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Original Research Article

Dietary butyrate, lauric acid and stearic acid improve gut morphology and epithelial cell turnover in weaned piglets

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ABSTRACT

This study was to evaluate the effects of the supplementation of saturated fatty acids with different chain lengths on growth performance, intestinal morphology, epithelial cell proliferation, differentiation and apoptosis in weaned piglets. Thirty-two weaned piglets (Duroc × Landrace × Yorkshire, BW = 7.81 ± 0.26 kg) were weaned at 21 d and randomly assigned to 1 of 4 experimental treatments: (1) a basal diet (control); (2) control + 0.3% butyrate (BT); (3) control + 0.3% lauric acid (LA); (4) control + 0.3% stearic acid (SA). All piglets were then slaughtered for tissue sampling after having been fed experimental diets for 28 d after weaning. Supplementation of BT increased the gain-to-feed ratio (G:F) ($P < 0.05$) compared to piglets fed the control diet from 14 to 28 d. In addition, the villus height (VH) to crypt depth (CD) ratio (VH:CD ratio) of the ileum were higher in the BT and LA diets than that of the control diet ($P < 0.05$). The SA-supplemented diet increased ileal VH ($P < 0.05$), whereas the BT-supplemented diet increased jejunal CD ($P < 0.05$). Compared to the control, diets supplemented with BT, LA, or SA all tended to increase jejunal proliferation (Ki67/crypt positive cells) ($P = 0.190$); diets supplemented with BT or SA significantly increased the number of ki67-positive cells in the ileal crypt ($P < 0.05$). Furthermore, in the jejunum, the protein expression of activated caspase 3 and villin were increased in piglets fed BT, LA, or SA diets compared to those on the control diet ($P < 0.05$). In the ileum, compared with the control diet, the BT diet tended to increase the protein level of mammalian phosphorylation target of rapamycin (p-mTOR, $P < 0.10$); LA or SA diets significantly increased p-mTOR protein expression ($P < 0.05$). These results show that dietary supplementation of BT, LA, or SA promotes jejunal cell renewal in weaned piglets. At the same time, increased proliferation of ileal crypt cells by promoting p-mTOR expression has beneficial effects on ileal morphology in weaned piglets.

1. Introduction

Weaning in piglets is a stressful event which involves nutritional stressors such as the abrupt switch from milk that is a high-fat, liquid diet to a solid diet that is low-fat and dry (van Beers-Schreurs et al., 1998; Moeser et al., 2007). Weaning stress is caused by low feed intake, high rates of diarrhea and damage to the intestinal mucosa in weaned pigs, which require high energy expenditure to maintain gut health (Moeser et al., 2007; Wang et al., 2022). Oils and fat-rich ingredients are thus used to achieve a high energy content in the feed.

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Fatty acids are classified into short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA), and long-chain fatty acids according to their carbon chain lengths. SCFA contain less than 6 carbon atoms and are also known as volatile fatty acids; MCFA contain 6 to 12 carbon atoms; long-chain fatty acids contain (LCFA) more than 12 carbon atoms. Previous studies have shown the effects that fatty acids, as important energy sources for intestinal cells, on growth performance as well as the immune system and intestine morphology of weaned piglets (Pluske et al., 1996; Ferrara et al., 2017; De Keyser et al., 2019). However, there is no difference in growth performance between post-weaning piglets fed diets supplemented with caproic and caprylic in the form of medium-chain glycerides (MCT 6/8) and control diets (De Keyser et al., 2019). Moreover, the supplementation of MCFA and SCFA also does not significantly affect jejunal morphometric data in weaned piglets (Ferrara et al., 2017). Further, gain and efficiency of feed were greater for the weaned piglets fed coconut oil, which is high in lauric acid, than for the pigs fed corn oil, which is rich in oleic acid, or lard, which is high oleic acid and palmitic acid (Lawrence and Maxwell, 1983).

While butyrate (C4) has been identified as a major energy source for enterocytes, its effects on weaned pig growth and intestinal morphology are still controversial (Kotunia et al., 2004; Biagi et al., 2007; Kien et al., 2007; Le Gall et al., 2009). In addition, Lauric acid (C12) has been shown to have antimicrobial, anti-inflammatory, and anti-viral properties (Kabara et al., 1972; Bartolotta et al., 2001; Nakatsuji et al., 2009). It has been reported to have positive effects on production parameters in late-finishing pigs (Pluske et al., 2018). On the other hand, stearic acid (C18) was found to have a potential role in increasing food intake (Wang et al., 2016). Previously, there were few reports on the effect of saturated fatty acids of different chain lengths on the turnover of intestinal epithelial cells in weaned piglets. Therefore, we chose butyrate, lauric acid and stearic acid to represent SCFA, MCFA and LCFA respectively, to study the effect of saturated fatty acids with different chain lengths on intestinal epithelial cell turnover.

2. Materials and methods

2.1. Animal ethics

All animal care and handling procedures were approved (Approval number 2016-093) by the Animal Care and Use Committee of Hunan Normal University, Changsha City, Hunan, China.

2.2. Animals and experimental treatments

A total of 32 Duroc × Landrace × Yorkshire piglets were weaned at 21 d of age. All weaned piglets (average BW = 7.81 ± 0.26 kg) were allotted to 1 of 4 dietary treatments based on BW, sex, and litter. Each dietary treatment was fed to 8 pens with 1 pig per pen. The experimental diets were as follows: (1) a basal diet (CTL, Table 1); (2) the basal diet + 3 g/kg butyrate (BT, sodium butyrate, 98%, King Techina, Hangzhou, China); (3) the basal diet + 3 g/kg lauric acid (LA, 96%, Acidchem International Sdn. Bhd, Malaysia); (4) the basal diet + 3 g/kg stearic acid (SA, 96%, Acidchem International Sdn. Bhd, Malaysia). BT, LA or SA were added during feed production. All weaned piglets were allowed free access to feed and water throughout the 28-d feeding trial. Piglets were weighed at 21, 35, and 49 d to determine weight gain. The feed consumed by each pig was also weighed daily to determine feed intake. At the end of the 28 d experiment, the average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G:F) were calculated for each pen.

Table 1
Ingredients and nutrient content of the basal diet for weaned piglets (%, as-fed basis).

Item	0 to 14 d	15 to 28 d
Ingredients		
Corn	25.14	40.35
Extruded corn	35.00	20.00
Soybean meal (44%)	9.00	21.00
Fishmeal (63%)	7.00	4.00
Plasma protein powder	5.00	0
Whey powder	9.00	5.00
Glucose	3.00	3.00
Soybean oil	3.50	2.70
Stone powder	1.05	0.60
$CaHPO_4$	0	0.90
Choline chloride	0.10	0.10
Antioxidants	0.05	0.05
Citric acid	0.50	0.50
Sodium chloride	0.10	0.10
Mineral premix[1]	0.15	0.15
Vitamin premix[2]	0.30	0.30
L-Lysine HCl	0.45	0.49
DL-Methionine	0.20	0.25
L-Threonine	0.14	0.17
L-Tryptophan	0.02	0.04
Fatty acid[3]	0.30	0.30
Total	100	100
Calculated nutrition level		
Net energy, Mcal/kg	2.44	2.47
Crud protein	19.70	18.62
Calcium	0.80	0.77
Available phosphorus	0.38	0.47
Lysine[4]	1.43	1.26
Methionine + Cyssteine[4]	0.81	0.77
Thronine[4]	0.81	0.78
Tryptophan[4]	0.20	0.20

[1] Provided the following minerals per kilogram diet: 100 mg $ZnSO_4$, 30 mg $MnSO_4$, 0.3 mg $CoSO_4$, 150 mg $FeSO_4$, 25 mg $CuSO_4$, 0.3 mg Na_2SeO_3, and 0.5 mg KIO_3.
[2] Provide the following vitamins per kilogram diet: 2,200 IU vitamin A, 17.5 μg vitamin B_{12}, 220 IU vitamin D_3, 16 IU vitamin E, 0.5 mg vitamin K_3, 3.5 mg riboflavin, 30 mg niacin, 10 mg D-pantothenic acid, 0.05 mg biotin, 0.3 mg folic acid, 1.0 mg thiamine, 7 mg pyridoxine, and 4.0 mg ethoxyquin.
[3] Basal diet group: soybean oil; butyric acid group: sodium butyrate; lauric acid group: lauric acid; stearic acid group: stearic acid.
[4] Standardized ileal digestible.

2.3. Sampling and processing

The piglets were anesthetized (with $C_3H_2ClF_5O$) and sacrificed by intravenous administration (jugular vein) of 4% sodium pentobarbital solution (40 mg/kg BW) (Yang et al., 2012a) to obtain intestinal tissues and digesta samples as described by Shen et al. (2009). Approximately 8 cm of intestinal tissue from the middle sections of the duodenum, jejunum and ileum were aseptically isolated. They were then flushed with 0.9% salt solution, and each segment was divided into 2 sections. One section was added into a 4% formaldehyde-phosphate buffer and kept at room temperature for microscopic assessment of mucosal morphology. The other was quickly frozen in liquid nitrogen and then stored at −80 °C for protein analysis of the mucosa by Western blotting.

2.4. Small intestinal morphology

To study small intestinal morphology, fixed intestinal segments were embedded in paraffin wax. Three cross-sections of 5 μm thickness of each intestinal segment were stained with hematoxylin and eosin. Villus height (VH) and crypt depth (CD) were determined at 40× magnification using an image processing and analysis system (Version 1, Leica Imaging Systems Ltd., Cambridge, UK), and the ratio of VH to CD was calculated At least 10 well-oriented intact villi and their associated crypts were measured for

each segment of each pig according to the procedure created by Yang et al. (2016).

2.5. Western blotting analysis

The jejunal and ileal mucosa was lysed in RIPA buffer (Beyotime Biotechnology, Shanghai, China) along with a protease inhibitor cocktail (Roche, Shanghai, China). The lysate protein was then analyzed using a standard Western blotting (WB) procedure (Liu et al., 2012). From each sample, 10 μg of the total protein was separated using 8% to 12% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred into polyvinylidene difluoride (PVDF) membranes. The membranes were incubated with the primary antibody at 4 °C during gentle overnight shaking. The primary antibody was then removed. Secondary antibodies (Anti-rabbit/mouse/goat IgG, horseradish peroxidase [HR]-linked antibody from Cell Signaling Technology [CST; Shanghai, China]) incubated the membranes for 1 h at room temperature. Finally, the blots were visualized using a chemiluminescence system from the Immobilon western chemiluminescence HRP substrate (Millipore, Shanghai, China). The following primary antibodies were used: cleaved-caspase 3 (CST; Shanghai, China), villin (CST; Shanghai, China), phosphorylated mammalian target of rapamycin (p-mTOR, CST; Shanghai, China), and β-actin (CST; Shanghai, China). The amount of target protein was normalized by β-actin.

2.6. Immunohistochemical analysis

The paraffin sections of the jejunum and ileum were heated at 60 °C for 30 min, deparaffinized twice in xylene for 10 min each time, and rehydrated through graded ethanol into phosphate buffer saline (PBS) for 3 min each time. Then, the sections were soaked with 3% H_2O_2 in methanol for 10 min in the dark to inactivate the peroxidase. Sections were heated up to a boil in a 10 mM sodium citrate buffer (pH 6.0) for 15 min and washed twice in PBS. Next, these sections were blocked in 5% bovine serum albumin (BSA) for 30 min at 37 °C. The Ki67 primary antibody (CST; Shanghai, China) was diluted in the PBS at a 1:500 and incubated overnight at 4 °C. The following day, the primary antibody was removed and washed thrice for 5 min with PBS. Samples were incubated with a secondary antibody (Anti-rabbit/mouse/goat IgG, HRP-linked antibody from Cell Signaling Technology [CST; Shanghai, China]) for 1 h at room temperature. DAB (3,3′-diaminobenzidine) was used as a chromogen to detect the antigen–antibody complex. Finally, these sections were dehydrated with increasing concentrations of ethanol followed by xylene as in conventional histology and sealed with a neutral resin.

2.7. Statistical analysis

All data were analyzed by one-way ANOVA for a randomized complete block design using SPSS statistical software. Statistical differences between diets were separated by Tukey's multiple range test and *t*-test and *P*-values were calculated using SPSS software. Significant differences among treatments at $P < 0.05$ have been indicated using asterisks (*) and different letters. Each pen or pig was used as experimental unit.

3. Results

3.1. Growth performance and small intestinal morphology

The 4 different diets had no significant effect on ADG, ADFI or G:F in weaned piglets from 1 to 14 or 28 d ($P > 0.05$, Table 2).

Table 2
Effects of dietary butyrate (BT), lauric acid (LA) and stearic acid (SA) on growth performance of weaned piglets.

Item	CTL	BT	LA	SA	SEM	*P*-value
0 to 14 d						
ADG, kg/d	0.14	0.12	0.10	0.14	0.01	0.172
ADFI, kg/d	0.39	0.37	0.39	0.43	0.02	0.554
G:F	0.32	0.34	0.27	0.33	0.02	0.691
15 to 28 d						
ADG, kg/d	0.35	0.39	0.36	0.39	0.01	0.234
ADFI, kg/d	0.71	0.67	0.68	0.71	0.01	0.596
G:F	0.53[a]	0.59[b]	0.51[ab]	0.55[ab]	0.01	0.025
0 to 28 d						
ADG, kg/d	0.24	0.24	0.22	0.26	0.01	0.258
ADFI, kg/d	0.55	0.51	0.53	0.57	0.01	0.476
G:F	0.44	0.48	0.43	0.46	0.01	0.639

SEM = standard error of the mean; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.
[a,b]Within rows, means that do not share the same letter are significantly different by Tukey's post hoc test ($P < 0.05$).

However, the G:F increased following dietary supplementation with BT compared to the CTL group from 15 to 28 d ($P < 0.5$, Tukey's, Table 2). Compared to piglets fed with the BT diet, piglets fed with the LA diet had increased VH:CD ratio in the jejunum ($P < 0.5$, Tukey's, Table 3). Compared to piglets fed with the CTL diet, piglets fed with the SA diet had increased VH in the ileum ($P < 0.5$, Tukey's, Table 3). The diet supplemented with LA increased ileal VH:CD ratio in piglets as compared to the CTL or SA diets ($P < 0.5$, Tukey's, Table 3). Compared to pigs fed with the CTL diet, pigs fed with BT or LA diets had increased VH as well as VH:CD ratio in the ileum ($P < 0.5$, *t*-test, Table 3). Piglets fed BT had increased jejunal CD ($P < 0.05$, *t*-test) compared to the CTL group in Table 3. However, the ratio of duodenal VH:CD ratio in piglets fed the LA diet was higher than that of the CTL diet ($P < 0.05$, *t*-test, Table 3).

3.2. Intestinal proliferation, differentiation, and apoptosis

Immunohistochemical technology was used to detect and assess the expression of Ki67, which is found in the nuclei of proliferating cells. Pigs fed BT ($P = 0.078$, *t*-test), LA ($P = 0.065$, *t*-test), or SA ($P = 0.091$, *t*-test) diets tended to have an increase in the number of Ki67 positive cells/crypt in the jejunum compared to those on the CTL diet in Fig. 1A–D, and I. Moreover, Ki67 positive cells per crypt in the ileum significantly increased following dietary supplementation with BT or SA compared with the CTL or LA diets ($P < 0.05$, Fig. 1E–H, and J). Furthermore, in comparison to the CTL diet, dietary supplementation with LA or SA significantly increased cleaved caspase 3 activation and villin expression in the jejunum ($P < 0.05$, Fig. 2A–C). Compared to piglets fed with the CTL diet, piglets fed the BT diet had increased cleaved caspase 3 ($P < 0.05$, Tukey's) activation and villin ($P < 0.05$, *t*-test) expression in the jejunum in Fig. 2A–(C. Piglets fed BT had decreased ileal cleaved caspase 3 expression ($P < 0.05$, Tukey's, Fig. 2D and F) compared with the CTL group. Furthermore, in comparison with the CTL or LA diets, dietary supplementation with SA significantly decreased caspase 3 expression in the ileum ($P < 0.05$, Tukey's, Fig. 2D and F). However, the expression of villin in the ileum did not differ between the four diets ($P > 0.05$, Tukey's, Fig. 2D and E).

3.3. Intestinal p-mTOR expression

LA or SA ($P < 0.05$, *t*-test) supplemented diets significantly increased p-mTOR expression, and BT ($P < 0.10$, *t*-test) supplemented diets tended to increase p-mTOR expression in the piglet

Table 3
Effects of dietary butyrate (BT), lauric acid (LA) and stearic acid (SA) on intestinal morphology of weaned piglets.

Item	CTL	BT	LA	SA	SEM	*P*-value
Villus height, μm						
Duodenum	276.99	314.77	311.68	298.78	7.87	0.341
Jejunum	378.77	380.71	409.94	390.66	8.85	0.608
Ileum	248.00^{a}	273.65^{ab}*	274.82^{ab}*	285.10^{b}	5.03	0.056
Crypt depth, μm						
Duodenum	380.52	375.02	332.65	379.69	13.57	0.573
Jejunum	290.17	327.05*	291.16	297.85	5.77	0.070
Ileum	249.76	235.83	235.69	263.28	7.22	0.479
VH:CD, μm:μm						
Duodenum	0.80	0.92	1.10*	0.89	0.04	0.052
Jejunum	1.41^{ab}	1.22^{a}	1.50^{b}	1.42^{ab}	0.04	0.055
Ileum	1.09^{a}	1.27^{ab}*	1.29^{b}	1.08^{a}	0.03	0.005

SEM = Standard error of the mean; VH:CD = Villus height-to-crypt depth ratio.
^{a,b,c}Within a row, means that do not share the same letter are significantly different by Tukey's post hoc test ($P < 0.05$).
*BT, LA or SA vs CTL group: $P < 0.05$, *t*-test.

ileum compared to the control diets in Fig. 3A and B. There were no differences in p-mTOR expression in the piglet ileum among the BT, LA or SA groups ($P > 0.05$, Tukey's, Fig. 3A and B).

4. Discussion

At 28 d post-weaning, there was no significant difference in the ADG, ADFI, and G:F between pigs fed BT, LA or SA diets and those on the CTL diet. However, between 15 and 28 d, post-weaning, the BT supplemented diet improved the G:F but did not affect the ADG and ADFI in piglets. Biagi et al. and Fang et al. demonstrated that sodium butyrate did not improve growth performance in weaned piglets (Biagi et al., 2007; Fang et al., 2014). Conversely, Piva et al. showed that sodium butyrate did in fact improve growth performance of weaned piglets (Piva et al., 2002). These differing results were due to differences in dietary composition, the dose of butyrate, and piglet weaning age (Partanen and Mroz, 1999). Further, Pluske et al. demonstrated that late-finishing pigs fed 12 to 13.5 g/kg LA in substitution for tallow diets increased ADG and ADFI, which is also inconsistent with our results that the LA supplemented diet did not affect weaned piglet production (Pluske et al., 2018). This may be due to differences in pig growth development and LA supplementation dosage. To the best of the author's knowledge (the authors have thoroughly searched the relevant literature) but could not find a single reference of the result in question. Furthermore, given that there are only 8 pigs per treatment, the results of growth performance may need to be validated in future large group animal trials.

Early weaning results in dysfunction in piglets with villi atrophy and crypt hyperplasia. In the present study, LA supplemented diets increased the ratio of VH to CD in the ileum compared with the CTL or SA diet in weaned piglets. Moreover, the LA supplemented diet increased the ratio of VH to CD in the jejunum compared with the BT diet in weaned piglets. In this case, Liu et al. showed that 10 mg/kg lauric acid improved the VH:CD ratio in the small intestinal mucosa in mice (Liu, 2021). Furthermore, Le Gall et al. (2009) demonstrated that the jejunal mucosa was thinner and jejunal villus height was lower with oral administration of 3 g/kg sodium butyrate in weaned piglets. In addition, Biagi et al. (2007) reported that different doses of sodium butyrate supplementation (from 0.1% to 0.4% of DM intake) had no effect on villi-crypt structure in 60 d weaned piglets. In conclusion, among the different chain length saturated fatty acids we evaluated, LA was the most effective in increasing the VH:CD ratio in the jejunum and ileum of weaned piglets.

Intestinal epithelial homeostasis is maintained by a strict equilibrium between cell proliferation in the crypt and cell

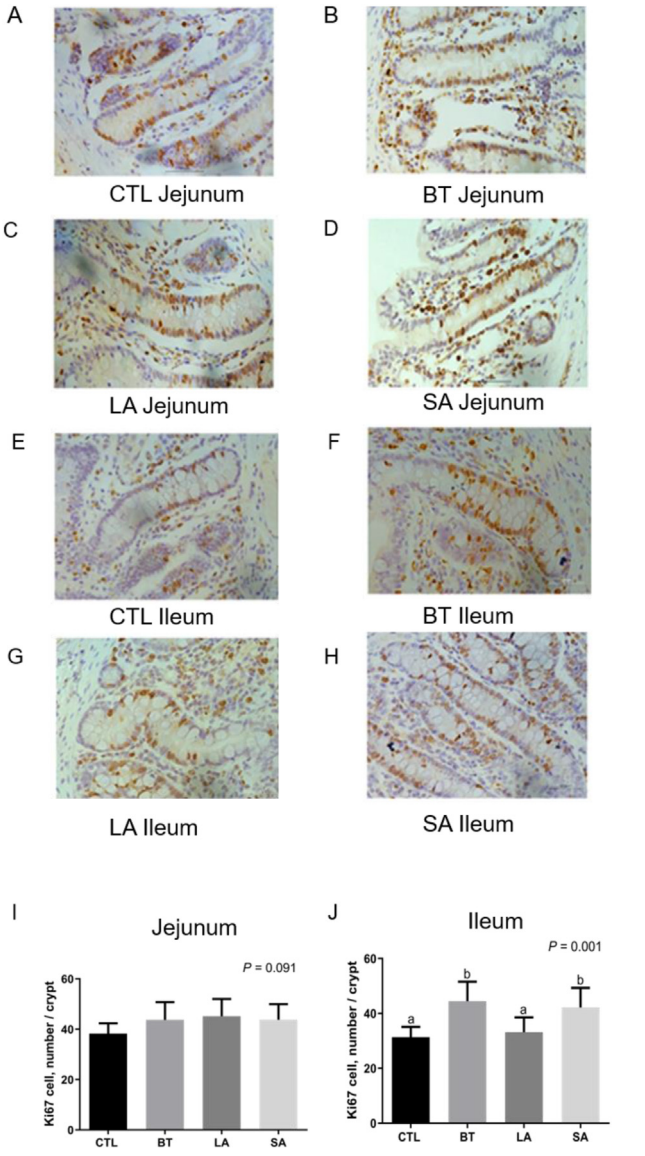

Fig. 1. Effects of dietary supplementation with butyrate (BT), lauric acid (LA), or stearic acid (SA) on the Ki67 expression in the jejunum and ileum. (A to D) Representative immunohistochemical images of Ki67-positive cells in the jejunum of piglets in CTL, BT, LA and SA groups, respectively. (E to H) Representative immunohistochemical images of Ki67-positive cells in the ileum of piglets in CTL, BT, LA and SA groups, respectively. (I) Quantitative analysis of Ki67-positive cells in CTL, BT, LA or SA jejunum. BT, LA or SA vs CTL group: $P < 0.10$, *t*-test. (J) Quantitative analysis of Ki67-positive cells in CTL, BT, LA or SA ileum. CTL: a basal diet. The error bar represents the standard error ($n = 6$). Means without a common letter differ significantly by Tukey's post-hoc test ($P < 0.05$).

shedding from the villus tip (Negroni et al., 2015). Proliferation of intestinal epithelial cells is typically assessed by the number of Ki67-positive cells in the crypts (Yang et al., 2012b; Yin et al., 2022). Therefore, we used Ki67 to assess cell proliferation in crypts. Our results showed that BT, LA, and SA supplemented diets all tended to all increase the positive Ki67 cells per crypt in the jejunum. Moreover, BT and SA supplemented diets both significantly increased the positive Ki67 cells/crypt in the ileum. This suggests that compared to the CTL diet, BT, LA, and SA supplemented diets can promote intestinal cell proliferation in the jejunum, while BT and SA supplemented diets can increase intestinal cell proliferation in the ileum. Further, our findings are consistent with a previous study by Kien et al. that ileal and

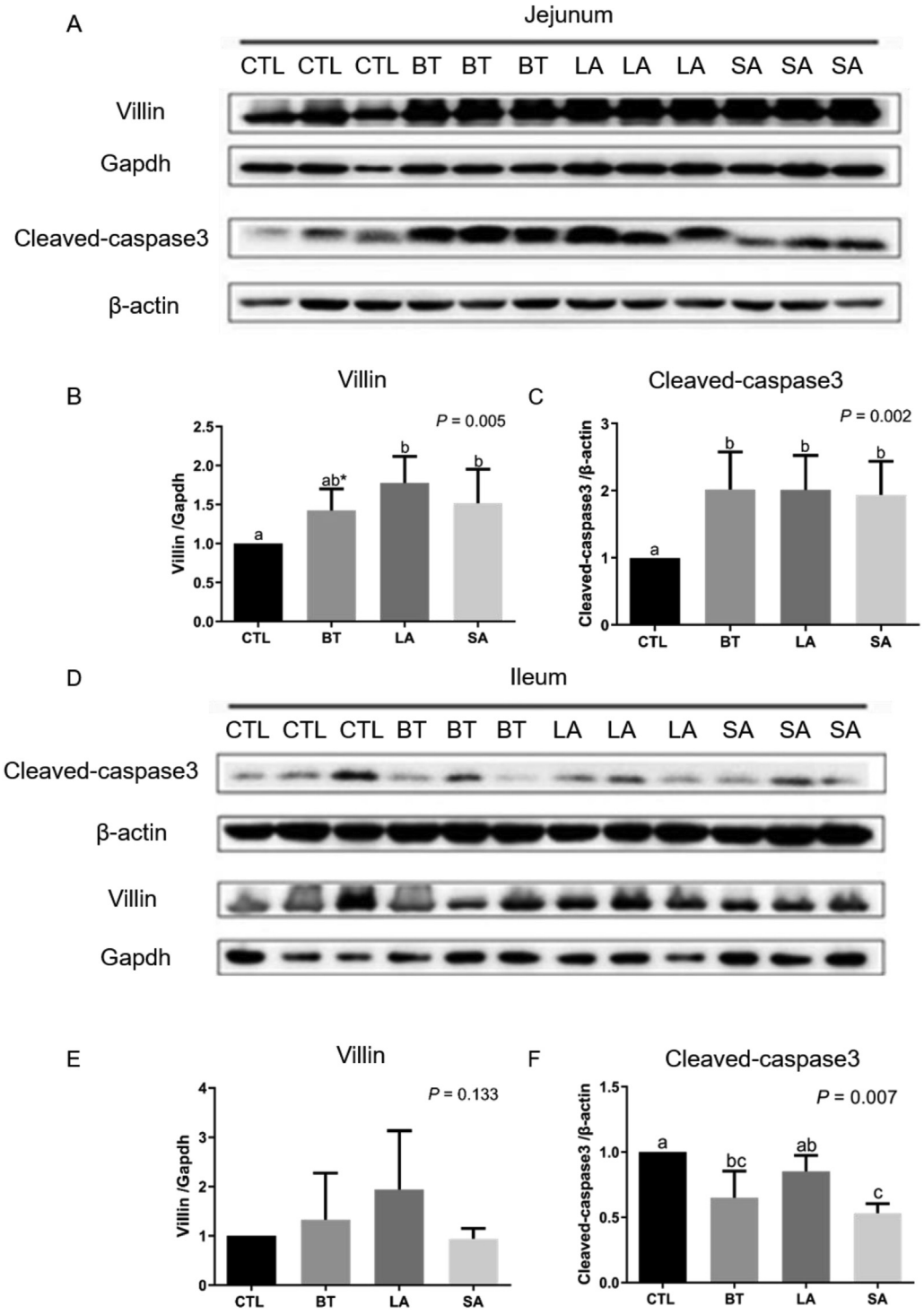

Fig. 2. Effects of dietary supplementation with butyrate (BT), lauric acid (LA), or stearic acid (SA) on the caspase 3 activation, and villin expression in the jejunum and ileum. (A) Image of villin and cleaved-caspase 3 Western blotting detection in the jejunum. (B) Normalized results of Western blot analysis to quantify villin expression in jejunal tissues of CTL, BT, LA and SA. BT vs CTL group: $P < 0.05$, *t*-test. (C) Normalized results of Western blot analysis to quantify cleaved-caspase 3 expression in jejunal tissues of CTL, BT, LA and SA. (D) Image of villin and cleaved-caspase 3 Western blotting detection in the ileum. (E) Normalized results of Western blot analysis to quantify villin expression in ileal tissues of CTL, BT, LA and SA. (F) Normalized results of Western blot analysis to quantify cleaved-caspase 3 expression in ileal tissues of CTL, BT, LA and SA. CTL: a basal diet. The error bar represents the standard error ($n = 6$). Means without a common letter differ significantly by Tukey's post-hoc test ($P < 0.05$). *Values are significantly different from the CTL by *t*-test at $P < 0.05$.

jejunal cell proliferation was increased by caecal infusion of butyrate in piglets (Kien et al., 2007).

Pigs fed BT, LA, and SA supplemented diets significantly increased caspase 3 activation and villin expression in the jejunum, but not in the ileum compared with the CTL diet. Cleaved-caspase 3 is an apoptosis marker as an executor and is involved in intestinal epithelial cell shedding (Negroni et al., 2015), while villin protein is a marker of intestinal epithelial cell differentiation (West et al.,

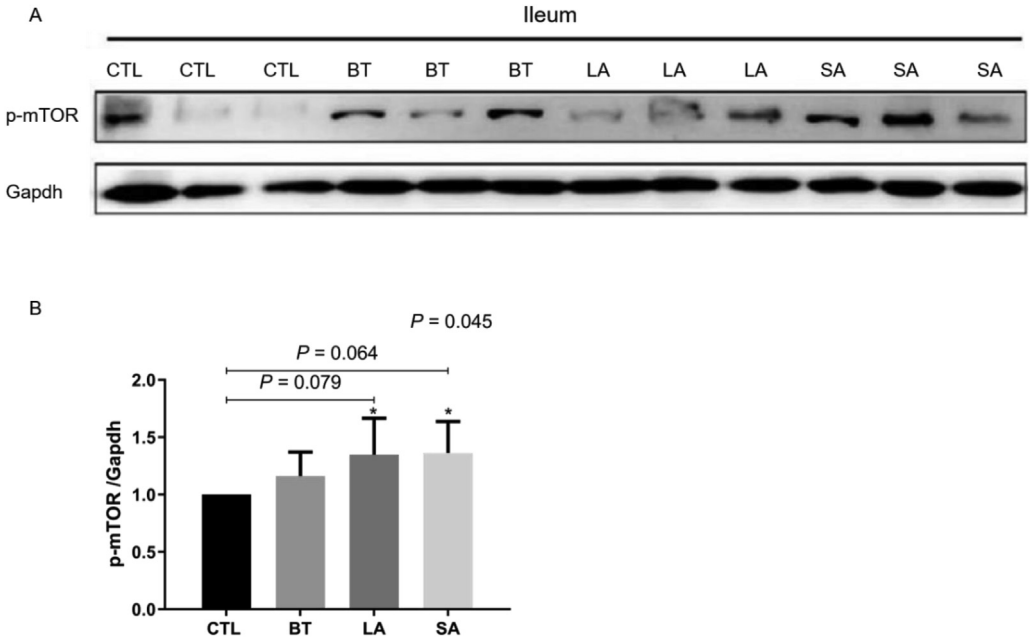

Fig. 3. Effects of dietary supplementation with butyrate (BT), lauric acid (LA), or stearic acid (SA) on the p-mTOR protein expression in the ileum. (A) The Western blots of ileal p-mTOR protein (B) p-mTOR protein abundance normalized to control. BT ($P < 0.10$), LA ($P < 0.05$) or SA ($P < 0.05$) vs CTL group, *t*-test. CTL: a basal diet. The error bar represents the standard error ($n = 6$). *Values are significantly different from the CTL by *t*-test at $P < 0.05$.

1988). Our results showed that BT, LA, and SA supplemented diets improved jejunal intestine cell proliferation, differentiation and apoptosis and suggested that they may promote jejunal intestine cell renewal in weaned piglets. Meanwhile, SA significantly improved the proliferation and apoptosis of ileal cells compared with the CTL or LA group. This implies that BT, LA, or SA supplemented diets may be beneficial for recovery from a pathological state in the intestines of piglets (Colom and Jones, 2016). Our study provides insights into the effects of dietary supplementation of BT, LA, or SA in on the growth performance and gut health in weaned piglets.

Furthermore, mTOR is activated by phosphorylation, which functions at the convergence point of a vast signaling network that senses fluctuations in extracellular and intracellular nutrients (Dibble and Manning, 2013), and plays a central role in protein synthesis, proliferation, and cell survival (Jacinto et al., 2006; Wang et al., 2022). Previous studies indicated that mTOR was activated to enhance the proliferation and migration of intestinal cells (Rhoads et al., 2004; Igarashi and Guarente, 2016). Interestingly, our results showed that the diets supplemented with BT, LA and SA all increased ileal p-mTOR activation and positive Ki67 cells/crypts in the ileum compared with the control diet. This suggests that p-mTOR may improve intestinal morphology by increasing the proliferation of epithelial cells, a result similar to that of Teufel et al. (2021).

Conclusions

Our results showed that different carbon-chain-length fatty acids exerted different effects on weaned piglets. We found that BT supplemented diets improved the G:F during 15 to 28 d, as well as the intestine morphology in the ileum of weaned pigs. Further, the ability of BT, LA, or SA supplemented diets to improve ileal morphology may be related to the promotion of epithelial cell proliferation following p-mTOR upregulation. Moreover, the LA supplemented diet significantly improved jejunal morphology compared with the BT diet.

Author contributions

Xianglin Zeng: Investigation, Data curation, Writing – original draft preparation. **Yuan Yang**: Investigation, Formal analysis. **Junmin Wang**: Data curation. **Zhaobin Wang**: Investigation, Validation. **Jun Li**: Validation. **Yulong Yin**: Supervision, Funding acquisition, Writing - review and editing. **Huansheng Yang**: Conceptualization, Funding acquisition, Methodology, Writing - review and editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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References

Bartolotta S, García CC, Candurra NA, Damonte EB. Effect of fatty acids on arenavirus replication: inhibition of virus production by lauric acid. Arch Virol 2001;146(4):777–90.
Biagi G, Piva A, Moschini M, Vezzali E, Roth FX. Performance, intestinal microflora, and wall morphology of weanling pigs fed sodium butyrate. J Anim Sci 2007;85(5):1184–91.
Colom B, Jones PH. Clonal analysis of stem cells in differentiation and disease. Curr Opin Cell Biol 2016;43:14–21.
Dibble CC, Manning BD. Signal integration by mTORC1 coordinates nutrient input with biosynthetic output. Nat Cell Biol 2013;15(6):555–64.
De Keyser K, Dierick N, Kanto U, Hongsapak T, Buyens G, Kuterna L, et al. Medium-chain glycerides affect gut morphology, immune- and goblet cells in post-weaning piglets: in vitro fatty acid screening with Escherichia coli and

in vivo consolidation with LPS challenge. J Anim Physiol Anim Nutr 2019;103(1):221–30.
Fang CL, Sun H, Wu J, Niu HH, Feng J. Effects of sodium butyrate on growth performance, haematological and immunological characteristics of weanling piglets. J Anim Physiol Anim Nutr 2014;98(4):680–5.
Ferrara F, Tedin L, Pieper R, Meyer W, Zentek J. Influence of medium-chain fatty acids and short-chain organic acids on jejunal morphology and intra-epithelial immune cells in weaned piglets. J Anim Physiol Anim Nutr 2017;101(3):531–40.
Igarashi M, Guarente L. mTORC1 and SIRT1 cooperate to foster expansion of gut adult stem cells during calorie restriction. Cell 2016;166(2):436–50.
Jacinto E, Facchinetti V, Liu D, Soto N, Wei S, Jung SY, et al. SIN1/MIP1 maintains rictor-mTOR complex integrity and regulates Akt phosphorylation and substrate specificity. Cell 2006;127(1):125–37.
Kabara JJ, Swieczkowski DM, Conley AJ, Truant JP. Fatty acids and derivatives as antimicrobial agents. Antimicrob Agents Chemother 1972;2(1):23–8.
Kotunia A, Wolinski J, Laubitz D, Jurkowska M, Rome V, Guilloteau P, et al. Effect of sodium butyrate on the small intestine development in neonatal piglets fed [correction of feed] by artificial sow. J Physiol Pharmacol 2004;55(Suppl 2):59–68.
Kien CL, Blauwiekel R, Bunn JY, Jetton TL, Frankel WL, Holst JJ. Cecal infusion of butyrate increases intestinal cell proliferation in piglets. J Nutr 2007;137(4):916–22.
Lawrence NJ, Maxwell CV. Effect of dietary fat source and level on the performance of neonatal and early weaned pigs. J Anim Sci 1983;57(4):936–42.
Le Gall M, Gallois M, Sève B, Louveau I, Holst JJ, Oswald IP, et al. Comparative effect of orally administered sodium butyrate before or after weaning on growth and several indices of gastrointestinal biology of piglets. Br J Nutr 2009;102(9):1285–96.
Liu FY, Tang XC, Deng M, Chen P, Ji W, Zhang X, et al. The tumor suppressor p53 regulates c-Maf and Prox-1 to control lens differentiation. Curr Mol Med 2012;12(8):917–28.
Liu ZH, Xie WW, Zan GX, Gao CQ, Yan HC, Zhou JY, et al. Lauric acid alleviates deoxynivalenol-induced intestinal stem cell damage by potentiating the Akt/mTORC1/S6K1 signaling axis. Chem Biol Interact 2021;348:109640.
Moeser AJ, Klok CV, Ryan KA, Wooten JG, Little D, Cook VL, et al. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am J Physiol Gastrointest Liver Physiol 2007;292(1):173–81.
Nakatsuji T, Kao MC, Fang JY, Zouboulis CC, Zhang L, Gallo RL, et al. Antimicrobial property of lauric acid against Propionibacterium acnes: its therapeutic potential for inflammatory acne vulgaris. J Invest Dermatol 2009;129(10):2480–8.
Negroni A, Cucchiara S, Stronati L. Apoptosis, necrosis, and necroptosis in the gut and intestinal homeostasis. Mediat Inflamm 2015;2015:250762.
Pluske JR, Williams IH, Aherne FX. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. Anim Sci 1996;62(1):145–58.
Partanen KH, Mroz Z. Organic acids for performance enhancement in pig diets. Nutr Res Rev 1999;12(1):117–45.
Piva A, Morlacchini M, Casadei G, Gatta PP, Biagi G, Prandini A. Sodium butyrate improves growth performance of weaned piglets during the first period after weaning. Ital J Anim Sci 2002;1(1):35–41.
Pluske J, Turpin D, Abraham S, Collins A, Dunshea F. 2C-125: lauric acid, a potentially new feed additive for the Australian pork industry. 2018. 2018.
Rhoads JM, Chen W, Gookin J, Wu GY, Fu Q, Blikslager AT, et al. Arginine stimulates intestinal cell migration through a focal adhesion kinase dependent mechanism. Gut 2004;53(4):514–22.
Shen YB, Piao XS, Kim SW, Wang L, Liu P, Yoon I, et al. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J Anim Sci 2009;87(8):2614–24.
Teufel C, Horvath E, Peter A, Ercan C, Piscuoglio S, Hall MN, et al. mTOR signaling mediates ILC3-driven immunopathology. Mucosal Immunol 2021;14(6):1323–34.
van Beers-Schreurs HM, Nabuurs MJ, Vellenga L, Kalsbeek-van der Valk HJ, Wensing T, Breukink HJ. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. J Nutr 1998;128(6):947–53.
West AB, Isaac CA, Carboni JM, Morrow JS, Mooseker MS, Barwick KW. Localization of villin, a cytoskeletal protein specific to microvilli, in human ileum and colon and in colonic neoplasms. Gastroenterology 1988;94(2):343–52.
Wang S, Xiang N, Yang L, Zhu C, Zhu X, Wang L, et al. Linoleic acid and stearic acid elicit opposite effects on AgRP expression and secretion via TLR4-dependent signaling pathways in immortalized hypothalamic N38 cells. Biochem Biophys Res Commun 2016;471(4):566–71.
Wang Z, Hu J, Yang X, Yin L, Wang M, Yin Y, et al. *N*-Acetyl-D-glucosamine improves the intestinal development and nutrient absorption of weaned piglets via regulating the activity of intestinal stem cells. Anim Nutr 2022a;8(1):10–7.
Wang M, Wang L, Tan X, Wang L, Xiong X, Wang Y, et al. The developmental changes in intestinal epithelial cell proliferation, differentiation, and shedding in weaning piglets. Anim Nutr 2022b;9(1):214–22.
Yang H, Fu D, Shao H, Kong X, Wang W, Yang X, Nyachoti CM, et al. Impacts of birth weight on plasma, liver and skeletal muscle neutral amino acid profiles and intestinal amino acid transporters in suckling Huanjiang mini-piglets. PLoS One 2012a;7(12):e50921.
Yang H, Li F, Kong X, Yuan X, Wang W, Huang R, et al. Chemerin regulates proliferation and differentiation of myoblast cells via ERK1/2 and mTOR signaling pathways. Cytokine 2012b;60:646–52.
Yang H, Wang X, Xiong X, Yin Y. Energy metabolism in intestinal epithelial cells during maturation along the crypt-villus axis. Sci Rep 2016;6:31917.
Yin L, Li J, Zhang Y, Yang Q, Yang C, Yi Z, et al. Changes in progenitors and differentiated epithelial cells of neonatal piglets. Anim Nutr 2022;8(1):265–76.