Genetic Relatedness Between Batur, Merino and Local Sheep Based on Random Amplyfied Polymorphism DNA Marker

Prayitno[1)*], T Hartatik[2)], R Pratiwi[2)] and WT Artama[2)]

[1)]Faculty of Animal Science, Jenderal Soedirman University, Jln. Dr. Soeparno No. 60, Purwokerto 53123, Central Java, Indonesia
[2)]Faculty of Animal Science, Gadjah Mada University, Jl. Fauna 3 Bulaksumur, Yogyakarta 55281, Indonesia
***Corresponding author email: pray.it@yahoo.com**

Abstract. RAPD analysis to determine the diversity, status and genetic close relatioship Batur with Merino, Garut, Thin Tail and Fat Tail sheep genomic DNA was used in 27 Batur, 15 Merino, 17 Garut, 15 Thin Tails and Fat Tails animals. The process of RAPD-PCR used five primers of 10 nucleotides. PCR results were electrophoresed with 2% agarose gel. Identified similarities and differences between individual RAPD bands one and cross-sample of the population, genetic distance and closeness of genetic relationship. The process 89 sample sheep RAPD-PCR generated of 4189 band from 100 to 1500 bp which consisted of 91 type. Batur sheep samples produced bands at most (1666 tape) and the lowest Fat Tails (493 bands). The number monomorfism of bands most of the Batur (891 bands) and the lowest Garut (170 bands), but the polymorphism band's highest of the Batur (775 bands) and the lowest Fat Tails (262 bands). Between individuals within populations have similar genetic Merino highest and the lowest Thin Tail. Cross-population genetic similarity is highest individuals in the population Batur and Merino, while the lowest Merino and Thin Tail. The closest genetic distance was Batur with Merino population and the most apart distant Merino with Thin Tail or Merino and Fat Tails. Batur sheep population has the closest genetic relationship with the Merino and the most apart distant is with Fat Tail.

Key Words: RAPD, genetic markers, genetic similarity, sheep

Introduction

Sheep reared in Batur village Banjarnegara District, has similar morphological features as well as Merino and a mix of Merino and local sheep. Batur sheep genetic status is unknown because they do not have a clear pedigree records. Study with specific genetic markers can be known genetic status and genealogical of the Batur sheep. Identification by morphological markers is difficult to distinguish homozygous and heterozygous individuals, so the results are not accurate. Measurement of morphology is also difficult to identify gene mutations, because mutant alleles can be detected only 0.1% (Urich, 1990). Genetic markers between populations can be to know the differences of population genetic structure, gene flow, genetic history and close relationship parents (Feral, 2002). Analysis by Amplyfied Random DNA Polymorphism Polymerase Chain Reaction technique (RAPD-PCR) can be found in specific genetic markers for identification of the genetic status of sheep Batur.

Marker Random Amplified Polymorphic DNA (RAPD) was used amplified DNA fragments of an unknown DNA nucleotide sequnce and function (Liu and Cordes, 2004). RAPD-PCR technique used primer have homology with the target locus is random and can be to amplify many loci so that the resulting DNA fragments potentially be used as genetic markers to identify and analyze the genome, diversity and close genetic relationship of animal sepsies (Tahmoorespur et al., 2008), DNA polymorphisms the level of inbreeding, the species and strains, the history of ancestor, to reconstruct a map of genes linkage, identification of major genes for a tool selection or Marker Asisted Selection abbreviated MAS (Liu and Cordes, 2004). RAPD technique was successfully used to estimate genetic diversity between one and different lineage or strains of

sheep (Kantanen et al., 1995; Ali, 2003), identifying the sex of the sheep embryo (Cushwa et al., 1996). RAPD marker when linked to Quantitative Trait Loci abbreviated (QTL) can be categorized as a genetic marker for the tool selection (marker-assisted selection) Pooled Dorset sheep (Malau-Aduli et al., 2006).

Materials and Methods

The research was conducted in Biotechnology Study Center Laboratorium, Gadjah Mada University with several step as follow: Blood sample of about 3 ml were coledted from viena jugularis 27 Batur, 15 Merino, 17 Garut, 15 Thin Tail and 15 Fate Tail sample sheep populations. Genomic DNA was extracted by phenol-chloroform extraction methods the protocol adopted from Sambrooke et al. (1989) methods with several modification. The pellet DNA was washed twice with cold ethanol absolute, after air dried and than subsequently dissolved in 100 µl TAE buffer. The isolated genomic DNA checked in 2% agarose gel electrophoresis. The consentartion and purity of the DNA measured by spectrofotometry on the absorbance 260 and 280 nm respectively. The purified DNA than storage in -200^{o}C until used for futhure RAPD-PCR analysis. RAPD-PCR was carry out with genomic DNA individual samples. A total five primer 10 mer of arbitary sequence (Frementas Coltd, Lithuania) were used. RA09 (CCT GGG ACT C), RA01(GGA AGC TCT C), RA08 (CGG GCA ACG T), Moh-4 (GCA TGC GAT C) and Moh-21(AAC CGC CGT CT) (SBS Genetech Co., Ltd, Cina) were used. The PCR process were cary out in a final volume 25 µl mixture containing 12,5 µl Mega Mix Royal PCR Mix (2MMR-10 Microzone Ltd, UK), 4 µl primer (100 picogram/ µl), 1 µl DNA sample (50 nanogram/µl) and 7,5 µl dH_2O. The PCR process consist 2 min of initial denaturation at 95^{o}C followed by 45 cycles of denaturation at 94^{o}C for 30 sec, annealing at 350^{o} C for 45 sec, extension at 72^{o} C for 1,5 min and final extension at 72^{o}C for 7 min (Thermocycler, Applied Biosystems. Gene Amp. PCR System 2400, PCMCIA RS232, Singapore). A volume of 25 µl was electroforesis on 2% agarose gel (Sigma Co Ltd, USA) that at constan voltage 5 volt cm-1 for 90 min. Profil RAPD bands were visualized and documented using digital camera (MPIX 5,0 M Zoom, Made in China) after staining with ethidum bromide. A 100 to 3000 bp DNA ladder (NL0403, VC 100bp Plus DNA Ladder, Vivantis) was used as know molecule size DNA marker. The RAPD profile was scored one for presence of a band and the absence was zero or nul scored. All score of a band constructed as a binary matrix for used compute genetic diversity, genetic similarity between individual in one and cross of the populations, genetic distance and genetic relatedness between population. Genetic similarity between individual within population was estimated based on bandsharing frequency by Lynch (1994) using the formula:

$$Bab = \frac{2Bab}{(ba + bb)}$$

Bab are everage band that similar a and b individual, whereas ba and bb the number of bands on a and b individual. 2Bab are number of bands that similar in a and b individual sample was compared. Genetic similarity between individual cross population population was estimated based on bandsharing frequency by Lynch (1990) using the formula:

$$B = 1 + B_{xy} - 0.5(B_x B_y)$$

Where, Bx and By are everage band that similar between individual within x and y populations. Bxy are everage band that similar between individual cross x and y populations that compared. Bandsharing frequency were used to estimate genetic similarity and also calculated genetic distance between populations. Genetic distance was estimated by Lynch (1991) using the formula :

$$D_{xy} = -\ln\left(B_{xy} \frac{B_{xy}}{(B_x B_y)^{0,5}}\right)$$

Where as Dxy are genetic distance, Bxy are average band that similar between individual cross x and y population, Bx and By are average band that similar between individual within x and y population comparared. Genetic distance value were used to cluster analysis by Unweighted Pair Group Method Using Arihtmetic Average (UPGMA software NTSYSpc2) for know closer genetic relationship between cross Batur, Merino, Garut, Thin Tail and Fate Tail sheep populatins.

Results and Discussion

RAPD profiles five breed of sheep

Amplification of DNA samples of 89 heads of sheep (Batur, Merino, Garut, Thin Tail and Fat Tail) with primers RA09, RA01, RB08, Moh Moh-4 and the resulting 3921-21 bands consisted of 91 type of bands monomorphism and polymorphism 100 to 1500 bp. Five primers can amplify 91 loci monomorphism and polymorphism. Merino samples has the most monomrphism loci (10 loci) for primer RA01 and the lowest locus Moh-4 in Fat Tail. In Batur sheep, there are presence seven loci monomorphism for primer RA09 or Moh-21, four locus Moh-4 in Garut, eight locus RA09 on Thin Tail and five loci for primer RA09 on Fat Tail sheep. Batur sheep samples produced most RAPD monomorphism and plomorphism bands (729 and 635 bands). The number and proportion of the monomorphism bands the lowest are Garut samples (204 bands and 31%). Although the proportion of the monomorphism bands Merino sample are highest (88%), but the number and proportion of polimorphism bands are lowest (90 bands and 12%), while the highest are Garut (69%). Batur sheep samples produced the most number of RAPD bands is possible because the number of samples more than the sample Merino, Garut, Thin Tail and Fat Tails. The results of this study showed differences when compared to the results of RAPD analysis of samples of sheep and goats by previous researchers. RAPD Analysis of samples of goat Kacang, Bligon and Peranakan Etawah with primers RA01, RA09 and RB08 produced 20 different bands from 300 to 2000 bp. The three primer resulted 2, 2 and 4 polymorphisms bands respectively (Katrina et al., 2003), but analysis the five local goat in China with the same primers produced 21 type of bands. Primer RA01, RA09 and RB08 produced 1, 2 and 1 polymorphism bands respetively (Luo et al., 2000). RAPD analysis of 108 samples from the three breed of local sheep in the Turkish using 15 different primers resulted 250 to 2,500 bps bands. Samples of sheep Kıvırcık, Go kcEada, and Sakız showed 82 polymorphic loci (Elmaci et al., 2007). RAPD analysis samples from five breeds of local sheep in Iran with 17 primers type, only 3 primers that produce polymorphic bands (Tahmoorespur et al., 2008). Results of research Stephen et al. (2009) reported that RAPD analysis of samples from five breeds (Ecotype) local sheep in Tanzania using five primers produced 30 types of band polymorphism. Results RAPD analysis of 238 samples from the five breeds of local sheep in Brazil using 19 primers produced 54 bands 339 to 2536 bp (Paiva et al., 2005).

Genetic similarity between individuals one population

Genetic similarity within one population was analyzed using the average bandsharing frequency of RAPD bands between individuals within a population. The average bandsharing frequency similarity indicates genetitic analysis showed similarities bandsharing is similarity (genetics homogeneity) between individuals within a population. Results RAPD highest

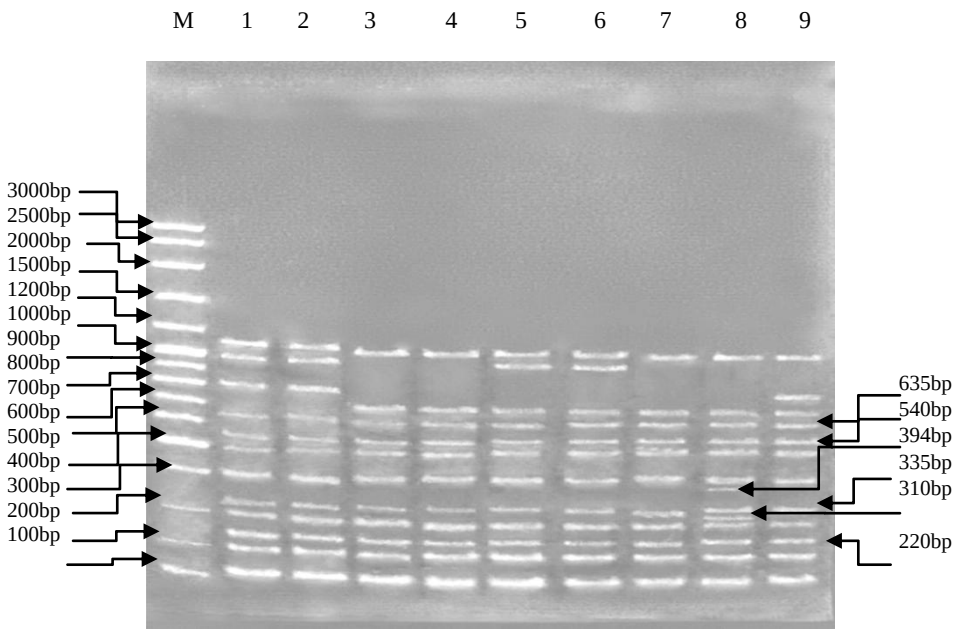

Figure 1. Sample of the RAPD bands profile Batur sheep from amplification by primer RA01

among individuals in samples of Merino (1 or 100%) produced by primer RA01 or RB08 and the lowest Thin Tail (0.66 or 66%) use of primer RB08. These results illustrate the highest genetic similarity (the genetic most homogen) are between individuals within populations of Merino. The level of genetic homogeneity in the population Batur, Garut and Thin Tail is the same i.e 83% respectively (Table 2). Presumably because the sample Merino is taken from Australia is coming from breeder that have pedigree records and make selection of candidates with both ewes and female so that the genetic merit of offspring uniform. Batur and Garut sheep is the result of crossbreeding of local sheep lineage (strain) Javanese Thin Tail and imports so that the genetic uniformity between individuals in both populations was lower than the Merino population. Thin Tail sheep and Fat Tails have long bred and spread to various regions, resulting in gene flow between populations due to the movement of sheep across districts, provinces or cross the island. Stephen et al. (2009) states, between individuals within a ecotype a separate by location (isolated) with other ecotype showed genetic uniformity (genetic similarity) is higher than that although ecotype breeding locations far apart but there easy movement of individuals between ecotype. In this Ecotype type not commonly found specific RAPD fingerprints for each of ecotype. Kantanen et. al. (1995) RAPD analysis of two local sheep in Finland show high genetic similarity. Yuangfang et al. (2002) RAPD analysised local and imports sheep in China shows the level of heterozygosity between individuals within a breed of sheep is higher than across breeds. Inter-local sheep have very close genetic relationship, although according to the history of local sheep in Brazil was the result of cross sheep were randomly various African and European origin. Presumably because of the breeding occurs genetic drift (Paiva et al., 2005). Katrina et al. (2003) RAPD analysis of sample goat Kacang, Bligon and Peranakan Etawah (PE) showed that the genetic similarity of individuals within a local goat population (Kacang goat) was lower than the Peranakan Etawah (PE), but individuals among the three

Table 1. The average bandsharing frequency RAPD bands between individual within a population produced by amplification using five primer 10 nucleotide

Primer Code	Breed of Sheep				
	Batur	Merino	Garut	Thin Tail	Fat Tail
RA09 (1)	0.86	0.99	0.93	0.92	0.87
RA01(2)	0.82	1	0.67	0.76	0.93
RB08(3)	0.89	1	0.89	0.66	0.89
Moh-4(4)	0.73	0.83	0.81	0.94	0.75
Moh-21(5)	0.86	0.97	0.85	0.89	0.93
Mean	0.83	0.96	0.83	0.83	0.87

populations of PE is almost the same genetic similarity. If traced their origins Merino are descendants of Mouflon (Ovis mussimon) in Europe, whereas Thin Tail and Fat Tail are descended Urial (Ovis orientalis) in Asia. Batur and Garut sheep are crosses of local sheep derive Urial with sheep imports derive Mouflon so that offspring have a combination of the two loci of his ancestors. Sheep in Southeast Asia are descendants Urial (Ovis orientalis), but also found in alleles of Argali (Ovis ammon) (Zeuner, 1963). George et al. (2002) states that Javanese Thin Tail sheep carrying genes Fecundety Java (FecJ), but Garut sheep carry the gene Fecundety Merino Booroola (FecB). Sheep breeding devoleped in the area of the Indonesian island and the Malay peninsula, thought to have come from Central Asia which now includes Tibet and Mongolia. The similarity can be observed in a Thin Tail sheep breeding in the area of Sumatra, has a hair (wool) similar to that breed of sheep in some Asian countries (Piper and Ruvinsky, 1997).

Genetic similarity between individual across populations

Individual genetic similarity across populations were analyzed using the average bandsharing frequency of RAPD bands between individuals across the two populations are compared. Bandsharing frequency of RAPD bands across the population to illustrate the genetic similarity (genetic homogeneity) between individual across of the population. High-bandsharing frequency similarity between individuals across populations have high genetic similarity. Bandsharing frequency is highest between Batur and Merino sheep, while the lowest (0.23) Merino and Thin Tail. Bandsharing frequency of RAPD bands between individuals across the population is smaller than within a population (Table 2 and 3).

The results of this study have similarities and differences with the results of RAPD analysis in goats and sheep that have been conducted by researchers previously. Results RAPD Kacang goat, Bligon and Peranakan Etawah with primers RA01, RA09 and RB08 showed bandsharing frequency between individuals within one of the population is greater than the cross population (Katrina et al. 2003), RAPD analysis with the same primer on the six breed of sheep in China (Boer, Saanen, Angora, Dairy and Shaanan Guanzhong White). Bandsharing frequency showed similarity between individuals within a population is more uniform than across the population (Luo et al., 2000).

RAPD analysis on the five-breed (ecotype) local sheep in Tanzania using five primers showed bandsharing frequency between individuals within ecotype of lower than cross-ecotype (Stephen et al., 2009). According Nei (1975) levels of heterozygosity in the population is spread locus polymorphism that causes genetic diversity. If the value of high heterozygosity the genetic diversity (diversity loci) is also high. Genetic similarity Batur and Merino sheep that high suggest one of parent (male or female) who derive Batur suspected breed of Merino, but also there are indications

Table 2. The average bandsharing frequency RAPD bands between individual across population produced by amplification using five primer 10 nucleotide

	Batur	Merino	Garut	Thin Tail	Fat Tail
Batur		0.86*	0.52*	0.38*	0.35*
Merino	0.03**		0.35*	0.23*	0.32*
Garut	0.46**	0.99**		0.41*	0.32*
Thin Tail	0.78**	1**	0.71**		0.51*
Fat Tail	0.89**	1**	0.87**	0.51**	

Note * = bandsharing frequency between individula across population. ** = genetic distance across population

(instructions) Thin Tail sheep as one of the ancestor of Batur sheep. Most of the individuals within Batur population has similar morphological with Merino than Thin Tail, while within Garut population close similarity with Thin Tail. According Merkens and Soemirat (1926), sheep Javanese Thin Tail Garut strain is the result of crossbreeding of local sheep Priangan, Merino and Kaapstad from Africa in 1865's. Merino sheep imported from Australia in 1860 and maintained in Garut district for producing wool, meat and manure.Male Merino sheep given to the Regent (the Duke) Limbangan (Garut) and some farmers in this area, then crossed with local sheep. Sheep result of cross breeding spread to the western part of Java island. Further stated that the gene Booroola Merino segregated into Java Thin Tail Garut srain, because of the results of DNA analysis found the sample Merino FecB allele, whereas Garut samples beside FecJ also found in allele FecB, but according Grant (1972) the possibility of gene Booroola originated from India. George et al. (2002) proposed the hypothesis that sheep Javanese Thin Tail sheep are crosses of local Javanese Thin Tail with a smallish Garoles from India. The results of DNA identification, sample the local sheep from Asia, Africa, Australia and New Zealand showed closeness genetic relationship with Merino, Garole or Javnese Thin Tail sheep Strain Garut.

Genetic distance between population sheep

Genetic distance is the estimate of the closeness of genetic relationship or phylogenetic between populations according to Nei standard. 1973 (Stephen et al., 2009). Genetic distance close to or equal to 0 (nul) is very close genetic relationship, but if the value is 1 (one) or more genetic relationship very apart distant. Result analysis of the average bandsharing frequency of RAPD bands between individuals across the population produced a variety of genetic distance values (Table 2). The closest genetic distance was populsi Batur and Merino, but also showed Merino farthest genetic distance with Tail Thin and Fat Tails. Three breeds of local sheep population (Garut, Thin Tail and Fat Tail) shows the genetic distance that far with Merino (respectively 0.99, 1 and 1). Batur sheep genetic distance is closer to Garut compared with Thin Tail or Fat Tails. Thin Tail or Fat Tail genetic distance with Garut or Batur almost the same, but the Fat Tail sheep more closely with Thin Tails compared with Batur or Garut. The results of this study indicate that the population Batur closer genetic relationship to imported sheep (Merino) compared with three breed of local sheep. Fellow local sheep population that has long been cultivated in Java (Garut, Thin Tail and Fat Tail) closeness of genetic relationship is almost the same, but Thin Tail sheep closer genetic relationship with fat tails compared to location of the develope (spread) of the breed Garut. Stephen et al. (2009) states that the (Ecotype) sheep in a country that is located far apart, can occur due to lack of genetic separation of individual contacts between regions so as to affect the genetic distances. Results RAPD analysis of Kacang goats and

goats crossing the descendants of local and imported (Bligon and Peranakan Etawah) shows genetic distance Kacang goat population is closer to Bligon compared with Peranakan Etawah. Fellow of Peranakan Etawah goat population, although different breeding locations closer genetic distance compared with Bligon (Katrina et al., 2003), while the fellow local sheep in Egypt closer genetic relationship when compared with commercial sheep from imports (Ali, 2003), genetic differences between the local population of one breed of sheep Brazil 9.27%, whereas between different breeds population 9.45%. Results across n fellow local sheep show a genetic relationship closer than the results of crossbreeding of local and imported sheep although imported into Brazil since 20 century ago (Paiva et al., 2005).

Genetic relatedness (phylogenetic) intra sheep population

Based on the results of UPGMA analysis using the genetic distance between populations construct a dendogram that shows a group of Batur adjacent with Merino and the Garut neighbour to the Thin Tail, but Fat Tail at separate group away with population Batur, Merino, Garut and Thin Tail (Figure 2). Individuals in the Batur population has the closest genetic relatedness with the Merino and the most distant with Fat Tails. Garut genetic relatedness closest to the Batur. Garut genetic relationship is also more closely with Thin Tail compared with Fat Tails. The most distant apart far genetic relatedness is Merino and Thin Tail, but Thin Tail genetic relatedness closest with Fat Tails. Dendogram and phylogeny tree constructed with RAPD markers by several researchers previously reported that the genetic relationship between local sheep which had long been grown in one country more closely than the imported or commercially sheep. Fellow local sheep in Egypt (Barki, Rahmani and Baladi) more closely than the Suffolk that comes from England (Ali, 2003). Five ecotype local sheep in Tanzania (Arushwa, Mwanza, Dodoma, Mtwar and Dwarf) closely related. Sheep Arushwa one group adjacent to Mwanza and Dodoma near Mtwara. Fellow local sheep from various regions in Tanzania has a close genetic relatedness. Dwarf sheep although originating from outside the local sheep Ecotype Tanzania (from West Africa) a closer genetic affinity with four groups of local Ecotype Tanzania compared with North Ronaldsay that derived from United Kingdom (Stephen et al., 2009).

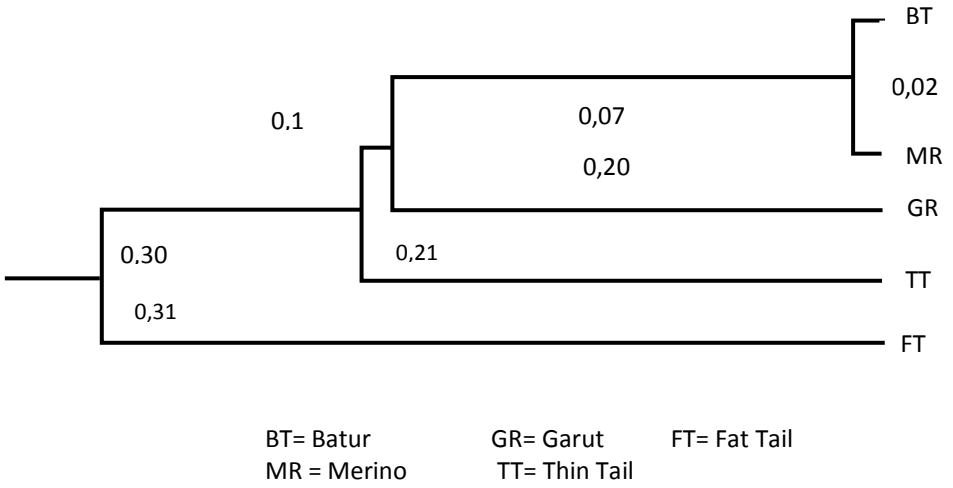

Figure 2. Dendogram philogeny tree closeness genetic relationship Batur, Merino, Garut, Thin Tail and Fat Tail Sheep

Conclusions

Individual genetic diversity in sheep populations Batur, Garut, Thin Tail and Fat Tail is almost the equally, but higher than Within Merino. Individuals in the sheep population Batur has the highest genetic similarity with the Merino and the lowest with Fat Tails. Batur sheep population has a closest relationship or genetic relatedness with Merino and most distant apart far with Fat Tail

Acknowledgement

Thanks to the Director General of Higher Education which has provided funds. Director of the Biotechnology Study Center and Biotechnology University of Gadjah Mada. Rector of the Jenderal Soedirman University and the Dean of the Faculty of Animal Science Jenderal Soedirman University. Supervisor dan co-supervisor of the guidance and advice.

References

Ali BA. 2003. Genetics similarity among four breeds of sheep in Egypt detected by random amplyfied polymorphic DNA markers. African J. Biotech. 2(7):194–197.

Cushwa WT, JF Medrano, E Beer and FA Ponce de Leon. 1996. Localization By in Situ Hybridization of A RAPD Marker to The Sheep Y Chromosome. XXVth International Conference on Animal Genetics, C155.

Elmaci C, Y Oner, S Ozis and E Tuncel. 2007. RAPD Analysis of DNA Polymorphism in Turkish Sheep Breeds. Biochem, Genet. 45:691–696.

Feral JP. 2002. How useful are the genetic markers in attempts to understand and manage marine biodiversity. J. Exp. Mar. Biol. Ecol. 268:121-145.

George HD, SM Galloway, IK Ross, SM Gregan, J Ward, BV Nimbkar, PM Ghalsasi, C Nimbkar, GD Gray, Subandryo, I Inounu, B Tiesnamurti, E Martyniuk, E Eythorsdottir, P Mulsant, F Lercerf, JP Hanrahan, GE Bradford and T Wilson. 2002. DNA test in prolific sheep from eight countries provide new evidence on origin of Booroola (FecB) mutation. Regular Article. Biological of Reprod. 66:1869-1874.

Grant B. 1972 (ed). Indonesia. Melbourne: Penguin Books

Kantanen J, J Vilkki, K Elo and A Mäki-Tanila. 1995. Random amplified polymorphic DNA in cattle and sheep: application for detecting genetic variation. Anim. Genet. 26:315–320.

Katrina A, M Astuti dan WT Artama. 2003. Studi Pertalian Genetik Kambing Lokal Berdasarkan Analisis Polimorfisme Dengan PCR-RAPD. Tesis. Program Studi Bioteknologi, Program Pascasarjana, Universitas Gadjah Mada.

Liu ZJ and JF Cordes. 2004. DNA marker technologies and their applications in aquaculture genetics. Aquaculture. 238:1-37

Luo J, ZG Liu, GS Yang and XM Zhen. 2000. Evaluation Of Genetic Relatedness And Diversity Using Randomly Amplified Polymorphic DNA (RAPD). Nortwest Sci-tech University of Agriculture and Forestry, Yangling, Shaanxi.

Lynch M. 1990. The similarity index and fingerprinting. Mol. Biol. Evol. 7:478-484.

Lync M and BG Maligan. 1994. Analysis of population genetic structure with RAPD marker. Molecular Ecology. 3:91-99.

Malau-Aduli AEO, CW Bignell, F Tavassoli-Salardini, A J Smolenski, A Palmer, J Bignell, S Burbury, R Batchelor, BS Malau-Aduli, SA Adediran, PA Lane and RJ Clark. 2006. Genetic Diversity and Breed Comparison of Crcass Traits In Tasmanian Corriedale and East Friesian Sheep By RAPD Markers. In: D Troy, R Pearce, B Byrne, J Kerry (Editors), Harnessing and Exploiting Global Opportunities', Proceedings of the 52nd International Congress of Meat Science and Technology, Ireland. 52:89-90.

Markens J and R Soemirat. 1926. Contribution to the knowledge of sheep breeding in the Dutch East Indies. Ned Indische Bladen Diergeneeskd, 38:395-414.

Nei M. 1975. Molecular Genetic Population And Evolution. pp. 128-170. Amsterdam, North-Holand Publishing.

Paiva SR, VC Silverio, AA Egito, C McManus, DA de Feria, AS Mariante, SR Castro, MSM Albuquerque and JA Degram. 2005. Genetic variability of Brazilian hair sheep breeds. Psq. Agropec. Bras. Brasilia. 40(9):887-893.

Piper L and A Ruvinsky. 1997. The Genetics of Sheep. CABI, Oxon, New York. pp 51-91.

Sambrook J, EF Fritsch and T Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, New York.

Stephen J, BA Clemens, Wollny and PS Gwakisa. 2009. Genetic Relationships Among Five Ecotype of Sheep in the United Republic of Tanzania. Community-based management of animal genetic resources. FAO Corporate Document Repository. http:www.fao.org/docrep/006/. Accessed Desember 28, 2009

Tahmoorespur M, MR Nassiry and A Mohammady.

2003. The use of 17 RAPD primers in some of Iranian sheep breeds. Dept. of Animal Science, College of Agric, Ferdowsi Univ. P.O.Box: 91775-1163, Mashhad, Iran. http://www.bsas.org.uk/ Accessed August 6, 2008.

Urich K. 1990. Comparative Animal Biochemistry. Springer-Verlag. Berlin, Gemrany. Heidelberg, New York.

Yuanfang G, L Xianglong, L Zhengzhu and L Jinquan. 2002. Studies of Random Amplyfied Polymorphic DNA (RAPD) of main indegenous breed in China. Hereditas. 24:423-426.

Zeuner FE. 1963. A History of Domesticated Animals. Hutchinson, London.