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Non-synonymous Single Nucleotide Polymorphism on *TLR1A* gene as a candidate for immune function of Indonesian indigenous chicken

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Abstract. *Indrijani H, Hilmia N, Anang A, Pangestu MSP. 2024. Non-synonymous Single Nucleotide Polymorphism on* TLR1A *gene as a candidate for immune function of Indonesian indigenous chicken. Biodiversitas 25: 2434-2441. TLR1A* is a candidate gene related to the immune system in chickens, which contributes to disease resilience, which could impact their productivity. This study aimed to determine mutations in the *TLR1A* gene in three indigenous Indonesian chickens namely, Pelung, Black Kedu and Sentul. A total of 127 DNA samples were used and consisted of 38, 41 and 48 respectively for Pelung, Black Kedu and Sentul chickens. DNA was isolated from white blood cells and amplification of *TLR1A* gene was conducted in part of exon 4 using PCR with specific primer. The sequencing results for the TLR1A gene were analyzed to identify mutations using the BioEdit and MEGA X programs. The results showed genetic polymorphisms in the *TLR1A* gene in Indonesian indigenous chicken. One synonymous mutation, g.710C>T, in which amino acid coded leusine at 207th, and four non-synonymous mutation, i.e., g.822A>G, altered amino acids coding from threonine to alanine (T245A), g.835C>T, which altered amino acids coding 249th from threonine to methionine (T249M), g.1015G>A that altered amino acids coding 309th from serine to aspargine (S309N) and g.1165T>G that changed amino acid from arginine to proline R359P. All mutation frequencies for the alleles are lower than those for the non-mutation alleles. The non-synonymous mutation of the *TLR1A* gene might alter the metabolism of this hormone related to the immune system in chickens.

Keywords: Altered amino acid, disease resistance, mutation, SNP, *Toll-Like Receptor 1A*

INTRODUCTION

The potential diversity and superiority of Indonesian indigenous chickens (*Gallus gallus domesticus*) genetic resources is an essential aspect that must be explored by using molecular technology. The use of this technology is expected to provide optimal benefits for the provision of animal protein sources. In addition, Pelung, Sentul, and Black Kedu chickens are Indonesian indigenous species with the potential to be developed due to their distinct advantages. Pelung chickens have been reported to have a significant body performance as well as a long and rhythmic crowing sound (Rusfidra and Arlina 2014; Asmara et al. 2023). Several studies have shown that Sentul chickens have relatively higher growth and egg production compared to local species (Indrijani et al. 2024), while Black Kedu chickens have attractive physical characteristics, distinguished visually because it has a black plumage color (Ismoyowati et al. 2012).

According to previous reports, these 3 native chickens offer various benefits, including resistance/immunity to disease caused by bacteria, viruses, fungi, or other microbes. Immunity is an essential function that prevents the detrimental effects of disease attacks on production efficiency, the environment, and consumer attitudes toward food products (Bovenhuis et al. 2022). Several studies have shown that the function is often facilitated by the genetic interaction between the host/animals and the pathogen causing the disease (Makepeace et al. 2021; Rajput and Thakur 2023). Based on these findings, the best and most appropriate approach to control infectious disease in chickens is to increase their genetic potential related to disease resistance. Chickens with resistance are known to have the ability to produce antibodies, which are affected by the capacity of their immune control genes and environmental influences, including feed, the type of infectious pathogen, and previous exposure to viruses/bacteria. Disease resistance genes typically encode antibodies, microRNAs, and other materials that help the host resist damage caused by pathogens (Dar et al. 2019). Furthermore, Doekes et al. (2023) revealed that based on Genome-Wide Association Study (GWAS) in Purebred layer chicken line, Natural Antibody (NAb) as an indicator for disease resistance, their heritability for IgG and IgM were 0.20 and 0.39, respectively. A previous study on NAb in Piglet showed genetically higher levels of NAb were associated with greater disease resilience (Chen et al. 2020).

In line with these findings, the identification of economically valuable and related genes can be directly used as Marker Assisted Selection (MAS) in selecting livestock for the next generation (Das et al. 2021). A widely known MAS with high applicability is Single Nucleotide Polymorphisms (SNPs), which is a favored marker for disease linked to specific genes due to their widespread presence, stability, and distribution throughout the genome of all organisms (Betts and Russell 2003).

The analysis of immune-related genes has shown the correlated effects of Toll-like receptor 1 family member A (*TLR1A*) on immunological status, particularly in relation to the concentration of Immunoglobulin Yolk (IgY) and NAb (Natural antibodies) (Bovenhuis et al. 2022). Toll-like receptors (TLRs) are known to play an important role in initiating both innate and acquired immunity. Previous study showed that genetic variations in TLRs could disrupt essential signaling pathways associated with these receptors, potentially increasing susceptibility to autoimmune disease (Zhang et al. 2021). Toll-like receptors (TLRs) form a set of sensory receptors adept at identifying microbial intrusions and triggering innate immune responses. The responses comprise inflammatory reactions observed in both immune and non-immune cells (Nihashi et al. 2019). In addition, polymorphism TLR1 type 1 and 2 genes in 9 breeds of chickens can facilitate the understanding of varied resistance among the breeds of chickens to disease (Ruan and Zheng 2011).

In a previous Genome-Wide Association Study (GWAS), a region of chicken genome was identified on the 4th chromosome with a significant effect on IgM NAb. This chromosomal region contained the Toll-Like Receptor 1 family member A (*TLR1A*) gene, where polymorphisms were identified as dominant variants affecting NAb levels (Berghof et al. 2018). In addition, the study of Bovenhuis et al. (2022) suggested that chickens in generation 5, 6, and 7 selection experiments showed an identified polymorphism in the *TLR1A* gene related to its role in the immune response, and positive selection was observed at this locus. Polymorphism *TLR1* type 1 and 2 genes in 9 breeds of chickens can help to further understand the varied resistance among the breeds of chickens to disease.

Despite the importance of this field of study, there are no reports on *TLR1A* gene polymorphism in indigenous Indonesian chickens. Therefore, this study aimed to identify SNPs in *TLR1A* gene as a candidate gene for disease resistance in indigenous Indonesian chickens. The results are expected to produce potential markers to accelerate selection and produce indigenous Indonesian chickens with disease resistance capacity.

MATERIALS AND METHODS

The samples used in this study were selected using the purposive sampling method, comprising 38 heads of Pelung, 41 Black Kedu, and 48 Sentul chickens in healthy and unvaccinated conditions. The samples of Pelung chicken were taken from enthusiasts in the Pelung chicken community in Soreang, Bandung, Indonesia. Sentul chickens were taken from BBPTU Jatiwangi, West Java, Indonesia, while Black Kedu were taken from BBPTU Maroon, Central Java, Indonesia. BBPTU Jatiwangi and Maroon have been implement closed breeding system.

Blood collection, DNA isolation and visualization

Blood samples were taken from the wing vein using a syringe (3 cc) and then stored in a Vaccutainer with Ethylenediaminetetraacetic Acid (EDTA) anticoagulant. In addition, DNA isolation was performed using the Genomic DNA Purification Kit brand Promega®. The results of DNA isolation were evaluated using electrophoresis 1.2% agarose gel for approximately 90 minutes with a voltage of 100 V and then visualized with an Ultra-Violet (UV) transilluminator. The well-isolated genome was clearly visible from the electrophoresis results.

DNA amplification of *TLR1A* gene

Polymerase Chain Reaction (PCR) comprises multiplying a target DNA molecule flanked by a pair of primers. Amplification of target genes was performed using PCR Green Master Mix from Promega®. Each PCR reagent contained a volume of 25 µL, consisting of 50 ng template DNA, 20 ng each of Forward and Reverse primers, and PCR Master Mix. The primers used were forward primer 5'GCCAATCTGTCAGGAATTTGGG'3 and reverse primer 5' GCTGGTCATGAAGCTCACCT'3. In addition, the primers were designed using Primer- Basic Local Aligment Search Tool (BLAST) from National Centre for Biotechnology Information (NCBI). The PCR machine used was LongGene Scientific Instruments, Model A600, with serial number 016-00357. Amplification results (PCR product) were then evaluated using 1.2% agarose electrophoresis and visualized with a UV transluminator, voltage 100 V for 60 minutes. *TLR1A* gene diversity was analyzed by performing direct sequencing of PCR products. Sequence results were aligned using Bioedit and MEGA X programs, and then analyzed for SNP position and amino acid alignment. Genotyping was performed based on the SNPs, and the Haplotype was compiled based on the mutations found in each chicken breed.

Data analysis

The allele and genotype frequency of each locus was calculated with the following equation Nei and Kumar (2000) that was analyzed by POPGEN.32 program.

RESULTS AND DISCUSSION

The *TLR1A* target gene was amplified using primers to detect SNPs in exon 4. The results showed that exon 4 of the *TLR1A* gene in chickens had 2454 bp of length, which coded 818 amino acids (NCBI access code NC_052535.1). The target gene could be amplified along 826 bp, as presented in Figure 1.

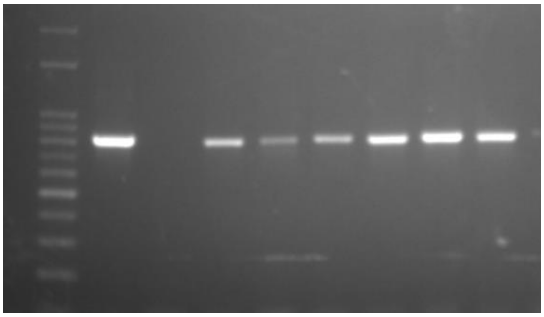

Figure 1. PCR product of *TLR1A* gene along 826 bp

Mutation analysis of *TLR1A* gene in Kedu, Pelung, and Sentul chickens

The results sequence of the *TLR1A* gene target with a length of 826 bp for each chicken breed was aligned using Bioedit and MEGA X programs. In this study, a total of 4 Single Nucleotide Polymorphisms (SNPs) was found, comprising 1 synonymous SNP g.710C>T, which did not alter amino acid leucine at 207th, and 4 non-synonymous SNPs (nsSNP) (g.822A>G, g.835C>T g.1015G>A and g.1165G>C). These non-synonymous mutations altered amino acid (AA) coding. g.822A>G altered the 245thAA from threonine to alanine (T245A), g.835 C>T changed the 249th AA from threonine to methionine (T249M), g.1015G>A altered the 309th AA from serine to asparagine (S309N), and g.1165G>C changed the 359th AA from arginine to proline (R359P). Synonymous and three non-synonymous mutations in this study were classified as transition mutations, except nsSNP g.1165G>C (R359P), which was considered a transversion mutation.

During the experiment, nsSNP g.1165G>C, which altered the coding of the 359th AA from arginine to proline was present in all breeds. g.835C>T mutation changed the 249th amino acid coding from threonine to methionine and was found in Kedu and Sentul chickens. Meanwhile, g.1015G>A mutation, which changed the coding of the 309th amino acid from serine to asparagine occurred in Pelung and Sentul chickens. Synonymous mutation g.710C>T (L207L) and non-synonymous mutation g.822A>G (T245A) altered amino acid from threonin to alanine and were only found in Sentul chickens. All SNPs observed in all breeds in this study are presented in Table 1.

The alignment results of the 5 mutations in the *TLR1A* gene nucleotide and its amino acid coding are presented in Figures 2, 3, 4, and 5. The findings of synonymous and non-synonymous SNP analysis were used for genotyping and haplotype compilation, as presented in Tables 2 and 3 (Black Kedu chickens), Tables 4 and 5 (Pelung chickens), and Tables 6 and 7 (Sentul chickens).

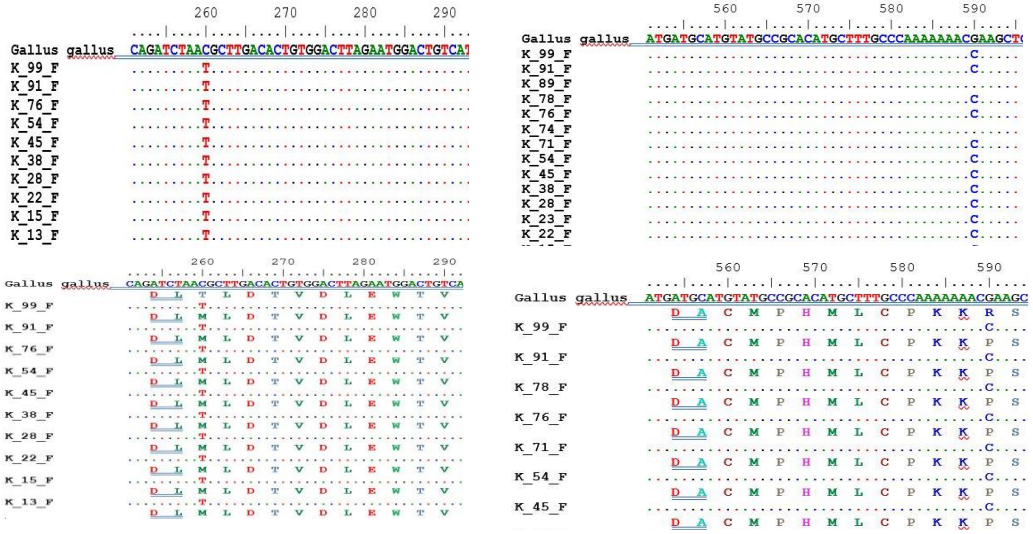

Figure 2. Alignment result of *TLR1A* gene mutation on g.835C>T (T249M) and g.1161G>C (R359P) in Black Kedu chicken

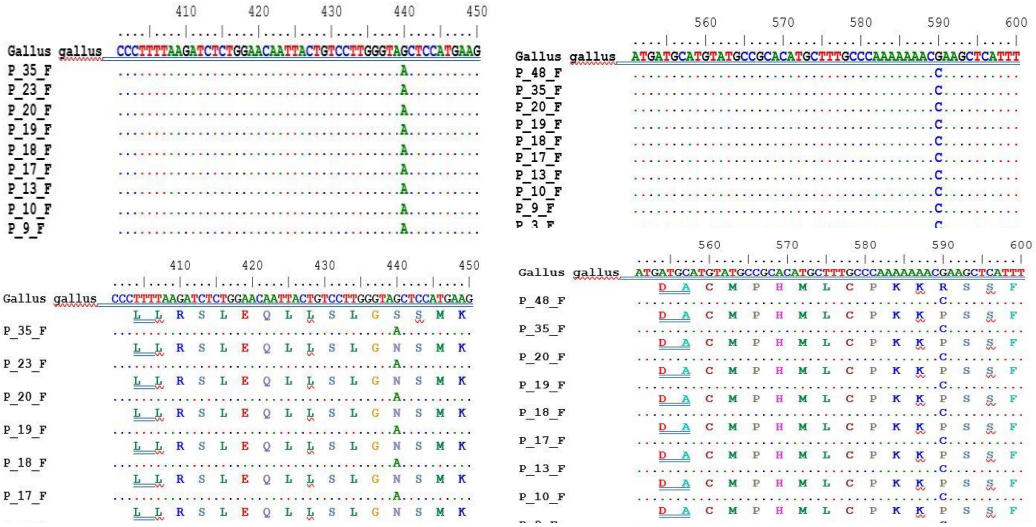

Figure 3. Alignment result of *TLR1A* gene mutation on g1035G>A (S309N) and g.1161G>C (R359P) in Pelung chicken

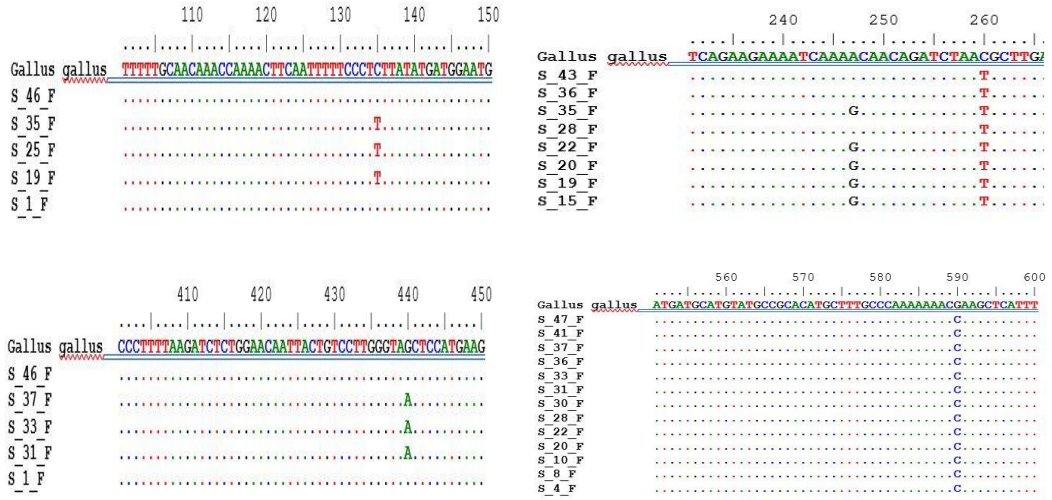

Figure 4. Alignment result of *TLR1A* gene mutation g.710C>T (L207L), g.822A>G (T245A), g.835C>T (T249M) g.1015G>A (S309N) and g.1165G>C (R359P) in Sentul chicken

Table 1. Mutation on *TLR1A* gene in Kedu, Pelung and Sentul chickens

Position	SNP	Breed	Mutation
g.710 C>T L207L	Synonymous Leucine	Sentul	Transition
g.822 A>G T245A	Non-synonymous Threonine to alanine	Sentul	Transition
g.835 C>T T249M	Non-synonymous Threonine to methionine	Kedu & Sentul	Transition
g.1015 G>A S309N	Non-synonymous Serine to asparagine	Sentul & Pelung	Transition
g.1165 G>C R359P	Non-synonymous Arginine to proline	All breed	Tranversion

Table 2. Genotype and allele frequency of *TRL1A* gene in Kedu chicken

Mutation/ SNP	SNP	Genotype frequency			Allele frequency	
g.835C>T	Non-synonymous	CC	CT	TT	C	T
T249M	Threonine to methionine	0.535	0.393	0.072	0.732	0.268
g.1165G>C	Non-synonymous	GG	GC	CC	G	C
R359P	Arginin to proline	0.357	0.481	0.162	0.598	0.402

Table 3. Haplotype polymorphisms of *TRL1A* gene in Black Kedu chicken

Haplotype	g.835C>T	g.1165G>C	Frequency (%)
Haplotype 1	C	G	60.98
Haplotype 2	C	C	14.63
Haplotype 3	T	C	24.39

Table 4. Genotype and allele frequency of *TRL1A* gene in Pelung chicken

Mutation/ SNP	SNP	Genotype frequency			Allele frequency	
g.1015 G>A	Non-synonymous	GG	GA	AA	G	A
S309N	Serine to sparagine	0.582	0.361	0.056	0.763	0.237
g.1165 G>C	Non-synonymous	GG	GC	CC	G	C
R359P	Arginine to proline	0.543	0.388	0.069	0.737	0.263

Table 5. Haplotype polymorphisms of *TRL1A* gene in Pelung chicken

Haplotype	g.440 G>A	g.590 G>C	Frequency (%)
Haplotype 1	G	G	71.05
Haplotype 2	G	C	5.26
Haplotype 3	A	G	2.63
Haplotype 4	A	C	21.05

Table 6. Genotype and allele frequency of *TRL1A* gene in Sentul chicken

Mutation/SNP	SNP	Genotype frequency			Allele frequency	
g.710 C>T	Synonymous	CC	CT	TT	C	T
L207L	Leucine	0.719	0.258	0.023	0.848	0.152
g.822 A>G	Non-synonymous	AA	AG	GG	A	G
T245A	Threonine to alanine	0.612	0.34	0.047	0.784	0.216
g.835 C>T	Non-synonymous	CC	CT	TT	C	T
T249M	Threonine to methionine	0.53	0.396	0.074	0.728	0.272
g.1015 G>A	Non-synonymous	GG	GA	AA	G	A
S309N	Serine to asparagine	0.874	0.122	0.004	0.935	0.065
g.1165 G>C	Non-synonymous	GG	GC	CC	G	C
R359P	Arginine to proline	0.515	0.405	0.08	0.717	0.283

Table 7. Haplotype polymorphisms of *TRL1A* Gene in Sentul chicken

Haplotype	g.710 C>T	g.822 A>G	g.835 C>T	g.1015 G>A	g.1165 G>C	Frequency (%)
Haplotype 1	C	A	C	G	G	60.87
Haplotype 2	C	A	C	G	C	13.04
Haplotype 3	C	A	C	A	C	6.52
Haplotype 4	C	A	T	G	C	4.35
Haplotype 5	C	A	T	G	G	2.17
Haplotype 6	C	G	T	G	G	2.17
Haplotype 7	C	G	T	G	C	4.35
Haplotype 8	T	A	C	G	G	2.17
Haplotype 9	T	G	T	G	G	4.35

Discussion

Immune response refers to how the body protects the normal metabolism system from attack or onset of foreign objects or microbes as well as bacteria, viruses, parasites, and protozoa, which could infect the body (Abbas et al. 2021). Innate immunity and Natural Anti Body (NAb) are antibodies present in healthy individuals, which can be detected without prior exposure to an external antigen (Berghof et al. 2018; Palma et al. 2018). In certain animal species, elevated levels of these antibodies were linked to better health and disease resistance (Grönwall et al. 2014; Elluru et al. 2015). In addition, a previous study by Regar et al. (2013) showed that livestock with high resistance (high total IgY) had good productive and reproductive performance.

Toll Like Receptor (TLR) is a hormone that was coded by *TLR* gene and plays a major role in immune system function in poultry (Ruan and Zheng 2011; Berghof et al. 2018; Velová et al. 2018; Nihashi et al. 2019; Rehman et al. 2021; Bovenhuis et al. 2022). Furthermore, Ruan and Zheng (2011), revealed that Toll-Like Receptors (TLRs) were essential components of the host immune response with the ability to recognize Pathogen-Associated Molecular Patterns (PAMPs). These patterns included lipopolysaccharides, lipopeptides, flagellins, double-stranded RNA, or CpG DNA motifs, thereby triggering immune responses. Polymorphisms of TLR gene and Single Nucleotide Polymorphism (SNP) have been reported to have the potential to affect their recognition of pathogen-associated molecular patterns, leading to varied host resistance and immune system (Ruan and Zheng 2011; Berghof et al. 2018; Bovenhuis et al. 2022). The rapid molecular and functional dissection of chickens' TLR repertoire was a major step toward the enhanced comprehension of the innate immune system and disease resistance of chickens (Keestra et al. 2013).

This study found synonymous mutation g.710C>T (L207L), which did not alter the amino acid coding of leucine. Several studies have shown that leucine (Leu) is one of the essential amino acids with a direct effect on protein biosynthesis. In addition, its interaction with lycine had a quadratic effect on the digestibility of leucine in broiler skeletal muscle (Amirdahri et al. 2023). Some Single Nucleotide Polymorphisms (SNPs), known as synonymous polymorphisms, did not cause changes in the amino acid sequence of proteins due to the redundancy of the genetic code (Yates and Sternberg 2013). Certain mutations were known to alter DNA sequences without changing the underlying protein sequence. Although it was long believed that these “synonymous” mutations were effectively neutral, half a century later, genome-wide analyses had begun to reveal both the evolutionary forces shaping synonymous mutations and their potential phenotypic effects (McGrath 2021). Furthermore, Kristofich et al. (2018) stated that synonymous mutations do not alter the specified amino acid but may alter the structure or function of an mRNA in ways that impact fitness.

This study found 4 nonsynonymous SNP, namely g.822A>G (T245A), g.835C>T (T249M), g.1015G>A (S309N), and g.1165G>C (R359P. In addition, 3 of these nsSNP were observed in this study, including g.822A>G (T245A), g.835C>T (T249M), and g.1165G>C (R359P) in line with Ruan and Zheng (2011) who found the component on 9 poultry breeds, except for nsSNP g.1015G>A (S309N). Non-synonymous SNPs typically lead to a modified amino acid that could potentially impact the structure or function of protein. This variability in non-synonymous SNPs could possibly influence the protein’s structural and functional attributes (Yates and Sternberg 2013). A previous study by Betts and Russell (2003) stated that non-synonymous Single Nucleotide Polymorphisms (nsSNPs) located on protein’s surface were often better tolerated, specifically when the side chain faced away from the protein and into the solvent, allowing for potential accommodation of other side chains. However, when nsSNP occurred in a crucial functional region on protein’s surface, it could disrupt the function. When nsSNP altered an amino acid engaged in protein-protein interface, it had the potential to hinder the interaction between proteins.

Alleles determination based on each synonymous and non-synonymous mutation showed that the frequency of mutated alleles on all SNP was smaller compared to non-mutated variants. This result was in line with a previous study by Betts and Russell (2003) who stated that non-synonymous mutation tended to occur at a lower frequency compared to synonymous, and minor allele frequency tended to be lower in non-synonymous SNPs. In this study, haplotype construction included synonymous mutations. Haplotype construction was based on mutations in a set of concurrently inherited genomes. A haplotype is a collection of DNA variants arranged along a single chromosome, commonly inherited as a unit. This tendency arose from their proximity on the chromosome, leading to infrequent

recombination events between these variants. Haplotypes could range from comprising a sole gene to being more extensive, including multiple genes within their structure.

Chickens TLR system had several unique properties both at the level of ligand specificity, the formation of TLR receptor complexes, and activated TLR signaling pathways. This knowledge opened novel opportunities for the rational design of immuno-modulatory agents for use in chickens, but also for a targeted search for desired natural variations in TLR function among breeds (Keestra et al. 2013). The results of some studies regarding TLR gene mutations in poultry were related to endurance. A previous report on antibody candidate gene polymorphism in chickens (Berghof et al. 2018) suggested that modifications in the form of SNPs in the *TLR1A* gene located on chromosome 4 were significantly associated with the concentration of KLH binding Ig Total/Nab (Natural Antibody) and IgM. Based on nsSNP F126L, which altered phenylalanine to leucine, there was a 0.65-point difference in antibody titers between CC and GG genotypes in the base population of chickens maintained at the Hendrix Genetics facility (Berghof et al. 2018). In a study by (Bovenhuis et al. 2022), 7th-generation chickens, High Line strains in commercial Purebred Elite White Leghorn chickens (Hendrix Genetic), the expected and observed frequencies of the C allele in the *TLR1A* gene were 0.77 and 0.66, respectively. Meanwhile, for the Low Line strain, the expected and observed frequencies of the C allele were 0.04 and 0.07. Several studies (Berghof et al. 2018) and (Bovenhuis et al. 2022) have also reported the main effect of *TLR1A* gene polymorphism on Ig Total and IgM, stating that its action could be a selection marker for general disease resistance.

A recent report by Ruan and Zheng (2011) was conducted on polymorphic sites of ChTLR1 type 1 and type 2 across different chicken breeds. The results showed the presence of variations in amino acids within the ChTLR1 type 1 and type 2 proteins. Based on the known correlation between TLR1 polymorphisms and susceptibility to bacterial infections in humans and mice, it was suggested that polymorphisms in ChTLR1 type 1 and type 2 could be linked to the resistance or susceptibility of chickens to infectious disease.

Several studies have been conducted on TLR gene polymorphism linked with feed consumption to determine the mode of alteration of TLR gene expression by nutrients. These interventions revealed the nutrigenomic potential of Toll-Like Receptors (TLRs) in improving the health and productivity of chickens through dietary supplementation with specific nutrients, particularly phytonutrients. In addition, variations in TLRs had been identified, suggesting potential associations with disease susceptibility and resistance in chickens. The genetic variations, including Single Nucleotide Polymorphisms (SNPs), were valuable for genomic selection to produce animals with enhanced genetic resistance and resilience against disease (Rehman et al. 2021). TLR ligands had been reported to have varying abilities to induce antiviral responses against avian influenza virus in chickens macrophages. The timing of treatment with TLR ligands and the dosage could influence the activation of macrophages and subsequent outcomes. Transcriptional analysis had shown that TLR2, TLR4, and TLR21 ligands could stimulate the expression of key immune response genes, such as IL1b, IFN-c, IRF7, and IFN-b in macrophages, which contributed to the control of avian influenza virus replication in the cells (Barjesteh et al. 2014).

In dairy cattle (*Bos taurus*), the results of Bhavaniramya et al. (2019) demonstated that Toll-Like Receptors (TLRs) were essential in initiating innate immune responses, particularly in identifying invading pathogens within mammary gland cells. Variability in gene expression of TLRs was notable, with several Single Nucleotide Polymorphisms (SNPs) associated with increased susceptibility to infectious disease. In addition, assessments using SIFT, PolyPhen2, and I-mutant tools revealed that non-synonymous SNPs in TLR2 were more detrimental, causing amino acid substitutions that subsequently impacted the protein's structure and function. Within TLR2 genes, significant mutations were detected at positions R563H and T605M. An analysis of available inhibitors showed that these drug molecules exhibited reduced binding affinity with the mutant forms of TLR2, as observed in the 3D models of both native and mutated structures. The comprehensive outcomes of this study suggested that mutations R563H and T605M within TLR2 could represent high-risk, non-synonymous SNPs, potentially impacting both the design and functionality of TLR2 (Bhavaniramya et al. 2019).

Human studies have shown that mutations in the TLR group were associated with disease. Several reports on humans also revealed that TLR1 and HLA-DRB1/DQA1 were significant susceptibility genes related to the likelihood of developing leprosy. In addition, leprosy, a chronic condition characterized by granulomas affecting the skin and peripheral nerves, was caused by *Mycobacterium leprae*. Analyses focusing on population differentiation revealed substantial differentiation at the TLR1 locus. The TLR1 1805G>T (Ser602Ile, rs5743618) polymorphism, a non-synonymous variant affecting the transmembrane domain of TLR, exhibited a strong Linkage Disequilibrium (LD) with TLR1−7202A>G. Individuals carrying the isoleucine allele showed heightened cell surface expression of TLR1 on peripheral monocytes (Johnson et al. 2007). SNP 1805G>T (Ser602Ile TLR1) had been observed to provide protection against leprosy within populations affected by this disease. In studies comprising affected patients and asymptomatic individuals from endemic regions, the 602Ser allele was notably less prevalent in the studied leprosy patients group.

TLRs are the main elements of the immune system and their function must be maintained to keep the integrity of innate immunity. Targeting of TLR signaling represented a new challenge for the treatment of many diseases (El-Zayat et al. 2019). Berghof et al. (2018) revealed that SNP in *TLR1A* generated fundamental role of *TLR1A* on regulation of IgM levels (i.e., KLH-binding IgM NAb, and total IgM concentration), or B cells biology, or both. Natural Antibodies (NAb) are antigen-binding antibodies present in individuals without previous exposure to this antigen. Selective breeding for Keyhole Limpet Hemocyanin (KLH)-binding NAb may increase survival by means of

improved general disease resistance (Berghof et al. 2018). In addition, TLRs tended to hold the potential to serve as a perfect target in the era of immunotherapies (Farooq et al. 2021)

Based on previous studies in chickens, cattle, and humans, mutations in the *TLR1A* gene were associated with disease and the immune system. The discovery of 5 non-synonymous SNPs in native Indonesian chickens was thought to affect the function of the *TLR1A* hormone in the metabolic system of disease resistance in chickens. The results of this study could serve as the foundation for further studies and to determine whether the detected nsSNP had the potential to be used as a Marker Assisted Selection (MAS) for disease resistance, specifically in local chickens in Indonesia.

In conclusion, there were genetic polymorphisms in the *TLR1A* gene in Indonesian indigenous chicken. One synonymous mutation, g.710C>T which amino acid coded leusine at 206th, and a non-synonymous mutation, g.822A>G, altered amino acids coding from threonine to alanine (T243A) were found only in Sentul chicken. Non-synonymous g.835C>T, that altered amino acids coding 247th from threonine to methionine (T247M) were found in Kedu and Sentul chicken, and g.1015G>A that altered amino acids coding 307th from serine to aspargine (S307N) were found in Sentul and Pelung chicken. Non-synonymous mutation g.1165T>G that altered amino acids from arginine to proline (R357P) was found in all chickens. The allele mutation frequency is lower than the non-mutation allele. The non-synonymous mutation may alter the *TLR1A* hormone in metabolism related to general immune system in indigenous chickens of Indonesia.

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REFERENCES

Abbas AK, Lichtman AH, Pillai S. 2021. Cellular and Molecular Immunology. 10th Edition. Saunders, Elsevier, Philadelphia.

Amirdahri S, Janmohammadi H, Taghizadeh A, Lambert W, Olyayee M, Assadi Soumeh E. 2023. The optimum ratio of digestible leucine: Lysine in wheat-based diets for female broiler chickens from 8-21 days of age. J Appl Poult Res 32 (2): 100289. DOI: 10.1016/j.japr.2022.100289.

Asmara IY, Hilmia N, Garnida D. 2023. The long-crowing chickens in Indonesia: A review. Open Agric 8: 20220220. DOI: 10.1515/opag-2022-0220.

Barjesteh N, Behboudi S, Brisbin JT, Villanueva AI, Nagy É, Sharif S. 2014. TLR ligands induce antiviral responses in chicken macrophages. PLoS One 9 (8): e105713. DOI: 10.1371/journal.pone.0105713.

Berghof TVL, Visker MHPW, Arts JAJ, Parmentier HK, van der Poel JJ, Vereijken ALJ, Bovenhuis H. 2018. Genomic region containing toll-like receptor genes has a major impact on total IGM antibodies including KLH-binding IGM natural antibodies in chickens. Front Immunol 8: 1879. DOI: 10.3389/fimmu.2017.01879.

Betts MJ, Russell RB. 2003. Amino acid properties and consequences of substitutions. In: Barnes MR, Gray IC (eds). Bioinformatics for Geneticists. John Wiley and Sons, England. DOI: 10.1002/0470867302.ch14.

Bhavaniramya S, Vanajothi R, Vishnupriya S, Al-Aboody MS, Vijayakumar R, Baskaran D. 2019. Computational characterization of deleterious SNPs in Toll-like receptor gene that potentially cause mastitis in dairy cattle. Biocatal Agric Biotechnol 19: 101151. DOI: 10.1016/j.bcab.2019.101151.

Bovenhuis H, Berghof TVL, Visker MHPW, Arts JAJ, Visscher J, van der Poel JJ, Parmentier HK. 2022. Divergent selection for natural antibodies in poultry in the presence of a major gene. Genet Sel Evol 54 (1): 24. DOI: 10.1186/s12711-022-00715-9.

Chen Y, Tibbs-Cortes LE, Ashley C, Putz AM, Lim K-S, Dyck MK, Fortin F, Plastow GS, Dekkers JCM, Harding JCS, Canada P. 2020. The genetic basis of natural antibody titers of young healthy pigs and relationships with disease resilience. BMC Genomics 21 (1): 648. DOI: 10.1186/s12864-020-06994-0.

Dar MA, Urwat U, Ahmad SM, Ahmad R, Kashoo ZA, Dar TA, Bhat SA, Mumtaz PJ, Shabir N, Shah RA, Heidari M. 2019. Gene expression and antibody response in chicken against *Salmonella typhimurium* challenge. Poult Sci 98 (5): 2008-2013. DOI: 10.3382/ps/pey560.

Das DN, Karuthadurai T, Gnanasekaran S. 2021. Genomic selection: A molecular tool for genetic improvement in livestock. In: Mondal S, Singh RL (eds). Advances in Animal Genomics. Academic Press, London. DOI: 10.1016/B978-0-12-820595-2.00010-2.

Doekes HP, Bovenhuis H, Berghof TVL, Peeters K, Visscher J, Mulder HA. 2023. Research note: Genome-wide association study for natural antibodies and resilience in a purebred layer chicken line. Poult Sci 102 (1): 102312. DOI: 10.1016/j.psj.2022.102312.

Elluru SR, Kaveri SV, Bayry J. 2015. The protective role of immunoglobulins in fungal infections and inflammation. Semin Immunopathol 37 (2): 187-197. DOI: 10.1007/s00281-014-0466-0.

El-Zayat SR, Sibaii H, Mannaa FA. 2019. Toll-like receptors activation, signaling, and targeting: An overview. Bull Natl Res Cent 43: 187. DOI: 10.1186/s42269-019-0227-2.

Farooq M, Batool M, Kim MS, Choi S. 2021. Toll-like receptors as a therapeutic target in the era of immunotherapies. Front Cell Dev Biol 9: 756315. DOI: 10.3389/fcell.2021.756315.

Grönwall C, Silverman GJ. 2014. Natural IgM: beneficial autoantibodies for the control of inflammatory and autoimmune disease. J Clin Immunol 34: S12-S21. DOI: 10.1007/s10875-014-0025-4.

Indrijani H, Anang A, Fadillah MF, Hilmia N, Fitriani M. 2024. Growth curve and heterosis effect of egg weight, doc weight, fertility and hatchability from crossing of Sentul Debu and Kulawu chicken at poultry breeding Jatiwangi. Adv Anim Vet Sci 12 (2): 363-370. DOI: 10.17582/journal.aavs/2024/12.2.363.370.

Ismoyowati I, Sukardi, Susanto A. 2012. Genetic diversity of kedu chicken based on phenotypic characteristics and microsatellite loci. Intl J Poult Sci 11 (9): 605-610. DOI: 10.3923/ijps.2012.605.610.

Johnson CM, Lyle EA, Omueti KO, Stepensky VA, Yegin O, Alpsoy E, Hamann L, Schumann RR, Tapping RI. 2007. Cutting edge: A common polymorphism impairs cell surface trafficking and functional responses of TLR1 but protects against leprosy. J Immunol 178 (12): 7520-7524. DOI: 10.4049/jimmunol.178.12.7520.

Keestra AM, de Zoete MR, Bouwman LI, Vaezirad MM, van Putten JPM. 2013. Unique features of chicken Toll-like receptors. Dev Comp Immunol 41 (3): 316-323. DOI: 10.1016/j.dci.2013.04.009.

Kristofich J, Morgenthaler AB, Kinney WR, Ebmeier CC, Snyder DJ, Old WM, Cooper VS, Copley SD. 2018. Synonymous mutations make dramatic contributions to fitness when growth is limited by a weak-link enzyme. PLoS Genet 14 (8): e1007615. DOI: 10.1371/journal.pgen.1007615.

Makepeace BL, Psifidi A, Robledo D, Xia D. 2021. Editorial: Genetics architecture and underlying molecular mechanisms in host-pathogen interactions. Front Genet 12: 695109. DOI: 10.3389/fgene.2021.695109.

McGrath C. 2021. Synonymous but not equal: A special section and virtual issue on phenotypic effects of synonymous mutations. Genome Biol Evol 13 (9): evab186. DOI: 10.1093/gbe/evab186.

Nei M, Kumar S. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, Oxford.

Nihashi Y, Ono T, Kagami H, Takaya T. 2019. Toll-like receptor ligand-dependent inflammatory responses in chick skeletal muscle myoblasts. Dev Comp Immunol 91: 115-122. DOI: 10.1016/j.dci.2018.10.013.

Palma J, Tokarz-Deptuła B, Deptuła J, Deptuła W. 2018. Natural antibodies facts known and unknown. Cent Eur J Immunol 43 (4): 466-475. DOI: 10.5114/ceji.2018.81354.

Rajput M, Thakur N. 2023. Editorial: Advances in host-pathogen interactions for diseases in animals and birds. Front Vet Sci 10: 1282110. DOI: 10.3389/fvets.2023.1282110.

Regar MN, Mutia R, Widhyari SD, Kowel YHS. 2013. Pemberian ransum kombinasi herbal dengan mineral zink terhadap performan ayam broiler yang diinfeksi *Escherichia coli*. Jurnal Zootek 33 (1): 35-40. DOI: 10.35792/zot.33.1.2013.3333. [Indonesian]

Rehman MS-U, Rehman SU, Yousaf W, Hassan F-U, Ahmad W, Liu Q, Pan H. 2021. The potential of toll-like receptors to modulate avian immune system: Exploring the effects of genetic variants and phytonutrients. Front Genet 12: 671235. DOI: 10.3389/fgene.2021.671235.

Ruan WK, Zheng SJ. 2011. Polymorphisms of chicken toll-like receptor 1 type 1 and type 2 in different breeds. Poult Sci 90 (9): 1941-1947. DOI: 10.3382/ps.2011-01489.

Rusfidra R, Arlina F. 2014. A review of "long crower chickens" as poultry genetic resources in Indonesia. Intl J Poult Sci 13 (11): 665-669. DOI: 10.3923/ijps.2014.665.669.

Velová H, Gutowska-Ding MW, Burt DW, Vinkler M. 2018. Toll-like receptor evolution in birds: Gene duplication, pseudogenization, and diversifying selection. Mol Biol Evol 35 (9): 2170-2184. DOI: 10.1093/molbev/msy119.

Yates CM, Sternberg MJE. 2013. Proteins and domains vary in their tolerance of non-synonymous Single Nucleotide Polymorphisms (nsSNPs). J Mol Biol 425: 1274-1286. DOI: 10.1016/j.jmb.2013.01.026.

Zhang J, Tan H, Cao Q, Su G, Yang P. 2021. Meta-analysis of miRNA variants associated with susceptibility to autoimmune disease. Dis Markers 2021: 9978460. DOI: 10.1155/2021/9978460.