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

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ABSTRACT

This experiment was done to investigate the influences of supplementary rumen-protected folic acid (RPFA) on ruminal fermentation parameters, microbial enzyme activity, cellulolytic bacteria and urinary excretion of purine derivatives in growing beef steers. Eight ruminally fistulated Jinnan beef steers (398.4 ± 1.9 kg) were used in a repeated 4 × 4 Latin square experimental design. The treatments were: control (without RPFA), low RPFA (LRPFA), medium RPFA (MRPFA) and high RPFA (HRPFA) with 0, 0.6, 1.2 and 1.8 g RPFA per steer per day, respectively. The dietary corn silage to concentrate ratio was 50:50 (dry matter [DM] basis). The dry matter intake was confined to 95% of voluntary intake. Mean ruminal pH was quadratically reduced with altering RPFA supplementation, and was the lowest for MRPFA and HRPFA, highest for the control, and intermediate for the LRPFA. Ruminal total VFA concentration was quadratically increased with increasing RPFA supplementation and was higher in MRPFA than in control. The ratio of acetate to propionate was quadratically increased due to the increased acetate concentration and the unchanged propionate concentration. Ruminal degradabilities of DM and neutral detergent fibre of corn silage, and DM and crude protein of concentrate mix increased quadratically with increasing RPFA supplementation. Ruminal enzyme activity of cellobiase, xylanase, pectinase and α-amylase quadratically increased and was higher in MRPFA than in control. The populations of *B. fibrisolvens*, R. *albus, R. flavefaciens and F. succinogenes* quadratically increased with altering the supplementary PRFA. Urinary excretion of purine derivatives quadratically increased with altering RPFA supplementation and was higher in HRPFA and MRPFA than in LRPFA and control. The results indicated that dietary supplements of RPFA improved ruminal fermentation, *in situ* ruminal degradation and urinary excretion of purine derivatives. It was suggested that the RPFA regulated the activity of rumen microbe or enzymes in a concentration-dependent manner. Under this experimental condition, the appropriate dose of RPFA was at 1.2 g/d for steer.

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

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1. Introduction

It is generally believed that the ruminal microorganisms could synthesize enough folic acid and meet their hosts requirements (Bechdel et al., 1928; Lardinois et al., 1944). However, literatures have demonstrated that folic acid administered by intramuscular injections or dietary supplementation improved the growth of weaned dairy calves (Petitclerc et al., 1999) and heifers (Dumoulin et al., 1991) and increased milk yields and milk component production in dairy cows (Girard and Matte, 1998; Graulet et al., 2007; Li et al., 2016). Furthermore, some literatures reported that the synthesis of folic acid in the rumen ranged from 16.5 to 21.0 mg/d in dairy cows (Santschi et al., 2005; Schwab et al., 2006), while the estimated folic acid requirement of a cow with 650 kg BW and 35 kg of fat-corrected milk yield was 35 mg/d (NRC, 2001). These results put into question the sufficient supply of folic acid from microorganisms for highly productive and rapidly growing ruminants. Since the growth and production performance has increased markedly over the last few decades, folic acid synthesized by rumen might be insufficient to meet the needs of ruminants and ruminal microorganisms.

Several studies have demonstrated the influences of supplementary folic acid on ruminal fermentation, microorganism and microbial protein synthesis where microorganisms, especially celluloytic bacteria, required folic acid (Slyter and Weaver, 1977; Girard et al., 1994; Wejdemar, 1996). Supplementary folic acid promoted the growth of celluloytic bacteria (Wejdemar, 1996), increased cellulose digestion (Hall et al., 1953; Ragaller et al., 2010), improved ruminal fermentation (Hayes et al., 1966) and increased the utilization of ammonia N for microbial protein synthesis (Wejdemar, 1996; Lee et al., 2000). However, other researches found limited influence of folic acid on ruminal fermentation (Clifford et al., 1967; Chiquette et al., 1993; Girard et al., 1994, 2009) and the utilization of ammonia N (Girard et al., 1994, 2009; Ragaller et al., 2010). The inconsistent influences of supplementary folic acid on ruminal fermentation and ammonia N utilization could be associated with diets with different ingredient and chemical composition, dose, physical form and way of administration of folic acid.

Research has demonstrated that more than 95% of folic acid supplemented in the dairy cow diets is directly degraded by rumen microbial populations (Santschi et al., 2005). Thus, the higher degradability of folic acid in the rumen could result in insufficient supply of folic acid for highly productive and rapidly growing ruminants. Furthermore, there is limited literatures on the influences of supplementary rumen-protected folic acid (RPFA) on ruminal fermentation, its effects on microorganisms and its activity. In addition, there are no data detailing the influences of folic acid on ruminal enzymes. Therefore, this experiment was undertaken to determine the influences of supplementary RPFA on ruminal fermentation, microbial enzyme activity, cellulolytic bacteria and urinary purine derivatives (PD) excretion in growing beef steer.

2. Materials and methods

2.1. Description of RPFA

Supplementary RPFA was a Chinese patent invented by Shanxi Agricultural University and was manufactured by Shanxi Jushuoyuan biological technology co., LTD., Taiyuan, China. The product contained 100 g/kg of folic acid, 550 g/kg of hydrogenated fat (ratio of C16:0–C18:0 = 2:1), 150 g/kg of bentonite powder and 200 g/kg of calcium stearate. The ruminal degradation rate of folic acid in RPFA was 0.304, which were determined by using nylon bag technique. Three samples, 5 replicates per sample, weighing approximately 5 g each were incubated in the rumen of each of four steers for 24 h. Residues were washed in cool water for 3 min using a washing machine, dried at low temperature in a forced air oven and dry matter determined by sample. Samples were then pooled within steer for further analysis of folic acid.

2.2. Animals and experimental design

The experimental scheme was authorized by the Animal Care and Use Committee of Shanxi Agriculture University. Eight ruminally fistulated Jinnan beef steers (398.4 ± 1.9 kg) were used in a repeated 4 × 4 Latin square experimental design. The experimental treatments were the control (without RPFA), low RPFA (LRPFA), medium RPFA (MRPFA) and high RPFA (HRPFA) with 0, 0.6, 1.2 and 1.8 g/d RPFA for each steer, respectively. Supplementary RPFA was artificially blended into the first third of the morning ration to guarantee the animals ingested completely. The dietary corn silage to concentrate ratio was 50:50 (dry matter [DM] basis, Table 1). Corn silage had been harvested at full ripening stage immediately after removal of the ear corn by rotary mower and forage harvester equipped with a pickup head attachment. Corn silage was ground through a tub grinder with a 6.35-cm screen and was ensiled. Dry matter intake of steers was confined to 95% of voluntary intake. Each experimental period included 25 days with 15 days of adaptive phase and 10 days of sampling period. Animals were raised in single pens (3.5 × 3 m) during adaptive phase and in metabolism cages for the sampling period. Animals were fed twice daily at 0700 and 1900 h and water was provided *ad libitum*. On days 13, 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 were measured.

2.3. Data collection and sampling procedures

Samples of feeds and refusals were collected once daily for DM determination. Feeds and orts were dried in an electrothermal blowing dry box at 55 °C for 48 h, and smashed to pass through a 1-mm screen with a mill (110, Qingdao Ruixintai