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Excretion of Endogenous Purine Derivatives in Male and Female Garut sheep

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ABSTRACT
The aim of this study was to determine the value of endogenous purine derivatives (PD) excreted in the urine Garut sheep. The endogenous PD excretion of male and female Garut sheep was compared using an independent student t-test. In this experiment, six males and six females Garut sheep of similar age and body weight (10 months and 25 kg, respectively) were used. The sheep are fed ad libitum with elephant grass and bran pollard in a 60:40 ratio during the adaptation period. The feed then was gradually reduced, starting at 90%, 60%, and 30% from initial amount, and then the sheep were fasted. The sheep were fasted for five days before the urine collection period began. Urine samples will be measured for endogenous PD excretion. The results showed that the excretion of allantoin, xanthine-hypoxanthine, and uric acid in fasted male Garut sheep were 0.040±0.005, 0.004±0.001, 0.014±0.001 mmol/$W^{0.75}$/day, respectively, while in fasted female Garut sheep were 0.035±0.005, 0.004±0.001, 0.013±0.001mmol/$W^{0.75}$/day, respectively. Endogenous PD excretion in male Garut sheep was 0.057±0.006 mmol/$W^{0.75}$/day, while it was 0.052±0.006 mmol/$W^{0.75}$/day in female Garut sheep. Based on the endogenous PD data, we obtained modified equations, which were $Y = 0.84X + (0.057W^{0.75}e^{-0.25x})$ for male Garut sheep and $Y = 0.84X + (0.052W^{0.75}e^{-0.25x})$ for female Garut sheep. In conclusion, sex differences in Garut sheep had no effect on the excretion of endogenous PD, but endogenous PD in male Garut sheep tended to be higher than in females.

Keywords: *Excretion, Garut sheep, Purine deriatives, Urine.*

1. INTRODUCTION

Ruminants get most of their protein from microbial protein in the rumen [2]. Estimating microbial protein synthesis in the rumen have been carried out using several methods, but the easiest and most effective method is by measuring PD in urine excreted by animals for 24 hours [3]. PD are metabolism products of nucleic acids in the animal's body. Feed nucleic acids are completely metabolized in the rumen, but microbial nucleic acids are resynthesized in the rumen and flow to the small intestine, where they are absorbed. PD derived from microbial nucleic acids are called exogenous PD. PD can also derived from the tissue of the animal itself, these are called endogenous PD [3].

The estimation of microbial protein synthesis in the sheep's rumen was calculated by the equation postulated by [1].

$$Y = 0.84X + (0.15W^{0.75}e^{-0.25X}) \quad (1)$$

This equation describes a positive relationship between total PD excreted in the urine (Y) and the purines absorbed in the intestine (X). In Equation (1), 0.84 is the proportion of PD that are excreted in the urine, 0.15 is the contribution of endogenous PD in sheep, ($W^{0.75}$) is the metabolic body weight of sheep, and ($e^{-0.25X}$) is the natural logarithm [3].

Although these equations have been found, these equations cannot be used for endogenous Indonesian animals. Differences in species and breeds of animals will cause differences in metabolism in the body, this occurs due to genetic influences, so that it can cause differences in the excretion of endogenous PD in the urine. Based on the results of research by [4] on endogenous ruminants in Indonesia which shows that endogenous PD excretion is strongly influenced by animal species and also animal breeds in one species. These results are also supported by the research results of [5] who obtained the results of differences in endogenous PD between Bligon and Kejobong goats, as well as the

results obtained by [6] which shows differences in endogenous PD between fat-tailed and thin-tailed sheep. Considering that Garut sheep are sheep that widely bred by many farmers in Indonesia, especially in West Java, it is necessary to know the endogenous PD contribution to the total PD in male and female Garut sheep.

Sex differences in animal will generally affect feed consumption, growth rate, and livestock productivity [7]. Male sheep tend to have a higher feed consumption, body size, and growth rate compared to female sheep. The difference in performance is certainly influenced by genetic and environmental factors, as it is known that metabolism runs enzymatically including nucleic acid metabolism. Therefore, it is possible that the model for estimating rumen microbial protein synthesis of male Garut sheep is different with the females.

2. MATERIAL AND METHOD

2.1 Material

2.1.1. Animals, Cage, and Feed

Total of six male and female Garut sheep, respectively, with similar age and body weight (10 months and 25 kg) were put in a metabolic cage equipped with a feed, urine and feces container. The sheeps were fed elephant grass (*Pennisetum purpureum*) and bran pollard with a 60:40 ratio.

2.2. Methods

2.2.1. Adaptation Period

The adaptation period was carried out for 14 days. Sheeps are fed ad libitum twice a day at 07.00 am and 04.00 pm.

2.2.2. Collection Period

Urine collection for determination of endogenous PD excretion was carried out by fasting sheeps. Feeding was reduced gradually for three days before the animals began to be fasted. On the first day, the feed was given as much as 90% of the initial amount of feed. On the second day, the feed was given as much as 60%, and on the third day, the feed was given as much as 30%. Sheeps began to be fasted on fourth day and during fasting urine samples were taken. Samples collection was carried out for five days. Fasting is stopped when ketone bodies are detected in the urine. Urine for 24 hours is collected in a plastic bucket containing 40 ml of 10% of sulphuric acid, so that the pH drops to less than three (acidic) to avoid microbial growth. Ninety ml of urine was taken and then divided by two to be put into 50 ml bottles and then stored in the chiller until analysis time. Urine samples will be measured for endogenous PD excretion, which includes allantoin, xanthine-hypoxanthine, and uric acid. The Fluitest UA kit was used to determine uric acid, while allantoin and xanthine-hypoxanthine were analyzed using the [1] method.

2.2.3. Statistic

The endogenous PD excretion of male and female Garut sheep was compared using an independent student t-test design.

3. RESULT AND DISCUSSION

3.1. Concentration of PD in fasted male and female Garut sheep

Concentration of PD when sheep were fasted both before and after divided by metabolic body weight is shown in Table 1.

Table 1. PD concentration in urine of fasted male and female Garut sheep (mean ± SE)

Concentration	Garut sheep	
	Male	Female
(in mmol/l)		
Allantoin**	0.822 ± 0.043^{c}	0.448 ± 0.038^{d}
Xanthine-hypoxanthine**	0.077± 0.004^{c}	0.050 ± 0.004^{d}
Uric acid*	0.306 ± 0.038^{a}	0.192 ± 0.031^{b}
Purine derivatives**	1.205 ± 0.081^{c}	0.690 ± 0.069^{d}
(in mmol/l/W$^{0.75}$)		
Allantoin**	0.074 ± 0.006^{c}	0.046 ± 0.006^{d}
Xanthine-hypoxanthine*	0.007 ± 0.001^{a}	0.005 ± 0.001^{b}
Uric acid^{ns}	0.028 ± 0.004	0.020 ± 0.004
Purine derivatives*	0.108 ± 0.009^{a}	0.070 ± 0.001^{b}

^{ab}: different superscripts on the same line show significant differences (P<0.05)
^{cd}: different superscripts on the same line show very significant differences (P<0.01)
^{ns}: not significantly different (P>0.05)

Based on the Table 1, the concentration of allantoin, xanthine-hypoxanthine, uric acid, and PD in fasted male and female Garut sheep before being divided by metabolic body weight indicated a very significant difference (P<0.01). After dividing by metabolic body weights, the concentration of allantoin in fasted male Garut sheep were significantly greater allantoin than in females (P>0.01). Uric acid concentration in male and

female Garut sheep were not significantly different after being divided by metabolic body weight, whereas xanthine-hypoxanthine and PD concentration in male Garut sheep after being divided by metabolic body weight were significantly higher than in females ($P<0.05$). The concentration of allantoin and PD showed significant differences in male and female Garut sheep. This may happen because the activity of the oxidase enzyme in male Garut sheep is different from that of the female. Previous study [8] found that male Marwari sheep have higher xanthine oxidase enzyme activity than female Marwari sheep.

3.2. Excretion of endogenous PD in male and female Garut sheep

Excretion of PD when sheep were fasted both before and after divided by metabolic body weight is shown in Table 2.

Table 2. PD concentration in urine of fasted male and female Garut sheep (mean ± SE)

Excretion	Garut sheep	
	Male	Female
(in mmol/day)		
Allantoin^{ns}	0.458 ± 0.063	0.363 ± 0.063
Xanthine-hypoxanthine^{ns}	0.042 ± 0.006	0.040 ± 0.007
Uric acid^{ns}	0.153 ± 0.009	0.132 ± 0.018
Purine derivatives^{ns}	0.652 ± 0.077	0.534 ± 0.084
(in mmol/l/day)		
Allantoin^{ns}	0.040 ± 0.005	0.035 ± 0.005
Xanthine-hypoxanthine^{ns}	0.004 ± 0.001	0.004 ± 0.001
Uric acid^{ns}	0.014 ± 0.001	0.013 ± 0.001
Purine derivatives^{ns}	0.057 ± 0.006	0.052 ± 0.006

^{ns}: not significantly different ($P>0.05$)

According to Table 2, the excretion of allantoin, xanthine-hypoxanthine, uric acid, and PD in fasted male and female Garut sheep before and after divided by metabolic body weight did not show significant differences, although the fact that PD excretion in male Garut sheep tends to be higher than in females. The endogenous PD excretion in this experiment is still within the normal range, [3] stated that the value of endogenous PD excretion in sheep can vary, ranging from 0.032-0.208 mmol/$W^{0.75}$/day. A previous study [4] reported higher endogenous PD excretion in thin tailed sheep and fat tailed sheep. Endogenous excretion of allantoin, xanthine-hypoxanthine, uric acid and PD in thin tailed sheep were 0.086, 0.025, 0.031 and 0.143 mmol/$W^{0.75}$/day, respectively, while in fat tailed sheep were 0.050, 0.027, 0.013 and 0.091 mmol/$W^{0.75}$/day, respectively. Another study [5] reported lower endogenous PD excretion in Kejobong and Bligon goats. The excretion of endogenous allantoin, xanthine-hypoxanthine, uric acid and PD in Kejobong goats were 0.011, 0.001, 0.009 and 0.019 mmol/$W^{0.75}$/day, respectively, while in Bligon goats were 0.013, 0.001, 0.007 and 0.019 mmol/$W^{0.75}$/day, repectively.

The proportions of allantoin, xanthine-hypoxanthine, and uric acid in endogenous PD excretion in male Garut sheep were 61.40%, 7.02%, and 24.56%, respectively, while in female Garut sheep were 76.92%, 7.69%, and 25%, respectively. The proportion of endogenous PD excretion in this study is still close to that reported by [4] who found that the proportions of allantoin, xanthine-hypoxanthine, and uric acid in endogenous PD excretion in thin tailed sheep were 61.40%, 7.02%, and 24.56%, respectively. Another study [5] reported that endogenous PD excretion in Bligon goat contained 64.77%, 0.36%, and 34.87% of allantoin, uric acid, and xanthine-hypoxanthine, respectively.

The results of endogenous PD excretion were used to modify the equation postulated by [1] which describes a positive relationship between total excretion of PD in the urine and purines absorbed in the small intestine. The excretion of endogenous PD of male and female Garut sheep were 0.057 mmol and 0.052 mmol, respectively. Based on the endogenous PD data, we got the modified equations for male and female Garut sheep. For male Garut sheep, we got the following equation:

$$Y = 0.84X + (0.057W^{0.75}e^{-0.25x}) \quad (2)$$

For female Garut sheep, we got the following equation:

$$Y = 0.084X + (0.052W^{0.75}e^{-0.25x}) \quad (3)$$

4. CONCLUSION

Sex differences in Garut sheep had no effect on the excretion of endogenous PD, but endogenous PD in male Garut sheep tended to be higher than in females. Based on the endogenous PD data, we obtained modified equations that can be used to estimate microbial protein synthesis in male and female Garut sheep, which are Equation (2) and Equation (3).

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