Simultaneous Determination of Purine Derivatives and Creatinine in Ruminant Urine by HPLC

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Key words: ruminant, purine derivatives, creatinine, microbial protein

Introduction Urinary purine derivatives (PD; allantoin, uric acid, xanthine and hypoxanthine) excretion rate is widely used to predict rumen microbial protein production in ruminant livestock (Tas *et al*, 2007). This paper describes a method of simultaneous determination of PD and creatinine in the urine of ruminant by Reversed Phase-HPLC.

Materials and methods The HPLC system (Varian, Inc., USA) equipped with a ProStar 335 Diode-array detector set 220nm was used. The analytical column used Varian C18 reversed-phase (250mm×4.6mm I.D., 5μm) with guard column (Varian). The stock standard solutions of uric acid (100μM), xanthine (50μM) and hypoxanthine (50μM) were prepared by dissolving in alkalinized (pH>10) hot water, and allantoin (300μM) and creatinine (100μM) were prepared in water. The stock standard solutions and urine samples diluted 1:10 were adjusted pH 6 with 0.01M H_3PO_4 and 0.01M KOH and kept at -20℃. The mobile phase was 25mM KH_2PO_4 solution (pH 4.7). The flow rate was 1ml/min. The column was maintained at 25℃, 20μl injection was adopted.

Results and discussion Allantoin and creatinine are constituents of urine excretion of ruminants with similar polarity. It is very difficult to separate these compounds in biological fluids. Figure 1 show chromatograms obtained for standard solutions and urine samples. The retention time for allantoin, creatinine, uric acid, hypoxanthine and xanthine in standards solution and sheep urine were 2.7, 3.5, 4.7, 5.7, 6.4 min, respectively. A linear relationship between the peak area and the concentration of standards in water was showed in Table 1. The recovery was determined by triplicate analysis of urine samples spiked with standards, which was satisfactorily recovered (96-102%). The within-day and day-to-day coefficients of variations (2.8-4.3%) were calculated by processing aliquots of spiked urine, while Table1 summarize the detection limits (LOD) at S/N of 3 in urine.

Figure 1 *Chromatograms of PD and creatinine in standards and sheep urine.*
1. allantoin; 2. creatinine; 3. uric acid; 4. hypoxanthine; 5. xanthine

Table 1 *Linear regression lines standards of PD and Creatinine.*

Component	*Regression equation*	r^2	*LOD(μM)*
Allantoin	y=0.0132x+0.0248	0.9999	0.06
Uric acid	y=0.0683x-0.0178	0.9994	0.02
Xanthine	y=0.0816x-0.0013	0.9999	0.01
Hypoxanthine	y=0.0362x+0.0012	0.9999	0.01
Creatinine	y=0.0431x+0.0050	0.9999	0.01

Conclusions A robust and precise method has been developed for the simultaneous determination of purine derivatives and creatinine in ruminant urine. Application of this method has the potential to facilitate development and implementation of less invasive studies of ruminant nitrogen metabolism.

Reference

Tas, B.M., Susenbeth, A., 2007. Urine purine derivatives excretion as indicator of in vivo microbial N flow in cattle: A review. *Livestock science* 111, 181-192.