J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
48(1):10-19, March 2023
DOI: 10.14710/jitaa.48.1. 10-19

Polymorphism of SNP g.8398A>G at prolactin gene and its effect on Indonesian Holstein dairy cow's milk performance and reproductive traits

Y.W. Setyorini[1,2], S. Sutopo[1*], E. Kurnianto[1], S. Sutiyono[1]
[1]*Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, 50275, Indonesia*
[2]*Balai Besar Pembibitan Ternak Unggul dan Hijauan Pakan Ternak Baturraden, Ministry of Agriculture of Indonesia, Banyumas, 53151, Indonesia*
**Corresponding e-mail: drsutopo36@gmail.com*

Received December 27, 2022; Accepted February 14, 2023

ABSTRACT

The objective of this study was to identify the polymorphism of Single Nucleotide Polymorphism (SNP) g.8398A>G at the prolactin gene exon 4 in Indonesian Holstein dairy cows and its effect on milk production, milk quality, and reproductive traits. A total of 140 blood samples were investigated to detect polymorphism by PCR-RFLP method using the RsaI restriction enzyme. Sequencing was performed for confirmation of SNP mutation points. The phenotype data collected were milk production, milk quality, and reproductive traits. Data were analyzed using a t-test. The results showed that polymorphism of SNP g.8398A>G at prolactin gene exon 4 was found in the study populations with AG and GG genotypes. Cows with the GG genotype indicated higher total milk yield and milk protein ($P<0.05$) than the AG genotype. Polymorphism was not associated with specific gravity, milk fat, milk lactose, total solid non-fat, and reproductive traits. SNP g.8398A>G at prolactin gene exon 4 showed potential as a genetic marker for selecting superior milk production traits.

Keywords: Milk production, PCR-RFLP, Prolactin gene, Single nucleotide

INTRODUCTION

The main goal of the selection program in dairy farming is to get better productivity by improving genetic quality. The efficiency and profitability of milk products can be more optimal by increasing both the amount and quality of milk produced, as it relates to the selling price of milk (Rahayu *et al.,* 2019). Reproduction is also an important factor affecting milk yield in dairy cows (Anggraeni *et al.,* 2015). Thus the selection effort is needed to make not only to obtain dairy cows with superior milk production but also those with good fertility. One of the applications of molecular genetic technologies for enhancing the genetic quality of cattle is a selection approach based on phenotypic data information paired with genetic markers that are related to the desired and inheritable economic and productivity properties (Archana, 2013; Damayanti *et al.*, 2022).

The prolactin (PRL) gene is a candidate

gene that affects milk traits (Brym *et al*., 2005; Agrawal *et al.*, 2020; Shah *et al.,* 2021). Prolactin is a hormone with a polypeptide structure which is about 10 kb in size and composed of 5 exons and 4 introns, it has been identified to be located on chromosome 23 near the quantitative trait loci in the bovine genome (Hallerman, 1988; Ozdemir, 2020). The bovine prolactin gene has five exons (GenBank Accession No: AF426315.1), namely exon 1 is 855-936 nt, exon 2 is 3661-3842 nt, exon 3 is 6186-6293 nt, exon 4 is 8321-8500 nt, and exon 5 is 9129-9388 nt and four introns which encoding a mature protein with 199 amino acids (Cao *et al.,* 2002; Ozdemir, 2020). The lactotrophic cells in the anterior pituitary gland diversity are primarily responsible for the synthesis and secretion of the hormone prolactin. Prolactin's primary function in animals is to control lactation (Bernard *et al*., 2019), development of the mammary glands and initiation of milk production (Dong *et al*., 2013), and also plays an important role in reproductive health in both males and females (Al-Fahham and Al-Nowainy, 2016).

Polymorphism in several regions of the prolactin gene has been reported, i.e. on exon 3 in Turkish native cattle breeds (Unal *et al*., 2015), Frieswal cow (Bukhari *et al*., 2013), *Bos indicus* in India (Agrawal *et al.*, 2020), American Swiss cattle in Mexico (Alfonso *et al.,* 2012), Gir and Kankrej cattle (Patel and Chauhan, 2017), on exon 4 at Holstein Friesian heifer in Turkey (Yasemin *et al*., 2017), on Exon 3 and 4 at Holstein cow in Turkey (Ozdemir, 2020), on partial intron 3 and 4 at Chinese Holstein cows (Dong *et al*., 2013), however, information on the identification of polymorphism of the exon 4 prolactin gene in Indonesian FH dairy cows is still rare.

Genetic polymorphism studies revealed a significant association between the genotype of the prolactin gene with milk traits in cattle (Brym *et al.,* 2005; Patel and Chauhan, 2017; Agrawal *et al.,* 2020; Hani *et al.,* 2021; Shah *et al.,* 2021), reproductive in cattle (Gayari *et al.,* 2020; Pytlewski *et al*., 2020). The prolactin gene has been observed for the milk trait in buffaloes (El-Magd *et al*., 2015; Nadeem and Maryam, 2016), milk trait in sheep (Gras *et al*., 2016; Ozmen and Kul, 2016; Padilla *et al.,* 2018), a reproductive trait in Awassi ewes (Al-Thuwaini, 2021), also reproductive trait in Alabio and Mojosari ducks (Damayanti *et al.,* 2022). Previous studies have examined the prolactin gene in the Indonesian Holstein cattle population in West Java and Central Java, but are limited to genotype variation (Agung *et al*., 2021) and the associations with the amount of milk yield (Hifni *et al.*, 2021). Thereby the diversity of prolactin genes and how they affect milk production, milk quality, and reproductive trait in Indonesian Holstein dairy cow populations need to be identified. Considering the important role of the PRL gene, this study intends to investigate the genotype diversity of the exon 4 of the prolactin gene in Indonesian Holstein dairy cows and its effect on milk and reproductive traits. The results of the study can be used as basic information on prolactin genes as genetic markers in the selection to obtain dairy cows with superior milk production and reproductive traits.

MATERIALS AND METHODS

Sample Collection and The Phenotypic Data

Blood samples from 140 Indonesian Holstein dairy cows were collected in this study, consisting of 100 samples from the breeding center of BBPTUHPT Baturraden and 40 samples from smallholder dairy farms. The criteria for cows used were those that have a record of reproduction and milk production of the first lactation. The procedures performed in this study relating to animals have been approved by the Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Number 58-06/A-7/KEP-FPP.

Five milliliters of blood were taken through the coccygeal vein using an 18 G vacutainer® BD flashback blood collection needle (BD vacutainer systems, UK) and inserted in a collection tube containing EDTA (BD vacutainer systems, UK). The collected blood samples were transported in a cooling box and stored in a refrigerator at 4°C until an analysis was performed. The phenotypic

data was taken from the records of BBPTUHPT Baturraden. The milk trait was in the form of the corrected milk yield in 305 days of mature equivalent at first lactation, Most Probable Producing Ability (MPPA) absolute, and milk quality. A total of 48 cows in the lactation period were sampled to test milk quality, including specific gravity, milk protein, milk fat, milk lactose, and total solid non-fat using a milk analyzer (Lactoscan MCC, UK). Reproductive trait records comprised age at first mating, service per conception in heifer, gestation period at first pregnancy, service per conception in parity I, gestation period at second pregnancy, and calving interval.

DNA Extraction, PCR Amplification, and Genotyping by RFLP Method

DNA analysis was performed using a kit extraction of Genomic DNA Mini (Geneaid, Taiwan) following the instructions in the standard-manufacturing protocol in the Division of Biology, Integrated Laboratory of Sebelas Maret University. The extraction result was coded and stored at -20ºC for use in the PCR reaction process. The quality of DNA was checked on 0.8% agarose gel (Thermo Scientific, Lithuania) stained with ethidium bromide (Promega, USA) and Tris Acetate EDTA (TAE) buffer (1stBase, Singapore) using submarine electrophoresis system Mupid-Exu (Advance, Japan).

The genotypes were analyzed by the Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) methods. PCR amplification of the PRL gene exon 4 was done using a pair of forward primers (5'-CCAAATCCACTGAATTATGCTT-3') and reverse primers (5'-ACAGAAATCACCTCTCTCATTCA-3'). The primers referred to the published nucleotide sequence of the Bos taurus PRL gene with GenBank Accession Number AF.426315.1 (Brym *et al*., 2005). A PCR reaction of 25 µL volume was obtained by mixing 9.5 µL of nuclease-free water (Promega, USA), 1 µL of forward primer and 1 µL of reverse primer, 12.5µL of 2xMyTaq HS Red Mix (Bioline, London) and 1 µL of DNA. Amplification was carried out using a thermal cycler machine (Bio-Rad, USA) with the following stages: predenaturation (at 95°C for 5 minutes); 35 cycles of denaturation (at 95°C for 30 seconds), annealing (at 54°C for 30 seconds), extension (at 72°C for 30 seconds), and final extension (at 72°C for 10 minutes). PCR products were performed electrophoresis at 2% agarose gel at 100 V for 35 min. Loading dye (Thermo Scientific) and 100 bp marker ladder (Geneaid, Taiwan) were used as DNA band size standards. Agarose gel was viewed using a gel documentation system (Glite UV, Pacific Image Electronics, Taiwan).

Genotyping of prolactin genes was carried out by digestion of PCR products using the RsaI restriction enzyme (Thermo Fisher Scientific). Each reaction was carried out by inserting 10 µL PCR product of the prolactin gene into the 0.2 mL tube, 1µL RsaI enzyme, 2 µL tango buffer and 18 µL nuclease-free water. The mixture was then incubated at a temperature of 37ºC for 2 hours. After incubation, 10uL of the mixture was then visualized with 2 % agarose gel. The results fragments were observed and compared with the size of the DNA marker. Sequencing was performed on samples representing each genotype to confirm the location of the mutation point.

Data Analysis

Analysis of genotypic frequency and allele distribution of prolactin genes was carried out following previous research methods (Nei and Kumar, 2000) with the formula:

Allele frequencies: $Xi = \frac{2n_{ii}+\sum n_{ij}}{2n}$

Description: Xi: frequency allele i; n_{ii}: number of genotype ii, n_{ij}: number of genotype ij, n: total samples.

Genotype frequencies: $Xii = \frac{n_{ii}}{n}$

Description: Xii:genotype frequencies ii; n_{ii}: number of genotype ii; n: total samples.

Hardy Weinberg Equilibrium was tested by the chi-square (Warwick *et al*., 1990):

$$X^2 = \sum (O - E)^2 / E$$

Description: X^2: Hardy Weinberg Equilibrium result; O: observed number of genotype ii or ij; E: expected number of genotype ii or ij

Heterozygosity was calculated as follows:

$$Ho = \sum \frac{Nij}{N}$$

Description: Ho: Observed heterozygosity; N_{ij}: the number of heterozygote individual; N: the total number of individuals observed

$$He = 1 - \sum_{i=1}^{q} Xi^2$$

Description: He : Expected heterozygosity; Xi : Allele frequencies; q : The total number of allele.

The mean and standard deviation of milk and reproductive traits were calculated for each genotype. There were two genotypes obtained in this study, so the effect of genotype on milk and reproductive traits of dairy cows was analyzed using an independent sample t-test in the Statistic Analysis Program on Demand for Academic (SAS, 2021) program. Basic Local Alignment Search Tool (BLAST) and MEGA X 11 version were used to determine the SNP mutation point on the sequencing results.

RESULTS AND DISCUSSION

Identification of SNP g.8398A>G Prolactin Gene Exon 4

A single band with a length of 294 bp was the result of the amplification of prolactin gene fragments using the PCR method in this study (Figure 1), with the position of the attachment and restriction point by the enzyme RsaI in the base 8398th located in exon 4 in the nucleotide sequence 8266 to 8559 (Figure 2) based on GenBank accession number No AF.426315.1. Identification using the RFLP approach on exon 4 recommended by Ozdemir (2020) was used in this study to detect genetic variation and its role as a marker in the milk trait.

Investigation of genetic variation by the RFLP PCR method using the RsaI (*Rhodopseudomonas sphaeroides*) restriction enzyme (Figure 3) obtained the genotypes of GG and AG. Determination of the genotype in the PRL gene based on the resulting fragments. Allele G did not did not displayed a restriction site with one fragment with a length of 294. Allele A has a RsaI enzyme restriction site (GT-AC) indicated by two fragments that are 162 bp and 132 bp, respectively. In allele A, there is a mutation of G-A in the nucleotide sequence 8398 that can be truncated by the restriction enzyme RsaI. The AA genotype was not found in the study population. Polymorphism is indicated by the RFLP results and reinforced by a chromatogram of the sequencing result at the restriction site (Figure 4). Mutation in this SNP is silent mutations where base substitution occurs that do not cause changes in amino acids (synonymous SNP). This result is in accordance with Brym *et al.* (2005) that the transition of the A-G SNP at position 8398 R in exon 4 of the prolactin gene revealed using SSCP and direct sequencing methods.

The alignment of nucleotide sequencing results with GenBank Accession Number AF.426315.1 showed the presence of SNP g.8398A>G and indicated the presence of other

Table 1. Genotype and Allele Frequencies on g.8398A>G at Prolactin Gene Exon 4 in Indonesian Holstein Dairy Cows

Population	N	Genotypes Frequencies		Alleles Frequencies		Ho	He	X^2
		GG	AG	G	A			
Population 1	100	0.740	0.260	0.870	0.130	0.2600	0.2262	2.23
Population 2	40	0.925	0.075	0.963	0.038	0.0750	0.0722	0.06
All samples	140	0.793	0.207	0.896	0.104	0.2071	0.1857	1.87

N = The number of samples; Population 1 = samples from the breeding center; Population 2 = samples from dairy smallholder farms; Ho = observed heterozygosity; He = expected heterozygosity; X^2 = chi-square test; X^2 standard $table_{0.05;1}$=3.841

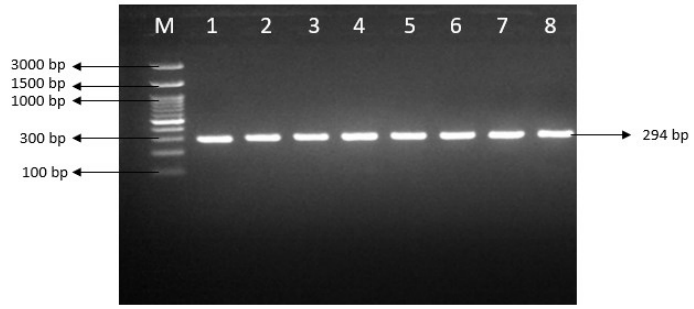

Figure 1. Amplification of prolactin gene exon 4 fragments in 0.8% agarose gel. M: Marker 100 bp; 1-8: Indonesian Holstein cow samples

Table 2. Total Milk Yield and Milk Quality with Different Genotypes on g.8398A>G at Prolactin Gene Exon 4 in Indonesian Holstein Dairy Cows

Parameters	Genotype			
	GG		AG	
	N	Mean±SD	N	Mean±SD
Total Milk yield 305 ME (kg/head/lactation)	74	5281.22±955.53[a]	26	4453.23±1046.60[b]
MPPA absolute (kg/head/lactation)	74	5173.58±477.77[a]	26	4759.59±523.30[b]
Specific gravity	38	1.0285±0.00040	10	1.0285±0.00029
Fat (%)	38	4.10±0.22	10	4.19±0.18
Protein (%)	38	3.07±0.10 [a]	10	3.00±0.84 [b]
Lactose (%)	38	4.32±0.14	10	4.32±0.21
Solid non-fat (%)	38	8.19±0.17	10	8.26±0.10

N = The number of samples; SD = standard deviations; Different superscripts in the same row show significant differences (P<0.05).

Table 3. Reproductive Traits with Different Genotypes on g.8398A>G at Prolactin Gene Exon 4 in Indonesian Holstein Dairy Cows

Parameters	Genotype			
	GG		AG	
	N	Mean±SD	N	Mean±SD
First mating (months)	74	17.67±2.90	26	18.82±3.33
Service per conception in heifer (times)	74	1.12±0.329	26	1.12±0.326
Gestation period at first pregnancy (days)	74	276.27±7.05	26	276.50±6.15
Service per conception in parity I (times)	74	1.49±0.546	26	1.46±0.582
Gestation period at second pregnancy (days)	74	280.08±8.51	26	283.19±13.13
Calving interval (days)	74	415.24±61.20	26	404.85±61.11

N = The number of samples; SD = standard deviations; Different superscripts in the same row show significant differences (P<0.05).

mutations in the order of the 8377th and 8362nd bases that were not truncated by the RsaI restriction enzyme (Figure 5).

Genotype and Allele Frequencies of SNP g.8398A>G

This study showed that the locus of prolactin gene exon 4 was polymorphic in both populations observed. Genotype variations (Table 1) showed that the G allele frequency in both populations was 0.896 and found to be more dominant than the A allele. The GG and AG genotype frequencies were obtained by 0.793 and 0.207, respectively. The homozygote GG genotype is present in the highest frequency in both populations, where the frequency in population II (0.925) is higher than the population I (0.740).

The genotype frequencies obtained in this study conformed with the FH cow genotype in Turkey which obtained 2 genotypes with a dominant GG genotype of 77.99% (Yasemin, 2017), but different in Jersey cattle in India with a predominantly heterozygote genotype frequency of 0.60 (Shah *et al.,* 2021). However, other studies showed that 3 genotypes with a predominant GG genotype (0.87) in the Indonesian Holstein Cattle population in West Java (Agung *et al.,* 2021). Genotypic differences with this study were likely influenced by the selection system, mating methods, and sires used in the populations. Polymorphism with 3 genotypes was also reported with the predominant GG genotype (0.56) in Holstein cows in Turkey (Ozdemir, 2020), whereas studies by Shah *et al.* (2021) on cross-breed FH cattle in India revealed that the dominant genotype was heterozygous (0.50). Variations of heterozygosity, allele, and genotype frequencies within cattle populations may be caused by differences in breed, sample size, location, or a variety of selections in the population (Bangar *et al.,* 2021).

The genotype variations of the PRL gene in other regions have also been revealed by earlier

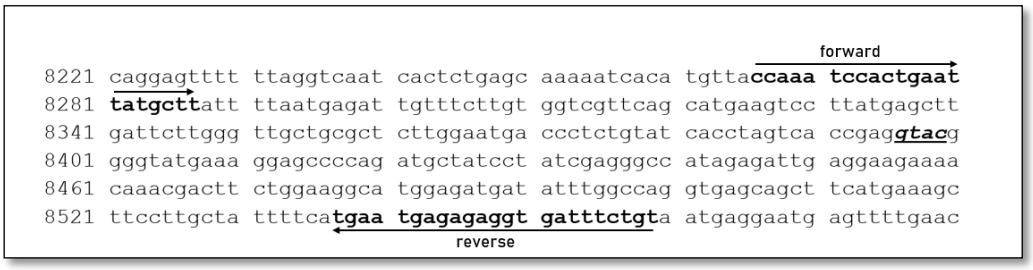

Figure 2. A specific DNA fragment sequence GenBank Acc. No. AF.426315.1 with a length of 294 bp at prolactin gene exon 4 and restriction site by RsaI enzyme

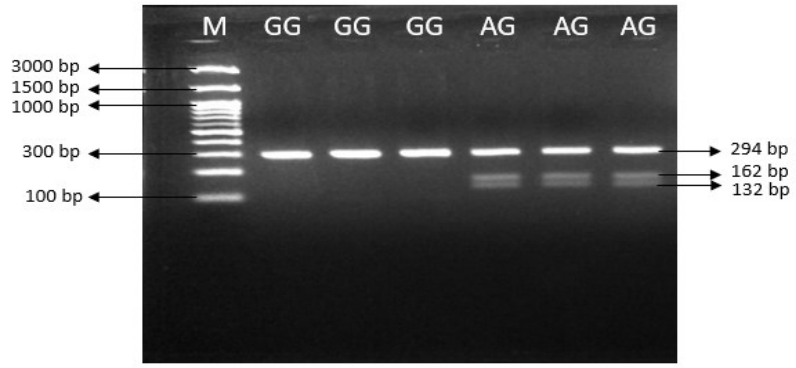

Figure 3. PCR-RFLP electrophoresis results of prolactin gene exon 4 after digesting by enzyme restrictions RsaI. M: 100 bp DNA ladder; GG and AG: genotype of prolactin gene exon 4.

research. The genotype frequencies at the PRL gene have been found by Pytlewski *et al*. (2020) in Polish Black and White Holstein Friesian cattle, i.e. GG, or in some other studies called AA, which was not digested by the RsaI enzyme (0.60), AG (0.37) and AA (0.03). While Frieswal cattle (Bukhari *et al*., 2013) and *Bos indicus* in India (Agrawal *et al.,* 2020) found dominant heterozygous genotype AG (or AB in another study) of 0.629 and 0.72, respectively. The frequency of GG, GA, and AA genotypes in the intron 3 and intron 4 regions were 0.76, 0.23, and 0.12; respectively, in Chinese Holstein cow (Dong *et al.,* 2013).

The observed heterozygosity (Ho) was 0.2071 higher than the expected heterozygosity (He), which was 0.1857. Heterozygosity of the study results showed low gene variation in both populations (Table 1). Estimation of heterozygosity values is necessary to determine genetic variability in populations. Low gene variety in a population is indicated by heterozygosity of less than 0.5 (Javanmard *et al.,* 2005). The low diversity of the prolactin gene in the study population is likely due to the already selected population and the limited number of males used (Agung *et al*., 2021). From the results of chi-square testing (Table 1), it is known that Hardy Weinberg Equilibrium (HWE) occurred in both populations (P>0.05) with chi-square value for population I (X^2=2.23) and population II (X^2=0.06) lower than X^2 of the standard table (3.841).

The Effect of SNP g.8398A>G at Prolactin Gene Exon 4 on Milk Performance and Reproductive Trait of Indonesian Holstein Dairy Cows

The result showed that cows with the GG genotype had a higher average milk production and were significantly different (P<0.05) from the AG genotype (Table 2). This is consistent with the previous study on crossbreed cattle in India (Shah *et al*., 2021), Gir and Kanrej cattle in India (Patel and Chauhan, 2017), and Chinese Holstein cows (Dong *et al.,* 2013). However, this result was disagreement with the study by Brym *et al*. (2005) in black and white cows and Jersey cows. Other researchers on Holstein cows found no association between the prolactin genotype

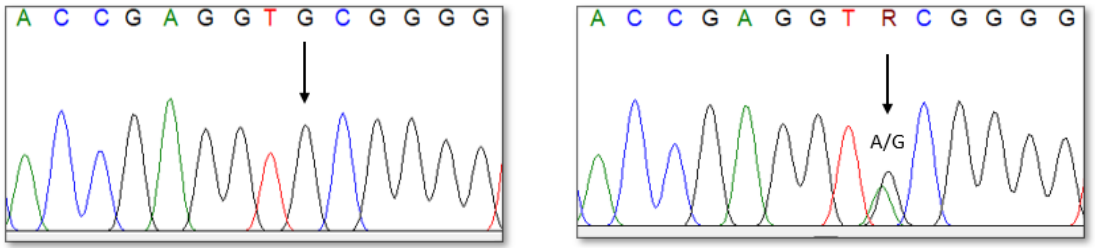

Figure 4. Chromatogram of sequencing results of prolactin gene exon 4. The arrows indicate the site of base mutation.

```
Query  1     TTGATTCTTGGGTTGCTGCGCTCCTGGAATGACCCTCTCTATCACCTAGTCACCGAGGTG  60
             ||||||||||||||||||||||| ||||||||||||| ||||||||||||||||||||
Sbjct  8339  TTGATTCTTGGGTTGCTGCGCTCTTGGAATGACCCTCTGTATCACCTAGTCACCGAGGTA  8398
Query  61    CGGGGTATGAAAGGAGCCCCAGATGCTATCCTATCGAGGGCCATAGAGATTGAGGAAGAA  120
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  8399  CGGGGTATGAAAGGAGCCCCAGATGCTATCCTATCGAGGGCCATAGAGATTGAGGAAGAA  8458
```

Figure 5. Alignment of sequence prolactin gene GenBank Accession Number AF.426315.1 with sample sequencing result.

and total milk production (Hifni *et al.,* 2021).

The results of statistical analysis showed that the GG genotype is significantly associated ($P<0.005$) with higher protein levels compared to the AG genotype. However, there was no association between the GG and AG genotypes on specific gravity parameters, fat levels, lactose levels, and solid non-fat (SNF) levels. Prolactin is a key regulator of milk production, mammary gland development, and milk protein gene expression (Shah *et al*., 2021). An association between genotype variations in the Prolactin gene and milk composting in *Bos indicus* has been discovered by Agrawal *et al.* (2020). The finding of this study was similar to the previous study in crossbreed cattle in India, which found the RR genotype is significantly associated with protein and fat; unfortunately, the genotype in Jersey cows was not associated with milk quality (Shah *et al.,* 2021). While in the Chinese Holstein cows genotype GG is lower in protein level than AG (Dong *et al.,* 2013).

Polymorphism of SNP g.8398A>G at prolactin gene exon 4 showed no significant differences in reproductive properties of first mating, service per conception in heifer, gestation period at first pregnancy, service per conception in parity I, gestation period at second pregnancy and calving interval (Table 3). The result is similar to studies reported by Yasemin *et al.* (2017), but in contrast to research in Iraqi Awassi ewes that found an association between the genotype of the prolactin gene with progesterone concentration, litter size, fecundity and prolificacy (Al-Thuwaini, 2021).

The GG genotypes of SNP g.8398A>G at prolactin gene exon 4 may be favourable among Indonesian Holstein dairy cows for better milk production and protein level percentage. This SNP could be a potential genetic marker, and selection with an increased frequency of GG genotypes is likely to escalate the milk production and protein levels.

CONCLUSION

The polymorphism of SNP g.8398A>G at prolactin gene exon 4 was found in Indonesian Holstein dairy cows populations with AG and GG genotypes, with the G allele frequencies showing higher than the A allele. The genotype of SNP in this study was associated with total milk yield and milk protein, however, the SNP was not correlated with the reproductive trait. SNP g.8398A>G at prolactin gene exon 4 could be the potential as a genetic marker for the selection of higher milk protein levels and superior milk production traits.

ACKNOWLEDGMENTS

This study was supported by the Ministry of Agriculture of the Republic of Indonesia. The author would like to thank the head of BBP-TUHPT Baturraden, Central Java and the dairy farmers in Yogyakarta for the permission to take samples.

REFERENCES

Agrawal, V. K., G. Gahlot, R. Joshi, G. S. Dhakad and S. Thakur. 2020. Assessment of genetic variability in prolactin gene and its impact on milk composition in Bos indicus cattle. Int. J. Curr. Microbiol. App. Sci. 9(6): 1514-1524.

Agung, P. P., A. S. Wulandari, W. P. B. Putra, T. R. Destianty, F. K. Muzaki, S. Hillary and E. M. Kaiin. 2021. Variation of prolactin and β-Lactoglobulin genes in the Indonesian FH Cattle. IOP Conf. Ser.: Earth Environ. Sci. 888 (1): 012023.

Al-Fahham, A. A. and H. Q. Al-Nowainy. 2016. The role of FSH, LH, and prolactin hormones in female infertility. Int. J. Pharmtech. Res. 6: 110-118.

Alfonso, E., R. Rojas, J. G. Herrera, M. E. Ortega, C. Lemus, C. Cortez, J. Ruiz, R. Pinto and H. Gómez. 2012. Polymorphism of the prolactin gene (PRL) and its relationship with milk production in American Swiss cattle. Afr. J. Biotechnol. 11(29): 7338-7343.

Al-Thuwaini, T. M. 2021. Novel single nucleo-

tide polymorphism in the prolactin gene of Awassi ewes and its role in the reproductive traits. Iraqi J. Vet. Sci. 35(3): 429-435.

Anggraeni, A. S., E. Kurnianto, S. Johari, S. Sutopo and Z. Shujun. 2015. Milk production and reproductive trait caused by LOC514211 gene mutation in dairy cows. J. Indonesian Trop. Anim. Agric. 40(4): 191-198.

Archana, V. 2013. Nucleotide sequence variability in exon 4 of prolactin locus in Murrah buffaloes (*Bubalus bubalis*). Res. J. Biotechnol. 8(6): 61-65.

Bangar, Y. C., A. Magotra, C. S. Patil and N. Jindal. 2021. Meta-analysis of genetic structure and association of prolactin gene with performance traits in dairy cattle in India. Biochem. Genet. 59: 668-677.

Bernard, V., J. Young and N. Binart. 2019. Prolactin A pleiotropic factor in health and disease. Nat. Rev. Endocrinol. 15(6): 356-365.

Brym, P., S. Kamiński and E. Wójcik. 2005. Nucleotide sequence polymorphism within exon 4 of the bovine prolactin gene and its associations with milk performance traits. J. Appl. Genet. 46(2): 179-185.

Bukhari, S., N. N. Khan, P. Gupta, A. K. Das, G. A. Raher, D. Chakraborty and A. Pandey. 2013. Prolactin gene polymorphism and its associations with milk production traits in Frieswal cow. Intl. J. of Molecular Zoology. 3(3): 10-13.

Cao, X., Q. Wang, J. B. Yan, F. K. Yang, S. Z. Huang and Y. T. Zeng. 2002. Molecular cloning and analysis of bovine prolactin full -long genomic as well as cDNA sequences. Acta Genet Sin. 29(9): 768-773.

Damayanti, I., S. Sudaryati and D. Maharani. 2022. The association of prolactin gene polymorphism with egg production traits in Alabio and Mojosari ducks. J. Indonesian Trop. Anim. Agric. 47(3): 184-191.

Dong, C. H., X. M. Song, L. Zhang, J. F. Jiang, J. P. Zhou and Y. Q. Jiang. 2013. New insights into the prolactin-RsaI (PRL-RsaI) locus in Chinese Holstein cows and its effect on milk performance traits. Genet. Mol. Res. 12(4): 5766-5773.

El-Magd, M. A., A. G. Zaki, K. A. Kahilo, M. E. S. Barakat and I. F. Hassan. 2015. Effect of SNPs in prolactin promoter on milk traits in Egyptian Buffalo. J. Adv. Dairy Res. 3(1):1-4.

Gayari, I., A. M. Ferdoci, A. Aziz, G. U. Zaman, F. Akhtar and R. Deka. 2020. Prolactin gene polymorphism in crossbred cattle of Assam and its association with productive and reproductive traits. Int. J. Chem. Stud. 8(2): 717-722.

Gras, M. A., G. C. Pistol, R. S. Pelmus, C. Lazar, H. Grosu and E. Ghita. 2016. Relationship between gene polymorphism and milk production traits in Teleorman Black Head sheep breed. Revista MVZ Córdoba. 21(1): 5124-5136.

Hallerman, E. M., J. L. Theilmann, J. S. Beckmann, M. Soller and J. E. Womack. 1988. Mapping of bovine prolactin and rhodopsin genes in hybrid somatic cells. J. Animal Genetics. 19(2): 123-131.

Hani, H. A., W. G. M. Al Bazi and H. A. Muhammed. 2021. Association of prolactin gene Polymorphysm with some biochemical and lactation traits in dairy cow in Karbala Province. Turk. J. Physiother. Rehabil. 32 (3): 1-7.

Hifni, B., G. Pambuko, R. E. P. Lestari, N. Herowati, R. Vanessa, S. Widayanti, N. Widyas and S. Prastowo. 2021. Study association of bPRL (Bovine Prolactin) gene and milk production trait in Indonesian Holstein dairy cattle. IOP Conf. Ser.: Earth Environ. 902(1): 012007.

Javanmard, A., N. Asadazadeh, M. H. Banabazi and J. Tavakolian. 2005. The allele and genotype frequencies of bovine pituitary-specific transcription factor and leptin genes in Iranian cattle and buffalo populations using PCR-RFLP. J. Iranian Biotechnol. 3: 104-108.

Nadeem, A. and J. Maryam. 2016. Genetic and genomic dissection of prolactin revealed potential association with milk production traits in Riverine buffalo. Trop. Anim.

Health Prod. 48(6): 1261-1268.

Nei, M. and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Pr. New York.

Özdemir, M. 2020. A Prl/RsaI polymorphism in exon 3 and 4 of prolactin gene in dairy cattle. Pakistan J. Zool. 52(1):1-4.

Ozmen, O. and S. Kul. 2016. Identification of novel SNPs of ovine PRL gene and their association with milk production traits. Russ. J. Genet. 52(9): 977-984.

Padilla, P., M. Izquierdo, M. Martínez-Trancón, J. C. Parejo, A. Rabasco, J. Salazar and J. A. Padilla. 2018. Polymorphisms of α-lactoalbumin, β-lactoglobulin and prolactin genes are highly associated with milk composition traits in Spanish Merino sheep. Livest. Sci. 217: 26-29.

Patel, J. B. and J. B. Chauhan. 2017. Polymorphism of the prolactin gene and its relationship with milk production in Gir and Kankrej cattle. J. Nat. Sci. Biol. Med. 8(2): 167.

Pytlewski, J., I. Antkowiak, E. Czerniawska-Piątkowska and A. Kowalczyk. 2020. A dependency between PRL gene polymorphism and breeding parameters in Polish Black-and-White Holstein-Friesian cows. Anim. Biotechnol. 32(4): 432-438.

Rahayu, A. P., T. Hartatik, A. Purnomoadi and E. Kurnianto. 2019. Association of single nucleotide polymorphisms in the fatty acid synthase, LOC514211, and fat mass and obesity-associated genes with milk traits in Indonesian-Holstein dairy cattle. Vet. World. 12(7): 1160-1166.

Shah, R. M., N. A. Ganai, F. D. Sheikh, S. Shanaz, H. M. Khan, S. Alam and M. A. Rather. 2021. Exon IV prolactin (PRL) gene polymorphism and its association with milk production traits in dairy cattle of Kashmir, India. J. Entomol. Zool. Stud. 9(2):521-524.

Unal, E. Ö., E. S. Kepenek, H. Dinc, F. Özer, G. Sönmez, I. Z. Togan and M. I. Soysal. 2015. Growth hormone (GH), prolactin (PRL), and diacylglycerol acyltransferase (DGAT1) gene polymorphisms in Turkish native cattle breeds. Turk. J. Zool. 39(5): 734-748.

Warwick, E. J. and J. E. Legates. 1990. Breeding and Improvement of Farm Animals. 8th ed. McGraw Hill Inc. Publishing Company. Singapore.

Yasemin, Ö. N. E. R., O. Yilmaz, O.K.U.T. Hayrettin, A. T. A. Nezih, G. Yilmazbasmecitoglu and A. Keskin. 2017. Associations between GH, PRL, STAT5A, OPN, PIT-1, LEP and FGF2 polymorphisms and fertility in Holstein-Friesian heifers. Kafkas Univ. Vet. Fak. Derg. 23(4):527-534.