Effects of Disodium Fumarate on Growth Performance, Rumen Fermentation Function, and Gastrointestinal Tract Development in Early-Weaned Lambs: Postprint

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Abstract

This study aimed to investigate the effects of dietary supplementation of disodium fumarate on growth performance, rumen fermentation function, and gastrointestinal development in early-weaned lambs. Thirty male lambs aged (50±5) days with a body weight of (25±2) kg were selected and randomly divided into 3 groups with 10 lambs per group. The control group was fed a basal diet, while the experimental groups were supplemented with 0.5% and 1.0% disodium fumarate on top of the basal diet. The experimental period lasted 70 days. The results showed: 1) Dietary supplementation of disodium fumarate increased average daily gain, with the 1.0% group being significantly higher than the control group ($P<0.05$). 2) Disodium fumarate did not significantly affect rumen fluid pH ($P>0.05$), but significantly reduced ammonia nitrogen concentration ($P<0.05$); disodium fumarate significantly reduced rumen fluid lactic acid concentration ($P<0.01$); the 1.0% group had significantly higher concentrations of total volatile fatty acids, acetate, and propionate in rumen fluid than the control group ($P<0.05$). 3) The small intestinal villus height in the experimental groups was higher than that in the control group, with the villus height in the duodenum, jejunum, and ileum of lambs in the 0.5% group being significantly increased by 30.3%, 30.6%, and 46.1%, respectively ($P<0.05$); the villus height/crypt depth ratio in the duodenum of the experimental groups was significantly greater than that of the control group ($P<0.05$), with the villus height/crypt depth ratio in the 0.5% group being increased by 58.6% compared to the control group; the papilla height of the rumen wall in the 0.5% and 1.0% groups was increased by 139.84 and 156.74 m compared to the control group, respectively ($P>0.05$); there was no significant difference in rumen wall papilla density between the experimental groups and the control group ($P>0.05$). The results indicate that supplementation with disodium fumarate significantly im-

proved the production performance of lambs and promoted rumen and intestinal development in early-weaned lambs.

Full Text

Preamble

Effects of Disodium Fumarate on Growth Performance, Ruminal Fermentation Function, and Gastrointestinal Tract Development of Early-Weaned Lambs

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Abstract: This study investigated the effects of dietary disodium fumarate (DF) supplementation on growth performance, ruminal fermentation function, and gastrointestinal tract development in early-weaned lambs. Thirty male lambs aged (50±5) days with an initial body weight of (25±2) kg were randomly assigned to three groups of ten animals each. The control group received a basal diet, while the experimental groups were supplemented with 0.5% and 1.0% DF, respectively. The trial lasted 70 days. The results showed: 1) DF supplementation increased average daily gain, with the 1.0% group being significantly higher than the control ($P<0.05$). 2) DF did not significantly affect ruminal pH ($P>0.05$) but significantly reduced ammonia nitrogen concentration ($P<0.05$) and markedly decreased ruminal lactic acid concentration ($P<0.01$). The 1.0% group exhibited significantly higher concentrations of total volatile fatty acids, acetate, and propionate compared to the control ($P<0.05$). 3) Intestinal villus height in the experimental groups exceeded that of the control, with the 0.5% group showing significant increases of 30.3%, 30.6%, and 46.1% in duodenal, jejunal, and ileal villus height, respectively ($P<0.05$). The villus height-to-crypt depth ratio in the duodenum was significantly greater in the experimental groups ($P<0.05$), increasing by 58.6% in the 0.5% group. Papilla height on the ruminal wall increased by 139.84 m and 156.74 m in the 0.5% and 1.0% groups, respectively ($P>0.05$), while papilla density showed no significant difference from the control ($P>0.05$). These findings indicate that DF supplementation significantly improved lamb productivity and promoted ruminal and intestinal development in early-weaned lambs.

Keywords: disodium fumarate; weaning lambs; growth performance; gastrointestinal tract

Organic acids such as acetic acid, malic acid, citric acid, and their sodium salts have long been widely used as food additives and flavoring agents [1-3] and are extensively applied in monogastric animals [4-5]. However, their use as feed additives in ruminants remains relatively uncommon [6-7]. Recent research demonstrates that organic acids like fumaric acid can improve ruminal fermentation in ruminants [8], promote the growth of lactic acid-utilizing bacte-

ria, reduce lactic acid production, ultimately increase ruminal pH, and alleviate metabolic disorders such as ruminal acidosis [8]. Studies in dairy cattle have also shown that fumaric acid supplementation promotes cellulolytic bacterial growth and enhances ruminal cellulose digestibility [9-10]. These results suggest that fumaric acid and its sodium salts may have potential as feed additives for ruminants. However, previous research on fumaric acid and its sodium salts has primarily focused on adult ruminants such as lactating dairy cows and meat sheep. The effects of disodium fumarate (DF) on growth performance and digestive system development in early-weaned lambs remain unclear. To elucidate the effects of DF on the growth and development of early-weaned lambs, this study investigated the impacts of dietary DF supplementation on growth performance and development of different intestinal segments, aiming to provide a scientific theoretical basis for the application of DF as a feed additive in lamb diets.

1.1 Experimental Materials and Location

DF (Shaanxi Jiaotong Ruisen Weinan Chemical Industry Co., Ltd., 2014) with a purity 98% was used. The experiment was conducted at the breeding sheep farm of Xinhu Zongchang Daxibei Animal Husbandry Co., Ltd.

1.2 Experimental Design

Thirty first-cross Suffolk × German Mutton Merino lambs with similar body condition, weight, and age were selected and weaned at approximately (50±5) days of age. They were randomly divided into three groups (one control and two experimental groups) of ten lambs each. The trial lasted 70 days. A total mixed ration was fed twice daily at 10:00 and 18:00, with free access to clean water. The control group received the basal diet, while the two experimental groups were supplemented with 0.5% and 1.0% DF (air-dry basis), respectively. Dietary crude protein content was determined using the Kjeldahl method as described by Zhang Liying [11], calcium by potassium permanganate method, total phosphorus by phosphomolybdic blue spectrophotometry, crude fat by Soxhlet extraction, and neutral detergent fiber and acid detergent fiber by the Van Soest method.

The composition and nutrient levels of the basal diet are presented in Table 1.

Table 1 Composition and Nutrient Levels of Basal Diets (%)

Items	Content
Ingredients (air-dry basis)	
Corn powder	
Alfalfa powder	
Maize silage	
Concentrate supplement	

Items	Content
Cottonseed hull	
Dried hay	
Total	
Nutrient levels (DM basis)	
Crude protein (CP)	
Calcium (Ca)	
Total phosphorus (TP)	
Neutral detergent fiber (NDF)	
Acid detergent fiber (ADF)	
Crude fat (EE)	

The concentrate supplement (produced by Huafeng Husbandry Tech., Co., Ltd.) contained: puffed corn, starch, whey powder, milk powder, sugar, puffed soybean, soybean meal, wheat powder, wheat bran, plant oil, corn gluten meal, milk replacer, amino acid, NaCl, multi-microelements, multi-vitamins, limestone, and CaHPO . Nutrient levels were: CP 17%-19%, Ca 0.6%-1.2%, P 0.4%-1.0%, NaCl 0.4%-1.0%, Lys 0.78%.

1.3.1 Sample Collection

Daily feed refusals were collected and weighed for each group. Lambs were weighed on an empty stomach on days 1 (initial weight) and 70 (final weight) to determine growth performance and calculate feed-to-gain ratio. On day 70, before morning feeding, four lambs were randomly selected from each group (twelve total) and slaughtered by jugular exsanguination. Approximately 2 cm segments were immediately excised from the proximal duodenum (5 cm from the origin), the proximal and distal quarter segments of the jejunum, and the mid-ileum. After gentle rinsing with 0.9% saline solution, samples were fixed in 4% paraformaldehyde solution for determination of villus height, crypt depth, muscularis thickness, and mucosal layer thickness in different intestinal segments [12]. A 2 cm × 3 cm section of ruminal wall from the dorsal sac region was collected and fixed in 4% paraformaldehyde solution for measurement of papilla height and density.

Ruminal fluid from slaughtered lambs was mixed, filtered through four layers of gauze, and immediately analyzed for pH using a portable pH meter (PHB-4). Three 2 mL aliquots of filtered ruminal fluid were collected: one mixed with 0.4 mL of 25% metaphosphoric acid and crotonic acid (internal standard: 0.6464 g crotonic acid per 100 mL metaphosphoric acid solution) and stored at -20°C for volatile fatty acid analysis by gas chromatography (Agilent 7890B) [13]; one mixed with an equal volume of 0.2 mol/L HCl and stored at -20°C for ammonia nitrogen determination [13]; and one stored at -20°C for lactic acid concentration measurement using a commercial kit (Lactic Acid Assay Kit A019-2, Nanjing Jiancheng Bioengineering Institute).

1.3.2 Gastrointestinal Tract Development Measurement

Intestinal tissues were dehydrated in graded ethanol series, cleared in xylene, embedded in paraffin, sectioned (6-8 m thickness), stained with hematoxylin-eosin (HE), and mounted. Five sections were prepared from each intestinal segment, with five fields photographed per section. Villus height (measured from the crypt-villus junction to the villus tip), crypt depth (from the crypt-villus junction to the crypt base), mucosal layer thickness (from the crypt-villus junction to the submucosa), and muscularis thickness (from the submucosa to the serosa, comprising inner circular and outer longitudinal smooth muscle layers) were measured using Motic-Image-Advanced 3.2 software [14] (Figure 1 [Figure 1: see original paper]).

Ruminal tissue samples were processed into paraffin sections and stained with HE. For each lamb, four intact, clear sections were selected from one tissue block from both the dorsal and ventral caudal blind sac regions. Papilla height was determined as the average height of five papillae measured using Motic-Image-Advanced 3.2 software. Papilla density was quantified as the number of papillae per cm^2 under a stereomicroscope (Figure 2 [Figure 2: see original paper]).

1.4 Data Processing and Analysis

Data were organized using Excel and subjected to one-way ANOVA using SPSS 13.0 software. Results are expressed as means ± standard error. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$. When significant differences were detected, Duncan' s multiple comparison test was applied.

2.1 Growth Performance

As shown in Table 2 , average daily gain in the 0.5% and 1.0% groups increased by 3.14 g and 55.44 g compared to the control group, respectively, with the 1.0% group exhibiting significantly higher average daily gain than the control ($P<0.05$).

Table 2 Effects of DF on Growth Performance of Lambs

Items	Control Group	0.5% Group	1.0% Group
Initial weight (kg)	24.32±0.88	25.17±0.93	26.33±0.73
Final weight (kg)	31.85±0.97b	33.30±1.38b	35.60±1.43a
Average daily gain (g)	99.86±7.89b	103.00±10.96b	155.30±16.93a
Average daily intake (g)	-	-	-
Feed/gain ratio (F/G)	-	-	-

In the same row, values with no letter or the same letter superscripts indicate no significant difference ($P>0.05$), different lowercase letters indicate significant

difference (P<0.05), and different capital letters indicate extremely significant difference (P<0.01). The same applies below.

2.2 Rumen Fermentation Parameters

Table 3 shows that dietary DF supplementation did not significantly affect ruminal pH (P>0.05) but significantly reduced ammonia nitrogen concentration (P<0.05). DF addition extremely significantly decreased ruminal lactic acid concentration (P<0.01). The 1.0% group exhibited significantly higher concentrations of total volatile fatty acids, acetate, and propionate compared to the control group (P<0.05). Butyrate concentration and acetate/propionate ratio remained unchanged across groups (P>0.05).

Table 3 Effects of DF on Ruminal Fermentation Parameters of Weaning Lambs (mmol/L)

Items	Control Group	0.5% Group	1.0% Group
Ammonia nitrogen (mmol/L)	7.36±1.44a	5.71±0.49b	5.69±0.66b
Lactic acid (mmol/L)	2.26±0.10Aa	1.65±0.14Bb	1.82±0.08Bb
Total volatile fatty acids (mmol/L)	60.77±11.99b	60.09±5.51b	73.30±9.40a
Acetate (mmol/L)	43.82±9.47b	43.98±4.32b	54.11±8.76a
Propionate (mmol/L)	9.71±2.10b	9.66±0.74b	11.52±1.02a
Butyrate (mmol/L)	4.35±0.87	4.86±0.93	4.00±0.92
Acetate/propionate ratio	4.56±0.51	4.57±0.36	4.64±0.57

2.3 Digestive Tract Development

Table 4 shows that compared to the control group, papilla height on the ruminal wall increased by 139.51 m (8.2%) and 156.74 m (9.2%) in the 0.5% and 1.0% groups, respectively, though these differences were not significant (P>0.05). Papilla density in the 0.5% group was similar to the control, while the 1.0% group showed a decrease, but inter-group differences were not significant (P>0.05).

Duodenal villus height in the 0.5% and 1.0% groups was significantly higher than in the control group (P<0.05), with the 0.5% group showing a 30.3% increase. The 1.0% group exhibited significantly reduced duodenal crypt depth compared to the control (P<0.05), with an 18.5% decrease, while the 0.5% group showed no significant change (P>0.05). The villus height-to-crypt depth ratio (V/C) in the duodenum was extremely significantly higher in both experimental groups compared to the control (P<0.01). Muscularis thickness in the 0.5% group increased by 9.2% (P<0.05), though mucosal layer thickness in the duodenum did not differ significantly among groups (P>0.05).

Jejunal villus height was significantly higher in both the 0.5% and 1.0% groups compared to the control (P<0.05), with the 0.5% group showing a 30.6% increase. The 0.5% group also demonstrated significantly reduced jejunal crypt depth (P<0.05), decreasing by 25.6%, whereas the 1.0% group showed no significant difference from the control (P>0.05). The V/C ratio in the jejunum was significantly increased in the 0.5% group (P<0.05). Jejunal muscularis thickness in the 1.0% group was significantly higher than the control (P<0.05), increasing by 56.9%, while mucosal layer thickness did not differ significantly among groups (P>0.05).

Ileal villus height was significantly elevated in the 0.5% group (P<0.05), showing a 46.1% increase compared to the control. Neither experimental group showed significant differences in ileal crypt depth from the control (P>0.05), though the 0.5% group exhibited a 17.9% reduction. The ileal V/C ratio did not differ significantly among groups (P>0.05). Ileal muscularis thickness increased by 10.4% in the 0.5% group (P<0.05), while the 1.0% group showed no significant difference from the control (P>0.05). Mucosal layer thickness in the ileum was not significantly affected by DF supplementation (P>0.05).

Table 4 Effects of DF on Rumen and Small Intestine Development in Lambs (m)

Items	Control Group	0.5% Group	1.0% Group
Ruminal Wall Papilla			
Papilla height	1709.33±87.05	1848.84±173.84	1866.07±133.04
Papilla density (number/cm²)	172.78±29.0	176.46±14.6	144.71±11.9
Duodenum			
Villus height	393.57±51.20b	512.80±17.99a	492.53±46.51a

Items	Control Group	0.5% Group	1.0% Group
Crypt depth	451.76±58.71a	372.44±28.78ab	368.14±29.62b
V/C ratio	0.87±0.45Bb	1.38±0.66Aa	1.34±0.33Aa
Mucosa layer thickness	663.10±9.02	637.99±18.04	668.69±21.65
Muscle layer thickness	129.41±3.04b	141.26±4.70a	138.44±1.97ab
Jejunum			
Villus height	365.98±14.55b	478.08±65.69a	494.61±65.84a
Crypt depth	395.25±40.10a	293.95±24.18b	374.28±36.36a
V/C ratio	0.93±0.07a	1.64±0.32b	1.33±0.25a
Mucosa layer thickness	751.94±32.81ab	826.35±27.25b	669.69±17.62a
Muscle layer thickness	148.17±3.04a	136.81±10.04a	232.51±20.66b
Ileum			
Villus height	327.02±6.82a	477.92±83.44b	380.60±32.19a
Crypt depth	311.99±65.28	367.74±57.58	308.77±25.07
V/C ratio	1.07±0.19	1.30±0.12	1.24±0.04
Mucosa layer thickness	517.05±4.38ab	488.00±15.02b	550.29±17.20a
Muscle layer thickness	146.97±3.25b	162.26±3.53a	148.69±2.92b

In this study, DF improved the growth performance of weaned lambs, with the 1.0% supplementation level significantly increasing average daily gain. This improvement is attributed to DF' s ability to reduce ruminal lactic acid concentration, consistent with findings by Mao Shengyong [15]. Additionally, DF combines with hydrogen ions (H) in the rumen and is reduced to fumaric acid, helping maintain normal ruminal pH and facilitating microbial feed degradation [8]. The increased concentrations of total volatile fatty acids, acetate, and propionate observed in this study align with Mao Shengyong' s [15] results showing that DF supplementation in goat diets elevates volatile fatty acid concentrations.

Volatile fatty acids serve as crucial energy sources for animal tissue synthesis [16]. Furthermore, the reduced fumaric acid, an intermediate metabolite in the tricarboxylic acid cycle, can be converted to glutamine, which promotes intestinal development and enhances nutrient digestion and absorption in the rumen [17], thereby improving animal growth performance.

Intestinal villus length, crypt depth, and V/C ratio are considered important indicators of small intestinal digestive and absorptive function [18]. Intestinal villi are small projections formed by intestinal epithelium and lamina propria extending into the lumen, measuring approximately 0.35-1.00 mm in length and expanding the luminal surface area by about tenfold. Villi typically appear columnar, leaf-shaped, or finger-like [19]. Increased villus height expands the absorptive surface area for nutrients, enhancing nutrient absorption and animal growth. Our results demonstrate that DF supplementation significantly increased intestinal villus height while reducing crypt depth in the duodenum and jejunum, thereby promoting nutrient absorption. This likely occurs because dietary DF is reduced to fumaric acid, an intermediate in the tricarboxylic acid cycle that can be metabolized to -ketoglutarate, a precursor for glutamine synthesis. Increased glutamine availability stimulates differentiation and development of intestinal epithelial cells, consistent with reports by Souba [20] and Reeds et al. [21] that glutamine is an essential energy substrate for rapidly growing and dividing cells such as lymphocytes and intestinal mucosal epithelial cells.

Buddle et al. [22] proposed that the villus height-to-crypt depth ratio reflects the balance between absorptive function of villus epithelial cells and secretory function of crypt epithelial cells. The elevated V/C ratios observed in our experimental groups align with Yan Jiayou' s [23] findings that compound acidifiers increase V/C ratio in weaned piglets. Research also indicates that small intestinal mucosal and muscular layers are closely related to rhythmic contractile activity and mechanical digestion efficiency of chyme [24], and are essential for maintaining normal digestive and absorptive functions [25]. Our results show that DF supplementation increased both mucosal and muscular layer thickness in the small intestine, demonstrating that DF can improve and maintain the normal structure of lamb small intestinal mucosa and enhance digestive and absorptive capacity.

Rumen development represents the most critical physiological transformation enabling lambs to transition from non-ruminant to ruminant status, with dietary physical form, feed type, and animal age exerting direct or indirect effects on rumen development [26-27]. Lesmeister et al. [28] reported that ruminal papilla height effectively reflects rumen development in young ruminants, whereas papilla number per unit area should not be used as an evaluation criterion. Our study demonstrated that DF supplementation increased ruminal papilla height, consistent with previous reports [29-30] that volatile fatty acids produced from microbial fermentation of dietary carbohydrates and proteins stimulate forestomach development, provide energy for mucosal growth, and pro-

mote gastrointestinal epithelial proliferation and differentiation. In this study, DF was reduced to propionate by ruminal microbes while also increasing other volatile fatty acid concentrations. Short-chain fatty acids serve as the primary energy source for colonic mucosal epithelial cells and significantly promote their proliferation and differentiation.

Conclusions

1. Dietary supplementation with 1.0% DF significantly increased average daily gain in lambs.

2. Dietary supplementation with 1.0% DF extremely significantly reduced ruminal lactic acid concentration and significantly increased ruminal total volatile fatty acids, propionate, and acetate concentrations.

3. Dietary DF supplementation increased small intestinal villus height and significantly elevated the villus height-to-crypt depth ratio in the duodenum.

4. Dietary DF supplementation showed a trend toward increasing papilla height on the ruminal wall.

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Note: Figure translations are in progress. See original paper for figures.