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Effects of rumen-protected pantothenate on ruminal fermentation, microbial enzyme activity, cellulolytic bacteria and urinary excretion of purine derivatives in growing beef steers

H.Q. Li[a], Q. Liu[a,*], C. Wang[a], G. Guo[a], W.J. Huo[a], S.L. Zhang[a], Y.L. Zhang[a], C.X. Pei[a], W.Z. Yang[a,b], H. Wang[c]

[a] College of Animal Sciences and Veterinary Medicines, Shanxi Agricultural University, Taigu, Shanxi 030801, PR China
[b] Agriculture and Agri-Food Canada, Research Centre, P. O. Box 3000, Lethbridge, AB, Canada
[c] Animal Husbandry and Veterinary Bureau of Yuci County, Yuci 030600, Shanxi Province, PR China

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ABSTRACT

This experiment was to evaluate the effects of rumen-protected pantothenate (RPP) on ruminal fermentation, microbial enzyme activity, bacteria population and urinary excretion of purine derivatives in growing beef steers. Eight ruminally cannulated first-generation crossbred (Blonde d'Aquitaine × Simmental) beef steers, averaging 12 months of age and 363 ± 7 kg of body weight (BW), were allocated into a replicated 4 × 4 Latin square design. Four treatments were control, low-RPP (LRPP), medium-RPP (MRPP) and high-RPP (HRPP) with 0, 0.32, 0.48 and 0.64 g RPP per kg dietary DM, respectively. Steers were fed a total mixed ration and dietary concentrate to corn silage ratio was 50:50 based on a dry matter (DM) basis. The experiment included four periods and lasted for 96 days, each period contained 14 days of adaptation and 10 days of data collection. Ruminal pH decreased linearly with increasing RPP supplementation and was lower for MRPP than for control. Ruminal total VFA concentration increased linearly with increasing RPP supplementation and was higher for MRPP than for control. The acetate to propionate ratio increased linearly due to the unchanged acetate molar proportion and the tendency towards decreased propionate proportion. Ruminal DM and neutral detergent fibre degradability of corn silage increased quadratically, whereas DM and crude protein degradability of concentrate mix increased linearly with increasing RPP supplementation. Activities of carboxymethyl-cellulase, cellobiase, xylanase and α-amylase increased linearly and was higher for MRPP than for control. Populations of *R. albus, R. flavefaciens, F. succinogenes, B. fibrisolvens, P. ruminicola* and *R. amylophilus* increased linearly and quadratically with increasing RPP supplementation. Urinary excretion of purine derivatives increased linearly with increasing RPP supplementation and was higher for HRPP and MRPP than for LRPP and control. The results indicated that dietary supplementary RPP improved ruminal fermentation, *in situ* ruminal degradation and urinary excretion of purine derivatives by stimulating bacteria growth and microbial enzymes secretion. It is suggested that supplementary RPP regulated bacteria growth and microbial enzymes secretion in a dose-dependent manner. Under the current experimental condition, the appropriate dose of RPP was at 0.48 g per dietary DM for growing crossbred beef steer.

1. Introduction

Either pantothenic acid (PA) or pantothenate plays an important role in intermediary metabolism by converting to coenzyme A (CoA) and acyl-carrier protein (ACP) through a series of enzymatic actions (Rock et al., 2000; Ball, 2006; Ragaller et al., 2011a). The CoA participates in the oxidation of fatty acid, carbohydrates, pyruvate, lactate, ketone bodies, and amino acids, as well as many synthetic reaction (Ball, 2006; Ragaller et al., 2011a). The ACP is required for the biosynthesis of long chain fatty acids (Ball, 2006; Ragaller et al., 2011a). Considering its important role of PA or pantothenate involved in intermediary metabolism, the effects of dietary supplementary PA

Abbreviations: ADF, acid detergent fibre; BW, body weight; CP, crude protein; DM, dry matter; ED, effective degradability; HRPP, high rumen-protected pantothenate; LRPP, low rumen-protected pantothenate; MRPP, medium rumen-protected pantothenate; aNDF, neutral detergent fibre; OM, organic matter; PD, purine derivative; TMR, total mixed rations; VFA, volatile fatty acid

* Corresponding author.
E-mail address: liuqiangabc@163.com (Q. Liu).

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has been evaluated extensively in non-ruminant animal (Grinstead et al., 1998; Groesbeck et al., 2007; Leeson et al., 2005). For a long time, it is believed that ruminal synthesized PA can meet the requirement of ruminants (Bechdel et al., 1928). According to NRC (2001), the requirement of a dairy cow for PA was estimated by tissue requirement (304 mg/d) and lactation requirement (121 mg/d), but ruminal net synthesized PA is about 38 mg/d and does not meet the requirement of high producing dairy cows (NRC, 2001). Furthermore, most of dietary supplementary PA is degraded by ruminal microorganisms (Zinn et al., 1987; Bonomi, 2000; Ragaller et al., 2011b). Then supplementation of rumen-protected pantothenate (RPP) is needed. Literature have demonstrated that supplementary PA improved rumen fermentation (Ragaller et al., 2011b; Völker et al., 2011), ruminal acid detergent fibre (ADF) degradability (Ragaller et al., 2011b), milk fat percentage and yield (Bonomi, 2000). However, other literature reported PA had no influences on milk yield, milk composition and component yield (Ferreira et al., 2015), ruminal microbial protein synthesis (Ragaller et al., 2011b; Völker et al., 2011) and serum glucose (Ragaller et al., 2011b). The difference in milk yield and milk composition might be due to the different dietary composition and the administration mode and dose of PA.

Considering the limited research as well as these inconsistent results about supplementary PA on ruminal fermentation and ruminal bacteria population, we hypothesized that as high producing dairy cows, ruminal synthesis of PA couldn't meet the requirements of growing steers. Hence, this experiment was undertaken to evaluate the effects of supplementary RPP on ruminal fermentation, microbial enzyme activity, bacteria population and urinary purine derivatives excretion in growing beef steers.

2. Materials and methods

2.1. Experimental design and animals

The experimental scheme was approved by Animal Protection and Welfare Committee of Shanxi Agriculture University (Taigu County, Shanxi Province). Eight ruminally cannulated first-generation crossbred (Blonde d'Aquitaine × Simmental) beef steers, averaging 12 months of age and 364 ± 7 kg of body weight (BW), were allocated into a replicated 4 × 4 Latin square design. Steers were equipped with rubber cannulas (inner diameter 7.5 cm; #4 C 3″, Bar Diamond, Inc. Parma Idaho, USA) in the dorsal sac of the rumen. Four treatments were control, low-RPP (LRPP), medium-RPP (MRPP) and high-RPP (HRPP) with 0, 0.32, 0.48 and 0.64 g RPP per kg dietary DM, respectively. Steers were fed a total mixed ration and dietary concentrate to corn silage ratio was 50:50 based on a dry matter (DM) basis (Table 1). The supplement of RPP containing 150 g D-calcium pantothenate per kg was manually mixed into the first one half of the morning ration. The supplement was produced according to the method of Wang et al. (2016) and manufactured by Shanxi Jushuoyuan biological technology Co., LTD., Taiyuan, China. The 24 h rumen degradability of RPP was 30%, as determined *in situ* using rumen cannulated cattle. The experiment included four period and lasted for 96 days, each period contained 14 days of adaptation and 10 days of data collection. Animals were raised in single pens (3×3.5 m) during adaption phase and in the metabolism cages for the data collection phase. All animals were fed at 07:00 and 19:00 h every day and free access to drinking water. On days 13 of each sub-period, animals were confined to 95% of their voluntary intake measured during the first 12 days to guarantee no residues at the collection periods. At the beginning and end of each period, the body weight of animals was measured.

2.2. Data collection and sampling

Samples of feeds and refusals were collected once every day for the determination of DM. Feeds and refusals were dried in an oven at 55 °C for 48 h, and milled to pass a 1-mm screen on a cutter mill (FZ102, Cubai laboratory instrument Co., Ltd., Shanghai, China) for later analysis. From day 24 to day 25 of each period, approximately 150 mL ruminal fluid was collected at 07:00, 10:00, 13:00 and 16:00 from reticulum, dorsal and ventral sac through the rumen fistula using a hand-operated vacuum pump. A total of 8 samples were taken from each animal in each period and pooled by steer and time for each period before analysis. Ruminal pH was immediately determined by a pH meter (PHS-2C, Gelaimo scientific instrument Co., Ltd., Wuhan, China). Then the ruminal fluid was filtered and divided into four portions: the first portion (5 mL) was combined with 1 mL of 250g/L (w/v) metaphosphoric acid for preservation and used to measure VFA. The second portion (5 mL) was combined with 1 mL of 20g/L (w/v) H_2SO_4 for preservation and used to measure NH_3. These two portions were kept frozen at –20 °C for later analysis. The third portion (40 mL) of filtrate was kept in a centrifuge tube and frozen at –80 °C for DNA extraction. The fourth portion (40 mL) of filtrate was used to measure the ruminal enzymes activity. During each period, the whole volume of urine excreted by each steer was gathered daily by using urine collection aprons into plastic containers (Wang et al., 2009). Urine samples were collected according to the proportion of 1% of the total urine output and placed into a glass reagent bottle with 10% sulphuric acid to ensure pH lower than 3.0, Urine samples were pooled by animal at the end of each period, and diluted five times by distilled water, then separated into two portion sand were kept frozen at –20 °C for later analysis.

Table 1
Ingredient and chemical composition of the basal diet.

Ingredients	Contents [in g/kg DM]
Corn silage	500
Corn grain, ground	246
Wheat bran	60
Soybean meal	40
Cottonseed cake	110
Calcium carbonate	13
Salt	5
Zeolite powder	20
Sodium bicarbonate	5
Mineral and vitamin premix[a]	1.0
Chemical composition	
Organic matter	940
Crude protein	156
Neutral detergent fibre	358
Acid detergent fibre	233
Calcium	5.7
Phosphorus	3.4
NEg[b], MJ/kg	5.1

[a] Contained per kg premix: 100 mg Co, 8500 mg Cu, 50,000 mg Fe, 30,000 mg Mn, 30,000 mg Zn, 300 mg I, 300 mg Se, 7500,000 IU vitamin A, 1200, 000 IU vitamin D, and 40, 000 IU vitamin E.
[b] Estimated according to the NRC (1996).

2.3. In situ ruminal degradability

Ruminal degradation kinetics of corn silage and concentrate were determined by nylon bag technique during day 16–18 in each period according to the method of Wang et al. (2009). The air-dried corn silage and concentrate mix were milled to 2.5 mm (FZ102, Cubai laboratory instrument Co., Ltd., Shanghai, China). About 3.2 g of corn silage and 4.5 g of concentrate mix were weighed in 8 × 12 cm nylon bags sewed by nylon cloth with a pore size of 36 ± 1.5 μm and heat-sealed. Individual bags were placed into the rumen through the ruminal fistula at 2 h after feeding (except the 0 h bags). The duplicated bags were suspended in the rumen of each steer for 0, 4, 8, 12, 24, 36, 48 and 72 h. An empty bag without sample for each incubation time was also incubated in the rumen as blank bag. The bags were removed at the end of the incubation period and were manually rinsed in cold tap water