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A study of major prolificacy genes in Malin and Dorper sheep in Malaysia

(Kajian gen kebiakan utama pada biri-biri Malin dan Dorper di Malaysia)

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Keywords: ovulation rate, litter size, mutation, prolificacy genes

Abstract

High prolificacy in some sheep breeds was due to mutation in *FecB* and *FecX* genes. The mutations of these genes were associated with an increase in ovulation rate, litter size and the number of follicles in the ovaries. The *FecB* gene was present in some high prolific breeds but absent in low prolific sheep breeds. The objective of this study was to verify the presence of these prolificacy genes and identify if the genes are polymorphic in Malin and Dorper sheep. Five prolificacy genes, *FecB, FecX*^{I}*, FecX*^{H}*, FecX*^{B} and *FecX*^{G} were screened in Malin and Dorper sheep. Four genes were successfully amplified by PCR except for *FecX*^{G}, which failed to amplify in all samples tested. Analysis of PCR products by Forced Restriction Fragment Length Polymorphism PCR (PCR RFLP) showed the absence of *FecB* and *FecX* gene mutations after the samples were digested with enzymes. From the analysis carried out, it was shown that Malin and Dorper sheep were homozygous non-carriers for Booroola *FecB* and *FecX* gene mutations. Based on these findings, both Malin and Dorper sheep breeds can be considered as low prolificacy sheep breeds.

Introduction

In Malaysia, the sheep industry is still small when compared to other sub-sectors of the livestock industry such as poultry and swine. Malaysia has targeted to increase the domestic production of goat and sheep meat from 9% to 35% which requires a reciprocal increase in the goat and sheep populations to 1.5 million heads by 2015 (Rafiu et al. 2012). To achieve this objective, the prolificacy of ewes was critical in order to increase the lambing percentage. Prolificacy refers to the ability of the female to produce multiple lambs through high ovulation rates and embryo survival.

Various major genes have been reported to affect prolificacy in sheep. These include three related oocyte-derived components, namely, the Growth Differentiation Factor 9 (GDF9), Bone Morphogenic Protein 15 (BMP15) and Bone Morphogenic Protein Receptor type 1B (BMPR-1B) genes (Kasiriyan et al. 2009). These three genes have been shown to be essential for ovulation rate and follicular growth (Kasiriyan et al. 2011). The GDF9 is

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an autosomal gene located on chromosome 5 (Sadighi et al. 2002). According to Davis et al. (2006), measurement from the small sample of ewes indicated that the effects of GDF9 mutation on the ovulation rate was greater than that of the BMP15 mutations. The GDF9 gene was known to control the fecundity of Belclare, Cambridge, Santa Ines, Moghani, Ghezel and Thoka ewes (Chu et al. 2011). On the other hand, the BMP15 gene that was important for ewe fertility (Galloway et al. 2000) consisted of four alleles, $FecX^H$, $FecX^I$, $FecX^B$ and $FecX^G$ (Davis 2005). These four alleles have been found in Romney, Belclare and Cambridge sheep (Galloway et al. 2000; Hanrahan et al. 2004).

The product of BMP15 gene is a growth factor that is exclusively expressed in the oocytes (Galloway et al. 2000). This gene, known as the *Inverdale* gene (*FecX*), is responsible for increasing the ovulation rate by about 1.0 but caused sterility in homozygous carrier females in Romney sheep (Davis et al. 1991, 1992). The point mutation in GDF9, BMP15 and BMPR-1B genes was reported to have an effect on ovulation rate in sheep (Hanrahan et al. 2004). The infertile ewes have small undeveloped 'streaked' ovaries that never ovulate. The ewes that carried all the BMP15 gene mutations have a litter size of about 0.6 higher than non-carrier ewes.

Davis et al. (2001) reported that crossbreeding of sheep carrying $FecX^I$ and $FecX^H$ produced daughters with one copy of each mutation which had similar infertile streaked ovaries as in ewes that were homozygous for the four BMP15 mutations. Similarly, Belclare and Cambridge ewes carrying a copy of the $FecX^G$ and $FecX^B$ genes were also infertile with streaked ovaries respectively (Hanrahan et al. 2004). However, crossbreeding of Booroola sheep carrying BMPR-1R mutation with Inverdale sheep mutation in BMP15 produced daughters with fully functional ovaries and high ovulation rates (Davis et al. 1999). Davis (2004) reported that ewes carrying a copy of BMP15, BMPR-1B and FecX2 mutations had normal ovaries and high ovulation rates (5 and 8 at 1.5 years, 12 at 2.5 years).

The BMPR-1B gene which was first identified in the 1980s (Davis 2004) influenced the fecundity of Booroola Merino sheep (*FecB*) and is responsible for ovulation rate and the litter size (Kumar et al. 2006; Kasiriyan et al. 2009). The Booroola gene (*FecB*) is a dominant autosomal gene with an additive effect on the ovulation rate whereby one copy of Booroola gene increases ovulation rate by about 1.5. The effect of this gene has been shown to be due to a mutation in BMPR-1B which is expressed in oocytes and granulosa cells (Wilson et al. 2001; Moore et al. 2003). The *FecB* gene has been found in some of prolific sheep breeds such as Huyang (Guan et al. 2007) and Small Tail Han (Chu et al. 2006) in China, Garole sheep in India (Kumar et al. 2006) and Javanese thin tail sheep in Indonesia (Davis et al. 2002) but absent in non-prolific breeds such as Tan and Xinjiang sheep in China (Davis et al. 2006; Guan et al. 2007; Hua and Yang 2009). The prolific Chinese merino breed has all three types of *FecB* genotypes. The average lambing rates of BB genotype (209.1%) and B+ genotype (208.7%) were significantly higher than ++ genotype. Introgression of *FecB* gene could improve the fecundities of low prolificacy flocks. Nonetheless, some researchers have indicated that this gene was not the only gene controlling fecundity in sheep. Therefore, the objective of this study was to verify the presence of prolificacy genes and identify if the genes are polymorphic in Malin and Dorper sheep.

Materials and methods

Sample collection and DNA extraction

Blood samples were collected in 4 ml-heparinised vacutainer tube (Becton Dickinson, USA) from Dorper (n = 39) and Malin (n = 20) sheep, the latter an indigenous breed in Malaysia. The Dorper was first brought in from South

Africa in the year 2010 and reared at MARDI Kluang, Johor. The DNA was extracted from the whole blood using a commercial kit (Promega, USA) following the manufacturer's instructions with minor modifications.

Synthesising oligonucleotides

Five prolificacy-related genes were amplified in the genomic DNA extracted from the blood samples by Polymerase Chain Reaction (PCR). The genes were *FecB, FecX^{I}, FecX^{H}, FecX^{B}* and *FecX^{G}*. The oligonucleotides were synthesized to introduce a point mutation resulting in PCR products where the *FecB* mutation contained an *Ava*II restriction site (G/GACC, Davis et al. 2002), *FecX^{H}* mutation with *Spe*I restriction site (A/CTAGT, Galloway et al. 2000), and *FecX^{I}* mutation generated *Xba*I restriction enzyme site (T/CTAGA, Galloway et al. 2000). The sizes of products containing mutations are shown in *Table 1*. On the other hand, the *FecX^{B}* and *FecX^{G}* could be cleaved by *Dde*I (C/TTAG, Hanrahan et al., 2004) and *Hinf*I (G/ACT, Hanrahan et al. 2004) respectively. However, the mutation type for these genes remained uncut against the digestions.

Amplification of prolificacy genes by PCR

The PCR amplification was carried out in 25 µl reaction mixtures including each primer (0.8 µl of a 10 µM solution), 200 µM dNTPs, 1X Buffer, 2.0 mM $MgCl_2$ and 2.5 U/µl *Taq* DNA polymerase (Promega, USA). However, the PCR programs for each gene were different. For the amplification, the program consisted of an initial denaturing step at 94 °C for 5 min, 35 cycles (94 °C for 30 s, 57 °C for 30 s and 72 °C for 30 s) and a final extension at 72 °C for 5 min in a DNA thermal cycler (GeneAmp® PCR System 9600 Perkin-Elmer). The PCR products were purified using a purification kit (Promega, USA).

Forced Restriction Fragment Length Polymorphism PCR (PCR RFLP)

The purified PCR products were subjected to forced PCR-RFLP as described by Galloway et al. (2000), Davis et al. (2002) and Hanrahan et al. (2004) using the restriction enzyme (RE) (*Table 1*) to detect a point mutation in the *FecB* and *FecX* genes. The products were incubated at 37 °C overnight and the reaction was stopped at 65 °C for 10 min. The product was separated in a 3% metaphor agar and stained with ethidium bromide.

Table 1. Oligonucleotides, enzymes, and sizes of genes before and after digestion

Gene	Primer sequence (5'→ 3')	Product size	RE	Digested products size
FecB	CCAGAGGACAATAGCAAAGCAAA CAAGATGTTTTCATGCCTCATCAACAGGTC	190 bp	AvaII	160 bp, 30 bp
FecX^{H}	TATTTCAATGACACTCAGAG GAGCAATGATCCAGTGATCCCA	240 bp	SpeI	218 bp, 22 bp
FecX^{I}	GAAGTAACCAGTGTTCCCTCCACCCTTTTCT CATGATTGGGAGAATTGAGACC	154 bp	XbaI	124 bp, 30 bp
FecX^{B}	GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA TTCTTGGGAAACCTGAGCTAGC	153 bp	DdeI	122 bp, 31 bp
FecX^{G}	CACTGTCTTCTTGTTACTGTATTTCAATGAGAC GATGCAATACTGCCTGCTTG	141 bp	HinfI	112 bp, 29 bp

RE = Restriction enzymes

Results and discussion

The *FecB* mutation in BMPR-1B and *FecX*^{B}, *FecX*^{H}, *FecX*^{I} genes in BMP15 were successfully amplified by PCR in all blood samples collected from the Malin and Dorper sheep except for the *FecX*^{G} gene. The DNA samples did not produce any fragment when amplified using *FecX*^{G} primers. However, the forced PCR-RFLP of *FecB*, *FecX*^{I} and *FecX*^{H} (*Figures 1 – 2*) remained uncut when digested with RE. In the case of *FecB* carriers (mutation), the individual has an *Ava*II restriction site (G/GACC) but non-carriers lacked this site. These conditions were similar in *FecX*^{H} and *FecX*^{I} alleles while the non-carriers remained uncleaved after digestion with *Spe*I and *Xba*I respectively. However, for non-carrier individuals with *FecX*^{B} gene, *Dde*I digested the products and produced 122 bp and 31 bp fragments (*Table 1*). These results showed that none of the samples carried the *FecB* mutation in BMP1R-1B gene similar to the absence of *FecX*^{B}, *FecX*^{H}, *FecX*^{I} mutations in BMP15 locus.

These results showed that FecB and BMP15 loci were monomorphic which was not reported in previous studies (Hanrahan et al. 2004; Guan et al. 2006; Chu et al. 2007). All the individuals were wild homozygous (Hua et al. 2008). The homozygote for three BMP15 alleles in ewes was infertile whereas the heterozygous individuals had greater ovulation rate (Shimasaki et al. 2003; Hanrahan et al. 2004). The prolific Chinese sheep breed of Small Tailed Han carried mutations in both BMPR-1B and BMP15 genes and had a greater litter size (2.61, Tu 1989; and 2.65, Wang et al. 1990) than those with either mutation alone (Chu et al. 2007). However, the Hu sheep which is known as the most prolific sheep breed in China has BB genotype in the *FecB* gene but has no mutation in the BMP15 gene (Guan et al. 2007).

Mutations in *FecB* and *FecX* genes were not the only factors responsible for high prolificacy (Guan et al. 2007) as Malin sheep which lacked mutations in these genes were highly prolific (prolificacy % per year, 0.6 – 1.45) (Wan Mohamed et al. 1988). Nevertheless, the ovulation rate of Malin and Dorper sheep was almost similar which was 1.04 (Azmi et al. 1993) and 1.0 (Greeff et al. 1993) respectively. The results in the present study are similar to those obtained in the Iranian sheep breed, Sangsari (Kasiriyan et al. 2009) and other Indian sheep breeds, Bannur, Deccani and Madras Red sheep (Wilson et al. 2001; Pardeshi et al. 2005). According to Kasiriyan et al. (2009), the mutant allele might be spontaneously

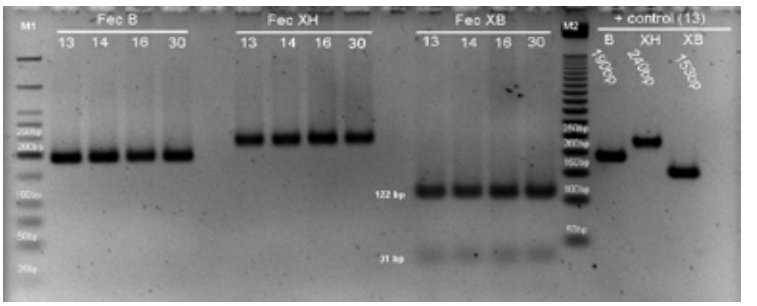

Figure 1. Amplification of PCR products of sample 13 (+ control) for the FecB, FecX^{H} *and FecX*^{B} *genes presenting a single fragment at a size 190 bp, 240 bp and 153 bp respectively. Lanes 13, 14, 16 and 30 are PCR-RFLP of each allele. FecB and FecX*^{H} *remained uncleaved but FecX*^{B} *produced two digested fragments at sizes 122 bp and 31 bp. Lanes M1 and M2 are 25 bp and 50 bp molecular weight markers (Promega, USA), respectively*

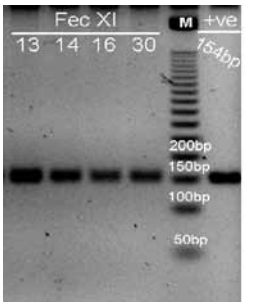

Figure 2. Lanes 13 – 30 shows forced RFLP-PCR of FecX^{I}*gene after digestion by XbaII. Lane +ve represents PCR product at 154 bp. Lane M is a 50 bp DNA marker (Promega, USA)*

occurring in inbreeding or via crossbreeding (Hua and Yang 2009). In addition, the environment also plays an important role in introducing a mutant allele into the organism. Malin or Dorper sheep which are highly adaptable to a harsh environment and highly resistant to diseases might have resulted in the failure in transmitting the mutant allele into these breeds (Kasiriyan et al. 2009).

In 1980s, Malaysia brought in various wool sheep breeds including the Suffolk, Border Leicester, Dorset Horn, Corriedale and commercial Merino x Border Leicester from Australia to increase the sheep population and improve the productivity of Malin by crossbreeding (Musaddin 1995). The results obtained in this study suggested that probably there was no natural avenue for the transfer of mutation genes from imported breeds to Malin (Pardeshi et al. 2005). It was reported in a previous study that the mutant *FecB* was not found in Tooka, Woodlens, Olcusa, Lacan, Belklir and Cambridge breeds reared in countries such as New Zealand, India, Philippines, Indonesia, Poland, France and Ireland (Kasiriyan et al. 2009).

However, the *FecB* gene is present in Garole (India) (Polley et al. 2012) and Javanese Thin tail (Indonesia) (Davis et al. 2002). It is doubtful whether the Booroola gene found in Javanese Thin tail was acquired from Merinos from Australia or via Garole sheep because Garole was proposed as the ancestor of Australia Booroola Merinos (Davis et al. 2002). The Awassi breed which had low lambing rate showed increased fertility rate from 1.2 – 2 lambs per lambing after the incorporation of the Booroola gene since 1986 (Baird and Campbell 1998).

On the other hand, the quality of feeds could also influence the expression of major genes and thus, affect the reproductive performance of sheep. Although Booroola and Garole breeds carry the same mutant allele, the ovulation rate and lambing in Booroola was higher than Garole (Piper and Bindon 1996). A study by Kumar et al. (2006) showed that a mutation of *FecB* gene was associated with twinning or high litter size in Garole ewes. In contrast, a study by Kumar et al. (2006) indicated that twinning in Marwari and Bharat Merino ewes was not linked to *FecB* mutation. This condition did not happen also in high prolificacy Irannian Sangsari sheep where BMP15 loci were not polymorphic (Kasiriyan et al. 2011).

Conclusion

The present study showed that the *FecB* and *FecX* genes were successfully amplified in Malin and Dorper sheep, however, no mutation occurred in these genes. The results indicated that the *FecB* and *FecX* genes in Malin and Dorper sheep were not polymorphic and thus, can be considered as low prolificacy sheep breeds. This finding is preliminary and therefore, further investigation of other major genes that may be involved in prolificacy should be carried out. A good breeding record is also required to determine the association of these genes with the prolificacy trait. In addition, due to the small sample size, there is a probability that the mutant genes were not detected in the samples tested. Thus, further research should be performed using a larger sample size for both Malin and Dorper sheep.

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Abstrak

Kebiakan yang tinggi pada kebanyakan baka biri-biri disebabkan oleh mutasi pada gen *FecX* dan *FecB*. Mutasi gen-gen ini dikaitkan dengan kadar ovulasi yang tinggi, bilangan anak dan bilangan folikel dalam ovari. Gen *FecB* hadir dalam kebanyakan baka yang prolifik tetapi tidak hadir dalam baka biri-biri yang kurang prolifik. Objektif kajian ini adalah untuk mengesahkan kehadiran gen-gen kebiakan dan mengenal pasti sama ada gen-gen ini polimorfik pada baka Dorper dan Malin. Lima gen kebiakan yang terdiri daripada *FecB*, $FecX^{I}$, $FecX^{H}$, $FecX^{B}$ and $FecX^{G}$ telah disaring pada biri-biri Dorper dan Malin. Empat gen tersebut berjaya diamplifikasi melalui PCR kecuali gen $FecX^{G}$ yang gagal diamplifikasi dalam kesemua sampel yang dikaji. Analisis produk PCR menggunakan PCR Polimorfisme Panjang Fragmen Pembatasan Paksa (PCR RFLP) menunjukkan ketiadaan mutasi gen *FecB* dan *FecX* selepas sampel dicerna dengan enzim. Analisis ini menunjukkan bahawa biri-biri Dorper dan Malin adalah bukan pembawa homozigot mutasi gen Booroola *FecB* dan *FecX*. Penemuan ini menunjukkan bahawa kedua-dua baka tersebut boleh dianggap sebagai baka biri-biri yang rendah kebiakannya.

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