Effects of Lactitol and Tributyrin on Growth Performance, Small Intestinal Morphology and Enzyme Activity in Weaned Pigs*

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ABSTRACT : One hundred and sixty crossbred pigs (6.62±0.36 kg) weaned at day 18±1 were used to investigate the effects of lactitol and tributyrin on performance, small intestinal morphology and enzyme activity. The pigs were assigned to one of five dietary groups (4 pens/diet with 8 pigs/pen) and were fed the negative control diet or the negative control diet supplemented with 10 g/kg glutamine (as a positive control), or 3 g/kg lactitol (β-D-galactopyranosyl-(1→4)-D-sorbitol), or 5 g/kg tributyrin (butanoic acid 1,2,3-propanetriyl ester), or 3 g/kg lactitol+5 g/kg tributyrin. Body weight and feed intake were measured weekly during the 4-week study. On day 7, four pigs per dietary treatment were sacrificed to examine small intestinal morphology and enzyme activity. The results showed that: (1) Compared with the negative control diet, the positive control diet improved weight gain and feed efficiency during weeks 1-2 and over the entire study ($p<0.05$), and also decreased duodenal and ileal crypt depth ($p<0.05$), but did not alter intestinal enzyme activity ($p>0.05$). Lactitol improved feed efficiency during weeks 3-4 and over the entire study ($p<0.05$), but did not improve weight gain and feed intake, intestinal morphology or enzyme activity ($p>0.05$). Tributyrin improved weight gain and reduced feed/gain during weeks 3-4 and over the entire study. Tributyrin significantly decreased crypt depth in the duodenum and ileum, and increased duodenal lactase and ileal maltase activity ($p<0.05$). Lactitol+tributyrin increased weight gain during weeks 3-4 and over the entire study, and improved feed efficiency during weeks 1-2 and 3-4 and over the entire study ($p<0.05$). Lactitol+tributyrin increased the jejunal villus height, and decreased the duodenal and ileal crypt depth ($p<0.05$). Lactitol+tributyrin also increased jejunal lactase and sucrase activity ($p<0.05$). (2) Compared with the positive control, tributyrin improved weight gain and reduced feed/gain during weeks 3-4 ($p<0.05$), decreased the ileal crypt depth, and improved the duodenal lactase and sucrase activity ($p<0.05$). Lactitol+ tributyrin improved weight gain during weeks 3-4, improved feed efficiency during weeks 3-4 and over the entire study, increased the ileal villus height, and increased jejunal lactase, sucrase and maltase activity ($p<0.05$). These results showed that tributyrin improved performance, intestinal morphology and enzyme activity, while the effect of lactitol was very limited. These results also showed that, compared with glutamine, tributyrin was more effective in improving intestinal morphology and enzyme activity, and tributyrin exerted a superior effect in improving performance as weaning progressed. These observations suggest that, as a chemical for repairing intestinal atrophy, glutamine and tributyrin should be used in the first and second periods of the starter phase, respectively. (**Key Words :** Glutamine, Lactitol, Tributyrin, Small Intestinal Morphology, Enzyme Activity, Weanling Pigs)

INTRODUCTION

It is well documented that weanling can result in a series of adverse changes in the digestive tract, including villus atrophy, crypt hyperplasia, and reduced enzyme activity (Lindemann et al., 1986; Miller et al., 1986). Weanling stress is often associated with growth retardation and diarrhea of piglets, and may increase economic loss for pig producers.

Some studies have shown that several compounds, such as glutamine, butyrate and lactitol, can prevent weanling stress of piglets by providing nutrients required for the animals and particularly for specific tissues, such as the gut (Luchansky, 2000; Pluske, 2001). These compounds are also required for maintenance of intestinal microbes (Piva et al., 2002). These compounds, defined as nutribiotics, affect the host by directly improving the overall trophic status of the digestive tract (Luchansky, 2000; Pluske, 2001; Piva et al., 2002).

Glutamine is one of the most abundant amino acids in

Table 1. Ingredient composition (%) of the basal diet (as-fed basis)

Item	%
Ingredient	
Corn	54.40
Soybean meal	22.50
Dried whey	8.00
Fish meal	6.50
Spray-dried porcine plasma	3.00
Soy oil	2.30
Dicalcium phosphate	1.20
Limestone	0.75
Salt	0.15
L-lysine.HCl	0.20
Vitamin and mineral premix[1]	1.00
Nutrient composition[2]	
Digestible energy (kcal/kg)	3,270
Crude protein	20.10
Calcium	0.94
Total phosphorus	0.75
Lysine	1.50
Methionine+cystine	0.72

[1] Provided the following amounts of vitamins and trace minerals per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D_3, 2,200 IU; vitamin E, 32 IU; vitamin K_3, 3.5 mg; vitamin B_{12}, 0.015 mg; riboflavin, 4.5 mg; niacin, 35 mg; pantothenic acid, 16 mg; choline chloride, 450 mg; folic acid, 0.8 mg; thiamin,1.5 mg; pyridoxine, 3.2 mg; biotin, 0.12 mg; Zn, 80 mg; Mn, 20 mg; Fe, 80 mg; Cu, 100 mg; I, 0.45 mg; Se, 0.32 mg.
[2] Digestible energy and amino acids were calculated; the other nutrients were analyzed.

animal blood and milk (Wu et al., 1994). It is an important energy substrate for intestinal mucosal cells, and plays an important role in repairing impaired enterocytes and maintaining local immune response (Souba et al., 1990; Lee et al., 2003). Tributyrin and lactitol are dietary and fermentable sources of butyrate, respectively (Piva et al., 2002). n-Butyrate is the main energy substrate for colonocytes (Roediger, 1980), and is an effective anti-proliferation and anti-differentiation agent in various cell lines (Scheppach et al., 1997; von Engelhardt et al., 1998; Schroder et al., 1999), thereby exerting an important impact on maintaining normal intestinal morphology (Piva et al., 2002).

Many studies have shown that glutamine can help maintain the normal structure and function of the small intestine in pigs (Ayonrinde et al., 1995; Wu et al., 1996; Liu, 2002; Liu et al., 2002; Shinzato et al., 2003; Lee et al., 2003). In contrast, little research has been conducted to investigate the effects of tributyrin and lactitol in weaned piglets. The objective of the present study was to determine the effects of tributyrin, lactitol and their combination as sources of specific nutrients for gut tissues on gastrointestinal structure and function as well as the performance of weanling pigs, with glutamine provided as a positive control.

MATERIALS AND METHODS

Animals and experimental design

The animal protocol for this research was approved by the Animal Care and Use Committee of Hubei Province. One hundred sixty crossed pigs (Landrace×Yorkshire) weaned at age 18±1 days were used in this experiment. From day 18 to 21 after birth, the pigs were fed on the negative control diet (Table 1). On day 21, the pigs (6.62±0.36 kg) were moved from the piggery to the nursery room, and assigned to one of five dietary treatments, balanced for initial body weight and ancestry. The negative control diet was formulated to meet or exceed NRC (1998) requirements for all nutrients. The five dietary treatments were as follows: 1) negative control diet (CTR); 2) negative control diet supplemented with 10 g/kg glutamine as positive control (GLN) (Chemical Abstract number 20030409, Jiangxi Zhicheng Biotechnic Company, Jiangxi, China); 3) negative control diet supplemented with 3 g/kg lactitol (LCT) (β-D-galactopyranosyl-(1→4)-D-sorbitol, Chemical Abstract number 20030305, Jiangxi Zhicheng Biotechnic Company, Jiangxi, China); 4) negative control diet supplemented with 5 g/kg tributyrin (TRB) (butanoic acid 1,2,3-propanetriyl ester, Chemical Abstract number 20030506, Wuhan Reagent Company, Wuhan, China); and 5) negative control diet supplemented with 3 g/kg lactitol and 5 g/kg tributyrin (LCT+TRB). Each dietary treatment was fed to four replicate pens (two pens of castrated males and two pens of females) with 8 pigs per pen. The pigs were housed in 1.80×2.5 m^2 pens with slatted floors, equipped with a feeder and a nipple waterer to allow pigs *ad libitum* access to feed and water. All the feed was pelleted. Room temperature was maintained at 25 to 27°C. Lighting was natural (April to May).

Measurement of body weight and feed consumption

The experiment lasted for four weeks. Body weight and feed intake were measured weekly. Pigs with diarrhea were recorded daily. Diarrhea incidence was calculated according to the method of Kelly (1990).

Tissue collection for morphology and enzyme assay

On day 7 after the feeding trial began (when pigs were 28 days old), two castrated males and two females from each dietary treatment were weighed and sacrificed with a 5 ml intra-cardiac injection of Euthestatew (pentobarbital sodium 200 mg/ml; Ceva Sante Animale B.V., Maasluis, The Netherlands). A midline laparotomy was performed. The abdomen was incised, and the small intestine was removed. The small intestine was cut into three 2×3 cm segments and another three 10 cm segments in length at 25%, 50% and 75% of total intestinal length (i.e, proximal, mid jejunum and distal ileum).

Table 2. Effects of lactitol and tributyrin on growth performance of piglets[1]

Item	Diets[2]					SEM[3]	p values[4]
	CTR	GLN	LCT	TRB	LCT+TRB		
Weeks 1-2							
Average daily gain (kg/d)	0.158 [a]	0.201 [b]	0.165 [a]	0.180 [ab]	0.187 [ab]	0.015	0.075
Average daily feed intake (kg/d)	0.292	0.303	0.299	0.300	0.266	0.020	0.384
Feed/gain (kg/kg)	1.85 [c]	1.52 [ab]	1.83 [c]	1.67 [bc]	1.42 [a]	0.09	0.001
Diarrhea incidence (%)	11.78 [b]	6.89 [a]	9.02 [a]	8.16 [a]	6.51 [a]	1.24	0.005
Weeks 3-4							
Average daily gain (kg/d)	0.458 [a]	0.482 [a]	0.481 [a]	0.520 [b]	0.527 [b]	0.016	0.002
Average daily feed intake (kg/d)	0.702 [ab]	0.713 [b]	0.665 [a]	0.717 [b]	0.693 [ab]	0.019	0.103
Feed/gain (kg/kg)	1.54 [b]	1.48 [b]	1.39 [a]	1.38 [a]	1.32 [a]	0.04	<0.001
Diarrhea incidence (%)	2.04	2.38	1.70	1.70	1.70	0.76	0.860
Weeks 1-4							
Average daily gain (kg/d)	0.308 [a]	0.341 [bc]	0.323 [ab]	0.350 [c]	0.357 [c]	10.5	0.002
Average daily feed intake (kg/d)	0.497	0.508	0.482	0.508	0.479	0.014	0.176
Feed/gain (kg/kg)	1.61 [c]	1.50 [b]	1.49 [b]	1.45 [b]	1.34 [a]	0.02	<0.001
Diarrhea incidence (%)	6.91 [b]	4.63 [a]	5.36 [ab]	4.93 [a]	4.10 [a]	0.86	0.049

[1] Data are means of four replicate pens of eight pigs each replicate.
[2] Negative control diet (CTR), CTR with glutamine (GLN, 10 g/kg, as a positive control) or tributyrin (TRB, 5 g/kg) and (or) lactitol (LCT, 3 g/kg).
[3] Standard error of the mean.
[4] Probability for contrast of five treatments by Duncan's Multiple Comparison.
[a, b, c] Values in the same row with different superscripts are different (p<0.05).

The 2×3 cm segments were processed, embedded, and stained according to the procedures of Luna (1968). The segments were flushed gently with ice-cold phosphate buffered saline (PBS, pH 7.4) and then fixed in 10% fresh, chilled formalin solution. The 10-cm intestinal segments were opened longitudinally and the contents were flushed with ice-cold PBS. The mucosa was scraped with a glass slide, snap-frozen in liquid nitrogen and then stored at -80°C for further analysis.

Intestinal morphology

After a 24-h fixation, the intestinal segments were taken out, and dehydrated using increasing concentrations of ethanol (70% to 100%) and chloroform. After dehydration, the segments were embedded in paraffin, and then placed in a refrigerator to make the paraffin sufficiently hard. Cross-sections of the segments were cut approximately 5 μm thick with a microtome (American Optical Co., Scientific Instrument Div., Buffalo, NY), and stained with haematoxylin and eosin. The method was according to Nabuurs et al. (1993). In each section, 10 fields were examined using a light microscope with a computer-assisted morphometric system (BioScan Optimetric, BioScan Inc., Edmonds, WA). The villus height and the associated crypt depth, mucosal thickness and villus width were measured, and then the ratio of villus height to crypt depth was calculated. Villus height is defined as the distance from the villus tip to crypt mouth, crypt depth from crypt mouth to base, mucosal thickness from mucosal epithelium to mucosal muscle layer, and villus width is the widest distance of the villus. Because of the similarity in the data of villous height, crypt depth, mucosal thickness and villus

width between sites and to reduce the amount of data, mean values were determined by averaging the measurements at the five sites along the small intestine.

Enzyme-specific activity

The mucosa was weighed and suspended in ice-cold NaCl solution (4:1, vol/wt). The mucosa was homogenized for 45 seconds, followed by centrifuging at 3,000 rpm for 15 min. The supernatant was collected and diluted with ice-cold NaCl solution (10:1, vol/vol). The protein concentration of the supernatant was determined using Folin-Phenol reagent. A volume of 100 μl diluted supernatant was dispensed into a glass test tube and incubated with respective substrate (lactose, sucrose or maltose) (100 μl, 0.056 mol/L) at 37°C for 60 min. The reaction was terminated by submerging the tubes in boiling water for 2 min. The tubes were cooled and the glucose concentration was determined (Sigma Diagnostics, St. Louis, MO, USA). Enzyme activity is expressed as micromoles of substrate converted/min/mg protein or U/mg protein.

Statistical analysis

All data were subjected to ANOVA analysis using the GLM procedures of SPSS 6.0 (SPSS Inc, 1993). The differences among group means were compared using Duncan Multiple Comparison based on the variance derived from ANOVA. Pen was used as the experimental unit for the performance data, whereas individual pig data were used as the experimental unit for intestinal morphology and enzyme-specific activity.

Table 3. Effects of lactitol and tributyrin on small intestinal morphology of piglets[1]

Item	Diets[2]					SEM[3]	p value[4]
	CTR	GLN	LCT	TRB	LCT+TRB		
Duodenum							
Villus height (μm)	369	304	303	329	384	36	0.132
Crypt depth (μm)	237[b]	163[a]	187[ab]	137[a]	165[a]	30	0.049
VH/CD[5]	1.54[a]	1.91[ab]	1.62[a]	2.53[b]	2.66[b]	0.36	0.019
Mucosal thickness (μm)	682[a]	676[a]	669[a]	619[a]	762[b]	13	0.009
Villus width (μm)	142[b]	144[b]	150[b]	113[a]	151[b]	33	0.048
Jejunum							
Villus height (μm)	299[ab]	367[bc]	240.3[a]	292.3[ab]	433[c]	46	0.007
Crypt depth (μm)	180[ab]	183[ab]	151.3[ab]	146.7[a]	200[b]	23	0.148
VH/CD[5]	1.67[ab]	2.00[a]	1.66[a]	2.03[ab]	2.14[b]	0.18	0.053
Mucosal thickness (μm)	683[ab]	669[ab]	604[a]	601[a]	698[b]	13	0.084
Villus width (μm)	128	103	111	115	122	40	0.053
Ileum							
Villus height (μm)	377[ab]	339[a]	385[ab]	413[ab]	427[b]	37	0.207
Crypt depth (μm)	198[c]	156[b]	198[c]	126[a]	145[ab]	11	<0.001
VH/CD[5]	1.90[a]	2.20[a]	1.95[a]	3.29[b]	2.96[b]	0.21	<0.001
Mucosal thickness (μm)	693	660	736	708	771	11	0.545
Villus width (μm)	151	130	136	127	133	67	0.226

[1] Data are means of four replicate pig of one pig each replicate.
[2] Negative control diet (CTR), CTR with glutamine (GLN, 10 g/kg, as a positive control) or tributyrin (TRB, 5 g/kg) and (or) lactitol (LCT, 3 g/kg).
[3] Standard error of the mean.
[4] Probability for contrast of five treatments by Duncan's Multiple Comparison.
[5] Villus height:crypt depth ratio
[a, b, c] Values in the same row with different superscripts are different (p<0.05).

RESULTS

Performance

Performance data are presented in Table 2. Compared with pigs fed the negative control diet, animals fed the positive control diet displayed a higher average daily gain, a lower feed/gain and a lower diarrhea incidence during 1-2 weeks (p<0.05), but no significant changes were observed during 3-4 weeks (p>0.05). When analyzed over the entire 28-day period, the effect of the positive control diet on weight gain, feed/gain and diarrhea incidence was also significant (p<0.05), but feed consumption was not affected by the positive control diet (p>0.05).

Compared with the negative control diet, the lactitol diet had no effect on weight gain and feed intake throughout the study (p>0.05). The lactitol diet reduced feed/gain during 3-4 weeks and over the entire study, and diarrhea incidence during 1-2 weeks (p<0.05).

Compared with the negative control diet, the tributyrin diet improved weight gain and reduced feed/gain during 3-4 weeks and over the entire study, and reduced diarrhea incidence during 1-2 weeks and over the entire study (p<0.05).

Compared with the negative control diet, the lactitol+ tributyrin diet improved weight gain during 3-4 weeks and over the entire study, and reduced feed/gain during 1-2 and 3-4 weeks, and over the entire study, and diarrhea incidence during 1-2 weeks and over the entire study (p<0.05).

Compared with the positive control diet, the lactitol diet reduced weight gain during 1-2 weeks and feed intake during 3-4 weeks, and increased feed/gain during 1-2 weeks and reduced feed/gain during 3-4 weeks (p<0.05). The tributyrin diet improved weight gain and reduced feed/gain during 3-4 weeks (p<0.05). The lactitol+tributyrin diet improved weight gain during 3-4 weeks, and reduced feed/gain during 3-4 weeks and over the entire study (p<0.05).

The weight gain and feed intake of pigs fed the lactitol+tributyrin diet had no difference from that fed the tributyrin diet (p>0.05), which means that lactitol and tributyrin didn't have an additive effect on weight gain and feed intake.

Small intestinal morphology

Data for small intestinal morphology are shown in Table 3. Compared with the negative control diet, the positive control diet decreased duodenal and ileal crypt depth (p<0.05). The lactitol diet did not have any effect on intestinal morphology (p>0.05). The tributyrin diet was effective in decreasing crypt depth and in increasing the ratio of villus height to crypt depth (VH/CD) in the duodenum and ileum (p<0.05). In addition, the tributyrin diet reduced duodenal villus width (p<0.05). The lactitol+ tributyrin diet increased jejunal villus height, decreased the duodenal and ileal crypt depth, and increased duodenal and ileal VH/CD and duodenal mucosal thickness (p<0.05).

Table 4. Effects of lactitol and tributyrin on disaccharidase activity (U/mg protein) of piglets[1]

Item	Diets[2]					SEM[3]	p value[4]
	CTR	GLN	LCT	TRB	LCT+TRB		
Duodenum							
Lactase	7.68[a]	8.75[a]	10.01[a]	13.39[b]	10.70[ab]	1.46	0.014
Sucrase	5.04[ab]	4.12[a]	3.49[a]	6.06[b]	3.59[a]	0.80	0.028
Maltase	23.03	18.47	21.08	22.98	16.67	3.65	0.357
Jejunum							
Lactase	9.30[b]	8.40[b]	3.80[ab]	6.46[ab]	13.95[c]	1.72	<0.001
Sucrase	6.25[a]	5.68[a]	6.49[a]	7.15[a]	13.56[b]	1.22	<0.001
Maltase	29.41[ab]	19.17[a]	29.00[ab]	25.92[ab]	31.98[b]	5.05	0.162
Ileum							
Lactase	1.08[ab]	1.21[ab]	0.52[a]	1.19[ab]	2.40[b]	0.78	0.247
Sucrase	7.86	8.02	8.35	7.93	11.93	1.78	0.157
Maltase	26.58[a]	34.54[ab]	25.20[a]	45.14[b]	27.65[a]	7.14	0.072

[1] Data are means of four replicate pig of one pig each replicate.
[2] Negative control diet (CTR), CTR with glutamine (GLN, 10 g/kg, as a positive control) or tributyrin (TRB, 5 g/kg) and (or) lactitol (LCT, 3 g/kg).
[3] Standard error of the mean.
[4] Probability for contrast of five treatments by Duncan's Multiple Comparison.
[a, b, c] Values in the same row with different superscripts are different ($p<0.05$).

Compared with the positive control diet, the lactitol diet decreased the jujunal villus height and increased the ileal crypt depth ($p<0.05$). The tributyrin diet decreased the ileal crypt depth, and increased the ileal VH/CD, and decreased the jejunal villus width ($p<0.05$). The lactitol+tributyrin diet increased the ileal villus height, the duodenal mucosal thickness, and the jejunal and ileal VH/CD ($p<0.05$).

Enzyme activity

The data for intestinal enzyme activity are presented in Table 4. Compared with the negative control diet, the positive control or lactitol diets had no effect on intestinal enzyme activity ($p>0.05$). However, the tributyrin diet increased duodenal lactase and ileal maltase activity ($p<0.05$). The lactitol+tributyrin diet increased jejunal lactase and sucrase activity ($p<0.05$).

Compared with the positive control diet, the lactitol diet had no effect on intestinal enzyme activity ($p>0.05$). The tributyrin diet improved the duodenal lactase and sucrase activity ($p<0.05$). The lactitol+tributyrin diet increased jejunal lactase, sucrase and maltase activity ($p<0.05$).

DISCUSSION

Tributyrin and glutamine, but not lactitol, improved performance of weaned pigs

Weaning is the most severe stress after birth. Poor growth and diarrhea of pigs observed immediately after weaning is usually associated with gastrointestinal dysfunction, demonstrated by villus atrophy, crypt hyperplasia and decline of enzyme activity in the small intestine (van Beers-Schreurs, 1996). Nutritional strategies may be able to manipulate the dysfunctional digestive tract. One of the strategies to reduce weanling stress is to provide specific nutrients, such as glutamine or short-chain fatty

acids, to directly feed gut tissues (Luchansky, 2000; Piva et al., 2002).

Glutamine is one of the most abundant amino acids in animal blood and milk (Wu et al., 1994). It is an energy substrate for intestinal mucosal cells (Wu et al., 1994), and plays an important role in repairing impaired enterocytes and maintaining local immune response (Souba et al., 1990; Lee et al., 2003). Weaned pigs are deprived of milk and have lower feed intake, probably leading to insufficient intake of glutamine. Glutamine deficiency, in turn, may result in intestinal dysfunction and poor growth. Previous studies have demonstrated that dietary glutamine supplementation is effective in repairing gastrointestinal dysfunction (Wu et al., 1996; Liu, 2002; Liu et al., 2002; Shinzato et al., 2003; Lee et al., 2003), and consequently improving performance. Therefore, in the current study, we used glutamine as the "positive control". In this study, we also demonstrated that glutamine improved pig performance during 1-2 weeks and over the entire study, which validates glutamine as a positive control.

N-Butyrate is a main energy substrate for enterocytes (Roediger, 1980). Tributyrin and lactitol are two dietary and fermentable sources of butyrate, respectively (Piva et al., 2002). In our study, we found that lactitol was of no use in improving weight gain and feed intake, and was of limited use in improving feed efficiency. In contrast, we found that the 0.5% tributyrin diet was effective in increasing weight gain during 3-4 weeks and over the entire study. Our results are in contrast to those of Piva et al. (2002), who found that tributyrin negatively affected performance of weanling pigs. The reason for this discrepancy may be associated with the dose of trybutyrin. The dose of tributyrin used in our study (5 g/kg) is only 50% of that of Piva et al. (2002). Piva et al. (2002) thought that some of the negative effects of the trybutyrin diet on growth parameters could be attributed to

too high a dose of trybutyrin (10 g/kg). Therefore, the lower dose tributyrin (0.5%) may stimulate growth of pigs effectively.

In the current study, lactitol was of no use in increasing weight gain, but tributyrin was effective in increasing weight gain. Furthermore, lactitol and tributyrin didn't show additive effects on weight gain and feed intake. Therefore, we recommend using not lactitol, but tributyrin. Moreover, in our study, glutamine was able to improve pig performance during 1-2 weeks. However, it was not able to further improve performance during 3-4 weeks. In contrast, the tributyrin diet was not able to improve pig performance during 1-2 weeks. However, it was able to improve performance during 3-4 weeks. Therefore, we recommend using glutamine in the first period of the starter phase, and using tributyrin in the second period of the starter phase.

Tributyrin and glutamine, but not lactitol, attenuated adverse changes of intestinal morphology associated with weaning stress

Small intestinal morphology has been widely used to mirror intestinal health and/or functions in pigs (Argenzio et al., 1990; Li et al., 1990; Zijlstra et al., 1996). Weaning is often coincidental with a reduction in villus height and an increase in crypt depth (Hornich et al., 1973; Gay et al., 1976; Kenworthy, 1976; Hampson, 1986a, b; Miller et al., 1986; Cera et al., 1988; Kelly et al., 1990, 1991a, b; Nabuurs et al., 1993; Pluske et al., 1996a, b), leading to fewer and less-differentiated enterocytes on the villi available for nutrient digestion. Abnormal intestinal morphology is usually associated with diarrhea and retarded growth of weanling piglets.

In agreement with the improved performance and decreased diarrhea incidence in weanling piglets, glutamine resulted in an amelioration of intestinal morphology, as indicated by the decreased duodenal and ileal crypt depth, which further validates glutamine as a positive control. Similar results were also reported by other investigators (Wu et al., 1996; Liu, 2002; Liu et al., 2002; Zhang and Gao, 2002; Shinzato et al., 2003; Lee et al., 2003). These studies showed that glutamine was an effective chemical in maintaining normal intestinal normal structure and function.

n-Butyric acid is a potent antiproliferative and differentiation agent in various cell lines (Scheppach et al., 1997; von Engelhardt et al., 1998; Schroder et al., 1999). It has been reported that n-butyric acid reduced the number of proliferating cells by 60% in the upper 40% of crypts (Scheppach et al., 1997). As a dietary or fermentable source of n-butyric acid, tributyrin has been shown to increase n-butyrate concentrations in the cecum (Piva et al., 1996). In the present study, the tributyrin diet resulted in a decrease of crypt depth, an increase in the ratio of villus height to crypt depth in the duodenum and ileum, and a decrease in villus width in the duodenum, suggesting that tributyrin may exert an antiproliferative effect on crypt cells in piglets by providing additional n-butyric acid. The reduced number of proliferating cells results in partitioning of nutrients away from the gastrointestinal tract towards growth (Piva et al., 2002), thereby improving the efficiency of nutrient utilization for growth.

Lactitol is considered as another substance to provide n-butyric acid *in vivo*. However, unlike tributyrin, we found that lactitol had no effect on intestinal structure. So, considering intestinal morphology, we recommended using not lactitol, but tributyrin. Moreover, compared with glutamine, tributyrin improved intestinal morphology more effectively. So, tributyrin could replace glutamine as an effective chemical in maintaining intestinal structure.

Tributyrin, but neither glutamine nor lactitol, increased intestinal enzyme activity of weaned pigs

Lactase activity in the small intestinal mucosa of the newborn pig is high at birth and declines gradually during the first 2 months of life (Manners and Stevens, 1972; James et al., 1987). By comparison, the activity of sucrase is generally absent, or present at very low levels, at birth but increases with age (Kidder and Manners, 1980). Gay et al. (1976) reported an apparent decline of lactase and sucrase activity attributable to weaning. The decline in lactase and sucrase activities after weaning is generally associated with a decrease in villus height and an increase in crypt depth (Hampson and Kidder, 1986). Brush-border lactase activity in the young pig has a more apical distribution on the villus than does sucrase (Dahlqvist and Nordström, 1966; Nordström and Dahlqvist, 1973; Kelly et al., 1991a, b) and shows a greater reduction in activity after weaning in response to a change in villus height (Pluske et al., 1996a, b). In the present study, the glutamine diet improved piglet performance and intestinal morphology, but it had no effect on intestinal enzyme activity. In contrast, Zhang et al. (2001) reported that glutamine could attenuate the decrease of lactase of postweaning pigs (Wu et al., 1994). The reason for this inconsistency is unknown.

Little research has been conducted to investigate the effect of tributyrin or lactitol on intestinal enzyme activity. In the present study, the lactitol diet had no effect on intestinal enzyme activity; but the tributyrin diet increased duodenal lactase and ileal maltase activity.

In summary, we found that, 0.5% tributyrin improved growth and feed efficiency during 3-4 weeks postweaning, and attenuated adverse changes of the intestinal structure and function associated with weanling stress. In contrast, lactitol had little effect. Moreover, tributyrin was more effective in improving intestinal morphology and enzyme

activity, and tributyrin exerted a superior effect in improving performance as weaning progresses compared with glutamine. These suggest that, as a chemical for repairing intestinal atrophy, glutamine and tributyrin should be used in the first period and the second period of the starter phase, respectively.

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