Association between Polymorphism of β-Lactoglobulin Gene on Milk Yield and Quality in Local Sheep at Jonggol Animal Science Teaching and Research Unit (JASTRU)

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ABSTRACT

The aims of this study were to identify the effects of polymorphism of β-lactoglobulin gene on milk yield and percentage of milk protein and fat in local sheep. A total number of 83 heads of lactating ewes raised under an extensive management at Jonggol Animal Science Teaching and Research Unit (JASTRU) of the Faculty of Animal Science, Bogor Agricultural University were studied. Research activities were carried out through some steps involving blood collection, DNA isolation, DNA amplification and separation of DNA fragments by electrophoresis with silver staining method. By using polymerase chain reaction (PCR) then genotyped by single strand conformation polymorphism technique (SSCP), it was successfully amplified a fragment length at 420 bp in β-lactoglobulin gene located on the exon 7. The electrophoresis pattern revealed 5 types of β-lactoglobulin gene and designated as A, B, C, D and E types. The proportion for those respective 5 types from the highest to the lowest were for A (27.71%), C (16.87%), D (12.05%), E (10.84%) and B (9.64%), respectively. There were no significant effect of polymorphisms in β-lactoglobulin gene on both milk yield and quality in local sheep at JASTRU.

Keywords : polymorphism, β-lactoglobulin gene, PCR-SSCP and local sheep

Introduction

Indonesian local sheep's are economically importance because of their many desirable characters such as to survive at low quality vegetation and to withstand seasonal shortages of food and water during dry season. Local sheep in Jonggol was originally from Jonggol area initially crossed with Garut sheep and continuously bred under an extensive management and a naturally selected toward hot and dry condition since 1980 (JASTRU, 1992). Genetic variation should be taken into consideration in order to put a guidance program of genetic conservation for this local sheep.

Polymorphism of β-lactoglobulin gene is valuable and well known due to its effects on quantitative traits and technological properties of milk. At DNA level, polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) allows for simultaneously typing of several alleles at casein loci, as well as for detection of unknown polymorphism.

In ruminants, genetic variability for milk protein is well known from which the variability has been shown to affect both milk composition and milk quality. Several studies have been done on cattle as reviewed by Braunschweigh *et al.* (2000), whilst the polymorphism in ovine milk protein has been less extensively investigated. An interesting and rather complex protein pattern was found in ovine milk, but a genetic control for polymorphism was observed only in few cases. Genetic polymorphism at a protein level was demonstrated for β-lactoglobulin consisting of 3 variants of A, B and C types (Erhardt, 1989) as

well as for α_{s1}-casein consisting of 3 variants of A, C, Welsh/D type (Chianese *et al.*, 1996). Some evidences have indicated that genetic variants in ovine affect physicochemical properties of milk (Pirisi *et al.*, 1999). Further, protein polymorphism was detected by electrophoresis methods at α_{s1}-casein (Chianese *et al.*, 1996), β-casein (Jann *et al.*, 2002) and α_{s2}-casein (Chessa *et al.*, 2003).

Among the DNA technologies, polymerase chain reaction-single-strand conformation polymorphism (SSCP) is a powerful tool for milk protein analysis, allowing for simultaneous typing of different alleles at α_{s1}-(*CSN1S1*), β-*CSN2*), and κ-(*CSN3*) casein loci. This method was successfully used for the analysis of bovine *CSN2* (Barroso *et al.*, 1999) and *CSN3* (Prinzenberg *et al.*, 2005). The same technique was used to differentiate *CSN1S1*B* from *CSN1S1*C* in cattle (Jann *et al.*, 2002). In sheep, Bastos *et al.* (2001) used PCR-SSCP to analyze *CSN1S1*, *CSN2* and *CSN3*. Polymorphic pattern was found at first two loci. Therefore, the present study was designed to analyze the effect of polymorphism of â-lactoglobulin gene on milk production and quality in local sheep under an extensive management at Jonggol Animal Science Teaching and Research Unit farm (JASTRU) of the Faculty of Animal Science, Bogor Agricultural University.

Materials and Methods

Sample collection and DNA isolation

A total number of local sheep used were 83 heads of lactating ewes collected at Jonggol Animal Science Teaching and Research unit (JASTRU) as a Unit Farm of the Faculty of Animal Science, Bogor Agricultural University. Individual milk yield was collected for 83 lactating ewes. Milk yield was measured 3 times a day for 2 months of lactation period. Milk production of individual ewe was obtained by subtracting the weight of the lamb after 15 min period of milking from pre milking previously fasted for 6 h period. Milk yield of individual ewe then was standardized to a mature equivalent production. Blood samples were collected randomly from all lactating ewes resulting for a total number of 83 DNA samples. The genomic DNA was extracted by a standard phenol-chloroform protocol through some modification by Sambrook *et al.* (1989).

PCR-SSCP analysis

By genotyping β-lactoglobulin gene, a fragment length at 420 bp on exon 7 of *CSN2* gene in sheep was amplified by a PCR method performed in a 12.5 il reaction mixture containing 1 µ l of DNA solution (100 to 150 ng), 10 pmol of each primer and 1 µ l (100 µ M) dNTP, 1 µ l (1.5 nM) $MgCl_2$, 10x PCR Promega *buffer* 1.25 µl and 0.15 µl supertaq. The primers used as described by Kumar *et al.* (2006) to amplify the fragment. Primers used were *forward* 52 CGGGAGCCTTG GCCCTCTGG 32 and *reverse* 52 CCTTTGTCGAGTTTGGGTGT 32. Polymerase chain reactions provided some steps: denaturizing at 94°C for 5 min; 30x cycles in (denaturizing at 94°C for 60 sec, annealing 56°C for 1 min, elongation 72°C for 2 min and final elongation 72°C for 7 min. SSCP analysis were carried out as follows : 6 µl of PCR product and 8 µl of denaturizing solution (0.05% xylene-cyanol, 0.05% bromophenol blue and 0.02 M EDTA in deionized formamide); heat denaturizing (95°C for 5 min) and a 15-h run (280V, 5°C) on 10% acrylamide : bisacrylamide gels (29:1) with 0.5% glycerol in 0.5x TBE buffer and silver staining by Tegelstrom method (1992) to visualize the bands.

Statistical design

Data were analyzed by a completely randomized design (Steel & Torrie, 1991) for identifying polymorphism effect of 5 alleles of A, B, C, D and E types of β-lactoglobulin gene on daily milk yield and percentage of milk protein and fat. Data of daily milk and percentage of milk protein and fat were collected from data recording at JASTRU. Mean differences were tested by Duncan test at a significant level of $\alpha = 0.05$. A mathematic model of completely randomized design was described as follows :

$$Y_{ij} = \mu + T_i + €_{ij}$$

Where:
Y_{ij} = observational value
μ = mean average
T_j = effect of β-lactoglobulin gene type-*i*
$€_{ij}$ = randomized error for a normal distribution

Frequency of each type of β-lactoglobulin gene was calculated by frequency formulation (Steel & Torrie, 1991) with a mathematical model:

$Y_i = n_i/N$

Where:
Y_i = gene frequency of β-lactoglobulin gene type-*i*
n_i = number of sample with β-lactoglobulin gene type-*i* in all population
N = total sample from population

Statistical analysis

Genetic variation for each sheep population was calculated in a form type of β-lactoglobulin gene according to Walpole (1995):

$$Xi = \frac{\sum_{i=1}^{n} ni}{N}$$

Where :
Xi = gene frequency of β-lactoglobulin gene type-*i*
ni = number of sample with β-lactoglobulin gene type-*i* in all population
N = total sample of population

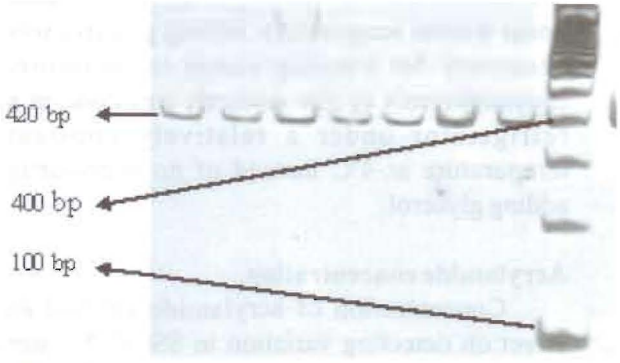

Figure 1. Results in amplifying β-lactoglobulin gene by PCR method at polyacrilamide 9 % gel (M: marker 100 bp DNA Ladder)

Results and Discussion

Amplification of β-lactoglobulin gene

β-lactoglobulin gene in local sheep at JASTRU was successfully amplified using primer pair as described by Kumar *et al.* (2006) through PCR method. Fitting primer pairs on the sequences of β-lactoglobulin gene on exon 7 conducted from Bank gene X12817 could identify estimation on successfully amplified fragment length. Results of β-lactoglobulin gene in local sheep successfully amplified were shown in Fig 1.

According to Kumar *et al.* (2006), the amplification of β-lactoglobulin gene by primer pair from Genbank at an accessing number of X12817 successively resulted in a fragment length at 426 bp (base pair). From this research, the successfully amplified fragment length of β-lactoglobulin gene was at 420 bp. Distinctive fragment lengths of β-lactoglobulin gene found in this research against that of arrangement by Kumar *et al.* (2006) was due to the later used the primer pair in amplifying β-lactoglobulin for goats in India. DNA samples used in this research were from sheep, therefore, distinction in species and original area resulted in distinctive amplification products.

Comparison on the sequences of amino acid in β-lactoglobulin genes among cattle, sheep and goats showed a high level of homology (over 95%) and identified only 6 positions in β-lactoglobulin gene in cattle leading to distinction in β-lactoglobulin between sheep and goats (Yahyaoui, 2001). Focusing on sheep and goat, it has been unknown yet the differences between amino acid sequences located in β-lactoglobulin gene. If there are distinctively different amino acid sequences between sheep and goats, they might cause distinctiveness of amplification products. Fig 2 showed the position of primer pair of AF 25 and 26 on the sequences in β-lactoglobulin in sheep.

Annealing temperature used in this research was 62°C. Annealing temperature was a range of temperature to lead primer complementing into DNA genome. Kumar *et al.* (2006) and Agung (2007) did the researches to amplify β-lactoglobulin gene at an annealing temperature of 65°C. Though there was a distinctively annealing temperature in amplifying β-lactoglobulin gene in this research against the former two, it would be no matter as β-lactoglobulin gene on exon 7 being the target of this research was still successfully amplified. The annealing temperature for amplification process is very substantial due to the process of lengthening DNA initially started from primer (Muladno, 2002).

Percentage of the achievement of amplified β-lactoglobulin gene was about 77% or equal to 64 samples successfully amplified from the total number of 83 samples. Less successfully amplifying DNA was likely because both attaining primer improperly leading to *in vitro* multiplication

Figure 2. The position of primer pair of AF 25 and 26 (underlined) in the sequences of β-lactoglobulin gene on exon 7 in sheep (Source: www.ncbi.nlm.nih.gov, accession number X12817, Harris *et al.*, 1988)

occurred ineffectively and extracting method unfavorably resulting for highly spotted substances. Succeeding in amplifying DNA, however, was determined by many factors such as concentration of DNA samples, polymerase tag, dinucleotide, ion Mg, buffer and primer. Moreover, heparin might be another eliminating factor during a multiplication process (Lestari, 2001). It was noted that by adding heparin up to 0.4 g/ml was able to decrease enzyme activity of polymerase taq by 60%. Various factors influence the result through PCR-SSCP method namely fragment length of DNA, temperature, acrylamide concentration, ratio between acrylamide and bisacrylamide and purity of DNA sample.

Fragment length

β-lactoglobulin gene resulted from the amplification in this research had a fragment length at 420 bp. According to Hayashi (1991) that sensitivity from PCR-SSCP method was 99% for that of DNA fragment length around 100-300 bp and 89% for DNA fragment length around 300-400 bp. As described previously, the fragment length for the resulting amplification in this research was at 420 bp, hence an accurate approach for PCR-SSCP was estimated approximately 89%.

Temperature

Electrophoresis temperature was carried out in a refrigerator at a temperature 4°C. Constant temperature strongly affect on both clarity and sharpness of resulted banding strand (Bastos *et al.*, 2001). If electrophoresis was done under a room temperature, adding glycerol was necessary for keeping stable temperature. Electrophoresis in this research was done in a refrigerator under a relatively constant temperature at 4°C instead of no necessarily adding glycerol.

Acrylamide concentration

Concentration of acrylamide gel had an effect on detecting variation in SSCP. A lower acrylamide concentration resulted in a softer and more sensitive gel in detecting variation (Hayashi, 1991). Barroso *et al.* (1999) found the best condition by using acrylamide concentration of 17%. Agung (2007) did use acrylamide concentration for 12%, while Ceriotti *et al.* (2004) used acrylamide concentration for 9.25%. Acrylamide concentration used in this research was for 8% (29:1) and 9% (59:1). By using dissimilar concentration in this research resulted in no differences in detection, but it spent more cost.

Composition between acrylamide and bisacrylamide

Composition in using acrylamide and bisacrylamide will affect on the matrix width passing through by DNA single probe. Barroso *et al.* (1999) conducted the ratio of 100:1 to remain the best condition of gel. Ceriotti *et al.* (2004) applied the ratio of 29:1, whilst Agung (2007) applied the ratio of 59:1.

Composition of acrylamide and bisacrylamide used in this study was 29:1 and 59:1, respectively. This dissimilarity resulted in no considerable effect of detection, as far as the form of DNA single strand was able to migrate into the gel matrix. Purifying DNA sample from the amplification was also an important factor of the achievement in PCR-SSCP method. During purifying process of DNA amplification, all substances instead of targeted DNA would be discarded at the time of separating supernatant by centrifugation. If DNA sample was inappropriately getting into purification, so there was still remaining PCR reaction that could cause other additional banding as a non-targeted DNA leading to a decreased accuracy for identifying types and determining genotype.

Detection on polymorphism of β– lactoglobulin gene by PCR-SSCP method

PCR-SSCP is a powerful method in detecting polymorphic DNA due to the existing changes on DNA fragments and in detecting a low rate of mutation (Yahyaoui, 2001). PCR-SSCP put the assumption that changes in nucleotide will affect conformation of single strand of DNA fragment (Bastos *et al.*, 2001) and migration rate during electrophoresis process. The migrating rate of single strand of DNA fragment at 9% gene of non-denaturized polyacrylamide was conversely comparable over diameter (size) of both DNA fragment and ion level in the gel (Muladno, 2002).

PCR-SSCP method had some steps. The first was for amplifying targeted DNA by *thermocycler* machine, the second was by purifying DNA sample and adding formaldehyde solution, and the third was for denaturizing PCR-produced DNA at temperature 94°C then coming into electrophoresis phase at 9% gel of nondenaturated polyacrylamide at a constant temperature. If an electrophoresis process was carried out at a room temperature, it was necessary for adding additive buffer such as glycerol into the gel to maintain a constant temperature. The electrophoresis process in this research was done under refrigerator temperature at 4°C, so without adding glycerol, temperature was kept constant. The constant temperature could affect the clarity and sharpness of banding product.

Polymorphism of β-lactoglobulin gene by SSCP method

This research found that β-lactoglobulin gene at 7 exon in local sheep at JASTRU Jonggol was polymorphic. This was dissimilarly against the study by Agung (2007) reported that β-lactoglobulin through PCR-RFLP method of analysis was identified as monomorphic in sheep. Kucinskiene (2005) by using PCR-RFLP method found two alleles in β-lactoglobulin gene in Lithuanian Native Coarsewooled sheep, namely alel A and B. In this study, DNA sample used originated from dual purpose sheep of meat-wool types. It was found 3 genotypes in this research, namely AA, AB and BB. Genotype BB had a strong correlation toward high milk production, while genotype AB and BB had a strong correlation with high protein and casein percentages.

Detecting polymorphism of β-lactoglobulin gene by the approached SSCP method resulted in 5 types of genes consisting of A, B, C, D and E. Fig. 3 illustrated the result of polymorphism of β-lactoglobulin gene detected by PCR-SSCP method. Fig. 4 illustrated the diagram of electrophoresis for each type of β-lactoglobulin gene. Peninsula type was done through the observation of migrating pattern of DNA single strand on a piece of nondenaturated polyacrylamide at 9% gel.

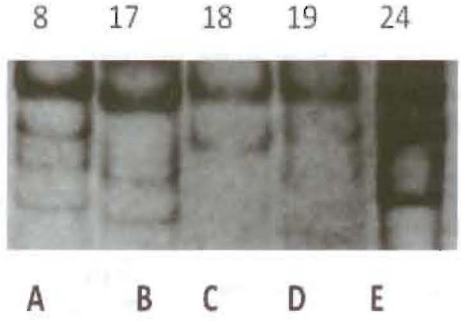

Figure 3. Results of polymorphism of β-lactoglobulin gene detected by PCR-SSCP method of nondenaturated polyacrylamide at 9% gel. There were 5 types of A (8), B (17), C (18), D (19) and E (24)

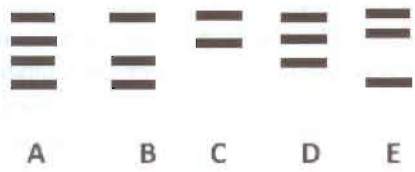

Figure 4. Electrophoretic diagrams of individual type of β-lactoglobulin gene

The success of detecting polymorphism by PCR-SSCP method in this research was 77%. Percentage of types of A, B, C, D and E were 27.71; 9.64; 16.87; 12.05 and 10.84%, successively. Frequency of each type was recognized by dividing the number of sample for a certain type over the total number of samples from all types. The frequency of all types in β-lactoglobulin gene were presented in Table 1.

Table 1 shows that the frequency of SSCP type A in β-lactoglobulin gene was the highest; on the contrary, SSCP type B was the lowest. SSCP type A had 4 bands whereas type B had 3 bands. In previous study by Agung (2007), β-lactoglobulin gene was amplified by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method and the result was monomorphic.

This was due to the application of PCR RFLP method through out the use of restricting enzyme of *SacII* was unable in recognizing cutting sites of that DNA fragment. No polymorphism in β-lactoglobulin gene in this study was also possible as local sheep came from 8 different areas namely Ciomas, Jonggol, Margawati, Indramayu, Donggala, Madura, Sumbawa and Rote. These local sheep had not been selected for high milk production and in Indonesia selection of local sheep was generally stressed on a meat type. Therefore, selection on local sheep in Indonesia has not been more focused on the identification of animals with better potency for both high milk production and high protein yield.

Table 1. Frequencies of individual type of β-lactoglobulin gene

SSCP type	Frequency (%)	Sample number (n)
A	42.28	27
B	12.50	8
C	21.87	14
D	7.81	5
E	15.62	10
Total	100	64

Detection of polymorphism of β-lactoglobulin by PCR-SSCP method can be divided based on the number of appearing band and migrating rate of DNA fragment. The types of β-lactoglobulin gene that were possible to be identified in this study had 2 to 4 bands. This result agreed with the study by Bastos *et al.* (2001) reporting that the maximum number of bands in a DNA sample by using SSCP method was 4 bands. The result from this study was still in types of β-lactoglobulin gene, hence it was still unable yet to identify possible alleles, determine genotypes and calculate heterozygosis values. To investigate the existence of allelic number in β-lactoglobulin gene in sheep was required to sequence the identified types. The distinction in detected polymorphism strongly depended on the changes of the pattern of DNA single strand. The factors of fragment length and its environment such as temperature, ion concentration, soluble concentration in the gel (Hayashi, 1991) affected the DNA single strand in a gel.

Effect of polymorphism in β-lactoglobulin gene on daily milk yield and milk composition

Polymorphism of β-lactoglobulin gene in ruminants has been reported from many studies. Ng Kwai Hang *et al.* (1986) and Bovenhuis (1992), for example, found polymorphism of β-lactoglobulin gene in dairy cattle relating to the percentage of milk protein and fat. Mroczkwski (2004) also found that polymorphism of β-lactoglobulin gene in Merino goat was associated with milk yield, protein percentage and fat percentage. Genotype BB had a tendency for producing high protein and fat percentages against genotype AA and AB.

Daily milk yields at the ages of 1, 2, 3 and 4 years were successively 368.30; 429.24; 519.96 and 462.98 g/hd/d, with the values of sd were 25.21; 137.24; 130.89 and 116.31 g/hd, respectively. Daily milk yields in this research were statistically ($p<0.05$) affected by the ages of ewes.

Polymorphism of the types in β-lactoglobulin at exon 7 identified in ewes at JASTRU Jonggol did not significantly affect daily milk yield, percentage of milk protein and percentage of milk fat. The existing distinction between this research against other researches conducted previously by Ng-Kwai-Hang *et al.* (1986), Bovenhuis (1992) and Mroczkwski (2004) was likely due to

Table 2. Effect of types of β-lactoglobulin gene on milk yield, protein and fat

Type	Milk Production			Protein (%)			Fat (%)		
	x ±sd	cv	n	x ±sd	cv	n	x ±sd	cv	N
A	474.5 ± 135.3	28.58	23	5.31 ± 0.625	11.77	4	3.07 ± 1.38	44.95	4
B	441.5 ± 129.8	29.39	8	5.35	-	1	3.56	-	1
C	483.8 ± 129.5	26.76	14	5.39 ± 0.506	9.38	7	3.91 ± 1.02	26.08	7
D	446.6 ± 70.5	15.78	10	6.09	-	1	3.27	-	1
E	493.3 ± 127.2	25.78	9	5.74 ± 1.12	19.51	2	4.74 ± 0.19	4.00	2

Note: χ = mean, sd = standard deviation, cv = coefficient of variation, n = sample number

many factors. Distinction of sequences in β-lactoglobulin in this research compared to variation of protein type in β-lactoglobulin studied by Ng-Kwai-Hang *et al.* (1986); Bovenhuis (1992) and Mroczkwski (2004), was caused by distinction in amino acid sequences produced by β-lactoglobulin gene. Ng-Kwai-Hang *et al.* (1990) pointed out that differences in number of population, dairy breed and environment could also be possible sources. Milk trait is one of quantitative traits controlled by many genes, an additive trait dominantly affected by environment. Nudda *et al.* (2003) pointed out that protein content in milk was affected by factors of genetic and environment.

Generally, local sheep in Indonesia was a meat type. It was likely that local sheep at JASTRU Jonggol has not been selected toward a high milk production, as a result, polymorphism in the types of β-lactoglobulin did not affect daily milk yield, percentage of milk protein and fat. By using PCR-SSCP method, it was known that β-lactoglobulin gene in local sheep at JASTRU was polymorphic. Five types of variant were identified in β-lactoglobulin gene at exon 7, namely type A (27.71%), B (9.64%), C (16.87%), D (12.05%) and E (10.84%). Type A was at the highest percentage compared to the other types. Based on the variant analysis showed that polymorphism of β-lactoglobulin identified by PCR-SSCP in this research resulted in no significant effect on daily milk yield, percentage of protein and percentage of fat in local sheep at JASTRU.

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References

Agung PP (2007) Identifiksi Keragaman Gen κ-Kasein dan β-Lactoglobulin pada Domba Lokal Indonesia. Skripsi. Fakultas Peternakan. Institut Pertanian Bogor, Bogor.

Barroso A, Dunner S & Canon J (1999) Technical Note: Use of PCR-Single Strand Conformation Polymorphism Analysis for Detection of Bovine β-Casein Variants A_1, A_2, A_3 and B. J Anim Sci 77: 2629-2632.

Bastos E, Cravador A, Azevedo J & Pinto HG (2001) Single Strand Conformation Polymorphism (SSCP) Detection in Six Genes in Portuguese Indigenous Sheep Breed "Churra da Terra Quente". Biotechnol Agron Soc Environ 5 (1): 7-15.

Bovenhuis H, Johan AM, Arendonk V & Korver S (1992) Associations between Milk Protein Polymorphisms and Milk Production Traits. J Dairy Sci 75: 2549-2559.

Braunschweig M, Hagger C, Stranzinger G & Puhan Z (2000) Associations between Casein Haplotypes and Milk Production Traits of Swiss Brown Cattle. J Dairy Sci 83: 1387-1395.

Ceriotti G, Chessa S, Bolla P, Budelli E, Bianchi L, Duranti E & Caroli A (2004) Single Nucleotide Polymorphisms in the Ovine Casein Genes Detected by Polymerase Chain Reaction-Single Strand Conformation Polymorphism. J Dairy Sci 87: 2606-2613.

Chessa S, Budelli E, Chiatti F, Cito AM, Bola P & Caroli A (2003) Short Communication: Predominance of β-Casein (CSN2) C Allele in Goat Breeds Reared in Italy. J Dairy Sci 88: 1878-1881.

Chianese L, Garro G, Mauriello R, Laezza P, Ferranti P & Addeo F (1996) Occurrence of Five alpha s1-Casein Variants in Ovine Milk. J Dairy Res 63(1): 49-59.

Erhardt G (1989) Evidence for a Third Allele at β-Lactoglobulin Locus of Sheep and Milk and its Occurrence in Different Breeds. Anim Genet 20: 197-204.

Harris S, Ali S, Anderson S, Archibald AL & Clerk AJ (1988) Complete Nucleotide Sequence of the Genomic Ovine beta-Lactoglobulin Gene. Nucleic Acids Res 16 (21): 10379-10380.

Hayashi K (1991) PCR-SCCP : a Simple and Sensitive Method for Detection of Mutations in Genomic DNA. PCR Method and Applications 1: 34-38.

Jann MW, Shirley KL & Small GW (2002) Clinical Pharmacokinetics and Pharmacodynamics of Cholinesterase Inhibitors. Clin Pharmacokinet 41 (10): 719-739.

JASTRU (1992) Annual Report Jonggol Animal Science Teaching and Research Unit. Fact. of Anim. Sci. Bogor Agric. Univ.

Kumar A, Rout PK & Roy R (2006) Polymorphism of β-Lactoglobulin Gene in Indian Goats and its Effect on Milk Yield. J Appl Genet 47 (1).

Kurcinskiene J, Vagonis G, Maliviciute J & Tapio I (2005) Genetic Polymorphism of β-Lactoglobulin in Lithuanian Blackface and Lithuanian Native Croarswooled Sheep. Vet Zootech 29: 90-92.

Lestari ID (2001) Analisis Keragaman Genetik Kura-kura Air Tawar (*Cuora ambionensis*) Berdasarkan Haplotipe mtDNA. Skripsi. Fakultas Matematika dan Ilmu Pengetahuan Alam. Institut Pertanian Bogor, Bogor.

Mroczkowski S, Korman K, Erhardt G, Piwczynski D & Borys B (2004) Sheep Milk Protein Polymorphism and its Effect on Milk Performance of Polish Merino. Arch Tierz Dummerstorf 47. Special Issue 114-121.

Muladno (2002) Seputar Teknologi Rekayasa Genetika. Pustaka Wirausaha Muda dan USESE Foundation. Bogor.

Ng-Kwai-Hang KF, Monardes HG & Hayes JF (1990) Association between Genetic Polymorphism of Milk Proteins and Production Traits during Three Lactations. J Dairy Sci 73: 3414-3420.

Ng Kwai Hang KF, Hayes JF, Moxley JE & Monardes HG (1986) Relationships between Milk Protein Polymorphisms and Major Milk Constituents in Holstein-Friesian Cows. J Dairy Sci 69: 22-26.

Nudda A, Feligini M, Battacone G, Pietro N, Macciotta P & Pulina G (2003) Effects of Lactation Stage, Parity, β-Lactoglobulin Genotype and Milk SCC on Whey Protein Composition in Sarda Dairy Ewes. Ital J Anim Sci 2: 29-39.

Pirisi A, Piredda G, Papoff CM, Di Saivo R, Pintus S, Garro G, Ferranti P & Chianese L (1999) Effect of Sheep alpha s1-Casein CC, CD and DD Genotypes on Milk Composition and Cheesemaking Properties. J Dairy Res 66 (3): 409-419.

Prizenberg EM, Gutscher K, Chessa S, Caroli A & Erhardt G (2005) Caprine ê-Casein (CSN3) Polymorphism: New Developments in Molecular Knowledge. J Dairy Sci 88: 1490-1498.

Sambrook J, Fritsch EF & Maniatis T (1989) Molecular Cloning: a Laboratory Manual. 2nd Ed. Cold Spring Harbor Laboratory Press, New York.

Steel RGD & Torrie JH (1991) Prinsip dan Prosedur Statistika. Cetakan Kedua. PT. Gramedia Pustaka Utama, Jakarta.

Tegelstrom H (1992) Mitochondrial DNA in Natural Population. An Improved Routine for Screening of Genetic Variation Based on Sensitive Silver Staining. Electrophoresis 7: 226-229.

Walpole RE (1995) Pengantar Statistika. Gramedia Pustaka Utama. Jakarta.

Yahyaoui MH, Coll A, Sanchez A & Folch JH (2001) Genetic Polymorphism of the Caprine kappa Casein Gene. J Dairy Res 78: 209-216.