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Polymorphisms and Associations of the NRAMP-1 and iNOS Genes on *Newcastle Disease* and *Salmonella enteritidis* Resistances in SenSi-1 Agrinak Chickens

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ABSTRACT

NRAMP-1 and iNOS genes were reported to be associated with a defense mechanism against bacteria and virus infections. This study aimed to identify NRAMP-1 and iNOS genes polymorphisms and their associations with the defense mechanisms against *Salmonella enteritidis* **and** *Newcastle Disease* **(ND) in SenSi-1 Agrinak chicken. The present study used a total number of 172 SenSi-1 Agrinak chicken. Identifications of NRAMP-1 and iNOS genes polymorphisms were performed by PCR-RFLP method. NRAMP-1 and iNOS genotypes were associated with immunoglobulin Y (IgY) concentration, specific antibodies against** *S. enteritidis* **and ND using General Linear Model (GLM). Immunity characteristics were further grouped into high, medium, and low categories. NRAMP-1 |** *Sac***I exon 11 and iNOS |** *Alu***I intron 24 in SenSi-1 Agrinak chickens were polymorphic. TC genotype has a higher immune response to infectious agents compared to TT and CC genotypes. The frequency of C allele was higher than the T allele in the concentration of immunoglobulin Y (IgY), antibodies titers against** *S enteritidis* **and ND. The TC genotype of NRAMP-1 gene was significantly associated with ND antibody titers, and the TT genotype of iNOS was significantly associated with** *S. enteritidis* **specific antibody. NRAMP-1 and iNOS genes can be used as potential candidate genes for immune traits in SenSi-1 Agrinak chickens.**

Keywords: disease resistance; iNOS gene; NRAMP-1 gene; polymorphism; SenSi-1 Agrinak chicken

INTRODUCTION

SenSi-1 Agrinak chicken breed was released from Indonesian Research Institute for Animal Production (IRIAP) to be used as a commercial meat type of local chicken in Indonesia. This breed was selected from the native breed of Sentul chicken, originating from Ciamis District of West Java Province. The Sentul male chickens were selected to achieve the average live weight of 1 kg/bird at ten weeks of age, with grey (G) and black-spotted white (BSW) plumage color and pea-comb type. SenSi-1 Agrinak was released as an improved local meat-type chicken breed after six generations of selection (Iskandar, 2018).

The advantage of this breed is its relatively fast growth rate and potential for meat production. The potential of the Sentul chicken makes it possible to be bred as a popular local chicken farm commodity. The challenge that is often faced by the local chicken farms in the extensive system is the low disease resistance (Pagala & Nafiu, 2012). The most prevalent diseases are caused by *Newcastle Disease* (ND) virus and *Salmonella enteritidis* (*S. enteritidis*).

Newcastle Disease (ND) is a disease caused by a virus and is an important limiting factor in chicken farming. *Newcastle Disease* (ND) is endemic in Indonesia, such as chicken Tolaki, which is susceptible to ND disease (Pagala & Nafiu, 2012). The period of ND virus is 4-6 days and the clinical symptoms are sudden death, drooping wings, weakness, loss of appetite, green diarrhea, and decreased egg production. *Newcastle Disease* (ND) virus can be spread through the air, feed, transport vehicles, wild birds, predators, clothing, and cage equipment (Pagala & Nafiu, 2012).

Salmonella is a bacteria that is easy to grow and can adapt to various forms of environmental conditions. Contamination of *Salmonella* sp. may be found in chicken eggs. *S. enteritidis* is one of the zoonotic bacteria that can be transmitted through food (*food-borne disease*). Ulupi *et al.* (2013) found that 3.12% of chicken eggs were positively contaminated with *S. enteritidis* in Bogor. The incidence of salmonellosis in humans is caused by con-

suming chicken eggs containing the bacteria *S. enteritidis* (Velge *et al*., 2005). Ulupi *et al*. (2014) state that it is important to guarantee that the eggs of Kampung chicken are free from salmonellosis so that the eggs are safe for consumption.

Prevention of diseases is commonly done by vaccination. Vaccination is rarely conducted by producers with a very small amount of chicken. These conditions expose the small-scale farms of domestic chickens to diseases infection leading to the death of the chicken. This problem can be controlled by selecting chickens that resistance to those infectious agents. Genetic improvement has been accomplished mainly through the approach of molecular-genetic selection. The identification of SNP-based polymorphism is commonly used to determine the genetic variation of genes encoding certain traits (Gunawan *et al.,* 2018). Many genes play roles in activating the resistance against diseases such as the Natural Resistance Associated Macrophage Proteins-1 (NRAMP-1) gene and Inducible Nitric Oxide Synthase (iNOS) gene.

The Natural Resistance Associated Macrophage Proteins-1 (NRAMP-1) gene and Inducible Nitric Oxide Synthase (iNOS) gene work in synergy with macrophage cells against pathogens. Macrophages and monocytes are part of an innate immune response and effectors and regulators of inflammation (Delgado *et al*., 2010). As an effector cell, monocyte equipped with chemokine receptors and pathogen recognition receptors. That can mediate the migration of monocytes from blood to tissues during infection. Monocytes can also differentiate into macrophages during inflammation. Migrations of monocytes to tissues and their further differentiation to macrophages are determined by the inflammatory milieu and pathogen-associated pattern of recognition receptors (Serbina *et al*., 2008). Monocytes will change shape and structure to become macrophage cells, which can immediately function as phagocytic cells. Phagocyte cells function to capture, swallow, kill, and destroy the antigens. Macrophages present these antigen particles to the surface as Antigen Presenting Cell (APC) (Wibawan & Soedjono, 2013; Wils-Plotz & Klasing, 2016). Subsequent reactions will induce the occurrence of specific immune responses mediated by helper T cells and B cells to produce specific antibodies (Abbas *et al*., 2017). The expression of the NRAMP-1 and iNOS genes in macrophage cells will facilitate the macrophages to engulf and kill pathogens or bacteria that successfully penetrate the tissue.

Several studies have shown that the NRAMP-1 and iNOS genes are associated with chicken resistance to *S. enteritidis* (Lamont *et al.,* 2002; Malek & Lamont, 2003; Tohidi *et al.,* 2013). The study of He *et al.* (2013) reported that mutations at site g.24101991 A>T of NRAMP-1 gene were associated significantly with chicken resistance to *S. enteritidis*. The expression of the high NRAMP-1 and iNOS genes were found in liver and intestinal tissue (He *et al*. 2013; Sundaresan *et al*. 2005). Also, several studies have shown that there are genes controlling chicken resistance to diseases, such as the NRAMP-1 and iNOS genes (Tohidi *et al*., 2013). Muhsinin *et al.* (2016) reported that NRAMP-1 and iNOS genes were associated with immune traits in Indonesian local chicken. However, no study reported that NRAMP-1 and iNOS genes were found in selected local chicken. Therefore, the aim of this study to identify NRAMP-1 and iNOS genes polymorphisms and their associations against *S. enteritidis* and ND in SenSi-1 Agrinak chicken.

MATERIALS AND METHODS

The present study used SenSi-1 Agrinak chickens from Indonesian Research Institute for Animal Production (IRIAP). This research used 172 SenSi-1 Agrinak chickens of 24 weeks of age, consisting of 38 males and 134 females. The DNA was extracted from the blood samples by phenolchloroform method (Sambrook *et al*., 2001) at the Animal Molecular Genetics Laboratory, Faculty of Animal Science, IPB University and biological assays were conducted at the Division of Medical Microbiology, Faculty of Veterinary Medicine, IPB University, Indonesia.

NRAMP-1 and iNOS Genotyping

Identifications of NRAMP-1 and iNOS gene polymorphisms were performed in four stages: DNA extraction, DNA amplification, PCR-restriction fragment length polymorphism (PCR-RFLP) analysis, and DNA fragment visualization (Sambrook *et al*., 2001). The SNPs and primers for NRAMP1 and INOS were used according to Muhsinin *et al.* (2016). The primers for the NRAMP-1 gene (F: 5′-CAATGAGACGGTGTCTGTGG-3′, R: 5′-CCCAGAAGAAATCTCCCTGC -3′) and INOS gene (F: 5′-CCAAGGACTTACAGGTGTGG-3′, R: 5′-CCAGGATGTTTGGGCTGTTG -3′) were annealed at 60°C for 20 sec, respectively. The SNP at position 421 bp (GAGCT|C) on chromosome 7 (exon 11) differentiated into different allelic transcripts of the NRAMP1 gene using the *Sac*I enzyme. While the SNP at position 449 bp (AG|CT) of chromosome 19 (intron 24) was differentiated into allelic transcripts of the INOS gene using the *Alu*I enzyme. The Gen Bank accession numbers for the NRAMP1 and INOS genes are AY072001.1 and AF537190.1, respectively. The PCR-RFLP products were visualized using agarose gel electrophoresis for the NRAMP1 and INOS genes with concentrations of 2.0%.

Biological Assays

Biological assays were used to analyze resistance factors in chickens, namely concentrations of immunoglobulin Y (IgY), Salmonella specific-antibodies (*S. enteritidis*), and ND antibodies titer (HI test). Immunoglobulin Y (IgY) concentrations were measured using indirect Enzyme-linked immunosorbent assays (ELISA), according to Mamutse *et al*. (2018). The analysis of Salmonella's specific-antibodies was carried out using the Clearance test. The clearance test is a method to measure the ability of antibodies to inhibit the growth of Salmonella bacteria. ND antibodies were measured using the haemagglutination inhibition to determine the antibodies titers again ND (Webster *et al.,* 2002).

Data Analysis

Genotype and allele frequencies observed and expected heterozygosities of the genotypes obtained via the PCR-RFLP method, Hardy-Weinberg equilibrium values, and polymorphic information contents (PICs) were calculated by POPGEN32 software. Association between the NRAMP-1 and iNOS genotypes with the immune properties of SenSi-1 Agrinak chickens was analyzed by analysis of variance (ANOVA) on the variables of immune properties analyzed using the GLM (General Linear Model) procedure with SAS 9.4 software (SAS Institute, Cary, NC, USA) and descriptive analysis with individual and categorized immune traits.

The association model used was as follows:

$$Y_{ijk} = \mu + G_i + S_j + C_k + \varepsilon_{ijk}$$

where Y_{ijk} was observations of immune traits, μ was population mean, G_i was effect of the genotype, S_j was effect of the sex, C_k was effect of the category, and ε_{ijk} was residual error.

The immune parameters were further categorized into three groups, i.e., low, medium, and high titers. The criteria used for the titer ranges for the respective groups of IgY were <5 (low), 5≤x≤8 (medium), and >8-15 μg/mL (high), whereas the *S*. antibody titers (Log10) were <4 (low), 4≤x≤6 (medium), and >6 cfu/mL (high), and the HI antibody titers (Log2) were <3 (low), 3≤x≤5 (medium), and >5 cfu/mL (high).

RESULTS

NRAMP-1 exon 11 and iNOS intron 24 genes of SenSi-1 Agrinak chickens were successfully amplified to produce PCR product lengths of 421 bp and 449 bp, respectively (Figure 1). The digestion of iNOS intron 24 fragments with *Alu*I restriction enzymes resulted in 3 different restriction patterns (Figure 2). The first pattern was a gene fragment truncated by the *Alu*I enzyme, indicated by two fragments at positions 310 bp and 139 bp designed the CC genotype. The second pattern had no *Alu*I restriction site resulting in one band at position 449 bp (TT genotype). The third pattern was a combination of all three fragments at positions 449, 310, and 139 bp (TC heterozygous genotype). The RFLP analysis using the *Sac*I restriction enzyme on the NRAMP-1 gene fragment in exon 11 resulted in 3 different fragments. Uncut Fragments by *Sac*I enzymes (421 bp) were TT genotypes, truncated fragments (258 bp and 163 bp) were CC genotypes, and combined fragments (421 bp, 258 bp, and 163 bp) or heterozygotes were TC genotypes (Figure 2).

The immune response of SenSi-1 Agrinak chickens for NRAMP-1 and iNOS genes were shown in Table 1. The average titers of IgY, *S. enteritidis* antibody, and ND antibody were 7.89±3.96 μg/mL, $3.5\times10^9\pm7.4\times10^9$ cfu/mL, and (Log 2) 3.11±2.19, respectively (Table 1). The genotype and allele frequencies of NRAMP-1 and iNOS in SenSi-1 Agrinak chicken were shown in Table 2. The distributions of genotype and allele frequencies were

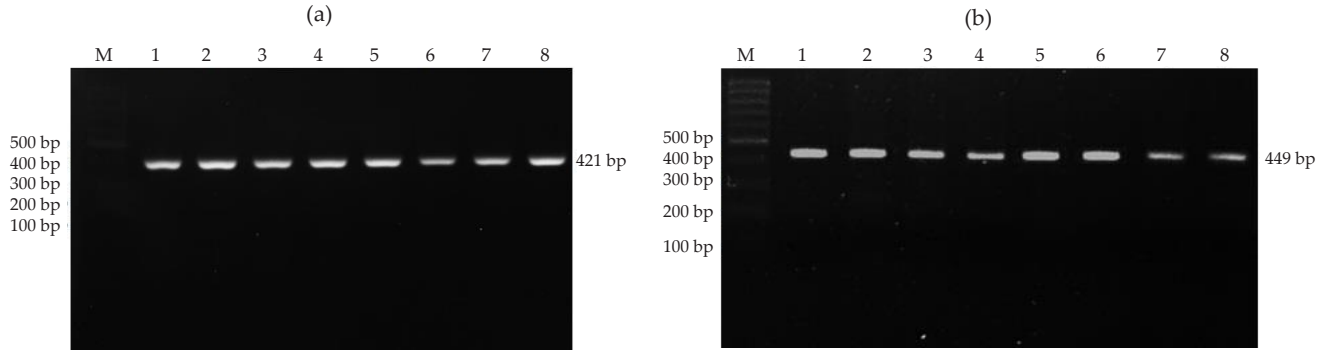

Figure 1. Amplification results of NRAMP1 gene exon 11 (a) and INOS gene intron 24 (b) in SenSi-1 Agrinak chicken. M= marker DNA 100 bp.

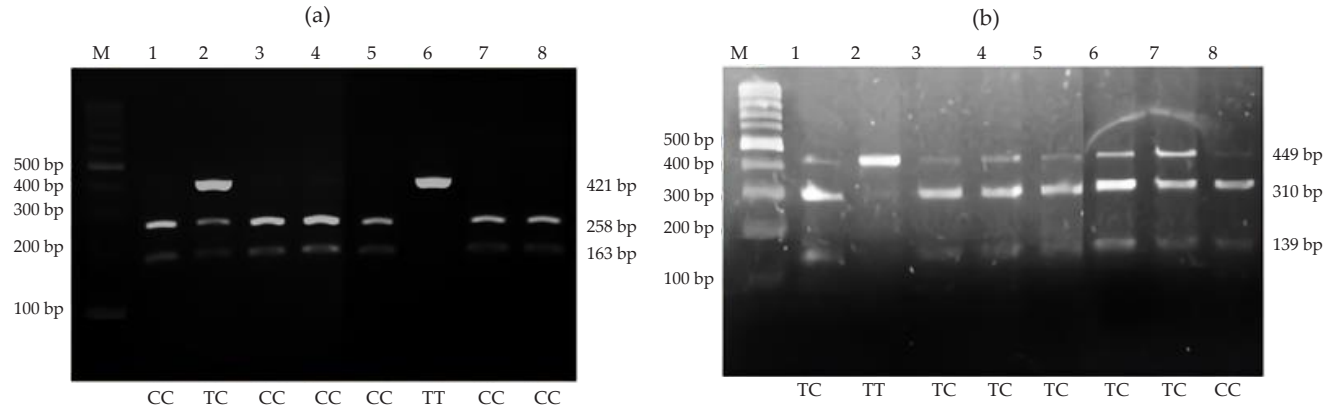

Figure 2. PCR-RFLP fragment results of NRAMP-1 gene exon 11 with restriction enzyme *Sac*I (a) and INOS gene intron 24 with restriction enzyme *Alu*I (b) in 2% agarose gel M= marker DNA 100 bp (SenSi-1 Agrinak chicken).

as follows: NRAMP-1 (TT=0.023, TC=0.238, CC=0.738, T=0.142, and C=0.858) and iNOS (TT=0.070, TC=0.733, CC=0.157, T=0.456, and C= 0.544).

Heterozygosity and polymorphic information contents of NRAMP-1 and iNOS in SenSi-1 Agrinak chicken are shown in Table 3. The values of Ho, He, and PIC showed for the NRAMP-1 were 0.238, 0.244, and 0.460, respectively, and for the iNOS were 0.773, 0.496, and 0.956, respectively. The calculated (Chi-square) values for the NRAMP-1 gene (0.101) was significantly smaller than the critical value (3.84), different from iNOS gene (53.634) that was significantly higher than the critical value (3.84).

Genotypes and allele frequencys of NRAMP-1|*Sac*I and INOS|*Alu*I of immune response categorized in SenSi-1 Agrinak chicken were shown in Tables 4, 5, 6, and 7. The CC genotypes of NRAMP-1 gene were higher in low and medium titers of IgY, different from TC genotype that was higher in high IgY titer (Table 4). The frequency of allele C of NRAMP-1 gene was higher than the frequencies of allele T in low, medium, and high titers of IgY (0.824, 0.914, and 0.680) (Table 4). However, there was higher allele T (0.514) in low category of iNOS gene (Table 5). The CC genotype, TC genotype, and allele C frequencies were high in categorized immune responses of NRAMP-1|*Sac*I (Table 6) and iNOS|*Alu*I (Table 7).

Association of the categorized immune responses of NRAMP-1|*Sac*I and iNOS|*Alu*I of categorized immune traits in SenSi-1 Agrinak chicken were shown in Table 8. The TC genotype of NRAMP-1|*Sac*I was significantly associated with ND antibodies/ HI titers. The association of the iNOS showed significantly associated with *S.enteritidis* antibodies. The TT genotype of the iNOS with *S.enteritidis* antibodies showed significantly higher than TC and CC genotypes (Table 8).

DISCUSSION

NRAMP-1 and iNOS gene fragments were amplified at an annealing temperature of 60 °C for 20 seconds using the ESCO Thermocycler machine. This result is in accordance with the result reported by Muhsinin *et al.* (2016), but different from the result reported by Liu *et al*. (2016) and Ramasamy *et al*. (2011) at annealing temperature that has been carried out for the primers of NRAMP-1 exon 11 and iNOS intron 24 gene fragments, each at 64 °C for 90 seconds and 55 °C for 1 minute.

The difference in annealing temperatures in both genes was caused by differences in the condition of the PCR machine and the mixture of PCR reagents. PCR optimization requires the right DNA polymerase. Harbison & Nguyen (2017) stated that the annealing temperature ranged from 55-72 °C. Besides that, the

Table 1. Descriptive statistics of immune traits of all investigated SenSi-1 Agrinak chickens

Variables	Mean	St Dev	Minimum	Maximum
IgY titer (μg/mL)	7.89	3.96	0.96	14.95
S. antibody titer (cfu/mL)	3.5×10^9	7.4×10^9	4.4×10^3	3.7×10^{10}
HI antibody titer (Log_2)	3.11	2.19	0	8

Table 2. Genotype and allele frequencies of NRAMP-1 dan INOS in SenSi-1 Agrinak chickens

Gene	N	Genotype frequency			Allele frequency	
		TT	TC	CC	T	C
NRAMP-1	172	0.023 (4)	0.238 (41)	0.738 (127)	0.142	0.858
INOS	172	0.070 (12)	0.733 (133)	0.157 (27)	0.456	0.544

Note: N= Total number of samples

Table 3. Heterozygosity and polymorphic information content of NRAMP-1 dan INOS in SenSi-1 Agrinak chickens

Gene	χ2 test	He	Ho	PIC
NRAMP-1	0.101^{ns}	0.244	0.238	0.216
INOS	53.634**	0.496	0.773	0.371

Note: ns= not significant, **= significant (p<0.01), x2 count<x2 table, 0.05;1=3.84. He= expected heterozygosity, Ho= observed heterozygosity, PIC= polymorphism information content.

Table 4. Genotype and allele frequency of NRAMP-1|*Sac*I with IgY antibody

Group of IgY antibody NRAMP-1	Total (N)	Genotype			Genotype frequency			Allele frequency	
		TT	TC	CC	TT	TC	CC	T	C
Low	37	1	11	25	0.027	0.297	0.676	0.176	0.824
Medium	93	2	12	79	0.022	0.129	0.849	0.086	0.914
High	25	1	14	10	0.040	0.560	0.400	0.320	0.680
	155	4	37	114	0.026	0.239	0.735	0.145	0.855

Table 5. Genotype and allele frequency of INOS|*Alu*I with IgY antibody

Group of IgY antibody INOS	Total (N)	Genotype			Genotype frequency			Allele frequency	
		TT	TC	CC	TT	TC	CC	T	C
Low	37	4	30	3	0.108	0.811	0.081	0.514	0.486
Medium	93	2	73	18	0.022	0.785	0.194	0.414	0.586
High	25	1	19	5	0.040	0.760	0.200	0.420	0.580
	155	7	122	26	0.045	0.787	0.168	0.439	0.561

Table 6. Genotype and allele frequency of NRAMP-1|*Sac*I with HI antibody

Group of HI antibody NRAMP-1	Total (N)	Genotype			Genotype frequency			Allele frequency	
		TT	TC	CC	TT	TC	CC	T	C
Low	74	2	6	66	0.027	0.081	0.892	0.068	0.932
Medium	53	1	22	30	0.019	0.415	0.566	0.026	0.774
High	26	1	8	17	0.038	0.308	0.654	0.192	0.808
	153	4	36	113	0.026	0.235	0.739	0.144	0.856

Table 7. Genotype and allele frequency of INOS|*Alu*I with HI antibody

Group of HI antibody INOS	Total (N)	Genotype			Genotype frequency			Allele frequency	
		TT	TC	CC	TT	TC	CC	T	C
Low	74	6	61	7	0.081	0.824	0.095	0.493	0.507
Medium	53	2	38	14	0.019	0.717	0.264	0.377	0.623
High	26	1	20	5	0.038	0.769	0.192	0.423	0.577
	153	8	119	26	0.052	0.778	0.170	0.441	0.559

Table 8. Antibody titers of IgY, *S. enteritidis*, and ND at difference genotypes of NRAMP-1|*Sac*I and INOS|*Alu*I genes

Gene	Genotype	Antibody					
		IgY	N	*S. enteritidis*	N	ND	N
NRAMP-1	TT	10.507±1.934	(4)	7.600±0.741	(5)	4.250±0.973^{ab}	(4)
	TC	9.666±0.636	(37)	8.083±0.276	(36)	5.055±0.324^{a}	(36)
	CC	7.212±0.362	(114)	7.500±0.153	(116)	2.606±0.183^{b}	(113)
INOS	TT	7.570±1.500	(7)	10.000±0.535^{a}	(7)	3.125±0.779	(8)
	TC	8.230±0.359	(122)	7.842±0.126^{b}	(127)	3.332±0.202	(119)
	CC	6.339±0.778	(26)	5.782±0.295^{c}	(23)	2.769±0.432	(26)

Note: N= total individual; means in the same column with different superscripts differ significantly ($p<0.05$).

optimal temperature of annealing was influenced by the concentration of $MgCl_2$ (Viljoen *et al*., 2005). Incorrect annealing temperature can cause no primary attachment. The timing of the annealing process is related to the primary length. The primary length of 18-22 bases is enough with 30 seconds, while for primary lengths more than 22 bases, annealing time of 60 seconds is required (McPherson & Moller, 2006).

Research results of Muhsinin *et al*. (2016) and Tohidi *et al*. (2013) showed that the genotype of the NRAMP-1 gene CC had a high frequency and that the TT genotype was found to be low in both chickens. In the iNOS intron 24 gene fragment, it was found high TC genotype frequencies. Different results were reported in Barnevelder and Broiler chickens (Kramer *et al*., 2003) and in Indonesian local chickens (Muhsinin *et al*., 2016).

The results of this study indicate that the NRAMP-1|*Sac*I exon 11 and iNOS|*Alu*I intron 24 in SenSi-1 Agrinak chickens are polymorphic because there are three types of genotypes found in each of these gene fragments and allele frequencies obtained are more than 0.01 (Nei & Kumar, 2000; Togashi & Lin, 2010; Allendorf *et al*., 2013). The high frequency of C allele in SenSi-1 Agrinak chicken populations was thought to be due to breeding selection and management. Selection made by breeders is to maintain chickens that have a C allele compared to chickens that have T alleles. According to Noor (2010), the factors that influence gene frequency are selection, mutation, population mixing, internal crossing, outer crossing, and genetic drift.

The value of heterozygosity is the average percentage of heterozygous loci of each individual or the average percentage of heterozygous individuals in the population (Nei & Kumar, 2000). The observed heterozygosity values (Ho) in SenSi-1 Agrinak chicken analyzed was 0.238 on NRAMP-1|*Sac*I and 0.773 on iNOS|*Alu*I. The value of expected heterozygosity (He) in SenSi-1 Agrinak chickens analyzed was 0.244 on

NRAMP-1|*Sac*I and 0.496 on iNOS|*Alu*I. At the NRAMP-1|*Sac*I locus exon 11 showed a lower Ho value than iNOS|*Alu*I. This result can be used as an indication of the degree of endogamy (*inbreeding*) as a result of an intensive selection process (Machado *et al.,* 2003; Ryan & Ray, 2014). A large difference between the values of Ho and He can be used as an indicator of the presence of genotype imbalances in the population analyzed (Tambasco *et al*., 2003; Harbison & Nguyen, 2017).

The PIC value is an ideal index to measure the polymorphism of allele fragments. According to Botstein *et al*. (1980), if the PIC value ≥ 0.50 indicates a very informative locus, the PIC value 0.25 <PIC <0.50 indicates a fairly informative locus, and the PIC value ≤ 0.25 indicates the locus has a low informative category. Based on the statement, the value of the NRAMP-1|*Sac*I gene exon 11 belongs to the medium category. The iNOS|*Alu*I gene intron 24, the PIC value, is in a low category that is very informative.

The results of the clearance test showed that the genotype of the NRAMP-1 and iNOS genes could kill *S. enteritidis* bacteria higher than TC and TT genotypes. Bacterial death on clearance tests was caused by phagocytic processes mediated by heterophil cells and antibodies in serum blood formed by lymphocyte cells (Cherayil, 2011; Pecoraro *et al*., 2017). The results of this study are similar to those reported by Tohidi *et al*. (2012) that CC genotypes in Malaysia local chickens were more resistant to *S. enteritidis* bacterial infections.

NRAMP-1 triggers a non-immune response that works on the host cells and expressed on phagocytic cells (Cellier, 2017). NRAMP-1 functions to modulate cytocidal proteins that are toxic to the cells, such as bacterial cells and transmembrane conserving. In addition to macrophages, NRAMP-1 can form on lymph cells and the liver because it easily recognizes *lipopolysaccharides* (LPS) from Salmonella so that the host becomes resistant to Salmonella (Yuniwarti *et al.,* 2013; He *et al.,* 2013; Hu *et al.,* 2015). The results of IgAMP on NRAMP1 both homozygous and heterozygous show high IgY. In the HI test, NRAMP1 did not associate with the ND virus. It happens because the HI test detects anti-ND antibodies, which are specific immunity, which is only formed if there is an induction of ND antigens both due to natural infection and vaccination.

iNOS can inhibit bacterial metabolism in *Clearance test*. This result is proven by the ability to neutralize the bacteria close to 100%. HI testing shows that iNOS is positively associated. This result is in accordance with its function because iNOS is an intracellular enzyme that has a function of oxidizing so that it will inhibit viral replication. Susceptibility to antibiotics for antimicrobial activity against *S. enteritidis* and *Enterococcus casseliflavus*, and ability to adhere to ileal cells of chicken (Hamida *et al.,* 2015; Guo *et al*., 2016; Li *et al*., 2017).

NRAMP-1 and iNOS genes are included in the natural immune system that is genetic. Several studies have revealed the association between NRAMP-1 and iNOS genes against disease resistance, including the associations with *tuberculosis* in humans (Medapati *et al*., 2017; Mahmud *et al*., 2017), *salmonella disease* in mice (Bauler *et al*., 2017; Reventun *et al*., 2017), *tuberculosis* in cattle (Pereira-Suárez *et al*., 2006; Delgado *et al*., 2010), the nature of immunity in pigs (Dai *et al*., 2017; Yan *et al*., 2017), *Rhodococcus equi pneumonia* in horses (Halbert *et al*., 2006), salmonella and viruses in goats and sheep (Lantier *et al*., 2012; Dincel & Kul, 2015;), and *S. enteritidis* in chickens (Kramer *et al*., 2003; Tohidi *et al*., 2013; Jin *et al*., 2015; Psifidi *et al*., 2016; Sun *et al*., 2017).

CONCLUSION

NRAMP-1|*Sac*I exon 11 and iNOS|*Alu*I intron 24 in SenSi-1 Agrinak chicken were polymorphic. TC genotype of NRAMP-1 gene was associated with ND antibody, and the TT genotype of iNOS associated with *S. enteritidis* antibodies. This study demonstrated that SNP of NRAMP-1 and iNOS might be used as potential candidate markers for the selection of high immune traits in local chicken.

CONFLICT OF INTEREST

Cece Sumantri and Asep Gunawan serve as editor of the Tropical Animal Science Journal, but have no role in the decision to publish this article. The authors also declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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