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Effects of supplementation of Simmental steers with 2-methylbutyrate on rumen microflora, enzyme activities and methane production

Y.L. Zhang[a], Q. Liu[a,*], C. Wang[a], C.X. Pei[a], H.Y. Li[a], Y.X. Wang[a], W.Z. Yang[a,b], Y.S. Bai[c], Z.G. Shi[c], X.N. Liu[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] Shanxi Province Ecology and Livestock Industry Management Station, Taiyuan, Shanxi, 030001, PR China

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ABSTRACT

The objective of this study was to evaluate the effects of 2-methylbutyrate supplementation on rumen microflora, enzyme activities and methane production in Simmental steers consuming a diet consisting of 600 g/kg corn stover and 400 g/kg concentrate (dry matter [DM] basis). Eight ruminally cannulated Simmental steers were used in a replicated 4 × 4 Latin square design. The treatments were: control (without 2-methylbutyrate), low 2-methylbutyrate (LMB), medium 2-methylbutyrate (MMB) and high 2-methylbutyrate (HMB) with 0, 8.4, 16.8 and 25.2 g 2-methylbutyrate per steer and per day, respectively. Dry matter intake (averaged 9 kg/day) was restricted to a maximum of 0.9 of *ad libitum* intake. Whether direct counts, roll-tube technique or real-time PCR quantification were used, populations of total bacteria, cellulolytic bacteria and anaerobic fungi linearly increased ($P<0.04$) and were higher ($P<0.05$) for MMB and HMB than for control and LMB, whereas those of protozoa and total methanogens linearly reduced and were lower for MMB and HMB than for control and LMB. With increasing 2-methylbutyrate supplementation, real-time PCR quantification of populations of *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Bufyrivibrio fibrisolvens* and *Fibrobacter succinogenes* linearly increased ($P<0.05$) and was higher for MMB and HMB than for control ($P<0.05$). Activities of CMCase, xylanase and β-glucosidase linearly increased ($P<0.05$) and were higher ($P<0.05$) for MMB and HMB than for control and LMB, whereas that of protease linearly reduced ($P<0.05$) and was lower for MMB and HMB than for control and LMB ($P<0.05$). Activities of α-amylase and α-glucosidase tended to be higher ($P<0.08$) for MMB and HMB than for control and LMB, but activity of urease tended to be lower for MMB and HMB than for control and LMB. Methane production linearly decreased ($P<0.01$) and was lower for MMB and HMB than for control ($P<0.05$) with increasing 2-methylbutyrate supplementation. Effective degradabilities of cellulose and hemicellulose of corn stover linearly increased ($P<0.01$) and were higher

Abbreviations: ADF, acid detergent fiber; BCVFA, branched chain volatile fatty acids; BW, body weight; CMCase, carboxymethyl-cellulase; CP, crude protein; DM, dry matter; ED, effective degradability; HMB, high 2-methylbutyrate; LMB, low 2-methylbutyrate; MMB, medium 2-methylbutyrate; N, nitrogen; aNDF, neutral detergent fiber; OM, organic matter; VFA, volatile fatty acid.

* Corresponding author. Tel.: +86 354 628 9115; fax: +86 0354 628 8052.
E-mail address: liuqiangabc@163.com (Q. Liu).

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for MMB and HMB than for control (P<0.05), whereas that of crude protein in concentrate linearly decreased (P<0.01) and was lower for MMB and HMB than for control and LMB (P<0.05) with increasing 2-methylbutyrate supplementation. The present results indicate that supplementation of diet with 2-methylbutyrate altered ruminal microbial populations and improved ruminal enzyme activities in a dose-dependent manner in steers fed high-roughage diet. In the experimental conditions of this trial, the optimum daily dose of 2-methylbutyrate at 16.8 g per steer is suggested.

1. Introduction

Branched-chain volatile fatty acids (BCVFA) such as isobutyric, isovaleric and 2-methyl butyric are mainly built up from the degradation products of branched-chain amino acids in the digestive tract of the ruminant and are in turn used for the biosynthesis of those amino acids or higher BCVFA (Andries et al., 1987), so that ruminal concentrations of BCVFA could limit microbial growth and activity when diets contain low protein content. A sufficient level of BCVFA is essential for efficient digestion of structural carbohydrates in the rumen. Most fiber-degrading microorganisms in the rumen require BCVFA (Bryant, 1973; Cummins and Papas, 1985). Early *in vitro* studies demonstrated that BCVFA are required by cellulolytic bacteria (Bryant, 1973). Supplementation of BCVFA to *in vitro* media has increased microbial protein synthesis (Cummins and Papas, 1985; Misra and Thakur, 2012), fermentation of fiber (Gorosito et al., 1985; Misra and Thakur, 2012), and dry matter (DM) digestion (Cummins and Papas, 1985). However, the responses to BCVFA supplementation from animal studies are inconclusive. The addition of BCVFA to diets containing urea increased feed intake, lactation persistency and weight gains (Palmquist, 1988). In other trials, BCVFA plus sulfur or nitrogen (N) improved the utilization of urea N and increased acetate production in the rumen (Brondani et al., 1991; Kamble and Thakur, 2004). Similarly, BCVFA supplementation increased *in situ* rumen fermentation, effective degradability (ED) and microbial protein synthesis (Liu et al., 2008, 2009; Wang et al., 2012). In contrast, several digestion and metabolic trials indicated that the apparent cellulose digestibility was rarely improved (Tuah and Tait, 1985; Gunter et al., 1990).

Research about BCVFA on rumen microbial populations, ruminal enzyme activities and methane emissions is limited and inconclusive. Moharrery and Das (2001) found that isoacids supplementation changed the pattern of enzymes in the rumen of sheep and increased cellulase activity. However, Suryapratama and Suhartati (2009) reported that lower dose of BCVFA supplementation was not 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; Machmüller and Kreuzer, 1999). Information on the effect of BCVFA supplementation on methane emissions in ruminants is scarce. Therefore, the aim of this work was to study the effects of increasing 2-methylbutyrate supplementation on rumen microflora, enzyme activities and methane production in Simmental steers fed a corn stover-based diet.

2. Materials and methods

2.1. Animals and experimental design

Eight ruminally cannulated Chinese Simmental steers averaging 2.5 years of age and 430 ± 15 kg of body weight (BW) were assigned to a replicated 4 × 4 Latin square design. The treatments were: control (without 2-methylbutyrate), low 2-methylbutyrate (LMB), medium 2-methylbutyrate (MMB) and high 2-methylbutyrate (HMB) with 0, 8.4, 16.8 and 25.2 g of 2-methylbutyrate per steer and per day, respectively. The analytical grade 2-methylbutyrate (985 g/kg of 2-methylbutyrate) was purchased commercially and was hand-mixed into the concentrate portion. Diets consisted of 600 g/kg corn stover and 400 g/kg concentrate (DM basis; Table 1). Corn was harvested at sealed storage, 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 3.6 kg concentrate was restricted to a maximum of 0.9 of *ad libitum* intake. 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 2-methylbutyrate within the Latin square design was hand-mixed into the concentrate portion increased by 0.25 and adaptation to the corn stover diet. Steers were housed in individual pens (3 m × 3 m) and were fed twice daily at 07:00 and 19:00 h, and fresh water was available throughout the experimental period. Refusals were measured daily at 16:00 h during the adaptation period, and the amount of feed offered was adjusted for a target of 0.05 refusals. On day 8, steers were restricted to 0.9 of their respective *ad libitum* intake determined during the prior 7 day in an attempt to assure no refusals during the sampling periods. The steers were weighed at the beginning and the end of each period. The experimental protocol was approved by the Animal Care and Use Committee of the Shanxi Agriculture University.