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Effects of tributyrin supplementation on *in vitro* culture fermentation and methanogenesis and *in vivo* dietary nitrogen, calcium and phosphorus losses in Small Tail ewes

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ABSTRACT

Butyric acid and its salt have been used as feed additives to improve feed efficiency, nutrient degradability and animal growth. In this study, an *in vitro* trial was conducted to evaluate the effects of tributyrin (**TB**) supplementation on fermentation, enzyme activity and gas production at dosages of 0, 2, 4, 6 and 8 g/kg dry matter (**DM**) basis in substrate, which was incubated for 36 h. In addition, an *in vivo* trial with the same concentrations of TB in diet as *in vitro* trial was conducted to assess the influence of TB addition on utilization of dietary nitrogen, calcium and phosphorus. Forty-five adult Small Tail ewes were randomly assigned to 5 treatments of 9 ewes each by initial body weight (55 ± 5 kg, mean ± SD). The *in vivo* trial lasted 18 days, and ewes had free access to water and the ration mixed with TB. The results showed that TB decreased *in vitro* molar proportion of acetate ($P < 0.001$), but increased propionate ($P = 0.004$), butyrate ($P < 0.001$) and valerate ($P < 0.001$). Tributyrin improved *in vitro* fermentation efficiency ($P = 0.003$) and enhanced activity of xylanase ($P = 0.008$), carboxymethyl cellulase ($P = 0.062$) and avicelase ($P = 0.006$). Tributyrin increased the *in vitro* apparent degradability of DM ($P = 0.002$), crude protein ($P < 0.001$), neutral detergent fiber ($P < 0.001$) and acid detergent fiber ($P = 0.056$). Tributyrin increased *in vitro* methanogenesis ($P < 0.001$), but decreased carbon dioxide production ($P < 0.001$). Besides, TB supplementation improved *in vivo* utilization of dietary nitrogen, calcium and phosphorus. The results indicated that TB addition had positive effects on not only *in vitro* fermentation but also *in vivo* dietary nutrient utilization, despite having an enhancing effect on methanogenesis.

1. Introduction

Butyric acid is one of the main products of ruminal fermentation in adult ruminant, with a varied proportion in the total fermentation acids from 5% to more than 20% (Aschenbach et al., 2011). During absorption in the rumen, around 90% of butyric acid is oxidized to ketone bodies through the ketogenic capacity of ruminal epithelium (Britton and krehbill, 1993). An increase in ketogenesis would supply ruminant with a large amount of ketone bodies to fulfill energy needs of the rumen growth, thus it is generally believed that intraruminal butyrate stimulates gene transcription and ultimately rumen maturation (Lane et al., 2002).

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Butyric acid and its salt also positively affect feed efficiency, degradability and growth in piglets and calves (Kotunia et al., 2004; Biagi et al., 2007; Guilloteau et al., 2009). Dengler et al. (2014) reported a positive effect of butyric acid supplementation on short-chain fatty acid absorption across the rumen epithelium. In particular, compared to propionate and acetate, butyrate stimulated Ca^{2+} net flux rate across the rumen wall epithelia of sheep (SchrÖder et al., 1999). Similar effects were reported in Mg^{2+} and Na^{+} transports across the ovine rumen wall (Gäbel et al., 1991; Leonhard-Marek et al., 1998).

Tributyrin (**TB**) is a rich form of butyric acid, which molecule can be metabolized into three free butyric acid molecules by rumen microbes. Compared with sodium butyrate, TB has more favorable pharmacokinetics, which is attributed to its more potent and direct effect on cells (Chen and Breitman, 1994). Additionally, because TB is more stable and less odorous, it is widely used in animal studies instead of butyric acid or sodium butyrate. However, little is known about the effects of TB in the adult ruminant so far. Thus, this study aimed at assessing the effects of TB on fermentation *in vitro* and dietary mineral utilization *in vivo* in adult Small Tail ewes.

2. Materials and methods

The animal experiments involved in this research were approved by the Animal Ethics Committee of Anhui Science and Technology University. *In vitro* trial was conducted at the key laboratory of College of Animal Science, Anhui Science and Technology University (Fengyang, China), while the *in vivo* trial was conducted at the Experimental Station of Anhui Province Modern Agriculture Technology System in Cattle and Sheep (Bengbu, China).

2.1. In vitro trial

Nine 12-month-old rumen-cannulated Small Tail ewes (initial body weight 55 ± 5 kg, mean ± SD) without pregnancy were housed in a 27 m^2 concrete-floor pen, with twelve feed bunks and two water troughs. Total mixed ration (**TMR**) was provided twice daily at 0700 and 1900 h, which ingredient and chemical composition is shown in Table 1. Ewes were provided with *ad libitum* access to the feed and water.

A completely randomized design was applied to five runs of *in vitro* batch cultures. In each run, rumen fluids collected 1 h before the morning feeding from the nine ewes were filtered through two layers of muslin, mixed in equal volumes and served as donor of mixed rumen microorganisms. Screw capped glass bottles (volume capacity of 140 ml) with roll tube stoppers were used as incubators. About 500 mg of substrate (DM basis) was grounded in a Wiley mill to pass through 2-mm screen before being weighed into the bottle (90 bottles per run, 18 bottles for each treatment). The substrate consisted in 40.0% ensiled corn stover, 20.0% peanut straw, 25.6% maize meal, 6.4% wheat bran, 5.12% soybean meal and 2.88% premix. Tributyrin (Wuhan Pan-China Biotechnology Co., Wuhan, Hubei, China) at 0 (control), 2, 4, 6 and 8 g/kg (DM basis) in substrate was respectively added to the treatments bottle, and this was followed by 25 ml of the filtered rumen fluid and 50 ml freshly prepared buffer solution (pH 6.85, Menke and Steingass, 1988). All the bottles were purged with N_2 for 5 s, and then sealed with butyl rubber stoppers and Hungate screw caps before being incubated at 39 °C for 36 h. In addition, three fermentation bottles without substrate and TB supplementation were used as blanks in each run. If necessary, through analyzing the concentrations of microbial protein and volatile fatty acid (**VFA**) in the blanks, the variation between runs caused by the different rumen fluid inoculum in different periods could be checked, else the trial would be

Table 1
Ingredient and chemical composition of the basal diet.

Item	% dry matter (DM)
Ingredient	
Ensiled corn stover	40.00
Peanut straw	20.00
Maize meal	25.60
Wheat bran	6.40
Soybean meal	5.12
Premix[1]	2.88
Analysed chemical composition of feed	
Metabolizable energy[2] (MJ/kg DM)	10.21
Crude protein (% DM)	9.01
Ether extract (% DM)	5.21
Neutral detergent fibre (% DM)	39.73
Acid detergent fibre (% DM)	24.74
Non-fibre carbohydrate[3] (% DM)	40.96
Ash (% DM)	5.09
Calcium (% DM)	1.19
Total phosphorus (% DM)	0.42

[1]Per kilogram of premix contained 154,400 IU of Vitamin A, 94,000 IU of Vitamin D_3, 338,200 IU of Vitamin E, 120.0 mg of I, 280.0 mg of Cu, 2240.0 mg of Fe,1740.0 mg of Mn, 1370.0 mg of Zn, 60.0 mg of Se, and 16.8 mg of Co.
[2]Metabolizable energy was based on calculated values (NRC, 2001).
[3]NFC (%) = 100-(NDF + CP + EE + Ash).