Animal Feed Science and Technology 216 (2016) 129–137

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Effects of 2-methylbutyrate supplementation on growth performance and ruminal development in pre- and post-weaned dairy calves

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ARTICLE INFO

Article history:
Received 5 February 2016
Received in revised form 23 March 2016
Accepted 24 March 2016

Keywords:
2-Methylbutyrate
Growth performance
Rumen development
Dairy calves

ABSTRACT

The objective of this study was to evaluate the effects of 2-methylbutyrate supplementation on growth performance and rumen development in pre- and post-weaned dairy calves. Fifty-six Chinese Holstein male calves with 15 days of age and 46.5 ± 0.37 kg of body weight (BW) were randomly assigned to one of four groups. The treatments were: control, low-2-methylbutyrate (LMB), moderate-2-methylbutyrate (MMB) and high-2-methylbutyrate (HMB) with 0, 3, 6 and 9 g 2-methylbutyrate per calf per day, respectively. Supplemental 2-methylbutyrate was hand- mixed into milk in pre-weaned calves and the concentrate portion of post-weaned calves. The study lasted 75 days, including a 15-day adaptation period and followed by a 60-day sampling period. Calves were weaned at 60 days of age. Six calves were chosen from each treatment at random and slaughtered at 30 and 90 days of age. Body weight, dry matter (DM) intake and stomach weight were measured, samples of ruminal tissues and blood were determined. DM intake increased linearly ($P < 0.03$) for post-weaned calves and overall DM intake with increasing 2-methylbutyrate supplementation and was higher for MMB and HMB than for LMB and control ($P < 0.05$). Average daily gain (ADG) increased linearly ($P < 0.05$) for pre-weaned calves, post-weaned calves and overall ADG with increasing 2-methylbutyrate supplementation, and was higher for MMB and HMB than for LMB and control ($P < 0.05$). Total stomach weight for pre- and post-weaned calves increased linearly ($P < 0.05$) and was higher for MMB and HMB than for LMB and control ($P < 0.05$). The ratio of rumen weight to total stomach weight tended to increase linearly ($P = 0.055$) for pre-weaned calves and increased linearly ($P = 0.019$) for post-weaned calves, whereas the ratio of abomasum weight to total stomach weight decreased linearly ($P < 0.05$) for pre-weaned calves and post-weaned calves with increasing 2-methylbutyrate supplementation. The ratio of total stomach weight to body weight for pre-weaned calves and post-weaned calves increased linearly ($P < 0.05$) and was higher for MMB and HMB than for LMB and control ($P < 0.05$). Both the length and width of rumen papillae, and the relative

Abbreviations: ACAT-1, acetoacetyl-CoA acetyltransferase 1; ADF, acid detergent fibre; ADG, average daily gain; BCVFA, branched chain volatile fatty acids; BHBA, beta-hydroxybutyrate; BW, body weight; CP, crude protein; DM, dry matter; FCR, feed conversion ratio; GH, growth hormone; GHR, growth hormone receptor; HMB, high 2-methylbutyrate; HMGCS1, 3-hydroxy-3-methylglutaryl-CoA synthase 1; IGF-1, insulin-like growth factor-1; LMB, low 2-methylbutyrate; ME, metabolic energy; MMB, medium 2-methylbutyrate; NDF, neutral detergent fibre; PCR, polymerase chain reaction; VFA, volatile fatty acid.

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http://dx.doi.org/10.1016/j.anifeedsci.2016.03.028

expression of mRNA for growth hormone receptor (GHR) and 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1) in rumen mucosa tended to increase linearly for pre-weaned calves, but increased linearly ($P < 0.05$) for post-weaned calves and was higher for MMB and HMB than for LMB and control ($P < 0.05$). Blood concentrations of BHBA increased linearly ($P < 0.05$) for post-weaned calves with increasing 2-methylbutyrate supplementation and was higher for MMB and HMB than for LMB and control ($P < 0.05$). Blood concentrations of GH and IGF-1 increased linearly ($P < 0.05$) for pre- and post-weaned calves with increasing 2-methylbutyrate supplementation and was higher for MMB and HMB than for LMB and control ($P < 0.05$). The present results indicated that 2-methylbutyrate accelerated growth of calves by improving rumen development and its ketogenesis. The 2-methylbutyrate stimulates the growth performance and rumen development of dairy calves in a dose-dependent manner. In the experimental conditions of this trial, the optimum 2-methylbutyrate dose was about 6.0 g 2-methylbutyrate per calf per day.

1. Introduction

In dairy farming practices, rumen development and its functional improvement in calves, especially rumen epithelium, plays a key role on nutrient delivery, absorption, and metabolism and have an important impact on calf health and future production performance (Bannink et al., 2008; Martens et al., 2012). With the developmental changes in morphology, metabolic function also changed, then the oxidation of glucose decreased companied with the increased oxidation of volatile fatty acids and the stepped-up ketogenesis of rumen epithelium. Ruminal fermentation products, one of the important factors affecting the development of rumen, not only provide energy for the growth of epithelial tissue and muscle contraction, but also directly affect the proliferation and differentiation of epithelial cells (Bannink et al., 2008; Martens et al., 2012). It is well documented that butyrate and, to a lesser extent, acetate and propionate, are the primary chemical stimulators of rumen development (Mentschel et al., 2001; Górka et al., 2011; Plöger et al., 2012). It is suggested that additives that produce more total VFA, especially butyrate concentrations, may be used to improve the development of rumen and growth performance.

The inclusion of branched-chain volatile fatty acids (BCVFA) in ruminant diets, including isobutyric, isovaleric and 2-methyl butyric, has shown increases in acetate and butyrate and reduction in propionate. Supplementation of BCVFA to *in vitro* media has increased microbial protein synthesis (Cummins and Papas, 1985), fermentation of plant cell walls (Kone et al., 1989), and dry matter (DM) digestion (Mir et al., 1986). Previous research has demonstrated that BCVFA improved rumen fermentation (Misra and Thakur, 2001; Wang et al., 2012; Zhang et al., 2015), rumen microbial enzyme activities (Yang, 2002; Liu et al., 2014; Zhang et al., 2015), the population of cellulolytic bacteria (Liu et al., 2014; Zhang et al., 2015), nutrient digestibilities (Misra and Thakur, 2001; Liu et al., 2009a), average daily gain in steers and sheep, and increased milk yields in dairy cows (Liu et al., 2009b; Val Neto et al., 2010). Moreover, our previous study reported that supplemental 2-methylbutyrate in pre- and post-weaned calves diets linearly increased rumen total VFA concentration, microbial enzyme activities of CMCase, cellobiase, xylanase, pectinase and amylase, and the relative quantity of *Ruminococcus albus, Ruminococcus flavefaciens, Butyrivibrio fibrisolvens* and *Fibrobacter succinogenes* (Li et al., 2015). Dietary 2-methylbutyrate could increase ruminal fermentation products, especially butyrate production. Dietary butyrate improve the development of rumen and small intestine (Guilloteau et al., 2010; Górka et al., 2011; Plöger et al., 2012). We hypothesized dietary 2-methylbutyrate could promote the growth of calf by increasing ruminal butyrate production to improve the development of rumen.

To the best of the authors' knowledge, no literature was found regarding dietary 2-methylbutyrate and its effects on rumen development and growth of pre- and post-weaned dairy calves. Therefore, the aim of this work was to evaluate the effects of 2-methylbutyrate on growth performance and ruminal development in pre- and post-weaned dairy calves.

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. Fify-six Chinese Holstein male calves with 15 days of age and 46.5 ± 0.37 kg of body weight (BW) were randomly assigned to one of four groups by using random numbers, 14 calves per group. The treatments were: control (without 2-methylbutyrate), low-2-methylbutyrate (LMB), moderate-2-methylbutyrate (MMB) and high-2-methylbutyrate (HMB) with 3, 6 and 9 g 2-methylbutyrate per calf per day, respectively. The supplement of 2-methylbutyrate analytical grade (98.5% of 2-methylbutyrate) was purchased commercially (Shanghai Aladdin biological technology co., LTD, Shanghai, China) and was hand- mixed into milk for pre-weaned calves and the concentrate portion of post-weaned calves. The study lasted 75 days, including a 15-day adaptation period and followed by a 60-day sampling period. Calves were weaned at 60 days of age. During the pre-weaned period, calves were offered whole milk (10% of BW) twice daily via nipple feeding at 0800 h and 1500 h for 25 days, from 26 to 30 day, daily offer of milk was decreased to half and calves were fed a commercial concentrate *ad libitum*. Post-weaned diets consisted of 60% alfalfa hay and 40% commercial concentrate (dry matter [DM] basis), and was