by UV transiluminator. A SSCP procedure was used to identify variation in the amplicon of CAST locus. The sheep that have been known to represent the CAST genotype then slaughter each genotype to identify their physical meat charactreristics.

Physical Meat Characteristics

Meat quality was measured based on the physical parameters which include: pH measurement by pH meter and measured after aging for 24 hours. Meat tenderness was shown by the enormous strength (kg/cm^2) required to cut the meat cores indicated by the needle cutter Warner Bratzler Shear Force (WBSF) that moves over the scale with a measurement sensitivity of 0.1 kg/cm^2. Water Holding Capacity was measured with a planimeter by finding out the amount of water (mg H_2O). Cooking loss was measured by substracting the initial weight with the weight after sample cooked at 80 °C for 1 hour.

Association of CAST gene polymorphims with physical meat quality was analyzed by t-test with the following statistical equation:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sigma \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \qquad \sigma = \sqrt{\frac{\sum_{i=1}^{n}(\bar{X}_i - \bar{X}_1)^2 + \sum_{i=1}^{n}(\bar{X}_i - \bar{X}_2)^2}{n_1 + n_2 - 2}}$$

Where :

$\bar{X}_1$ and $\bar{X}_2$ = Mean value in genotype 1 and genotype 2
n_1 and n_2 = Number of sample in genotype 1 and genotype 2
σ = Total varians

Physical meat quality data corrected in advance using the following statistical equation:

$$X_i \ correction = \frac{\bar{X} standard}{\bar{X} \ observed} x \ X_i \ observed$$

Where :
X_i correction = value of physical meat characteristics after being corrected by sex and age

$\bar{X}$ standard = mean of physical meat characteristics of standard population

$\bar{X}$ observed = mean of physical meat characteristics of observed population

X_i observed = value of physical meat characteristics before being corrected by sex and age

Results and Discussion

Differences of physical meat characteristic

Means value of physical meat quality from different CAST genotypes on the local sheep are shown in Table 1. There was no significant difference (P>0.05) either in tenderness, water holding capacity, cooking loss and pH from three different CAST genotyes (CAST-11, CAST-12 and CAST-22) in local sheep. Similar results with a previous study by Zhou *et al*. (2008), who reported that all allelic variation (CAST-1, 2 dan 3) or variations of genotypes were identified in the CAST locus did not significantly affect the lamb tenderness.

Tenderness value of research results in the range 2 - 3 in a tender category, but no differences among the three genotypes. In contrast to some previous studies that identified a significant association between CAST variation with beef tenderness. In cattle, CAST gene variations have been used commercially as genetic markers. Two markers are currently available commercially were *GeneSTAR Tenderness* and *Igenity TenderGENE*. *GeneSTAR* using SNP G/A in 3'UTR region (Barendse 2002),

Tabel 1. Mean value of physical meat characteristics from various CAST genotypes of local Thin Tail Sheep

Physical meat characteristics	Genotypes					
	CAST-11 (n=4)	CV(%)	CAST-12 (n=10)	CV(%)	CAST-22 (n=7)	CV(%)
Tenderness (Kg/ cm^2)	3.16±0.72	22.78	2.98±0.79	26.51	2.49±0.71	28.81
Cooking loss (%)	49.54±3.24	6.54	43.76±3.61	8.25	46.32±6.53	14.10
Water Holding Capacity (WHC) (MgH_2O)	117.31±13.70	11.68	99.46±19.83	19.94	103.73±19.27	18.58
Persentage of WHC (% MgH_2O)	39.10±4.56	11.66	33.15±6.60	19.91	34.57±6.42	18.57
pH_{ult} (24 h)	5.57±0.07	1.26	5.75±0.31	5.39	5.80±0.32	5.54

Note: CV = Coefficient of variation (standard deviation/mean x 100%)

while *Igenity TenderGENE* using SNP G/C in intron 5 region (Van Eenennaam *et al*. 2007).

Differences in CAST gene effect sheep meat tenderness and beef probably caused by the fact that sheep meat is more tender due to the rate of myofibrils proteolysis of sheep meat is faster than beef (Koohmaraie *et al*. 1991), therefore, the differences in sheep meat tenderness has smaller effect on meat tenderness.

Conclusion

There was no difference between physical meat quality with CAST genotype variation in local sheep.

Acknowledgements

This research was funded by DP2M Dikti through *Hibah Kompetensi* project with contract No: 224/SP2H/PP/DP2M/III/2010 and DIPA IPB through *Hibah Disertasi Doktor* with contract No : 10/I.3.24.4/SPK/PDD/2011.

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