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Dietary supplementation of tannic acid modulates nitrogen excretion pattern and urinary nitrogenous constituents of beef cattle

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ABSTRACT

The objectives of the trial were to investigate the effects of dietary supplementation of tannic acid (TA) on nitrogen (N) excretion pattern and urinary nitrogenous constituents of beef cattle. Four adult Simmental male cattle (initial BW 376.0 ± 9.0 kg) were used as experimental animals. Four levels of TA, i.e. 0, 6.5, 13.0 and 26.0 g/kg dry matter (DM), were added to a basal ration consisted of concentrate mixture and corn silage in a 4 × 4 Latin square design as experimental treatments, respectively. Each experimental period consisted of a 12-day adaptation phase followed by a 3-day sampling phase. The results showed that supplementing TA did not affect the N retention, but shifted the N excretion from urine to faeces, regulated the urinary N components by decreasing urea and increasing hippuric acid.

1. Introduction

Urea in cattle urine accounts for 52.1–93.5% of total urinary nitrogen (N) (Dijkstra et al., 2013). It is the major loss of dietary N. Urea can be rapidly broken down to ammonia (NH_3) by microbial urease in urine patches and NH_3 can be transformed to potent greenhouse gas nitrous oxide (N_2O) (Ravishankara, 2009). The nitrogenous compounds in faeces are more stable than that in urine of cattle (Bussink and Oenema, 1998). Hippuric acid, which is a nitrogenous component in cattle urine, could inhibit N_2O formation in urine patches (Kool et al., 2006; Groenigen et al., 2006; Bertram et al., 2008). Therefore, reducing urinary urea excretion, shifting urinary N to faecal N and increasing urinary hippuric acid excretion of cattle would be beneficial to dietary N utilization and mitigating N_2O emission.

It was reported that the N loss in urine was decreased when sheep were fed 75 g chestnut hydrolysable tannin (HT)/kg dry matter (DM) (Deaville et al., 2010) and supplementing HT extracted from chestnut at 15.3 g/kg DM increased the faecal N excretion and decreased the urinary N excretion in sheep (Wischer et al., 2014). The results showed that the N excretion pattern in sheep could be modulated by supplementing HT. However, it is unclear if supplementing HT could affect the urinary outputs of urea and hippuric acid in sheep.

Tannic acid (TA) is a typical HT composed of 8 to 10 molecules of gallic acid per molecule of glucose. The objectives of the trial were to study the effects of dietary supplementation of commercial pure TA on N excretion pattern, focusing on the urinary excretions of urea and hippuric acid and verify if TA would be feasible in modulating urinary nitrogenous components of beef cattle.

2. Materials and methods

All experimental procedures were approved by The Animal Care and Use Committee of China Agricultural University.

2.1. Animals and treatments

Four Simmental male cattle (376.0 ± 9.0 kg initial BW) were used as experimental animals. Four levels of TA (purity 99.9%, Sinopharm Chemical Reagent Company, Shanghai, China), i.e. 0 (Control), 6.5, 13.0 or 26.0 g TA/kg DM, were supplemented to the basal ration of the cattle as experimental treatments in a 4 × 4 Latin square design. The basal ration was composed of corn silage 497.9 g, corn 184.6 g, corn gluten meal 213.7 g, soybean meal 68.0 g, wheat bran 19.4 g, sodium chloride 5.5 g and sodium bicarbonate 10.9 g per kg DM. The chemical composition (determined values) of the basal ration contained organic matter (OM) 916.8 g, crude protein (CP) 132.3 g, neutral detergent fiber (NDF) 514.7 g and acid detergent fiber (ADF) 238.1 g per kg DM.

The cattle were fed in stall with individual feed bunks. Each cattle was offered 5.4 kg DM of total mixed ration (TMR) daily,

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supplying major nutrients at the level of about 1.1 times of maintenance requirements (Feng, 2000). The ration was divided into two equal meals and provided to the cattle at 0700 h and 1700 h, respectively. TA was added to the ration and well mixed before feeding. The cattle had free access to drinking water.

2.2. Sampling

Each experimental period consisted of a 12-day adaptation phase and a 3-day consecutive sampling phase. The cattle were weighed in the morning of the first day of each experimental period. During each sampling phase, the faeces were completely collected daily using a plastic bucket placed behind each cattle. The faeces were weighed and 3% of the total was sampled. The urine was completely collected using a rubber funnel connected to a plastic tube and a plastic bucket and 2% of the total was sampled. The feed samples were also taken during the sampling period. On the second day of each sampling phase, 10 ml blood was taken through the jugular vein using evacuated K_2EDTA tubes (Greiner Bio-one, Frickenhausen, Germany) from each cattle 2 h after morning feeding. The blood was centrifuged at 2000 g at 4 °C for 15 min to obtain plasma for later analysis. All samples were kept in a refrigerator at −20 °C.

2.3. Measurements and chemical analysis

The urine pH was measured using a pH meter (pH-HJ90, Aerospace Computer Company, Beijing, China). The DM, ash and ether extract (EE) of feed and faecal samples were determined according to AOAC (1990) using the methods no. 934.01, 920.39 and 924.05, respectively. The ADF and NDF were analyzed on an Ankom A200i Fiber Analyzer (ANKOM Technology Co., New York, USA) using the methods of Van Soest et al. (1991). The N of feeds, faeces and urine samples was determined using the Kjeldahl method (AOAC, 1990). The CP content was calculated as N × 6.25.

The allantoin and uric acid in urine samples were analyzed on a spectrophotometer (UV-1801, Beijing *Beifen Ruili* Analytical instrument Co., Beijing, China) using the methods of Young and Conway (1942) and Fujihara et al. (1987), respectively. The urea and creatinine in urine samples were analyzed on an automatic biochemical analyzer (AU400, Olympus Co., Tokyo, Japan) using the glutamate dehydrogenase and the sarcosine oxidase methods, respectively. The plasma urea N (PUN) was analyzed on an automatic biochemical analyzer (7160, Hitachi Co., Tokyo, Japan) using a commercial colorimetric kit (BioSino Bio-technology and Science Co. Ltd., Beijing, China).

The hippuric acid was analyzed according to China Hygenic Standard (WS/T 52-1996) using the following procedures. One ml urine sample was diluted with 4 ml distilled water and added with 0.6 ml quinoline (purity ≥99.0%, Sinopharm Chemical Reagent Company, Shanghai, China) and 0.2 ml benzenesulfonyl chloride (purity ≥95.0%, Sinopharm Chemical Reagent Company, Shanghai, China). After completely mixed, the mixture was kept in dark for 30 min at 30 ± 2 °C and then added with 3.7 ml ethanol (purity ≥99.7%, Sinopharm Chemical Reagent Company, Shanghai, China) and kept for 30 min at 30 ± 2 °C. The absorbance of the mixture was measured at room temperature at wavelength of 470 nm on an UV-1801 spectrophotometer (Beijing *Beifen Ruili* Analytical Instrument Co., Beijing, China).

2.4. Calculations and statistical analysis

The rumen microbial N supply to the cattle was predicted according to the equations of Chen and Gomes (1992):

$$Y = 0.85X + \left(0.385BW^{0.75}\right)$$

where X refers to the total absorbed purine derivatives (PD), mmol/d; Y, the total urinary PD, mmol/d; $BW^{0.75}$, the metabolic body weight (BW) of cattle, kg; 0.85, the slope representing the recovery of absorbed purines as urinary PD; 0.385, the endogenous PD excretion of cattle, mmol/kg $BW^{0.75}$/d.

$$\text{Rumen microbial N (g/d)} = X\ \text{(mmol/d)} \times 70/0.116 \times 0.83 \times 1000 = 0.727X.$$

where, 70 refers to the N content of purines, mg N/mmol; 0.83, the digestibility of microbial purines; 0.116, the ratio of purine N/total N in mixed rumen microbes.

The N retention and the N retention rate were calculated using the equations:

$$\text{N retention}(g/d) = \text{Feed N}\,(g/d) – \text{Faecal N}(g/d) – \text{Urinary N}\,(g/d)$$

$$\text{N retention rate}(\%) = N\ \text{retention}(g/d)/\text{Feed N}(g/d) \times 100$$

The data were analyzed using the general linear model procedure of SAS 8.1 (Inst. Inc., Cary, NC, USA, 2001)using the model: $Y_{ijk} = \mu + T_i + P_j + C_k + \varepsilon_{ijk}$, where Y_{ijk} refers to the observation from cattle; μ, overall mean; T_i, diet treatment (i=1, 2, 3 and 4); P_j, period (j=1, 2, 3 and 4); C_k, cattle (k=1, 2, 3 and 4); ε_{ijk}, residual error. Differences between the means of treatments were determined by Dunnett's test. Linear and quadratic effects due to TA addition were determined using polynomial contrasts. Differences between treatments were considered to be significant at $P \leq 0.05$ and as a trend toward significance at $0.05 < P < 0.10$.

3. Results

3.1. N excretion

The results in Table 1 showed that supplementing TA increased the faecal N excretion, the ratio of faecal N/N intake ($P < 0.001$) and tended to decrease the ratio of urinary N/N intake ($P = 0.054$) at 6.5, 13.0 or 26.0 g/kg DM, increased the ratio of faecal N/total N excretion ($P < 0.001$) and decreased the ratio of urinary N/total N excretion ($P < 0.001$) at 13.0 and 26.0 g/kg DM and decreased the urinary N excretion ($P < 0.001$) at 26.0 g/kg DM. Significant linear responses were also found in these parameters ($P < 0.05$). The results also showed that supplementing TA did not affect the total N excretion, the N retention and the N retention rate at 6.5, 13.0 or 26.0 g/kg DM ($P > 0.05$), but decreased the PUN concentration ($P < 0.05$) at 13.0 or 26.0 g/kg DM and the effects tended to be significant in a linear manner ($P = 0.057$).

3.2. Urinary pH, urinary N constituents and rumen microbial N flow

The results in Table 2 showed that the urinary pH of all treatments were within 7.80–8.48. Supplementing TA up to 26.0 g/kg DM did not significantly affect the urinary pH ($P > 0.05$) but tended to decrease the urinary pH in a linear manner ($P = 0.080$). Supplementing TA at 6.5, 13.0 or 26.0 g/kg DM decreased the urinary urea excretion ($P < 0.001$). The effects were significant in both linear ($P < 0.003$) and quadratic manners ($P < 0.05$). Supplementing TA a 13.0 or 26.0 g/kg DM decreased the ratio of urea N/total urinary N ($P < 0.05$) and the effects tended to be significant both linearly ($P = 0.083$) and quadraticly ($P = 0.077$). Supplementing TA increased the urinary hippuric acid excretion ($P < 0.01$) at 6.5, 13.0 or 26.0 g/kg DM and increased the ratio of hippuric acid N/total urinary N at 26.0 g/kg DM ($P < 0.05$). Supplementing TA at 26.0 g/kg DM increased the ratio of creatinine N/urinary N ($P < 0.05$) but did not affect the urinary creatinine excretion ($P > 0.05$).