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Amino acid diversity on the basis of cytochrome b gene in Kacang and Ettawa Grade goats

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ABSTRAK

Tujuan penelitian adalah untuk mengidentifikasi dan mengkaji keragaman asam amino gen Cytochrome B (Cyt b), penanda genetik dan karakteristik asam amino spesifik pada kambing Kacang dan Kambing Peranakan Ettawa (PE).Materi penelitian menggunakan 19 ekor kambing Kacang dan 12 ekor kambing PE. Sample ditentukan dengan *purposive sampling*. DNA total diisolasi menggunakan kit Genomic DNA Mini Kit (Geneaid) dan gen Cyt b diamplifikasi dengan metode PCR menggunakan primer CytbCapF dan CytbCapR dan dilakukan sekuensing. Hasil menunjukkan terdapat 2 asam amino spesifik yang membedakan kambing Kacang dan PE dengan *C. hircus* dan *C. aegagrus* dan 4 asam amino spesifik yang membedakan dengan *C. falconeri*, namun tidak ada asam amino spesifik yang dapat membedakan kambing Kacang dengan PE. Dapat disimpulkan bahwa asam amino spesifik pada gen Cyt b dapat digunakan sebagai penanda genetik yang membedakan antara kambing Kacang dan PE dengan 3 kambing pembanding lainnya.

Kata–kata kunci: asam amino, cytochrome b, kambing lokal, penanda genetik

ABSTRACT

The objectives of study were to identify and assess the amino acid diversity of Cytochrome b (Cyt b) gene, genetic marker and characteristic of specific amino acid in Kacang and Ettawa Grade goat. Nineteen heads of Kacang goat (KG) and twelve heads of Ettawa Grade goat (EG) were purposively sampled. The genomic DNA was isolated by Genomic DNA Mini Kit (Geneaid) and amplified Cyt b using PCR method with CytbCapF and CytbCapR primers and was sequenced. The results showed that there were two specific amino acids that distinguish KG and EG goat with *C. hircus* and *C. aegagrus* and four specific amino acids that distinguish KG and EG goat with *C. falconeri,* but there were no specific amino acids can be used as a genetic marker to distinguish between Kacang and EG goat. In conclusion, specific amino acids in Cyt b gene can be used as a genetic marker among KG and EG goat with 3 goat others comparator.

Keywords : amino acid, cytochrome b, genetic marker, local goat

INTRODUCTION

Indonesia has a high diversity of local animal genetic resources. Local goats constitute an important genetic resources of the country. The Ministry of Agriculture (2016) reported that total goat population in Indonesia in 2015 was about more than 19 milion heads, the highest population in Central Java province as many as about more than 4 million heads, or 21,40% of the total population in Indonesia. Sodiq and Zainal (2008) stated that Central Java is the province with the largest population of goats. Kacang and Ettawa Grade (EG) goats are meat type goats that occur widely in the Central Java province. Kacang goat is an Indonesian native goat which are scattered in

various regions in Indonesia (Prawirodigdo *et al*., 2003). While EG goat is result of cross between Kacang and Ettawa breeds, and it looks similar to Ettawa but smaller and has two or three coat color patterns, namely black striped, brown striped, mottled white and black (Batubara *et al*., 2009).

Understanding the genetic diversity of a population is very important conservation and breeding program. For conservation, it is necessary to know the genetic status of a population in order to design a conservation program to avoid extinction and help to develop management plans for animal survival. Genetic diversity reflects the genetic resources needed for ecological adaptation in evolution (Poerba and Yuzammi, 2008). High genetic diversity will greatly help a population to adapt to changes that occur in their surroundings and have a high breeding rate. Also genetic diversity information is required in selection during animal breeding (Santoso *et al.,* 2006). Long-term breeding programs that utilize germplasm to improve traits of a livestock should be based on the accurate estimation of genetic determination, therefore determination of an individual as material can be done properly in genetic improvement (Karsinah *et al.,* 2002).

Genetic diversity information can be determined in two ways, through phenotype and genotype traits. Through genotype traits, it can be done by mitochondrial DNA (mtDNA) analysis. It is one of many methods that is used to study the origin of domesticated animal (Machugh and Bradley, 2001). Cytochrome B gene (Cyt b) that contained in mitochondrial DNA, is a gene that is involved in the transport of electrons in the formation of energy respiratory chain (Anderson *et al.,* 1981). Cyt b gene is inherited maternaly and used widely for research or phylogeny identification and genetic relationship among the species of the same genus or family. It is because Cyt b gene is one of coding regions for protein in mitochondrial genome and the region is conserve or do not change much or do not experience bases mutation, thus it will be more sensitive to be used as a genetic marker for identifying the purity of the species (Manceau *et al.,*1999; Widayanti *et al*., 2006). Research on using Cyt b gene as genetic marker for genetic information analysis on goat was done in Chinese goat (Chen *et al.,* 2006; Liu *et al.,* 2009), Vietnamese goat (Hassanin *et al.,* 2010), Turkish goat (Kul and Ertugrul, 2011) and Indonesian goat (Jiyanto *et al.,* 2014; Pakpahan *et al.,* 2016a; Pakpahan *et al.,* 2016b). The objective of this study was to identify and assess the amino acid diversity of Cyt b gene, genetic marker and characteristic of specific amino acid in Kacang and EG goat.

MATERIALS AND METHODS

Sample Collection

Materials of this research were 31 DNA goats from blood that were taken from *vena jugularis*. These were obtained from two breeds of local goat, which consisted of 19 heads of Kacang goat (KG) from Grobogan regency and 12 heads of Ettawa Grade goat (EG) from Kendal regency. Sample was determined using purposive sampling, based on criteria that the research location were selected based on regions with the largest population and development area of Kacang goat and EG goat; the goat must be 1-2 years old and did not have a genetic relationship between one sample to other samples.

DNA Extraction, PCR Amplification and Sequencing

Total genomic DNA was extracted from blood by using Genomic DNA Mini Kit (Geneaid) with Sambrook *et al.* (1989) method with modification following the protocol. The results of DNA extraction were visualized using 1% gel Agarose electrophoresis. Electrophoresis was run on 100 V condition for 45 min and whole genome DNA result could be seen on UV light.

The complete mitochondrial Cyt b gene sequences (1140 bp) were amplified by the forward primer CytBCapF (5'-tggaatctaaccatgaccaatg-3') and reverse primer CytBCapR (5'-ggctattctccttttctggttt-3'). PCR amplification was conducted in 50 µL volume, containing 25 µL Kappa ready mix, 3 µL DNA template, 1 µL forward primer, 1 µL reverse primer and 20 µL ddH_2O. The PCR amplification was conducted using a Infinigen Thermal Cycler based on program, initial denaturation at 94°C for 5 min, followed by 35 cycles, each consisting of 30 sec denaturation at 94°C, 45 sec primers annealing at 49°C, 90 sec elongation at 72°C, then 5 min elongation at 72°C for the final stage and were stored at 4°C. The PCR product was visualized using 1% gel Agarose. Electrophoresis was run on 100 V condition for 20 min and the amplification result could be seen on UV light. The PCR products were sequenced by 1st Base-Asia, Malaysia.

Data Analysis

Cyt b gene sequence was analyzed using Clustal W (Thompson *et al.,* 1994) in Molecular Evolutionary Genetics Analysis (MEGA6) program (Tamura *et al*., 2013). Alignment of Kacang and EG goat Cyt b gene sequence was conducted through multiple alignment with *C. hircus* (D84201.1), *C. aegagrus* (AB004069.1) and *C. falconeri* (AB044309.1) as comparator which were obtained from GenBank. The nucleotide sequence then was translated in the form of amino acids by Mitochondrial Vertebrate genetic code.

RESULTS AND DISCUSSION

Cyt b gene amplification result was 1261 bp, so it can be obtained the complete Cyt b gene sequences throughout 1140 bp and 377 amino acids that begin with start codon (ATG) and end with stop codon (AGA). This is consistent with the results of some research that stated the length of Cyt b gene in goat was 1140 bp and preceded with start codon that encoded by ATG and end with stop codon that encoded by AGA (Pietro *et al*., 2003; Guang-Xin *et al.,* 2015). Comparison result showed, there were differences in the characteristics of the amino acids.

The difference of the amino acid sequences within Kacang and EG goat (Table 1-4) showed one synonymous amino acids (codon site 164th), two non synonymous amino acids (site codon 16th (KG4, EG6, EG11, EG12) and 231st (EG7), and 374 conserve amino acids. A comparison of the amino acid sequences of Kacang and EG goat with *C. hircus* (Table 1-2) showed 13 synonymous amino acids (codon site 16th (except KG4, EG6, EG11, EG12), 102th, 131st, 132nd, 146th, 148th, 149th, 164th, 197th, 231st (except forEG7), 240th, 283rd and 355th), four non synonymous amino acids (site codon 16thth (only for KG4, EG6, EG11, EG12), 190th, 214th, 231st) and 362 conserve amino acids. While comparison of Kacang and EG goats with *C. aegagrus* (Table 3-4) showed seven synonymous amino acids (codon site 16th (only for KG4, EG6. EG11. EG12); 102nd, 164th, 197th, 231st (except for EG7); 283th and 355th) and three non synonymous amino acids (codon site 16th (except for KG4, EG6, EG11, EG12), 214th and 231st (only for EG7)) and 369 conserve amino acids. Major differences were between Kacang and EG goats with *C. falconeri* (data not presented) with as many as 43 synonymous amino acids (codon site 5th, 20th, 25th, 37th, 41st, 43th, 62nd, 72nd, 77th, 95th, 102nd, 105th, 121st, 123rd, 131st, 139th, 149th, 164th, 165th, 170th, 173rd, 188th, 196th, 197th, 204th, 206th, 215th, 229th, 232nd, 246th, 259th, 266th, 267th, 272nd, 283th, 304th, 325th, 334th, 355th, 357th, 367th, 373rd and 375th) and six non synonymous amino acids (codon site 16th, 97th, 189th, 214th, 231st and 303rd) and 328 conserve amino acids. Conserve amino acid is the most found in this study. The present result is consistent with the character of Cyt b gene that it is converse, does not change much or does not experience mutation, thus it will be more sensitive to be used as genetic marker for identifying species purity, grouping based on breed and determining genetic relationship (Manceau *et al.,* 1999; Widayanti *et al.,* 2006).

Amino acid sequence of Kacang and EG goats had least difference with *C. aegagrus* (Table 5 and Table 6). There were three amino acids at codon site 17th (except sample KG4, EG6, EG11 and EG12) and 215th (Threonine turned Alanine), and site codon 232nd (sample EG7) Alanine turned Valine. The differences of amino acid between Kacang, EG and *C. hircus* were four amino acids at codon site 17th (sample KG4, EG6, EG11 and EG12) that Alanine turned Treonine, 191st (Glycine turned Alanine) and 215th (Threonine turned Alanine), and site codon 232nd (sample EG7) that Alanine turned Valine. When compared between Kacang and EG to *C. falconeri*, there were 5 differences of amino acid. It was at codon site 17th (sample KG4, EG6, EG11 and EG12) that Alanine turned Threonine, 98th (Valine turned Isoleucine), 190th (Alanine turned Threonine), 215th (Threonine turned Alanine), 232nd (only sample EG7) that Alanine turned Valine and 304th (Methionine turned Valine). There were two specific amino acid differences in Kacang and EG goat on codon site 191st and 215th when compared to *C. hircus*, and on codon site 17th and 215th when compared with *C. aegagrus.*While there were four spesific amino acid differences in Kacang and EG goat when compared to *C. falconeri,* there were on codon site 98th, 190th, 215th and 304th. It differences can be used as genetic markers to distinguish Kacang and EG goat with *C. hircus*, *C. aegagrus* and *C. falconeri,* but it cannot be used as a genetic marker for level intra-species to distinguish Kacang and EG goat. Based on the number of amino acid differences that can be presumed that Kacang and EG goat have a closer genetic relationship with *C. aegagrus* compared to *C. hircus* and *C. falconeri*. *C. aegagrus* (Bezoar) is a wild goat which is the ancestor of

Table 1. Difference in Codon Site of Amino Acid among Kacang and EG Goats with *C. hircus*

Sample	Codon site on														
	16	102	131	132	146	148	149	164	190	197	214	231	240	283	355
*C.hircus**	GCA	TAT	GTC	CTA	ACC	CTC	CTC	TGA	GGC	CTC	ACA	GCC	CTA	ATC	ATT
KG1	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG2	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG3	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG4	A..	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG5	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG6	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG7	…	..C	..T	T..	..T	..T	..T	..G	.C.	..T	G..	…	T..	..T	..C
KG8	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG9	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG10	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG11	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG12	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG13	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG14	…	..C	..T	T..	..T	..T	..T	..G	.C.	..T	G..	…	T..	..T	..C
KG15	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG16	…	..C	..T	T..	..T	..T	..T	..G	.C.	..T	G..	…	T..	..T	..C
KG17	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG18	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
KG19	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
EG1	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
EG2	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
EG3	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T.	..T	..C
EG4	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
EG5	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
EG6	A..	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
EG7	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	.T.	T..	..T	..C
EG8	…	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
EG9	…	..C	..T	T..	..T	..T	..T	..G	.C.	..T	G..	…	T..	..T	..C
EG10	…	..C	..T	T..	..T	..T	..T	..G	.C.	..T	G..	…	T..	..T	..C
EG11	A..	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C
EG12	A..	..C	..T	T..	..T	..T	..T	…	.C.	..T	G..	…	T..	..T	..C

* from GenBank; KG: Kacang goat; EG: Ettawa Grade goat
(.) same with *C. hircus*
Codon (Nucleotide base) :A (Adenine), C (Citosine), G (Guanine), T (Timine)

Table 2. Differences in Amino Acid Site among Kacang and EG Goats with *C. hircus*

Sample	Amino Acid Site on														
	16	102	131	132	146	148	149	164	190	197	214	231	240	283	355
*C. hircus**	A	Y	V	L	T	L	L	W	G	L	T	A	L	I	I
KG1	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG2	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG3	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG4	T	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG5	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG6	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG7	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG8	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG9	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG10	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG11	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG12	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG13	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG14	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG15	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG16	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG17	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG18	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
KG19	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG1	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG2	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG3	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG4	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG5	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG6	T	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG7	.	.	.	.	.	.	.	.	A	.	A	V	.	.	.
EG8	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG9	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG10	.	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG11	T	.	.	.	.	.	.	.	A	.	A	.	.	.	.
EG12	T	.	.	.	.	.	.	.	A	.	A	.	.	.	.

* goat breed was taken from GenBank; KG: Kacang goat; EG: Ettawa Grade goat
(.) same with *C. hircus*
Amino acid :A (Alanine), G (Glycine), I (Isoleucine), L (Leucine), T (Threonine), V (Valine), W (Tryptophan), Y (Tyrosine)

Table 3. Differences Codon Site of Amino Acid among Kacang and EG Goats with *C. aegagrus*

Sample	Codon site on							
	16	102	164	197	214	231	283	355
*C. aegagrus**	ACA	TAT	TGA	CTC	ACA	GCC	ATC	ATT
KG1	G..	..C	…	..T	G..	…	..T	..C
KG2	G..	..C	…	..T	G..	…	..T	..C
KG3	G..	..C	…	..T	G..	…	..T	..C
KG4	…	..C	…	..T	G..	…	..T	..C
KG5	G..	..C	…	..T	G..	…	..T	..C
KG6	G..	..C	…	..T	G..	…	..T	..C
KG7	G..	..C	..G	..T	G..	…	..T	..C
KG8	G..	..C	…	..T	G..	…	..T	..C
KG9	G..	..C	…	..T	G..	…	..T	..C
KG10	G..	..C	…	..T	G..	…	..T	..C
KG11	G..	..C	…	..T	G..	…	..T	..C
KG12	G..	..C	…	..T	G..	…	..T	..C
KG13	G..	..C	…	..T	G..	…	..T	..C
KG14	G..	..C	..G	..T	G..	…	..T	..C
KG15	G..	..C	…	..T	G..	…	..T	..C
KG16	G..	..C	..G	..T	G..	…	..T	..C
KG17	G..	..C	…	..T	G..	…	..T	..C
KG18	G..	..C	…	..T	G..	…	..T	..C
KG19	G..	..C	…	..T	G..	…	..T	..C
EG1	G..	..C	…	..T	G..	…	..T	..C
EG2	G..	..C	…	..T	G..	…	..T	..C
EG3	G..	..C	…	..T	G..	…	..T	..C
EG4	G..	..C	…	..T	G..	…	..T	..C
EG5	G..	..C	…	..T	G..	…	..T	..C
EG6	...	..C	…	..T	G..	…	..T	..C
EG7	G..	..C	…	..T	G..	.T.	..T	..C
EG8	G..	..C	…	..T	G..	...	..T	..C
EG9	G..	..C	..G	..T	G..	…	..T	..C
EG10	G..	..C	..G	..T	G..	…	..T	..C
EG11	...	..C	…	..T	G..	…	..T	..C
EG12	...	..C	…	..T	G..	…	..T	..C

* goat breed was taken from GenBank; KG: Kacang goat; EG: Ettawa Grade goat
(.) same with *C. aegagrus*
Codon (Nucleotide base) :A (Adenine), C (Citosine), G (Guanine), T (Timine)

Table 4. Differences Amino Acid Site among Kacang and EG goats with *C. aegagrus*

Sample	Amino Acid Site on							
	16	102	164	197	214	231	283	355
*C. aegagrus**	T	Y	W	L	T	A	I	I
KG1	A	.	.	.	A	.	.	.
KG2	A	.	.	.	A	.	.	.
KG3	A	.	.	.	A	.	.	.
KG4	.	.	.	.	A	.	.	.
KG5	A	.	.	.	A	.	.	.
KG6	A	.	.	.	A	.	.	.
KG7	A	.	.	.	A	.	.	.
KG9	A	.	.	.	A	.	.	.
KG9	A	.	.	.	A	.	.	.
KG10	A	.	.	.	A	.	.	.
KG11	A	.	.	.	A	.	.	.
KG12	A	.	.	.	A	.	.	.
KG13	A	.	.	.	A	.	.	.
KG14	A	.	.	.	A	.	.	.
KG15	A	.	.	.	A	.	.	.
KG16	A	.	.	.	A	.	.	.
KG17	A	.	.	.	A	.	.	.
KG18	A	.	.	.	A	.	.	.
KG19	A	.	.	.	A	.	.	.
EG1	A	.	.	.	A	.	.	.
EG2	A	.	.	.	A	.	.	.
EG3	A	.	.	.	A	.	.	.
EG4	A	.	.	.	A	.	.	.
EG5	A	.	.	.	A	.	.	.
EG6	.	.	.	.	A	.	.	.
EG7	A	.	.	.	A	V	.	.
EG8	A	.	.	.	A	.	.	.
EG9	A	.	.	.	A	.	.	.
EG10	A	.	.	.	A	.	.	.
EG11	.	.	.	.	A	.	.	.
EG12	.	.	.	.	A	.	.	.

* goat breed was taken from GenBank; KG: Kacang goat; EG: Ettawa Grade goat
(.) same with *C. aegagrus*
Amino acid :A (Alanine), I (Isoleucine), L (Leucine), T (Threonine), V (Valine), W (Tryptophan), Y (Tyrosine)

Table 5. Differences of Codon Site on Cytochrome b among Kacang and EG goat with Three Comparator Goats

Sample	Codon site on						
	16	97	189	190	214	231	303
*C. hircus**	GCA	ATC	ACA	GGC	ACA	GCC	GTA
*C. aegagrus**	A..	…	…	.C.	…	…	…
*C. falconeri**	…	G..	G..	.C.	…	…	A..
KG1	…	…	…	.C.	G..	…	…
KG2	…	…	…	.C.	G..	…	…
KG3	…	…	…	.C.	G..	…	…
KG4	A..	…	…	.C.	G..	…	…
KG5	…	…	…	.C.	G..	…	…
KG6	…	…	…	.C.	G..	…	…
KG7	…	…	…	.C.	G..	…	…
KG8	…	…	…	.C.	G..	…	…
KG9	…	…	…	.C.	G..	…	…
KG10	…	…	…	.C.	G..	…	…
KG11	…	…	…	.C.	G..	…	…
KG12	…	…	…	.C.	G..	…	…
KG13	…	…	…	.C.	G..	…	…
KG14	…	…	…	.C.	G..	…	…
KG15	…	…	…	.C.	G..	…	…
KG16	…	…	…	.C.	G..	…	…
KG17	…	…	…	.C.	G..	…	…
KG18	…	…	…	.C.	G..	…	…
KG19	…	…	…	.C.	G..	…	…
EG1	…	…	…	.C.	G..	…	…
EG2	…	…	…	.C.	G..	…	…
EG3	…	…	…	.C.	G..	…	…
EG4	…	…	…	.C.	G..	…	…
EG5	…	…	…	.C.	G..	…	…
EG6	A..	…	…	.C.	G..	…	…
EG7	…	…	…	.C.	G..	.T.	…
EG8	…	…	…	.C.	G..	…	…
EG9	…	…	…	.C.	G..	…	…
EG10	…	…	…	.C.	G..	…	…
EG11	A..	…	…	.C.	G..	…	…
EG12	A..	…	…	.C.	G..	…	…

* goat breeds were taken from GenBank; KG: Kacang goat; EG: Ettawa Grade goat
(.) same with *C. hircus*
Codon (Nucleotide base) :A (Adenine), C (Citosine), G (Guanine), T (Timine)

Table 6. Differences of Amino Acid Site on Cytochrome b among Kacang and EG goats with Three Comparator Goats

Sample	Amino Acid Site on						
	16	97	189	190	214	231	303
*C. hircus**	A	I	T	G	T		V
*C. aegagrus**	T	.	.	A	.		.
*C. falconeri**	.	V	A	A	.		M
KG1	.	.	.	A	A		.
KG2	.	.	.	A	A		.
KG3	.	.	.	A	A		.
KG4	T	.	.	A	A		.
KG5	.	.	.	A	A		.
KG6	.	.	.	A	A		.
KG7	.	.	.	A	A		.
KG8	.	.	.	A	A		.
KG9	.	.	.	A	A		.
KG10	.	.	.	A	A		.
KG11	.	.	.	A	A		.
KG12	.	.	.	A	A		.
KG13	.	.	.	A	A		.
KG14	.	.	.	A	A		.
KG15	.	.	.	A	A		.
KG16	.	.	.	A	A		.
KG17	.	.	.	A	A		.
KG18	.	.	.	A	A		.
KG19	.	.	.	A	A		.
EG1	.	.	.	A	A	.	.
EG2	.	.	.	A	A	.	.
EG3	.	.	.	A	A	.	.
EG4	.	.	.	A	A	.	.
EG5	.	.	.	A	A	.	.
EG6	T	.	.	A	A	.	.
EG7	.	.	.	A	A	V	.
EG8	.	.	.	A	A	.	.
EG9	.	.	.	A	A	.	.
EG10	.	.	.	A	A	.	.
EG11	T	.	.	A	A	.	.
EG12	T	.	.	A	A	.	.

* goat breed was taken from GenBank; KG: Kacang goat; EG: Ettawa Grade goat
(.) same with*C. hircus*
Amino acid :A (Alanine), G (Glycine), I (Isoleucine), M (Methionine), T (Threonine), V (Valine)

Table 7. Amino Acid Characteristic on Cytochrome b Gene of Kacang and Ettawa Grade (EG) Goats Compared to 3 Comparator Goats

Characteristic	Within Kacang and EG Goats	Kacang and EG Goats Compared to		
		C. hircus	*C. aegagrus*	*C. falconeri*
Synonymous amino acid	1	11	5	43
Non synonymous amino acid	2	4	3	6
Converse amino acid	374	362	369	328
Codon mutation				
-1st codon	1	4	2	6
-2nd codon	1	2	1	1
-3rd codon	1	9	5	42

the domestic goat and *C. falconeri* (Markhor), and it has contributes to several species of goats in Asia (Mason, 1984; Takada *et al.*, 1997; Pidancier *et al.*, 2006). Naderi *et al.* (2008) stated that an early domestication center of *C. aegagrus* was in the Central Iranian Plateau (Yazd and Kerman Provinces) and in the Southern Zagros (Fars Province). While *C. hircus* (domestic goat) is a domestication goat of wild goat (*C. aegagrus*), which distribute to Africa (South Africa), Slovenia, Europe (Switzerland), Asia (Malaysia). According to Shackleton (1997), the distribution of *C. aegagrus* were include Afghanistan, Armenia, Azerbaijan (Nakhichevan), Lebanon (extinct), Russia (East Caucasus), Turkey, Georgia and Iran while distribution of *C. falconeri* were include India and Pakistan.

The most codon mutations occurred in third, second and first codon (Table 7). This is because the first and second codon in Cyt b gene b had a low gamma value, so the possibility of substitution mutation was very low. Conversely, the third codon had a high gamma value with the result that the possibility of substitution mutation is high (Farias *et al.,* 2001). However, despite the third codon have a high substitution value, mostly substitution is saturation effect. Saturation effect is a change of nucleotide bases that are not followed by amino acid changes, which itis called synonymous amino acids (Kocher *et al.,* 1989). Slechtova *et al.* (2006) and Doadrio and Perdices (2006) stated that the most varied nucleotide was at third codon, whereas most conserve nucleotides were at second codon.

CONCLUSION

Specific amino acids in Cyt b gene can be used as a genetic marker among KG and EG goat with 3 goat comparator. There were several specific amino acid that distinguish Kacang and EG goat with *C. hircus*, *C. aegagrus* and *C. falconeri*. But there were not amino acids can be used as a genetic marker to distinguish between Kacang and EG goat. Mutations in Cyt b gene mostly were in third codon and the most differences were found between kacang and EG goat with *C. falconeri*.

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