Plasma Allantoin Concentration and Urinary Purine Derivatives to Creatinine Ratio are not Sensitive Estimator of Microbial Protein Supply in Calves

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Urinary excretion of purine derivatives (PD), xanthine, hypoxanthine, uric acid and allantoin, by ruminants has been proposed as a good estimator of the intestinal flow of microbial protein synthesized in the rumen^{8,15}. A series of our studies have shown that urinary PD could apply to calves, except for calves just after early-weaning^{1,3}.

Recently, CHEN *et al.*⁷ suggested that both plasma concentration of allantoin and the urinary PD : creatinine ratio might be also suitable for use as an alternative to daily PD excretion to give an indication of microbial protein production in steers, although the sensitivity remained to be unclear. Because of a single-time measurement without collecting urine for all day, these parameters might be able to be useful under practical farm condition. However, PUCHALA and KULASEK¹³ reported that urinary allantoin : creatinine ratio had rather large variation within a day in sheep, suggesting the unreliability for estimating microbial

protein production. This study was conducted to evaluate the sensitivity of plasma allantoin concentration and urinary PD : creatinine ratio in calves.

Materials and Methods

Animals and feeds : Plasma allantoin concentration and urinary excretion of PD and creatinine were determined in two experiments using each 9 male Holstein calves (*ca.* 140 kg) in a 3×3 Latin square design. To maintain the functioning of esophageal groove closure for casein administration into the abomasum, the suspension was given by a nipple method under a constant auditory environment in addition to routine noises in succession to the suckling period of milk replacer.

Treatments compared in Exp. 1 were as follows ; (A) basal diet consisted of 10.0% rice straw, 88.2% flaked corn, 0.45% NaCl, 0.90% CaCO₃, and 0.45% trace mineral and vitamin mixture (CP : 7.9% ; TDN : 74%), (B) 1.44% urea

血漿アラントイン濃度および尿中プリン誘導体-クレアチニン比は仔牛の微生物態蛋白質供給量の鋭敏な指標ではない : 入来常德・舟場正幸・畑野元子・阿部又信 (麻布大学獣医学部, 相模原市, 229)

supplement in place of flaked corn of the basal diet, and (C) iso-nitrogenous casein administration into the abomasum instead of urea, in addition to (A). Treatments compared in Exp. 2 were as follows; (D) basal diet consisted of 10.0% rice straw, 79.2% flaked corn, 7.65% soybean meal, 0.90% NaCl, 1.80% CaCO₃, and 0.45% trace mineral and vitamin mixture (CP : 10.4% ; TDN : 73%), (E) 1.17% urea supplement in place of flaked corn of the basal diet, and (F) iso-nitrogenous casein administration into the abomasum instead of urea, in addition to (D). In both experiments, the calves were given diets daily at 2.7% of initial BW in each period, resulting in the CP intake 2.1 g/kg BW for (A), 3.2 g/kg BW for (B) and (C), 2.8 g/kg BW for (D), and 3.7 g/kg BW for (E) and (F). Calves were offered the diets and casein suspension at 08 : 30 and 16 : 30.

Trials and analyses : Urine was collected for last 4 d of 2 wk period into 300 ml of 20% (v/v) H₂SO₄, and blood was taken by jugular vein puncture on the last day of each period at 08 : 00, 10 : 30, 12 : 30, 14 : 30 and 16 : 30. Urinary allantoin was determined by the method of YOUNG and CONWAY¹⁶⁾. Plasma allantoin was also analyzed after deproteinization and defatted by the same procedure. Uric acid was determined using a commercial kit (Uric Acid C-Test, Wako, Tokyo). Because hypoxanthine and xanthine were not usually detected in urine of cattle⁴⁾, the sum of uric acid and allantoin was regarded as PD.

Data were analyzed by analysis of variance for Latin square design using the GLM procedure of SAS¹⁴⁾. When the effect of treatment was significant ($P < 0.05$), means among the treatment groups were compared by Duncan's multiple range test ($\alpha = 0.05$). Further, to examine the interaction of the changes in plasma allantoin concentrations within a day and treatments, factors considered were treatment, period, animal, sampling time, and interaction of treatment and sampling time.

Results and Discussion

Table 1 shows CP intake related to BW, N-intake, urinary excretion of PD and plasma allantoin concentration in calves different in daily N-intake. The intakes of CP from the basal diet were 2.1 and 2.8 g/kg BW, and the intakes of supplemental CP by urea or casein were 1.1 and 0.9 g/kg BW in Exp. 1 and 2, respectively, leading to the variable CP intake (2.1 to 3.7 g/kg BW). According to NRC¹²⁾, calves weighing 150 kg require 639 g dietary CP for 1.0 kg of daily gain (4.3 g CP/kg BW). Our previous report²⁾ also showed that urea supplement to the basal diet consisted of corn and soybean meal increased N-retention up to the intake of 3.3 g CP/kg BW in calves weighing 120–180 kg. At least calves fed only the basal diet (*i.e.*, (A) and (D) group in Exp. 1 and 2, respectively) are thought to be unable to meet N-requirement. Therefore, the increase in microbial protein production due to urea supplement (*i.e.*, (B) and (E) group) would improve N-balance.

In fact, urinary PD increased 1.38 and 1.41 times by urea supplement in calves, compared with those fed the basal diet and those administered casein, respectively, in Exp. 1. When calves were given the basal diet at 2.8 g CP/kg BW in Exp. 2, it also caused the increase in urinary PD, suggesting the reliability of urinary PD as an estimator of microbial protein supply. Further, urinary PD tended to be higher in calves ingested 2.8 g CP/kg BW than in those ingested 2.1 g CP/kg BW, although the comparison could not be evaluated statistically. MATSUOKA *et al.*¹⁰⁾ showed the positive relationship between DCP intake and urinary allantoin excretion in sheep fed the low to moderate CP diets. MATSUMOTO *et al.*⁹⁾ reported that urinary excretion of allantoin was increased not only by the elevation of dietary CP levels but also by the higher degradability in goats.

Urea supplement to the basal diet caused a

Table 1. Daily CP intake related to BW, N-intake, urinary excretion of purine derivatives (PD) and the ratio to creatinine (C), and plasma allantoin concentration in calves supplemented urea to the diet or administered casein into the abomasum

	Exp. 1				Exp. 2			
	A	B	C	SEM	D	E	F	SEM
Daily CP intake (g/kg BW)	2.1	3.2	3.2		2.8	3.7	3.7	
N-intake (g/d)	52.3 ^b	81.2 ^a	78.7 ^a	0.9	61.5 ^b	83.1 ^a	81.2 ^a	0.6
From basal diet	52.3	52.4	49.9	0.2	61.5	61.9	60.6	0.1
From urea		28.8		0.1		21.2		0.1
From casein			28.9	0.1			20.6	0.1
Urinary PD (mmol/d)								
Uric acid	1.6 ^a	1.8 ^a	1.3 ^b	0.1	1.7	2.0	1.8	0.1
Allantoin	38.3 ^b	53.3 ^a	37.4 ^b	1.2	52.3	57.8	52.1	1.7
PD	39.9 ^b	55.1 ^a	39.1 ^b	1.2	54.0 ^b	59.8 ^a	53.9 ^b	1.7
PD/C (%)	1.14 ^b	1.45 ^a	1.11 ^b	0.04	2.04 ^a	2.07 ^a	1.87 ^b	0.05
Plasma allantoin (μ mol/l)								
08:30	150 ^{b,*}	186 ^a	143 ^b	9	167	158	157	8
10:30	130	153	121	9	212	194	188	7
12:30	147	158	136	8	171	179	164	10
14:30	125 ^b	176 ^a	158 ^b	9	157	153	152	9
16:30	163 ^{a,b}	181 ^a	148 ^b	7	167	157	169	11
Daily mean	143 ^b	171 ^a	141 ^b	6	175	168	166	5

^{a,b} Means that have a common letter in the same row of the experiment did not differ.

* Treatment $\times$ sampling time interaction, $P < 0.08$.

higher ratio of urinary PD : creatinine in Exp. 1, similar to urinary PD. However, the ratio was comparable between (D) and (E) groups and the ratio of (D) was unexpectedly higher than that of (F) in Exp. 2, as well as all the ratios in Exp. 1. It might not be able to rely urinary PD : creatinine ratio as the estimator of microbial protein production, because protein-energy nutrition has been reported to affect not only endogenous allantoin excretion⁶⁾ but also urinary creatinine¹¹⁾.

The fluctuations of plasma allantoin concentrations with the lapse of time were relatively higher in both experiments (within *ca.* 20% of daily mean), although CHEN *et al.*⁷⁾ observed small changes within a day even in steers under the condition of once daily feeding. The reason of the inconsistent results was not clear. The daily mean of plasma allantoin

concentrations and the concentrations at 08:30, 14:30 and 16:30 were significantly higher in calves supplemented urea in Exp. 1, and the time-course of the concentrations also tended to be different among the treatment groups (treatment $\times$ sampling time interaction, $P < 0.08$). Also, calves fed the basal diets at 2.1 g CP/kg BW (*i.e.*, (A) and (C) groups) tended to show the lower concentrations than those fed the basal ration of 2.8 and 3.7 g CP/kg BW, although it could not be evaluated statistically. In Exp. 2, however, urea supplement to the basal diet hardly affected plasma allantoin concentrations, in spite of the increase in urinary PD. CHEN *et al.*⁵⁾ suggested that urinary excretion of allantoin could not be deduced from plasma allantoin concentration at any one particular time of the day because of the diurnal variation in the flow of microbial matters from

the rumen and into the small intestine.

Not only urinary PD:creatinine ratio but also plasma allantoin concentration was suggested to be not so sensible as urinary excretion of PD as an estimator of intestinal flow of microbial protein. Plasma allantoin as the estimator might be applicable only in case of quite large differences of microbial protein production among calves having a similar weight, because a linear decline of the plasma allantoin concentration related to age was shown in our study (FUNABA *et al.*, submitted). To clarify the factors affecting allantoin metabolism, plasma allantoin concentration and urinary PD:creatinine ratio will be needed to be examined in calves different in microbial protein production but similar protein 1-1 energy status.

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