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RUMINAL BUTYRATE INFUSION INCREASED PAPILLAE SIZE AND DIGESTA WEIGHT BUT DID NOT CHANGE LIQUID FLOW RATE IN THE RUMEN OF GOATS

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ABSTRACT

This study investigated the effects of ruminal infusion of butyrate on digesta weight and liquid flow in rumen. Goats (n=15) fed concentrates 200 g/day and hay *ad libitum*, were assigned into two groups: control (C, n=7) and butyrate (B, n=8) and were intraruminally infused with 0.3 (B group) or 0 (C group) g/kg.BW/day sodium butyrate, dissolved in potassium buffer (50 ml), before morning feeding for 4 weeks. Liquid flow in the rumen was determined by pulse dosing of cobalt-ethylene diamine tetra acetic acid (Co-EDTA) into the rumen. The papillae height and the thickness of epithelium increased ($P < 0.05$) in B compared to C. The full weight of rumen and the weight of rumen digesta, expressed as percent of total stomach weight, were greater in B than in C ($P = 0.04$ and 0.06, respectively), but the full weight of omasum and the weight of omasal digesta were lower in B than in C ($P = 0.05$ and 0.07, respectively). Ruminal volume, liquid turn over and liquid outflow rate did not differ ($P > 0.05$) between groups. It was concluded that ruminal infusion of butyrate increased papillae size and digesta weight but did not change liquid passage rate in the rumen of goats.

Key words: rumen papillae; rumen digesta, liquid passage rate; goat;

INTRODUCTION

Short chain fatty acids (SCFAs) are produced in the rumen through microbial fermentation of diet and absorbed by rumen papillae (Remond *et al*., 1996). Rumen absorbs the energy substances i.e. SCFAs, which fulfill 70 - 80% of energy requirement for growth, maintenance and production in the ruminants (Bergman *et al*., 1990).The papillae size (surface area) is directly related to food intake (Susan and Rosa, 1973), digesta weight in rumen (Clauss *et al*., 2009), rate of fermentation (Amaral *et al*., 2005), and weight of rumen (Roth *et al*., 2009). The diet influences rumen papillae surface area and eventually the rumen efficiency (Gabel *et al*., 1987; Bannink *et al*., 2008) and animal health. Butyrate has been shown to have potent effects on papillae size (Sander *et al*., 1959; Tamate *et al*., 1962) through increasing epithelial proliferation and reducing cell apoptosis (Kauffold *et al*., 1977; Sakata and Tamate, 1978; Mentschel *et al*., 2001). Increasing ruminal butyrate concentration through dietary manipulation led to increase papillae size, feed intake, rumen fermentation rate and nutrient absorption in sheep and goats (Gabel *et al*., 1987; Shen *et al*., 2004). Dietary supplement of butyrate has been shown to improve growth performance in young calves, which is proposed to be mediated at least in part, by improved rumen efficiency (Sander *et al*., 1959; Tamate *et al*., 1962; Vidyarthi and Kurar *et al*., 2001; Guilloteau *et al*., 2009). SCFA are removed (clearance) from the reticulorumen by 2 processes: absorption through the wall and passage with the rumen fluid through the reticulum-omasum orifice (Peters *et al*., 1990). The extent of digesta retention and the rate of liquid passage in the rumen are two important factors which determine the efficiency of rumen (Allen *et al*., 1997; Le Gall *et al*., 2009). Butyrate treatment extends the digesta retention in stomach of pigs ((Le Gall *et al*., 2009) and increases digestive efficiency (Clauss *et al*., 2009). However, it has not been completely understood that how butyrate influences the rumen efficiency. The purpose of present study was to evaluate the effects of ruminal butyrate infusion on papillae morphology, digesta weight and liquid passage rate in rumen.

MATERIALS AND METHODS

The experimental design and procedures were approved by the Animal Care and Use Committee of Nanjing Agricultural University under the act "The State Science and Technology Commission of P. R. China, 1988". All chemicals and reagents used in this study were purchased from Shanghai Shiye Chemical Reagent Co. Ltd., Shanghai; except otherwise stated.

Animals, feeding and infusion procedure: Fifteen ruminally-fistulated (Boer × Yangtze River Delta White) goats of age approximately 120 days and average body weight 20 kg at the commencement of experiment were used in this study. Goats were fed concentrates 200 g/day divided into two portions on 0800 and 1700 and hay *ad libitum*. The concentrate was composed of ground corn,

soybean meal, cottonseed bran, wheat bran, fish meal, calcium phosphate, trace mineral salt and vitamin premix (vitamin A, vitamin D and vitamin E). The metabolizable energy (ME) content (MJ/kg DM) was 10.85 and 6.96 for concentrate and hay respectively. Water was always available for animals. Goats were randomly assigned into two groups viz control (C, n=7) and butyrate (B, n=8). The goats in both group were given 50 ml of 0.1 M potassium phosphate buffer intraruminally with sodium n-butyrate (Merck, Hohenbrunn, Germany) at the dose rate of 0.3 $g.kg.BW^{-1}.d^{-1}$ (B) or without butyrate treatment (C). After infusion the rumen content was thoroughly mixed through fistula to ensure the uniform distribution of infusates throughout the rumen. The infusion was done within 10-15 sec at 0700 h every day, 1 h before morning feeding and lasted for 28 days.

Sampling and measurements: Rumen fluid samples were taken on d 14, just before infusion as 0 and 0.5, 1.0, 1.5, 2.5, and 3.5 h after the infusion. Rumen fluid was strained and pH was measured immediately. The sample was then stored frozen for VFA analysis after addition of 1ml of 10% $HgCl_2$ to 10 ml rumen fluid (Robert *et al*., 1962). Animals were slaughtered on d 28. Immediately after slaughter the abdomen was cut opened and the complex-stomach was exteriorized and the full weights were recorded. The contents collected from rumen, omasum and abomasum were weighed. Rumen was washed with PBS and rumen tissues from the atrium ruminis were collected and fixed in 4% paraformaldehyde solution until analyzed for papillae morphology. To evaluate the histo-morphology the anterior rumen tissue were collected from six goats of each group. The samples were fixed in 4% paraformaldehyde overnight, dehydrated, cleared and embedded in paraffin. Sections of 5-7µm thickness were cut and stained by H&E procedure. Four papillae were determined for each goat, and three visual fields for each papilla. The morphometry was done using Image-Pro Plus 6.0, Media Cybernetic Inc.). The height and thickness of papillae and the thickness of epithelial layer were measured. Ruminal SCFAs were determined by chromatograph HP6890N (Agilent Technologies, Delaware, USA) as described by Yang *et al*. (2012). Nitrogen (99.99% purity) was used as carrier gas with a constant flow rate of 2.8 ml/min and a split ratio of 1:30. Capillary column temperature was set to 140°C for 4 min and then raised at 25°C /min to 240°C. The temperature of the injection port and the FID were set to 180°C and 250 C. Tiglic acid was used as an internal standard.

Ruminal liquid passage: Liquid passage from rumen was estimated on d 26 by Cobalt-ethylene diamine tetracetatic acid (Co-EDTA) pulse dosing method (Penner *et al*., 2009). Each goat received a 20 ml solution containing 167.5 g Co-EDTA buffered to pH 6.8 with NaOH. The Co-EDTA solution was thoroughly mixed with ruminal contents. An initial sample was collected immediately before the marker dose (time 0), and subsequent ruminal fluid samples were collected at 0.25, 0.5, 1, 2, 3, 6, 12, 18, and 24 h after the dose. Individual samples of ruminal fluid were analyzed for Co concentration. Ruminal Co concentration was determined by atomic absorption (Perkin Elmer Optima 2100 DV). The exponential rate of decay for Co was calculated as described by Penner *et al*. (2009) using the equation: $R_t = R_0 \times e^{-k \times t}$, where: R_t = concentration at a given time, R_0 = concentration at time 0, k = fractional rate of clearance and t = time, h.

Statistical analysis: Data were presented as Mean ± SEM. Differences considered significant at $P < 0.05$ were determined by Student's t test using statistical software SPSS12.0 (StatSoft, Tulsa, OK, USA)

RESULTS AND DISCUSSION

Ruminal fermentation pattern is shown in figure 1. At 30 minutes after infusion the molar concentration of butyrate significantly increased ($P < 0.01$) and remained elevated for 3.5 h. Molar concentration of acetate and propionate remained unchanged between the groups. Molar concentration of total SCFA increased ($P < 0.05$) in B for 1 h and then returned to normal. The pattern of rumen fermentation exhibited by goats after butyrate infusion in our study was similar to that of cattle that received intraruminal infusion of butyrate (Shen *et al*., 2005).

The size of rumen papillae is greatly influenced by the SCFA in the rumen and this effect depends upon the type and the concentration of specific SCFA to which they are exposed (Bannink *et al*., 2008). Sander *et al*. (1959) and Tamate *et al*. (1962) compared the effects of acetate, propionate and butyrate on rumen of young calves. They found that intraruminal infusion of individual SCFA increased papillae size, but the effect of butyrate infusion was comparatively greater than acetate or propionate infusion. In present study, the histomorphometric analysis (Table 1) showed that papillae height increased in B (2598 ± 49) compared to C (2362 ± 19; $P < 0.005$) and the mean distance between two papillae decreased in B (773 ± 18) compared to C (958 ± 23; $P < 0.05$). These results indicate that ruminal butyrate induced the papillae growth by causing the enlargement of the papillae size and the papillae density. The positive effect of butyrate on rumen papillae size was confirmed by the previous studies on bulls (Shen *et al*., 2005), sheep and goats (Gabel *et al*., 1987; Shen *et al*., 2004). The change in papillae size is usually associated with change in epithelial tissue mass. It has been observed that butyrate treatment causes increase of rumen epithelial thickness (Kauffold *et al*., 1977; Gorka *et al*., 2009). In the present study, the wall thickness of papillae

in B (387 ± 16) was not different from that in C (413 ± 27; P = 0.434) however the thickness of epithelium increased in B (218 ± 3) compared to C (199 ± 4; P < 0.05). The synchronous increase of papillae height, thickness and its density in epithelium of B shows an increase of papillae absorptive area.

Full weights of complex-stomach expressed in kg and expressed as percentage of final body weight were not different (P > 0.05) between the groups. However, expressed as percentage of total stomach the full weight of rumen increased in B (89.09 ± 0.71 %) compared to C (86.71 ± 0.62 %; P < 0.05) and the full weight of omasum decreased in B (4.26 ± 0.09 %) compared to C (4.92 ± 0.28 %; P = 0.07) as shown in Table 2. The digesta weights in complex-stomach were also affected by the butyrate infusion (Table 3). Full weights (kg) of digesta were not different (P > 0.05) between the groups. However, expressed as percentage of complex-stomach the digesta weight in rumen in B (91.72 ± 0.59 %) was higher than in C (89.81 ± 0.54 %; P = 0.06). The full weight of omasum was lower in B (3.27 ± 0.12 %) compared to C (4.04 ± 0.30 %; P = 0.05). Previous report showed that butyrate caused heavier digesta weight in colon of pigs (Nofarias *et al*., 2007). The lower omasum digesta weight in B compared to C indicated the decreased solid digesta flow from rumen and increased digesta retention time in the rumen. The extent of digesta retention is one of the important factors that determine the efficiency of digestion in various sections of the GIT (Van Soest, 1994). Zimmerman *et al*. (2006) observed the higher rumen digesta weight associated with improved papillae surface enlargement factor (SEF), papillae length and width in the deer. This agreed with our observation that rumen papillae size increased and rumen digesta retention time prolonged in butyrate infused group. The dietary butyrate supplement has been shown to increase digestibility in young calves (Vidyarthi and Kurar, 2001; Guilloteau *et al*., 2010). Le Gall *et al*. (2009) reported that oral administration of butyrate increased feed intake and extend the digesta retention in stomach of pigs. Clauss *et al.* (2009) reported that longer retention time in herbivores increases digestive efficiency. It is possible that ruminal butyrate infusion improved the rumen efficiency by extending the digesta retention in rumen and by enlarging the rumen papillae absorptive area.

Ruminal liquid parameters i.e. ruminal volume, liquid turn over / h) and liquid outflow were not different between the groups (table 4). The rate of liquid passage is an important characteristic which affects the quantity of SCFA absorbed from the rumen. SCFAs are removed from the rumen by absorption across the rumen wall and by passage from the rumen through the omasal orifice (Allen, 1997). Although some acids pass through the omasal orifice with particulate matter, liquid flow is much greater than particle passage because of a larger pool size and a faster turnover rate in liquid flow (Allen, 1997). It has been suggested that the fractional passage of SCFA from rumen is equal to the liquid passage rate (Penner *et al*., 2009). In the present study the concentrations of butyrate and the total SCFAs were higher in B compared to C during the time 1-3.5 h after butyrate infusion. Since the liquid passage rate did not differ between two groups, the absorption of SCFA from rumen wall should be greater in goats of B group. This is consistent with Krehbiel *et al*. (1992), who observed that increased ruminal butyrate concentration led to increase of SCFA absorption from rumen of sheep.

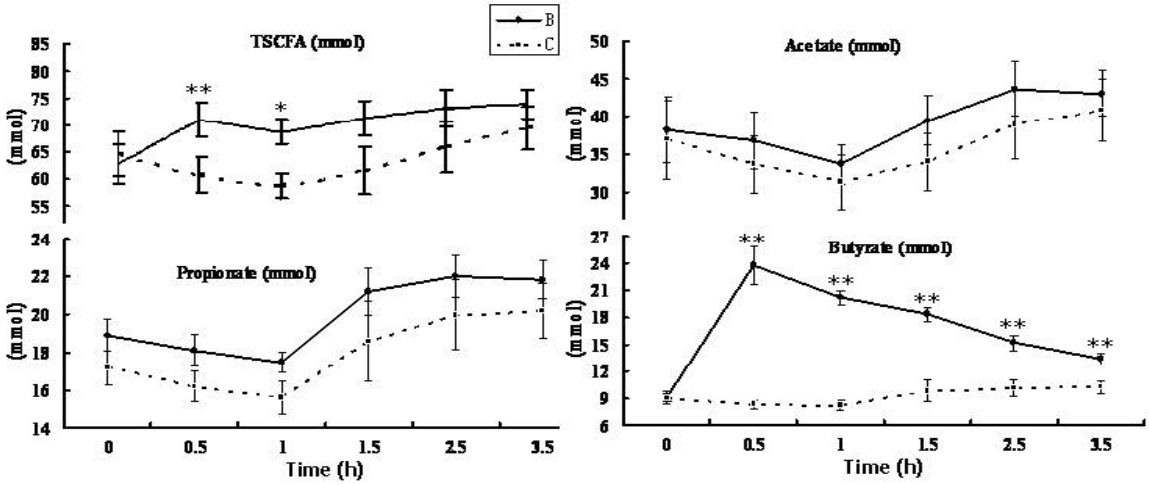

Figure 1. Effect of ruminal butyrate infusion on ruminal SCFA (Molar) concentration at different time points in goats. Goats were infused with sodium butyrate at 0.3 (B, n = 8) or 0 g/kg.BW/day (C, n = 7) in 0.1 M potassium buffer (50ml) over 28 d. Values are presented as mean±SEM Significance was considered at P < 0.05

Different from C, ∗P < 0.05, ∗∗P < 0.01

Table 1. Effects of ruminal butyrate infusion on histomorphometric parameters of papillae in rumen of goats

Papillae characteristics, µm	B	C	P-value
Papillae height	2598.03 ± 48.8	2362.38 ± 19.02	0.004
Papillae thickness	386.53 ± 16.37	413.19 ± 27.28	0.434
Inter-papillae distance	772.93 ±17.55	957.53 ± 23.15	0.001
Epithelial thickness	218.24±3.53	199.69 ± 4.41	0.017

Goats were infused with sodium butyrate at 0.3 (B, n = 8) or 0 g/kg.BW/day (C, n = 7) in 0.1 M potassium buffer (50ml) over 28 d. Rumen papillae samples from 6 goats of each group were used for morphological evaluation.
Values are means ± SEM. Significance was considered at $P < 0.05$

Table 2. Effects of ruminal butyrate infusion on full weights of various compartments of complex stomach of goats

Items	B	C	P-value
Full weights, kg			
Rumen	4.71 ± 0.35	4.21 ±.32	0.34
Omasum	0.23 ± 0.01	0.24 ± 0.01	0.58
Abomasum	0.37 ± 0.01	0.41 ± 0.04	0.43
Total stomach	5.31 ± 0.36	4.85 ± 0.36	0.40
Full weights (% of Final body weight)			
Rumen	22.37 ± 0.86	20.59 ± 1.65	0.38
Omasum	1.09 ± 0.02	1.14 ± 0.02	0.20
Abomasum	1.66 ± 0.13	1.99 ± 0.20	0.22
Total stomach	25.09 ± 0.84	23.73 ± 1.82	0.52
Full weights (% of total stomach weight)			
Rumen	89.09 ± 0.71	86.71 ± 0.62	0.04
Omasum	4.26 ± 0.09	4.92 ± 0.28	0.07
Abomasum	7.09 ± 0.47	8.38 ± 0.57	0.13

Goats were infused with sodium butyrate at 0.3 (B, n = 8) or 0 g/kg.BW/day (C, n = 7) in 0.1 M potassium buffer (50ml) over 28 d. Values are presented as mean±SEM Significance was considered at $P < 0.05$

Table 3. Effect of ruminal butyrate infusion on digesta weights in various compartments of complex stomach of goats

Items	B	C	P-value
Digesta weights, kg			
Rumen	4.40 ± 0.35	3.96 ± 0.30	0.37
Omasum	0.16 ± 0.01	0.18 ± 0.00	0.15
Abomasum	0.24 ± 0.01	0.28 ± 0.05	0.46
Total stomach	4.80 ± 0.36	4.41 ± 0.35	0.47
Digesta weights (% of Final body weight)			
Rumen	20.94 ± 1.00	19.35 ± 1.47	0.40
Omasum	0.77 ± 0.02	0.81 ± 0.03	0.19
Abomasum	1.13 ± 0.06	1.35 ± 0.23	0.39
Total stomach	22.81 ± 0.96	21.55 ± 1.67	0.54
Digesta weights (% of total stomach weight)			
Rumen	91.72 ± 0.59	89.81 ± 0.54	0.06
Omasum	3.27 ± 0.12	4.04 ± 0.30	0.05
Abomasum	5.02 ± 0.48	6.15 ± 0.77	0.26

Goats were infused with sodium butyrate at 0.3 (B, n = 8) or 0 g/kg.BW/day (C, n = 7) in 0.1 M potassium buffer (50ml) over 28 d. Values are presented as mean±SEM Significance was considered at $P < 0.05$

The development and renewal of rumen papillae depend on adequate energy and protein intake. An increase of dietary energy intake leads to an enlargement of rumen papillae surface area and nutrient absorptive rate in goats (Shen *et al*., 2004), However, the inducement of papillae growth by high dietary energy intake needs 4 – 6 w (Shen *et al*., 2004) of concentrate feeding, which also leads to increase of the financial cost in animal production and the deposition of fat in body of ruminant animals. Butyrate now has been considered as a potent regulator in epithelial cells growth for its roles in increase of cell proliferation and reduction of apoptosis (Mentschel *et al*., 2001). The effect of butyrate has been recognized through the epigenetic mechanism of inhibiting the histone deacetylation (Daroquiand and Augenlicht, 2010). In rumen epithelial cells, increases of cyclin D1 mRNA expression has been observed within 3 h and cell proliferation observed 24 h following butyrate treatment (data not shown). If the butyrate can be applied to calves and early lactation cows, it might cause a rapid rumen papillae growth and consequently improve the nutrients and energy substances absorption in animals.

Table 4. Effects of ruminal butyrate infusion on volume, liquid flow and turn over in rumen of goats

Item	B	C	P value
Rumen liquid Volume, l[1]	2.12 ± 0.09	2.03 ± 0.06	0.440
Rumen Liquid flow, l/h[3]	0.36 ± 0.02	0.35 ± 0.009	0.543
Rumen turn over, / h	0.083 ± 0.01	0.087 ± 0.04	0.73

[1]estimated as Co (dose in mg) infused in the rumen divided by ruminal Co (mg/l) concentration at time zero (Cr 0), [2] estimated as the fractional clearance rate of Co (h^{-1}) ×Rumen liquid volume (l), [3] equal to the fractional clearance rate of Co (h^{-1}).
Goats were infused with sodium butyrate at 0.3 (B, n = 8) or 0 g/kg.BW/day (C, n = 7) in 0.1 M potassium buffer (50ml) over 28 d. Values are presented as mean±SEM Significance was considered at $P < 0.05$

In conclusion, the present study showed that the ruminal infusion of butyrate caused changes in rumen: an increase of papillae absorptive area and digesta weight accompanied by an increase of SCFAs concentration, but did not change liquid passage rate from rumen to omasum. Taken together, these data indicated an increase of SCFA absorption from the rumen.

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