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Paper

Effect of Rare Earth Elements on Feed Digestibility, Rumen Fermentation, and Purine Derivatives in Sheep

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Abstract

The experiment was conducted to evaluate the effect of rare earth elements (REE) on feed digestibility, rumen fermentation, and urinary purine derivatives (PDs) in sheep. Eight sheep (44.58±2.9 kg of body weight) fitted with ruminal cannulas were used in a replicated 4×4 Latin square design 20-day experiment. Sheep were fed a basal diet

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nd 300 mg REE-citrate per kg dry matter (DM). Mixture of REE rium (56.8%), lanthanum (35.0%) and praseodymium (6.5%).

decreased, whereas total volatile fatty acids concentration was linearly increased with increasing REE supplementation (P<0.05). The ratio of acetate to propionate was linearly (P<0.01) and quadratically (P<0.01) decreased due to increase of propionate concentration (P<0.05). *In situ* ruminal neutral detergent fibre (aNDF) degradation of *Leymus chinensis* was improved (P<0.01), but the *in situ* ruminal crude protein (CP) degradation of soybean meal was decreased by feeding REE (P<0.01). Moreover, digestibility of DM, organic matter, aNDF, acid detergent fibre and CP in the total tract and urinary excretion of PD were also linearly (P<0.01) and quadratically (P<0.01) increased with increasing REE addition. In conclusion, supplementation of the basal diet with REE improved rumen fermentation and feed digestion in sheep. It was suggested that REE stimulated rumen microbial activity, digestive microorganisms or enzyme activity in a dose-dependent manner. The optimum supplemental dose of REE was about 200 mg/kg dietary DM in sheep.

Key Words: Rare earth elements, Sheep, Feed digestibility, Rumen fermentation, Urinary purine derivatives

Introduction

Rare earth elements (REE) including lanthanum (La), cerium (Ce) and other lanthanides are a group of elements which have similar physical and chemical properties. China has the largest reserve of REE in the world. Therefore, China is the major supplier of REE for the world market. Nowadays, REE are widely applied to metallurgy, chemical industry, electronics, medicine, and agriculture (Rambeck and Wehr, 2005; Richter, 1996).

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REE have been successfully used as new natural feed additives in many years. Numerous studies indicated that proper

al., 1991; He *et al.*, 2001, 2010; Wang and Xu, 2003). For monogastric animals, many reports concerning performance enhancing effects of REE existed. He and Xia (1998) found that REE increased the BW gain and the feed conversion by 4 to 23% in weaned piglets with a BW of about 7 kg. The average daily gain and feed conversion ratio were increased by 12.95 and 6.78% respectively with the supplementation of 100 mg/kg dry matter (DM) La in growing pigs (Wang and Xu, 2003). In broilers, it was found that the supplementation of low doses of REE-citrates (70 mg/kg DM) can improve growth performance (He *et al.*, 2009). For ruminants, He *et al.* (1994) reported that daily gain of beef cattle improved by 7.3 and 8.2% with addition of 300 and 500 mg REE/kg of DM in diet, respectively. Liu *et al.* (2008) found that supplementation of diet with 900 mg $LaCl_3$ per steer per day significantly improved rumen fermentation and feed digestion. In addition, studies considering the effect of dietary REE supplementation on residues in tissues of animals were also conducted. He *et al.* (2001) and Rambeck *et al.* (2004) reported that muscle, liver and kidney show a little accumulation of REE contents during feeding trials performed on pigs with addition of REE in diet.

Thus, REE may well be of interest in animal production as a new, safe and inexpensive alternative feed additive. Prior studies indicated that not only do REE improve digestibility and utilisation of nutrients (Lu and Yang, 1996; Xu *et al.*, 1998), but it also improves rumen fermentation (Liu *et al.*, 2008; Yang *et al.*, 2009). However, data considering the effect of dietary REE supplementation on rumen fermentation as well as nutrient digestibility in sheep is still rare. The objectives of this work were to investigate the effects of REE on feed digestibility, rumen fermentation, and urinary excretion of purine derivatives (PDs) in sheep.

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Eight ruminal cannulated Dorset×Small Tail Han×Tan sheep with BW of 44.5±12.9 kg were used in a replicated 4×4 Latin square design. The four treatments were: control, REE-low, REE-medium and REE-high with 0, 100, 200 and 300 mg REE-citrate per kg of diet DM, respectively. Rare earth elements were purchased commercially and mainly contained cerium (56.8%), lanthanum (35.0%) and praseodymium (6.5%). The supplement of REE was added to the concentrate portion when it was pelleted in the feed mill. The experiment lasted 20 days with 10 days for adaptation and 10 days for sampling. Diets contained 300 g/kg DM of concentrates and 700 g/kg DM of forage to meet maintenance nutrition requirements for sheep, and there were not feed residuals throughout the experiment. The chemical composition of basal diet is shown in Table 1. Sheep were confined individually in metabolism cages and were fed as two equal meals at 07:00 and a.m. and p.m., and had *ad libitum* access to water throughout the experimental period. Diets were sampled once weekly and composed by period, and then stored in plastic bags for chemical analysis.

Apparent digestibility in the total tract

Each sheep was dosed with 1 g of chromic oxide in a paper capsule in two equal proportions at 07:00 a.m. and 07:00 p.m. on days 6-15 of each period for use as an indigestible marker (Harris *et al.*, 1967). Initial five days were used for uniform chromic oxide excretion and the last five days were used for collection of faeces. Faecal pellets were collected from the polyester cloth bags fastened over the anus of the each sheep two times daily (08:00 a.m. and p.m.), and the representative samples (10% of daily collected faecal output) were pooled for the 5 d collection periods. After being dried at 60°C, the samples were ground to pass a 1-mm screen for chemical analysis. Dry matter excreted in faeces was evaluated by dividing chromium input by chromium concentration in the faeces. Excretion of other nutrients in the faeces was evaluated by multiplying DM flow by their concentration in faecal DM.

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cannula of each sheep at 07:00 and 10:00 a.m., 01:00 and 04:00 p.m. It was filtered through four layers of cheesecloth and pH was determined immediately by using an electric pH meter (Sartorius Basic pH Meter PB-20; Sartorius AG, Göttingen, Germany). An aliquot (5 mL) was mixed with meta-phosphoric acid (1 mL, 250 g/L) for determination of volatile fatty acids (VFA), and filtrate was mixed with 20 g/L (w/v) H_2SO_4 at 5:1 ratio for determination of NH_3. The samples were stored at -20°C for further analyses.

In situ ruminal degradability

Ruminal degradation kinetics of soybean meal and *Leymus chinensis* was measured using nylon bag technique on day 11 to 13 of each period. Bags (6×10 cm) were made of monofilament Pecap polyester (Guangzhou Minyuan Business Co., Ltd., Guangdong, China) with a mean pore size of 47±2 μm and heat-sealed. The samples were milled (2.5 mm), and bags containing 3.5 g of *Leymus chinensis* and 4.5 g of soybean meal were suspended in the rumen of each sheep 2 h after feeding. Samples were incubated separately in duplicate bags. The bags were removed after 0, 6, 12, 24, 48 and 72 h of incubation, and rinsed in a mini-washing machine for 20 min. All bags were subsequently dried for 12 h at 65°C, and then at 105°C for 24 in order to calculate DM disappearance.

Ruminal nutrient degradability was determined using the model of McDonald (1981):

$y=a+b(1-e^{-c(t-L)})$, for $t>L$

where a is the soluble fraction; b is the slowly degradable fraction; c is the fractional degradation rate constant at which b is degraded; L is the lag time (h); and t is the time

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non-linear parameters a, b and c were estimated using the non-
edure of SAS (1996).

Orskov and McDonald (1979).

$$ED=a+[(b\times c)/(c+k)]$$

where k was the particulate passage rate out of the rumen and it was 0.02/h for *Leymus chinensis* and 0.052/h for soybean meal according to our measurements.

Urine collection and purine derivative measurements

During the 11 to 20 days of each period, urine was collected daily into containers with 100 mL/L sulfuric acid sufficient to maintain the pH below 3. One percent of daily urine output was retained and composed over the 10-day collection period per sheep. At the end of each experimental period, 20 mL of urine samples was diluted in 100 mL with distilled water, and frozen at -20°C until analysis. Total urinary PD excreted (mmol/day) were estimated as the sum of uric acid, allantoin, xanthine and hypoxanthine. Excretion of endogenous PD was represented as $0.150W^{0.75}\ e^{-0.25X}$ for each sheep (Chen and Gomes, 1992).

Chemical analyses

The oven-dried samples were ground through a 1-mm sieve in preparation for DM (ID No. 934.01; AOAC, 1990), organic matter (OM) (ID No. 942.05), and crude protein (CP) (ID No. 984.13; AOAC, 1990) analysis. The neutral (aNDF) and acid detergent fibre (ADF) (both inclusive of residual ash) of dried samples were determined as described by Van Soest *et al.* (1991). Heat-stable α-amylase and sodium sulfite were used in the analysis of aNDF. Ruminal VFA concentration was determined by using gas

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nonia N content of ruminal samples was determined as
d Gao (1993) by using spectrophotometry. Allantoin and uric

Statistical analyses

The data were analysed by using the PROC MIXED procedure of SAS (1996) to account for effects of square, period within square, animal within square and treatment. For the analysis, the Latin square design had treatment as fixed effects, and square, period within square, and animal within square as random effects. Rumen fermentation data were summarised by sampling time and analysed using the same mixed model with a repeated measures statement. Linear and quadratic orthogonal contrasts were tested to determine the influence of increased dietary REE levels on the response variables. Significance was accepted at $P<0.05$ unless otherwise indicated.

Results

Ruminal pH and fermentation

As shown in Table 2, mean ruminal pH value was linearly ($P<0.01$) and quadratically ($P<0.01$) decreased with increasing REE supplementation. Similarly, ruminal ammonia N content was quadratically ($P<0.05$) decreased with increasing REE supplementation. No difference ($P>0.05$) was observed in the acetate concentration in rumen fluid, whereas that of propionate was linearly ($P<0.02$) and quadratically ($P<0.04$) increased with increase in dietary REE. As a result, ratio of acetate to propionate was linearly ($P<0.01$) and quadratically ($P<0.01$) decreased. Molar proportion of butyrate was not affected ($P>0.05$), but total VFA was linearly ($P<0.05$) increased as increasing REE supplementation.

ıe total tract

(P<0.01) increased with increasing REE supplementation.

Effective ruminal degradability

In situ ruminal digestion kinetics and ED of *Leymus chinensis* and soybean meal are shown in Table 4. For *Leymus chinensis*, the soluble fraction, slowly degradable fraction and the ED of DM and aNDF were linearly (P<0.01) and quadratically (P<0.01) increased, whereas fractional degradation rate of *Leymus chinensis* DM and aNDF was linearly (P<0.02) and quadratically (P<0.01) decreased with increasing REE supplementation.

For the soybean meal, the soluble fraction and fractional degradation rate of DM were linearly (P<0.01) and quadratically (P<0.02) decreased, whereas a linear (P<0.01) and quadratic (P<0.01) increase in slowly degradable fraction was observed with increasing REE supplementation. The ED of DM was linearly (P<0.01) decreased with increasing REE addition. Regarding the digestion kinetics of CP, with increasing REE supplementation, the soluble fraction was linearly (P<0.03) and quadratically (P<0.01) increased, but slowly degradable fraction and ED of CP were linearly (P<0.01) and quadratically (P<0.01) decreased. The fractional degradation rate was linearly (P<0.01) decreased as the level of supplemental REE increased.

Urinary purine derivatives

Urinary PDs are shown in Table 5. No difference was observed in xanthine and hypoxanthine (P>0.05). However, daily urinary excretion of uric acid was quadratically (P<0.03) increased. Urinary excretion of allantoin and total PD were increased linearly (P<0.01) and quadratically (P<0.01) with increasing REE supplementation.

Ruminal pH, as an important indicator, can reflect the rumen microbial ecosystem. Low ruminal pH appeared to have negative effect on attachment of bacteria to plant cell walls and fibre digestion (Cheng *et al.*, 1984). In our study, ruminal pH was decreased with the addition of REE, average rumen pH ranged from 6.53 to 6.72, and these values were in the optimum range for cellulolytic bacteria activity (Russell and Wilson, 1996). The quadratic decrease of ammonia N concentration was similar to the findings of Liu *et al.* (2008) who reported a decrease of ammonia N content by La supplementation. Prior studies suggested that REE promote animal growth by influencing the development of bacterial species in the gastrointestinal tract, yet these effects seem to be dose-dependent (Muroma, 1958; Rambeck and Wehr, 2005). Our study also confirmed that high dose of REE addition (300 mg/kg DM) has a negative effect on the development of several rumen bacteria, and thus decreases the ammonia N utilisation.

Rumen fermentation pattern was switched from acetate to propionate as shown by the reduction in the ratio of acetate to propionate with increasing REE supplementation in diets of sheep (Table 2). Furthermore, the reduction in ration of acetate to propionate and increase of total VFA concentration were mainly resulted from the increase in concentration of propionate. The increase in the total ruminal VFA concentration was consistent with the response of ruminal pH to REE supplementation. This results were similar with findings of Liu *et al.* (2008) who observed a linear and quadratic increase of total VFA and propionate with increasing La supplementation from 0, 450, 900 to 1800 mg per head per day in Simmental steers. In contrast, Yang *et al.* (2009) observed no effect of REE supplementation (adding 400 g/kg and 800 mg/kg DM REE in diluted rumen fluid) on ruminal VFA concentration in continuous culture trial. Prior studies have confirmed that REE affected the growth of ruminal bacteria dose-dependently (Ruming *et al.*, 2002). Muroma (1958) reported that high concentration (10^{-4} to 10^{-2} mol/L) leads to

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l growth, while at concentration of 10^{-5} mol/L (*i.e.* ~1.4 mg/L),

ance bacterial growth. It is suggested that changes of ruminal

support bacterial growth. The quadratic response to REE supplementation showed that 300 mg/kg DM was not beneficial to further improving feed fermentation in the rumen (Table 2).

Apparent digestibility in the total tract and *in situ* ruminal degradability

The linear increase of *in situ* ruminal ED of *Leymus chinensis* DM and aNDF was in line with linear increase of ruminal total VFA (Table 2) and nutrients digestibility in total tract (Table 3) with increasing REE supplementation. Also, Liu *et al*. (2008) found a linear (P<0.01) and quadratic (P<0.01) increase digestibility of aNDF by supplementation with 450, 900 and 1800 mg $LaCl_3$ per steer per day. Yang *et al*. (2009) observed that a linear increased ruminal true digestibility of NDF when REE supplementation was increased from 0, 400 g/kg and 800 mg/kg DM using dualflow continuous culture fermentors. The quadratic response to REE addition indicated that 300 mg/kg DM did not increase nutrient digestibility in the total tract and *in situ* ruminal ED of *Leymus chinensis* aNDF. This observation was in accordance with Schwabe *et al*. (2011), who observed no significant influence on digestibility of nutrients by addition of 300 mg/kg DM REE.

Furthermore, the digestibility of CP was also improved with REE supplementation. However, it was not supported by an increase of *in situ* ruminal CP degradability of soybean meal (Table 4). Digestibility of CP was significantly improved by REE supplemented in pigs (Ming *et al*., 1995; Hu *et al*., 1999). Liu *et al*. (2008) found that La supplementation improved the digestibility of CP linearly (P<0.01) and quadratically in steers (P<0.01). Several studies from the Chinese and European scientific community suggested that REE improved growth and feed effiency of pigs (Halle *et al*., 2003), beef cattle (Shen *et al*., 1991), and rats (He *et al*., 2003). Liu

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ted that performance enhancing effects of REE may be ascribed
in the digestive tract.

on the study in monogastric animal, the potential modes of action by REE in the digestive tract have been researched. Ou *et al.* (2000) suggested that REE may promote the secretion of digestive fluids in animal stomachs. Besides, Prause *et al.* (2005) reported that REE may influence the permeability of intestines or enhance the activities of certain enzymes involved in the digestive tract, and thus enhance digestibility. Similarly, Rambeck and Wehr (2005) indicated that REE may possess certain antibacterial properties and promote animal growth by selectively influencing the development of bacterial species in the gastrointestinal tract. Moreover, Zhang *et al.* (2000) and Liu *et al.* (2004) demonstrated that REE was able to inhibit the growth of several bacteria dose-dependently. In the present study, high dose of REE (300 mg/kg) supplementation led to no further improvement of total digestibility, and *in situ* ruminal degradation confirmed that REE modulate the digestive microorganisms or enzymes in a dos-edependent manner.

Table 1. The ingredients and chemical composition of the basal diet.

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Table 2. Effects of rare earth elements supplementation on ruminal pH and fermentation in sheep.

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Table 3. Effects of rare earth elements supplementation on nutrient digestibility in the total tract of sheep.

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CSV Display Table

Table 5. Effects of rare earth elements supplementation on urinary purine derivatives in sheep.

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Urinary purine derivatives

Total urinary excretions of PD were used to estimate rumen microbial protein synthesis in ruminants (Chen and Gomes, 1992). In the present study, the increased urinary excretion of PD suggested that increase of REE supplementation would increase the microbial protein production in the rumen. The improvement in nutrient digestibility (Table 3) and *in situ* ruminal degradation of *Leymus chinensis* (Table 4), and reduction of ruminal ammonia N concentration (Table 2) also supported an enhanced ruminal microbial protein synthesis by REE supplementation. Cellulolytic bacteria obtain their N exclusively from ammonia N (Russell *et al.*, 1992). It was suggested that REE addition may enhance activity of ruminal fibrolytic bacteria because *in situ* ruminal ED of *Leymus chinensis* aNDF was linearly and quadratically increased (Table 4). However, proteolytic activity in the rumen was likely reduced by REE supplementation in the present study, while protease activity post-ruminally was increased (Table 3) since it showed a linear decrease of ruminal ED of soybean meal.

Conclusions

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tation of REE in sheep altered rumen fermentation pattern from *In situ* ruminal aNDF degradation of *Levmus chinensis* was

supplementation. Based on nutrients digestibility and rumen fermentation, the optimum dose of REE was about 200 mg/kg dietary DM in sheep, while 300 mg/kg DM was not beneficial to improve the feed utilisation under the present experimental conditions.

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