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“Industry based on Knowledges”

17th-19th November 2016, Convention Hall, Andalas University

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Animal Science Faculty of Andalas University
Co-organized by :
Alumni Center of Universiti Putra Malaysia

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Biosciences and Social Science 2016**

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**17th – 19th November 2016, Convention Hall, Andalas
University, Padang, West Sumatera, Indonesia**

Organized by:

**Animal Science Faculty of Andalas University
and
Alumbi Center of Universiti Putra Malaysia**

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Identification Polymorphisms of Inos Gene and Association with Body Resistance Trait in Kampung Chicken

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Abstract

iNOS gene is one of the genes that play important factor in immunity related to resistance disease through non specific immune response in chicken. The aim of this study was to identify the polymorphism of iNOS gene in kampung chicken and it is association with resistance to the bacteria *Salmonella pullorum*. Genetic polymorphism of chicken was investigated using PCR RFLP method. There were three stages of identification: extraction of DNA genom, PCR amplification of iNOS gene (with size 449 bp), and RFLP method using restriction enzyme (*AluI*). The data were analyzed include of genotype and allele frequencies, heterozygosity values and Hardy-Weinberg equilibrium (HW). The result showed that iNOS gene was showed polymorphic in kampung chickens population. It were found two alleles (T and C), and three genotypes (TT, TC and CC). The chi square test revealed the locus of iNOS was deviated in Hardy Weinberg equilibrium. Association analysis indicated that CC genotype was significantly associated ($P < 0.05$) with disease resistance than TC and TT genotypes in Kampung chicken, that's mean TC and TT genotype has high disease resistance than CC. In conclusion the polymorphism of chicken iNOS gene could be potential to the candidate gene in breeding programe for increasing genetic resistance against *S. pullorum* in kampung chickens.

Keywords: chicken, iNOS gene, *Salmonella pullorum*, Hardy-Weinberg (HW)

1. Introduction

Kampung Chicken is Indonesian native chickens, that it have been domesticated. It is different with other chicken, Kampung chicken does not have typical characteristic and spread the territory of Indonesia [1]. Local chicken, including kampung chicken, have a high resistance to virus infections [2]. Referensi [3] were reported kampung chicken resistance to *Salmonella enteritidis* infection.

S. enteritidis is one of *Salmonella* sp bacterium which are zoonotic. The *S. enteritidis* could be caused salmonellosis in humans who consume eggs by chickens that infection. The effect of *S. enteritidis* is very different from the *S. pullorum*.

S. pullorum is pathogenic to chickens and can cause death [4]. *S. pullorum* bacteria

can cause illness defecation lime. The disease attacks the digestive organs (Oliveira et al. 2000) [5]. Once the importance of this disease, the government made a decree that all breeding chicken in Indonesia must be certified free from *S.* [6].

The resilience of the living body is influenced by genetic and environmental as well as the interaction of the two. There are several genes that control the body's resistance chicken, one of which is a gene inducible nitric oxide synthase (iNOS). iNOS gene is a gene that synthesizes the iNOS protein. This protein acts as cytosolic enzymes, which catalyze L-arginine into nitric oxide (NO). NO is one of the reactive oxygen species that can act as cytotoxic compounds to kill bacteria that infect immune cells [7].

iNOS gene in chickens located on chromosome 19. The structure of iNOS gene is promoter region, 28 exon, 27 introns and flanking region with a length of 22.028 bp (Ensembl, NOS2 ENSGALG00000038096). Results of previous studies mentioned that iNOS gene is polymorphic in local chicken [8], on Broiler chickens [9] and at Leghorn chickens [10].

The purpose of this study is to identify the genotype diversity iNOS gene as controlling disease resistance in chicken and to test for association with disease resistance properties caused by the bacterium *S. pullorum*.

2. Material and Methods

The study was conducted from July to Oktober 2016. The research was conducted at Field Laboratory, Laboratory of Molecular Animal Breeding and Genetics, Department of Animal Production and Technology Science, Faculty of Animal Science, Bogor Agriculture University and Laboratory of Medical Microbiology, Faculty of Animal Veterinary Bogor Agriculture University. The number of samples were used 44 of chicken blood. For the association with body resistance in chicken were used 9 of blood samples that represent each genotype.

DNA extraction using procedure phenol-chloroform method [11], involves preparation samples, protein degradation, degradation of organic material, precipitation of DNA and DNA stored at -20 ° C.

The PCR amplification were used to amplify iNOS gene with length products 449 bp. Primer were designed using Primer Designing Tools Program. The PCR reactions were performed in 0.5 mL of chicken DNA sample, 10.85 mL DW, 0.3 mL of each primer, 0.05 mL of each Taq polymerase, 1.5 mL of each buffer, 0.3 mL of each dNTP and MgCl 2 mL 1:00. The mixture is inserted into the PCR machine with the conditions of initial denaturation temperature of 95 ° C for 5 min, 35 cycles consisting of denaturation 95 ° C

for 10 seconds, annealing 60 ° C and 72 ° C final extension for 5 minutes.

RFLP method was used to determine the genotype iNOS gene. PCR result of the TLR4 gene fragment was cut by *AluI* restriction enzyme (0.3 mL) for 6 hours at 37 ° C. Visualized by electrophoresis using a 2% agarose gel with 0.5 TBE buffer (Tris Borate EDTA). The data were analyzed Allele frequencies, genotypic frequencies, Hardy-Weinberg equilibrium and heterozygosity.

The polymorphism of iNOS genes associated with body resistance trait, challenge test with *S. pullorum*. Challenge testing clearance test method [12]. includes Bacteria Culture Preparation, chicken blood examination and interpretation of results.

The data were analyzed using *analysis of variance* (ANOVA) with completely randomized design. iNOS gene genotype was as treatment and observation data were as response.

$$Y_{ij} = \mu + P_i + \epsilon_{ij} \quad [13]$$

Where Y_{ij} is the trait observed on the genotype, μ is the overall mean, P_i is effect of the single nucleotide polymorphism genotypes, and ϵ_{ij} is the random residual effect.

3. Result and Discussion

iNOS gene in kampung chickens located on chromosome 19. The structure is promoter region, 28 exon, 27 introns and flanking region with a length of 22.028 bp (Ensembl, NOS2 ENSGALG00000038096). Allele frequencies and genotype frequencies iNOS gene in kampung chicken showed in Table 2.

The results showed indicate two alleles in iNOS gene, namely T and C allele which cutting by enzymes *AluI*. The size product PCR T allele of 310 bp and 139 bp and C allele of 449 bp. Frequency T and C allele in kampung chickens in this study respectively 0.602 and 0.398.

Table 1. Primer sequences, PCR product size for identification of polymorphism in Inos.

Gene	Genbank accession No	Primer Sequence (5'-3')	SNP	PCR Product
INOS	NOS2 ENSGALG0000005693	F:CCAAGGACTTACAGGTGTGG R: CCAGGATGTTTGGGCTGTTG	Intron 21 (T→C)	449 bp

Tabel 2. The Genotypic and Allele Frequencies of iNOS in Kampung Chickens.

Chicken	N	Frekuensi alel		Frekuensi genotipe		
		T	C	TT	TC	CC
Kampung	44	0.602	0.398	0.455 (20)	0.295 (13)	0.250 (11)

Tabel 3. The Observed, and Expected Heterozygosity for in Kampung Chickens.

Chicken	N	He	Ho	χ^2
Kampung	44	0.479	0.295	6.464

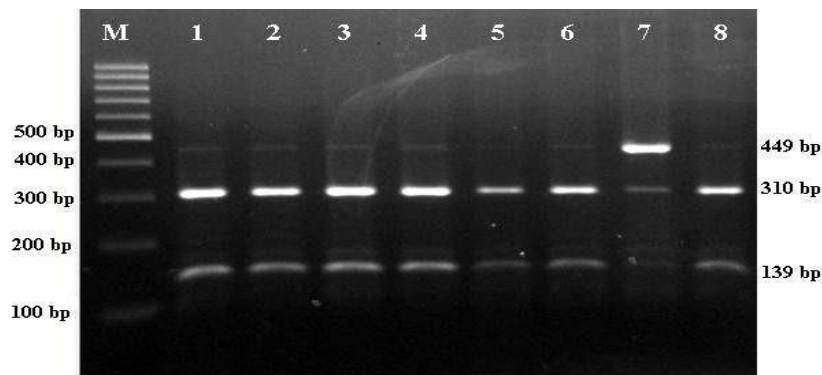


Fig. 1 PCR-RFLP electrophoresis pattern of *AluI* digested PCR products from Inos gene with 2% agarose gel

Table 4. Association Between iNOS Gene *AluI* with Resistance Against to *Salmonella pullorum*

Genotype INOS gene	First Konsentration (cfu/ml)	Final Konsentration (cfu/ml)	The Mortality Rate of Bacteria (%)
TT	6.8×10^{10}	4.96×10^8	99.516 ^a
TC	6.8×10^{10}	2.87×10^8	99.830 ^a
CC	6.8×10^{10}	7.4×10^8	98.910 ^b

Same latter means in the same column with different superscripts differ significantly ($P < 0.05$).

Mutation of the allele T / C on the fragment to 310 bp, it is consistent with the statement referensi[8] that there is a mutation of the allele T / C similar to that reported previously that the digested fragments are due to a T/C substitution at position 173 bp in intronic region for iNOS.

In the iNOS gene was founding three genotypes namely TT, TC and CC (figure 1). Genotype frequencies was obtained TT (0.455), TC (0.295) and CC (0.250). the result genotyping iNOS gene in kampung chicken locus *AluI* has polymorphic. According to referensi[8] that iNOS gene has polymorphic at Malaysia native chicken.

Heterozygosity Dan Genotype Equilibrium iNOS Gene

Observed and expected genotypic and allele frequencies for the the candidate genes are displayed in Table 3. The observed and expected heterozygosity for iNOS was 0.479 and 0.295. Heterozygosity value is the average percentage of heterozygous loci per individual or the average percentage of heterozygous individuals in the population [14].

Genotype equilibrium iNOS gene in the population (Hardy-Weinberg equilibrium), were analyzed by chi-square (χ^2). The test results of gene iNOS on *AluI* locus can be seen in Table 3. The chi square test revealed the locus of iNOS was deviated in Hardy Weinberg equilibrium.

Association of iNOS gene genotype and the expression of resistance against *S. pullorum* infection in Kampong chicken

Polymorphism OF Inosgene were associated with resistencetraith to *S. pullorum* bacterium in kampong chicken. Challenge test: was used clearance test method [12]. Association between iNOS gene *AluI* with resistance against to salmonella pullorum represented in table 4.

The clearance test to *S. pullorum* in kampong chicken showed TT, TC and CC genotype can be killed the *S. pullorum* bacterium. The clearance test showed 99.516% at TT genotype, 99.830% at TC genotype and 98.910% at CC genotype. According to Statistical analysis, mortality of bacterium level was Associated significantly with genotype of iNOS gene. Genotype of TT and TC was higher than CC genotype. Referensi [10], reported that Inos gene were associated with response to *S. enteritidis* in leghorn chicken.

Conclusion

This study concluded that TT and TC genotype was significantly associated ($P <$

0.05) with disease resistance than CC genotypes in Kampung chicken. Polymorphism of chicken iNOS gene could be potential to the candidate gene in breeding programme for increasing genetic resistance against *S. pullorum* in kampung chickens.

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