รายงานผลการวิจัย
ทุนวิจัยงบประมาณแผ่นดิน ปี พ.ศ. 2533

เรื่อง

การศึกษาเมตาบอลิสมของยูเรียในกระบือปลักในภาวะปกติและภาวะที่ได้รับการฉีดสารละลายยูเรีย

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STUDIES ON UREA METABOLISM IN SWAMP BUFFALOES GIVEN EXOGENOUS UREA INFUSION

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บทย่อ

ศึกษาเมตาบอลิสมของยูเรียในกระบือปลักในภาวะปกติและภาวะที่ได้รับการฉีดสารละลายยูเรียโดยใช้กระบือปลักเพศเมีย จำนวน 5 ตัว น้ำหนักระหว่าง 200-290 กิโลกรัม การทดลองแบ่งออกเป็น 2 ระยะ ระยะแรกเป็นระยะควบคุม ระยะที่ 2 เป็นระยะที่มีการศึกษาในขณะฉีดสารละลายยูเรียเข้าหลอดเลือด การวัดการหมุนเวียนของยูเรียและการนำกลับยูเรียมาใช้ภายในร่างกายโดยใช้สารรังสี ^{14}C-urea มาศึกษาในทั้ง 2 ระยะ จากผลการศึกษาพบว่าขณะฉีดสารละลายยูเรียเข้าร่างกาย ความเข้มข้นของยูเรียในพลาสม่าเพิ่มขึ้นอย่างมีนัยสำคัญ ปริมาณยูเรียในร่างกายเพิ่มจาก 85 ลิตร เป็น 92 ลิตร อัตราการหมุนเวียนของยูเรียลดลง ในขณะที่ช่วงเวลาครึ่งชีวิตของสารละลายยูเรียภายในร่างกายไม่มีความแตกต่างระหว่างระยะควบคุมและขณะฉีดสารละลายยูเรียเข้าสู่ร่างกาย อัตราการนำกลับยูเรียมาใช้ไปยังกระเพาะส่วนรูเมนมีแนวโน้มลดลงในขณะที่ฉีดสารละลายยูเรียเข้าสู่ร่างกาย อัตราการขับทิ้งยูเรียทางไตเพิ่มขึ้น แต่อัตราการขับปัสสาวะและอิเลคโทรไลต์ไม่มีการเปลี่ยนแปลง จากผลการทดลองสรุปได้ว่าการเก็บสารละลายยูเรียภายในร่างกายของกระบือปลักรวมทั้งการทำงานของไตเพื่อเก็บยูเรียไว้ในร่างกายเป็นผลมาจากปัจจัยหลาย ๆ อย่างรวมกัน

Summary

Five female swamp buffaloes, weighing between 200-290 kg, were used in the experiment. The experiment was conducted into two series. The first series was performed as control. In the second series, the animal was given an exogenous urea infusion. The turnover of plasma urea and quantitative aspect of urea recycle were investigated in both series using ^{14}C-urea. The results of experiments showed that the concentration of plasma urea increased significantly during exogenous urea infusion. Urea space increased from 85 L to 92 L. Turnover rate of urea decreased while the biological half life time showed no difference after exogenous urea infusion. The urea recycled into the rumen tended to decrease during exogenous urea infusion. Renal urea excretion increased while the urine flow rate and electrolyte excretion were not affected during period of urea infusion. It can be concluded that the ability of the buffalo to retain endogenous urea is influenced simultaneously by several factors and renal urea retention can not be interpreted as the effect of a single parameter.

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Introduction

Many studies in ruminant show that urea is direct transferred via blood or saliva to the rumen (Houpt, 1959 ; Somer, 1961) where it is dissociated into ammonia and carbondioxide by the action of microbial urease. This ammonia is used in microbial protein synthesis in the rumen, but it is also absorbed from the rumen into the portal blood stream and resynthesized into urea in the liver (Lewis et al, 1958). In consequence, urea is in recycling and undergoes dissociation and resynthesis within the ruminant body. It has been shown that blood urea transfers to the rumen is an important aspect especially during a time of protein shortage and kidney should be able to conserve urea by decreasing the renal urea excretion (Schmidt Nielsen et al, 1958). An endogenous urea recycle is postulated for these phenomena. Although the phenomena have been studied in other ruminating animals e.g. goat, sheep (IDe, 1975) but, little is know about the urea recycle in buffalo.

The previous study in the buffalo suggested that the limitation of renal urea reabsorption would be attributed to changes in either plasma pool size of urea or changes in body metabolism of nitrogenous substance (Chaiyabutr et al., 1992). It seems to remain in qualitative understanding. Changes in urea pool size would relate to the extent of passage of endogenous nitrogen into the rumen and these factors that alter recycled nitrogen have not been established in buffalo. It has been reported that isotope tracer experiment might be a very useful approach for urea kinetic study. Therefore, the present studies were conducted with ^{14}C-urea for the purpose of clarifying the quantitative pattern of the endogenous urea recycling in the buffalo and the extent to which this was altered by an exogenous urea infusion.

Materials and Methods

The experiment was conducted in five female buffaloes weighing between 200-290 kg. Two series of experiments were carried out. The animals were fed with paragrass and water hyacinth ad lib throughout the experiment of each series. At least 2 weeks elapsed between two series experiments on the same animal. On the day of experiments, the animal was withheld of food and drinking water while it was tethered with standing position in the room.

Animal preparation :

On the day before studies began, polyethylene catheters (PE 200) were placed in both jugular veins to facilitate both infusion and blood sampling. The balloon catheter (gauge 24) was inserted into the urinary bladder for urine collection.

Experimental procedures :

The experiment was conducted into two series.

Series I

In the first series of experiment, the animal received a priming dose of para-aminohippuric acid (PAH) 1 gm and inulin 2 gm in 40 ml of normal saline followed immediately by substaining solution of PAH 25 mg/4ml/min and inulin 50 mg/4 ml/min. The solution was infused at a constant rate throughout the experiment using peristaltic pump (EYELA, MP-3). Renal clearance measurements were started about an hour after injection of the priming dose. Urine sample was collected over an accurately timed period about 15-20 min. To ensure each accurate collection, the urine sample was started after voidness the bladder. Blood sample was collected at the mid point period of urine collection.

After renal clearance measurements, each animal was injected intravenously with 100 μci/animal of ^{14}C-urea in 20 ml of normal saline. Five ml of blood samples was collected with a heparinized syringe from the jugular vein at the following interval 5,10,15,20,30,60,90,120,180 and 240 min after the injection. Blood samples were centrifuged at 3000 rpm for 10 minutes and plasma was kept at-20°C and used for subsequent analyses.

Series II

The second series was conducted after the first series of the experiment at least 2 weeks interval. In the second series of experiment, the short period infusion of urea was carried out in each animal by intravenous infusion of exogenous urea with the priming dose of solution containing 3 gm of urea in 30 ml of normal saline following immediately by continous infusion of urea 50 mg/min/4 ml of normal saline solution throughout the experimental period. After 1 hr. of exogenous urea infusion, the renal clearance measurements and urea metabolism using ^{14}C-urea injection were carried out as in the series I of the experiment.

Analytical method

The concentration of inulin in plasma and urine was determined by Anthrone method (Young and Raisz, 1952). The concentration of PAH in plasma and urine was determined by method of Bratton and Marshall as described by Smith (1962). Urea concentration in plasma and urine was determined by using diacetylmonoxime method (Coulombe and Favreau, 1963). Sodium, potassium and chloride ion concentrations in plasma and urine were determined by flame photometer.

Plasma urea-^{14}C determination :

Each of 0.5 ml of plasma was diluted with 0.5 ml of distilled water and plasma protein were precipitated with 0.5 ml of 10% tricarboxylic acid. After centrifugation at 3000 rpm, 0.5 ml of supernatant was mixed with 3.5 ml

of scintillation fluid (1 L of scintillation fluid contained 5 g of 2,5-diphenyloxazole (PPO), 0.25 g of 1-4 bis (2-(4-metyl-5 phenyloxazolyl) benzene) (POPOP), 500 ml of Toluene and 500 ml of Triton X-100). Its radioactivity was determined by scintillation counter (Liquid scintillation counter, 1214 Racbeta, LKB Wallac) with gain and window set appropriately for ^{14}C.

Calculations

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were obtained using the clearance of inulin and clearance of PAH respectively. The tubular reabsorbed urea was obtained by the difference between the glomerular filtered and the renal excreted urea.

The plasma ^{14}C-urea specific radioactivity were plotted on logarithmic scale against time. The biological half-life time of urea expressed as $t^1/_2$ was calculated from the slope of the decrease in the activity of ^{14}C. The urea space was determined by dividing the total ^{14}C-urea radioactivity injected by the radioactivity per ml of plasma at zero time extrapolated from the regression curve. Urea pool size was estimated by multiplying the urea space by plasma urea concentration. The turnover rate of urea was calculated by multiplying the urea pool size by $0.693/t^1/_2$.

An estimation of the urea recycled into the rumen for microbial protein synthesis could be calculated in the following equation :

$$\Delta U_P = \Delta U_S - \Delta U_E - \Delta U_R$$

where ΔU_P = the change in the total body urea pool size (turnover rate of urea).

ΔU_S = the amount of urea synthesized by the liver

ΔU_E = the amount of renal urea excretion

ΔU_R = the amount of endogenous urea returned to the rumen

By rearrangement of equation :

$\Delta U_P + \Delta U_E = \Delta U_S - \Delta U_R$

Therefore $\Delta U_S - \Delta U_R$ = an estimation of the urea recycled into the rumen for microbial protein synthesis

Statistical analysis

All the results were statistically analysed by paired t-test. Data are presented as means and standard errors of means (S.E.)

Results

Turnover pattern of ^{14}C-urea in the buffalo.

Figure 1 shows the representative regression curves for plasma ^{14}C-urea which had been administered to a buffalo in either control or during urea infusion. They decreased exponentially with the elapse of time after intravenous injection. Straight line were well fitted for both control and during urea infusion which had been plotted on logarithmic scales. It was noted that the decreased pattern was the same. No difference was observed in the half life ^{14}C-urea pattern between control and during exogenous urea infusion.

Quantitative aspect of urea turnover

The results for quantitative aspect of urea turnover are presented in table 1. The concentration of plasma urea increased significantly ($P<0.05$) during intravenous infusion of urea for 4 h, corresponding to the urea space of 85 L at the control period to 92 L during urea infusion ($P<0.05$). The urea pool size changed proportionally with the plasma urea concentration ($P<0.05$). The biological half-life time determined with ^{14}C-urea was found no difference between control and during exogenous urea infusion.

It was found that there was a decrease of the turnover rate of urea by approximately 33% during urea infusion period when compared to the control period. An estimate of the urea recycled into the rumen tended to decrease during urea infusion when compared to the control period, but the differences were not significant.

Renal urea excretion

During intravenous infusion of urea, no change in glomerular filtration rate was observed. Therefore, an increase in filtered urea was due to an elevation of plasma urea concentration (Table 2). Renal urea excretion slightly increased during period of urea infusion. The renal urea reabsorption slightly increased which coincided with an elevation of the ratio between reabsorption and filtered urea.

Plasma concentration and excretion rates of sodium, potassium and chloride ion

Exogenous urea infusion had no effect on plasma concentration of sodium, potassium and chloride ion. Excretion rates of these electrolytes were also not affected by exogenous urea infusion.

Figure 1. Representative regression curves of ^{14}C-urea in plasma of buffalo #01 in control and during exogenous urea infusion.

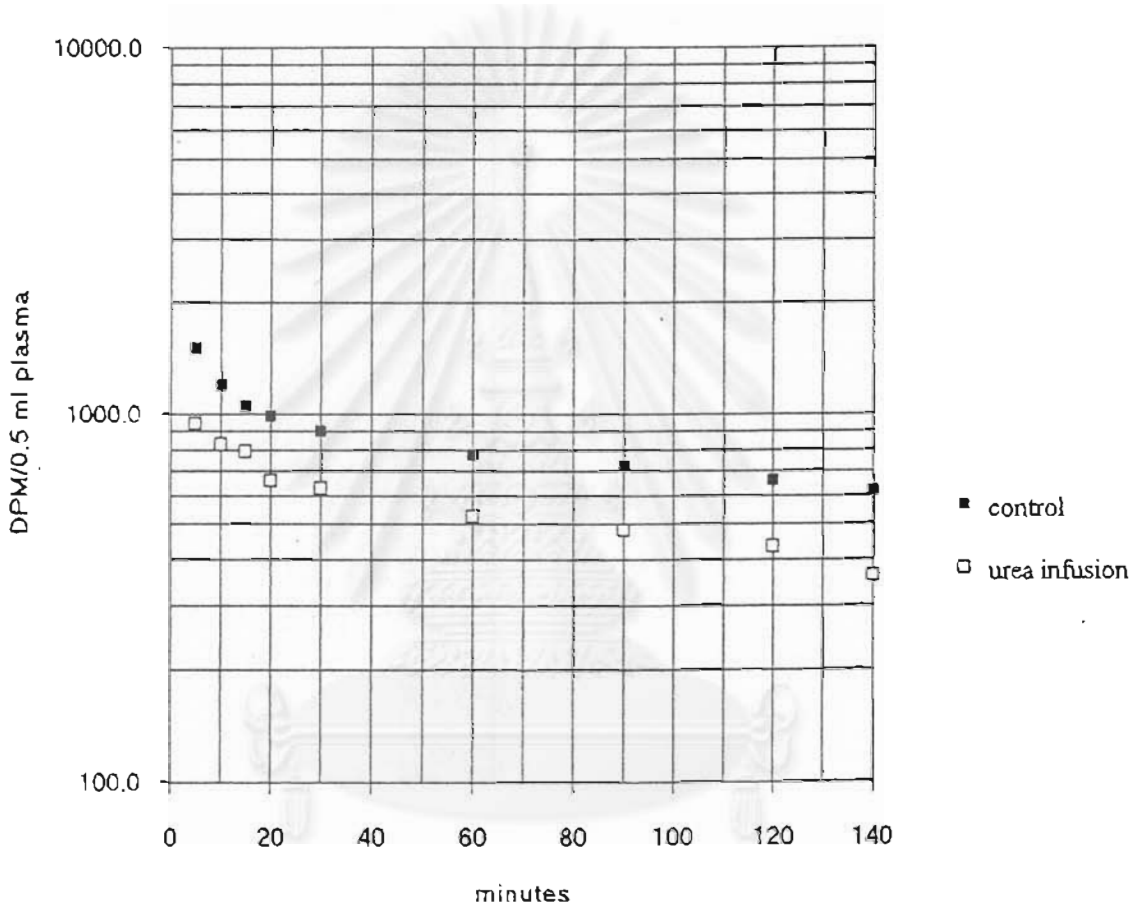

Table 1. Plasma urea concentration, urea distribution space, urea pool size and half-life of urea in buffaloes studied. (Mean ± S.E.)

	control	exogenous urea infusion	control VS urea infusion
Plasma urea (mg/100 ml)	21.3 ± 2.1	29.0 ± 4.7	$P < 0.05$
Urea space (L)	84.7 ± 9.4	92.4 ± 13.3	$P < 0.05$
Urea pool (g)	17.5 ± 0.8	23.6 ± 2.4	$P < 0.05$
Half-life ^{14}C-urea (min)	98.6 ± 36.1	111.3 ± 22.7	NS
Urea turnover (mg/min)	235.1 ± 96.1	157.9 ± 22.0	NS
Endogenous urea recycling (mg/min)	252.3 ± 97.0	181.9 ± 26.2	NS

Table 2. Renal urea excretion in buffaloes studied. (Mean ± S.E.)

	control	exogenous urea infusion	control VS urea infusion
Renal urea excretion (mg/min)	17.2 ± 2.3	24.1 ± 0.5	$P < 0.05$
Urea filtered (mg/min)	29.4 ± 5.0	41.2 ± 6.1	$P < 0.05$
Renal urea reabsorption (mg/min)	12.2 ± 3.6	17.2 ± 2.3	NS
Reabsorption/filtered (%)	38.1 ± 10.1	42.6 ± 4.3	NS

Table 3. Plasma concentration, renal excretion rate of sodium, potassium and chloride ion in buffaloes studied. (Mean ± S.E.)

	control	exogenous urea infusion	control VS urea infusion
Sodium			
Plasma (mEq/L)	130 ± 1.6	132 ± 0.7	NS
Excretion (μEq/min)	1635 ± 215	1432 ± 178	NS
Potassium			
Plasma (mEq/L)	3.8 ± 0.2	3.7 ± 0.2	NS
Excretion (μEq/min)	998 ± 210	1007 ± 36	NS
Chloride			
Plasma (mEq/L)	9.0 ± 1.3	98 ± 2.4	NS
Excretion (μEq/min)	1008 ± 261	785 ± 230	NS

Discussion

The results of the present experiments show that levels of the urea pool size and plasma urea concentration consistently increased during exogenous urea infusion. However, signs or symtoms associated with the urea toxicity during exogenous urea infusion were not apparent. This result confirms to those observed by Chaiyabutr and co-workers (1992) for the buffalo given exogenous urea infusion in high ambient temperature. In the present experiment, the kinetic parameters of urea were calculated by intravenous administration of small amount of radioactive tracer ^{14}C-urea. It was found that the rate of urea turnover decreased by approximately 32% while the biological half life time of ^{14}C-urea was considerably longer during elevation of plasma urea concentration. It was estimated from the results obtained that an exogenous urea intravenous infusion might not be transferred to alimentary canal, including rumen, where microorganism are capable of utilizing urea. The excretion of urea would be restricted by its endogenous recycling and microbial assimilation. These results were compatible with a decrease in the values of urea recycling (ΔU_S - ΔU_R) and an increase in urinary urea excretion during urea infusion (Table 2). However the present results contradict with those reports of Macfarlane (1964) and Schmidt-Nielsen et al (1957) which showed that infusion of urea to ruminants caused a recycling of urea to the rumen. It may be related to many factors for example low protein diets intake and water restriction which has been reported to affects the amount of urea recycling. The reabsorption of urea into the alimentary tract of ruminants has been shown to carry water with it (Houpt and Houpt, 1968 ; Vercoe, 1969). In the present study, the large positive values of ΔU_S - ΔU_R obtained in either normal or during exogenous urea infusion indicate the occurance of urea utilization for non protein nitrogen. The buffalo in the present study had been fed with paragrass and water hyacinth which were

composed of the lower nitrogen diet. It is possible to presume the net recycling of blood urea nitrogen to the rumen still occurred in either control period or during exogenous urea infusion. This may be of particular important and interest from the view point of buffalo nutrition.

As shown in table 1 and 2, there is a large difference between the turnover rate of urea and that of urea excretion in all buffaloes studied. This indicates that the urea synthesized is in general much larger in amount than the urea excreted in the buffalo. This phenomenon shows the same as other ruminating animals (Ide, 1975). It was roughly estimated from the results obtained that more than a half of the quantity of blood urea might be transferred to the rumen and undergo dissociate in this organ. With an increase the plasma urea concentration by exogenous urea infusion, the urea turnover rate and endogenous urea recycling decreased while renal urea excretion increased. An increase in renal urea excretion did not relate to unchanges of urine flow rate and electrolyte excretion, although a positive correlation between rate of urine flow, fractional potassium excretion and urea excretion has been previously reported (Chaiyabutr et al., 1992). These results can be concluded that the ability of the buffalo to retain endogenous urea is influenced simultaneously by several factors and renal urea retention can not be interpreted as the effect of a single parameter.

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