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THE EFFECTS OF PROTEIN CONTENTS ON HYDROXIPROLINE CONTENT AND PHYSICAL QUALITY OF RABBIT SKIN

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ABSTRACT

Collagen is a major structural protein in skin and bones of all animals. Collagen has an important effect on physical strength of skin as well as influences penetration of chemical use on leather tanning. Hydroxiproline is an amino acid present only in collagenous protein; therefore, it can increase skin quality and improve leather tanning process. The research was conducted to evaluate the effect of different protein contents in rabbit feed that were 13 % (P1), 16 % (P2) and 19 % (P3). The feed rations consist of concentrate BR-1, BR-2, water spinach (*Ipomoea sp*), and cassava. All treatments were isoenergy that was 2740,8 cal. Drinking water was given *ad libitum*. The treatment was given to all rabbits for three months. After that, the animals were slaughtered and their skins were removed and sun-dried. Samples for hydroxiproline and physical quality test were taken from croupon area. The research used Completely Randomized Designs (CRD) and the differences among treatments were tested by Duncan's Multiple Range Test. The results showed that the hydroxiproline content and physical quality of rabbit skins differed significantly ($P<0,01$) among treatments. The hydroxiproline content in skins of rabbits given feed containing 19 % protein was higher than that containing 16 % and 13 %. The values of hydroxiproline were 11,8 % (19 % CP), 11,5 % (16 % CP) and 9,8 % (13 % CP). The tensile strength and elongation differed significantly ($P<0,01$). The values of tensile strength were 219,85 kg/cm^2 (19 % CP), 158,97 kg/cm^2 (16 % CP) and 152,27 kg/cm^2 respectively. The values of the elongation were 23,47 % (19 % CP), 21,33 % (16 % CP) and 20,10 % (13 % CP) respectively.

Keywords : Hydroxiproline, Protein, Physical Quality Of Rabbit Skin.

INTRODUCTION

Indonesia is a country rich of natural and human resources. One of the potential natural resources is livestock industry with all its products including leather. The development of leather and leather products industry can give significant benefits for the people especially to increase their incomes as well as to reduce the amount of unemployment by creating small and medium enterprises in leather and leather products industry.

Skin is a by-product commodity of livestock industry with a high economic value. Meat, skin, and other by-products can be produced from rabbit. As a matter of fact, many people love rabbit skin due to its attractive fur. In leather industry, skin is the main raw material to be processed into leather. Recent standard of leather quality test

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requires all leather product manufacturers to find a way to pass the standard particularly on the physical quality.

Main amino acids constructing collagen are glycine (33.5%), proline (12%) and hydroxiproline (10%). The rest consists of other amino acids. Interestingly, collagen contains only a few sulphate and tyrosine. Collagen is also the only protein containing a significant amount of hydroxiproline. Therefore, the amount of collagen in a skin tissue can be determined by measuring the hydroxiproline content in it. The other unique amino acid in collagen is hydroxiline. Collagen is the highest amount of protein in body consisting 30% of total protein in the body. Protein sub-unite prolimerising to form collagenous fibril is a long molecule chain called tropocollagen (Junqueira and Carneiro, 1980).

In relation with the significant benefits of leather industry, continuous efforts need to be done to improve leather manufacturing including its raw materials and processes. The existence of collagen greatly influences the physical strength of skin and mass transfering process as well as chemical reaction in skin tanning. Hydroxiproline is the amin amino acid constructing collagen. Thus, to increase skin quality and improve leather manufacturing, there is a need to find out the corelation between protein content in feed and hydroxiproline content in the skin.

The physical strength is determined by tissue structure and chemical composition in the skin. Feeding animal with higher protein contents will result in its thicker skin. Thicker skin means thicker fibril, fibre weave, and fibre bundle, making the skin become thicker and its physical quality become higher. The thickness of animal skin is determined by its species, genus, sex, age, and environment including care and feeding.

MATERIALS AND METHODS

The research utilized 18 local male rabbits aged 2.5 to 3 months and weight 802.5 to 92.5 grams grouped into three. Each group was given a different content of protein in feed: 13%, 16%, and 19% with isoenergy that was 2740.8 cal. Type of cages used was raised cages. The dimensions were 0.45 metres high, 0.90 metres wide, and 1.15 long and from the ground to the cage floor was 0.30 metres. Each cage was equipped with feed container, drinking water container, termometer, and a neon of 20 watts.

The research used balance electro cuisine scale with the accuracy of 1.00 gram to measure the animal and feed weight, scalpel knife, analytic scale acculab with accuracy of 0.01 grams. The feed given consisted of four types: BR-1, BR-2, from PT Japfa Comfeed, water spinach, and cassava.

The tensile strength and elongation test used Tensile Strength Tester.

Rabbit Care and Dressing

All the eighteen rabbits were equally grouped into three groups. First group was given feed with 13% protein, second group 16%, and third group 19% with isoenergy of 2740.8 cal. The amount of feed was based on 5% diet and increased in parallel with the increase body weight per week. Feed were given twice a day (at 08.00 a.m. and 17.00 p.m.), the rations of which could be seen in Table 1. Drinking water was given *ad libitum*.

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Table 1. Rations of feed

Treatment (%Protein)	Feed rations (DM Basis)			
	BR I	BR II	Cassava	Water Spinach
13%	13,57%	52,96%	22,69%	10,78%
16%	37,56%	38,46%	9,61%	14,37%
19%	88,93%	-	-	11,07%

Weighing of feed and its left-overs was done every day using feed scale. Weighing of body weight was done once a week to measure its increasing. The results were devided by the number of days to measure daily increasing of body weight.

Slaughtering of the rabbits was done at the end of the caring period. Before being slaughtered, they were fasted for 12 hours and their live weights were scaled. Dressing rabbit applied abdomen cut method and rabbits were hung.

Skin Drying

Principally, skin drying is a process of reducing water content in the skin to more or less 15%. The process was done by exposing streched skins under direct sunlight. The duration of sun drying depended on the temperature. It was done approximately one week when it was sunny or 2 weeks when it was cloudy. The sun drying was ended when skins looked transparent.

Measurement of Hydroxiproline Content

Materials utilized: rabbit skin and meat samples, NaCl 10%, Aqua Destillata, Petroleum benzene, solution of HCl 6 N, solution of NaOH 10 N, Buffer pH 6, Chloramine T, n-Propanol, Standard Solution of Hydroxiproline. The apparatus was: Spectrophotometer, Soxhlet Unite, Erlenmeyer 250 mL, digital analytic scale, Sentrifuge instruments, Filtering paper.

Samples were cut into 4 grams and added with 100 ml HCl and lime stone. After that, they were heated in waterbath for 16 hours. The hydrolized liquid was filtered afterward. The filtrate, then, was diluted to 200 ml. From this liquid, 50 ml was taken and pH was set 5.5 – 6.5 by adding NaOH bit by bit. Then, 4 ml was taken and put into 10 ml test-tube and added with 2 ml of Chloramin – T. The tube was wrapped with alluminium foil and incubated in waterbath at 60°C for 15 minutes. The absorbance was scaled with spectrophotometer. The hydroxiproline of the sample was determined using standard curv.

Result of the Research and Analysis

Feeding rabbit with higher protein content (19%) affected hydroxiproline contents significantly ($P<0.01$) compared with rabbit fed with lower protein content (13% and 16%). The benefit of increased protein content in feed was faster growth of rabbit body, so that carcass weight of rabbits fed with higher protein contents could be gained faster (Damayanti, 1994).

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Table 2. Result of Statistical Analysis and DMRT Test showed that ydroxiproline content in meat and skin differed significantly

Parameter Hydroxyproline	% Protein		
	13%	16%	19%
Skin	9.827^{b}	11.503^{c}	11.832^{c}
Meat	4.743^{a}	4.455^{a}	4.408^{a}
Average	7.285^{ns}	7.979^{ns}	8.120^{ns}

a,b,c = different superscripts in the same colomn and row showed significant differences (P<0.05)
ns (non significant) in the same colomn showed no significant differences
s (significant) in the same row showed significant differences (P<0.05)

The result of statistical analysis (table 2) showed that hydroxiproline content in meat and skin differed significantly. It was obvious that there was a hydroxiproline amino acids distribution between meat and skin. The higher the protein content in the feed was, the higher the hydroxiproline content in the skin would be. When collagenous protein content was high, the hydroxiproline content in collagen fibre was also high (Stryer, 1981).

Figure 1 showed a photomicrograph of rabbit skin from rabbit fed with 13% protein content. In the stratum papillary and reticulare, thick collagen fibres could be seen. Figure 2 showed thicker collagen fibres compared to Figure 1. Figure 3 showed thicker collagen fibres compared to figure 2 and showed compact and solid collagen fibres.

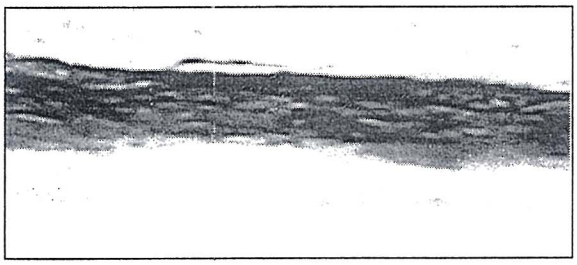

Figure 1. Microscopic cross section of skin with 13% protein content of magnification 400x and Mallory dye method

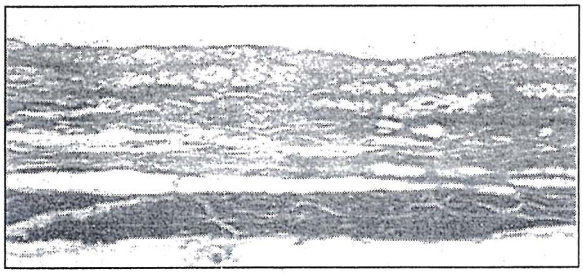

Figure 2. Microscopic cross section of skin with 16% protein content of magnification 400x and Mallory dye method

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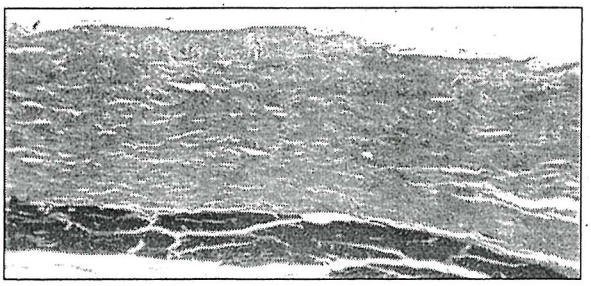

Figure 3. Microscopic cross section of skin with 19% protein content of magnification 400x and Mallory dye Method

According to Djojowidagdo (1999), thicker skin resulted in thicker fibril diameter, fibre weave diameter, and thicker fibre bundle, creating higher physical strength of the skin. The physical strength of a tissue structure depended on fibril diameter, fibre weave diameter, thickness of fibre bundle, interwoven angle, and thickness of skin (raw, preserved, or tanned skin). The thickness of animal skin and characteristics were determined by its species, genus, sex, age, and environment including care and feeding. Results of the research illustrated the fact that rabbits fed with higher protein contents (19%) would produce higher tensile strength compared to rabbit fed with lower protein contents (13% and 16%). The result also showed that feeding rabbit with 13% protein content would make its skin have tensile strength of 152.27 kg/cm² and elongation 20.10%. This was lower than rabbit skin fed with 16% protein content which had 158.97 kg/cm² of tensile strength and 21.33% elongation. Nevertheless, this was also lower than rabbit skin fed with 19% protein content that produced 219.85% tensile strength and 23.47% elongation. The above data showed that higher protein content fed to rabbit would make hydroxiproline content in its skin become higher, and this increased tensile strength and elongation of the skin.

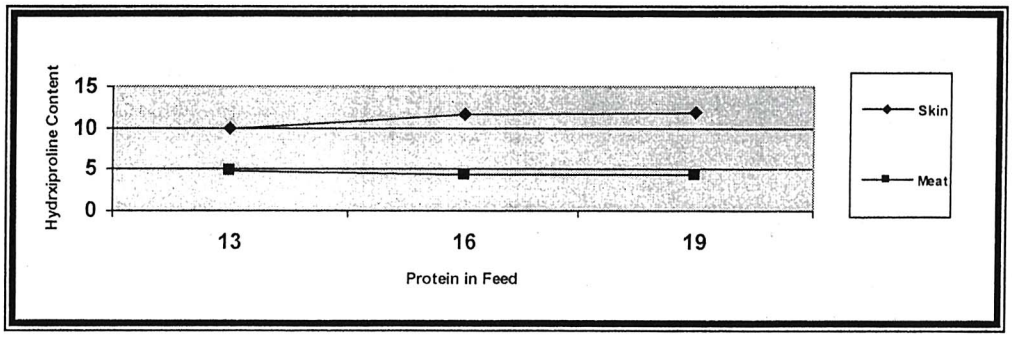

Figure 4. Graphic of the Defference of Hydroxiproline content in Rabbit Skin and Meat with rabbit fed protein content 13%, 16% and 19%.

Figure 4. illustrated the fact that the higher the protein content was, the higher the hydroxiproline content in rabbit skin would be. It showed that corium is the majority layer (98%) of the skin; the content of collagenous protein in corium in form of collagen fibres was also higher. Roddy (1997) said that the majority of protein in skin was collagenous fibres with the amount of 80% to 90% from total protein. The content of hydroxiproline in the skin increased in parallel with the increasing number of protein contents in feed. It also affected the increasing values of tensile strength and elongation.

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Collagenous protein was different from other proteins in general. The characteristics of the collagenous protein were: it contained higher content of glycine amino acids that was about 33%. The test of skin physical quality in this research included composite test of tensile sterength and elongation from six samples of every treatment. Each variable was taken from croupon area of rabbit skin. Average results of skin physical test could be seen in figure 2.

Table 3. Average Values of Skin Physical Test

Parameter	% Protein		
	13%	16%	19%
Tensile Strength (Kg/Cm^2)	$152,27^a$	$158,97^b$	$219,85^c$
Elongation (%)	$20,10^a$	$21,33^b$	$23,47^c$

a,b,c, different superscripts in the same row showed significant results ($P<0.01$)

The increase of protein contents in feed caused rabbit weight gain to be increasing. The benefit of the increase protein content in feed was faster growth of rabbit body, so that the carcass weight of the rabbit given higher protein contents could be gained faster (Damayanti, 1994). Feeding rabbit with higher protein content (19%) resulted in significantly thicker skin ($P<0.01$) compared with rabbit fed with lower protein contents (13% and 16%). According to Djoyowidagdo (1999), thicker skin resulted in thicker fibril diameter, fibre weave diameter, and fibre bundle, creating higher physical strength of the skin. The physical strength of a tissue structure depended on fibril diameter, fibre weave diameter, thickness of fibre bundle, interwoven angle, and thickness of skin (raw, preserved, tanned skin). The thickness of animal skin and its characteristics were determined by its species, genus, sex, age, and environment including care and feeding. The results of the research showed that rabbit fed with higher protein content (19%) would produce higher tensile strength compared to rabbit fed with lower protein contents (13% and 16%). The result also showed that feeding rabbit with 13% protein content would make its skin have tensile strength of 152.27 kg/cm^2 and elongation 20.10%. It was lower than rabbit skin fed with 16% protein content which had 158.97 kg/cm^2 of tensile strength and 21.33% elongation. Nevertheless, this was also lower than rabbit skin fed with 19% protein content that produced 219.85% tensile strength and 23.47% elongation. The above data showed that higher protein content fed to rabbit would result in higher hydroxiproline content in its skin, and this increased tensile strength and elongation of the skin.

CONCLUSION

1. Protein content of 19% in feed resulted in 11.8323% hydroxiproline content, 219.85 kg/cm^2 tensile strength and 23.47% elongation.
2. The higher the hydroxiproline content in skin was, the higher the tensile strength and elongation would be.
3. Feeding rabbit with higher protein content increased hydroxiproline content in rabbit skin but decreased hydroxiproline content in its meat.

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