Correlation Between Ratio of Purine Derivative: Creatinine Concentrations in Spot Urine Sampling with Total Urinary Excretion of Purine Derivative in Garut Rams and Ewes

Titi Widya Ningrum[1], Chusnul Hanim[1,*], Lies Mira Yusiati[1]

[1]*Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia*
[*]*Corresponding author. Email: c. hanim@ugm.ac.id*

ABSTRACT
This study aimed to determine the correlation between ratio of purine derivative:creatinine concentrations by spot sampling method with total urinary excretion of purine derivative (PD) in Garut rams and ewes, as well as knowing the right sampling time to predict microbial protein synthesis in Garut rams and ewes. There were six Garut rams and six Garut ewes placed in metabolic cages and fed with elephant grass and bran pollard with a ratio of 60:40 (based on the dry matter). This research was conducted 14 days for the adaptation period and five days for the collection period. The urine samples in the collection period used the spot sampling method by taking urine periodically at intervals of three hours a day (24 hours). Urine samples obtained were analyzed for creatinine and purine derivatives content (allantoin, uric acid, and xanthin-hypoxanthine). The data was analyzed *using an Independent Student t-test* design between Garut rams and ewes. The ratio of purine derivatives (PD): creatinine (PDC index) was correlated with the total daily excretion of purine derivatives (PD), hence the best time for spot sampling could be implemented. Based on the research, concentration of urinary PD and creatinine in Garut rams were higher than ewes. Excretion of allantoin, xanthinhypoxanthine and creatinine in Garut rams were also higher than ewes. The concentration nor excretion of urinary PD in rams and ewes before being divided by metabolic body weight showed significant differences. The best time for spot sampling applied to Garut rams was six to nine hours after afternoon feeding with the equation Y=6.802X-26.04, while for Garut ewes was six to nine hours after afternoon feeding with the equation Y=3,630X+43,79. In conclusion, the concentration and total excretion of urinary PD in Garut rams were higher than the ewes. There were differences in the regression equation formula, to predict PD excretion based on PD concentration corrected by creatinin content of urine spot sampling with the similar best sampling time at the range of six to nine hours after afternoon feeding.

Keywords: *Purine Derivative Excretion, Spot Sampling, Rumen Microbial Protein Synthesis, Garut Sheep*

1. INTRODUCTION

Ruminants have advantages over other livestock, namely the presence of biological processes by microbes in the rumen. Rumen microbes are the largest source of protein for ruminants. The protein contributions percentage in the livestock body through rumen microbial protein is about 60 – 80% [1]. Given this, it is necessary to study the microbes' contribution in providing protein for ruminants to develop feeding strategies to increase productivity.

Microbial protein synthesis can be measured with *in vivo* method equipped with the use of an internal marker, namely RNA (*ribonucleic acid*), DNA (*deoxyribonucleic acid*), and DAPA (*diaminopilemic acid*) as well as using isotopes such as 35S, 15N, dan 32P [2,3]. *In vivo* method equipped with a marker has a weakness that requires rumen fistulation and duodenal cannulation, where it is not in line with animal welfare. One of the simpler and more innovative methods is the measurement of excreted purine derivatives in the urine for 24 hours [4].

However, determining purine derivatives in urine has advantages and disadvantages, it requires a total urine collection for 24 hours. This is considered less effective, so a more practical method is needed, namely spot sampling. To prevent collecting urine for 24 hours, with spot sampling method will help to know the urine collection time which can describe the level and total PD excretion. Spot sampling is a method of measuring purine derivatives at a certain time which is considered to be correlated with the total collection. Spot sampling method requires urine creatinine concentrations combined with purine derivative concentrations, in the form of a PD:C ratio called purine derivative–creatinine index (PDC index). The spot sampling method will save time and money when the total urine collection is less effective.

Purine derivatives are the result of nucleic acid metabolism in the livestock body. The nucleic acid content in feed ingredients is predominantly degraded in the rumen, hence the nucleic acid entering the duodenum comes from rumen microbes. Different enzyme activities can affect nucleic acid metabolism, resulting in the different excretion of purine derivatives. Each livestock species and breed tends to differ in the process of nucleic acid metabolism, this occurs because of genetic influences on livestock. The excretion of PD in Ongole crossbred cattle, Bali cattle, Friesian Holstein Crossbred cattle, and buffalo was different [5]. The total PD excretion of Kejobong goats was higher than Bligon goats. Moreover, the best spot sampling time for Kejobong goat (range 14.00 to 17.00) was different from Bligon goat (range 11.00 to 14.00) [6]. The best spot sampling time for thin-tailed sheep was 07.00 to 10.00 (0 to 3 hours after morning feeding). Based on several previous studies, each species and breed has a different excretion value and the best time for spot sampling [7]. The total PD excretion in Kejobong goats and Bligon goats was different (18.85 vs 19.33 μmol/$W^{0.75}$/day) [8].

Garut sheep is a livestock commodity that is widely raised in Indonesia, especially in West Java and is Indonesian sheep biodiversity that should be preserved. Therefore, it is necessary to study the estimation of rumen microbial protein synthesis using the spot sampling method in Garut sheep. Sex differences between Garut rams and ewes affect feed consumption, growth rate, and livestock productivity. Garut rams generally have a higher body weight, feed consumption, and growth rate than the ewes. Garut rams DM consumption was 765-1403 g/head/day, while Garut ewes DM consumption was only 455-585.81 g/head/day [9]. The increase in microbial biomass is in line with the increase in consumption, so the PD excretion pattern in Garut rams and ewes will be different.

The estimation of rumen microbial protein synthesis using the spot sampling method on Garut rams and ewes needs to be further investigated. It is hoped that this research can be used as a reference in optimizing the contribution of microbial protein to its host livestock. However, determining purine derivatives in urine has advantages and disadvantages, it requires a total urine collection for 24 hours. The total urine collection for 24 hours is considered less effective, so a more practical method is needed, namely spot sampling. This method is useful for the application of the estimation of rumen microbial protein synthesis based on the excretion of purine derivatives in the urine of Garut sheep at the farmer level or in the field. Furthermore, it will be easier to evaluate the level of nutrient adequacy or evaluate the quality of the Garut sheep ration at the farmer level. This research is expected to be useful in increasing livestock productivity and will support the preservation of Indonesian sheep biodiversity. Therefore, it is necessary to study the estimation of rumen microbial protein synthesis using the spot sampling method in Garut sheep.

2. MATERIALS AND METHODS

2.1. Materials

This study used 6 Garut rams and 6 Garut ewes, each aged 10 months with an average initial weight of 25 kg. Garut rams and ewes were placed in metabolic cages equipped with feed, drinking, urine, and fecal containers. The sheep were fed with elephant grass and bran pollard with a ratio of 60:40 (based on the dry matter) *ad libitum*. Table 1 presents the nutrient composition of the feed.

2.2. Methods

2.2.1. Adaptation period

Garut rams and ewes were weighed to determine initial body weight which was used as a basis for determining feed requirements. The first feeding was 3% of body weight, then the consumption was evaluated. If the feed were running out, the feed amount was increased the next day, until it was given ad libitum feeding. The adaptation period was carried out for 14 days with *ad libitum* feeding. The livestock daily consumption was recorded to ensure the daily consumption has reached *ad libitum*. Feeding was done 2 times a day, at 08.00 and 16.00.

Table 1. Feed nutrient composition

Feed Ingredients	DM (%)	OM (%DM)	CP (%DM)	EE (%DM)	CF (%DM)	NFE (%)	TDN (%)
Elephant Grass	20.13	87.25	9.86	2.09	31.42	43.88	56.29
Bran Pollard	87.17	93.67	15.45	4.59	9:30	64.33	76.33
Feed nutrition (ratio of elephant gr ass and bran pollard was 6 0 %:40%)							
	28.78	89.76	12.05	3.07	22.77	51.88	89.76

2.2.2. Collection period

During the collection period, livestock was given ad libitum feeding for 5 days. During the collection period, the total volume of urine was recorded. Urine that was excreted 24 hours was kept in a urine container and added with an acid solution of H2SO4 10%. This aimed to reach lower pH to less than or equal to 3 and avoid damage to purine derivatives. The collected urine was measured in volume, then filtered to remove contaminants in the urine. The filtered urine was then put in bottles and ready to be stored in the refrigerator until the analysis time.

Spot urine samples were collected for 5 days, periodically at intervals of 3 hours a day (24 hours). For one day, there were 8 times of spot urine sample collection, at 07.00 to 10.00, 10.00 to 13.00, 13.00 to 16.00, 16.00 to 19.00, 22.00 to 01.00, 01.00 to 04.00, and 04.00 to 07.00. Excreted urine every 3 hours was collected in a container containing an acid solution of H2SO4 10%. Spot urine samples were prepared with the same way as the total colletion urine Then, creatinine and purine derivatives (PD) concentrations (allantoin, xanthine and hypoxanthine) were analyzed [10], and uric acid using kit *FluiteUA.*

2.2.3. Data Analysis

Data on concentrations and total purine derivatives (PD) excretion, which included allantoin, uric acid, xanthine and hypoxanthine, and creatinine, were compared between Garut rams and ewes with *independence sampling T-test*. Purine derivatives (PD) and creatinine (C) concentrations from spot urine sampling were taken every 3 hours and analyzed by calculating the ratio of PD:C in the form of a PDC *index*.

$$PDC\ index\ = \frac{[DP]}{[Creatinine]} \times W^{0,75} \quad (1)$$

where W is body weight (kg), [PD] and [*Creatinine*] was the concentration of purine and creatinine derivatives (µmol/L) [11]. *PDC index* or the calculated PD:C ratio, then tested for regression correlation with PD excretion from the total urine collection. The equations are used for rams and ewes to describe the quantitative relationship between absorption of microbial purines (X µmol/day), and excretion of PD in urine (Y µmol/day).

3. RESULTS AND DISCUSSION

Table 2. Purine derivative concentrations of Garut rams and ewes with ad libitum feeding (mean ± SE)

Concentrations	Garut Sheep	
	Rams	Ewes
In µmol/L		
Allantoin*	1552.24 ± 237.26^{a}	1207.09 ± 135.48^{b}
Uric acid*	384.66 ± 33.57^{a}	332.15 ± 24.14^{b}
Xanthine-Hypoxanthine**	345.42 ± 60.05^{c}	237.14 ± 24.41^{d}
Purine Derivatives**	2282.33 ± 322.90^{c}	1776.38 ± 172.76^{d}
Creatininens	174.38 ±19.77	158.93 ± 12.78
In µmol/L/W$^{0.75}$		
Allantoinns	141.52 ±20.72	126.52 ± 25.56
Uric acidns	21.82 ± 7.09	18.97 ± 3.70
Xanthine-Hypoxantinens	29.73 ± 3.97	25.03 ± 4.54
Purine Derivativesns	208.08 ±28.20	186.16 ± 36.05
Creatininens	15.88 ± 1.54	16.62 ± 2.87

ab : Different superscripts on the same line showed significant differences (P<0.05)
cd : different superscripts on the same line showed significant differences (P<0.01)
ns : non-significant (P>0.05)

3.1. Urinary purine derivative concentrations in Garut rams and ewes

Concentrations of allantoin, uric acid, xanthinehypoxanthine, PD, and creatinine in the urine excreted Garut rams and ewes with *ad libitum* feeding before and after divided with metabolic body weight are presented in Table 2.

Based on Table 2, the concentrations of allantoin, uric acid, xanthine-hypoxanthine, and PD in Garut rams and ewes before being divided by metabolic body weight showed significant differences. The concentrations of allantoin, uric acid, xanthine-hypoxanthine, PD and creatinine in Garut rams and ewes urine when the unit was divided by metabolic body weight showed no significant difference. The concentrations of allantoin, uric acid, xanthin and hypoxanthine, PD (µmol/L) in Bligon goats fed with peanut straw were not significantly different from Kejobong goats ($P<0.05$). Garut rams and ewes were given the same feed during the study, which aimed to avoid potential differences in PD concentrations caused by feed factors. Feed could influence DP concentrations. Moreover, other factors, such as the source of energy and protein in the feed and the balance of its availability in the rumen [6].

The data analysis results showed no difference in allantoin, uric acid, xanthine-hypoxanthine, and DP concentrations (µmol/L/$W^{0.75}$) between Garut rams and ewes. However, the PD level of Garut rams was significantly higher than the Garut ewes both in µmol/head/day and after correcting with metabolic body weight (µmol/L/$W^{0.75}$). Allantoin, uric acid, and PD concentrations in Garut rams and ewes were in the normal range. The concentrations obtained were close to the results on Bligon goats which were 1,178.41; 227.90; and 1,418.40 µmol/L and the Kejobong goat which were 1.335.94; 197.96; and 1,547.40 µmol/L [6]. Xanthine-hypoxanthine concentrations in this study were in the normal range, hypoxanthine xanthine concentrations in thin-tailed sheep (DET) were 329.45 µmol/L and fattailed sheep (DEG) were 205.91 µmol/L [7].

Based on Table 2, creatinine concentrations were not significantly different in Garut rams and ewes. Urinary creatinine is not affected by feed consumed and the physiological status of livestock [12]. However, the PD level of Garut rams was significantly higher than the Garut ewes both in µmol/head/day and after correcting with metabolic body weight (µmol/L/$W^{0.75}$).

3.2. Urinary purine derivative excretion in Garut rams and ewes

Total purine derivatives excretion (allantoin, uric acid, xanthine-hypoxanthine, and creatinine) during *ad libitum* feeding both before and after divided with metabolic body weight are presented in Table 3.

Based on Table 3, allantoin, xanthine-hypoxanthine, PD, and creatinine excretion of Garut rams was significantly higher than Garut ewes ($P<0.05$), but when divided into units of metabolic body weight, those excretions were not significantly different. Uric acid concentration and excretion in both µmol/head/day and mol/$W^{0.75}$/day of Garut rams were not significantly different from Garut ewes. Sex differences in Kejobong goats also did not perform significant differences in uric acid and purine derivatives excretion in urine. However, the total PD excretion of Garut rams was significantly higher than the ewes by 2259.86 and 1484.26 (µmol/head/day) [13].

The allantoin, uric acid, and purine derivatives excretion in this study were higher. The allantoin, uric acid, and purine derivatives excretion value in Bligon goats of 917.26; 165.92; and 1,092.40 (µmol/head/day) and the Kejobong goat of 1.325.60; 174.81; and 1,512 (µmol/head/day) [6]. Another study reported a higher purine derivatives excretion compared to this study, thintailed sheep (DET) had allantoin, uric acid, and purine derivatives excretion values of 4430.70; 436.75; and 5302.53 µmol/head/day, respectively [7]. The xanthinehypoxanthine excretion results in this study were higher. The xanthine-hypoxanthine excretion values in Bligon goats and Kejobong goats were as much as 9.22 and 11.63 µmol/head/day. Xanthine-hypoxanthine excretion in goats is lower than in sheep with the same feed [14].

The difference in allantoin excretion showed a significant difference between Garut rams and ewes. It indicated a difference in the xanthine oxidase enzyme in the metabolic process. The difference in allantoin excretion between sheep and goats may be due to higher xanthine oxidase activity in goats when compared to sheep. The xanthine oxidase enzyme degrades xanthine and hypoxanthine into uric acid and allantoin before being excreted in the urine [14]. Based on several studies, it is known that allantoin has the largest proportion of total PD, the proportion of allantoin in purine derivatives ranges from 59%-85% [11]. Based on Table 4, the proportions of allantoin, uric acid, and xanthinehypoxanthine in Garut rams were 76.47%, 21.43%, and 2.15%, respectively, while the proportions of allantoin, uric acid, and xanthine- hypoxanthine in Garut ewes were 73%, 24.65%, and 2.35%, respectively. The proportion of uric acid was higher than xanthine-hypoxanthine. This was in line with several studies which stated uric acid proportion is greater than the xanthine-hypoxanthine proportion.

Table 3. Excretion of allantoin, uric acid, xanthine hypoxanthine, and PD in Garut rams and ewes urine with ad libitum feeding (mean ± SE)

Concentrations	Garut Sheep	
	Rams	Ewes
In µmol/head/day		
Allantoin*	1705.42 ± 479.68[a]	1089.46 ± 382.26[b]
Uric acidns	509.50 ± 169.81	367.64 ± 151.29
Xanthine-Hypoxanthine*	44,95 ± 11,81[a]	27,15 ± 9,32[b]
Purine Derivatives*	2259.86 ± 64.34[a]	1484.26 ± 540.91[b]
Creatinine* In µmol/W0.75/day	110.91 ± 2.84[a]	106.83 ± 1.77[b]
Allantoinns	128.40 ± 31.62	104.18 ± 31.86
Uric acidns	21.82 ± 7.10	18.97 ± 3.70
Xanthine-Hypoxantinens	5,21 ± 1,77	3,88 ± 1,22
Purine Derivativesns	155.43 ± 35.45	127.03 ± 35.35
Creatininens	10.13 ± 0.79	11.12 ± 1.09

ab : different superscripts on the same line showed significant differences (P<0.05)
cd : different superscripts on the same line showed significant differences (P<0.01)
ns : non-significant (P>0.05)

3.3. Correlation between ratio of PD:C concentrations in spot urine sampling with PD excretion in total urine collection of Garut

Determination of correlation was done by making a linear regression between the PD excretion in total urine collection with the ratio of PD:C concentrations from each urine sample which were collected every three hours a day (24 hours). The ratio of PD:C concentrations in each spot urine sample collection becomes the X-axis, while the total collection of PD excretion becomes the Yaxis. This was done to obtain the strongest correlation between PD concentrations of spot urine sampling with PD excretion in total urine collection. Correlation data at each spot sampling time was presented in Table 4 for Garut rams and Table 5 for Garut ewes.

Based on Table 4, linear regression analysis between PD concentrations of spot sampling urine and total collection urine in Garut rams generally showed a low correlation. However, it can be seen that there were three times spot sampling which had a significant correlation with that found in the urine daily collection. These times included 6 to 15 hours after the afternoon feeding at 22.00 to 01.00; 01.00 to 04.00; and 01.00 to 04.00 to 07.00. However, between the three collection times of spot sampling, it was known that the strongest spot sampling correlation found in the time range 22.00 to 01.00 with (P<0.05) and R^2 = 0.8371 followed by the time range 04.00 to 07.00 with (P<0.05) and R^2= 0.7445, then the time range 01.00 up to 04.00 with (P<0.05) and R^2= 0.4386.

The study results were different. The strongest correlation of PD concentrations in urine spot sampling with total urine collection in Kejobong goats was during the day with a period of 14.00 to 17.00. The timespan of spot sampling will be more reliable when associated with the timing of feeding [6]. There are variations in the PD concentration in one day. This PD concentration variation could be induced by diurnal variations in the absorption of microbial purines in the intestine caused by the frequency of feeding livestock. Thus, spot sampling time was better calculated based on the feeding time [10,12].

When related to spot sampling time with feeding time, it is known that spot sampling time in Garut sheep had the strongest correlation with the total collection of 6 to 9 hours after feeding in the afternoon, from 22.00 to 01.00. The PD concentration compared to creatinine had a significant correlation in sample collection of 0 and 6 hours after feeding [12]. The significant correlation of spot sampling time was 12 hours after feeding [15]. Kejobong goats had a significant correlation in the total collection in the time range of 0 to 7 hours after afternoon feeding. Hence, the spot sampling time with the strongest correlation in Garut rams [6].

Linear regression analysis between PD concentrations of spot urine sampling and total urine collection presented in Garut ewes performed a low correlation. However, strongest correlation with the total collection was at night from 22.00 to 01.00 with a time of 6 to 9 hours after afternoon feeding when associated with feeding time.

Table 4. Correlation between ratio of PD:C concentrations in spot urine sampling with PD excretion in total urine collection of Garut rams

Spot sampling time	N treatment	P	R^2	Regression linear equation
07.00 – 10.00	6	>0.05	0.043	Y= 4.5265X + 55.39
10.00 – 13.00	6	>0.05	0.001	Y= - 0.2414X + 160.66
13.00 – 16.00	6	>0.05	0.652	Y= 4.6118X + 28.50
16.00 – 19.00	6	>0.05	0.655	Y= 5.8158X - 4.37
19.00 – 22.00	6	>0.05	0.003	Y= - 0.5454X + 169.97
22.00 – 01.00*	6	<0.05	0.837	Y= 6.8022X – 26.04
01.00 – 04.00*	6	<0.05	0.439	Y= 3.8133X + 63.39
04.00 – 07.00*	6	<0.05	0.745	Y= 6.0351X - 2.53

* significant (P<0.05)

based on Table 5, there were four times of spot sampling which had a significant correlation with the daily urine collection (P<0.01 and (P<0.05). These time ranges included 3 to 6 hours after morning feeding at 10:00 to 13:00; and 3 to 15 hours after afternoon feeding from 19.00 to 22.00; 22.00 to 01.00; and 04.00 to 07.00. The strongest correlation of spot sampling in Garut ewes, namely the time range from 22.00 to 01.00 (P < 0.01) and R^2 = 0.881, time range 19.00 to 22.00 (P < 0.05) and R^2 = 0.788, time range 04.00 to 07.00 (P<0.05) and R^2= 0.667, then the time range 10.00 to 13.00 (P<0.05) and R^2= 0.521. Spot sampling time in Garut ewes which had the

4. CONCLUSION

Based on this research, it could be concluded that the concentration and total excretion of PD in Garut rams were higher than the ewes. There were differences in the regression equation formula, to predict PD excretion based on PD concentration corrected by creatinin content of urine spot sampling with the similar best sampling the time at the range of six to nine hours after afternoon feeding. It was known that the regression equation for Garut rams was Y= 6.8022X - 26.04; while for Garut ewes was Y = 3.630X + 43.79.

Table 5. Correlation between ratio of PD:C concentrations in spot urine sampling with PD excretion in total urine collection of Garut ewes

Spot sampling time	N treatment	P	R^2	Regression linear equation
07.00 – 10.00	6	>0.05	0.058	Y= 1.6055X + 94.86
10.00 – 13.00	6	<0.05	0.521	Y= 4.8831X + 44.51
13.00 – 16.00	6	>0.05	0.132	Y= 2.5665X + 75.03
16.00 – 19.00	6	>0.05	0.111	Y= 2.9817X+ 63.55
19.00 – 22.00*	6	<0.05	0.788	Y= 4.4918X + 22.16
22.00 – 01.00*	6	<0.05	0.881	Y= 3.630X + 43.79
01.00 – 04.00*	6	>0.05	0.572	Y= 3.6062X + 59.86
04.00 – 07.00*	6	<0.05	0.667	Y= 8.0051X - 37.99

* significant (P<0.05)

** significant (P<0.01)

ACKNOWLEDGMENTS

On this occasion, the author would like to thank Universitas Gadjah Mada for the grant under the scheme of Rekognisi Tugas Akhir.

REFERENCES

[1] Uddin, M.J. Khandaker, Z.H. Khan, M. Jasimuddin, M.M.H. Khan, Dynamic of microbial protein synthesis in the rumen, A Review. *Annals Vet. Anim. Sci.*, 2(5) (2015) 117 – 131.

[2] Z.C.J. Csapo, J. Schmidt, T.G. Martin, Quantitative determination of protein of bacterial origin, *TrAC*, 20 (2001) 42-48.

[3] S.K. George, M.T. Dipu, A.K. Vermam, P. Singh, U.R. Mehra, Species differences in the concentration of purine derivates and creatinine in spot urine samples, *JIVA*, 9 (2011) 24-26.

[4] X.B. Chen, M.J. Gomes, Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives. An Overview of the Technical Details. Rowett Research Institute, Bucksburn, Aberdeen, 1995.

[5] L.M. Yusiati, Development of Rumen Microbial Protein Synthesis Method Using Excretion of Purine Derivatives in Urine of Various Indonesian Ruminant Livestock. Dissertation, Faculty of Animal Science, Yogyakarta, 2005.

[6] D. Putra, L.M. Yusiati, R. Utomo, Estimation of rumen microbial protein synthesis using the excretion of purine derivatives in urine using technique spot sampling on Bligon goats and Kejobong goats, *Buletin of Anim. Sci*., 40(3) (2016) 178-186.

[7] T.L. Aulyani, Estimation of Rumen Microbial Protein Synthesis Based on Purine Derivatives in Urine By Method Spot Sampling in Thin and Fat Tailed Sheep and Its Application at the Breeder Level. Thesis, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, 2016.

[8] C.S. Purwati, L.M. Yusiati, S.P.S. Budhi, The contribution of basal purine excretion on the total excretion of purine derivates in the urine of Bligon and Kejobong goats, *Buletin Peternakan*, 37(1) (2013) 6-11.

[9] G.H. Wijaya, M. Yamin, H. Nuraini, A. Esfandiari, Production performance of Garut and Jonggol sheep treated with bean sprouts and omega-3 waste, *Indones. Vet. J*., 17(2) (2016) 246 – 256.

[10] X.B. Chen, G. Grubic, E.R Orskov, P. Osuji, Effect of feeding frequency on diurnal variation in plasma and urinary purine derivatives in steers, *Anim. Sci.*, 55 (2) (1992) 185-191.

[11] X.B. Chen, E.R Orskov. Research on Urinary Excretion of Purine Derivatives in Ruminant: Past Present and Future. International Feed Resources Unit, Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen, 2003.

[12] S.K. George, M.T. Dipu, U.R. Mehra, A.K. Verma, and P. Singh, Influence of level feed intake on concentration of purine derivatives in urinary spot samplings and microbial nitrogen supply in crossbred bulls. *Asian-Aust. J. Anim. Sci*., 19 (2006) 1291-1297.

[13] L. Yusiati And C. Hanim, Estimation of rumen microbial nitrogen supply based on purine derivates excreted in the urine of Kejobong and Bligon goat fed by king grass and peanut straw. Proceeding 3rd AINI International Seminar ISBN: 978-602-969348-5, Padang, September 24th, 2013.

[14] M.D. Carro, G. Cantalapiedra-Hijar, M.J. Ranilla, E. Molina-Alcaide. Urinary excretion of purine derivates, microbial protein syntesis, nitrogen use, and ruminal fermentation in sheep and goats fed diets of different quality, *Journal Animal Science*, 90(11) (2012) 3963-3971.

[15] M.T. Dipu, S.K. George, P. Singh, A.K. Verma, U.R. Mehra. Measurement of microbial protein supply in Murrah buffaloes using urinary purine derivatives excretion and pdc index, *Asian-Aust. J. Anim. Sci.*, 19 (2006) 347-355.