Bulletin of Animal Science, Special Edition, 1995 *ISSN 01260 - 4400*

Blood Protein Polymorphism on Javanese Fat Tailed Sheep and Javanese Thin Tailed Sheep

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ABSTRACT: The aim of this study is to know the genotype characteristic of Javanese Fat Tailed Sheep and Javanese Thin Tailed Sheep through electrophoresis methods. Sixty sheeps were used, which comprised are 5 male and 25 female Javanese Fat Tailed Sheeps; 5 male and 25 female Javanese Thin Tailed sheeps. Polyacrylamide gel electrophoresis was used in this study. The result of study indicates that Javanese Fat Tailed Sheep and Javanese Thin Tailed Sheep has a different band patterns.

Key Words: Sheep, Protein Polymorphism, Electrophoresis

Introduction

Sheep is a kind of small ruminant that potential to be protein source. Sheep is very popular to small farmers because its good tolerance to bad condition good reproduction and relatively needs small investment compared to other ruminants. The demand oF sheep meat is also high.

Javanese Fat Tailed Sheep ("DEG") and Javanese Thin Tailed Sheep ("DET") are breds that commonly raised by farmers at East and Central Java. The DEG which are promated to be a meat source, have big and long tail sharpenning at its end, white hair, and the male have no horn (Sumoprastowo, 1987). The DET, which also a meat source are prolific breed. The DET smaller that the DEG. It have white, or black/brown spotted white hair, the male have horn (Sumoprastowo, 1987 ; Direktorat Jenderal Peternakan , 1991).

The development of sience and technology has contribution in the development of animal breeding, particulary on electrophoresis method. The electroforesis method is a method that can determine a given characteristic of protein (Walker and Doonan, 1992). The electrophoresis method is also used for determining protein polymorphism of body liquid and other tissue (Herlina and Salundik, 1994). Protein is inheritance gene produced. Therefore, the determination of protein fraction of animal body liquid or other tissues can also determine the genotype characteristic of the animal. Discribed by Tjahjaningsih (1991) that the variation of blood protein band pattern is resulted from the variation of genes the regulate the characteristic which is expressed by the protein bands; so that some qualitative characteristic are physically influenced by expression of the genes action.

Gahne et al. (1977) wrote that electrophoresis method using the media polyacrilamide gel has a high separation capability. Gahne et al. (1977) also described that a simple method of horizontal polyacrilamide gel produced transferin, post transferin, albumin and post albumin.

The objective of this study is to know the genotype characteristic using the DEG and DET blood protein polymorphism analysis.

Materials and Methods

The blood sample was taken at the Animal Breeding & Genetics Laboratory Faculty of Animal Science Diponegoro University, while the analysis processes were done at Animal Breeding & Genetics Laboratory Faculty of Animal Science Bogor Agriculture University.

Materials

Sixty sheep were used in this study, comprised 5 males and 25 females of DEG and 5 males, 25 females of DET.

Method

The electrophoresis method applied in this study was vertical polyacrilamide gel electrophoresis (Ogita & Markert, 1979). The analysis procedure of blood plasma was done gradually ie: the collection

of blood sample, the preparation of blood sample, the making of material for analysis, the making of electrophoresis gel, the dropping of blood sample, colouring, and destaining.

The collection of blood sample. Five cc of sheep blood was taken from vena jugularis using venoject heparin tube and than was kept in the ice flask

The preparation of blood sample. The blood was centrifuged 3000 rpm during 15 minutes, to separate the haemoglobine from the blood plasm. The blood plasm was placed into small bottle, and the bottle then was closed by aluminium foil, and then was kept in the refrigerator on 4°C.

The making of chemical analysis materials. The making of the running gel, the stacking gel, the electrode buffer and the sample materials were based on the Gahne et al. (1977), while the staining and destaining solution were based on Thinnes et al. (1975).

Material I, Running gel

Material IA : 39.0 g accrylamide, 1.0 g Bis, and 20.0 ml glyserol, added by aquades to reach 100 ml.

Material IB : 9.15 g Tris, 3.0 ml HCl, added by aquades to reach 100 ml.

Meterial IC : 0.2 g amonium persulfat, added by aquades to reach 100 ml.

Material ID : 400 ul TEMED, added by aquades to reach 100 ml.

Material II, Stacking gel

Material IIA: 38.0 g acrylamide, 2.0 g Bis, 20.0 g glyserol, added by aquades to reach 100 ml. Material IIB: 1.5 g Tris, 1.0 ml HCl, added by aquades to 100 ml.

Material IIC: 0.4 g amonium persulfat added by aquades to reach 100 ml.

Material IID: 200 ul TEMED , added by aquades to reach 100 ml.

Material III, Electrode buffer comprise are 1.5 g Tris, 7.2 glysin, added by aquades to reach 100 ml.

Material IV, Material for sample blood comprise are 1.64 g Tris, added by HCl to 25 ml, 40 ml glyserol, 20.0 ml 0.01% brom blue fenol added by aquades to reach 15 ml.

Material V. Staining material comprise are 90 g TCA, added by 1200 ml aquades, 300 ml methanol, 105 ml acetic acid and 37.5 ml 1 % commasie blue.

Material VI. Destaining material comprise are 800 ml aquades, 250 ml methanol, 100 ml acetic acid.

The materials I - IV were kept in a refrigerator, while materials V and VI were kept in dark bottle.

The making of electrophoresis gel. This material consisted of running gel, and stacking gel. Running gel consisted of 4 ml material IA, 5 ml material IB, 2.5 ml material IC, 2.5 ml material ID and 6 ml aquades. The formed running gel then was poured into electrophoresis slab glass to reach appoximatelly 3 cm heigth from electrophoresis slab level. To make the surface of running gel flat, some isobuthanol was dropped in the running gel. The isobuthanol was kept still until the running gel was solid. The isobuthanol then was separated out from the electrophoresis slab. The stacking gel solution comprised 2 ml material II A, 5 ml material II B, 2.5 ml material II C, 2.5 ml material II D and added 8 ml aquades. The formed stacking gel then was poured into electrophoresis slab above the running gel until surface of electrophoresis slab. The blood plas was placed on a comb like plastic ruler, and was put on the stacking gel before it become solid. Wait about 30 minutes until the gel was formed, and then polace electrophoresis slab in refrigerator during 45 minutes.

The dropping of blood sample. After gel was formed to make mixture for sample place comprise 0.7 µl material IV added 0.3 ul blood plasm. The mixture of sample blood then was placed into the blood sample, that was formed in the electrophoresis slab. The electrophoresis slab device then was connected to power supply on 250 volt- 25 mA. The protein separation process to from the blood protein polimorphism needed 150 minutes.

Colouring process. Gel then was taken out from the electrophoresis with help solution staining (material IV). The gel then was submerged in staining solution and then was placed in incubator during 2 hours on 4°C.

Destaining process. The last phase of the blood protein band pattern identification process was the destaining process. The destaining process was done 3 times to make the protein band pattern clearer.

The identification of the electrophoresis result was done immediately after the destaining process

ISSN 01260 - 4400

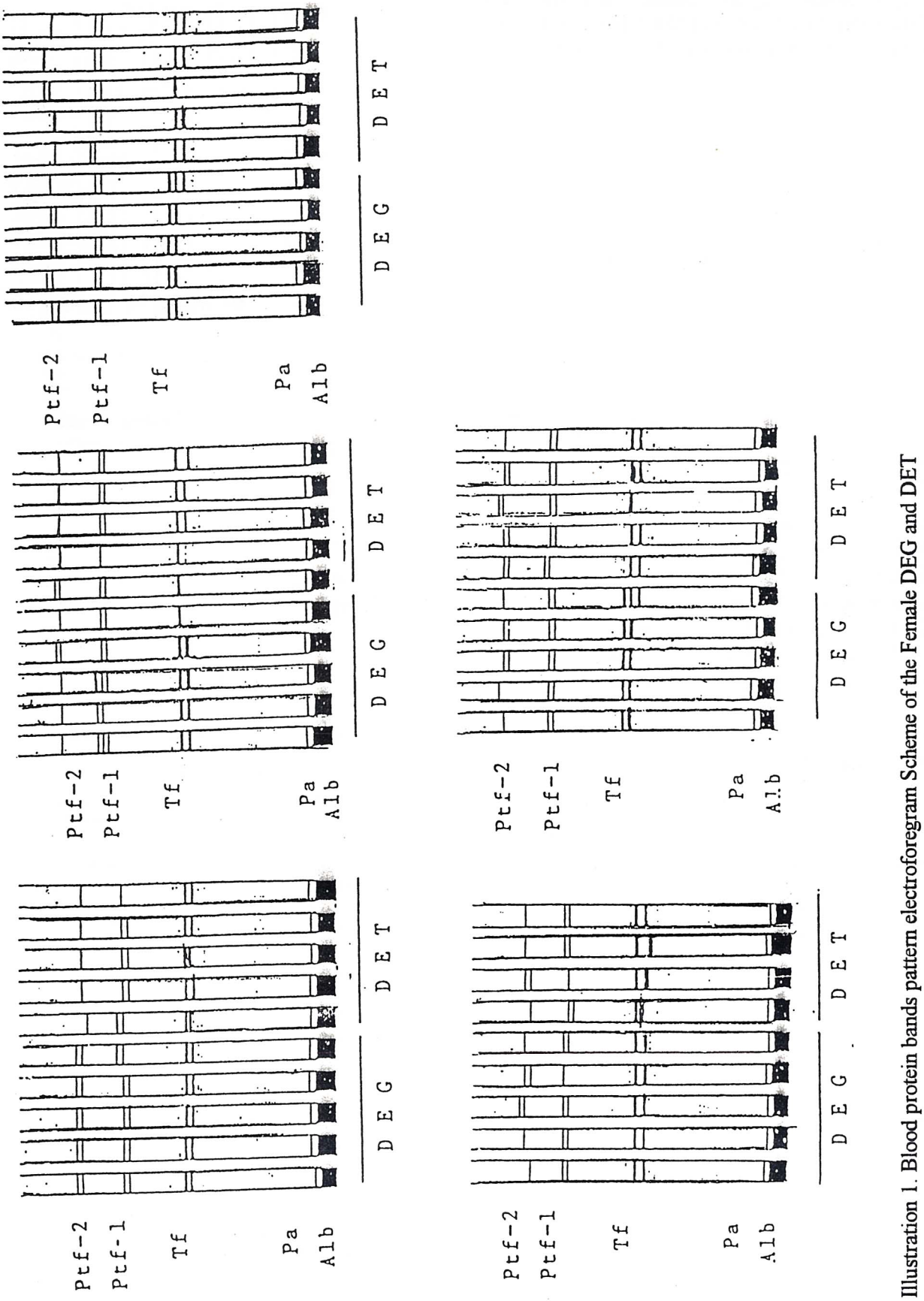

Illustration 1. Blood protein bands pattern electroforegram Scheme of the Female DEG and DET

using a electroforegram scheme. The identification of the sheep blood protein polimorphism was based on the genetic monitoring method of Gahne et al. (1977).

Result and Discussion

The Blood Protein Polimorphism

The identification of the DEG and the DET genotype characteristic was done by observing the blood protein polimorphism. The blood protein that was observed comprised 5 kinds, ie : PTf-1 (Post transferin-1), PTf-2 (Post transferin-2), Tf (Transferin), Pa (Post albumin), Alb (Albumin). The result of the analysis of the individual can be read on the illustration 1 and 2.

The Illustration 1 showed the blood protein polymorphism of female DEG and DET. PTf-2 on DEG consisted of 1 - 2 bands; while PTf-1 consisted 1 - 3 thin bands; the Tf consisted of 1 - 3 thick and clear bands; the Pa consisted of 1 band , while the Alb did not showed a clear band, because it was blocked by a dark area. The total of DEG protein bands were 6 to 7 bands. The result of the PTf-2, PTf-1 and Tf protein polimorphism were equal to the Herlina and Salundik (1994) is report. Whereas the Pa band polimorphism was not syncron with the Herlina & Salundik (1994), result which identified 2 bands. The results may caused by kinds of destaining material used in the respective study. Herlina and Salundik (1994) used amido black, while this study used coomassie blue.

The result of DET blood protein polimorphism showed 1 - 2 bands of PTf-2, 1 - 2 thin bands of PTf-1; 1 - 2 thick and clear bands of Tf; 1 Pa band while Alb band can not be read. The were 4 to 6 protein bands of the DET. The result of PTf- 2, PTf-1 and Tf of the DET was equal to the Herlina and Salundik (1994) report, while the Pa band was not. The used of different destaining material caused the different of the Pa band polymorphism.

The Illustration 2 showed the blood protein polymorphism of the male DEG and DET. On the DEG, there were 2 - 3 bands of PTf-2; 1 - 2 bands of PTf-1; 1 - 2 thick bands of Tf; 1 bands of Pa; while the Alb can not be identified. The total protein bands were 7 - 8. On the DET, there were 1 - 2 bands of PTf-2; 2 bands PTf-1; 2 thick bands of Tf; 1 band of Pa; while the Alb can not also be identified. The total protein bands was 6 to 7.

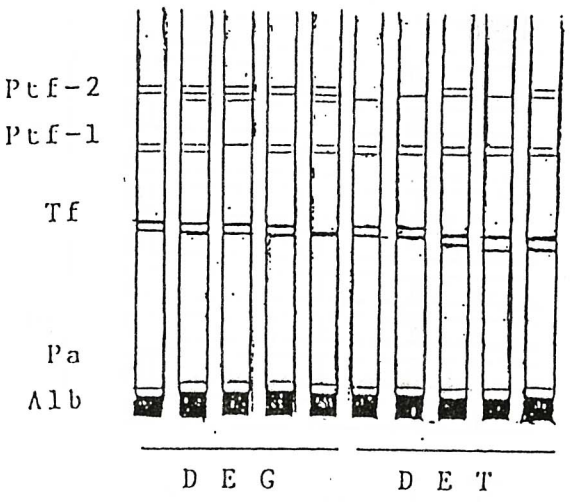

Illustration 2. Blood Protein Bands Pattern Electroforegram Scheme of the male DEG and DET.

Conclusion

The result of study indicateds that Javanese Fat Tailed Sheep (DEG) and Javanese Thin Tailed Sheep (DET) has a different blood protein band pattern.

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