Nitrogen Kinetics in Growing Sheep Consuming *Leucaena leucocephala, Gliricidia sepium* or *Calliandra calothyrsus* as a Sole Diet

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ABSTRAK

WIDIAWATI, Y., M. WINUGROHO and E. TELENI. 2012. Kinetik nitrogen dalam pemeliharaan domba yang diberi pakan tunggal *Leucaena leucocephala, Gliricidia sepium* dan *Calliandra calothyrsus. JITV* 17(3): 215-220.

Pemanfaatan protein tanaman pakan oleh ternak sangat dipengaruhi oleh proses pemecahan protein di dalam rumen dan saluran pencernaan. Jumlah protein yang dapat terdegradasi di dalam rumen dipengaruhi oleh bentuk protein dan kehadiran senyawa sekunder. Penelitian bertujuan untuk mengetahui banyaknya protein dalam ketiga leguminosa yang dapat dimanfaatkan oleh ternak. Penelitian tentang pemanfaatan protein tanaman leguminosa telah dilakukan dengan melihat nitrogen kinetik pada domba yang diberi pakan daun *Leucaena leucocephala, Gliricidia sepium, Calliandra callothyrsus* dan hay dari rumput Rhodes dengan menggunakan 24 ekor domba (BB 23 kg). Pakan diberikan setiap 2 jam dimulai jam 07 pagi. Ke-24 ekor ternak dibagi menjadi 4 grup perlakuan pakan yaitu grup LL, GS, CC dan CG, dimana masing-masing diberi pakan daun Leucaena, Gliricidia, Calliandra dan hay dari rumput Rhodes. Rumput Rhodes diberikan sebagai pakan kontrol. Penambahan 1,4% urea pada rumput dilakukan untuk meningkatkan nitrogen yang diberikan kepada ternak yaitu sebesar 150 g/hari. Sehingga setiap kelompok ternak menerima jumlah nitrogen yang sama setiap harinya. Pengamatan dilakukan terhadap penggunaan pakan dan nitrogen kinetik pada ternak perlakuan. Hasil menunjukkan bahwa jumlah nitrogen yang dikonsumsi kemudian tidak di degradasi dalam rumen dan tidak dicerna dalam usus secara nyata lebih tinggi pada kelompok CC (P < 0,01) kemudian diikuti oleh kelompok LL, GS dan CG. Nilai untuk kelompok CC adalah 67% kemudian diikuti oleh kelompok LL (35%) dan GS (33%). Jumlah nitrogen yang didegradasi dalam rumen kemudian dieksresikan lewat urin secara nyata lebih tinggi pada ternak yang mengkonsumsi Gliricidia dan Leucaena dibandingkan dengan yang mengkonsumsi Calliandra (P < 0,01), dengan nilai 46 % untuk kelompok GS dan LL dan hanya 16% untuk kelompok CC. Jumlah nitrogen yang termanfaatkan oleh ternak sama untuk semua kelompok perlakuan yang mengkonsumsi legum yaitu antara 17-21% (P > 0,05). Plasma urea konsentrasi 0,86 ; 0,8 ; dan 0,46 mg/ml (P < 0,01); *urea entry rate* 79; 78; 50 g/d (P < 0,01) dan jumlah urea dalam urin 26.7; 24.5; 7.3 g/d (P < 0,01) sama untuk ternak di kelompok LL, GS dan CC. Kesimpulan dari hasil ini adalah kebanyakan nitrogen dalam Calliandra tidak didegradasi dalam rumen dan juga tidak dicerna didalam usus tetapi di keluarkan lewat feses. Sementara lebih banyak protein dalam Leucaena dan Gliricidia yang didegradasi dalam rumen kemudian dikeluarkan lewat urin.

Kata Kunci: Leucaena, Gliricidia, Calliandra, Nitrogen-Kinetik, *Urea Entry Rate*

ABSTRACT

WIDIAWATI, Y., M. WINUGROHO and E. TELENI. 2012. Nitrogen kinetics in growing sheep consuming *Leucaena leucocephala, Gliricidia sepium* or *Calliandra calothyrsus* as a sole diet. *JITV* 17(3): 215-220.

The utilization of protein feed by animals are influenced by the process of its degradation in the rumen and its digestion in the intestine. The extent of its degradation and digestion are influenced by the form of protein and the present of secondary compound in the plant. The aim of the study was to determine the amont of protein in the three leguminouse shrubs utilized by the animals. The study on the utilization of protein from leguminouse shrub was undertaken by investigate the nitrogen kinetic in 24 head of growing sheep (BW 23kg) consuming *Leucaena leucocephala, Gliricidia sepium, Calliandra callothyrsus* leaves and Rhodes grass hay. The diets were fed every two hours starting at 07:00 am. The animals were divided into four groups of dietary treatment namely LL, GS, CC and CG groups which were fed by Leucaena, Gliricidia, Calliandra dan Rhodes grass leaves, respectively. The Rhode grass, as control diet, was mixed with 1.4% of urea to increase the nitrogen offered to the animals up to 150 g/day. Thus all the four diets were offered the same amount of nitrogen/day. Measurements were undertaken on feed utilization and nitrogen kinetics in experimental animals. The amount of nitrogen consumed then undegraded in the rumen as well as undigested in the intestine was significant higher in CC group (P < 0.01) then those in LL, GS and CG groups. The value was 67% for CC group then followed by 35% for LL group and 33% for GS group. The amount of nitrogen degraded in the rumen thus lost in urine was significantly higher in animals fed GS and LL leaves compared to those in animals fed CC diet (P < 0.01). The values were 46% for GS and LL group and only 16% for CC group. The amount of nitrogen retained by the animals was similar among the three groups being 17-21% (P > 0.05). The Plasma urea concentration, *urea entry rate* and urea in urine animals were similar in LL and GS groups but higher than those in CC group. The values were 0.86; 0.8; 0.46 mg/ml (P < 0.01) and 79; 78; 50 g/d (P < 0.01) and 26.7; 24.5; 7.3 g/d (P < 0.01) for LL, GS and CC groups, respectively. The conclusion is most of nitrogen from Calliandra was un-degraded and un-digested in the rumen and intestine thus excreted through feces, while most of the protein in Leucaena and Gliricidia was degraded in the rumen thus excreted through urine.

Key Words: Leucaena, Gliricidia, Calliandra, Nitrogen-Kinetics, *Urea Entry Rate*

INTRODUCTION

Protein quality has a relationship with the amount of protein degraded in rumen or escape from rumen degradation thus made available for the absorption in small intestine. The extent of degradation of feed CP in the rumen would depend on the forms of the proteins and on the presence or otherwise of secondary compounds. There are two general forms of protein present in a plant material, namely, water-soluble and non water-soluble proteins. The former is rapidly degraded and the latter is more slowly broken down in the rumen. Leucaena, Gliricidia and Calliandra have larger fractions of water-soluble CP (24-38%) compared to that on grass being 16% (WIDIAWATI, 2004).

Secondary compounds are present in most of the shrub legume leaves. The most compounds that are found in the shrub legumes such as Leucaena, Gliricidia and Calliandra are mimosine, coumarine and tannin, respectively (MUELLER and HARVEY, 2006; MAKKAR *et al.*, 2007). The rate of protein degradation in the rumen might be influenced by the tannin present in the plant. The amount of CP degraded in the rumen was observed to decline by 39% when forages contained >10% tannin, and declined by only 18% when forages contained <7% tannin (MULLEN, 1996). Therefore, there migh some protein in Calliandra undegraded in the rumen thus used as protein by pass by animal (MCSWEENEY *et al*., 1999).

The ammonia (NH_3) released when proteins are broken down in the rumen is useful to rumen microbes when present in a certain amount, but becomes toxic to the host animal when present in excess. Rumen bacteria activities and growth in the rumen require the ammonia in the rumen. Thus, ammonia has important role in the production of protein microbial, which is used as one of protein source for ruminant animals. Excess of ammonia in the rumen would be through rumen wall and goes into the urea pool.

Urea entering the blood pool arises from two sources, namely, from rumen NH_3 and from amino acids catabolised at tissue level. The contribution of each source to urea concentration in blood, therefore, would depend on factors that would influence the rate of absorption of rumen NH_3 and on factors that would influence the extent of amino acid catabolism in the body.

Increases in daily N intake generally increase blood urea concentration and *urea entry rate*. Rumen NH_3 is the major contributor to the plasma urea pool. About 60% of N in plasma urea may be derived from rumen NH_3 (MARINI *et al*., 2004). A positive correlation between NH_3 concentration in the rumen and plasma urea concentration has been reported by some workers (MIN *et al*., 2003.) The NH_3 absorbed from the rumen is transferred through the blood stream to the liver, where urea synthesis occurs. The extent of NH_3 absorption from the rumen is related to its concentration in the rumen and the pH of the rumen (ABDOUN *et al*., 2007).

The rate of protein degradation in the rumen has a profound influence on the concentration of urea in the plasma. Less degradable protein in the rumen results in a lower rate of urea synthesis in the liver (LAPIERRE and LOBLEY, 2001). Conversely, a higher protein degradation rate in the rumen would increase the rate of urea synthesis in liver, resulting in increased plasma urea concentration as well as urea entry rate.

The different amount of secondary compound present in the three shrub legumes might has an effect on protein degradation in the rumen as well as the amount of ammonia converted to urea then goes into the urea pool. Therefore the aim of the study was to determine the contribution of the nitrogen in the three shrub legumes available by ruminant animals or loss as waste both in urine or feces. These parameters then can be used as indicators to determine the amount of nitrogen from the feed used by the animals.

MATERIAL AND METHODS

Twenty-four sheep, approximately five to six months old were used in a Complete Randomized Design (DANIEL, 1991). They were divided into four equal groups, evenly matched for LW. Each group was allocated at random to the following dietary treatments, namely Leucaena(LL), Gliricidia(GS), Calliandra(CC) and Rhodes grass - CG) as control group. Mean (±SE) initial LW of animals in each treatment group were 23.1 ± 0.78; 23.1 ± 0.85; 23 ± 0.78 and 23.1 ± 0.78 kg for LL, GS, CC, and CG groups, respectively. During the pre-experimental period, the animals were kept in individual pens on a wooden slatted floor or in metabolism cages. Each sheep had free access to their respective diets and clean drinking water.

The feeds used in the experiment were fresh leaves of Leucaena, Gliricidia and Calliandra and chopped Rhodes grass hay which was mixed with 1.4% urea. The addition of urea was to increase the N content of the hay up to 1.28% N. In order to increase the dry matter offered and consumed by the animlas in control group, Rhodes grass used was in the dry form (hay) instead of fresh materials. The average age of the shrub legumes varied between five and six months of re-growth. The three leguminouse were offered to the animals to supply the equivalent of 150 g N per day (NRC, 1985). Each daily feed was divided into 12 equal portions, where each portion was given at 2 hourly intervals from 07:00 h to 15:00 h.

The experimental period consisted of a 10-day preliminary period and a 10-day measurement period of feed utilization and urea kinetics. *Urea entry rate* was calculated by using continuous infusion of [^{14}C]urea into the left jugular venous catheter of each animal at a constant rate of 0.237mL/min for eight hours. Ten ml of blood sample was withdrawn from the right jugular vein of each animal at half-hourly intervals during the last three hours of the infusion period. The plasma then was analyzed for urea concentration and *urea entry rate*.

All raw data were tabulated using Microsoft®Excel 2000 for Windows 2000 (Microsoft Corporation, USA) and analysed using SPSS Version 7.0 for Windows 95 (SPSS Inc., USA). Data were analysed using ANOVA for Complete Randomized Design (DANIEL, 1991). Where significant effects from treatments were observed, differences among mean values were examined by using Tukey's test.

RESULTS AND DISCUSSION

Feed utilisation and N balance

Data on feed utilisation and N balance of sheep fed the different diets are summarised in Table 1. The animals in the LL, GS and CC groups consumed all of their respective feeds on offer each day, thus the DM and OM intake were similar but higher than those of animals in CG group ($P < 0.05$). Animals in the GS and CC groups ate the amount of N as calculated, while animals in the LL group consumed 5.5% more N, although this amount was not significantly higher.

The nitrogen content of Rhodes grass hay was increased through addition of urea to 8% but the intake of DM, OM and N by animals in the CG group still was significantly lower than those in sheep fed the shrub legumes.

Dry matter intakes of shrub legumes were 3.1, 2.9 and 3.2% of body weight for animals fed the Leucaena, Gliricidia and Calliandra, respectively and 2.5% for sheep fed the Rhodes grass hay. These levels of DM intake were in the range normally observed in growing ruminant animals (NRC, 1985). The animals fed the shrub legumes ate all their respective feeds each day while those in the CG group ate less, even though the hay was made available to the group on an *ad libitum* basis. It should be noted that a primary interest of the current experiment was to examine animals consuming the same amounts of nitrogen per day and if possible, also the same amounts of feed DM per day. This was largely achieved with animals on the shrub legumes diets but not with those on the hay diet.

The N required for maintenance by sheep used in the current experiment was estimated to be 7-8 g/d, while for a moderately growing sheep, growing at 250 g LW/d, the requirement was estimated to be 26 g N/day (NRC, 1985). This level of N intake was achieved by the animals fed the shrub legumes diets, but not by the animals fed the hay diet.

About 67% of N eaten by the animals fed Calliandra was not digested thus was excreted in the feces. While the animals fed Rhodes grass hay excreted the highest proportion of N intake via urine (62%). The animals fed the Gliricidia had the highest amount of N retained as percentage of N intake (21%), although this was not significantly different from values for animals fed the Leucaena (19%) and Calliandra (17%). There was almost no net N retention observed in animals fed Rhodes grass hay.

There were inefficiencies in the utilisation of nitrogen in each of the three shrub legumes when they used as sole diets. The inefficiencies were due largely to the imbalance of N and ME intake or as expressed in another way, to the sub-optimal ratios of NI:DOMI which varied from 0.06 to 0.07 for the three shrub legumes. This also is reflected in the low apparent Biological Values [(BV); N retained/Digestible N intake] of the shrub legumes; i.e., 0.29, 0.31 and 0.5 for Leucaena, Gliricidia, and Calliandra, respectively. However, these values can be misleading, particularly with reference to that for Calliandra. In terms of Biological Value, that for Calliandra was the highest for the feeds examined; but this does not take into account the digestibility of the feed. In terms of Protein Efficiency Ratio [(PER); LWG (g)/CP intake], the values for Leucaena, Gliricidia and Calliandra were 0.596, 0.600 and 0.100 respectively; highlighting the very low PER for Calliandra. It would appear that the problem with this shrub legume is the low availability of protein for digestion and absorption due to high tannin content. High content of tannin in Calliandra (> 8.1%; JAYANEGARA *et al*., 2011) might protect protein from rumen degradation.

There is no report on the effect of mimosine and coumarine on the breakdown of CP in the rumen. On the other hand, the presence of tannin in forages had been reported to reduce CP degradation in the rumen (MCSWEENEY, 2001; JAYANEGARA and PALUPI, 2010). This was suggested to be due to tannin-protein complexes being formed (SILANIKOVE *et al*., 2001). The amount of CP degraded in the rumen was observed to decline by 39% when forages contained more than 10% tannin, and declined by only 18% when forages contained less than 7% tannin (MULLEN, 1996). Not all proteins in Calliandra are associated with tannin (MCSWEENEY *et al*., 1999).

Urea kinetics

The mean values of plasma urea concentration, *urea entry rate*, urinary urea, urine output and water intake of sheep fed the different diets are presented in Table 2.

Plasma urea concentrations, *urea entry rates* and the amounts of urea excreted through urine were similar in animals fed Gliricidia and Leucaena. These were significantly higher than corresponding values in animals fed Calliandra and Rhodes grass hay. Animals in the CG group had the lowest *urea entry rate* among the treatment groups. It seems that addition of urea in CG group increased the availability of nitrogen in the rumen then used by rumen microbes activities. Thus might just small amount of urea absorbed through rumen wall.

There were significant differences among groups in the water consumed and urine outputs. The animals fed Leucaena and Rhodes grass hay had significantly higher water intakes then animals fed Gliricidia and Calliandra. The corresponding value in the GS group was the lowest among the treatment groups. Animals fed Rhodes grass hay had the lowest urine output per day, while the highest value was observed in sheep fed Leucaena.

In general, plasma urea concentration and *urea entry rate* increase as protein intake increases. The exception to this was observed in animals fed Calliandra. Although the animals in the shrub legumes groups consumed similar amounts of nitrogen, the mean *urea entry rate* in sheep fed Calliandra was 36% and 37% lower than those in animals fed Gliricidia and Leucaena, respectively, but similar to those in sheep fed Rhodes grass hay. Low N digestibility (32%) and low protein degradability in the rumen (48%) in the Calliandra (WIDIAWATI, 2004), would underpin the observation on the difference of Calliandra from the other two shrub legumes. The difference on the protein degradation in the rumen among the three shrub legumes might has relationship whit the difference in tannin content. Tannin content in Leucaena and Gliricidia was reported less than 5.8%, while in Calliandra the content was more than 8.1 % (JAYANEGARA *et al*., 2011). High tannin presence in forages had been reported to reduce CP degradation in the rumen (MCSWEENEY, 2001; JAYANEGARA and PALUPI, 2010).

Table 1. Dry matter (DM) and organic matter (OM) intake, ratio of nitrogen (N) to digestible organic matter intake (DOMI) and N balance of sheep fed Leucaena (LL), Gliricidia(GS), Calliandra(CC) and Rhodes grass (CG) diets during the 10-day measurement period

Variables	Treatments				
	LL	GS	CC	CG	± SE
Live weight (kg)	23.1	23.1	23	23.1	
Intake					
DM total (g/d)	755[a]	746[a]	760[a]	573[b]	61
OM total (g/d)	693[a]	690[a]	709[a]	520[b]	55
N balance (g/d)					
N intake (g/d)	26[a]	24[a]	24[a]	8[b]	1.9
N faeces (g/d)	9[b]	8[b]	16[a]	3[c]	1.0
(% of NI)	35[c]	33[c]	67[a]	38[b]	3.2
N urine (g/d)	12[a]	11[a]	4[b]	5[b]	0.6
(% of NI)	46[b]	46[b]	16[c]	62[a]	5.1
N retention (g/d)	5[a]	5[a]	4[a]	0.3[b]	1.4
N retention (% NI)	19[a]	21[a]	17[a]	3.7[b]	5.5
NI:DOMI	0.06[b]	0.06[b]	0.07[a]	0.03[c]	0.002

Within rows, means with different superscript differ significantly ($P < 0.05$)

The significantly positive relationship between N intake and *urea entry rate* or urinary urea suggests an increasing rate of catabolism of amino acids as N intake was increased. What is unclear is the quantitative source of the urea-N observed. A possibility is the rumen NH_3-N formed from the degradation of dietary CP and the subsequent deamination of the amino acids released in the rumen. The NH_3-N released from amino acid breakdown is absorbed from the rumen and converted to urea in the liver. Such a possibility is quite likely since the NI:DOMI in the rumen varied from a high 0.06 to 0.07 in the shrub legumes diets; values which almost doubled those (0.03 to 0.04) considered optimal for rumen microbial activity.

Ammonia probably would be absorbed from rumen, then goes into the bloodstream. The amount of NH3-N absorbed from the rumen is mainly determained by the protein degradaded in the rumen (REYNOLDS and KRISTENSESN, 2008). Under the wide variety of diet and physiological condition of animals, the amount of NH3-N absorption from the rumen was for about 42 % of dietray N intake (FIRKINS and REYNOLDS, 2005). The other possible sources of plasma or urinary urea-N are amino acids (dietary or tissue) oxidised in the liver or skeletal muscle due to sub-optimal ratios (at the tissue level) of amino acids:ME or amino acids:amino acids for lean growth. A significant proportion of the carbon skeleton of such amino acids may be used for glucose synthesis. This possibility may be examined by using the relationship between the amounts of undegraded CP in the rumen and *urea entry rates* of Leucaena, Gliricidia and Rhodes grass. Out of Calliandra the data indicated that there was a very strong positive relationship (R^2 = 0.89) between the rumen undegraded protein and *urea entry rate*. This suggests that a significant proportion of the amino acids apparently absorbed from the intestine were deaminated, most probably in the liver.

The dietary treatments significantly affected water intake by the sheep. Since the animals were maintained under comfortable temperature and humidity levels, the variation in water intake could reflect variations in the moisture and crude fibre contents of the diets or osmotic load in blood. For example, the animals fed the CG diet, which contained the lowest moisture (13% on DM basis; observed in this study) and the highest crude fiber (44% on DM basis; KARDA and DRYDEN, 2001) drank the greatest amount of water. Thus the highest water intake of the animals in CG group was as a result of the lowest moisture content of the CG diet compared to the shrub legumes diets.

Results of experiment indicated that ammonia available in the rumen was exceed the optimum level for rumen bacterial growth thus converted to urea then excreted through urine when the three shrub legumes offered as sole diet. To optimized the utilisation of ammonia in the rumen by rumen bacterial, the energy in the form of VFA must be available. Such energy is produced when fibre diet is degraded in the rumen. Therefore the three shrub legumes might offered to animals in mixed diet with high fibre feed.

CONCLUSION

Most of nitrogen from Calliandra was un-degdraded and un-digested in the rumen and intestine thus excreted through feces (67%). While similar amount of nitrogen in Leucaena and Gliricidia was degraded in the rumen thus excreted through urine (46%). The amount of nitrogen retained and used by animals in the three shrub legumes was similar being 21, 19 and 17% for Gliricidia, Leucaena and Calliandra, respectively.

Table 2. Means values of plasma urea concentration, *urea entry rate*, urinary urea, water intake, urine output and urea-N as percentage of urinary-N in sheep fed Leucaena (LL), Gliricidia (GS), Calliandra(CC) and Rhodes grass (CG) diets during the 10-day measurement period

Variables	Treatments				
	LL	GS	CC	CG	± SE
Plasma urea (mg/mL)	0.86[a]	0.80[a]	0.46[b]	0.48[b]	0.4
Urea entry rate (g/d)	79[a]	78[a]	50[b]	38[c]	3.1
Urinary urea (g/d)	26.7[a]	24.5[a]	7.3[b]	9.6[b]	1.9
Water intake (mL/d)	1461[a]	1162[b]	981[b]	1915[a]	286
Urine output (mL/d)	984[ab]	513[c]	671[bc]	497[c]	185
Urea-N in urinary-N (%)	98	98	85	92	12

[*] Within rows, means with different superscript differ significantly ($P < 0.05$)

In application to the animals, the Leucaena, Gliricidia and Calliandra must be offered in the combination with energy sources or high fibre diet to optimize the utilization of ammonia in the rumen.

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