α_{s1} -Casein gene polymorphism in Katjang, Jamnapari, Boer and Boer-feral goats in Malaysia

(Kepolimorfan gen $\alpha_{\rm s1}$ -casein di dalam kambing Katjang, Jamnapari, Boer dan Boer-Feral di Malaysia)

A.B. Amie Marini*, B. Abdul Rashid**, K. Musaddin* and I. Zawawi***

Keywords: α_{s1} -casein gene, polymorphism, goat, Malaysia

Abstract

Casein and milk serum or whey proteins are the two major proteins (about 95%) in mammalian milk. The casein protein comprises $\alpha_{s1}, \alpha_{s2}, \beta$, and κ -casein while β -lactoglobulin and α -lactalbumin make up the whey protein. A study was carried out to characterise and determine the α_{s1} -casein protein gene polymorphism and genetic relationship in goat breeds available in Malaysia. A total of 115 heads of goats including Katjang, Boer, Boer Feral and Jamnapari were used in the study. Genomic DNA was extracted from the blood samples and amplified by PCR. Genetic variability was calculated using Popgene Software computer program. This study showed that casein gene exists in goat breeds found in Malaysia. All local goat breeds showed high frequency of α_{s1} -casein A, B and C alleles, which are related to high casein content in milk. The highest frequency of α_{s1} -casein A alleles was found in Katjang goats with a value of 0.61. The dendogram constructed based on genetic distance matrix using neighbour-joining method showed the genetic relation between the four breeds of goats. Boer and Boer-Feral goats were grouped together in one cluster whereas Katjang and Jamnapari populations were grouped in another cluster. The Boer goats differed genetically from the local Katjang or Jamnapari goats in α_{s1} -casein gene.

Introduction

The goat population in Malaysia has increased over the years since 2000. In 1999, the total population was 190,539 which increased to 398,031 in 2008 of which about 90% were for chevon production and 10% for goat milk production. (http://data.dvs.gov.my/shares/UMUM/ STATISTIK/TERNAKAN2007-2008/DVS. POPULATION.pdf). Furthermore, the demand for chevon and mutton has steadily increased. However, the self-sufficiency rate of chevon and mutton in 2005 was estimated at about 8% and demand for these meats was met through importation from Australia and New Zealand. This has created interest in goat farming in Malaysia.

In mammals, about 95% of the milk proteins are made up of casein and whey proteins. The casein protein comprises α_{s1} , α_{s2} , β and κ -casein. In goats, α_{s1} -casein locus is characterised by seven alleles associated with four quantitative levels of the corresponding protein. A, B and

50774 Kuala Lumpur, Malaysia

^{*}Strategic Livestock Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301,

^{**}MARDI Station, Kluang, Locked Bag 525, 86009 Kluang, Johor, Malaysia

^{***}National Institute of Animal Biotechnology, Jerantut, Pahang, Malaysia

Authors' full names: Amie Marini Abu Bakar, Abdul Rashid Baba, Musaddin Kamaruddin and Zawawi Ismail Email: amieza@mardi.gov.my

[©]Malaysian Agricultural Research and Development Institute 2011

 α_{s1} -casein gene polymorphism in Malaysian goats

C alleles are associated with high casein content at 3.6 g/litre, E allele is intermediate with 1.6 g/litre, low level of casein in D and F alleles at 0.6 g/litre and O which is a true null allele (Grousclaude et al. 1987; Mahé and Grousclaude 1989). The differences in the α_{s1} -casein protein are mainly due to the occurrence of amino acid substitutions in A, B, C and E alleles and deletion of some amino acids in D and F alleles.

Polymorphism of the α_{s1} -casein alleles has been observed in several breeds of goats (Grousclaude et al. 1987; Jordana et al. 1991; Ramuno et al. 1991; Grousclaude et al. 1994). The significance of the α_{s1} -casein alleles is in its effect on the goat cheese making process in terms of clotting property and overall cheese yield. It was observed that goats with homozygous A allele yields 7% more cheese when compared to homozygous E and relatively even higher in comparison with homozygous F (Maria et al. 2005).

The type and frequency of caprine α_{s1} -casein has not been studied in Malaysia. Thus, the objective of this study was to characterise and determine the polymorphism of α_{s1} -casein protein gene in the Malaysian indigenous goat and introduced goat breeds.

Materials and Methods Genomic DNA Extraction

Blood samples were collected in heparinized or Ethylene Diamine Tetra acetic Acid (EDTA) tubes from 115 goats which comprised Boer (n = 24), Boer-Feral (n = 39), Katjang (n = 11) and Jamnapari (n = 41). Genomic DNA was extracted and purified from whole blood samples using Wizard Genomic DNA Purification Kit (Promega, USA). Samples were then quantified using UV-Vis Spectrophotometer (ThermoSpectronic, USA) at Abs 260 and 280.

Polymerase Chain Reaction (PCR)

PCR amplification was performed in a 50 μ l reaction mixture consisting of

50 ng genomic DNA, 1 U of Taq DNA polymerase, 0.2 mM dNTPs and 20 pmol of the specific primers for each allele (Table 1). Thermal cycling conditions included a denaturation step of 95 °C for 4 min, followed by 32 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 2 min and a final extension at 72 °C for a 2 min. PCR products were subjected to electrophoresis on a 2% agarose gel and stained with ethidium bromide. The Quantity One Software of the Gel Documentation System (USA) was used to visualize and estimate the size of the bands representing each allele. Genetic variability and distances within and between populations were calculated using Popgene Software Version 1.31.

Results and discussion

The identification of the α_{s1} -casein gene, genotyping was restricted to the A, B and C alleles which were found more frequently in all the breeds studied. The D and E alleles were not detected in local breeds of goat. The frequency of α_{s1} -casein A alleles were found to be higher in Katjang and Jamnapari compared to the Boer and Boer-Feral goats (*Table 2*).

Katjang goats also showed the highest frequency of α_{s1} -casein A, B and C alleles

Table 1. List o	of primers	used in	the PCR	mixture

System	Primers
Allele E	BT99 5'-ctatcatgtcaaaccattctatcc-3'
	BT72 5'- caatttcacttaaggatgttacac-3'
Allele F	BT58 5'-aagtttcatggttgtcaagat-3'
	BT73 5'-gaatteettgateateaacceage-3'
System 3	BT51 5'-gagaacatcaatgaactgatgaag-3'
	BT32 5'-cagctggggcacgttgtattttttcag-3'
System 5	BT25 5'-gaagatgtgccctctgagctgtac-3'
	BT74 5'-cagatggggcacgttgtattttttcag-3'
A3-O1	BT33 5'-gatattgggagtgaatcaactgag-3'
	BT66 5'-ctcacttgacgaactgcttccagc-3'
B3-C	BT66 5'-actcactggagagagtccttggat-3'
	BT97 5'-gtggctgttgctcttgccaggcc-3'
A2-0	BT25 5'-gaagatgtgccctctgagcgttac-3'
	BT60 5'-ctctttcatactgtgaagttgttc-3'
A0-A1-D	BT65 5'-ctcagggtagaagtaggccag-3'
	BT36 5'-gaacaacttcacagtatgaaagag-3'

followed by Boer and Boer-Feral, compared to Alpine, Saanen (Grousclaude et al. 1987; Ramuno et al. 1991), Canaria (Jordana et al. 1991) and Hungarian milk (Veress et al. 2004) as shown in Table 3. Ramuno et al. (1991) and Grousclaude et al. (1987) reported that Saanen goats had higher frequency of α_{s1} -case in E which ranged from 0.41–0.46 while the highest frequency of α_{c1} -case in F alleles were found in Alpine goats. Both alleles were associated with low α_{s1} -case protein. The presence of high frequency of α_{s1} -casein A in the indigenous Katjang goats indicates their ability to produce milk containing high α_{s1} -casein protein.

Distinct breeds within the indigenous goat populations of Malaysia are generally not recognized, although some local populations have been isolated to some extent and thus may have become genetically differentiated. It is important to establish whether large genetic differences among these populations for milk production traits especially casein protein exists, since it

would be advantageous to use the population of goat breeds with the trait of interest as a basis for genetic improvement programmes. The genetic distance was measured using the allele differences at locus α_{s1} -casein between populations. The genetic distance and genetic similarity values were inversely correlated (Table 4). The highest genetic distance was observed between Katjang and Boer populations with a value of 0.7809, while the lowest genetic distance was found between Boer and Boer-Feral with a value of 0.0194. The genetic similarities of these two populations were also high which was expected as the Boer-Feral goats used in this study had the Boer blood line with different parentage.

The (UPGMA) based phylogenetic tree constructed from the genetic distance, grouped Boer and Boer-Feral in one cluster while Katjang and Jamnapari were grouped in another cluster (*Figure 1*). The Katjang and Jamnapari goats were also genetically similar in α_{s1} -casein gene probably due to

1	2	\$1		0				
Breeds	Ν	Alleles						
		А	В	С	D	Е	F	NULL
Boer	24	0.15	0.10	0.71	_	_	0.04	_
Boer-Feral	39	0.22	-	0.68	_	-	0.03	0.08
Katjang	9	0.61	0.11	0.17	_	_	-	0.11
Jamnapari	41	0.51	0.10	0.28	-	-	0.10	0.01

Table 2. Frequency of α_{s1} -case in alleles in local goat breeds

Table 3. Allele frequencies of α_{s1} -casein gene in Katjang, Jamnapari, Boer and Boer-Feral in comparison to some European breeds

Breeds (numbers)	Alleles		References	
	A+B+C+D+O	Е	F	
Boer (24)	0.96	_	0.04	
Boer-Feral (39)	0.98	-	0.03	
Katjang (9)	1	-	_	
Jamnapari (41)	0.9	-	0.10	
Alpine (213)	0.14	0.34	0.41	Grousclaude et al. (1987)
Alpine (80)	_	0.35	0.59	Ramuno et al. (1991)
Saanen (159)	0.07	0.41	0.43	Grousclaude et al. (1987)
Saanen (70)	0.03	0.46	0.46	Ramuno et al. (1991)
Canaria (74)	0.028	0.20	-	Jordana et al. (1991)
Hungarian milk (109)	0.61	0.08	0.31	Veress et al. (2004)

 α_{s1} -casein gene polymorphism in Malaysian goats

Table 4. The genetic distance (below diagonal) and genetic similarity (above diagonal) measured on the locus a_{s1} -casein between different populations

Population	Boer	Boer-Feral	Katjang	Jamnapari
Boer	****	0.9807	0.4580	0.6548
Boer-Feral	0.0194	****	0.5393	0.7095
Katjang	0.7809	0.6174	****	0.9462
Jamnapari	0.4234	0.3432	0.0554	****



Figure 1. Genetic distance among 4 populations of goat breeds based on Nei's Dendogram (1972)

their close geographical locations and both are tropical breeds.

Conclusion

It was generally observed that the main α_{s1} -case in alleles identified in the local goat breeds were A, B, C and F with the absence of D and E alleles. The study showed the genetic relationship between the four breeds of goats i.e. Boer and Boer-Feral were grouped together in one cluster while Katjang and Jamnapari were grouped in another cluster. The Boer goats differed genetically from the local Katjang or Jamnapari goats in α_{s1} -casein gene. Future studies will be carried out to establish the correlation of the A, B and C alleles with concentration of casein protein in the milk of these goat breeds. These α_{s1} -case alleles may be a useful genetic marker for selection of goats for high casein content in milk.

Acknowledgement

This project was part of the Malaysian-Hungary Bilateral S&T Cooperation programme organized and sponsored by the International Division, Ministry of Science, Technology and Innovation, Malaysia (MOSTI). The authors express their sincere thanks to the members of the Animal Molecular Biology Laboratory and staff of the Small Ruminant Unit, Strategic Livestock Research Centre, MARDI, Serdang, for all their support and cooperation.

References

- Grosclaude, F., Mahe, M., Brignon, G., Stasio,
 L. and Juenet, R. (1987). A Mendelian
 polymorphism underlying quantitative
 variation of goat α_{s1}-casein. *Genet. Sel. Evol.*19: 399–412
- Grousclaude, F., Ricordeau, G., Martin, P., Remeuf, F., Vassal, L. and Bouliion, J. (1994). From gene to cheese: the polymorphism of the caprine α_{s1} -casein, its effects and evolution. *INRA Prod. Anim.* 7: 3–19
- Jordana, J., Sanchez, A., Jansa, M., Mahe, M. and Grousclaude, F. (1991). Comparison between Spanish goat breeds at αs1-casein genetic variants. *Inf. Tec. Econ. Agrar.* 11: 598–600
- Mahè, M.F. and Grosclaude, F. (1989). α S1-Cn^D, another allele associated with a decreased synthesis rate at the caprine α_{S1} -casein locus. *Genetics Selection Evolution* 21(2): 127–129
- Maria, F., Silvia, F., Vlatka, C. C., Ada, B., Pietro, P., Ino, C., Gian, F.G. and Giuseppe, E. (2005). Caprine α_{s1}-casein polymorphism: Characterisation of A, B, E and F variants by means of various biochemical and molecular techniques. *Food Technol. Biotechnol.* 43(2): 123–132
- Ramuno, L., Rando, A., Di Gregorio, P., Massari, M., Blassi M. and Masina, P. (1991). Structtura genetica di alcune populazioni caprine allevate in Italian al locus della caseina α s1. (Genetic structure of some local Italian goats at α_{s1} -casein locus). *Proc. IX*. *Congress NazASPA*, p. 579–589
- Veress, G., Kusza, S., Bosze, Z., Kukovics, S. and Javor, A. (2004). Polymorphism of the α_{s1}casein, κ-casein, β-lactoglobulin genes in the Hungarian Milk Goat. *South African J. Anim. Sci.* (34): 20–23

Abstrak

Casein dan serum susu atau dadih susu merupakan dua protein utama (sebanyak 95%) dalam susu mamalia. Protein casein terbahagi kepada α_{s1} , α_{s2} , β dan κ-casein manakala protein wei pula terdiri daripada β-laktoglobulin dan α-laktalbumin. Kajian ini dijalankan untuk menciri dan menentukan kewujudan gen α_{s1} -casein yang polimorfik dan saling kait genetik baka kambing yang terdapat di Malaysia. Sebanyak 115 ekor kambing yang terdiri daripada baka Katjang, Boer, Boer-Feral dan Jamnapari telah dikaji. Genom DNA telah diekstrak daripada sampel darah dan diperbanyakkan melalui teknik PCR. Kepelbagaian genetik telah dianalisis menggunakan program komputer Popgene. Kajian menunjukkan terdapat gen α_{s1} -casein dalam baka kambing di Malaysia. Semua baka kambing tempatan menunjukkan kadar frekuensi alel α_{e1} -casein A, B dan C yang tinggi dan ia bersaling kait dengan kandungan casein yang tinggi di dalam susu. Frekuensi alel α_{s1} -casein A yang tinggi dijumpai di dalam kambing Katjang dengan nilai 0.61. Dendogram yang dibina berasaskan matrik jarak genetik dengan menggunakan kaedah neighbour-joining menunjukkan pertalian genetik antara empat baka kambing yang telah dikaji. Kambing Boer dan Boer-Feral digabungkan di dalam satu kumpulan, manakala kambing Katjang dan Jamnapari digabungkan di dalam kumpulan yang lain. Kambing Boer didapati berbeza secara genetik daripada kambing Katjang atau Jamnapari tempatan berdasarkan gen α_{s1} -casein.