Scientific Papers. Series D. Animal Science. Vol. LVI
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN-L 2285-5750

IDENTIFICATION OF PIT-1 GEN USING PCR-RFLP AND GENETIC EVALUATION OF HACHING WEIGHT USING PATERNAL HALB SIB ON INDEGENOUS BREED SINGING COCKEREL PELUNG

Sri Bandiati Komar PRAJOGA[1], Kundrat HIDAYAT[1], Perman IMAN[2]

[1]Animal Breeding and Reproduction Laboratory, Animal Husbandry Faculty, Universitas Padjadjaran, Bandung, Indonesia
[2]Biochemistry Laboratory, Mathematics and Natural Sciences Faculty, Universitas Padjadjaran, Bandung, Indonesia

Corresponding author email: komarsri@yahoo.com

Abstract

This research was conducted at the Laboratory of Animal Breeding and Reproduction of Animal Husbandry Faculty, and Laboratory of Biochemistry of Mathematic and Natural Sciences Faculty Universitas Padjadjaran at June 2012. The objectives of this research were to know variation of Pit-1 gen of Pelung singing cockerel and evaluate genetic parameter of Hatching Weight (HW). The data comprised of 76 HW-records as progeny from 5 cocks and 15 hens of Pelung using Paternal Halb Sib and 29-blood samples. Variance component and heritability were estimated by Restricted Maximum Likelihood (REML) using Animal Model with the program of VCE 4.2. Fixed effect was sex and hatching period. Variation of chicken Pit-1 gen was analyzed using PCR-RFLP and used 5 primers (PR1, PR2, PR3, PR4 and PR5) and 4 restriction enzymes. The average hatch weight was 33.83 ±2.42 gr. The heritability value was 0.5 ±0.05 as high category. The genetic respond to selection was 2.55 gr. The accuracy of selection of was 0.707. While selection intensity was 1.92% for 3 hens and 1 cock (sex ratio 1 ♂3 ♀. Presence or absence of deletions in a PCR fragment of the result can be distinguished by differences in the electrophoresis migration of the fragment. The result showed that there was deletion of 57 bp of insertion fragment length of 387 bp. In this experiment the difference migration did not occur in all samples, that was implicated with reverse and forward primer (PR1).

Key words: *Artificial Insemination, Paternal Halb Sib, Pit-1 Gen, Singing Cockerel.*

INTRODUCTION

Pelung Chicken is one of the local chickens, which were developed by the community with the objective being to sound melodious crowing roosters, easy listening, big, long, playing and rhythmic. However, if it is not supported with good stamina, the sound quality may not appear as expected.

Although the criteria of performance and body weight are ignored, in the future the characteristics of Pelung Chicken (big body) will be lost.

On a semi-intensive maintenance Pelung adult, can achieve weight of 3.37 kg for cocks and 2.52 kg for hens, while the DOC body weight is 30.7 g for males and 31.6 g for females. The usefulness of Pelung Chicken as a source of animal protein, have higher productivity than other types of local chicken in Indonesia. The roosters have loud rhythmic and long floating crow. Pelung chicken do not have a specific pattern of feather color, many of them have a mixture color of red and black, yellow and white, and also shiny green color mix, but the most often found is the mixture of red and black.

Genetic diversity is important in breeding programs for the genetic optimization of the acquisition of certain properties can be achieved when there is enough opportunity for the selection of genes or the desired properties.

Beside that, genetic diversity plays an important role in the survival of the population. The loss of genetic diversity can reduce the chance of survival of the population.

Growth is weight gain until they reach adult size or per additional body mass per unit time. Specific growth occurs in young animals which is formed by the large bone, protein, and water networks.

Through a complicated on differentiation phase of the anterior pituitary and the regulation of prolactin gene (PRL), growth hormone (Growth hormone-GH) and thyroid-stimulating

hormone-ß (TSH-ß), then Gen Chicken PIT1 is recommended as a candidate gene to the nature of the production.

Somatic cells residing on fur or white blood cells can be used as a source for the DNA analysis. In the microsatellite study to obtain superior genes for the purification of Pelung chicken, so that the origin of the nation can be standardized based on Polymerase Chain Reaction (PCR) and Restriction Fragment Polymorphism (RFLP) (Bandiati, 2006).

Therefore, based on the availability of the genetic resources, it is essential to form seeds of Pelung chicken, which have criteria in accordance with the will of the breeder, for purposes of standardization and certification. The purpose of this study was to determine the variation of Pit-1 gene in blood samples and the response to selection in Pelung chickens base on hatching weight.

MATERIALS AND METHODS

1. Blood sampling as many as 29 samples, grouped into group A (A1, 2, 3, 4, 5); B (B1, 2, 3, 4, 5); C (C1, 2, 3, 4, 5); D (D1, 2, 3, 4, 5); E (E1, 2, 3, 4); F (F1, 2, 3, 4) and G1.
2. Pre-treatment sample storage (using va-cuumtainer containing EDTA anticoagu-lant).
3. Isolation of DNA samples using the Genomic DNA Purification Kit (Fermentas).
4. PCR analysis for each of the isolated DNA with four pairs of primers using Taq Dream Green PCR Master Mix.
5. Electrophoresis of PCR results to determine the quantity of DNA and determine the approximate size of the fragment amplification results using 1% agarose electrophoresis, specific to MR1 marker electrophoresis performed using 2% agarose electrophoresis to determine the insertion and deletion, another marker proceed to the next stage of analysis.
6. Cutting PCR results using four different restriction enzymes.
7. Electrophoresis of restriction enzyme cutting results using 1.5% agarose electrophoresis.

Table 1. Detailed Information of the Marker MR1-MR5 on PITI Gene in Chickens

Marker	Variation	location	Size	restrictions Enzymes	Primer	Statement analysis of variation
MR1	Insersions/ delesions 57 pb	Intron 2	387/ 330 pb	-	PR1 forw/ Rev	Analysis of insertions / deletions (display size of the ribbon electrophoresis)
MR2	C/T	Intron 5	599 pb	TaqI	PR 2 forw/ rev	RFLP
MR3	A/G	Intron 5	599 pb	MspI	PR 2 forw/ rev	RFLP
MR4	C/T	Intron 5	442 pb	EcoRI	PR 3 forw/ rev	RFLP
MR5	C/T	Ekson 6	483 pb	TasI	PR 4 forw/ rev	RFLP

Table 2. Order of Primer (forward/reverse 5′-3′)

Primer Name	Order of Primer		
PR1	Forw	:	gtcaaggcaaatattctgtacc
	Rev		tgcatgttaatttggctctg
PR 2	Forw	:	ggaccctctctaacagctctc
	Rev		gggaagaatacagggaaagg
PR 3	Forw	:	ggggattttgccactttaggg
	Rev		tgggtaaggctctggcactgt
PR 4	Forw	:	tgggaagaacagtttatggc
	Rev		tggctagcttgtaagggaatc

Isolation Method Using Genomic DNA Purification Kit (Fermentas) Component Kit:

- Lyses solution
- Precipitation solution (10x concentration)
- NaCl Solution (1.2 M)

Working Step:

1. Mix the 200 µL of blood samples of chicken (upper blood that has been stored on the vacumtainer with anticoagulants EDTA) with 400 µL lyses solution, pipette up and down, 10 minute incubation at 65°C (to be turned back several times during incubation).
2. Adding 600 µL of chloroform, inversion (alternating-turn tube) 3-5 times, 2 min centrifugation at 11,000 rpm.
3. Remove top aqueous phase, which contained DNA into new tubes had contained 800 µL 1x precipitation solution (80 µL precipitation 10x solution is added 720 µL sterile aqubidest), mix for 2 minutes (using a vortex), 2 min centrifugation at 11,000 rpm, discard supernatant.

4. Dissolve the pellet with 100 µL NaCl solution.
5. Add 300 µL of absolute ethanol cold, let the DNA precipitate for 10 minutes at-20°C, centrifuged for 4 min at 11,000 rpm, discard the supernatant. Can be added to the leaching method using 70% ethanol and then dried.
6. Dissolve the DNA in 100 mL sterile aquabidest Furthermore, the results of PCR was cut with restriction enzymes to determine the mutations found in fragments.

Marker	Variation	Restriction enzyme	Incubation Temp RFLP
MR1	Insertion/ delusion 57 bp	-	-
MR2	C/T	TaqI	65°C
MR3	A/G	MspI	37°C
MR4	C/T	EcoRI	37°C
MR5	C/T	TasI	65°C

Mixed cutting reaction by restriction enzyme (RFLP)

ddH_2O	9 µl
Buffer enzyme 10x	3 µl
Enzyme (10 u/ µl)	3 µl
DNAHasil PCR	15 µl
Final volume	30 µl

Electrophoresis of PCR Product:

Resulted DNA from electrophoresis purification used 1% polyacrylamide with silver staining colouring to see the pattern of band microsatellite, that was amplificated by primer.

Especially for MR1 marker electrophoresis performed using 2% agarose electrophoresis to determine the insertions and deletions, another marker proceed to the next stage of analysis.

PCR product was cut using four kinds of restriction enzymes, for this analysis used electrophoresis 1.5% agarose.

Genetic Evaluation:

Parameters were analyzed using REML method with repeated measurement pattern Animal Model (Grouneveld, 1998). Response selection by using the formula $R = i\, h^2\, s_P$

RESULTS AND DISCUSSIONS

Variations Polymorphism Pit-1 Gen:

At the holding cell isolation method optimazation with the Genomic DNA Purification Kit (Fermentas), used as a comparison sample of whole blood and uppers (serum). It was found that isolation by using whole blood (mixture) at stage 3 there will be no separate phase 3 well (the upper (aqueous phase), the middle (cell debris), and the lower (organic phase) as in isolation by using serum.

Isolation using whole blood at stage 3 there will be only two phases, namely solid phase (gel-like) on the top and the organic phase. Because it was later used as an isolated sample is part of the chicken blood serum. Furthermore, by using the PR1, all samples showed deduction Variations of PR1 primers in reverse and forward showed that the migration of the same relative to all samples and estimated (based on comparison with a marker) that is the size of the fragments between 250-500 bp, primer design based amplification size was 387 bp (when insertions) or 330 bp (when deletions). The results of further analysis to look for mutations that occur in fragment, that MR1 using PR1 showed 100% truncated insertions and deletions, MR2, that treated combination between PR2 with tag1 enzyme cut only 37% of total (11 samples out of 29 samples), MR3 that PR2 with MspI enzyme cut only 48.27% (14 samples from 29 samples), MR4 is PR3 with Eco RI enzymes cut 89.65% (26 samples out of 29 samples) and MR5 is a PR4 with Tas1 enzymes cut only 31.03% (9 samples out of 29 samples). According to Nie et al (2008) that the PIT1 haplotypes were associated with Hatching Weight Significantly ($P = 0.0252$), Body Weight at 28 days ($P = 0.0390$) and Shank Diameter at 56 days ($P = 0.0400$).

Structure of Research Data:

Structure of research data from 76 heads Pelung DOC consisting of 33heads females and 43 heads males, who are a offspring of the 5 sires (cocks) with 15 dams (hens). The structure of research data are listed in (Table 3). From (Table 3), shows the average of HW was 33.83 ± 2.42 gr with a coefficient of variation (CV) was 7% lower than the CV all ages, which means that the condition of the data into a uniform.

Table 3. Average Weekly Gain (AWG) Pelung Chicken. Absolute Growth Rate (AGR), Relative Growth Rate (RGR).

Age	N	Average	Sd	Min.	Max.	CV
Week		Gram				
0	80	33.83	2.42	27.06	38.50	7.00
1	80	57.88	5.42	46	70	9.37
2	80	96.17	9.44	75	115	9.82
3	80	146.57	19.88	110	202	13.56
4	80	215.78	25.61	158	267	11.87
5	80	299.15	34.03	206	366	11.38
6	80	396.89	45.62	259	491	11.49
7	79	502.35	57.53	332	631	11.45
8	79	622.54	73.94	413	782	11.88
9	79	751.78	94.87	501	962	12.62
10	77	891.70	112.75	578	1 157	12.64
11	76	1 029.34	133.32	697	1 337	12.95
12	76	1 157.77	153.46	759	1 463	13.25

In terms of average body weight gain is achieved per week, then it can be followed in (Table 4), in order to determine the point of inflection of postnatal growth in Pelung chickens:

Table 4. Average Weekly Gain (AWG) Pelung Chicken. Absolute Growth Rate (AGR), Relative Growth Rate (RGR).

N	Age	BW	AWG	LPR	RGR
		gram			
80	0	33.83			
80	1	57.88	24.05	3.44	0.055
80	2	96.17	38.29	5.47	0.052
80	3	146.57	50.40	7.20	0.042
80	4	215.78	69.21	9.89	0.039
80	5	299.15	83.37	11.91	0.033
80	6	396.89	97.74	13.96	0.028
79	7	502.35	105.46	15.07	0.023
79	8	622.54	120.19	17.17	0.021
79	9	751.78	129.24	18.46	0.018
77	10	891.70	139.92	19.99	0.017
76	11	1 029.34	137.64	19.66	0.014
76	12	1 157.77	128.43	18.35	0.011

In terms of body weight per week at make the diagram as the Y axis (ordinate) and age serve as its X axis, it will get the intersection between these two variables forming a growth curve, it is when in Generate growth curve using nonlinear regression exponential ($Y = ae^{bx}$), then when followed up to the age of adulthood (puberty) will look sigmoid shape, however in this study preferred only see the point of inflection on the growth of chickens Pelung by limiting the growth phase to the acceleration phase.

To be clear inflection point in sight, it can be showed to on a growth curve (Figure 1).

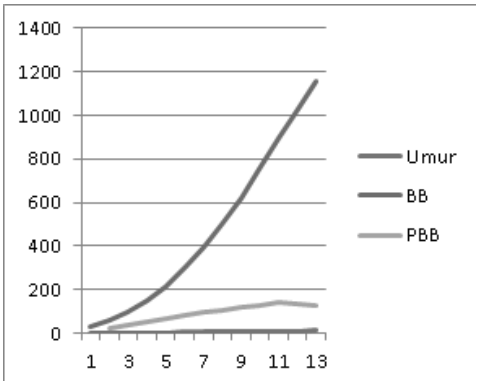

Figure 1. The Growth Curve since Hatched until 12 weeks old

The highest Average Weekly Gain (139.92 gr) was achieved at the age between 10 weeks old before the 11 weeks old. In the 12 weeks of absolute growth rate ranged (RGR) from 3.44 gr to 18.35 gr.

This value will continue to grow during puberty and feeding chickens, when it passed the fat accumulation.

In contrast to the relative growth rate obtained by dividing the absolute growth rate by half of the initial weight and final weight, which ranged from 0.055 gr to 0.011 gr will be changed to a negative value when animals are no growing any more, it is in accordance with the statement of Broody (1945).

Components and Heritability Body Weight range up to age 3 months.

The results of data analysis using REML with Animal Model with repeated measurement pattern (Table 5).

Table 5. Variance Component Ve, Va, VP and Heritability of HW, BW4, BW12 dan CW.

Hatching Period	Avg of BW of 0, 4, 12 and 1-12 weeks			
	0	4	12	1-12
V_e	2.95	328.25	11.775,15	4,218.681
V_a	2.95	328.25	11.775,15	699.752
V_P	5.80	656.49	23.550,31	4,918.433
Heritability	0.50	0.50	0.50	0.142

The highest selection intensity achieved on sex ratio of 1 male with 3 females, which is based on the highest rank in the genetic population,

the increasing HW on future generations is 2.55 gr above the population average.

The number of males used will also affect the fertility of the eggs, however, when the AI reproductive technologies was used, the problems will not be encountered. In this technology not only the proportion between males and females was considered, but also the quality of spermatozoa will make determination.

According to Iskandar (2005) Pelung chicken, Kedu chicken and Sentul chicken has an average spermatozoa number of 2.26 billion sperm per millimeter. Apart from that hen, the HW also affected by the weight of the hen. The too young or too old hen will produce eggs which did not have optimal weight for hatching eggs, because for HW occupies about 61-76% of the egg weight (Latour, et al, 1998).

CONCLUSIONS

Variation of reverse and forward primers PR1 showed that migration is relatively the same for all the samples and estimated (based on comparison with a marker) that is the size of the fragments between 250-500 bp, amplification size based on primer design was 387 bp (during insertions) or 330 bp (during deletions).

Variance components for HW trait consist of a variance of additive genetic (Vga) of 699.75. Variance of environment (Ve) was 4218.67; The Variance of phenotype (VP) is 4918.43. Increased HW on future generations is 2.55 gr above population average.

ACKNOWLEDGEMENTS

Thanks to DP2M DIKTI (Ministery of Higher Education) for supporting this research project.

REFERENCES

Bandiati S.B.K., 2005. Interaksi gen, Buku Ajar Genetika ternak, Fakultas Peternakan Universitas Padjadjaran. Bandung.

Bandiati S.B.K., 2006. Laporan Hibah Penelitian A3 "Analisis Marka-DNA Terciri pada Ayam Pelung", Fakultas Peternakan Universitas Padjadjaran. Bandung.

Barker J. S. F., W. G. Hill, D. Bradley, M. Nei, R. Fries and R. K. Wayne,. 1998. Measurment of domestic animal Diversity (MORAD) Original working group report, FAO, Rome.

Broody, 1945. Bioenergetik.

Direktorat Jenderal Bina Produksi Peternakan, 2004. http://www.deptan.go.id/infoeksekutif /nak/2004

Groeneveld, E., 1998. VCE4 User's Guideband Reference Manual Version $.2 Institute of Animal Husbandry and Animal Behaviour, Federal Agriculture Research Center. Germany.

Iskandar, S. dan Triana Susanti, 2007. Karakter dan Manfaat Ayam Pelung di Indonesia. Balai Penelitian Ternak. PO Box. 221. Bogor. Indonesia.

Qinghua Nie, Meixia Fang, Liang Xie, Min Zhou, Zhangmin Liang, Ziping Luo, Guohuang Wang, Wensen Bi, Canjian Liang, Wei Zhang and Xiquan Zhang, 2009. The PIT1 gene polymorphisms were associated with chicken growth traits.

Raman, H., A. Karakousis, S. Moroni, R. Raman, B. Read, 2002. Development, validation and allele diversity of microsatellite markers closely linked with the Alp locus associated with Alumunium tolerance in barley. Proceedings of The 10th Australian Barley Technical Symposium. The Regional Institute Ltd. Australia.

Rasyaf M., 1989. Pengelolaan Produksi telur. Kanisius. Yogyakarta.

Sambrook J., E. F. Fritsch and T. Maniatis, 1989. Molecular Cloning: A Laboratory Manual 2nd ed, Cold spring Harbour, Cold Spring Laboratory Press, NY.

Sudiro F., 1993. Aneka Ayam Hias dan Piaraan. Cetakan Ke 2. Kanisius. Yogyakarta. Hal 1-6.

Suparno I., 1994. Ilmu dan Teknologi Daging, Cetakan ke 2. Gadjah Mada University Press. Yogyakarta. Hal 35.

Tanudimadja K., 1974. Anatomi Veteriner VII: Anatomi dan Fisiologi Ayam. Diktat Kuliah. FKH-IPB. Bogor.

Tillman A.D., 1991. Ilmu Makanan Ternak Dasar. Gadjah Mada University Press. Yogyakarta. Hal 161-179.

Toth G., Z. Gaspari, J. Jurka, 2000. Microsatellites in different eukaryotic genomes: Survey and analysis. Genome Research. 10 (7) : 967-981.

Warwick R.E., J.M. Astuti dan W. Harjosubroto, 1990. Pemuliaan Ternak, Gajah Mada University Press. Yogyakarta.

Winter A.R., E. M. Funk, 1960, Poultry Science and Practice. Fifth Edition. J.B. Lippincott Company.