IS THERE ANY DIFFERENCE ON THE RECYCLING UREA BETWEEN BUFFALOES AND CATTLE

Kustantinah[1]

ABSTRACT

Indigenous of the urea recycled to the rumen could be as a source of nitrogen for microbes that would be digested in the intestines. Most of research work done in ruminant nutrition were all conducted in the stage of maintenance to know the effect of feed composition on the flux variation of indigenous urea to the rumen, and very little work on the recycling variation in the ruminal of indigenous animal in Indonesia. The first study concerning urea recycling was that there was evidence of urea found in the saliva and years before this, it was observe that the urea transferred to the rumen was via rumen wall. Based on this finding, some research worker then treat to observe that urea produced in the organism was not totally excreted in the urine, on the contrary part of it will transferred to the digestive tract. About 50-80% of the total urea entering the digestive tract would be degraded especially in the large intestine. Development of technique using radio isotope marker could determine the urea traffic in the digestive tract (rumen and intestine). Rumen is the major place of urea utilization, urea are transferred to the microbes as it has the capability of using nitrogen in that kind of form. The urea will enter rumen together which saliva or directly penetrate the rumen wall. Regulation and control of these mechanisms are still in discussion, there is probably a role of kidney in the regulation. The more important thing to determine is whether the kidney function has a role in the process of filtration in the glomerulus of the recycling. This question should be answer since from the previous preliminary study in our laboratory, with the difference species of animal namely buffaloes was much higher (30.75 mg/dl) compared to that of the cattle (19.2 mg/dl). For the data obtained, should have been a major key in detecting the difference of urea recycled. That differences of urea recycled probably was caused by the role of kidney glomerulus filtration that is different between species of animal.

Key words: Difference, Recycling-urea, Buffaloes, Cattle

INTRODUCTION

Urea, which is transported from the blood to the tractus digestivus, could be an important resource of nitrogen for the microbes protein synthesis in the reticulo-rumen as well as in the caecum. The first investigation about the urea recycling developed after determining the urea content in the salive.

Starting at 1957, the urea transfer through the rumen wall was known from the research using different methods of urea concentrations in the arterio-vena and isolated rumen epithelium. Based on these findings worker from that moment have a hypotheses that urea which was produced in the organism was not totally excreted in the urine, but part of it could be transferred in the tractus digestivus. Development of the technique using labeled radio-active marker could determine the location of the transferred urea in the tractus digestivus (rumen, intestinum).

The direct measurement of the recycled urea have been used in *invitro* using clean rumen and was already free from microbes and suspended in NaCl solution and that added with urea from out side the rumen and the transferred urea will be determined with measurement of the urea quantity in the inside of the rumen.

[1] Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia

Urea Transfer

Rumen is a major place for the utilization of urea transferred. In the rumen the microbes would use the formed nitrogen. This urea will go into the rumen together with salive or directly penetrate the rumen wall.

The regulation and control of this transfer until now is still being in discussion, there is a possibility of kidney role (specific on the role of glomerulation filtration) on the urea transfer regulation.

Activity of the transferred in the rumen caused by 2 things, namely it's a passive diffusion and active diffusion. On the reference of Chaoutenne (1991) it was explained that for the measurement of the transferred urea in the passive diffusion system a study has developed using isolated rumen and other part of the tractus digestivus and replace the content with salt solution (physiological-serum) than it will be found the ammonia in the salt solution, also Engelhard and Niche (1965) using rumen epithelium noted that transferred urea was caused by passive diffusion.

The hypothesis concerning active diffusion connected with the role of horned layer of the rumen wall, the transferred urea value in the rumen should be multiplied by 50, based on the destruction of this layer wall due to the alkaline solution treatment, the cornification part of the rumen epithelium was suggested as a limitation on the nitrogen in the form of NH_4.

Urea, which is transferred in the rumen, will immediately be hydrolyzed by urease of the microbes. Urease is an enzyme which is produced by rumen bacteria, will degrade urea which is transferred in the rumen, yield of CO2 and ammonia, and this ammonia is available for microbes proteosynthesis.

For the estimation of the cycle urea many method could be adapted, formula of Ford and Milligan (1970) from the regression for estimating of the transferred urea in the rumen (Y) in the fraction of the uremia (blood urea content) (X), $Y = 0.117 + 0.37\ X$.

Whilst Kennedy and Milligan (1980) showed that equation to estimate the transferred urea of the blood from the rumen for sheep, in their function of the organic matter, ammonia concentration in the rumen and with the blood urea.

Transferred N-urea = $-0.0991\ (NH_3) + 0.00236\ (NH_3)_2 + 0.0658\ (DMO) - 0.000771\ (DMO)_2 + 0.0931$ (Nblood urea) $- 0.000288$ (N plasma urea)$_2 - 7.58$. Which r2 = 0.72, n= 15, NH_3 = concentration of ammonia in the rumen (mg/l), DMO = Apparent digestibility of the Organic Matter in the rumen, blood/plasma-n = Concentration of the urea –N in the blood (mg/l), Transferred urea-N = Urea-N through on the rumen (g/day/LW).

In the ruminant, urea, which is degraded in the tractus digestivus could also be, calculated by subtraction the synthesis-urea by the excreted urea in the urine, which values varied between 10-90% for the sheep. Whilst Obara and Shimbayashi (1987) stated that the value was about 28% for sheep and Bunting *et al* (1987) found that the value was about 67% for calves. About 50-80% of all the urea that entered into the tractus digestivus will be degraded especially in the large intestines. Nolan *et al* (1976) stated that after intra-vena urea injection (15N) about 30% of the urea will be degraded in the tractus digestivus (a transfer of about 1.3 g N/day) about 15-20% will enter into the rumen and other part will be degraded in the post-ruminal tractus and 19% of 15N will be found in the feces. Whilst Dixon and Nolan (1986) showed that utilization of marked 14C urea about 38% of coecum N-NH_3 was originated from blood urea, this value was similar with the transfer of 1.4 g N/day.

From the research data available, it was obvious that urea quantity that enter into the tractus digestivus (apart the rumen) will be degraded due to the fermentation or limited microbes synthesis (colon, coecum) where urea will be absorbed freely and reused for urea synthesis. In the large intestines, about 10% of the urea synthesized were originated from ammonia absorption.

Salive role

The relation ship between the transferred urea in the rumen and the concentration (content) of the urea in the

blood was influenced especially by the urea in the salive. For cattle and sheep, urea concentration in salive about 60% was from the blood urea. Whilst the salive quantity excrete have a relation ship quite closed with the DM consumption and also with the proportion of the forage and concentrate fed to the animal.

About 60% of the urea concentration in the salive were originated from the plasma urea and as for sheep, which was fed with alfalfa, the concentration of the salive-urea could reach 67% of the urea in the blood. Whilst Norton *et al* (1982) stated that in sheep, concentration of salive-urea was about 48 to 57% of the blood urea, if the animal was fed with hay.

It was also shown that the concentration of urea in the salive would increase if salive quantity decreased. Leng and Nolan (1984) stated that the low cycle condition due to the limitation of the N-feeding, part of the urea which enter into the rumen was via salive, whereas for the higher cycle, the part of the urea in the rumen came from the increasing of the urea that directly penetrate the rumen wall.

The salive quantity excreted daily was dependent on the type of the animal and also the type of their feed for Goat the value is about 8-19 liter, whilst for cattle could reach 100-190 liter. The quantity of the excretion of salive could increase if the feed is rich with the fibre, which condition would also increase the rumination and secretion will be decreased if the feed was rich with concentrate.

In the non-lactating Goat, Obara and Shimbayashi (1980 and 1987) showed that the transfer of salive was related quite close with the urea concentration in the blood and the consumption of nitrogen.

We can see that by increasing the protein digestibility, the urea concentration in the blood will be increased and the urea transferred by salive is also increased quite high.

Factor That Influence the Blood Urea Transfer into the Rumen

Rumen wall permeability

The different of the rumen wall permeability were specified among species. Houp since 1968 stated that the rumen wall permeability was higher in goat than in sheep. Whilst Engelhardt and Nickel (1965) signed that the rumen epithelium permeability in goat was about 1.7 times more higher than in calve. From the observation concerning the different permeability of the rumen wall could be due to the different of the surface diffusion (papillae were less developed) This hypotheses has to be confirmed.

Feeding effect

The urea cycling measured in goat receiving several different kinds of feeding (Mc Rae and Reed, 1980).

Mc Rae and Reed (1980) estimation that the recycling in sheep in the maintenance state for different feeding (Table 2) were based on the following equation:

Table 1. The relationship between the salive-urea transfer and the concentration of the urea in the blood (uremia)

Feed	1a	2a	3b	4b
Digestibility of the protein/Kg LW	0.6	1.2	1.9	3.8
Concentration of plasma urea (mgN/l)	58	106	172	360
Urea transfer in the rumen (gN/day)	1.1	1.86	0.63	0.84
Urea transfer in the salive (gN/day)	0.17	0.37	0.42	0.70
Salive transfere in the total transfer	14	20	66	83

a : Obara and Shimbayashi, 1970
b : Obara and Shimbayashi, 1987

Table 2. The recycling urea in sheep in the maintenance state for different feeding

Feeding	N consumption (gN/day)	NH_3 absorption	N-urea recycling
Alfalfa	16	7.0	1.5
Hay	11	3.5	1.5
Ensilage	19.5	10.0	1.5

N-Duodenum + NH_3 transferred through rumen wall = (N-endogen + N-urea) recycling + N-consumption. In this research the quantity of the endogenous nitrogen-protein which recycled were higher than the urea, the part of the recycle was originated from the gastric secretion/tractus digestivus, waste of the salive and desquamation of the rumen epithelium.

Estimation which was done by Mac Rae and Reed (1980) was based on the ration which composed by brome hay indicating that for the N recycling of non urea of 11 g, 1.5 g was originated from salive protein, 6.0 g was originated from epithelium cel destruction of the rumen.

The stadia of the transferred urea nitrogen in the rumen were varied, dependent on the nitrogen quantity in the feed, urea transfer varied from 1.6-5.6 g N/day, if the nitrogen consumption by the goat increased from 2.8 to 35 g N/day, whilst Grantley and Oldham (1982) showed that in bull, the increasing of the nitrogen feeding (corn silage and concentrate) from 56 to 88 g N/day caused increasing on urea transfer from 17 to 35 g N/day, whilst Moctar and Phiffer (1981) showed that feeding in maintenance state for sheep, which containing less soluble N (500g Hay and 22 g sugar) and in the same time infused with casein into the abomasum. In this condition, the quantity of N-urea, which was recycling in the rumen, could reach 13.5 g N/day. Also Chalmers *et al* (1975) stated that utilization of the low quality forage (containing low of N) by sheep was followed by relatively lower of N-urea cycle in the rumen, namely about 0.5 – 2.3 g N/day.

From the researches that have been conducted it was concluded that for sheep receiving low quality forage would give lower N-urea transfer in the rumen.

Obara and Shimbayashi (1980) showed that goat in production condition, when receiving low N-feeding, would give a low concentration of the blood urea, about 58 mg/l and the transfer in the rumen was about 48.6 mg N/hour, but if the blood urea increased (106 mg N/l) it would give a transfer of about 77.7 mg N/hour.

Urease enzyme

The urease enzyme which was produced by bacteria in the rumen, have an essential function in the urea cycle processed, the role of the urease were to converse the urea into ammonia.

In the ruminant species, the urease activity is quite higher and dependent on the restriction of the rumen epithelium and would be associated with the population of proteolitic bacteria.

Urease activity in the rumen was starting to in the preruminant period for the goat, whilst for calves, the activity was already higher since they were born. And when they mature, the urease activity would dependent on the feeding, would be higher if the feeds were rich in the organic matter that could be easily fermented.

Urease activity are also dependent on the consumption of N. Jovorsky *et al* (1987) showed that the activity of urease would become important if N content in the feeds were low.

The mentioned researcher think that urease activity would be profitable in the feeding containing law in nitrogen and would make a reasonable high retention of urea-nitrogen. Whilst Rybosova *et al* (1984) noted a individual variation on urease activity in the cell wall of rumen, that was about 30 – 800 nKat/25 cm^2 rumen papilla in the goat which consumed N about 6.2 gN/day. Rybosova *et al* (1984) also noted that urease activity

Table 3. The relationship between the urease activity sand feed nitrogen (Javorski *et al* 1987) in sheep

Consumption of N	Urease activity nkat*/mg Bacteria **		
G N/day	Rumen wall	Rumen liquid	Particle
3.7	13.25	8.96	5.69
21.0	3.81	3.76	1.92

nkat * : nano Katal
Bacteria ** : Bacteria dry matter

would be highest in the rumen wall and followed by the rumen liquid and then by feed particle, accordingly to the results published by Javorsky *et al* (1987).

The role of urease to facilitate the urea transfer from the blood into the rumen have been demonstrated by Houpt and Houpt (1968) cited by Whitelaw *et al* (1991), this researcher used a combination of antimicrobia, enzyme inhibitor to eliminate the urease activity of the rumen. Obtained observation showed that N-urea transfer from blood into the rumen would be very quick in the absence of urease.

The inhibition of the urease activity in vivo could complete information of the effect on urea recycling in the economy of the host animal mentioned process. How ever attempts to use this experimental approach in animal consuming normal feeds have been obstructed by the lack of a suitable inhibitor urease of the better known compounds which affect this property (e.q. Quinone, dihydric phenols, Phenyl mercuric acetate, boric acid) most are toxic irritable for longterm use in vivo whilst Acetohydroxyamate is an exception, but the use of this compound to inhibit the breakdown rate of urea given as a feed supplement to sheep has been so un successful because of the rapid adaptation of rumen microbes to the inhibitor (Jones and Milligan 1975).

The discovery that phosphoramidate a structural analogue of urea, produced reversible inhibition of urease (Dixon *et al*, 1975 cited by Whitelaw *et al* 1991) has been introduced in recent years for the synthesis of a compound that would inhibit of urease activity (Liao and Raines, 1985) and it was stated that phenyl phosphoryldiamidate (PPDA) has proved to be one of the most potent compounds.

Voigt *et al* (1980) examined the use of PPDA in the diet of dairy cows. Only a slight reduction in urease activity was achieved although the rate of urea hydrolysis and the concentration of NH_3 in rumen fluid both decreased during the early part of the 24 week treatment period and Piatkowski and Voigh (1981) observed that no improvement in milk yield was obtained when PPDA was given to dairy cows at levels of 370 or 740 mg/day.

PPDA was clearly a very potent and reversible inhibition of rumen bacterial urease. The most obvious in urea metabolism in response to PPDA addition to the rumen was the accumulation of urea in rumen fluid and it's rapid equilibration with plasma urea concentration.

Table 4. Effect of the urease inhibitor (Whitelaw *et al.*, 1991)

Treatment	Control	PPDA to rumen	PPDA to abomasum	Statistical significance
No Observation	6	4	4	
Live weight (kg)	34.8	35.0	34.5	NS
Plasma urea - Concentration (mg/l)	197.5	213.4	228.5	NS

The relationship between means plasma and rumen urea concentrations for the six animals are clearly rectilinear throughout the rage of plasma urea concentration encountered (Whitelaw *et al* 1991). This response to urease inhibition is fully consistent with the hypothesis that the urea transfer from blood to rumen occurred rapidly by simple diffusion. Urea content with in the rumen at equilibrium thus simply enlarges the whole body urea pool and urea space (Table 4).

The addition of PPDA to the abomasum in the experiment of Whitelaw *et al* (1991) caused urease activity in rumen fluid decreased about 58% but there was no significant effect on the urea metabolism. Nevertheless the effect in the rumen was due to absorption of PPDA into the bloodstream and consequently the flowing back to the rumen, or due to the result of simply from occasionally reverse peristaltic of abomasal content was not known.

The absence of response to abomasal PPDA probably due to the inactivation of the inhibitory effect at the existing pH in the abomasum. This hypothesis was in accordance to Austin *et al* (1984). They showed that hydrolysis of phenylphosphoramidate (PPDA) at pH value of 2 to 3 were very rapid hydrolysis to form NH3 and PPA was also unstable in acid solution. They also showed that PPDA was totally decomposed to form phenylphosphate and NH_3 in about 20 minutes at pH 2. Whitelaw *et al* (1991) stated that PPDA was potential to inhibition of bacteria urease in the rumen so the utilization in the rumen and post rumen on the recycling urea could be measured qualitatively.

Inhibition of urea in the rumen yields an accumulation of the urea concentration in the rumen very fast and this would balance with the urea in the plasma. This matter showed that the urea transfer in the rumen could be caused by the simple diffusion.

Effect of stade of physiology

Concentration of the blood urea (urea in blood = uremia). Chaoutenne (1991) using non lactating goat, showed that the blood-urea concentration will always be maximized in the morning (figure 1). The coefficient of variation between physiological states were not important, it varied between 5 to 11% and the bigger variation will be found in the dry condition, at early state of pregnancy and middle state of the pregnancy and the values

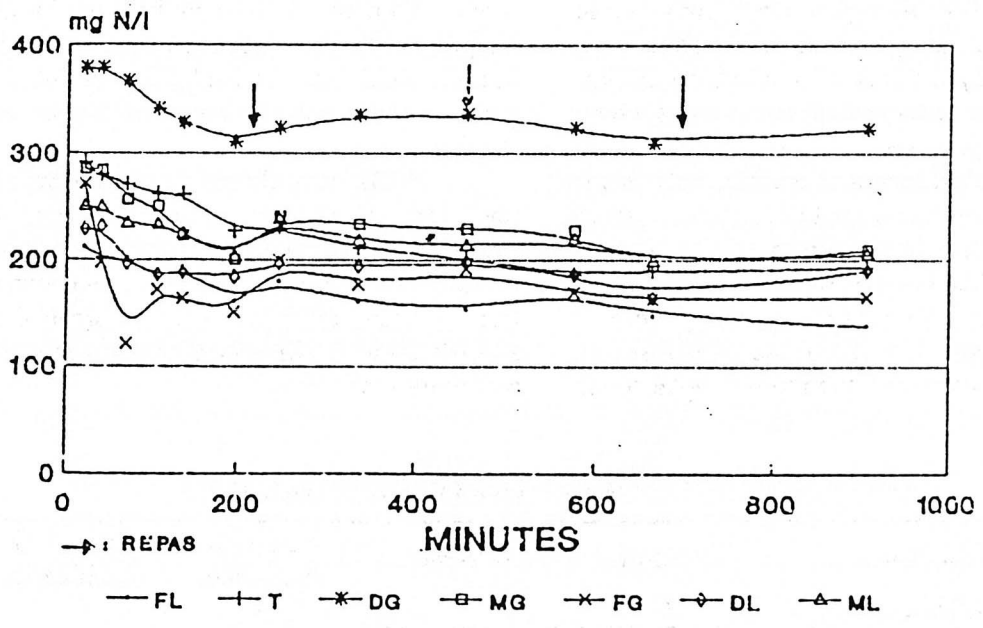

Figure 1. Concentration of the blood-urea goat in the different physiological states (Chaoutene, 1991)

Tabel 5. The consumption of the cattle and buffaloes for ten days observation from 7 species of animals

Animal	Metabolic body weight (kg)	Consumption (kg DM/day)
Cattle	46.98	3.96
CV (%)	7.8	14.9
Buffaloes	56.65	5.38
CV (%)	8.5	10.2

of the uremia varied between 115 mg N/l in the middle of the pregnancy and 243 mg N/l by the end of the pregnancy state.

Uremia in the dry state were lower than those recorded by Nolan and Leng (1972) for sheep which value was about 561 mg N/l and as for Obara and Shimbayashi (1987) using goat in the non lactating condition was about 36.8 mg/dl and for young sheep was about 36.8 mg/dl and for Young sheep was about 26.2 mg/dl (Bunting *et al* 1987). Whilst Oddy, Gooden and Annison since 1983 working with dairy tocks stated that the recycling urea of the dry young sheep, pregnancy and in production conditions 3 types of the ration fed to each recycling urea was maximum for the animal similar state of physiology, found that the which received the feeding regime with more content of energy or more lack in nitrogen. For the same regime, the measurement of the recycling urea of the dry female or pregnant was similar. For the lactating sheep, the recycling were more high about 23 g urea / days for the animal received 13.3 MJ and 51.4 g nitrogen and only 5 g for the same regime (5.56 MJ and 21.36 g N) for the dry sheep. These results have confirmed that the physiological conditions of the animals on the affect the recycling.

Effect of the Kidney on the recycling urea regulation

In accordance to introductions indication that the urea which produced in the organism were not totally excreted in the urine but part of it would be transferred in the tractus digestivus, this mean that the role of kidney will be very important.

In this trial, feeding was conducted for 14 days for adaptation period and the consumption data were obtained for 10 days

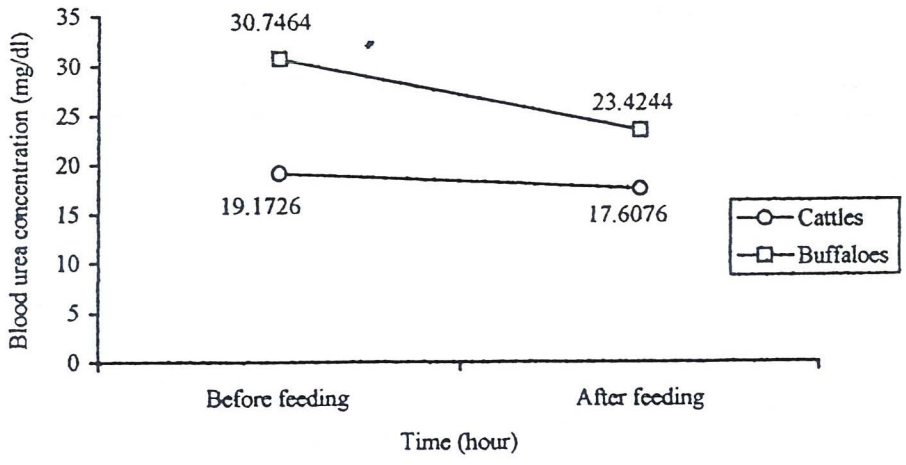

Figure 2. Evolution of the concentration of blood urea, the value has been observed from average value of 7 cattle and 7 buffaloes

Table 6. Urea blood concentration for Cattles and Buffaloes before and after feeding (mg/dl)

	Before feeding (mg/dl)	After feeding (mg/dl)
Cattles	19.2	17.6
Buffaloes	30.7	23.4

after the adaptation periods. The rice bran was given about 2 Kg for the cattle and 1 Kg for Buffaloes. The withdrawal of the blood was done in the eleventh day after the adaptation period in the morning before feeding and 6 hour after the feeding. The concentrations of the urea plasma are shown in Figure 2.

From the data available showed that the uremia will always still be high after feeding in the morning, for the buffaloes gave a value of about 19.17 mg / dl and for cattle gave a value of about 30.75 mg/dl. After 6 hour of feeding in the morning, urea blood concentration obviously decreased for cattle and also for buffaloes, the value was about 17.61 mg/dl for the cattle and 23.42 mg/dl for the buffaloes, have decreasing value about 1.6 point for the cattle and reach 7.3 point for the buffaloes.

Observation shown also that for the buffaloes, after feeding or 6 hour after feeding, the uremia concentration were always higher than cattle, this matter must be explained which factor could control the regulation the different urea cycle between species. Chaoutene (1991) using sheep in different states of physiology found that variation of uremia in the different state of physiology (Figure 1) it was also stated that there was also a variation among individual animal, this matter perhaps due to the intrinsic factor (coming from the animal them self) this should be analysed further.

The hypothesis concerning the different between individual or species, besides from feeding and physiological condition, there were also urease inhibitor, permeability, concentration of the rumen ammonia, probably it could be caused to the different function of the kidney of each species. The most important role is glomerulair filtration rate (GFR).

The filtrate volume of the glomerulair per time is called GFR (Glomerulus Filtration Rate), for the measurement of GFR which is found in the blood could be used and have the following characteristics: The substance should be easily filtrated in the free form, the quantity of the filtrated substance should not be absorbed, should not be secreted by the tubular cell, so they should not be destructed and should not also be produced by the kidney, the substance should not have any effect to the kidney function.

There conditions could be found in Inulin and in many conditions if Inulin could not be measured, we could use another endogenous substance namely creatinin, which usually could be found in the blood.

The quantity of the substance which could be filtrated per unit time (A) could be calculated based on the plasma substance concentration which would be measured Pin (g/l) multiplied by GFR (ml/mn), based on the characteristics given namely the filtrated substance quantity should not be absorbed and secreted by the tubular cell, so it has a constant condition and the substance in the blood should not be destructed or produced by the kidney. The quantity of filtrated substance per unit time would not be varied as it passes through the nephron. Consequently is that the substance quantity, which is found in the urine per the same interval unit time will be the same. This substance quantity which could be filtrated per unit time will be found by multiplying the urine volume per minute: Vu (ml/mn) with the urine substance concentration which is measured in Uin (Inulin urine, g/l) or Pin x GFR = Uin x Vu, which GFR = (Uin/Pin) x Vu (ml/mn) (Figure A). Which Pin is a plasma concentration of Inulin, GFR is a volume of filtration glomerulus, Vu is urine volume per minute (ml/mn) and Uin is urine concentration and Inulin substance (g/l).

GFR value is usually about 120 ml/mn/1.73 m2 of body area, or about 180 l/day and Widiyono *et al* (1998) stated that for younger sheep (in pra ruminant condition) have a GFR similar to a mature sheep with value of about 1.99 ml/mn/BW.

The study of GFR or regulation of the kidney could found a mechanism regulation to order the different on urea recycling of each species of animals.

REFERENCES

Austin, E.R., T.J. Bradford and M.S. Lupin. 1984. High performance liquid chromatographic determination and hydrolysis studies of phenyl phosphorodiamidate, a urease inhibitor. *Journal of Agriculture and Food Chemistry*, 32.

Bunting, L.D., M.D. Howard, R.B. Muntifering, K.A. Dawson, J.A. Boling. 1987. Effect of feeding frequency on forage fiber and nitrogen utilization in sheep. *J. Anim. Sci.*, 64.

Chaoutene, H. 1991. Recyclege d'uree endogene chez les chevres laitiers: Efft du stade physiologique. *These de Doctorat. ENSAIA-INPL.* France.

Dixon, R.M. and J.V. Nolan. 1986. Nitrogen and Carbon flows between the coecum, blood and rumen in sheep given chopped lucerne (*Medicago sativa*) hay. *Br. J. Nutr.*, 55.

Engelhard, W.V. and W. Nickel. 1965. Die permeabilitat der pansenwand fur harnstoff, antipyrin un waster. *Arch. Ges. Physiol.*, 286.

Ford, A.L. and L.P. Milligan. 1970. Tracer studies of urea recycling in sheep. *Can. J. Anim. Sci.*, 50.

Jones, G.A and J.D. Milligan. 1975. Influence on some rumen and blood parameters of feeding acetohydroxamic acid in a urea-containing ration for lambs. *Can. J. Anim. Sci.*, 55.

Javorsky, P., E. Rybosova, I. Harvassy, K. Korsky, V. Kmet. 1987. Urease activity of adherent bacteria and rumen fluid bacteria. *Physiol. Bohemoslov.*, 36.

Kennedy, P.M and L.P. Milligan. 1980 The degradation and utilization of endogenous urea in the gastrointestinal tract of ruminants : a review. *Can. J. Anim. Sci.*, 60.

Liao, C.F H and S.G. Raines. 1985. Inhibition of soil urease activity by amino derivatives of phosphoric and thiophosphoric acids. *Plant and Soil*, 85

Nolan, J.V., R.A. Leng. 1972. Dynamic aspect of ammonia and urea metabolism in sheep. *Br. J. Nutr.*,27.

Norton, B.W., A.N. Jones, D.G. Arsmstrong. 1982. The effect of intra ruminal infusion of sodium bicarbonat, ammonium chloride and sodium butyrate of urea metabolism in sheep. *Br. J. Nutr.*, 48.

Obara, Y.and K. Shimbayashi. 1980 The appearance of recycled urea in the digestive tract of goats during the final third of a once daily feeding of low-protein ration. *Br. J. Nutr.*,44.

Rybosova, E., P. Javorsky, I. Havassy, K. Horsky. 1984. Urease activity of adherents bacteria in sheep rumen. *Physiol. Bohemolov.*, 33

Whitelaw, F.G., J.S. Milne and S.A. Wright. 1991 Urease (Ec 3.5.1.5) inhibition in the sheep rumen and it's effect on urea and nitrogen metabolism. *Br. J. Nutr.*, 66.