RENAL REGULATION OF UREA EXCRETION IN SWAMP BUFFALO FED WITH HIGH PROTEIN SUPPLEMENTATION

N. Chaiyabutr[1], S. Chanpongsang and P. Loypetjra

Department of Physiology, Faculty of Veterinary Science
Chulalongkorn University, Thailand

Summary

The effect of supplemented high protein diet intake on renal urea regulation in swamp buffalo was carried out in the present experiment. Five swamp buffalo heifers weighing between 208-284 kg were used for this study. The animals were fed with a supplementary high protein diet and renal function and kinetic parameters for urea excretion were measured. This was compared to a control period where the same animals had been fed only with paragrass and water hyacinth. For 2 months the same animals were fed a mixed of paragrass, water hyacinth plus 2 kgs of a high protein supplement (protein 18.2% DM basis) per head per day. In comparison to the control period, there were no differences in the rate of urine flow, glomerular filtration rate (GFR), effective renal plasma flow (ERPF), plasma urea concentration and filtered urea. In animals supplemented with high protein intake mean values of urea clearance, excretion rate and the urea urine/plasma concentration ratio markedly increased ($p < 0.05$) while renal urea reabsorption significantly decreased from 40% to 26% of the quantity filtered. In this same study group urea space distribution and urea pool size increased which coincided with an increase in plasma volume ($p < 0.05$). Plasma protein decreased while plasma osmolarity increased ($p < 0.05$). Both urea turnover rate and biological half-life of ^{14}C-urea were not affected by a supplementary high protein intake. The results suggest that animals supplemented with high protein diets are in a state of dynamic equilibrium of urea which is well balanced between urea excreted into the urine and the amount synthesized. The limitation for renal tubular urea reabsorption would be a change in extra-renal factors with an elevation of the total pool size of nitrogenous substance.

(Key Words : Renal Urea Excretion, High Protein Diet, Buffalo)

Introduction

Urea reabsorption is important to the ruminant since blood urea nitrogen can be used in a protein regeneration cycle (Houpt, 1959). Many studies have demonstrated a relationship between urea excretion and urea plasma concentration which has been shown to be sigmoid (Cocimano and Leng, 1967) or linear (McIntyre and Williams, 1970). The restriction of renal urea excretion in sheep which have been fed low protein diet has shown that urea reabsorption by the renal tubules is related to plasma urea concentration and the amount of filtered urea with no alteration of glomerular filtration rate (GFR) (Schmidt-Nielsen et al., 1958). In sheep reduction in dietary protein affects the GFR (Gans and Mercer, 1962; Rabinowitz et al., 1973). An increase in the fraction of filtered urea reabsorbed has been noted at low urine flow when sheep were fed with a low protein diet (Scott and Mason, 1970). In buffalo, which had received an intravenous infusion of exogenous urea, an increase in filtered urea reabsorption and reduction in the urine flow rate have also been demonstrated. These changes were not observed in heat stressed buffalo (Chaiyabutr et al., 1992). Chaiyabutr and co-workers (1992) suggested that the limitation of renal urea reabsorption in heat stressed buffalo could be attributed to an increase in plasma pool size of nitrogenous substances.

Urea excretion in the urine and the blood urea level under different nutritional conditions in buffalo is poorly understood due to the variability of renal urea reabsorption. The present study was undertaken to clarify the possible effects of renal urea excretion and reabsorption in buffalo after increasing dietary protein.

[1]Address reprint requests to Dr. N. Chaiyabutr, Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Thailand.

Received August 23, 1994
Accepted January 21, 1995

Materials and Methods

Animals and feeding procedures

Experiments were conducted on five swamp buffalo heifers weighing between 208-284 kg. The experiments consisted of a pre-experimental period followed by a two months experimental period. In the pre-experimental period (control) which ranged from 2 to 3 years, all animals had been feeding *ad libitum* on paragrass and water hyacinth only. In the experimental period each animal was fed a mixed ration consisting of paragrass, water hyacinth and a supplement of high protein concentrate once daily. The buffalo was fed with a single allowance of 2 kg (moist weight) of the concentrated mixture per head per day. This concentrated mixture was designed to provide a high protein diet for animals. Composition of this mixture (percent DM basis) was moisture 10.8%, protein 18.2%, fat 4.3%, crude fiber 5.7%, ash 19.7% and NFE 41.4%. During the experiment, all animals were allowed free access to drinking water.

Animal preparations

All animals were trained to become accustomed to the procedures. On the study day before renal clearance measurements, the buffaloes were tethered in the pen under a metal roof, the right and left jugular veins were catheterized with polyethylene tubes (O.D. 1.5 mm). One catheter was used for taking blood samples and the other for the infusion of solutions. The bladder was catheterized with a self-retaining urethral catheter (24 gauge balloon catheter) for urine collection. The animals were neither fed nor watered during measurements.

Experimental procedures

On the day of the experiment two series of measurements were performed. First, renal function and plasma volume were measured after the balloon catheter had been passed into the bladder. The priming dose of the clearance solution containing 2 gm of inulin and 1 gm of PAH (para-aminohippuric acid) in 40 ml of normal saline was injected intravenously followed by infusion of a sustaining solution at the rate of 100 mg / 2 ml / min of inulin and 50 mg / 2 ml / min of PAH using peristaltic pump (EYELA, MP3, Tokyo). In the second hour after the start of the infusion, duplication of urine collections were performed at 20 min intervals and blood samples were taken at the mid point of each urine collection for chemical determinations and clearance calculations. To ensure accurate collection, the urine sample was started after emptying of the bladder. Plasma volume was measured by dye dilution technique using T-1824, as previously described (Chaiyabutr et al., 1980). In brief, a bolus of 20 ml T-1824 (0.5%) was injected into the jugular vein and serial samples of venous blood were collected for plasma volume determination from the contralateral jugular catheter. In the second series of the experiment a urea kinetic study was carried out. This part of the experiment was performed after the measurements for renal function and plasma volume determinations had been made. Each animal was injected intravenously with 100 μci / animal of ^{14}C-urea (Radiochemical Center, Amersham, U.K.) in 20 ml of normal saline. Blood samples (5 ml) were taken with a heparinized syringe via the contralateral jugular catheter at -2, 5, 10, 15, 20, 30, 60, 90, 120, 180 and 240 min after intravenous administration. Plasma was separated and kept at -20℃ to the time of analysis.

Chemical analysis

The concentration of substances in plasma and urine were analysed by the following procedures : urea by using the diacetyl monoxime method (Coulombe and Favreau, 1963), inulin by anthrone method (Young and Raisz, 1952) and PAH by the method of Bratton and Marshall as described by Smith (1962). Sodium, potassium and chloride in plasma and urine were measured by Flame photometry and Chloridometer respectively. Total plasma protein concentration was measured by Biuret method. Plasma osmolality was measured by osmometer. Packed cell volume (PCV) was determined by a micro capillary method.

Plasma ^{14}C-urea determination

Each of 0.5 ml of plasma was diluted with 0.5 ml of distilled water. Plasma protein was precipitated with 0.5 ml of 10% tricarboxylic acid. Supernatant (0.5 ml) was separated by centrifugation and was mixed in 3.5 ml of scintillation fluid (1 L of scintillation fluid contain 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.

Calculation

Based on the Fick Principle inulin clearance (Cin) was used to measure glomerular filtration rate (GFR) and PAH clearance (CPAH) was used to measure effective renal plasma flow (ERPF). Renal blood flow (RBF) was obtained by dividing ERPF by 1-packed cell volume. Filtration fraction (FF) was obtained by dividing GFR by ERPF. Fractional excretion (%FE) was obtained by dividing clear-

ance of either electrolyte or urea by GFR. The tubular reabsorbed urea was calculated from the difference between the glomerular filtered and the renal excreted urea.

The plasma ^{14}C-urea specific radioactivity was plotted on a semi-logarithmic scale against time. The biological half-life of urea expressed as t1/2 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 / t1 / 2.

Statistical analyses

Results are shown as mean values of measurements for five animals. A paired t-test was used for evaluation of differences between the control and high protein intake period.

Results

Packed cell volume, body weight, plasma protein concentration, plasma osmolarity and plasma volume

Table 1 shows that the level of plasma protein concentration decreases in animals supplemented by a high protein diet, while plasma osmolarity and body weight markedly increased. Packed cell volume was not affected by the supplementary high protein diet. The absolute value of plasma volume significantly increased ($p < 0.05$) while plasma volume with regard to body weight was not affected in animals fed with a high protein diet. Increasing the dietary protein intake in the buffalo heifer had no significant effect on plasma concentrations of sodium, potassium and chloride. Renal excretion rates of sodium and chloride slightly decreased which coincided with the decrease in fractional excretion. The renal excretion rate and fractional excretion of potassium did not change after a supplementary high protein intake.

TABLE 1. EFFECTS OF SUPPLEMENTARY HIGH PROTEIN INTAKE (HP) ON BODY WEIGHT, PACKED CELL VOLUME, PLASMA PROTEIN CONCENTRATION, PLASMA OSMOLARITY, PLASMA VOLUME AND RENAL ELECTROLYTE EXCRETIONS IN SWAMP BUFFALOES

	Control	High protein diet	Control vs HP
Body weight (kg)	247 ± 34	295 ± 40	$p < 0.05$
Packed cell volume (%)	28.1 ± 1.7	29.7 ± 2.5	NS
Plasma protein (gm%)	8.82 ± 0.29	7.98 ± 0.31	$p < 0.05$
Plasma osmolarity (mOsm/kg)	266 ± 3	276 ± 2	$p < 0.05$
Plasma volume (L)	11.14 ± 1.02	13.32 ± 1.69	$p < 0.05$
Plasma volume (ml/kg)	45.3 ± 3.2	45.2 ± 4.0	NS
Plasma Na (mmol/L)	130.5 ± 3.3	131.5 ± 1.4	NS
Na-Excretion (μmol/min)	1,757.6 ±431.6	1,241.1 ±388.3	NS
Plasma K (mmol/L)	3.6 ± 0.4	3.7 ± 0.3	NS
K-Excretion (μmol/min)	1,145.3 ±420.4	1,157.6 ±272.1	NS
Plasma Cl (mmol/L)	94.5 ± 2.6	98.0 ± 4.7	NS
Cl-Excretion (μmol/min)	1,166.1 ±527.9	875.3 ±480.1	NS

Values represent Mean ± S.D.
Significant differences ($p < 0.05$) between control and high protein intake (HP) were determined using paired t-test.
NS = not significant.

Renal hemodynamics and renal urea clearances

Urine flow slightly increased (by approximately 12%) in animals fed with a high protein supplemented diet. Mean values of clearance showed that glomerular filtration rate (GFR), effective renal plasma flow (ERPF), the filtration fraction (FF) and renal blood flow (RBF) were not significantly affected by the supplementary high protein intake. Mean values of urea clearance, excretion rate and the urea U / P concentration ratio increased by approximately 36% ($p < 0.05$) when animals were fed with a high protein diet. Renal urea reabsorption decreased from 40% to 26% of the quantity filtered when dietary protein was supplemented (table 2).

Kinetic parameters for urea and plasma urea concentration

Table 3 shows that the plasma urea concentration was not affected by supplementary high protein feeding. The kinetic study of urea showed an exponential decrease of ^{14}C-urea with the elapse of time after intravenous injection. Straight line was well fitted for both control period and the period of supplementary high protein intake and therefore, the biological half life of ^{14}C-urea and urea turnover rate of both periods showed no significant differences. The values of urea space distribution and urea pool size were significantly higher ($p < 0.05$) while the plasma urea concentration / pool size ratio was markedly lower ($p < 0.05$) in animals supplemented with a high protein diet.

TABLE 2. EFFECTS OF SUPPLEMENTARY HIGH PROTEIN INTAKE (HP) ON RENAL HEMODYNAMICS AND RENAL UREA CLEARANCES IN SWAMP BUFFALOES

	Control	High protein diet	Control vs HP
Urine flow (ml/min)	7.4 ± 2.0	8.3 ± 4.3	NS
GFR (ml/min)	135.5 ± 36.5	150.5 ± 22.3	NS
ERPF (ml/min)	1,066.1 ±176.1	1,102.3 ±179.4	NS
RBF (ml/min)	1,487.3 ±273.9	1,579.2 ±312.1	NS
FF (%)	12.8 ± 2.0	13.1 ± 0.8	NS
Renal urea clearance (ml/min)	82.1 ± 22.8	113.3 ± 30.1	$p < 0.05$
Filtered urea (mg/min)	28.74± 7.26	31.46± 8.54	NS
Renal urea excretion (mg/min)	17.18± 4.53	24.05± 9.66	$p < 0.05$
Renal urea reabsorption (mg/min)	11.56± 7.41	7.42± 1.38	$p < 0.05$
Reabsorbed/filtered (%)	40.2 ± 6.5	25.6 ± 10.2	$p < 0.05$
Urine/Plasma urea ratio	11.2 ± 1.6	15.1 ± 4.1	NS

Values represent Mean ± S.D.
Significant differences ($p < 0.05$) between control and high protein intake (HP) were determined using paired t-test.
NS = not significant.

TABLE 3. KINETIC PARAMETERS FOR UREA AND PLASMA UREA CONCENTRATION IN SWAMP BUFFALOES FED WITH HIGH PROTEIN DIET SUPPLEMENTATION (HP)

	Control	High protein diet	Control vs HP
Plasma urea (mg%)	21.25± 4.12	20.68± 3.22	NS
Urea space (L)	110.8 ± 19.3	134.4 ± 7.2	$p < 0.05$
Urea pool size (g)	23.8 ± 7.9	28.4 ± 5.7	NS
Plasma urea / Urea pool ratio	0.92± 0.16	0.73± 0.04	$p < 0.05$
Biological T1 / 2 ^{14}C-urea (min)	355.5 ±175.0	383.7 ±115.0	NS
Urea turnover (mg/min)	52.7 ± 21.4	53.9 ± 14.8	NS

Values represent Mean ± S.D.
Significant differences ($p < 0.05$) between control and high protein intake (HP) were determined using paired t-test.
NS = not significant.

Discussion

In the present experiment, the level of plasma protein concentration decreased while plasma osmolarity increased when buffaloes were given a high protein diet. This indicates that the level of plasma osmolarity might not be created by the level of plasma protein concentration. It is mainly determined by the plasma electrolyte concentrations (Tasker, 1971). This is slightly increased in plasma sodium, potassium and chloride concentrations. Many investigators showed that blood urea levels depend on protein intake and give a useful measure of the protein status. However, in the present study the unchanged plasma urea concentration may be due to the attraction of water by an increase in urea pool size which reflected increase in plasma volume. These results may offset both the plasma protein concentration and the hemoconcentration.

In the present study, the renal hemodynamics of the

buffalo were not affected by supplementary high protein intake. This result was different from the nonruminant where it has been shown that an increase in the GFR in dogs was apparent after a protein rich meal (O'Conner and Summerill, 1976). Since the level of plasma urea concentration was not affected throughout the period of study, the elevation in renal urea excretion of the buffalo fed with a high protein diet was not the consequence of a change in the quantity of urea filtered at the glomeruli. The reduction in the fraction of filtered urea reabsorbed in buffaloes given high protein diets was not attributed to either the change in urine flow or urinary electrolyte excretion. However a marked increase in the fraction of filtered urea reabsorbed was demonstrated at low urine flow when sheep were fed with a low protein diet (Schmidt-Nielsen et al., 1958) or in buffaloes after an increase in blood urea level (Chaiyabutr et al., 1992). The urine over the plasma (U / P) ratio at relatively constant urine flow in the present study should be an indicator of the regulatory state of the kidney.

It has been shown that an elevation of the concentrating ability of the mammalian kidney coincides with an increase in urea excretion. The high concentration of urea in the medullary interstitium restricts the passive reabsorption of urea from urine undergoing water reabsorption in the collecting duct and so ensures high concentration of urea in the final urine (Livinsky and Berliner, 1959). In ruminants, the excretion of urea is being restricted principally by endogenously recycled and by microbial assimilation. Excretion is facilitated by the reabsorption of urea from the renal tubule (Ide, 1971). The plasma urea concentration has been shown to be a major determinant of urinary urea excretion in both cattle and sheep (Thornton, 1970). However, in present study the plasma urea concentration of the buffalo remained almost constant during the supplementary high protein intake. This suggests that other factors rather than the blood urea concentration are limiting the reabsorption of urea in the kidney. During a supplementary high protein intake, the decreased renal urea retention may be caused first by decreased tubular urea reabsorption from the glomerular system. In the present study the decrease in the ratio of plasma urea level to urea pool size occurred in animals supplemented with high protein intake which would indicate an uneven urea distribution in body fluids. An increase in either urea space distribution or urea pool size of animals supplemented with high protein intake may be a reliable index of the lower quantitative transfer of urea from tissue to blood. However, Cocimano and Leng (1967) reported that the urea transport from the tissue into the blood increased at a lower nitrogen intake in sheep. In this study, the urea turnover rate was not significantly decreased whereas plasma urea concentration remained relatively constant. This could reflect the state of dynamic equilibrium of urea in the body; a balance between urea excreted into urine and amount of urea synthesis. The intensity of the urea transport probably depends on the adaptation of the buffalo to the supplementary high protein diet and the decreased ability of kidneys to retain endogenous urea.

Acknowledgements

This study was funded by the Government Budget CU / 2532. The authors wish to thank Miss Hathaithip Parkinsee for her assistance in the preparation of the manuscript.

Literature Cited

Chaiyabutr, N., C. Buranakarl and P. Loypetjra. 1992. Renal regulation of urea excretion during urea infusion in acute heat exposed buffaloes. Asian-Australasian J. Anim. Sci. 5(1):81-90.

Chaiyabutr, N., A. Faulkner and M. Peaker. 1980. Effects of starvation on the cardiovascular system, water balance and milk secretion in lactating goat. Res. Vet. Sci. 28:291.

Cocimano, M. R., and R. A. Leng. 1967. Metabolism of urea in sheep. Br. J. Nutr. 21:253-371.

Coulombe, J. J., and L. Favreau. 1963. A new simple semimicromethod for colorimetric determination of urea. Clin. Chem. 9:107-108.

Gans, J. H. and P. F. Mercer. 1962. Effect of osmotic diuretics and vasopressin on renal function in the sheep. Am. J. Vet. Res. 23:230-235.

Houpt, T. R. 1959. Utilization of blood urea in ruminants. Am. J. Physiol. 197:115-120.

Ide, Y. 1971. Observation on plasma urea turnover and renal urea excretion in goats. Jap. J. Vet. Sci. 33:179-185.

Livinsky, N. G. and R. W. Berliner. 1959. The role of urea in the urine concentrating mechanism. J. Clin. Invest. 38:741-748.

McIntyre, K. H. and V. T. Williams. 1970. The role of the kidney in nitrogen conservation in sheep. Australian J. Exp. Biol. and Med. Sci. 48:81-91.

O'Connor, W. J. and R. A. Summerill. 1976. The effect of a meal of meat on glomerular filtration rate in dogs at normal urine flows. J. Physiol. 256:81-91.

Rabinowitz, L., R. A. Gunther, E. S. Shoji, R. A. Freedland and E. H. Avery. 1973. Effects of high and low protein diets on sheep renal function and metabolism. Kidney Int. 4:188-207.

Schmidt-Nielsen, B., H. Osaki, H. V. Murdaugh Jr. and R. O'Dell. 1958. Renal regulation of urea excretion in sheep. Am. J. Physiol. 194:221-228.

Scott, D. and G. D. Mason. 1970. Renal tubular reabsorption of urea in sheep. Ot. J. Exp. Physiol. 55:275-283.

Smith, H. W. 1962. Principle of Renal Physiology. London, Oxford Univ. Press. pp. 212-213.

Tasker, J. B. 1971. Fluid, electrolytes, and acid base balance. In: Clinical Biochemistry of Domestic Animals. Vol. II (Kaneko, J. J. and Cornelius, C. E. ed.) Academic Press, New York, London, pp. 62-107.

Thornton, R. F. 1970. Urea excretion in ruminants, I. studies in sheep and cattle offered the same diet. Aust. J. Agric. Res. 21:323-336.

Young, M. K. and L. G. Raisz. 1952. An anthrone procedure for determination of inulin in biological fluids. Proc. Soc. Exper. Biol. and Med. 80:771-774.