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Microsatellite Marker LEI0258 Variability in Six Indonesian Local Chicken Populations

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ABSTRACT
The microsatellite LEI0258 is a genetic marker for chicken MHC haplotypes and can be used to study the influence of population genetics on immune responses. In this study, we investigated the variability of the LEI0258 locus in 173 chickens from six Indonesian local chicken populations (Black Kedu Pelung, Sentul, Merawang, Nunukan, and Gaga). The LEI0258 locus was found to be polymorphic in overall populations with a total of 25 alleles. The observed and expected heterozygosity values ranged from 0.565 (Gaga) to 0.967 (Pelung) with an overall mean of 0.855 and from 0.715 (Gaga) to 0.879 (Merawang) with an overall mean 0.829, respectively. The decreasing heterozygosity indicated that the population's genetic diversity had decreased. The average PIC values ranged from 0.687 (Gaga) to 0.870 (Merawang), overall studied chicken was 0.811. All the chicken populations were in Hardy Weinberg equilibrium, except for the Gaga chicken.

Keywords: *Microsatellite; Marker; LEI0258; MHC; Indonesian local chicken*

1. INTRODUCTION

Indonesia is a country rich in biodiversity, one of which is Indonesian local chicken. Several breeds of Indonesian local chicken are indigenous animal genetic resources that have yet to be tapped for their potential as meat, egg, and fancy chicken. To date, more than 31 distinct breeds of indigenous chickens have been identified as Indonesian local chickens [1]. Indigenous chickens are noted for their outstanding resistance to poultry diseases and their adaptability to local climatic conditions [2].

The major histocompatibility complex (MHC) genes play an essential role in host immunity in chickens. MHC also has a significant impact on disease resistance or susceptibility, production, and essential economic traits. Moreover, MHC is linked to disease resistance against a variety of pathogens, including bacteria and susceptibility to certain poultry diseases [4–7].

MHC is a set of immune response genes discovered on chromosome 16. It is divided into two regions: B and RFP-Y. Complex B comprises three functionally different loci: BF, BL, and BG region [7]. The MHC is associated with a broad spectrum of immune responses, making it a good predictor of disease etiology and outcome. The MHC genes are of particular importance, a polymorphic multigene system whose products have a significant role in antigen presentation to T lymphocytes during humoral and cell-mediated immune responses.

The LEI0258 microsatellite marker is located on the MHC between the BG and BF regions [8]. LEI0258

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locus can be used as a valuable marker to show MHC variability in different chicken populations. It can also be used to study genetic diversity in chickens, especially on the MHC gene. The LEI0258 marker is noteworthy because of the vast number of alleles found and the wide variety of allele sizes. These substantial allelic differences are identifiable using very low-cost electrophoretic size separation methods, prompting research into this marker as an MHC haplotype indication. Sequence analysis was used to investigate the underlying reason for this variability [9].

Recently, many studies have been carried out to determine the value of genetic diversity of chickens using microsatellite as Marker Assisted Selection (MAS) reported and proven to be used by this panel for biodiversity studies. Previous studies have been done by Ismoyowati et al. [10], Ashari et al. [11], Saputra et al. [12]. However, no one has utilized the LEI0258 microsatellite to characterize the Indonesian indigenous chickens. It appears that defining MHC haplotypes for populations subjected to selective breeding is worthwhile. The Indonesian local chicken populations (Black Kedu, Pelung, Sentul, Merawang, Nunukan, and Gaga chicken) are being selectively bred to increase meat and egg production. Other than that, the Indonesian chicken population could be indigenous genetic resources for disease resistance genes.

This study aimed to establish allelic and genetic diversity between 6 Indonesian local chicken populations based on MHC-linked LEI0258 marker typing. The results from this study, in combination with information on their phenotypic and production attributes, form a preliminary database based on which further investigation, future comparison, production improvement, and well-informed conservation strategies can be made. However, until far, study on MHC in indigenous Indonesian chickens has been limited in comparison to commercial chickens and other chickens.

2. MATERIALS AND METHODS

2.1. Ethical approval

The experimental method was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada (00033/EC-FKH/Eks./2021).

2.2. Study area

Six Indonesian indigenous chicken breeds, namely Black Kedu, Gaga, Merawang, Nunukan, Pelung, and Sentul, were sampled from different regions shown in Table 3.

2.3. Experimental animals and their management

A total of 173 samples were randomly sampled from the six Indonesian local chicken populations. Black Kedu, Pelung, Sentul, Merawang, Nunukan (30 samples each) and Gaga (23 samples) were used in this study. The breeds had a varied origin, breeding strategies, and selection criteria had been raised under different management systems. Thus, we categorized them into three main management systems (extensive, semi-intensive, and intensive) based on the prevailing production systems of the different provinces in Indonesia (Table 3.).

2.4. DNA isolation

Before DNA isolation, blood samples were drawn from the brachial wings vein and preserved in vacuum tubes containing EDTA at -20oC. DNA isolation was carried out using a DNA extraction kit (gSYNCTMDNA Extraction Kit, #GS004, GS100, GS300; Geneaid Biotech Ltd., Sijhih, Taiwan). The DNA samples were then stored at -20oC before being used in the PCR. The DNA quality and quantity were checked using 1% agarose gel electrophoresis and a spectrophotometer NanoDrop 2000C (Thermo Scientific, Waltham, MA, USA).

2.5. PCR and genotyping

The LEI0258 locus was determined using the polymerase chain reaction (PCR) with forward (5'-CACGCAGCAGAACTTGGTAAGG-3') and reverse (5'-AGCTGTGCTCAGTCCTCAGTGC-3') primer pair described elsewhere [9].

Reagent volume of the PCR in a final volume of 20 µL was as follows: 1.5 µL of 25 ng/µL genomic DNA, 10 µL HS prime Taq premix, 0.4 µL of 5 pmoles each forward and reverse primer, and 7.7 µL of triple distilled water. The optimized PCR cycling conditions for this marker were: an initial denaturation step at 95°C for 3 min, followed by 32 cycles at 94°C for 45s, annealing at 61°C for 45 s, 72°C for 45 s, and a final extension of 20-30 min at 72°C. The PCR products for each sample were verified with 3% agarose gel containing ethidium bromide.

Each diluted PCR (1 µL) product was mixed with 0.1 µL Gene-Scan LIZ 500 size standards and

9.9 µL of Hi-Di formamide solution for genotyping. The sample was heated at 95°C for 5 minutes and then immediately kept on ice and analyzed by capillary electrophoresis with an ABI-3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA) at the Neogene laboratory (Korea).

2.6. Population genetics analysis

Genotype data from 173 chickens that have been read and summarized using GeneMapper ver. 4.1. A test for deviation from the Hardy–Weinberg equilibrium (HWE) was conducted with Genepop ver. 1.2 and GenAlex ver 6.501 [13]. The number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) for each population were calculated using GenAlex software. The polymorphic information content (PIC) using the Cervus program [14].

3. RESULTS

Analysis of allelic variability by capillary electrophoresis revealed a total of 25 different alleles across local chicken, with sizes ranging from 193 to 509 bp. Nine private alleles were detected across the local chicken. Each private allele was specific to only one local chicken: alleles 237, 241, 285, and 395 bp (Merawang chicken), 323 and 489 bp (Nunukan), 344 and 509 (Sentul), and 421 bp (Pelung). The frequencies of private alleles were low, seven alleles (0.29% each). On the other hand, 18 alleles (with their frequencies in parentheses): 205 (15.61%), 369(9.54%), 261 (8.96%), 193 (8.38%), 249 (8.09%), 309 (7.23%), 217 (7.237%), 443 (5.7805%), 333 (5.20%), 295 (4.05%), 307 (3.76%), 207 (3.18%), 321 (2.89%), 357 (2.60%), 489 (2.02%), 285 (1.73%), 283 (1.16%), and 417 (0.58%) were the most frequent across local chicken. Only one allele (309 bp) was common to all six local chicken populations (Table 1.; Figure 1).

The number of alleles per local chicken ranged from 8 (Gaga) to 17 (Merawang), with an average of 11.83 alleles per local chicken. However, the Merawang chicken had the highest effective number of alleles (8.295), and the Gaga chicken had the lowest (3.503). Analyses of data from capillary electrophoresis indicated overall PIC at this marker to be 0.811. The overall means of observed (Ho) and expected (He) heterozygosity were 0.855 and 0.829, respectively. Pelung chicken showed the highest Ho (0.967), and Gaga chicken showed the lowest Ho (0.565). Merawang chicken showed the highest He (0.879), whereas Gaga chicken showed the lowest value (0.715) with an overall mean of 0.829. Sentul and Gaga chicken had lower Ho than He, whereas the reverse was true for the Merawang, Pelung, Black Kedu, and Nunukan chicken. Merawang chicken has the highest (0.870) for the PIC value, whereas Gaga chicken has the lowest (0.687). The average PIC value in this study was 0.811. From 6 population Indonesian local chicken breeds, only Gaga chicken was in HWE ($P<0.05$) (Table 2).

4. DISCUSSION

The range of the LEI0258 allele in this study was 193-509 bp. This is different from the study of Manjula et al. [15] identified 38 LEI0258 allele range of 193-539 bp. In Chinese indigenous chicken strains, 69 alleles were identified, ranging from 193-489 bp [16], 26 alleles in North American and European layer- type chickens [9], and 22 alleles in 2 commercial layer and five non-commercial layer chickens, range 182 to 552 bp [8]. Many of these alleles were found to be common among chicken populations.

The alleles 207, 217, 237, 283, 323, 344, 395, 417, 421, 489, and 509 were not addressed in prior studies by Nikbakht et al. [17], Izadi et al. [8], Lwelamira et al [18], and Fulton [9]. Several others (93, 205, 261, 295, 307, 321, 357, 443) were discovered in the studies mentioned. As a result, we can conclude that this study discovered 11 novel alleles that have never been reported before. Izadi et al. [8] Furthermore, because the detection tools and methodologies used in the various studies differed, results had to be confirmed using sequencing.

The Pelung, Sentul, and Gaga chicken populations had the highest allelic frequency of 205 (0.150, 0.267, and 0.478, respectively), and the Black Kedu, Nunukan, and Merawang had the lowest (0.00- 0.067). This allele was one of the common thatfound in 5 of the six populations in this study. Compared to previously study, the frequency of allele 205 was very low in the Marandi chicken populations [17] and not found in Tanzanian chicken populations [19]. Lwelamira et al. [18] reported that allele 205 was associated with a strong antibody response to the Newcastle vaccine. In case in this study, the allelic frequency of 205 was low in Merawang and Nunukan populations and absented in Gaga chicken

populations. Differences in allele frequencies for some LEI0258 alleles among different chicken populations are probably linked to their disease selection history. The LEI0258 allele has a high serological correlation with specific haplotype B haplotypes. However, not all alleles are unique to a certain MHC-B serotype haplotype, as multiple serological MHC-B haplotypes have reported identical allele sizes [9, 18].

The observed heterozygosity (Ho) levels were 0.967 (Pelung), 0.933 (Merawang and Black Kedu), 0.900 (Nunukan), 0.833 (Sentul), and 0.565 (Gaga) (Table 2.). The Ho value of Pelung, Merawang, and Black Kedu chickens are closed with the result in Kandy ecotype and Karuwalgeswawa from Sri Lanka (0.947 and 0.943) and Umuobasi ecotype from Nigeria (0.950). However, this study is higher than those reported from Korean native chicken (KNC) such as KNC Gray Brown and KNC Black (0.820), KNC Red Brown (0.739), KNC White (0.731), KNC Yellow Brown (0.755), KNC NIAS Ogye (0.633), and KNC Yeonsan Ogye (0.738). Moreover, Ho from Bangladesh chicken such as Hilly Chicken (0.821), Non-descript common deshi (0.780), Naked neck (0.674), Aseel (0.667), Red Jungle Fowl (0.579) is lower compared to this study and Korean Native chicken.

Sentul dan Gaga chicken had Ho values lower than He values according to the Ho and He values. Mwambene et al. [19] stated that if the Ho is lower than He, thereby pointing to a possible departure from random mating. Based on the expected heterozygosity (He), we know that all Indonesian local chicken populations in this study have high He values. Its mean diversity on the LEI0258 locus has a high value in all chicken populations. Several populations have higher Ho values (heterozygous excess), namely Merawang, Pelung, Black Kedu, and Nunukan. In contrast, the population of Sentul and Gaga chickens showed a heterozygous deficiency, which was indicated by the value of Ho being smaller than the value of He. The heterozygous deficiency found in both populations can be caused by one or more of the following factors: null alleles, Wahlund effect, errors in scoring (heterozygotes are considered homozygous), and inbreeding [21]. Haryono et al. [22] stated that the disequilibrium mainly was produced by heterozygote inadequacies resulting from inbreeding and/or the Wahlund effect (population substructure). The existence of the Wahlund effect can be ruled out because the samples were obtained from the same flock for each population. Heterozygous deficiency can also be caused by technical factors such as genotyping errors. This genotyping error can be caused by poor DNA quality [23].

From this study, we know that the PIC values varied from 0.687 (Gaga) to 0.870 (Merawang), with an average of 0.811. The PIC value with microsatellite marker LEI0258 in this study (Table 2) was relatively lower when compared to Tanzanian chickens at 0.935 [19] and chickens from various countries (Sri Lanka, Bangladesh, Nigeria, South Korea) 0.912 [24]. Seo et al. [25], the number of alleles, He, and PIC are essential indicators that can be used to distinguish individuals and populations within a species. The PIC value can be used to evaluate the informativeness of a marker to determine between individuals and breeds [22]. Mateescu et al. [26] classified the PIC value into three groups: the uninformative group with a PIC value <0.3, quite informative with a value of 0.3-0.59, and very informative with a PIC value > 0.6. The results of this study found that the microsatellite marker LEI0258 is very inf ormative because it has a value (PIC>0.6), so it is highly recommended to be used in the analysis of genetic diversity in indigenous chicken populations in Indonesia. Hariyono [27], it should be noted that the word "informative" is defined as the power of a marker in distinguishing the individuals being analyzed. The more polymorphic a marker is, the higher the marker's ability to distinguish between individuals and between populations being studied.

This observation was further augmented by the presence of 1 out of 6 Indonesian local chicken populations with significant deviations from HWE at this locus. The gaga chicken population significantly deviated from HWE, with the Ho value being higher than the He value. This result indicates that non-random mating occurs with a crossbreeding marriage system. This statement is strengthened by rearing Gaga chickens by backyard scavenging and comes from smallholder farmers (Table 3). Mwambene et al. [19], stated that although the specific cause of reported heterozygote deficiencies and deviations from HWE at this locus is difficult to predict, excess heterozygosity in some populations may be due to the preliminary intro duction of novel cockerels from other breeds for crossbreeding with native chickens.

Table 1. Frequencies of LEI0258 alleles in six Indonesian local chicken populations.

Allele (bp)	Population						Overall Freq	Pop
	Merawang	Pelung	Black Kedu	Sentul	Nunukan	Gaga		
193	0.033	0.000	0.083	0.083	0.250	0.043	0.084	5
205	0.067	0.150	0.000	0.267	0.050	0.478	0.156	5
207	0.000	0.000	0.000	0.100	0.000	0.109	0.032	2

217	0.033	0.067	0.100	0.067	0.150	0.000	0.072	5
237	0.017	0.000	0.000	0.000	0.000	0.000	0.003	1
241	0.017	0.000	0.000	0.000	0.000	0.000	0.003	1
249	0.250	0.217	0.000	0.000	0.000	0.000	0.081	2
261	0.067	0.133	0.283	0.017	0.000	0.022	0.090	5
283	0.017	0.017	0.000	0.033	0.000	0.000	0.012	3
285	0.100	0.000	0.000	0.000	0.000	0.000	0.017	1
295	0.083	0.067	0.083	0.000	0.000	0.000	0.040	3
307	0.033	0.067	0.033	0.017	0.000	0.087	0.038	5
309	0.150	0.067	0.100	0.050	0.033	0.022	0.072	6
321	0.017	0.050	0.033	0.017	0.050	0.000	0.029	5
323	0.000	0.000	0.000	0.000	0.017	0.000	0.003	1
333	0.017	0.117	0.000	0.033	0.000	0.174	0.052	4
344	0.000	0.000	0.000	0.017	0.000	0.000	0.003	1
357	0.017	0.000	0.017	0.117	0.000	0.000	0.026	3
369	0.000	0.017	0.250	0.167	0.067	0.065	0.095	5
395	0.017	0.000	0.000	0.000	0.000	0.000	0.003	1
417	0.000	0.017	0.017	0.000	0.000	0.000	0.006	2
421	0.000	0.017	0.000	0.000	0.000	0.000	0.003	1
443	0.067	0.000	0.000	0.000	0.267	0.000	0.058	2
489	0.000	0.000	0.000	0.000	0.117	0.000	0.020	1
509	0.000	0.000	0.000	0.017	0.000	0.000	0.003	1

Note: Freq = Frequency; Pop = number of populations sharing the allele

Tabel 2. Analysis of genetic diversity in six Indonesian local chicken populations.

Population	**N**	**Na**	**Ne**	**Ho**	**He**	**uHe**	**PIC**	**HWE**
Merawang	30	17	8.295	0.933	0.879	0.894	0.870	ns
Pelung	30	13	8.182	0.967	0.878	0.893	0.866	ns
Black Kedu	30	10	5.573	0.933	0.821	0.834	0.799	ns
Sentul	30	14	7.143	0.833	0.860	0.875	0.847	ns
Nunukan	30	9	5.538	0.900	0.819	0.833	0.797	ns
Gaga	23	8	3.503	0.565	0.715	0.730	0.687	*
Total	173	71						
Mean	28.833	11.833	6.372	0.855	0.829	0,843	0.811	

Note : N: number of individuals; Na: number of alleles per locus; Ho: observed heterozygosity; He: expected heterozygosity; uHe: unbiased expected heterozygosity = (2N / (2N-1) × He; PIC: Polymorphism information content; HWE: test for Hardy-Weinberg equilibrium, P< 0.05, *: Significant, ns: non-significant.

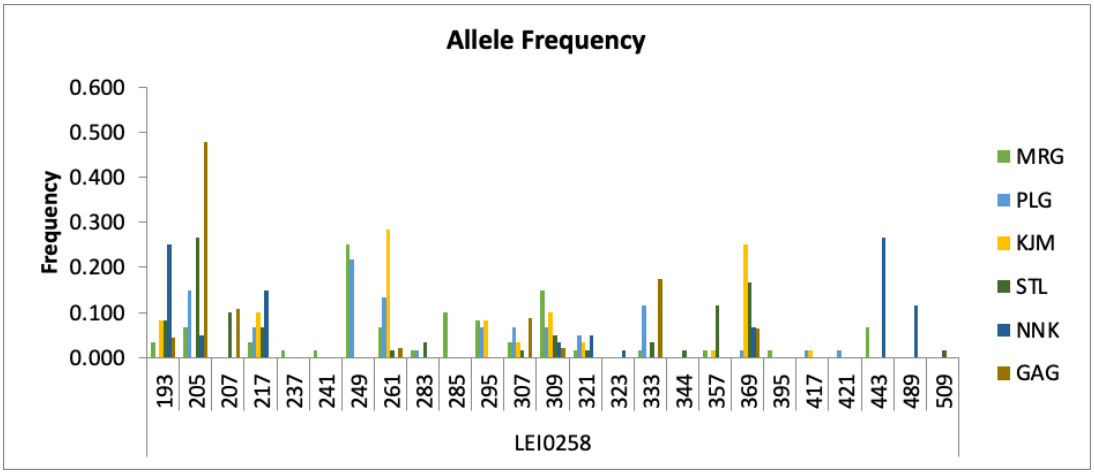

Figure . 1 Allele frequencies distribution in six Indonesian local chicken populations

Note : MRG: Merawang; PLG: Pelung; KJM: Black Kedu; STL: Sentul; NNK: Nunukan; GAG: Gaga.

Table 3. Characteristics of the farming systems.

Breed	Origin	Sampling Location	Latitude	Longitude	Management Systems	Flock Size	Source of Day Old Chicken	Access to Veterinery Service	Biosecurity/ disease control
Merawang	Bangka Belitung Island	Bangka Belitung Island	2°12'30.0"S	105° 58'20.7"E	Semi-Intensive	5-25	Artifical incubation	High	Maximum
Pelung	West Java Province	Cianjur District, Wes Java Province	6°49'23.5"S	107° 07'13.1"E	Semi-Intensive	5-30	Artifical incubation	High	Maximum
Black Kedu	Central Java Province	Temanggung District, Central Java Province	7°17'48.2"S	110° 10'47.6"E	Semi-Intensive	5-30	Artifical incubation	High	Maximum
Sentul	West Java Province	Majalengka District, West Java Provinci	6°43'36.3"S	108° 16'41.0"E	Semi-Intensive	5-30	Artifical incubation	High	Maximum
Nunukan	Nunukan Island, North Kalimantan	Nunukan Island, North Kalimantan	4°05'48.5"N	117° 42'23.9"E	Semi-Intensive	1-8	Artifical incubation	High	Maximum
Gaga	South Sulawesi Province	2 location : Bantul district, Yogyakarta Kendal District,	7°50'50.6"S 6°55'30.2"S	110°23'48.7"E 110°09'10.8"E	Backyard scavenging (small holder farmer)	1-5	Natural incubation	Zero or rarely	Minimum

5. CONCLUSION

We find that the microsatellite marker LEI0258 can be utilized to type local chicken populations using MHC. In Indonesian local chicken populations, the approach revealed a significant level of allelic variety. The findings will aid in the conservation of genetic resources as well as marker-assisted selection in chicken breeding programs.

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