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Diacylglycerol Acyltransferase1 (DGAT1) Gene Polymorphism in New Zealand Holstein Friesian Cattle under Dairy Breeding Station and Its Correlation with Milk Quality

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ABSTRACT: Functional single nucleotide polymorphism (SNPs) in major genes can be potential to be used genetic assisted selection (GAS) to improve considered traits. Using GAS into breeding program could give more accurate and efficient results. Diacylglycerol acyltransferase1 (DGAT1) gene is one of potential candidate major genes to improve milk components. The occurrence of dinucleotide substitution of AA to GC at exon 8 in the DGAT1 gene causes the change of lysine to alanine in amino acid (K232A). The aims of the study were to study *Diacylglycerol Acyltransferase1 K232A (DGAT1 K232A)* gene polymorphism and its association with milk quality components (fat, protein, SNF, lactose, DM) in Holstein Friesian (HF) dairy cattle imported from New Zealand. A total of 72 cows were investigated from Baturraden Excellent Dairy Cattle Breeding Station (Baturraden EDCBS). Genotyping of the DGAT1 gene by PCR-RFLP technique using *EaeI* restriction enzyme resulted in a fragment length by 411 bp. Genotype frequencies of KK, KA and AA were respectively 0.24, 0.76 and 0.00; while allele frequencies of K and A were 0.61 and 0.39. The result showed that the DGAT1 was polymorphic but its genotypes (KK and KA) were not significantly associated with individual milk components. Effect of DGAT1 polymorphism on milk quality should further be confirmed in a wider investigation.

Keywords: DGAT1 gene, Holstein Friesian, PCR-RFLP, milk quality.

INTRODUCTION

DGAT1 (diacylglycerol acyltransferase) gene is located in the centromic region of chromosome 14 (BTA14) consist of seventeen exons and sixteen introns. Recently, a quantitative trait locus (QTL) mapping study in dairy cattle resulted the identification of DGAT1 gene which is a enzyme in triglyceride synthesis and has strong effect in milk fat percentage and other milk production characteristic (Grisart *et al*. 2002). Studies led to discovery of a non-conservative dinucleotide substitution in exon 8 at positions of 10433 and 10434. The substitution is a change from AA to GC which at the protein level, result in a Lysine (Lys, K) to Alanine (Ala, A) substitution at amino acid number 232, therefore this polymorphism is commonly K232A (Grisart et al. 2002 a). Since its identification, the K2323A substitution has been associated with milk fatty acids and milk yield and composition in various dairy cattle breeds, such as German Holstein Friesian, Dutch Holstein Friesian, New Zealand Holstein Friesian, Brasilian Holstein and Polish Holstein Friesian (Grisart *et al.* 2002); Spelman *et al.* 2002 a; Thaller *et al*. 2003; Gautier *et al.* 2007; Schennink *et al.* 2007). The lysine encoding allele (K) has been shown to increase milk fat synthesis and to some extent and protein content (Grisart *et al.* 2002 a; Winter *et al.* 2002; Thaller *et al.* 2003; Schennink *et al.* 2007). The lysine allele (K) has also been reported to cause a decrease in protein and milk yield. Thus, both functional and positional data made DGAT1 a promising candidate gene for milk fat percentage and milk production in cattle. The effect of the DGAT1 mutations in fatty acids profile had been reported (Asmarasari, 2014), so in this paper, the authors will report the results of the research on the correlation of DGAT1 gene in milk quality traits of dairy cattle in dairy breeding station. The use of the DGAT1 gene as a marker gene needs to be verified mainly on HF breeding dairy cattle under environment in indonesia.

The objective of this research was to examine genetic polymorphism that have been identified and associated with milk production and milk quality traits of the DGAT1 gene of New Zealand Holstein Friesian dairy cattle in Central Java, Indonesia.

MATERIALS AND METHODS

Genotypes

The study was conducted in excellent national dairy cattle breeding stations (Baturraden EDCBS) in Central Java from March-April 2012. Blood samples for DNA isolation were collected from 72 cows New Zealand Friesian Holstein. Extraction procedure followed the phenol-chloroform method that was modified by Andreas *et al.* (2010). Genotyping of the DAT1 polymorphism was performed using PCR-RFLP.(Applied Byosistem 9700). The primers were designed based on the DGAT1 sequence (AY065621): forward 5'-GCACCATCCTCTTCCTCAAG-3'and reverse 5'-GGAAGCGCTTTCGGATG-3' (Cardoso *et al.* 2011; Winter *et al.* 2002). PCR cycling conditions were 95ºC for 5 min, followed by 40 cycles of 95ºC for 30 s , annealing at 60ºC for 1 min and extension at 72ºC for 1 min and the final extension at 72ºC for 5 min. The amplified product or amplicon had the length of 411 bp. Restriction enzyme used was *Eae*I which recognized restriction sites *GGCA.

Phenotypes

Data of milk yields and milk quality in their first lactation were collected from 72 HF cows to which blood samples were collected. A 0.5 liter milk was sampled from each cow twice a day, in the morning and the evening milking time from March - April 2012. Milk quality (protein percentage, fat percentage and lactose) were analyzed using MilkoScan Minor (Australia).

The Analysis Data

DGAT1|*Eae*I locus were allele frequency, genotype frequency, degrees of heterozygosity observation (H_0) and heterozygosity expectation (H_e) were tested using PopGene32 software ver. 1.31(Yeh and Boyle 1997). The associations were analyzed by the General Linear Model (GLM) using SAS software ver 9.1 (2002).

RESULT AND DISCUSSION

Result of polymorphism of DGAT1 gene were not significantly effect on every trait measured (Table 1).

Table 1. Effects of DGAT1 polymorphism on milk production and milk quality traits

Trait	Genotype		Probability
	KK (n=20)	KA (52)	
Daily milk yield (liter)	11.32±6.50[a]	12.33±4.80[a]	0.984[ns]
Protein (%)	3.12±0.28[a]	3.06±0.14[a]	0.797[ns]
Fat (%)	3.32±0.44[a]	3.20±0.43[a]	0.517[ns]
Lactose (%)	4.63±0.46[a]	4.50±0.15[a]	0.616[ns]

ns = non significant s = significant

Daily milk production of New Zealand FH cow that maintained in under Indonesian environment much different from FH cows reared in New Zealand, native country as well as when compared to other breed (Table 2). Milk production is one of quantitative traits that are controlled by polygenes and also influenced by environment and generally controlled by external factors (external) and internal factors. External factors are factors that come from outside the body of livestock such as climate, the amount and quality of feed, diseases and parasites (Indrijani, 2001),

poor management practices and may be improved simply through better nutrition (Smaragdov 2006) . Whilst internal factors are genetic factors, the period of lactation, milking frequency, age and body size of livestock, dry period, the estrous cycle and pregnancy, ketosys and milk fever (Sudono *et al.*, 2005).

Table 2. Mean values and standard deviation of the analysed traits.

Breed	Daily mild yield (kg)	Fat (%)	Protein (%)
Friesian	30.01 ± 2.81	3.63 ± 0.53	3.26 ± 0.15
Jersey	23.03 ± 4.50	4.52 ± 0.73	3.26 ± 0.15
Piedmontese	10.86 ± 2.58	3.81± 0.72	3.64 ± 0.41
Valdostana	12.04 ± 5.00	3.57 ± 0.31	3.28 ± 0.34

Testing the effect of DGAT1 gene variant genotypes on milk production was done on Baturraden Excellent Dairy Cattle Breeding Station (Baturraden EDCBS), where the station is to implement an intensive maintenance management. Objective observations made at this station are to minimize the influence of the environment and maintenance management to milk production. Genetic variation occuring between the K allele and the A allele was due to a mutation at exon 8 by the existing dinucleotide substitution AA→GC that was identified for causing K232A (lysine K to Alanine A.

The association of variant genotype DGAT1 gene to the average protein content of milk was not statistically significantly different. Cows with KK genotype produce milk protein content of 3.12% and cows with KA genotype produce milk protein content of 3.06%. Milk protein is formed by three main sources, namely peptides derived from blood, plasma proteins and free amino acids. Milk protein content is relatively fixed during lactation, because these proteins are synthesized in the udder glandular epithelial cells is controlled by the genes.

Estimated Effects of The DGAT1 K232A Polymorphism on fat percentage, protein percentage and yield traits in this study, however contrasted with previous studies (Spelman *et al.,* 2002; Grisart *et al.,* 2002; Thaller *et al.,* 2003;Strzalkowska *et al*., 2005).

Table 3. The K232A polymorphism in the DGAT1 gene of various cattle breeds and its association with milk production traits (N: number of individuals, FY:Fat yield, MY: Milk yield, PY:Protein yield, F%:Fat percentage, P%:Protein percentage).

Population (N)	Allele Frequency	Association	Reference
New Zealand Holstein-Friesian bulls (1527)	K:0.60 A:0.40	K: Increases FY, decreases MY and PY	Spelman *et al.,* 2002
Fleckviech bulls (833)	K:0.07 A:0.93	K: Increases FY, F% and P%; decreases MY and PY	Thaller *et al.,* 2003
German Holstein bulls (858)	K:0.55 A:0.45	K: Increases FY, F% and P%; decreases MY and PY	Thaller *et al.*, 2003
Montbeliarde bulls	K:0.04 A:0.96	K: Increases FY, F% and P%; decreases MY and PY	Gautier *et al.*, 2007

Dutch Holstein Friesian (1762)	K:0.40 A:0.60	K: Increases FY, F% and P%; decreases MY and PY	Schennink *et al.*, 2007
Polish Holstein Friesian (177)	K: 0.40 A:0.60	-	Strzalkowska *et al*. ,2005
This study (New Zealand under dairy breeding station (72)	K:0.61 A:0.39	K:insignificant MY, P%, F%	Asmarasari *et al*., 2014

CONCLUSION

Research on DGAT1 gene polymorphism does not affect the production and milk quality traits. Verification could do more with increasing the number of samples and genotype association studies to provide information early in the selection program.

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