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Effects of isovalerate supplementation on microbial status and rumen enzyme profile in steers fed on corn stover based diet

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ABSTRACT

The objective of this study was to evaluate the effects of isovalerate supplementation on microbial status and ruminal enzyme activities in steers fed on corn stover based diet with a low proein. Eight ruminally cannulated Simmental steers were used in a replicated 4 × 4 Latin square experiment. The treatments were control (without isovalerate), LIV, MIV and HIV with 8.4, 16.8 and 25.2 g isovalerate per steer per day, respectively. Isovalerate was hand- mixed into the concentrate portion. Diet consisted of 60% corn stover and 40% concentrate (dry matter [DM] basis). Dry matter intake (averaged 9 kg/day) was restricted to a maximum of 90% of *ad libitum* intake. Whether direct counts, cultured using a roll-tube technique or real-time PCR quantification, population of total bacteria, cellulolytic bacteria and anaerobic fungi were linearly increased, whereas that of protozoa and total methanogens was linearly reduced with increasing isovalerate supplementation. Real-time PCR quantification of population of *R. albus, R. Flavefaciens, B. Fibrisolvens* and *F. succinogenes* was linearly increased ($p<0.04$) with increasing isovalerate supplementation. Activities of CMCase, xylanase and β-glucosidase were linearly increased ($p<0.05$), whereas that of protease was linearly reduced ($p=0.005$) with increasing isovalerate supplementation. Methane production was linearly decreased ($p<0.001$) with increasing isovalerate supplementation. Effective degradabilities of cellulose and hemicellulose of corn stover was linearly increased ($p<0.001$), whereas that of crude protein in diet was linearly decreased ($p<0.001$) with increasing isovalerate supplementation. The present results indicate that supplementation of diet with isovalerate improved microbial status and ruminal enzyme activities in steers. It was suggested that the isovalerate stimulated the digestive microorganisms or enzymes in a dose-dependent manner based on corn stover diet with a low protein. In the experimental conditions of this trial, the optimum isovalerate dose was about 16.8 g isovalerate per steer per day.

1. Introduction

Branched-chain volatile fatty acids (BCVFA), mainly including isobutyrate, isovalerate and 2-methylbutyrate, which are naturally produced in the rumen and arise from the catabolism of amino acids (valine, isoleucine and leucine), can be used for the biosynthesis of branched-

Abbreviations: ADF, acid detergent fiber; BCVFA, branched chain volatile fatty acids; BW, body weight; CMCase, Caboxymethyl-cellulase; CP, crude protein; DM, dry matter; ED, effective degradability; HIV, high isovalerate; LIV, low isovalerate; MIV, medium isovalerate; NDF, neutral detergent fiber; VFA, volatile fatty acid

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chain amino acids or increase in concentration of BCVFA (Andries et al., 1987). Hence, when diets contain low concentrations of plant protein, ruminal concentrations of BCVFA could limit microbial growth and activity. A sufficient level of BCVFA is essential for efficient digestion of structural carbohydrates in the rumen and synthesis of microbial protein. Most cellulolytic bacteria in the rumen require BCVFA (Bryant, 1973; Cummins and Papas, 1985). Supplementation of BCVFA to *in vitro* media has increased microbial protein synthesis (Cummins and Papas, 1985; Misra and Thakur, 2012), fermentation of plant cell walls (Gorosito et al., 1985; Misra and Thakur, 2012), and dry matter (DM) digestion (Cummins and Papas, 1985; Mir et al., 1986).

However, the results from animal studies are inconclusive. The addition of BCVFA to diets containing urea increased feed intake and lactation persistency and tended to increase weight gains (Palmquist, 1988; Formigoni et al., 1995). Balance studies indicated that the BCVFA improved the utilization of urea N in the rumen (Felix et al., 1980; Kamble and Thakur, 2004). In other trials, BCVFA plus sulfur or nitrogen increased acetate production in the rumen (Brondani et al., 1991; Quispe et al., 1991). Similarly, BCVFA supplementation increased *in vivo* rumen fermentation, effective degradability and microbial protein synthesis (Liu et al., 2008a,b; 2009a,b; Wang et al., 2012). In field trials, BCVFA increased milk yields when given with a variety of practical ratios (Formigoni et al., 1995; Liu et al., 2009b; Val Neto et al., 2010). In contrast, the apparent cellulose digestibility was rarely improved according to several digestion and metabolic trials (Hefner et al., 1985; Tuah and Tait, 1985). Similarly, Gunter et al. (1990) observed that supplemental BCVFA had no effect on digestion and fermentation of grass hay.

Research about BCVFA on rumen microbial status, ruminal enzyme activities and methane emissions is limited and inconclusive. Moharrery and Das (2001) found microbial production was enhanced by BCVFA supplementation, and the pattern of enzymes changed in the rumen of sheep, and then cellulase activity was increased compared with the control. However, Suryapratama and Suhartati (2009) reported lower dose of BCVFA supplementation was not strong enough to influence the growth of rumen bacteria and protozoa. Moreover, medium chain fatty acid supplementation in *in vitro* and *in vivo* studies significantly suppressed methane production in ruminants (Dong et al., 1997; Machmuller and Kreuzer, 1999). Whether BCVFA supplementation would be reduce methane emissions in ruminants? Therefore, the aim of this work was to study the effects of isovalerate supplementation on microbial status and rumen enzyme profile in steers fed on corn stover based diet with a low protein.

2. Materials and methods

2.1. Animals and experimental design

The experimental protocol was approved by the Animal Care and Use Committee of the Shanxi Agriculture University. Eight ruminally cannulated Chinese Simmental steers averaging 2.5 years of age and 430 ± 15 kg of body weight (BW), were assigned to a duplicate 4 × 4 Latin square design, and the duplicate Latin squares were conducted simultaneously. The treatments were: control (without isovalerate), LIV, MIV and HIV with 8.4, 16.8 and 25.2 g isovalerate per steer per day, respectively. The supplement of isovalerate analytical grade (98.5% of isovalerate) was purchased commercially and was hand- mixed into the concentrate portion. Diets consisted of 60% corn stover and 40% concentrate (dry matter [DM] basis; Table 1). Corn stover had been harvested at dough stage immediately after removal of the ear corn by rotary mower and forage harvester equipped with a pickup head attachment, then air-dried and baled. Corn stover was ground through a tub grinder with a 6.35-cm screen before feeding. Feed intake including 5.4 kg forage and 4.6 kg concentrate. Experimental periods were 21 days with 11 d of adaptation and 10 d of sampling. During the adaptation periods, from day 1 to 4, different levels isovalerate within the Latin square design was hand- mixed into the concentrate portion increased by 25% and adaptation to the corn stover diet. Steers were housed in individual pens (3 m × 3 m) for adaptation period and in metabolism cages during the collection period. Steers were fed twice daily at 07:00 and 19:00 h and fresh water was available throughout the experimental period, and feed were provided in proportion to the diet and then converted to amounts per day. The animals were weighed at the beginning and the end of each period.

Table 1
Ingredient and chemical composition of the basal diet (in g/kg dry matter).

Ingredients	
Corn stover	600
Corn grain, ground	208
Wheat bran	40
Soybean meal	66
Cottonseed cake	48
Rapeseed meal	20
Calcium carbonate	5
Salt	4
Dicalcium phosphate	3.5
Mineral and vitamin mix[a]	5.5
Chemical composition	
Organic matter	943.2
Crude protein	101.1
Neutral detergent fiber	565.1
Acid detergent fiber	355.9
Calcium	8.6
Phosphorus	5.2

[a] Contained 42 mg/kg Co, 3500 mg/kg Cu, 20,000 mg/kg Fe, 12,000 mg/kg Mn, 12,000 mg/kg Zn, 1200 mg/kg I, 600 mg/kg Se, 3,000,000 IU of vitamin A, 500,000 IU of vitamin D, and 15,000 mg of vitamin E per kg premix.

2.2. In situ ruminal degradability

Ruminal degradation kinetics of the corn stover and the concentrate mixture used in the present study was measured using nylon bag technique on days 12–14 of the experimental period. The corn stover and concentrate diet were ground to pass a 2.5-mm screen with a mill (FZ102,