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Effects of branched-chain volatile fatty acids supplementation on growth performance, ruminal fermentation, nutrient digestibility, hepatic lipid content and gene expression of dairy calves

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ABSTRACT

This study evaluated the effects of branched-chain volatile fatty acids (BCVFA) supplementation on growth performance, ruminal fermentation, nutrient digestibility, hepatic lipid content and gene expression of dairy calves. Forty-eight Chinese Holstein male calves (10 months of age; 345 ± 6.4 kg of body weight [BW]) were assigned randomly to four treatments with 0, 60, 120 and 180 mg BCVFA per kg BW per day for each calf. Supplemental BCVFA was hand-mixed into the top third of the daily ration and fed to calves for 100 days to the experimental treatments. Supplementation of BCVFA in calves ration increased ADG and feed conversion linearly. Dry matter (DM) intake was unaffected, but digestibilitiy of DM, organic matter, crude protein, ether extract, neutral detergent fibre and acid detergent fibre increased linearly with increasing levels of BCFVA supplementation. Similarly, Supplementation of BCVFA linearly decreased ruminal pH and ammonia-N but increased total VFAs. Molar proportion of acetate linearly increased, whereas propionate linearly decreased and the ratio of acetate to propionate increased linearly with increasing BCVFA in rations. Relative mRNA expressions of peroxisome-proliferator-activated receptor α and carnitine palmitoyl transferase-1 linearly increased with BCVFA levels. The results suggested that BCVFA supplementation improved ruminal fermentation, nutrient digestibility and growth of calves with promoted hepatic lipid oxidation. Due to the unsignificant difference between 120 and 180 mg groups for all parameters, the optimum supplementary dose of BCVFA was 120 mg per kg BW per day.

1. Introduction

Ruminal volatile fatty acid (VFA, including acetate, propionate and butyrate) derived from feed carbohydrate degradation is an important energy source for ruminants. Ruminal acetate, which accounts for 70%–75% of total VFA, enteres into the liver via portal vein to generate energy through tricarboxylic acid cycle or synthesize fatty acid (Bergman, 1990; Li et al., 2013). Fushimi et al. (2006) found that the dietary acetate supplementation decreased blood total triacylglycerols (TG) concentration and mRNA levels of sterol regulatory element binding protein-1 (*SREBP1*) and fatty acid synthase (*FAS*), and inhibited lipogenesis in the liver of rats.

Abbreviations: *ACACA*, acetyl-coenzyme A carboxylase α; 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; *CPT1*, carnitine palmitoyl transferase-1; EE, ether extract; *FAS*, fatty acid synthase; FCR, feed conversion ratio; NDF, neutral detergent fibre; NEFA, non-esterified fatty acid; PCR, polymerase chain reaction; *PEPCK*, phosphoenolpyruvate carboxykinase; *PPARα*, peroxisome-proliferator-activated receptor α; *SREBP-1*, sterol regulatory element binding protein-1; TG, triacylglycerols; VFA, volatile fatty acid

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Kondo et al. (2009) reported that acetate administration inhibited hepatic lipid accumulation by upregulating mRNA expressions of peroxisome-proliferator-activated receptor α (*PPARα*) and carnitine palmitoyl transferase-1 (*CPT-1*) in the liver of mice. Moreover, Li et al. (2013) found that the expressions of *PPARα* and *CPT1* elevated, but *SREBP1*, acetyl-CoA carboxylase α (*ACACA*) and *FAS* decreased in the acetate treated bovine hepatocytes. Therefore, acetate could influence dietary energy utilization and hepatic lipid metabolism by regulating gene expression.

Ruminal branched-chain volatile fatty acids (BCVFA, a mixture of isobutyrate, isovalerate and 2-methylbutyrate) is derived from feed protein degradation, and is an essential nutrient for the growth of ruminal cellulolytic bacteria (Eugène et al., 2004). The supplementation of BCVFA increased populations of *R. albus, R. flavefaciens, B. fibrisolvens* and *F. succinogenes* and activities of caboxymethyl-cellulase, cellobiase, xylanase and pectinase (Liu et al., 2014; Wang et al., 2015; Zhang et al., 2015). Thus, ruminal total VFA concentration and acetate production increased with BCVFA supplementation in steers (Liu et al., 2009a,b) or calves (Liu et al., 2016a). Moreover, the increased average daily gain (ADG) observed in these studies (Liu et al., 2016a; Wang et al., 2016) might be associated with an increased energy utilization.

Considering the effects of acetate on hepatic gene expression and the positive role of BCVFA supplementation on ruminal acetate production, it was hypothesized that the increased acetate production with BCVFA supplementation could improve growth performance and energy utilization of calves by regulating hepatic lipid metabolism. Therefore, the objective of the study was to evaluate the effects of BCVFA supplementation on dry matter (DM) intake, ADG, feed conversion rate (FCR), ruminal fermentation, nutrient digestibility, blood parameters and relative mRNA expressions of *PPARα*, *SREBP1*, *CPT1*, *ACACA*, *FAS* and phosphoenolpyruvate carboxykinase (*PEPCK*) in the liver of calves.

2. Materials and methods

2.1. Animals and experimental design

The experimental protocol was approved by the Animal Care and Use Committee of Shanxi Agriculture University. Forty-eight Chinese Holstein male calves (10 months of age; 345 ± 6.4 kg of body weight [BW]) were assigned randomly to four treatments with 0, 60, 120 and 180 mg BCVFA per kg BW per day for each calf, respectively. The supplement of BCVFA was a mixture of isobutyrate, isovalerate and 2-methylbutyrate (analytical grade, 0.985 g/g isobutyrate, isovalerate and 2-methylbutyrate, Yangxi Spice Factory, Guangdong, China) according to the ratio of 1:1:1 and hand-mixed into the top third of the daily ration. The amount of BCVFA fed was determined from recent work of Liu et al. (2008) who found that isobutyrate (16.8 or 25.2 g/steer/day) supplemented to the ration improved ruminal fermentation and nutrient digestion in beef cattle. The study lasted 100 days, including a 10-day adaptation period and followed by a 90-day sampling period. Calves were fed ad libitum a total mixed ration (TMR). The TMR was formulated based on NRC (2001) recommendations for 350 kg dairy calves with weight gain of 1.3 kg/d. The ingredients of the TMR (g/kg DM) were corn stover (600 g/kg), corn grain (ground, 200 g/kg), wheat bran (40 g/kg), soybean meal (82 g/kg), cottonseed cake (40 g/kg), rapeseed meal (20 g/kg), calcium carbonate (5 g/kg), salt (4 g/kg), dicalcium phosphate (3 g/kg), and mineral and vitamin mix (5 g/kg). The mineral and vitamin mix contained 42 mg/kg Co, 1600 mg/kg Cu, 10,000 mg/kg Fe, 6000 mg/kg Mn, 8000 mg/kg Zn, 120 mg/kg I, 60 mg/kg Se, 500,000 IU of vitamin A, 300,000 IU of vitamin D, and 5000 mg of vitamin E per kg premix. Chemical composition of the TMR included 935.6 g/kg organic matter (OM), 120.4 g/kg crude protein (CP), 534.2 g/kg neutral detergent fibre (NDF), 347.3 g/kg acid detergent fibre (ADF), 8.2 g/kg calcium, and 5.3 g/kg phosphorus per kg DM. Calves were housed in individual pens (2.5 m × 3 m) and fresh water was available throughout the experimental period.

2.2. Data collection and sampling procedures

Body weights were recorded on two consecutive days before and at the end of the collection period. Feed offered and refusals were recorded daily through the experimental period to calculate DM intake. Samples of feed and refusals were collected once weekly for DM determination. Feed and refusals were dried in an oven at 55 °C for 48 h, and ground to pass a 1-mm screen with a cutter mill (110, Qingdao Ruixintai instrument Co., Ltd., Qingdao, China) for chemical analysis.

During d 77–87 of the experimental period, calves were dosed orally twice daily with 5 g of chromic oxide in gelatin capsule per day per calf in two equal proportions at 07:00 and 19:00 h as a digesta marker. Fecal samples (approximately 200 g wet weight) were collected from the rectum three times daily at various times (3 h intervals) during d 87–97 of the period. At the end of the experiment, samples were thawed, composited by calf on an equal wet weight basis. After being dried at 55 °C for 48 h to constant weight, samples were ground to pass a 1 mm sieve in a cutter mill (110, Qingdao Ruixintai instrument Co., Ltd., Qingdao, China) for chemical analyses. Dry matter excreted in feces was calculated by dividing chromium input (grams of chromium per day) by chromium concentration (grams of chromium per kilogram of DM) in the feces (Ferret et al., 1999). Excretion of other nutrient in the feces was calculated by multiplying DM flow by their concentration in fecal DM.

For all calves, ruminal fluid was sampled 3 h after the morning feeding on day 98 via the esophagus using a stomach tube (outside diameter 1 cm, inside diameter 0.8 cm, length 300 cm) connected to a vacuum pump (Speedivac 2, Edwards High Vacuum, Crawley, UK) from several sites within the rumen (Jacobson et al., 1957). Ruminal pH was immediately measured using an electric pH meter (PHS-3C, Shanghai Leijun experimental instrument Co., Ltd., Shanghai, China). Samples were then strained through four layers of cheesecloth. Five milliliters of filtrate was preserved by adding 1 mL of 250 g/L meta-phosphoric acid to determine VFA, and 5 mL of filtrate was preserved by adding 1 mL of 20 g/L (w/v) H_2SO_4 to determine NH_3–N. Samples were subsequently stored frozen at −20 °C until analyses.