Estimation of Rumen Microbial Nitrogen Supply Based on Purine Derivatives Excreted in The Urine of Male and Female Garut Sheep Fed Ad Libitum

Mutiara Mustika Putri Mahanani[1], Chusnul Hanim[1], Kustantinah Kustantinah[1], Zaenal Bachruddin[1], Budi Prasetyo Widyobroto[1], and Lies Mira Yusiati[1,*]

[1] *Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281*
**Corresponding author. Email: liesmira@ugm.ac.id*

ABSTRACT
This experiment aimed to compare the rumen microbial nitrogen supply in male and female Garut Sheep. Six male and female Garut sheep were put in the metabolism cages, fed *ad libitum* with *Pennisetum purpureum* and bran pollard with a ratio 60 : 40. This study begins with an adaptation period of 14 days. Urine collection was carried out for seven days. Urine samples be measured for purine derivatives (PD), consisting of allantoin, uric acid ,and xanthine-hypoxanthine. During the collection period, samples of feed, uneaten feed, and feces were taken out for dry matter and organic matter analysis. The total urinary PD excretions data were used to estimate microbial nitrogen supply (EMNS) based on the equation postulated with modification in endogenous PD excretion for male and female Garut sheeps. Data obtained were analyzed using the Independent Student T-Test design. The results showed that urinary PD excretion in male Garut sheep was higher than in females (0.160 vs 0.127 mmol/$W^{0.75}$/day). EMNS in male Garut sheep also tended to be higher than in females (1.14 vs 0.74 g N/day). In conclusion, the excretion of PD and EMNS in male Garut sheep tended to be higher than in females.

Keywords: rumen microbial, nitrogen supply, purine derivate, Garut sheep

1. INTRODUCTION

Most of the protein supply in ruminants comes from microbial protein. Around 60% to 80% of the protein sources absorbed by ruminants come from microbial protein [3]. Estimating microbial protein synthesis in the rumen have been carried out using several methods, i.e., markers of ribonucleic acid (RNA), diaminopimelic acid (DAPA), or using isotopes such as ^{35}S, ^{15}N and ^{32}P[4]. These methods have many disadvantages because they are quite complicated and require cannulated animal. A simple method has been found for estimating microbial protein synthesis, using the measurement of PD (allantoin, uric acid, xanthine, and hypoxanthine) in urine excreted by livestock for 24 hours. This method is based on the positive relationship between microbial nucleic acids absorbed in the small intestine and the PD excreted in the urine [5].

Purine derivatives are metabolism products of purine bases or nucleic acids in the body of livestock. Most of the dietary nucleic acids are metabolized in the rumen, while most of the microbial nucleic acids are recycled in the rumen, then flow to the intestines, and are absorbed by the animal's body [6]. The urinary excretion of PD consists of exogenous PD and endogenous PD (basal excretion). Endogenous purine derivatives are derived from the tissue or epithelial cells of the animal itself. The estimation of rumen microbial protein synthesis was calculated based on a positive relationship between the excretion of PD in the urine (Y) and the absorbed purines (X). The relationship was expressed by the equation $Y=0{,}84X+(0{,}15W^{0.75}e^{-0.25X})$ [6].

Metabolism in livestock is influenced by several factors, one of which is the sex of animals. Gender differences between male and female of Garut sheep will generally affect feed consumption, growth rate, and livestock productivity [7]. Male cattle tend to have a

higher body size, feed consumption, and growth rate than female cattle. The sex differences will also show differences in the excretion of purine derivatives. Based on the explanation above, research is needed to formulate an estimation model of microbial protein synthesis for Garut Sheep with different sexes.

2. MATERIALS AND METHOD

2.1. Animals and Biological Trial

Six male and female Garut sheeps aged 10 months with an average weight of 25 kg were put in a metabolic cage equipped with a feed, urine, and feces container. The sheep were fed *ad libitum* twice a day at 07.00 am and 04.00 pm. The sheep were fed elephant grass (*Pennisetum purpureum*) and bran pollard with a ratio of 60:40.

This study begins with an adaptation period of 14 days. The collection period was carried out for seven days. During the collection period, 200 g of feed samples were taken daily as well as 10% of the individual uneaten feed. The samples were dried, bulked, and grinded. Subsamples were taken for nutrient analysis, including dry matter (DM), organic matter (OM) analysis according to the [8] method. Daily individual feces samples as much as 5% of total excretion were taken and put in the fridge through the collection period. The samples were made into a composite and sub-samples as 10% of the total samples were taken for DM and OM analysis.

Urine was collected daily into a plastic bucket placed under the cages. The bucket contained 10% sulfuric acid solution to reach the urine pH below 3 to avoid microbial growth. Ninety ml of urine was taken and then divided by 2 to be put into 50 ml bottles and then stored in the chiller until analysis time. Urine was then analyzed for DP content consisting of allantoin, uric acid, and xanthine-hypoxanthine. Allantoin and xanthine-hypoxanthine were determined using the Chen and Gomes method [1], while uric acid was determined using the Fluitest UA kit's spectrophotometric method.

Microbial protein synthesis was estimated using the Chen and Gomes [1] equations with a modification in endogenous PD excretion for male dan female Garut sheep [2]. For male Garut sheep using the following equation:

$$Y = 0.84X + (0.057\ W^{0.75}e^{-0.25X}) \quad (1)$$

For female Garut sheep using the following equation:

$$Y = 0.84X + (0.052\ W^{0.75}e^{-0.25X}) \quad (2)$$

The above equations are used to describe the quantitative relationship between the absorption of microbial purines (X mmol/d) and excretion of PD in urine (Y mmol/d). After knowing the excretion of purine derivatives from the above equation, the microbial nitrogen supply can be estimated by the following equation:

$$EMNS = \frac{X\ (mmol/hari)\times 70}{0{,}83\times 0{,}116\times 1000} \quad (3)$$

EMNS is the estimated microbial nitrogen supply, (70) is N content in purine, (0.8)3 is the rumen microbial purine digestibility, and (0.116) is the ratio of purine N: total rumen microbial N.

The efficiency of the microbial protein synthesis in the rumen was expressed as grams of microbial N per kilogram of digestible organic matter digested in the rumen (DOMR).

2.2. Statistical Analysis

The data obtained were statistically analyzed using the Independent Student Test-T design to compare the excretion of purine derivatives and estimated microbial nitrogen supply (EMNS) between male and female Garut sheep.

3. RESULT AND DISCUSSION

3.1. Purine Derivatives Concentration in Urine of Male and Female Garut Sheep

The concentration of allantoin, uric acid, xanthine-hypoxanthine, and PD in the urine excreted by male and female Garut sheep before and after dividing metabolic body weight are shown in Table 1.

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

Concentration	Garut sheep	
	Male	Female
(in mmol/l)		
Allantoin^{ns}	1.444 ± 0.064	1.269 ± 0.058
Uric acid*	0.321 ± 0.029^{a}	0.227 ± 0.022^{b}
Xanthine-Hipoxanthine^{ns}	0.058 ± 0.001	0.048 ± 0.004
Purine derivatives*	1.823 ± 0.090^{b}	1.545 ± 0.082^{b}
(In mmol/l/W$^{0.75}$)		
Allantoin^{ns}	0.132 ± 0.007	0.133 ± 0.011
Uric acid	0.029 ± 0.003	0.024 ± 0.003
Xanthin-Hipoxanthin^{ns}	0.005 ± 0.001	0.005 ± 0.001
Purine derivatives^{ns}	0.167 ± 0.001	0.162 ± 0.014

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

PD concentrations were obtained from the sum of allantoin, uric acid, and xanthine-hypoxanthine concentration. Based on Table 1, the concentration of uric acid and PD of male and female Garut sheep before divided by metabolic body weight showed a significant difference ($P<0.05$). However, when it was divided by metabolic body weight, both allantoin, uric acid levels xanthine-hypoxanthine, and PD in the urine of male and female Garut sheep were not significantly different. The PD concentration that was not significantly different was reported in a previous study [9] on male and female Kejobong goats.

Allantoin and PD concentration in male and female Garut sheep were still in the normal range. The allantoin concentrations in this study were close to the results reported by [10] using fat tail sheep which was 1.830 mmol/l. A previous study reported that the concentration of allantoin in the urine of Bligon and Kejobong goats were 1.178 mmol/l and 1.336 mmol/l, respectively, while the concentration of PD in the urine of Bligon and Kejobong goats were 1.418 mmol/l and 1.547 mmol/l, respectively [11]. Another study reported that allantoin concentration in Sri Lanka goats ranged from 1.145 to 2.834 mmol/l [12].

3.2. Purine Derivatives Excretion In Urine of Male and Female Garut Sheep

Table 2. The excretion of allantoin, uric acid, xanthine-hypoxanthine, and PD in the urine of male and female Garut sheep (mean ± SE).

Excretion	Garut sheep	
	Male	Female
(in mmol/day)		
Allantoin^{ns}	1.402 ± 0.131	1.016 ± 0.142
Uric acid*	0.284 ± 0.019^{a}	0.186 ± 0.019^{b}
Xanthine-Hipoxanthine^{ns}	0.058 ± 0.007	0.041 ± 0.004
Purine derivatives*	1.745 ± 0.153^{a}	1.242 ± 0.159^{b}
(In mmol/W$^{0.75}$/day)		
Allantoin^{ns}	0.128 ± 0.013	0.104 ± 0.013
Uric acid	0.026 ± 0.002^{a}	0.019 ± 0.002^{b}
Xanthin-Hipoxanthin^{ns}	0.005 ± 0.001	0.004 ± 0.001
Purine derivatives^{ns}	0.160 ± 0.015	0.127 ± 0.014

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

The excretion of urinary PD, which includes excretion of allantoin, uric acid, and xanthine-hypoxanthine during *ad libitum* feeding, are shown in Table 2, both before and after divided by metabolic body weight.

Based on Table 2, the excretion of allantoin and xanthine-hypoxanthine in the urine of male and female Garut sheep both before and after being divided by metabolic body weight showed significant differences. Excretion of uric acid in male Garut sheep before and after dividing metabolic body weight was significantly higher than that of female Garut sheep ($P<0.05$). The excretion of purine derivatives in Garut male sheep before being divided by metabolic body weight was higher than the female sheep. However, after dividing the metabolic body weights, the excretion of PD showed no significant difference even PD excretion in males tended to be higher than in females. Sex differences in Kejobong goats also did not show significant differences in the excretion of allantoin, uric acid, and purine derivatives in urine, even PD excretion in males tended to be higher than in females [9].

The excretion of allantoin, uric acid, xanthine-hypoxanthine, and purine derivatives in this study is close to [11], who reported that Bligon goats had excretion of allantoin 1.326 mmol/day, uric acid 0.175 mmol/day, xanthine-hypoxanthine 0.012 mmol/day, and DP 1.512 mmol/day. The results of the study are also close to those of [13], who reported that the excretion of allantoin, uric acid, xanthine-hypoxanthine, and purine derivatives in Kejobong goats was 0.905 mmol/day, 0.268 mmol/day, 0.064 mmol/day, and 1.180 mmol/day, respectively. Another study reported higher purine-derived excretion than this study that thin-tailed sheep and fat-tailed sheep had purine-derived excretion values of 3.527 mmol/day and 2.905 mmol/day, respectively day [10].

Uric acid excretion showed significant differences in male and female Garut sheep, possibly due to differences in the activity of the xanthine oxidase enzyme. A previous study [14] stated that the activity of the xanthine oxidase enzyme in male Marwari sheep tends to be higher than that of female Marwari sheep. Allantoin has the largest proportion in PD compared to uric acid and xanthine-hypoxanthine same as reported that the proportion of allantoin in purine derivatives ranges from 59%-85% [5]. Based on Table 2 above, the proportions of allantoin, uric acid, and xanthine-hypoxanthine in male Garut sheep were 80%, 16.25%, and 3.13%, respectively, while the females were 81.89%, 14.96%, and 3.15%, respectively. The results of this study are close to the results reported that the proportions of allantoin, uric acid, and xanthine-hypoxanthine in Dorper sheep fed forage: concentrate 60:40 were 82.01%, 14.06%, and 4.34%, respectively [15].

3.3. Estimation of Microbial Protein Synthesis in Male and Female Garut Sheep

Estimation of microbial synthesis (EMNS), digested organic matter intake (DOMI), degraded organic matter in the rumen (DOMR), and microbial protein efficiency (EMNS/DOMI and EMNS/DOMR) in male and female Garut sheep are shown in Table 3.

Table 3. Estimation of microbial synthesis (EMNS), degraded organic matter in the rumen (DOMR), digested organic matter intake (DOMI), and microbial protein efficiency (EMNS/DOMI and EMNS/DOMR) in male and female Garut sheep (mean ± SE)

Nilai	Garut sheep	
	Male	Female
EMNS^{ns} (g N/day)	1.14 ± 0.15	0.74 ± 0.15
DOMI^{ns} (g/day)	443.29 ± 21.76	396.04 ± 39.25
DOMR^{ns} (g/day)	288.14 ± 14.14	257.43 ± 25.51
EMNS/DOMI^{ns} (g N/kg DOMI)	2.65 ± 0.42	1.88 ± 0.34
EMNS/DOMR^{ns} (g N/kg DOMR)	4.08 ±0.64	2.90 ± 0.52

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

Based on Table 3, the calculation results of EMNS, DOMI, DOMR, EMNS/DOMI, and EMNS/DOMR in male and female Garut sheep were not significantly different even EMNS/DOMR in males tends to be higher than in females. Another study reported the same results, EMNS/DOMR in males tends to be higher than in females, EMNS and EMNS/DOMR values in male and female Kejobong goats were not significantly different [9]. The male and female Garut sheep did not differ in the efficiency of microbial synthesis, possibly because the use of the same feed caused the work of microbes to synthesize microbial protein with the same ability. Previous studies reported that differences in feed caused differences in microbial protein synthesis in goats even at the same level of feed consumption [16], that the same feed did not cause differences in EMNS in goats, despite using two different breeds of goats [9].

The EMNS results of male and female Garut sheep obtained in this study were close to Kejobong goats at 1.221 g N/day [11] and in fat tailed sheep at 1.66 g N/day [10]. The EMNS of male and female Garut sheep is lower than the study reported by [17] using castrated sheep of 3.90 g N/day and [18] who used sheep with hay feed, which was 10.73 g N/day. Another study [19] explained that the level of EMNS is influenced by microbes to form microbial protein in the rumen.

DOMR values obtained from DOMI in male and female Garut sheep were multiplied by 0.65. Based on the research that has been done, the DOMR value for male Garut sheep is 288.14 g/day and female Garut sheep is 257.43 g/day. The DOMR value in this study is close to the DOMR value in the [20] study in Thai goats, which is 247.33 g/day. The DOMR results close to this research are also reported by [10] on thin tailed sheep and fat tailed sheep were 331.50 g/day and 378.33 g/day.

The value of microbial protein efficiency or EMNS/DOMR in this study is close to the results of [9] on male Kejobong goats of 4.11 g/kg DOMR and Kejobong goats of 2.49 g/kg DOMR. Another study [10] reported higher EMNS/DOMR results in thin tailed sheep and fat tailed sheep, namely 7.33 g N/kg DOMR and 5 g N/kg DOMR.

4. CONCLUSION

The excretion of PD, EMNS, and EMNS/DOMR in male and female Garut sheep was not significantly different, but excretion of PD, EMNS, and EMNS/DOMR in male Garut sheep tended to be higher than in females.

ACKNOWLEDGMENTS

The authors acknowledge to Hibah Penelitian Program Pascasarjana 2020 from Faculty of Animal Science, Universitas Gadjah Mada for financial support.

REFERENCES

[1] X. B. Chen and E. R. Ørskov. 2004. Estimation of Microbial Protein Supply in Ruminants Using Urinary Purine Derivatives: Research on Urinary Excretion of Purine Derivatives in Ruminants: Past, Present and Future (Springer, Berlin, 2004)

[2] M. M. P. Mahanani, C. Hanim, Kustantinah, Z. Bachruddin, B. P. Widyobroto, L. M.Yusiati. Endogenous Purine Derivatives in Urine of Male and Female Garut Sheep. in Proceedings of the 9th International Seminar on Tropical Animal Production, ISTAP, Indonesia (to be published)

[3] Uddin, M. J. Khandaker, Z. H. Khan, M. Jasimuddin and M. M. H. Khan. Dynamics of microbial protein synthesis in the rumen - A Review. Annals of Vet. and Anim. Sci. 2, 5 (2015)

[4] S. K. George, A. K. Verma, U. R. Mehra, M. T. Dipu, and P. Singh. Evaluation of purine metabolites - creatinine index to predict the rumen microbial protein synthesis from urinary spot samples in Barbari goats. J. Anim. and Feed Sci. 20, 4 (2011).

[5] X.B. Chen and E. R. Ørskov. Estimation of Microbial Protein Supply in Ruminants Using Urinary Purine Derivatives: Research on Urinary Excretion of Purine Derivatives in Ruminants: Past, Present and Future. (Springer, Berlin, 2004)

[6] T. Fujihara and M. N. Shem. Metabolism of microbial nitrogen in ruminants with special reference to nucleic acids. Anim. Sci. J. 82, 2 (2011)

[7] G. H. Wijaya, M. Yamin, H. Nuraini, dan A. Esfandiari. Performans Produksi dan profil metabolik darah domba garut dan jonggol yang diberi limbah tauge dan omega-3. J. Vet.17, 2 (2016)

[8] AOAC. Official Method of Analysis of the Association of Official Analitycal Chemist.18th ed. (Maryland: AOAC International, USA, 2005)

[9] L. M. Yusiati and. C. Hanim. Estimation of rumen microbial nitrogen supply based on purine derivatives excreted in the urine of kejobong and bligon goat feed by king grass and peanut straw, in Proceeding 3rd AINI International Seminar, Indonesia (2013)

[10] P. A. Dughita. Kontribusi ekskresi basal purin terhadap total ekskresi derivat purin dalam urin domba ekor gemuk dan domba ekor tipis. Thesis. Faculty of Animal Science. Universitas Gadjah Mada, Yogyakarta, Indonesia (2016)

[11] D. Putra, L. M. Yusiati, dan R. Utomo. Estimasi sintesis protein mikrobia rumen menggunakan ekskresi derivat purin dalam urin dengan teknik spot sampling pada kambing bligon dan kambing kejobong. Buletin Peternakan. 40, 3 (2016)

[12] Marapana, R.A.U.J. and T. Seresinhe. Effect of feeding regime on growth, digestibility and excretion of purine derivatives in goats, in Proceedings of the fourth academic sessions, (2007)

[13] C. S. Purwati. Kontribusi ekskresi basal purin terhadap total ekskresi derivat purin dalam urin kambing bligon dan kejobong. Thesis. Faculty of Animal Science. Universitas Gadjah Mada, Yogyakarta (2012)

[14] R. Maan and N. Kataria. Serum xanthine oxidase profile in stressed Marwari sheep from arid tracts in India. J. Stress Phys. & Biochem. 8, 3 (2012)

[15] T. Ma, K.-D.Deng, Y.Tua, N.-F.Zhang, C.-G.Jiang, J.Liu, Y.-G.Zhao, and Q.-Y.Diao. 2014. Effect of dietary forage to concentrate ratios on urinary excretion of purine derivatives and microbial nitrogen yields in the rumen of Dorper crossbred sheep. Small Ruminant Research. 160, 1 (2014)

[16] M. R. Mbewe, V. R. Hamandishe, V. E. Imbayarwo-Chikosi, and B. Masunda. Nitrogen balance and rumen microbial protein synthesis in goats fed diets containing soaked and roasted Mucuna bean (Mucuna pruriens). Online Journal of Animal and Feed Research. 4, 1 (2014)

[17] A. Natsir. Ekskresi Derivat Purin dan Estimasi Suplay Protein Mikrobia pada Ternak Domba yang Mendapat Suplemen Protein Berbeda. Jurnal Ilmu Ternak dan Veteriner. 12, 3 (2007)

[18] S. Ghasemi, A. A. Naserian A , R. Valizadeh A , A. M. Tahmasebi A , A. R. Vakili A and M. Behgar. Effects of pistachio by-product in replacement of lucerne hay on microbial protein synthesis and fermentative parameters in the rumen of sheep. Animal Production Science. 52, 11 (2012)

[19] G. Mahesti. Pemanfaatan Protein pada Domba Lokal Jantan dengan Bobot Badan dan Aras Pemberian Pakan yang Berpengaruh. Thesis. Fakultas of Animal Science, Universitas Diponegoro, Semarang, Indonesia (2009)

[20] P. Paengkoum and M. Wanapat Utilization of Concentrate Supplements Containing Varying Levels of Sunflower Seed Meal by Growing Goats Fed a Basal Diet of Corn Silages. Pakistan Journal of Nutrition. 8, 8 (2009)