

Polymorphisms of aquaporin 7 (*AQP7*) gene in Madura bulls (*Bos indicus*)

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Madura cattle (*Bos indicus*) are one of the Indonesia's native cattle from Madura Island. This cattle has been decided as the Indonesian native cattle through decision of the Indonesian Ministry of Agriculture No: 3735/Kpts/HK.040/11/2010 (Kementan 2010). The Madura cattle was kept by the farmers for meat production and drought purposes. Besides this, cattle are also maintained for bull racing (Karapan festival) and cow's contest (Sonok festival). As the beef cattle in Indonesia, the Madura cattle have to reach the adult body weight (20 months of age) of 192.11±63.71 kg in male and 190.57±61.24 kg in female (Hartati and Putra 2021). In addition, the carcass weight of Madura cattle (10-24 months of age) was 227.67±11.59 kg in male and 211.88±46.66 kg in female (Prihandini *et al.* 2020). Recently, the frozen sperm (straw) of Madura bull was produced in many National Artificial Insemination Centers of Indonesia. Generally, the bulls for straw production were selected based on Indonesian National Standard (SNI) for sperm quality (SNI 4869.1 2017) and Madura breed (SNI 7651.2 2013). However, the candidate bulls can be evaluated and selected based on their molecular genetic information mainly on genes governing fertility. The aquaporin 7 (*AQP7*) gene is one of candidate genes used for molecular selection for sperm quality traits in bulls (Ma *et al.* 2011, Kumar *et al.* 2014).

AQP7 gene is important to produce *AQP7* protein in the germ cell that is responsible for spermatid development in mammals (Ishibashi *et al.* 1997, Kageyama *et al.* 2001). The bovine *AQP7* gene (GenBank: NC_037335.1) is located at chromosome 8 and consist of nine exons. Recently, two mutation sites of c.N8S (A264G) and c.E36Q (G371C) were detected in the bovine *AQP7* gene at exon 2 and exon 3 regions, respectively (Ma *et al.* 2011, Kumar *et al.* 2014). In buffalo, 6 mutation sites in exonic region (Kumari *et al.* 2018a) and 15 mutation sites in intronic region were detected in the bubaline *AQP7* gene

with SSCP analysis (Kumari *et al.* 2018b). The study to evaluate the polymorphism in *AQP7* gene of Madura bulls has not been reported. Hence, this study was carried out to identify the polymorphism in *AQP7* gene of Madura bulls raised at Indonesian Beef Cattle Research Station (IBCRS) with PCR-RFLP method.

Total 83 blood samples of Madura bulls (*Bos indicus*) at Indonesian Beef Cattle Research Station (IBCRS) were used in this study for DNA extraction with Zymo Biomix DNA Kits (Zymo Research, USA) following the manufacturer's instruction. The PCR analysis was performed in 20 µL volume of PCR sample including 3 µL of DNA sample, 0.4 µL of each primer (1 pmol), 10 µL of PCR mix (Bioline, USA) and 6.2 µL of double distilled water (DDW).

The exon 2 region of *AQP7* gene (221 bp) was amplified with a primer pair of Forward: 5'- TGA GGT CTG ATG GGA ATG AGG -3' and Reverse: 5'- CTG CCA TGA GGG AGG AGC TA -3'. The exon 3 region of *AQP7* gene (199 bp) was amplified with a primer pair of Forward: 5'- TCA CCT GAT CTC ATT TCT GCC -3' and Reverse: 5'- AGT CTG CTC ACC CTG TAC CC -3' (Ma *et al.* 2011). The PCR thermal profile in each gene consisted of pre-denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 15 sec, annealing at 60.4°C for 30 sec, extension at 72°C for 10 sec and final extension at 72°C for 5 min. The RFLP analysis was performed in 10 µL volume of reaction mix/sample/gene including 3 µL of PCR product, 0.2 µL of restriction enzyme, 1 µL of 10× buffer Tango and 5.8 µL of DDW. A *TaqI* restriction enzyme (ACN*GT) was used to detect two mutation sites of c.N8S and c.E36Q in *AQP7* gene. Therefore, the genetic diversity parameters such as genotypic frequency, allelic frequency, observed heterozygosity (H_o), expected heterozygosity (H_e), polymorphic informative content (PIC), number of effective allele (n_e) and Chi-square (χ^2) values were computed according to Nei and Kumar (2000). The general linear model of SAS software (SAS Institute Inc., USA) was employed to associate the high polymorphic SNPs with four semen quality traits (sperm volume, progressive motility, sperm concentration and daily sperm viability).

In the present study, two mutation sites of c.N8S (exon 2)

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and c.E36Q (exon 3) were observed through PCR-RFLP analysis. Therefore, three genotypes of *AQP7/Taal* gene were identified according to the c.N8S and c.E36Q polymorphisms (Figs 1 and 2). A mutation of c.N8S in



Fig. 1. RFLP result in *AQP7/Taal* gene to detect a mutation site of c.N8S in Madura bulls on 2% of agarose gel showed polymorphic with three genotypes of AA (221 bp), BB (92 bp) and AB (221 bp & 92 bp). M: DNA ladder 50 bp.

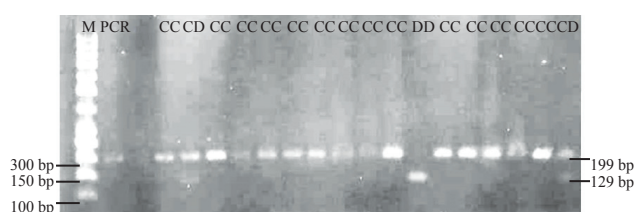


Fig. 2. RFLP result in *AQP7/Taal* gene to detect a mutation site of c.E36Q in Madura bulls on 2% agarose gel showed polymorphic with three genotypes of CC (199 bp), DD (129 bp) and CD (199 bp & 129 bp). M: DNA ladder 50 bp.

AQP7/Taal gene of animal studied exhibited the moderate PIC value (0.30) as shown in Table 1. Meanwhile, a mutation of c.E36Q in *AQP7/Taal* gene revealed the low PIC value (0.07). Low PIC value in mutation of c.E36Q indicated that this mutation can not be used for the molecular selection because of low genetic diversity.

Botstein *et al.* (1980) stated that the PIC value has three categories viz. low (<0.30), moderate (0.30-0.50) and high (>0.50). Ma *et al.* (2011) and Kumar *et al.* (2014) obtained two genotype of CC and CD in *AQP7/Taal* gene (c.E36Q) in bulls with the CC as the common genotype. Therefore, CC genotype (c.E36Q) has the higher sperm volume,

sperm motility and post-thaw motility traits in Frieswal bulls (Kumar *et al.* 2014), frozen sperm viability and frozen sperm motility traits in *Bos taurus* bulls rather than CD genotype. Ma *et al.* (2011) and Kumar *et al.* (2014); reported two genotypes of AA and AB in *AQP7* gene (c.N8S) in bulls with AA as the common genotype. Thus, the AA genotype in *AQP7/Taal* (c.N8S) exhibited higher sperm motility in Frieswal (Kumar *et al.* 2014) and frozen acrosome integrity ratio and frozen motility in *Bos taurus* bulls (Ma *et al.* 2011) than AB genotype. Interestingly, the BB (c.N8S) and DD (c.E36Q) genotypes of *AQP7/Taal* were observed in Madura bulls with very low frequency and could be included as a rare genotype. In this study, 15 selected Madura bulls were used for the association study between c.N8S polymorphism and sperm quality traits. These bulls was selected based on phenotypic traits (SNI 7651.2 2013) and estimated breeding value (EBV) of growth traits. Unfortunately, there were no significant association between mutation of c.N8S with sperm quality traits of Madura bulls as shown in Table 2. However, this results need to confirm with the large number of animal samples and sperm quality data.

SUMMARY

In this study, a PCR-RFLP method was employed to investigate the evidence of two mutation sites in exon 2 (c.N8S) and exon 3 (c.E36Q) regions of *AQP7/Taal* gene in Madura bulls. Both mutation sites were polymorphic with presence of three genotypes. However, a mutation of c.N8S had the moderate PIC value (>0.30). Hence, it can be suggested that the mutation of c.N8S can be used for the genetic marker of sperm quality traits in Madura bulls. However, the association study with 15 selected Madura bulls revealed that there are no significant association between c.N8S and sperm quality traits. However, the mutation of c.N8S in animal studies was not associated with sperm quality traits of sperm volume, progressive motility sperm concentration and daily sperm viability. In future, a further research with large number of animal samples and records data are important to obtain the genetic markers for productivity traits accurately.

Table 1. Genetic diversity in *AQP7/Taal* gene from 83 Madura bulls

Mutation	Genotypic frequency			Allelic frequency		H _o	H _e	n _e	PIC	χ ²
c.N8S	AA (0.64)	BB (0.13)	AB (0.23)	A (0.75)	B (0.25)	0.23	0.37	1.59	0.30	12.28
c.E36Q	CC (0.95)	DD (0.02)	CD (0.02)	C (0.96)	D (0.04)	0.02	0.07	1.07	0.07	35.52

H_o, observed heterozygosity; H_e, expected heterozygosity; n_e, number of effective allele; PIC, polymorphic informative content; χ², Chi-square.

Table 2. Association between *AQP7/Taal* (c.N8S) and sperm quality traits of selected Madura bulls

Sperm quality	Genotype (n)			P value
	AA	AB	BB	
Sperm volume (mL)	6.42±0.68 (11)	6.95±0.78 (2)	6.95±0.78 (2)	0.449
Progressive motility (%)	47.18±19.63 (11)	21.50±12.16 (2)	48.00 (1)	0.253
Sperm concentration (×10 ⁶)	1077.50±899.57 (11)	754.00±724.08 (2)	697.00 (1)	0.839
Daily sperm viability (%)	66.18±15.44 (11)	40.50±14.85 (2)	68.00 (1)	0.134

n, number of observation.

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