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GENETIC EVALUATION OF PRIANGAN SHEEP USING MULTIVARIATE MATERNAL GENETIC EFFECT AND THEIR VARIATION OF SHEEP MITOCHONDRIAL-DNA

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Abstract: This research was conducted at the Margawati Station for Breeding and Development of Priangan Sheep, and the Farmers' Group of Sub District of Garut as well as and in the Sub District of Bandung on June 2008. The objectives of this research were to evaluate genetic parameter, correlated response to selection (indirect selection Weaning Weight (WW) base on Birth Weight (BW) and to take out the variation of sheep Mitochondrial-DNA (*mt*-DNA). The data comprised of 605 BW- records and 605 WW -records of progeny from 23 sires and 355 dams of Priangan sheep and 36-blood sample from different area. Variance component, heritability and genetic correlation were estimated by Restricted Maximum Likelihood (REML) using Maternal Genetic Effect Model with the program of VCE 4.2. Best Linier Unbiased Prediction (BLUP) using the PEST program estimated Breeding Values of BW. Fixed effect was sex, parity of dams and litter size. Variation of sheep *mt*-DNA was analyzed using PCR and the complementary sequence reads were combined using Phred / Phrap software. Heritability for BW and WW were 0.096 ±0.05 and 0.280 ±0.09, as low category. The genetic correlation between BW and WW was 0.502 ±0.261. The accuracy of selection of BW and WW were 0.309 and 0.529. The breeding Value of BW was 0.41-(-0.40) gram. The highest correlated response to selection was 0.412 kg. While selection intensity was 6.86% for 20 ewes and 0.40% for 1 ram (sex ratio 1 ♂: 20 ♀). The DNA-region (control region) of *mt*-DNA of Priangan sheep was 1447 *nt*. Variation of sheep *mt*-DNA has been analyzing for DNA sequencing.

Key words: correlated respond to selection, mitochondrial-DNA, polymerase chain reaction (PCR)

Introduction

The Priangan sheep is called also as Garut sheep; they are belonging to family *Ovidae*, genus *Ovis*, and species *Ovis aries*. They are variety of the Javanese Thin-tailed. Priangan sheep has been identified as new local breed in the regency of Garut as product of crossbreeding between local sheep, Merino and Cave came from Africa in the past. The first offsprings of cross breeding between Merino and local Garut sheep were used as wool, meat and manure production by Regent of Limbangan and by other sheep breeders around Garut. They were then distributed from Sub District Priangan to around West Java Island in the 19th century *(Merken and Soemirat, 1926, cited by FAO)*. Mt-DNA is power tool for tracking ancestry through females and has been used in this role to track the ancestry of many species back hundreds of generation. In sexually reproduction organisms, mitochondria are normally inherited exclusively from mother.

Priangan sheep generally has convex face. Ewes look polled while rams have big horns. Due to their big horns and aggressiveness, many farmers raise these rams for traditional ram fighting contest, a popular activity in West Java, Indonesia. The Association of Sheep and Goat Farmer (HPDKI) has identified Garut sheep since the year of 2002. The qualitative trait of Priangan sheep are listed as follow: lack of external ears (*rumpung*) to medium (*ngadaun hiris*), hairy throat ruff and the slight fat at the base of tail, the color is extremely varied from black, black with little white, black and white pied, white with little black, white, grey or grey and white, tan or tan and white. The horns are heavy pronounced at cross ridges and sharp angles.

The Margawati Station for Breeding and Development of Priangan Sheep is established, as Government owned Institution to improve Garut sheep breeding quality. In addition, sheep Farmers smallholder also have tried to select the available better Garut Sheep to produce elite ewes in order to improve genetic quality. The superior character of female reproduction of Garut sheep is prolific. It is supposed to be not easy, especially in term of money and time problems. However, selection has been used to improve their genetic progress to produce exotic animal with high production and reproduction abilities.

The productivity and reproduction are quantitative traits as expression of combination between genetic factor and environment. The average of birth weight (BW) of Priangan sheep was 2.75 kg for male and 2.35 kg for female. The average of weaning weight (WW) was 11.5 kg for male and 9.11 kg for female *(Gunawan, 2006)*.

The genetic parameter can be estimated by using half sib correlation with nested experimental design, full sib and offspring-parent regression *(Falconer and Mackay, 1995)*. In the year, 2002 *Anang* et al. have developed computer program for Cumulative Model (CM), Multiple Trait Model (MTM), Fixed Regression

Model (FRM) and Random Regression Model (RRM) using statistical analysis animal model for REML to estimate genetic parameter. They initially need the pedigree information before designing experiment.

The objectives of this research were to evaluate genetic parameter, correlated response to selection (indirect selection Weaning Weight (WW) base on Birth Weight (BW) and to take out the variation of sheep Mitochondrial-DNA (*mt*-DNA).

Materials and Methods

The object of this research was 605 Priangan Sheep (312 male and 293 female lambs) as progeny from 23 sires and 355 dams, which was recorded from November 2005 to November 2007 by the Margawati Station for Breeding and Development of Priangan Sheep. Meanwhile, descriptive analyses method was utilized for this research.

The estimation of variance component and correlation genetic used Animal Model for REML, and the program used Variance Component Estimation – VCE 4.2 *(Groeneveld, 1998)*. Breeding value is was estimated by Multiple Trait Model (MTM) Best Linier Unbiased Prediction (BLUP) using Maternal Genetic Effect Model for PEST programs. Fixed effect was sex, litter size, parity of dams and year season.

The statistic formula for Maternal Genetic effect Model:

$$Y_1 = X_1\, b_1 + Z_1\, u_1 + W\, m_1 + e_1 \qquad \textit{and} \qquad Y_2 = X_2\, b_2 + Z_2\, u_2 + Wm_2 + e_2$$

Where:

Y_1 and Y_2 = vector of observations of BW and WW
X_1 and X_2 = design matrix connecting the observations to fixed effects of BW and WW
Z_1 and Z_2 = design matrix connecting the observations to random effects of BW and WW
W_1 and W_2 = design matrix connecting the observations to maternal effects of BW and WW
b_1 and b_2 = vector of fixed effects of BW and WW
u_1 and u_2 = vector of random effects of BW and WW
m_1 and m_2 = vector maternal effect of BW and WW
e_1 and e_2 = vector residuals of BW and WW

Correlated response to selection between BW and WW can be estimated by multiplying total intensity of selection with the accuracy of selection for BW, accuracy of Selection for WW, genetic correlation between BW and WW and phenotype standard deviation of WW ($CR_2 = i\ h_1\ h_2\ r_{g12}\ \sigma_{p2}$, where CR_2 = response to selection of BW and WW, i = total intensity of selection [(i rams + i ewes)/2}, h_1 = accuracy of individual selection for BW records ($\sqrt{h_1^2}$); h_2 = accuracy of individual selection for WW ($\sqrt{h_2^2}$); r_{g12} = genetic correlation between

BW and WW, σ_{p2} = phenotype standard deviation of WW *(Falconer and Mackay, 1996)*.

Sheep Mitochondrial-DNA isolation:

The blood samples were taken aseptically from sheep's vena much as 2 ml. The samples were then collected using EDTA as anticoagulant. Furthermore, the samples were taken in to laboratory utilizing cool box with the temperature was kept in the range of 0-5^{O} *(Sambrook et al., 1989)*.

Amplification of *mt*-DNA for PCR analyses:

PCR was done in the level of as much as 0.05 μl from the total of *mt*-DNA with DyNAZyme II standard volume of 50 μl (Jennifer et al. 2007). The list of primer can be described as follows: OARCR 15339-15410 L, OARCR 15412-15435 L, OARCR 16363-16391 H, OARCR 25-48 H.

Electrophoresis of PCR Product

Resulted *mt*-DNA from electrophoresis purification used 6% polyacrylamide with silver staining coloring to see the pattern of band microsatellite that was amplification by primer.

Data Fluorogram Analyses

Fluorogram Analyses utilized *Cimarron* 3.12 base-caller implemented in MegaBACE Sequence Analyzer (Amersham Biosciences). The complementary sequence reads were combined using Phred/Phrap software *(Ewing et al., 1998)*.

Results and Discussion

BW and WW in the laboratory of Cooperative of Indonesian Pigs Holder i is recorded every 0 and 100 days after partus. The data comprised 605 BW records and 605 WW records of progeny from 23 sires and 355 dams. Recording started from body weight of zero day to mature weight. After all data get tabulated and given code according to REML, then it can be provided in first data description structure as a result from Excel, as written on Table 1.

The structure of data shows, that BW was higher and WW was higher in compare to the result of several references. *Gunawan (2006)* has published that male and female BW and WW of Priangan Sheep were 2.35 kg and 9.10 kg. There were deference result causes of management and year season.

Table 1. Structure data of BW and WW records in the Station of Breeding and Development Priangan Sheep Margawati.

Female						Male					
1	2	3	4	5	6		7	8	9	10	11
Trait	*N*	Average (kg)	min. (kg)	Max. (kg)	CV (%)		*N*	Average (kg)	min. (kg)	Max. (kg)	CV (%)
BW	293	2.49±0,62	0.99	2.31	25.16		312	2.68±0,60	1.01	2.09	22.38
WW	293	10.71±2.78	9.50	20.00	26.95		312	11.76±3.26	9.00	19.00	27.76

Where: CV= Coefficient Variation; *N* = total data.

For the better data should’t have the big deviation standard, so the selection process won’t loose so much lambs. But, to select a group of lambs with the big Coefficient Variation (CV) of data will give the effective result, if only not less than 10% or closer to 0, because the genetics progress will be very small and intend to reach the selection plateau.

In a line with *Falconer and Mackay (1996)*, that to wait the selection process the correlated recording character, it needs big budget for measurement, then selection has to be done with correlated indication.

Table 2. Heritability, accuracy of selection, standard deviation and genetic correlation

Trait	Heritability (h^2) ± se	Accuracy ($\sqrt{h^2}$)	Maternal effect (m^2) ± se	$r_{g\ (BW\text{-}WW)}$
BW	0.096 ±0.023	0.309	0.261 ±0.062	0.502 ±0.083
WW	0.280 ±1.218	0.529	0.074 ±0.324	

According to *Dalton (1981)* if the selection is held, the high heritabilty will be better for the effectiveness of the process.

The decision will be made not only based on the heritability value, but also the genetics correlation between both the variables, if selection will be done indirectly. The heritability of the two characters and genetics correlation should be known. The point is agreed by *Falconer* and *Mackay (1996)* who come to public with the experiment called *double selection experiment*.

The genetics correlated value between BW and WW can be seen on Table 2. This genetic correlation is needed to select the lambs indirectly. The value shows the hightness relationship between this two kinds of characters that get the influence by the breeding value. The genetics progress in BW will give the progress of genetics to the WW. The genetics correlation value of BW and WW provides the highest value (0.502 ±0.083), including the high positive category.

Breeding value is the value from lambs genetics quality for a certain character that is given relatively based on the individu in population. The estimation of this value is the main factor to evaluate the excelent swine genetics because a half of the value will be given to the next generation. The research result shows that the range of positive breeding value of BW is between 0.41-(-0.40) gram. In BLUP for PEST program, this value has the position of positive and negative and if zero (0) is equivalent the average of population.

Based on the analisys there were 140 ewes (47.78%) that were more than the average and selected as the dams. There are 7 heads (2.24%) of selected rams based on their breeding value that were over the population average. Obviously, the genetics potency for growth character for rams is higher than the ewes. According to this ranking, will be selected some rams that will get involved in the process, to gain the intensity of selection. The respond to selection for BW and WW are 0,096 kg and 0,716 kg, while the correlated respond to selection between BW and WW is 0.123 kg.

The intensity of selection is the average of deviation for all selected sheep in one phenotypic standard deviation unit. In simply, intensity of selection is excess average of selected ewes in the population average. It depends on number of selected rams. If selection involves big number of sheep, it will make intensity of selection and genetic progress run slowly. It refers to *Kinghorn (1992)* that the intensity of selection inversely proportionate with the number of the selected sheep.

Analysis of Variation Sheep *mt*-DNA Using PCR

The Figure 1 shows the character of electrophoresis analysis PCR product using agarose 2%. As can be seen in Figure 1, after being purified as shown in the result of electrophoreses, the result of PCR showed that the banding was for base pairs of 1.447. According to PCR's current result that shows the place of band's pattern is in the same place of 1.447 *pb*. It means that the postulate from previous researchers should be revised since the form of lack external ears in Priangan sheep is qualitative traits that is determined by only one or two pairs of gen. This is not influenced by environment and this does not have any relation with production.

From the result of sequencing read shows that only AT code sample describing variation like deletion in the 75 *pb* long that is placed in the position of 15790 to 15864 *pb* of *mt*-DNA.

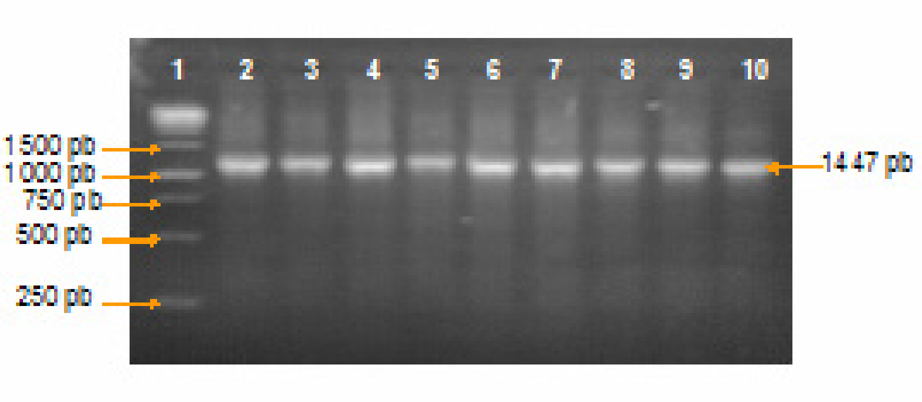

Figure 1. Electrophoresis characteristic result using agarose 2%

Where :
Lable 1 : *marker 1000 bp*
Lable 2 : sample 1 O
Lable 3 : sampel 5 O
Lable 4 : sampel 2 OM
Lable 5 : sampel 4 OK
Lable 6 : sampel 6 AT
Lable 7 : sampel 1 MW
Lable8 : sampel 1 VD
Lable 9 : sampel 2 VD
Lable10: sampel 3 VD

Conclusion

The highest response to selection of BW (0.096 kg) and WW (2. 152 kg) is reached at 6.82 % (20 heads) selected ewes and 0.32 % (1 head) selected ram, in ratio 1 ram: 20 ewes. However, it is recommended that the ratio for sheep is 4 rams: 80 ewes (sex ratio 1 ♂: 20 ♀) to avoid inbreeding. In this research, the similar ratio value, this has response to selection 0.024 kg for BW and 1.073 kg for WW. At the same intensity, the correlated response between BW with WW is 0.181 kg. The relative efficiency value of indirect selection for WW according to BW is 0.168.

Only 10 samples of 36 PCR products could show good bands in the position of 1447 *bp*. The variation of sheep *mt*-DNA of Priangan sheep gave indication of deletion (75 *bp*) at the position of 15790 to 15864 *bp* of *mt*-DNA.

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Genetska evaluacija priangan rase ovaca korišćenjem multivarijantnog maternjeg genetskog uticaja i njihova varijacija mitohondrijalne DNK ovaca

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Rezime

Priangan ovca je identifikovana kao nova lokalna rasa u regionu Garut kao proizvod ukrštanja lokalnih rasa, merino i cave iz Afrike u prošlosti. Ocena komponenti varijanse i genetske korelacije korišćenjem Animal Model for REML, i programa Variance Component Estimation-VCE 4.2 *(Groeneveld, 1998)*. Odgajivačka vrednost je ocenjivana pomoću Multiple Trait Model (MTM) Best Linier Unbiased Prediction (BLUP) korišćenjem programa Maternal Genetic Effect Model for PEST. Fiksni uticaji su bili pol, veličina legla, paritet majki i sezona. Variranje *mt*-DNK ovce je analizirano korišćenjem PCR *(Jennifer i sar., 2007)*. Lista prajmera se može opisati na sledeći način: OARCR 15339-15410 L, OARCR 15412-15435 L, OARCR 16363-16391 H i OARCR 25-48 H. Heritabiliteti za

masu na rođenju (BW) i odbijanju (WW) su bili 0.096 ±0.05 i 0.280 ±0.09, kao niža kategorija. Genetska korelacija između BW i WW je bila 0.502 ±0.261. Tačnost selekcija na BW i WW je bila 0.309 i 0.529. Priplodna vrednost za BW je bila 0.41-(-0.40) grama. Reakcija na selekciju u korleaciji (indirektna selekcija WW masa pri odbijanju na bazi BW mase na rođenju je bila niža od direktne baze za selekciju na WW). Korelativna reakcija na selekciju se ne preporučuje. Variranje *mt*-DNK ovce rase priangan je imalo indikaciju delecije (75 *bp*) na poziciji 15790 do 15864 *bp mt*-DNK.

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