PRELIMINARY RESULTS OF INVESTIGATED URINE PURINE DERIVATIVES AND MICROBIAL NITROGEN SYSTHESIS OF DOMESTIC HERBIVORES SELECTED IN MEKONG DELTA OF VIETNAM

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ABSTRACT

The objective of the investigation was to determine different potentials of urine purine derivatives (PD) and estimated microbial nitrogen synthesis (MNS) in domestic herbivores raised in the Mekong Delta for future applications. The domestic herbivores used in the study included 4 buffaloes (284 ± 12.3 kg), 4 cattle (146 ± 8.6 kg), 6 goats (21.1 ± 3.76 kg), 6 sheep (21.5 ± 4.27 kg) and 10 rabbits (1.88 ± 0.023 kg). Diet used for feeding buffalo was rice straw, natural grass, *sesbania grandiflora* and urea-molasses, for cattle was rice straw mixed with molasses and minerals, for goat was Para grass, for sheep was *panicum maximum* and concentrate and for rabbit was Para grass, sweet potatoes vines and concentrate. The investigation period was 3 weeks with 2 weeks for dietary adaptation and one week for measurements.

The results showed that daily excretions of allantoin, uric acid and purine derivatives (mmol/kgW$^{0.75}$) in urine and estimated MNS (gN/kgW$^{0.75}$) of the animals were variable among the herbivore species. The MNS (g/kgW$^{0.75}$) were 1.11, 0.479, 0.123, 0.112 and 0.038 for buffaloes, cattle, sheep, rabbits and goats. Although the CP intake (g/kgW$^{0.75}$) of rabbits was higher than cattle, sheep, buffalo and goats, the PD (mmol/kgW$^{0.75}$) were higher for the cattle (0.705) and buffaloes (0.384) compared to the others. Especially in case of buffalo the urine PD excretion and MNS produced could be driven by the characteristics of its physiology and nutrient metabolisms. It was preliminarily concluded that PD excretion and the estimated MNS of the herbivores were crucially influenced by different species and protein intake level.

Keywords: *excretion, ruminals, feed intake, digestion*

INTRODUCTION

Herbivores are able to convert low-quality forages into high quality protein products, howerver the use of high fiber and low protein roughages is constrained the animal performance due to high neutral detergent fiber with low digestibility. The protein from feed ingredients is an important factor influencing ruminal proteolysis and amount of ammonia, amino acid, peptides, and volatile fatty acids available for microbial growth and proliferation (Zadeh and Kor, 2013). Optimization of microbial protein synthesis should increase the efficiency of nitrogen utilization and reduce N urinary excretion (Singh et al., 2018) and then improve the feeds and feeding benefits. Zhang et al. (2020) also found that a higher degree of synchronization of energy and nitrogen release leads to active ammonia assimilation and higher microbial protein synthesis, and better animal performance. The purines from the rumen microbes are metabolized and excreted in the urine as their end products including allantoin, uric acid, xanthine and hypoxanthine, in which larger amounts of urine allantoin and uric acid are commonly used to evaluate the quantity of purine derivatives (Chen and Ørskov, 2004). Vo Thi Kim Thanh and Ørskov (2006) suggested that the excretion of purine derivatives in urine can be used to measure microbial protein supply in ruminants, since there is a relationship between microbial nucleic acids reaching the intestine from the rumen and excretion of purine derivative in the urine. Thus the herbivores such as buffaloes, cattle, goats, sheep and rabbits could efficiently utilize low quality diets with fibrous feeds to produce more protein for the hosts. However, the understandings of urinary excretion of purine derivatives, namely allantoin and uric acid in different herbivores have been still limited in the Mekong delta of Vietnam. Therefore the objective of the present study was to investigate potential differences of the urinary excretion on purine derivatives and estimated microbial nitrogen synthesis of the herbivore species for future research applications.

MATERIALS AND METHOD

Location anf time

The experiment was carried out at the experimental farm in Long Hoa village, Binh Thuy district, Can Tho City. The chemical analyses were done at the laboratory E205 of Department of Animal Science, College of Agriculture of Can Tho University. The experimental duration was 5 months from January to May, 2017.

Experimental animals (Mean ± SE), diets and feedings

The domestic herbivores in the growing stage used for investigation included 4 local buffaloes (284 ± 12.3 kg), 4 local cattle (146 ± 8.6 kg), 6 Bach Thao goats (21.1 ± 3.76 kg), 6 Phan Rang sheep (21.5 ± 4.27 kg) and 10 crossbred rabbits (1.88 ± 0.023 kg). The live weights of the animals were weighed at the begining of the experimental period.

They were individually kept in the cages as the digestibility trials for measuring the feeds, refusals, faeces and urine. Diets used for feeding the herbivores were dependent on the experimental farm conditions. The dietary ingredients for the buffalo was rice straw, natural grass, *sesbania grandiflora* and urea-molasses; for the cattle was rice straw mixed with molasses and minerals; for the goats was Para grass; for sheep was *panicum maximum* and concentrate and for rabbit was Para grass, sweet potatoes vines and concentrate. The dry matter and other nutrient intakes were showed in Table 2 based on metabolic weight ($kgW^{0.75}$) of the animals.

The animals were fed twice a day at 7:30 AM and 2:30 PM with the supplements being given prior to the forages offered, while fresh water was alway available within 24 h. The observations for each herbibore species was three weeks including two weeks for dietary adaptation and one week for sample collection.

Measurements taken

The measurements were:

Feeds and nutrient intakes. Feeds offered and refusals were individually and daily measured for analyses of dry matter (DM), organic matter (OM), crude protein (CP), and ash following the procedure of AOAC (1990), while neutral detergent fiber (NDF) was employed by the method of Van Soest et al. (1991).

Apparent nutrient digestibility. Apparent DM, OM, CP, and NDF digestibility were employed by measuring the nutrient intakes and feces, which were daily collected and weighed according to McDonald et al. (2010).

N retention was implemented by analyzing urinary nitrogen according Pathoummalangsy and Preston (2008) following the formula: $N_{Retention} = N_{Intake} - (N_{Feces} + N_{urine})$.

Allantoin was determined by Young and Conway (1942), uric acid was measured automatic meter (Roche Diagnostics). The purine derivative is determined by the total amount of allantoin and uric acid excreted in the urine estimated by Singh et al. (2007).

The microbial nitrogen synthesis (MNS) was estimated from urinary excretion of purine derivatives (PD).

In which:

PD = Allantoin + uric acid (Chen and Gomes, 1992)

Cattle: $PD = 0.85X + 0.147*W^{0.75}$ (Pimpa et al., 2001)

Buffalo: $PD = 0.12X + 0.2* W^{0.75}$ (Liang et al., 1999)

Goat, sheep, rabbit: $PD = 0.84X + (0.15W^{0.75} * e^{-0.25X})$ (Chen and Gomes, 1992)

MNS (g N/day) = 0.727 * X, (Chen and Gomes, 1992)

X: the purines of rumen microorganisms are absorbed.

Data analysis and report

The data were analyzed by using descriptive statistics with Mean ± standard error (SE) of Minitab Reference Manual Release 16.1 (Minitab, 2010). Then the results were primarily reported and explained by the characteristics of different herbirore species and diets offered.

RESULTS AND DISCUSSION

Chemical composition of feeds

The chemical composition of feeds used in the experiment was showed in Table 1.

Table 1. Chemical composition of feeds used in the experiment

Herbivore species	Feeds	DM, %	OM, %	CP, %	NDF, %
Rabbit	Para grass	15.6	88.6	11.2	69.3
	Sweet potatoes vines	10.1	89.6	16.4	41.4
	Concentrate	92.1	87.4	16.4	40.3
Sheep	*Panicum maximum*	16.0	88.5	13.4	74.0
	Concentrate	86.1	87.3	13.3	37.8
Goat	Para grass	17.1	88.2	9.00	68.6
Cattle	Rice straw with molasses and minerals mixture	64.0	83.3	11.3	41.2
Buffalo	Molasses	68.5	94.6	1.75	-
	Urea	-	-	288	-
	Natural grasses	21.4	90.7	8.98	70.1
	Sesbania grandiflora	21.9	91.5	26.2	24.8
	Rice straw	83.9	85.6	3.33	69.7

Table 1 showed that the DM and CP (%) of concentrate in the experiment were 86.1-92.1 and 13.3-16.4, respectively. They were similar to those findings of Nguyen Thi Vinh Chau and Nguyen Van Thu (2014) being 90.7% and 15.7%, respectively. The DM of sweet potatoes vines was 10.1% lower than Para grass (15.6-17.1%) but the CP content of Para grass was lower (9.0-11.2%), while this was 16.4% in sweet potatoes vines. Truong Thanh Trung et al. (2016) reported that DM and CP of sweet potato vines were 10.5 and 20.1%. The CP and NDF of rice straw in the present study were lower than the report of Nguyen Trong Ngu et al. (2019) being 4.60% and 74.2%. These were reported by Ho Thanh Tham (2018) were 5.20% and 68.9%, respectively. Feedstuffs used for feeding the herbivores in the experiment included forages and concentrate, *Sesbania grandiflora,* urea and molasses as supplements to improve the dietary nutrition except for the goats which were fed only Para grass.

Feeds and nutrient intakes

Feeds and nutrient intakes of the experimental animals were showed in Table 2.

Table 2. Metabolic live weight ($W^{0.57}$) and daily feed and nutrient intakes (Mean ± SE) of the berbivores in the experiment

Herbivore Species	Live weight ($KgW^{0.75}$)	DM (g/kg $W^{0.75}$)	OM (g/kg $W^{0.75}$)	NDF (g/kg $W^{0.75}$)	CP (g/kg $W^{0.75}$)
Rabbit	1.61 ± 0.02	105 ± 2.42	92.4 ± 4.31	48.2 ± 2.12	17.0 ± 0.821
Cattle	42.0 ± 1.67	88.0 ± 0.62	74.2 ± 0.52	36.5 ± 0.31	9.90 ± 0.923
Buffalo	69.3 ± 2.73	87.8 ± 1.65	85.5 ± 1.63	58.0 ± 1.11	6.80 ± 0.961
Sheep	9.97 ± 0.22	63.0 ± 2.74	55.7 ± 2.42	43.4 ± 1.92	8.80 ± 0.212
Goat	9.84 ± 0.12	51.2 ± 2.23	45.1 ± 1.94	34.3 ± 1.82	5.30 ± 0.194

Average DM, OM, NDF, and CP intake of rabbits were 105, 92.4, 48.2, and 17.0 g/kg $W^{0.75}$, respectively (Table 2). These results of CP intake in the present experiment was higher than that reported by Phan Thuan Hoang and Nguyen Van Thu (2010) being from 8.00 to 9.98 g/kg$W^{0.75}$ for the growing Californian rabbits. However, the CP intake (12.0 g/kgW0.75) was higher than that reported by Truong Thanh Trung and Nguyen Thi Kim Dong (2016) with crossbred rabbits fed diets (15-23%CP) on the rabbits with 6.88 to 9.19 g/kg $W^{0.75}$. The current study showed that supplementation of concentrate would result in a higher amount of CP as compared with other supplements. Although it is fact that naturally not all tree forages contain high CP content of all types of grasses because it depends on the type of leaves and grasses and their chemical composition (Rahman et al., 2015).

The DM intake (g/kg $W^{0.75}$) was 105, 88.0, 87.8, 63.0 and 51.2 for rabbits, cattle, buffaloes, sheep and goats, respectively. The value of goats was lower than values being 74.1-81.6 g/kg $W^{0.75}$ reported by Yulistiani et al. (2014), but it was not similar to findings of Carro et al. (2012) being 50.3-54.7 g/kg $W^{0.75}$. This could be explained by different feeds offered, the goats in the experiment were fed only Para grass. Feeding a supplement to overcome protein and mineral deficiency could increase the intake of low-quality forage (Yulistiani et al., 2014). In general, differences between sheep and goats were more pronounced for the medium-quality diet compared with those of high quality diet (Carro et al., 2012).

In cattle DM, NDF and CP intakes (g/kg $W^{0.75}$) were 88.0, 36.5 and 9.90, respectively. The DM intake of cattle was lower than the values of 92.2 reported by Nguyen Trong Ngu et al.

(2019) with beef cattle fed rice bran (1.5 kg/head/day), grass (1.0 kg DM/100 kg BW/day) and rice straw *ad libitumm*, while the feeds in the study were rice straw with molasses and minerals mixture. However, it was similar to the findings of Wanapat et al. (2013) being 87.0-90.7 g/kg $W^{0.75}$. The CP intake of cattle of the present study was in agreement with that presented by Paengkoum et al. (2013) (6.15-7.55 g/kg $W^{0.75}$). DM intakes between cattle and buffaloes in the experiment were similar, however according to the findings by Widyobroto and Budhi (2012) in cattle was lower than buffalo. The DM intakes of buffalo (g/kg$W^{0.75}$) was 87.8 and it was higher than that found by Pham Tan Nha et al. (2008) being 74.1 g/kg$W^{0.75}$. Finally, the result in Table 3 showed that the CP intake was orderly from high to low values being in rabbits, cattle, sheep, buffaloes, and goats.

Apparent nutrient digestibility and nitrogen retention

The apparent nutrient digestibility and nitrogen retetion of the herbivores was summarized in Table 3.

Table 3. Apparent nutrient digestibility (%) and nitrogen retention (N ret, g/$W^{0.75}$) of herbivore species in the study

Herbivore species	DM, %	OM, %	NDF, %	CP, %	N ret, g/$W^{0.75}$
Rabbit	73.0 ± 2.16	78.5 ± 3.63	44.4 ± 4.72	79.3 ± 0.870	0.93 ± 0.071
Sheep	65.1 ± 1.51	66.1 ± 1.64	68.5 ± 1.87	84.1 ± 1.47	0.82 ± 0.067
Goat	71.3 ± 2.52	71.6 ± 2.41	72.5 ± 2.57	77.0 ± 1.53	0.27 ± 0.039
Cattle	57.0 ± 1.87	60.9 ± 3.62	63.8 ± 2.18	58.2 ± 3.62	0.68 ± 0.027
Buffalo	60.0 ± 0.763	66.7 ± 0.792	62.1 ± 1.88	68.7 ± 1.42	0.43 ± 0.032

In Table 3 generally, digestibilities (%) of DM, OM and NDF in goats were higher than buffaloes, cattle, and sheep due to goats were feed only Para grass, while cattle and buffaloes were fed rice straw, and sheep were fed *Panicum maximum* grass. However in rabbits DM and OM digestibilities were higher than those above except the NDF digestibility, because they were fed the easily digestible weet potato vines and Para grass. CP digestibility of cattle was lower than that of the other species. Although Carro et al. (2012) concluded that digestibility of nutrients was similar in the goats and sheep species, this could only occur when they were fed the same feedstuffs in diets. The CP digestibility (%) of goats was 77.0 and it was close to the report of Nguyen Van Thu (2017) being 78.8-82.3%. The DM and NDF digestibilities (%) of sheep were 65.1 and 66.1 and they were similar to the values of 61.3-69.8% and 61.0-65.3% stated by Nguyen Thi Huyen et al. (2019).Yulistiani et al. (2014) indicated that an adequate and sustained provision of nitrogen to the rumen microbes is crucial for optimum plant cell digestion and high microbial protein synthesis.

The DM and OM digestibility (%) of cattle was lower than those of buffalo (Table 3). Vo Thi Kim Thanh (2014) reported that in cattle DM digestibility was from 36.9 to 81.9%, while in buffalo was from 50.8 to 82.6%. Similarly, Widyobroto and Budhi (2012) found that the DM digestibility of buffalo was better than cattle. Tran Van Thang et al. (2018) showed that the NDF digestibility from 49.9 to 68.1% and CP digestibility from 60.6 to 75.7%. The CP digestibility (%) of cattle was 58.2, while this was 68.7 in buffaloes. Similarly, Singh et al. (2018) indicated that buffaloes were better utilization of nutrients as compared to crossbred cattle and the digestibilities of main nutrients were significantly higher (P<0.05) for buffalo than cross bred cattle.

Nitrogen retention (g/kgW$^{0.75}$) of rabbits was the highest of 0.93 and this was 0.82, 0.68, 0.43 and 0.27 for sheep, cattle, buffaloes and goats, respectively. Similarly, Truong Thanh Trung and Nguyen Thi Kim Dong (2016) found that nitrogen retention of crossbred rabbits was from 0.66 to 0.91 g/kgW$^{0.75}$. While this was 0,27±0,04 g/kgW$^{0.75}$ for goats, which was much lower than the values of growing goats from 0.80 to 1.10 g/kgW$^{0.75}$ reported by Nguyen Thi Kim Dong and Nguyen Van Thu (2020). This could be explained that goats in the study was fed low protein diet of Para grass only.

In short, results of Table 3 indicated that the nutrient digestibilities of buffaloes were better than cattle. The goats were better utilization for the green forages, while the nitrogen retention of rabbits was higher than the ruminants.

Allantoin, uric acid, purine derivatives and microbial nitrogen synthesis

Daily purine derivatives excretion (mmol/head) and microbial nitrogen synthesis (g/head) in the experimental were showed in Table 4.

Table 4. Daily excretion of allantoin, uric acid and purine derivatives (PD) in urine and estimated microbial nitrogen synthesis (MNS) in ruminants

Herbivore species	Allantoin (mmol/head)	Uric acid (mmol/head)	PD (mmol/head)	MNS (g/head)
Rabbit	0.33 ± 0.013	0.10 ± 0.002	0.43 ± 0.013	0.18 ± 0.011
Sheep	1.27 ± 0.009	0.98 ± 0.006	2.25 ± 0.061	1.23 ± 0.023
Goat	0.77 ± 0.029	0.43 ± 0.012	1.21 ± 0.015	0.37 ± 0.005
Cattle	24.4 ± 1.45	5.16 ± 0.051	29.6 ± 1.46	20.1 ± 1.30
Buffalo	18.9 ± 1.65	7.64 ± 0.099	26.6 ± 1.71	77.1 ± 8.06

Daily purine derivatives (mmol/head) of sheep and goat were 2.25 and 1.21mmol/head, respectively. According to Dos Santos (2018) daily purine derivative excretion in sheep and goat were 2.10 and 1.55 mmol/day. In the study daily MNS (g/head) of goat (0.37) was lower than sheep (1.23) due to low protein content in diet, while in case of the adequate crude protein diets Nguyen Van Thu (2017) found that this was from 4.03-5.24 g/day with some numerical improvements in estimated MNS for the CP6.0, CP6.5 and CP7.0 (g/kgLW/day) treatments.

The daily allantoin (mmol/head) of cattle was 24.4 and higher than in buffalo, sheep, goat, and rabbit (18.9, 1.27, 0.77, and 0.33, respectively). In the present study, in buffaloes this was higher than the result stated by Vo Thi Kim Thanh and Ørskov (2006) being 7.55, however Khorshidi et al. (2012) found that it was from 15.8 to 21.8 following the intakes from 15% to 45% concentrate (DM) in diets by each buffalo and was 13.2 mmol/day in the forage diet only. The daily PD (mmol/head) was higher for the buffalo (26.6) compared to sheep, goat, and rabbit (2.25, 1.21 and 0.43, respectively), while in cattle this was the highest of 29.6. Vo Thi Kim Thanh and Ørskov (2006) reported that daily PD excretion (mmol/head) in buffaloes and cattle calves after 3 months of age were only 0.26 and 0.69. Increasing the concentrate levels in the diets of swamp buffaloes would increase the amount of microbial protein synthesized in the rumen (Khorshidi et al., 2012). The difference in purine derivative excretion occured only after rumen development (Vo Thi Kim Thanh and Ørskov, 2006), thus the important role of microbial nitrogen synthesis in rumen clearly indicates the urinary PD excretion. Our results in the present study confirmed the finding of Khorshidi et al. (2012) of daily MNS (g/head) concentration in buffalo fed concentrate as mentioned above was from 60.4 to 99.4. Nguyen Van Thu (2017) indicated that ruminants released energy and nitrogen likely influenced the microbial protein synthesis (MPS), but only in diets containing high concentrations of readily fermentable carbohydrate where bacterial storage of intracellular polysaccharides may be limiting. Dang Van Phuc (2021) and Nguyen Phuong Nam (2020) found that there were improvements of PD, MNS and growth performance of growing goats when increasing crude protein and soluble carbohydrate as energy source and in case of only soluble carbohydrate supplemented by ground maize in beef cattle diets also improved PD and MNS production (Lai Quoc Khanh, 2020). Similarly, a higher degree of synchronization of energy and nitrogen release leads to active ammonia assimilation and higher microbial protein synthesis (Zhang et al., 2020).

The excretion of allantoin, uric acid and purine derivatives in urine and estimated microbial nitrogen synthesis (MNS) of the animals based on metabolic live weight ($W^{0.75}$) were showed in Table 5.

Table 5. The daily excretion of allantoin, uric acid and purine derivatives (mmol/kg$W^{0.75}$) in urine and estimated MNS (g/kg$W^{0.75}$) of the animals

Herbivore species	**CP (g/kg$W^{0.75}$)**	**Allantoin (mmol/kg$W^{0,75}$)**	**Uric acid (mmol/kg$W^{0,75}$)**	**PD (mmol/kg$W^{0,75}$)**	**MNS, (g/kg$W^{0.75}$)**
Rabbit	17.0 ± 0.821	0.205 ± 0.082	0.062 ± 0.021	0.267 ± 0.121	0.112 ± 0.031
Sheep	8.80 ± 0.212	0.127 ± 0.093	0.098 ± 0.009	0.226 ± 0.002	0.123 ± 0.021
Goat	5.30 ± 0.194	0.078 ± 0.009	0.044 ± 0.087	0.123 ± 0.018	0.038 ± 0.009
Cattle	9.90 ± 0.923	0.581 ± 0.047	0.123 ± 0.092	0.705 ± 0.048	0.479 ± 0.038
Buffalo	6.80 ± 0.961	0.273 ± 0.094	0.110 ± 0.019	0.384 ± 0.019	1.11 ± 0.011

Results of daily excretion of allantoin, uric acid and purine derivatives (mmol/kg$W^{0.75}$) in urine and estimated MNS (g/kg$W^{0.75}$) of the animals was variable among the herbivore species. Although the CP intake (g/kg$W^{0.75}$) of rabbits was higher than cattle, sheep, buffalo and goat, the PD (mmol/kg$W^{0.75}$) were higher for the cattle (0.705) and buffaloes (0.384) compared to the others (Table 5). Khorshidi et al. (2012) reported that there was a high and significant correlation between DM intakes with excreted purine derivatives and produced microbial nitrogen protein in the buffalo rumen. However, in the present study the PD excretion of buffalo was lower than cattle, but the estimated microbial nitrogen synthesis (g/kg$W^{0.75}$) of buffalo was higher than cattle (1.11 vs 0.479). Vo Thi Kim Thanh and Ørskov (2006) showed that glomerular filtration rate may be lower in buffaloes than cattle, leaving more time in the blood, thus more time for recycling to the rumen and metabolized by bacteria or the permeability from the blood to the rumen is greater in buffaloes than cattle. In addition the MNS (g/kg$W^{0.75}$) were 0.123; 0.112 and 0.038 for sheep, rabbits and sheep in this experiment. In summary the results indicated that based on the metabolic live weight (kg$W^{0.75}$) there were variable in PD and MNS values among the herbivore species and the CP intakes, which could essentially impact on them. Especially in case of buffalo the urine PD excretion and MNS produced could be driven by the characteristics of its physiology and nutrient metabolisms.

CONCLUSION AND IMPLICATION

Based on the preliminary results of this investigation, it could be concluded that:

Urine PD excretion and the estimated MNS of the herbivores were crucially influenced by different species and protein intake level. Based on the metabolic live weight, buffaloes and cattle produced higher PD (mmol/kg$W^{0.75}$) and MNS (g/kg$W^{0.75}$) than the others.

Buffaloes produced higher MNS (g/kg$W^{0.75}$) than in cattle, due to physiological and nitrogen metabolism characteristics.

The synchrony of protein and soluble carbohydrate as energy source in diets and feeding practice should be considered for applied studies.

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