POLYMORPHISM OF INSULIN-LIKE GROWTH FACTOR 1 GENE IN KEDAH-KELANTAN CATTLE USING PCR-RFLP TECHNIQUE

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ABSTRACT. The Kedah-Kelantan cattle (KK) is an indigenous cattle breed and is mainly kept for meat production in Malaysia. Due to lack of information about polymorphism of growth traits in these cattle, Insulin-like Growth Factor 1 (IGF-1) was chosen to be the candidate gene in this preliminary study. The aim of this study is to investigate the polymorphism of IGF-1 gene in KK and to show that the PCR-RFLP technique can be used as a basis for use as molecular markers in cattle. A total of 46 KK blood samples were collected for DNA extraction performed using a commercial kit. The exon 1 of IGF-1 gene was amplified to produce a 249 bp fragment. The amplified fragments were digested with *Eco105I* restriction endonuclease and then subjected to electrophoretic separation in Fluorosafe stained 2.5 % agarose gel. The result revealed two alleles, A and B. Three genotypes were observed: AA, AB and BB. Frequencies were 0.07, 0.13 and 0.80 for AA, AB and BB, respectively. This gives frequencies of 0.13 and 0.87 for A and B alleles. It is concluded that the population is in Hardy-Weinberg disequilibrium ($p<0.05$). It is possible that this gene has been exposed to selection.

***Keywords*:** Kedah-Kelantan cattle, Growth factor, IGF-1 gene, PCR-RLFP

INTRODUCTION

Indigenous breeds are important assets to countries for many reasons, but particularly because, over time, they have developed unique combinations of adaptive traits to best respond to pressures of the local environment (FAO, 2000 and Abdelwahid *et al*., 2009). Farm owners prefer to choose crossbreds or imported exotic breeds compared to indigenous breeds, which are often neglected and not fully evaluated in terms of genetic improvement. In a crossbreeding programme, indigenous breeds are often used as the dam breed is crossed with other breeds to create a population which can meet the targeted traits which need to be maintained. (Abdelwahid *et al.,* 2009). These traits, may perish over time if they are not maintained and monitored and concerted efforts are needed to recognize and exploit these traits.. Thus, it is important to evaluate the genetic structure and variation to ensure that the breed can be preserved.

The Kedah Kelantan (KK) is an indigenous breed of Malaysia, a beef-type cattle and is mainly kept for meat production (Abdelwahid *et al.,* 2009). The body size

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is small, compact and well adapted to the local tropical environment making them easy to be maintained. The population size of purebred KK is fast decreasing and most of the commercial populations are actually crossbreds (Abdelwahid *et al.,* 2009). There is also a lack of information on the genetic polymorphism of KK on growth traits. According to Yazdanpanah *et al.* (2013) genetic polymorphism in native breeds is a major concern considering the necessity of preserving genetic resources. This is important for planning a breeding program for improvement and conservation of native breeds especially breeds that have potential for economic industry.

Recent developments in molecular genetic techniques have made it possible to identify genetic variation at specific loci and the association between variation at gene affecting quantitative traits (quantitative trait loci, QTL) and production traits (Chung & Kim, 2005). Many studies on genetic improvement on cattle involved with the traits of interest such as growth, carcass, and milk production traits have been conducted using certain candidate genes and anonymous genetic markers. Thus, Insulin-like Growth Factor 1 (IGF-1) has been selected as it is commonly used as effective markers for growth traits (Ge *et al*., 2001; Li *et al.,* 2004; Siadkowska *et al.,* 2006 and De la Rosa Reyna *et al.,* 2010). The gene IGF-1, plays an important physiological role in the growth and development of mammals (Ge *et al.,* 2001 and Chung & Kim, 2005). In bovine, IGF-1 locus is located in chromosome 5, as reported by other studies (Siadkowska *et al.,* 2006; De la Rosa Reyna *et al.,* 2010; Mirzaei *et al.,* 2012 and Jeanmas *et al.,* 2013).

Since, there is also a lack of information on IGF-1 polymorphism in indigenous Kedah-Kelantan (KK) compared to other breeds, research in this aspect needs to be conducted. The objectives of this study thus, is to identify the allele and genotype frequencies of IGF-1 gene and to prove that PCR-RLFP technique can serve as a basis for studies on the use of molecular markers in cattle.

MATERIALS AND METHODS

This study was carried out on 46 Kedah-Kelantan cattle at Insitut Biologi Veterinar Kebangsaan (IBVK) farm in Jerantut, Pahang, Malaysia. These animals were originally from Pusat Ternakan Haiwan Pantai Timur, Tanah Merah, Kelantan, Malaysia.

A total of 5 ml of EDTA blood was collected from the 46 KK cattle and storedin -20 °C before further processing. Genomic DNA was isolated by using commercial DNA Extraction Kit (Genedirex, Bio-Helix Co., Ltd, Taiwan). The extracted DNA was appropriately labelled and stored at -20 °C for analysis. The study sample was concentrated on a 249 bp fragment in the 5'- flanking region of IGF-1 gene. Primers sequences for PCR were established by Ge *et al*. (2001); Li, *et al.* (2004); Laureano *et al.* (2009) and Yazdanpanah *et al.* (2013) (**Table 1**).

The PCR reaction volume of 50 µl contained 1 X PCR buffer, 3 mM $MgCl_2$, 0.2

mM dNTPs, 1.25 U Taq DNA polymerase (Fermentas,USA), 0.5 µM of each primer and 1.0 µl of DNA templates. PCR was carried out using Biometra thermocycler with the following conditions: initial denaturation at 94 °C for 3 mins, followed by 32 cycles, denaturation at 94 °C for 30 s, annealing at 61 °C for 40 s and extension at 72 °C for 60 s. A final extension at 72 °C for 5 mins at the end of the amplification cycles was included. The PCR products were analysed by agarose gel 2.5 %. The positive reaction products were used for enzymatic digestion by *Eco105I* (TAC. GTA) restriction endonuclease.

The digestion reaction contained 5 µl of PCR product, 2 µl Buffer 10 x, 5 U of *Eco105I* and H_2O up to a total volume of 20 µl which is then were incubated at 37 °C for 3 hours and followed by 20 min of inactivation at 65 °C. The digested PCR products were analysed by agarose gel 2.5 % in 1 X TBE buffer for 2 hours at 70 volt. The gels were stained with Fluorosafe (1st Base, First BASE Laboratories Sdn. Bhd., Selangor) and visualized under UV light on a transilluminator. The frequencies of genotype, alleles, mean expected, mean observed and Hardy-Wienberg equilibrium test were calculated using Popgene (version 1.32) software and Pearson's chi-square test.

Table 1. Primer used in the amplification of bovine IGF-1 gene

Locus/Primer	Primer sequence
IGF-1-F	ATT ACA AAG CTG CCT GCC CC
IGF-1-R	ACC TTA CCC GTA TGA AAG GAA TAT ACG T

RESULT AND DISCUSSION

The Insulin-like Growth Factor 1 (IGF-1) gene amplified by PCR technique in KK cattle resulted in the DNA fragment with 249 bp including the sequences spanning over exon 1 region (**Figure 1**). In the current study, PCR-RFLP digestion of the 249 bp fragment obtained from the 5'- flanking region of the IGF-1 gene with *Eco150I* resulted in three migration patterns (**Figure 2**). In line with our results, Ge *et al.* (2001) concluded that the digested samples that resulted in three fragment 249 bp; namely 226 bp and 23 bp correspond to genotypes AB, two fragments 226 bp and 23 bp correspond to genotypes AA and undigested samples with fragment of 249 bp is genotype BB. The fragments resulted due to a transition of T (allele A) to C (allele B) and identified at 512 bp 5'to the first codon (ATG) of the first exon.

Table 2. Genotypes (observed) and allele frequencies (observed) and allele frequencies (expected) of Kedah-Kelantan population

Genotypes frequencies (Observed)			Allele frequencies (Observed)		Allele frequencies (Expected)		X^{2*}
AA	AB	BB	A	B	A	B	9.2453
0.07	0.13	0.80	0.135	0.865	0.1304	0.8696	

*$\chi^2_{0.05}$ value compared critical value=3.841

Kedah-Kelantan experimental group shows 37 out of 46 samples have the same electrophoretic migration pattern, called genotypes BB while AA and AB were 3 and 6, respectively. The genotypes of all animals were used to determine the allele frequencies. The A and B expected allele frequencies are based on Popgene software calculated as 0.1304 and 0.8696, respectively and were similar to other studies (Laureano *et al.,* 2009; De la Rosa Reyna *et al.,* 2010 ; Yazdanpanah *et al.,* 2013 and Jeanmas *et al.,* 2013).

The allele B was found to be more dominant than allele A. Curi *et al.* (2005) suggested that the B allele is characteristic of *Bos indicus* because they found it fixed allelic frequencies of B allele in Nelore population (1.00). In addition, Ge *et al*. (2001) reported Angus beef cattle showed the allelic frequency of A and B was 0.64 and 0.36, respectively. Other, De la Rosa Reyna *et al.* (2010) reported for Beef master populations (crossbred *Bos taurus* and *Bos indicus*) and showed the allele B was almost fixed (0.97). Since Kedah-Kelantan cattle are Zebu type (or *Bos indicus*) cattle, the data shows high frequency in B allele (0.8696) which is in accordance to other studies. The results obtained from this study shows a contrast with those reported in the study of Ge, *et al*. (2001) in which the B allele showed a lower frequency in a *Bos taurus* population. This fact may indicate this allele (B) is characteristic of *Bos indicus* animals (Laureano, *et al.,* 2009). The high frequency of the allele B suggests that this allele variant might have been favoured by selection for production traits (De la Rosa Reyna *et al.,* 2010 and Yazdanpanah *et al.,* 2013).

The observed frequency of genotypes AA, AB and BB detected by RFLP was 0.07, 0.13 and 0.80 respectively (**Table 2**). Kedah-Kelantan population was in Hardy-Weinberg disequilibrium ($p<0.05$). In other words, the gene frequencies are changing over time and thus evolving.. In this population, the observed and expected heterozygosity were 0.13 and 0.2268 respectively (**Table 3**). From this study, the heterozygosity is low and inbreeding level is estimated at 0.4268 based on population inbreeding coefficient, f which is probability due to mating between relatives or the size of population is small. The population inbreeding coefficient formula was used as mentioned by Kent (2012) is as follows:

f = 1- [observed heterozygosity/expected heterozygosity]

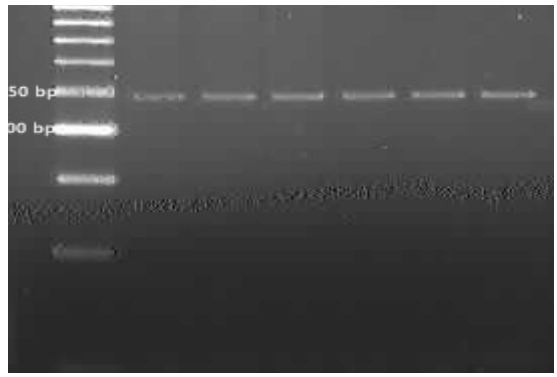

Figure 1. Gel electrophoresis of PCR products of IGF-1 (Size obtained: 249 bp)

Table 3. The observed and expected heterozygosity in IBVK population

Region	Observed heterozygosity	Expected heterozygosity
IBVK	0.13	0.2268

Figure 2. IGF-1 genotyping by PCR-RFLP technique.

CONCLUSION

The Kedah-Kelantan cattle breed shows good polymorphism whereby the IGF-1 locus, based on results showed the electrophoretic migration pattern but further studies need to be conducted to clarify these findings as there is a lack of information on polymorphism of IGF-1 gene in Kedah-Kelantan cattle. The number of samples should be increased or expanded to obtain a solid base for findings. The data provides evidence that the population is evolving over time. It was found thatBesides, the inbreeding level is approximately 43% for the population. The IGF-1 gene can be used as a potential gene which opens interesting prospects for future selection programs, especially marker assisted selection between different genotypes of different locus for other Quantitative Loci Traits (QTLs) such as growth, reproductive, milk production, weight gain, carcass, meat and others. The present results show that PCR-RFLP is one of the appropriate tools for evaluating genetic variability. It is suggested that further studies be carried out to investigate the relationship between different genotypes in KK cattle and growth performance as this will enhance the cattle industry by leaps and bounds.

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