Polymorphisms of the Thy-1 Gene in IPB D2 Chicken Line: Association with IgY and ND Antibody

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ABSTRAK

Putri NT, Murtini S, Ulupi N, Khaerunnisa I, Sumantri C. 2022. Keragaman gen Thy-1 pada galur ayam IPB D2: Asosiasi dengan antibody IgY dan ND. JITV 27(4):186-194. DOI: http//dx.doi.org/10.14334/jitv.v27i43029.

Gen Thy-1 berperan penting dalam respon imun, terutama dalam aktivasi sel T. Gen ini mengkodekan protein Thy-1 (CD90) yang berukuran 25–37 kDa. Gen Thy-1 memainkan peran penting dalam respon imun, terutama dalam aktivasi sel T. Tujuan penelitian ini mengidentifikasi keragaman gen Thy-1 dan asosiasinya dengan konsentrasi total IgY dan titer antibodi ND. Sebanyak 123 sampel ayam IPB D2 terdiri dari ayam IPB D2 G0 (generasi awal) 38 sampel dan ayam IPB D2 G2 (generasi kedua) 85 sampel umur 21 minggu digunakan pada penelitian ini. Identifikasi keragaman gen dilakukan dengan teknik PCR dan sekuensing DNA, sedangkan ELISA dan uji HI dilakukan untuk analisis IgY dan titer antibodi ND. Analisis data terdiri dari frekuensi genotipe, frekuensi alel, heterozigositas pengamatan, heterozigositas harapan, dan keseimbangan Hardy-Weinberg. Metode General Linear Model (GLM) dan uji Duncan digunakan untuk mengetahui asosiasi antara keragaman gen dengan konsentrasi IgY dan titer antibodi ND. Hasil penelitian menunjukkan dua titik SNP pada intron posisi basa g.2139 C>T dan g.2542 A>G. SNP g.2139 C>T dan g.2542 A>G bersifat polimorfik, namun tidak berada pada keseimbangan Hardy-Weinberg karena adanya seleksi. SNP g.2139 C>T signifikan berasosiasi ($P<0.05$) dengan konsentrasi total IgY. Semua titik SNP tidak berasosiasi ($P>0.05$) dengan titer antibodi ND. Tidak terdapat asosiasi antara haplotipe dengan konsentrasi total IgY dan titer antibodi ND pada penelitian ini. Kesimpulannya, hasil penelitian ini menemukan bahwa SNP g.2139 C>T bersifat polimorfik dan bisa dijadikan kandidat marker untuk konsentrasi total IgY tinggi. Namun, hal ini perlu divalidasi dengan analisis ekspresi gen (qRT-PCR) dan pada populasi yang lebih besar.

Kata Kunci: IgY, Imunitas, Ayam IPB D2, Titer Antibodi ND, SNP, Gen Thy-1

ABSTRACT

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The Thy-1 gene plays a crucial role in immunological response, particularly in the activation of T cells. This gene encodes a Thy-1 protein (CD90) with 25–37 kDa of size. This study aimed to find a variation in the Thy-1 gene in IPB D2 chickens and its association with the total IgY concentration and ND antibody titers. A total of 123 head of 21 week old IPB D2 chickens consisting of 38 IPB D2 G0 (first generation) chickens and 85 IPB D2 G2 (second generation) chickens were used. PCR methods and direct-DNA sequencing were used to identify the polymorphism of Thy-1 gene. ELISA and HI test were used to analyze total IgY concentration and ND antibody titers. Genotype frequency, allele frequency, observed heterozygosity, expected heterozygosity, and Hardy-Weinberg equilibrium were analized. The General Linear Model and Duncan's Multiple Range Test were used to evaluate association between gene polymorphism and IgY concentration and ND antibody titers. Results discovered two SNPs on the intron (g.2139 C>T and g.2542 A>G). Four haplotypes were created by combining two SNPs. The g.2139 C>T and g.2542 A>G were polymorphic, but not in the Hardy-Weinberg equilibrium because of selection. The SNP g.2139 C>T were significantly associated ($P<0.05$) with total IgY concentration. All SNPs were not associated ($P>0.05$) with ND antibody titer. There was no association between the haplotype polymorphism of the Thy-1 gene with the total IgY concentration and ND antibody titer. It was identified that SNP g.2139 C>T was polymorphic and could be used as a candidate marker for high total IgY concentration. However, further analysis in large population and a validation through gene expression (qRT-PCR) are needed to prove this hypothesis.

Key Words: IgY, Immunity, IPB D2 Chickens, ND Antibody Titer, SNP, Thy-1 gene

INTRODUCTION

IPB D1 chickens are composite local chickens designed by crossing male offspring from F1 Pelung-Sentul chickens with female offspring from F1 kampung chicken-broiler parent stock strain Cobb. The selection of the three types of local chickens (pelung, sentul, and kampung) and broiler chickens for crossbreeding was based on the genetic potential of each breed, including meat production, egg production, disease resistance, and rapid growth (Ulupi et al. 2016; Sumantri et al. 2020). The IPB D1 chickens were officially released as a new line of composite local chickens by the Ministry of Agriculture of the Republic of Indonesia by Decree number 693/KPTS/PK.230/M/9/2019. The purpose of IPB D1 chicken production is to spread it to the local community. One of the initiatives to generate IPB D1 chickens with strong disease-resistance features was the creation of the IPB D2 chicken line. The IPB D2 chicken is a candidate for a new line created by selecting IPB D1 chickens based on immunocompetence and body resistance traits including IgY concentration and ND antibody titer. Candidate IPB D2 chicken line has the advantage of a high IgY concentration above 10 mg/mL, and has an ND antibody titer above 3 log 2 HI units.

Immunoglobulin Yolk (IgY) and Newcasle Disease (ND) antibody are important indicators of disease resistance in chickens. IgY is the main antibody found in poultry. The IgY protein is mostly present in blood and the fluid portion of eggs in chickens. Similar to mammalian IgG, it serves to protect the chicks against pathogens (Munhoz et al. 2014; Gaetani et al. 2017). ND antibody titers are specific antibodies to neutralize ND virus infection. Antibodies can neutralize the virus by tying up with ND virus particles and preventing the virus from adhering to host cells (Kapczynski et al. 2013). Newcastle disease (ND) is a disease in poultry caused by avian paramyxovirus serotype 1 (APMV-1) viruses. The biggest impact of ND is more in rural areas and the production of chickens reared on a free range. In developing countries, local chickes is a very important asset to meet the protein needs of meat and eggs (Alexander 2000; Rahman et al. 2017).

The rearing of local chickens in the community usually uses a free-range pattern that pays less attention to biosecurity. This causes chickens to be more susceptible to viruses and bacteria that may cause disease and even death. The existence of IPB D2 chicken line which has superior disease resistance is very important and may help in improving the genetic quality of Indonesian local chickens. Efforts to improve the genetic quality of Indonesian chickens have been carried out with various breeding programs, such as by selection. Selection of disease resistance traits conventionally is less effective because the heritability value for disease resistance traits in chickens is relatively low (0.20) (Touko et al. 2021). Selection of a molecular approach by utilizing candidate genes as genetic markers is one way to increase the effectiveness of breeding programs (Psifidi et al. 2016). In the selection program and genetical point of view, the concentration of IgY and ND antibody titers can be employed as indicators. Several genes regulate the synthesis of IgY and ND antibodies (Al-Habib et al. 2020).

The Thy-1 (Thymocyte Differentiation Antigen 1) gene or also known as CD90 (Cluster of Differentiation 90) is one of the gene that play a role in antibody production. The Thy-1 gene encodes the protein Thy-1 (CD90), which is a glycoprotein with a molecular size 25-37 kDa. This glycoprotein is expressed on the outside of cell membranes, in a wide variety of cell types, including human fibroblasts, neurons, blood stem cells and endothelial cells, and murine T cells (Lung et al. 2010). The Thy-1 gene in chicken is situated on chromosome 24, measuring 4279 bp and consisting of a promoter region, four exons, three introns, and a flanking region, according to information from the National Center of Biotechnology (NCBI) with access number GenBank NC 006111.4. Thy-1 gene in mice plays a role in T cell activation. Thy-1 gene is similar to CD28 in its capacity to stimulate mouse T cell activation. Activation of mouse T cells via Thy-1 requires an intact and functional TCR/CD3 complex, as Thy-1 triggers do not activate TCR/CD3-deficient T cells (Haeryfar & Hoskin 2004). CD3 molecules are involved in activating T cytotoxic cells (Tc) and T helper cells (Th). Th cells are T cells that play a role in humoral immunity, which can activate naive B cells into plasma cells that are ready to produce specific antibodies against antigens (Wibawan & Soejoedono 2013).

The identification of the Thy-1 gene polymorphism and its association to disease resistance in candidate IPB D2 chicken line has never been reported . This study aimed to analyze the Single Nucleotide Polymorphism (SNP) of the Thy-1 gene and their associations with disease resistance traits, i.e. the total IgY concentration and ND antibody titer in the candidate IPB D2 chicken line. The results of this study are expected to be a candidate for genetic markers (marker assisted selection) to select and accelerate the production of IPB D2 chicken lines that have disease resistance.

MATERIALS AND METHODS

Animals and phenotypic parameters

This study obtained the approval of ethical clearance and animal welfare from the Animal Care and Use Committee (ACUC) of IPB University (Access No: 224-2021 IPB). In this study a total of 38 IPB

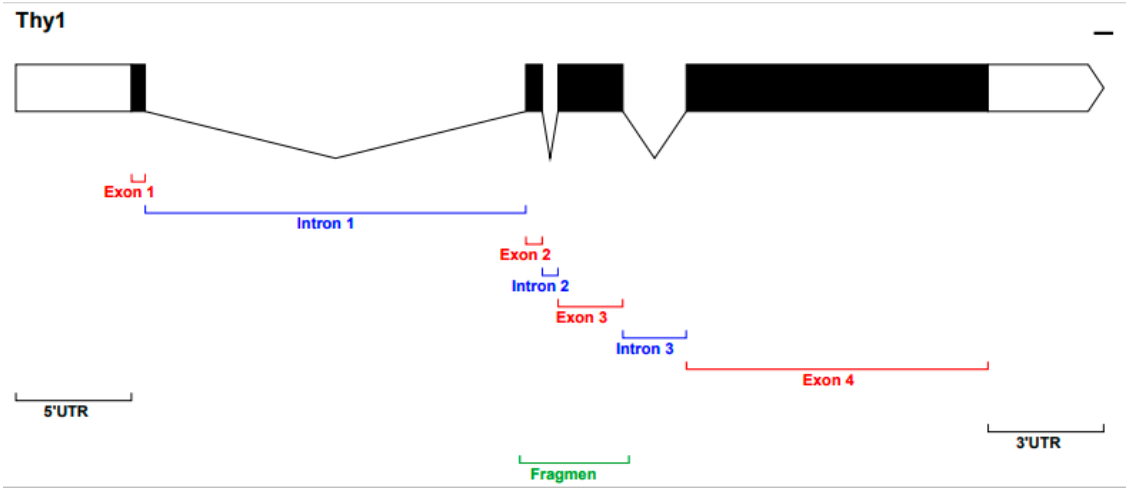

Figure 1. Reconstruction of Thy-1 gene structure in GenBank with NCBI access code: NC_006111.4 and the fragment target of this study (green)

D2 G0 (basic generation) and 85 IPB D2 G2 (second generation) chicken blood samples were collected from the Animal Breeding and Genetic Division of the Faculty of Animal Science, IPB University. Blood samples were collected at 21 weeks old. Those blood samples were drawn from the brachialis vein using a 3 mL syringe, then placed in a tube with 1.5 mL EDTA. The total IgY concentration was analyzed using indirect ELISA method (Hnasko & McGarvey 2015). The ND antibody titer were tested using HI test according to the OIE Manual of Standard Diagnostic Tests (Afonso et al. 2012).

DNA extraction

The phenol-chloroform extraction technique was used to extract DNA. Blood samples were put into a 1.5 mL tube (50 µL), then added with 0.2% NaCl (1,000 µL), and allowed to stand for 5 minutes. The supernatant was discarded after centrifuging samples that had been left to stand for 5 minutes at 8,000 rpm.

The resulting precipitate was added with 20 µL of proteinase K (5 mg/mL), 40 µL of 10% sodium dodecyl phosphate (SDS), and 30 µL 1×STE. The solution mixture was incubated for 2 hours at 55ºC and slowly homogenized. The mixed solution was added with 400 µL of phenol, 400 µL chloroform isoamyl alcohol (CIIA), and 40 µL 5 M NaCl while homogenized for 1 hour at room temperature. The solution combination was then centrifuged at 12,000 rpm for 5 minutes until a distinct DNA phase appeared.

The DNA phase was transferred to a new tube (1.5 mL) of 400 µL, then 800 µL of absolute ethanol and 40 µL of 5 M NaCl were added and stored in the freezer for one night. The DNA samples were centrifuged again for 5 minutes at 12,000 rpm to separate absolute ethanol. The supernatant was removed and the ethanol was allowed to evaporate fully at room temperature. After that, 100 µL of 80% TE were added, and the DNA sample was kept at -20ºC.

Primer design and DNA amplification

The primer used to amplify the THY-1 gene was designed using the Primer Designing Tools Program (http://www.ncbi.nlm.nih.gov/tools/primerblast). The primer sequences in this study were as follows: forward primer (F): 5'-CCTGGCTCACCACATCTCTC-3' and reverse primer (R): 5'-GAACTGGAGGCCATACCCTG-3'. The primer can amplify the exon 2 and 3 of Thy-1 gene along 566 bp, from 2615 bp (intron 1) to 3161 bp (intron 3) (Figure 1).

A total amount of 25 µL was used for DNA amplification. The amplified DNA sample was placed in a 0.2 mL tube with a sample volume of 0.3 µL. The DNA sample was then mixed with 11.9 µL of NFW, 0.15 µL of forward primer, 0.15 µL of reverse primer (all from IDT DNA, Singapore), and 12.5 µL of MayTaq HS RedMix (Bioline Reagents Ltd., London, UK). On an AB System PCR equipment (GeneAmp® PCR System 9700, Applied Bio Systems, Foster City, USA), the PCR procedure was carried out in four phases. The PCR conditions used have been modified, namely initial denaturation at a temperature of 95 ºC for 1 minute and carried out for one cycle. The second through fourth steps are repeated 35 times, with each cycle consisting of a 15-second denaturation process at 95 degrees Celsius, a 10-second annealing process at 60 degrees Celsius, and a 10-second extension process at 72 degrees Celsius.

Electrophoresis

A 45 g agarose powder was mixed with 30 mL 0.5 x TBE solution and microwaved for 3 minutes to make a 1.5 percent agarose gel. After cooling the agarose

solution for 2 minutes at 50 rpm with a magnetic stirrer, 1 µL of fluorosafe was added until the solution was homogenous. The gel solution was then poured into the gel tray for 30 minutes until it solidified into a gel. The gel was put into the electrophoresis machine, which was filled with 0.5 x TBE buffer. 3 µL amplicon was inserted into the gel well and migrated with 100 bp marker as much as 3 µL with a voltage of 100 V for ±35 minutes. The electrophoresis findings were then viewed using a UV Transilluminator equipment (Alpha Imager, Alpha Innotech, Santa Clara, USA). The PCR products of IPB D2 chicken samples were sequenced using the services of Macrogen, South Korea.

Statistical analysis

The results of the sequencing of the THY-1 gene fragment were analyzed using MEGA version 10.0., Bio Edit, and Finch TV programs. The sequencing results were then aligned based on the Gen Bank sequence reference, Gen Bank access code: NC_006111.4. Genetic polymorphism was analyzed using PopGen32 software based on allele and genotype frequencies, Hardy-Weinberg equilibrium, and heterozigosity. The haplotype was analyzed using DNAsp version 6.12.01 software. The association of the SNPs and haplotype of Thy-1 gene with total IgY concentration and ND antibody titer were analyzed by General Linear Model (GLM) procedure using SAS 9.2 software (SAS Institute, Cary, NC, USA) and the least means square values for genotypes and haplotypes were compared by Duncan's Multiple Range test (Zhang et al. 2019). Significant association were declared when P<0.05. The mathematical model follows:

$$\boldsymbol{Yij = \mu + Gi + eij}$$

where Yij is the dependent variable (total IgY concentration and ND antibody titer), µ is the mean square value, Gi is the effect of the I genotype, and eij is the random error.

RESULTS AND DISCUSSION

Detection of Thy-1 gene mutation of candidate IPB D2 chicken line

A single nucleotide polymorphism (SNP) is a pattern in the DNA sequence that displays variations in the base of a single nucleotide, generally one of two potential nucleotides at a given place. SNPs provide several benefits over other forms of polymorphisms when it comes to the genetic dissection of complex characteristics and disorders, as well as investigating population-based genes as they are found across the genome, such as in exons, introns, intergenic regions, promoters, and enhancers. SNPs in the coding area impact protein translation directly, SNPs in the intron region affect splicing, and SNPs in the promotor position affect gene expression (Asaf et al. 2014).

Identification of SNPs from the results of Thy-1 gene sequencing in IPB D2 chicken line candidate found two SNP points with nucleotide changes, i.e, g.2139 C>T and g.2542 A>G (Table 1). Both g.2139 C>T and g.2542 A>G are intron 2 and intron 3, respectively. The part of DNA translated in the protein synthesis process is the exon part, while the intron has an important role in the splicing process (Rogozin et al. 2012). Splicing is the process of removing all noncoding base sequences and combining coding base sequences to obtain mature mRNA molecules, and this process occurs after the transcription process. In the eukaryote genome, there is an alternative splicing phenomenon that aims to increase genome efficiency. Alternative splicing is the main mechanism that causes protein differences and regulates gene expression (Gunawan et al. 2017). According to Maston et al. (2006), Locus control Regions (LCRs) are a group of regulatory elements involved in regulation loci or entire gene cluster. LCRs have the ability to control gene expression from a distance and work regardless of place, and they can also be found inside an intron of the gene. Although the SNPs found in the intron region and introns are not translated into amino acids, mutations that occur in the intron region can also affect gene expression.

Genotype frequency, allelic frequency, and hardy-weinberg equilibrium Thy-1 gene

Table 2 shows the genotype frequency, allele frequency, and Hardy-Weinberg equilibrium of the Thy-1 gene in the candidate IPB D2 chicken line. The Thy-1 gene in the candidate IPB D2 chicken line was polymorphic, according to the findings. According to Allendorf & Luikart (2007), a gene is polymorphic if the allele frequency in a large population is below 0.99 and below 0.95 in a small population.

SNPs g.2139 C>T and g.2542 A>G positions had 3 genotypes. The frequency of the GG genotype at position g.2542 A>G (0.53) in the IPB D2 G0 population showed the highest value in all observed SNPs, it can be said that the probability of the emergence of the GG genotype was higher than other genotypes. The frequency of the CC genotype at position g.2139 C>T (0.54) in the IPB D2 G2 population showed the highest value in all observed SNPs. The difference in genotypes and alleles between the G0 and G2 populations is thought to be due to selection based on disease resistance indicators, thus affecting genetic diversity.

The Hardy-Weinberg equilibrium of the Thy-1 gene in the population was examined using chi-square (χ^2) to

see if the observed data diverged or did not depart from the predicted ratio according to the Hardy-Weinberg equilibrium law. The results showed that SNPs g.2139 C>T and g.2542 A>G were not in Hardy-Weinberg equilibrium. If the estimated value of χ^2 is less than the table value of χ^2, the population is considered to be in Hardy-Weinberg equilibrium. Due to the random merger of gametes in large populations, SNPs in Hardy-Weinberg equilibrium reveal that allele and genotype frequencies remain constant from generation to generation (Allendorf et al. 2013). If there is no selection, migration, mutation, or genetic drift, a big enough population will not change from generation to generation (Noor 2010). The imbalance that occurs indicates the existence of selection in the population. The maintenance of IPB D2 chickens is carried out intensively and selected based on disease resistance indicators so that the possibility of random mating from generation to generation is small.

Heterozygosity value Thy-1 gene

Heterozygosity is one of the essential parameters in determining genetic diversity in a population. High heterozygozygosity suggests a high level of genetic variety, whereas low heterozygozygosity indicates a low level of genetic variation. Inbreeding has occurred in a population when the observed heterozygosity value (Ho) is lower than the predicted heterozygosity value (He) (Zhao et al. 2019). SNPs g.2139 C>T and g.2542 A>G had a lower Ho value than the He value, indicating that inbreeding occurred. This is also consistent with the value of χ^2, which is not in Hardy-Weinberg equilibrium (Table 2). The observed heterozygosity value (Ho) of the three SNPs in this study was found to be low, which is below 50%. Javanmard et al. (2005) explained that a heterozygozygosity score of less than 50% suggests poor gene diversity in the studied population. The difference between the value of Ho and the value of He in the analyzed population can be used as an indicator of an imbalance of genotypes in the population. It is suspected that there is a degree of endogamy (marriage in groups) because it is the result of an intensive selection process (Machado et al. 2003). Inbreeding in a population could be make genetic defect. The number of individuals in a population greatly affects the level of inbreeding, where the smaller the population, the

Table 1. The position of the Thy-1 gene SNPs in the target sequence

No	SNPs position	Location in Thy-1 Gene	Mutation type
1	g.2139 C>T	Intron 2	Transition
2	g.2542 A>G	Intron 3	Transition

Table 2. Genotype frequency, allele frequency, and chi-square value of Thy-1 gene in candidate IPB D2 chicken line

No	SNP/Population	N	Genotype Frequency			Allele Frequency		χ^2
1	g.2139 C>T		CC	CT	TT	C	T	
	IPB D2 G0	38	0.40 (15)	0.13 (5)	0.47 (18)	0.46	0.54	21.30*
	IPB D2 G2	85	0.54 (46)	0.21 (18)	0.25 (21)	0.65	0.35	25.05*
2	g.2542 A>G		AA	AG	GG	A	G	
	IPB D2 G0	38	0.42 (16)	0.05 (2)	0.53 (20)	0.45	0.55	31.28*
	IPB D2 G2	85	0.49 (42)	0.05 (4)	0.46 (39)	0.52	0.48	70.65*

N= Number of samples, *significantly different at $\chi^2_{(0,05;1)} = 3,841$

Table 3. The value of observed heterozygosity (Ho) and expected heterozygosity (He) of the Thy-1 gene in the candidate IPB D2 chicken line

No	SNP/Population	Ho	He
1	g.2139 C>T		
	IPB D2 G0	0.132	0.504
	IPB D2 G2	0.212	0.460
2	g.2542 A>G		
	IPB D2 G0	0.053	0.501
	IPB D2 G2	0.047	0.502

Ho= Observed heterozygosity, He= Expected heterozygosity

greater the inbreeding pressure on a trait. The number of individuals in a population is related to the effective population size, especially those capable of reproduction (Praharani et al. 2018). The maintenance IPB D2 chickens for future breeding it can be done by increasing the population and interbreeding between generations. It aims to reduce inbreeding pressure and increase herterozygosity.

Association of the Thy-1 genotype with total IgY concentration in IPB D2 chickens

Immunoglobulins are glycoproteins secreted by plasma cells in response to exposure to antigens and are considered to be the product that largely influences humoral immunity (Amro et al. 2018). Chicken has three main classes of immunoglobulins (Ig), namely IgM, IgY (IgG), and IgA. Immunoglobulin Yolk (IgY) is the dominant systemic antibody in chicken blood. The association of Thy-1 genotype diversity with total IgY concentration is presented in Table 4.

The result (Table 4) showed that the total concentration of IgY in IPB D2 chickens ranged from 11–13 mg/mL. According to (Oberländer et al. 2020), the average concentration of IgY in blood serum ranges from 5–15 mg/mL, IgM is around 1–2 mg/mL, and IgA antibodies are around 3 mg/mL. Based on the average IgY concentration, IPB D2 chickens had a higher antibody concentration than native chickens (10.07 mg/mL) and purebred chickens (7.89 mg/mL) (Setiani 2016).

Statistical test results showed that SNP g.2139 C>T genotype CT was significantly different from genotype CC and TT ($P<0.05$) in G2 population while in G0 population was not significantly different ($P>0.05$). The CT genotype showed a higher total IgY concentration than the CC and TT genotypes. The change in base C to T did not cause an increase in the total IgY concentration in the TT genotype but in the CT genotype. SNP g.2139 C>T was associated with IPB D2 G2 chickens, while the G0 population had no association. This is presumably due to the selection process that has been carried out from G0 to produce G2 based on disease resistance indicators, so that the action of the Thy-1 SNP gene g.2139 C>T genotype CT appeared in IPB D2 G2 chickens. SNP g.2139 C>T has the potential to be a candidate genetic marker. However, this should be validated by further research through gene expression analysis (qRT-PCR). SNP g.2542 A>G in the two populations based on statistical tests were not significantly different ($P>0.05$) between genotypes, with the AA genotype having a higher total IgY concentration than the AG and GG genotypes.

Table 4. Association of the Thy-1 genotype diversity with total IgY concentration

SNP/Population	N	Total IgY Concentration (mg/mL) (N)		
g.2139 C>T		CC	CT	TT
IPB D2 G0	30	11.77±1.15 (12)	11.45±0.57 (4)	11.75±1.41 (14)
IPB D2 G2	81	$12.56±1.71^{b}$ (45)	$13.63±0.99^{a}$ (16)	$12.56±1.70^{b}$ (20)
g.2542 A>G		AA	AG	GG
IPB D2 G0	31	11.98±1.36 (12)	11.52±1.72 (2)	11.66±1.13 (17)
IPB D2 G2	83	12.96±1.77 (40)	12.48±0.75 (4)	12.60±1.80 (39)

N= number of samples; Numbers in the same line and followed by different superscripts indicate that they are significantly different ($P<0.05$), n: number of samples

Table 5. Association of Thy-1 genotype with ND antibody titers

SNP/Population	N	Titer Antibodi ND (log 2 HI unit) (N)		
g.2139 C>T		CC	CT	TT
IPB D2 G0	35	2.86±2.51 (14)	2.00±0.82 (4)	3.82±2.79 (17)
IPB D2 G2	77	1.58±1.45 (40)	1.22±1.44 (18)	1.37±1.50 (19)
g.2542 A>G		AA	AG	GG
IPB D2 G0	36	4.07±2.40 (14)	4.00±5.66 (2)	2.80±2.55 (20)
IPB D2 G2	77	1.59±1.60 (39)	0.67±1.15 (3)	1.34±1.28 (35)

N= number of samples

Table 6. Haplotype frequency in 566 bp Thy-1 gene amplicons in candidate IPB D2 chicken line

Haplotype	Nucleotide position		Frequency (%)	
	g.2139 C>T	g.2542 A>G	IPB D2 G0 (n)	IPB D2 G2 (n)
Haplotype 1	T	G	23.68 (9)	23.53 (20)
Haplotype 2	T	A	31.58 (12)	16.47 (14)
Haplotype 3	C	G	31.58 (12)	23.53 (20)
Haplotype 4	C	A	13.16 (5)	36.47 (31)

N= number of samples

Table 7. Association of the Thy-1 gene haplotype with IgY concentration and ND antibody titer in IPB D2 chickens

Haplotype	IPB D2 G0		IPB D2 G2	
	IgY mg/mL (N)	ND Antibody Titer (log 2 HI unit) (N)	IgY mg/mL (N)	ND Antibody Titer (log 2 HI unit) (N)
Haplotype 1	11.37±1.02 (7)	3.33±2.12 (9)	13.13±1.73 (30)	1.71±1.61 (28)
Haplotype 2	11.86±1.47 (10)	3.42±2.75 (12)	12.16±1.68 (20)	1.35±1.22 (17)
Haplotype 3	11.84±1.32 (10)	3.27±3.00 (11)	12.51±0.98 (11)	1.23±1.54 (13)
Haplotype 4	12.13±1.73 (4)	3.5±3.42 (4)	12.99±1.84 (20)	1.26±1.37 (19)

N= number of samples

Association of the Thy-1 genotype with ND antibody titer in IPB D2 chickens

An ND antibody titer of above 4 log 2 HI units was categorized as a protective level for vaccinated chickens, while an ND antibody titer below 3 log 2 HI units was categorized as non-protective (Rahman et al. 2017). Based on the results of the study (Table 5), the IPB D2 chicken with vaccination at the age of 3 days and 3 weeks obtained below protective level, except AA genotype and AG genotype of SNP g.2542 A>G in population IPB D2 G2. The second generation (G2) IPB D2 chickens had lower ND antibody titers than the basic generation (G0) or were below the protective level.

In some cases, ND antibody titers were found under the protective category. Lower ND antibody titers may be due to vaccination failure or may be due to the presence of parental antibodies being passed on to the offspring that neutralize the vaccine virus. The higher the ND antibody titer, the lower the susceptibility to clinical infection with the ND virus (Rahman et al. 2017). The relationship between Thy-1 gene polymorphism and ND antibody titer based on statistical tests, showed that the two SNP points found had no significant effect (P>0.05) or were not associated. This study showed that genotype diversity did not affect the ability of IPB D2 chickens to producing specific ND antibodies.

Haplotype

A haplotype is a nucleotide sequence that reflects the genetic variation of each individual within a species (Permana et al. 2015). Two SNP points of the Thy-1 gene were found in IPB D2 chickens, forming four haplotypes. The frequency of the Thy-1 gene haplotype in IPB D2 chickens can be seen in Table 6. Haplotype 4 is a nucleotide sequence that is the wild type according to the Gene Bank (NC_006111.4) with a frequency of 13.16% (IPB D2 G0) and 36.47% (IPB D2 G2). The highest haplotype frequency in IPB D2 G0 was found in haplotype 2 and 3 (31.58%), while the lowest haplotype frequency was found in haplotype 4 (13.16%). The highest haplotype frequency in IPB D2 G2 was found in haplotype 4 (36.47%), while the lowest haplotype frequency was found in haplotype 2 (16.47%). The g.2139 C>T base mutation was found in haplotypes 1 and 2. The g.2542 A>G base mutation was found in haplotypes 1 and 3. Haplotype 1 showed a combination of SNPs at bases g.2139 C>T and g.2542 A>G.

Statistical test results showed that there was no relationship between haplotype and total IgY concentration and ND antibody titer in both G0 and G2 populations (P>0.05) or not associated. Haplotype 1 in population G2 is the haplotype with the highest total concentration of IgY and ND antibody titer and is the haplotype with a combination of SNPs that undergoes mutations, while in population G0 haplotype 4 is the haplotype with the highest total concentration of IgY and ND antibody titer and is the haplotype with the combination of SNPs. without mutation or wild type.

According to (Setyawati et al. 2019), the total IgY concentration is categorized as high if the value is 9.3 mg/mL, while according to Rahman et al. (2017), the ND antibody titer is said to be protective if the antibody titer value is 4 log 2 HI units. Based on Table 6, the results of the average concentration of total IgY on

haplotypes 1, 2, 3, and 4 were in the high category. The average ND antibody titer in the G0 population was close to the protective level, while in the G2 population the ND antibody titer decreased or was below the protective level. Antibodies can be obtained from maternal antibody or direct exposure to the disease. The low value of ND antibody titer in IPB D2 G2 chickens does not mean that the chicken is not resistant to ND disease, but it is suspected that there is no exposure to ND virus in chicken rearing so that it does not trigger the production of ND antibodies. According to Bernardini et al. (2017), the maternal antibody will progressively decline with age and then rise again, depending on the exposure to the illness from the environment. Antibody production is induced by exposure to disease through intricate cell signalling (APC, T cells, and B cells). The development of antibodies and the immunological response of chickens can both be affected by communication problems or mistakes (Al-Habib et al. 2020).

The Thy-1/CD90 gene belongs to the CD (Cluster of Differentiation) group. CD is a protein that functions as a receptor or ligand for agents that expose the body. Not all CD molecules function in cell signaling (communication between cells), some CD molecules only play a role in cell adhesion (Wibawan & Soejoedono 2013). The function of the Thy-1 gene is not fully understood, especially in chickens. Chen et al. (2005) stated that the Thy-1 gene can also function as a cell adhesion molecule. The Thy-1 gene in mice has been implicated in mediating thymocyte adhesion to thymic epithelial cells, regulating thymocyte apoptosis, and in modulating neurite growth in neurons.

CONCLUSION

The results showed two SNPs of the Thy-1 gene in the candidate IPB D2 chicken line, i.e, g.2139 C>T and g.2542 A>G in intron 2 and intron 3. All SNPs are formally defined as polymorphic. The CT genotype of SNP g.2139 C>T can be used as a candidate marker for high total IgY concentration. However, further analysis is needed, namely validation through gene expression (qRT-PCR). No SNPs were associated with ND antibody titer. The combination of 2 SNPs formed four haplotypes. There was no association between the haplotype polymorphism of the Thy-1 gene with the total IgY concentration and ND antibody titer in IPB D2 chickens in this study.

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