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SUPERIORITY OF FILIAL RABBITS DERIVED FROM PUREBRED AND INDONESIAN LOCALBRED BASED ON PHENOTYPE AND GENOTYPE

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ABSTRACT

In Indonesia, rabbits have developed as small-scale businesses in tourist areas in the form of ornamental rabbits or meat rabbits. The rabbits are kept various kinds of purebred or crossbreeding between purebred and local Indonesian rabbits. The purpose of this study was to observe the genotype F1 crossbreed through the molecular analysis of various rabbit breeds that had been carried out by rabbit breeders as genotype diversity. This research method was a case study and experiment using various rabbits that many farmers raised. The purebred of rabbits used include Rex, Satin, Lion, Flemish Giant, New Zealand White, local Indonesian rabbits. The rabbit crossed include Rex with Satin, Lion with Indonesian Local-bred, Flemish Giant with Local. The Phenotype analysis for F1 derived from purebred and Local-bred under the same management. Molecular analysis was carried out using Polymerase Chain Reaction (PCR) and Polymerase Chain Reaction-Restricted Fragment Length Polymorphism (PCR-RLPF) with GH primers with HaeIII restriction enzymes. Descriptive analyzed was used for describing research data. The results showed that all samples carried out PCR with GH5 were amplified at 289 bp. PCR results showed that almost all local rabbits were AB-type (85% of the total sample) while all rabbits were purebred and F1 crossbreed with Local-bred had BB type, and phenotype superiority F1 to the dam was 18.51-7.41 %. The conclusion from this study was the F1 derived from purebreds, and Local-bred had a superiority genotype and phenotype compared to the dams (Local-bred).

Keywords: Rabbit, GH, HaeIII, PCR-RFLP, crossbreeding

1. INTRODUCTION

In contrast to other agricultural commodities, livestock has a role and a very complex function in the social and cultural life of Indonesian society. Economic development and globalization have encouraged people to consume animal protein, including meat consumption more (Dwiyanto and Priyanti, 2009).

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This leads to the world of farming in Indonesia is very dependent on other countries, especially meet the food demand of materials of animal meat such as beef and chicken meat and eggs so that less food sovereignty and create food dependency on other countries is very high. The uniqueness of the rabbit business was a small business with middle-class consumers, due to the price of rabbit meat 2-4 times the price of chicken meat with revenues rabbit breeders with the scale of more than 100 individuals was approximately 5 million per month (Mudawamah et al., 2014). Indonesian Local rabbits (Lepus Negricollis Cuvier) maintained by many people because it is easy to maintain. Besides, the price of local rabbits was also relatively inexpensive and resistant to disease because it had been a long time to adapt to the environment. To increase the production of rabbit meat from local farms had been a cross between superior rabbit to local rabbit (Maj et al., 2009; Ouyed and Brun, 2008; Al-Saef et al., 2008). One such effort by crossing local rabbit females to males Lyon and Flemish Giant. Superior type rabbits of Lyon and Flemish giant are widely available in Indonesia.

Therefore, one of the issues on rabbit farms that were derived from imported rabbits and local Indonesian rabbits should be supported by molecular research related to growth. Research on gene hormone (GH) with PCR-RFLP related to meat type is widely used in ruminants (Seavagan et al., 2017; Shareef, 2018), poultry (Yurnalis et al., 2017). Unfortunately, similar research through PCR-RFLP with GH for meat type of rabbit does not exist. So, the crossbred for their growth potential as meat rabbits.

2.MATERIALS AND METHODS

This research method was case study and experiment using a variety of purebred rabbit that was widely raised by farmers in Indonesia including Rex, Satin, Lion (L), Flemish Giant (FG), New Zealand White (NZW), and Indonesian Local-bred (Local). Besides that, samples also used F1 crossbreeding derived from Rex x Satin (Reza), Lion x Local (LLocal) and Flemish Giant x Local (FGL). Data analysis was descriptive with percentage analysis and NTSYS (Numerical Taxonomy and Multivariate System) software. Superiority F1 to Dam.

Superiority F1 to dams. The data was taken by observing litter size and F1 birth weight and weight gain for one month to the dam which raised the same management, with formula as followed:

Superiority F1 to Dam = {(% deviation litter size F1 to Local) + (% deviation birth weight F1 to Local) + (% deviation weight gain for one month F1 to Local)}/3.

DNA Isolation. The samples used the down hair from F1 various breed rabbits were then put in a 1.5 ml Eppendorf tube containing CTAB (Cetyltrimethylammonium bromide) buffer extracted 300 µl and stored in a refrigerator if the sample was not directly isolated. DNA isolation with the following steps: the down hair from rabbits was vortexed and incubated at 60oC for 1 hour, then cooled at room temperature, after that added 300 µl CI (chloroform: isoamyl alcohol = 24: 1) and vortexed about 20 seconds. The solution was centrifuged at a speed of 10,000 rpm for 10 minutes at 25o C. The supernatant was transferred to a new 1.5 ml Eppendorf tube, 2-propanol was added

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and gently shaken and incubated at -20o C for 20 minutes. Then centrifuged at a speed of 10,000 rpm for 10 minutes at 4o C, removing the supernatant and washing the pellets with 70% ethanol. The pellets were dried at 55o C, and 50 µl TE (Tris-HCl and EDTA) were added and stored at -20o C.

Polymerase Chain Reaction (PCR). Molecular detection of this study used Growth Hormone primer (Malveiro et al., 2001) in the following sequence: GH5F: 5'-AAA GGA CAG TGG GCA CTG GA-3' GH5R: 5'-CCC TTG GCA GGA GCT GGA AG-3' PCR cocktail used with the composition of dh2O was 2 µl, primer F and primer R were 1 µl, 2x go Taq green 5 µl, DNA 1 µl, whereas PCR-RFLP using the enzyme HaeIII (GG ↓ CC) with the composition of PCR-RFLP cocktail was 4 µl of PCR DNA, 10 µL of BSA buffer 1 µl, 4.5 µl of dH2O, 0.5 µl of enzyme and cocktail PCR-RFLP incubated at 37 ° C and stop the reaction at 65 ° C for 10 minutes. The PCR program was predenaturation at 95o C for 5 minutes, denaturation at 95o C for 30 seconds, 55o C annealing for 30 seconds, final extension of 72o C for 10 minutes with a cycle of 35 times. DNA electrophoresis results from PCR and PCR-RFLP with agarose 2% and 4%.

3.RESULTS AND DISCUSSION

PCR Results From the PCR results with GH5 in F1 rabbits from crossbreeding, purebred rabbits and local breed showed that all samples were successfully amplified at 289 bp (Figure 1). Many studies were similar, but in other animals with GH1, GH 2 and GH 3 were amplified at 436 bp, 891 bp, and 441 bp (India buffalo, Janmeda and Vataliya, 2017), 214 bp and 365 results of amplification with GH4 and GH5 on Local Sheep (Seevagan et al., 2017).

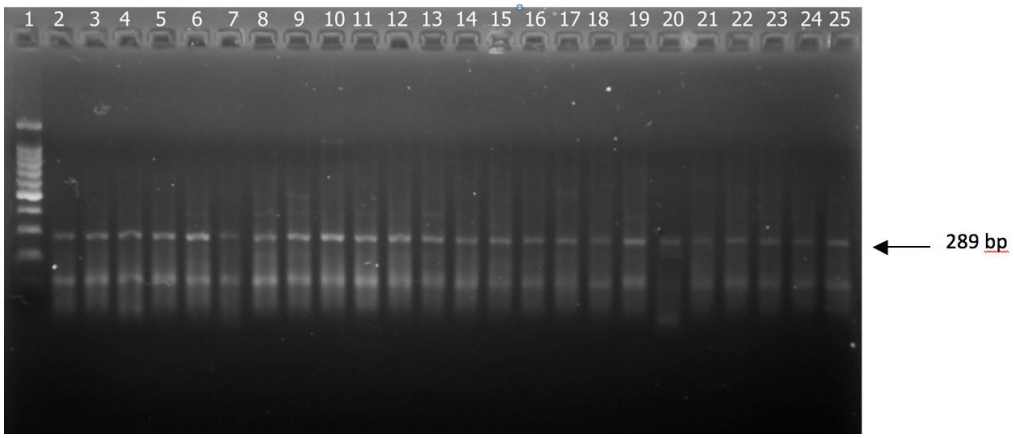

Figure 1. Amplification PCR result of All Samples at 289 bp

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Based on the data Tabel 1 showed that the local rabbit (Local) had AB and BB genotypes in the exon GH gene five after cutting HaeIII enzyme, AB genotype consists of A and B alleles (Figure 2, Tabel 1).

Table 1. The Genotype of the PCR-RFLP results in various breed rabbits

Breeds	n	Genotypes		Genotypes (%)	
		AB	BB	AB	BB
Local	7	6	1	85.71	25
NZW	3	-	3	0	100
L	3	-	3	0	100
Local	1	-	1	0	100
FG	3	-	3	0	100
FGL	2	-	2	0	100
Rex	4	-	4	0	100
Reza	5	1	4	20	80
Satin	4		4	0	100
Total	32	7	25		

PCR-RFLP results (Table 1) showed that the genotype of all purebred rabbit and F1 crossbreed between purebred and Indonesian local breeds were BB type (100% of the total sample), except for F1 crossbreeding between purebred Rex and Satin was 80% with BB type and 20% with type AB. On the contrary, almost all local rabbits were AB-type (85.71% of the total sample) which was different from the results of PCR-RFLP on purebred and F1 crossbred purebred with Local-bred, only 15% were BB genotype. The results of this study were supported by research on PCR-RFLP with GH genes in goats only producing 2 types of genotypes (Ilham et al., 2016) also in sheep using GH1 to produce 2 genotypes namely AA and AB but in GH5 they produce three genotypes namely GG, GH and HH (Marini et al., 2015).

Phylogenetic features based on the results of PCR-RFLP with GH5 gene and HaeIII restriction enzyme in rabbit purebred and crossbreeding could be seen in Figure 3.

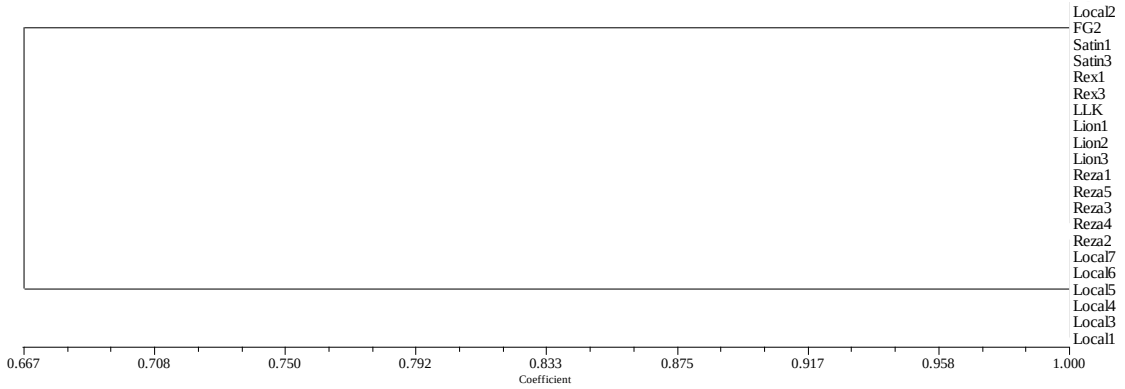

Figure 1. Rabbit dendogram based on PCR-RFP GH gene with HaeIII cutting in rabbit purebred and crossbreeding obtained from UPGMA

Based on the data above shows that all purebred rabbits and F1 crossbreeding with Local-bred had genetic distance values of 0.667 with all samples of local rabbits (except local2) and Reza2. The difference in genetic distance was caused by the A allele (289 bp) in local rabbits that decreased productivity than the purebred and crossbred rabbits. This statement is proven in F1 data which showed superiority percentage F1 derived from purebred and Local-bred than its dams (Table 2).

Table 2. F1 Superiority percentage than its dams

No	F1 Crossbred	n	F1 Superiority than its dams (%)
1	F1 Flemish Giant x Local	5	18.51
2	F1 Lyon x Local	5	7.40

Table 2 showed that the superiority of F1 resulting from a crossbreeding between purebred and Local-bred had a positive superiority value of phenotype to its dam breeds (Local-bred) seen from litter size, birth weight, and weight gain until the age of one month. Besides that, F1 derived from purebred and Local-bred had also genotype superiority supported by PCR-RFLP data which showed a difference in F1 crossbreed genotype between purebred and Local breeds which were 100% BB-type compared to local livestock which 85% were AB type.

4.CONCLUSIONS

F1 derived from purebreds and Local-bred had phenotype and genotype superiority compared to the dams (Local-bred).

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