

GASTROINTESTINAL BIOLOGY

Effect of dietary folate level on organ weight, digesta pH, short-chain fatty acid concentration, and intestinal microbiota of weaned piglets

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Abstract

Folate is increasingly thought to promote gastrointestinal health and regulate the diversity of gut microbiota to alleviate weaning stress in piglets. The present study was conducted to investigate the effects of folate on organ weight, digesta pH, short-chain fatty acids (SCFAs) concentration, and intestinal microbiota in weaned piglets. A total of 28 piglets (6.73 ± 0.62 kg) were allocated to four dietary treatments consisting of a control group, 3, 9, and 18 mg/kg of folate supplementation in a 14-d feeding trial. The results showed that piglets fed with 9 and 18 mg/kg of folate supplementation had greater ($P < 0.05$) average liver and spleen weight than the control group. Folate supplementation (9 and 18 mg/kg) can significantly increase ($P < 0.05$) the stomach pH and tend ($P < 0.10$) to decrease the cecum pH. Folate treatment (9 and 18 mg/kg) had a positive effect on the metabolism of SCFAs in piglets, in particular, compared with the control group, and the content of acetic acid (AA) and valeric acid was markedly increased ($P < 0.05$) in the cecum and colon, respectively. Moreover, isobutyric acid, butyric acid, and isovaleric acid were tended ($P < 0.10$) to increase in the colon. Cecum contents samples were used to determine bacterial community diversity by 16S rRNA gene amplicon sequencing. At the genus level, in the cecum, there was a higher ($P < 0.05$) relative abundance of *Lactobacillus reuteri*, *Lactobacillus salivarius*, and *Lactobacillus mucosae* in the 9 mg/kg folate supplementation group. The functional pathways analysis predicted that folate may modify nutrient metabolism by changing the gut microbiota function of weaned piglets. Furthermore, the data showed that *Lactobacillus* was positively correlated with AA in the cecum. Overall, these findings suggested that folate treatment could increase the organ weight and the stomach pH of weaned piglets and had beneficial effects on gut health, which might be attributed to the alteration in intestinal microbiota induced by folate and the interaction of the intestinal microbiota with SCFAs.

Key words: digesta pH, folate, intestinal microbiota, organ weight, short-chain fatty acid, weaned piglets

Abbreviations

AA	acetic acid
BA	butyric acid
BW	body weight
DCs	dendritic cells
IBA	isobutyric acid
IL	interleukin
IVA	isovaleric acid
OUT	operational taxonomic unit
PCoA	principal co-ordinates analysis
ROS	reactive oxygen species
SCFA	short-chain fatty acids
TNF- α	tumor necrosis factor- α
VA	valeric acid

Introduction

Early weaning stress syndrome in piglets is an important event in the large-scale breeding of pigs, causing huge economic losses to the agriculture enterprises every year. A series of studies have proved that weaning can lead to atrophy of piglet intestinal villi, digestive and absorption disorder, and inflammation, which induce the release of a large number of reactive oxygen species (ROS) free radicals, eventually causing intestinal dysfunction and diseases (Xiong et al., 2015; Zou et al., 2017). Evidences have shown that diet supplementation of appropriate doses of some functional nutrients, such as vitamins, can adjust or improve the immune function and capability of disease resistance and reduce the risk of disease infection or inflammatory responses of weaned piglet (Chen et al., 2019; Li et al., 2019; Wang et al., 2020b).

Folate is considered as a form of the water-soluble vitamin B9 and plays an important role in the growth, health, and maintenance of animal metabolism (Bässler, 1997; Ragaller et al., 2009; Sesay et al., 2017). Folate metabolism also plays an important role in DNA synthesis, chromosomal stability, and integrity via regulated spindle assembly checkpoint signaling (Guo et al., 2017). Mammals, including rats and pigs, cannot synthesize folate, and folate in their bodies is mainly derived from food. Previous studies have shown that folate deficiency can elevate oxidative stress, impair antioxidant enzymatic activities, increase ROS generation, reduce mtDNA biogenesis, and promote mitochondrial oxidative decay (Huang et al., 2001; Hsu et al., 2013). A series of studies from animal models have shown that folate supplementation during critical periods in early development induces different metabolic phenotypes by regulating the promoter DNA methylation status of some specific genes and subsequent mRNA expression abundance. Folate plays a key part in enhancing the immune capacities and resistance against bacterial infection of animals. Folate deficiency was also associated with impaired maturation of dendritic cells (DCs), decreased cytokines (interleukin-6 [IL-6], IL-12, and tumor necrosis factor- α [TNF- α]) secretion by DCs stimulated with lipopolysaccharide and inhibit differentiation of CD4(+) T lymphocytes into Th17 cells (Wu et al., 2017). Grieshop et al. (2001) reported that the daily supplementation of folate to pregnant sows can affect the percentage of peripheral lymphocytes and the secondary antibody response level of the piglets at the weaning process. Moreover, Wang et al. (2011) found that supplementation of folate (100 mg/kg) in the diets of lactating sows can increase milk production, improve milk quality, and increase the growth performance of piglets. Supplementation of folate in

the diet can resynthesize methionine for protein synthesis during moderate methionine deficiency in piglets (McBreairty et al., 2016).

It is possible and necessary to add a higher dose of folate in the diet of weaned piglets to meet their needs and promote immune function, improve the capability of disease resistance, and relieve inflammation. However, little or no data exist presently quantifying folate supplementation on physicochemical effects in the gastrointestinal tract of weaned piglets. Therefore, the purpose of the present study was to determine the effect of dietary supplementation of folate on organ weights, digesta pH, short-chain fatty acid (SCFAs) concentration, and intestinal microbiota of weaned piglets. This may provide valuable insights into exploring the optimum supplementary dose of folate in weaned piglets.

Materials and Methods

The animal experimental design and procedures used in the present study were approved by the Animal Protocol Review Committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences (Changsha, China).

Animals and treatments

A total of twenty-eight piglets (Duroc \times [Landrace \times Yorkshire]; 6.73 ± 0.62 kg) weaned at 21 d of age were randomly assigned by body weight (BW) and gender to four treatments, and each treatment included seven replicate pens and one piglet per pen. Each treatment consisted of a basal diet supplemented with different dosages of folic acid. Treatments A, B, C, and D groups were supplemented with 0, 3, 9, and 18 mg/kg of folate, respectively, 0, 10, 30, and 60 times according to the NRC's (2012) recommendation. The basal diet composition is presented in Table 1 was formulated to meet the nutrient specifications for 7 to 11 kg BW piglets (NRC, 2012). The folate product used in the present study was composed of 80% folate and 20% carrier (Beijing, Blooming Biotechnology Co., Ltd.). The piglets were allowed to consume diets ad libitum and free access to water for 14 d. Previous studies reported that after weaning there are acute phase (first 5 to 7 d) and adaptive phase (second week after weaning), since it takes about 7 d for weaned pigs to learn how to eat out of a feeder and resume an intake that is comparable to that during the preweaning period (Pluske et al., 1997; Le Dividich and Seve, 2000). Therefore, the present study conducted a 14-day animal experimental period.

Organ weight and digesta pH

On day 14 of the trial, all piglets were sacrificed by captive bolt penetration under general anesthesia via intravenous injection of 4% sodium pentobarbital solution (40 mg/kg BW). Subsequently, piglets were immediately eviscerated from sternum to pubis, and the small intestinal tract and organs (liver, spleen, and kidney) were removed, and, finally, the weight (emptied from digesta and blood) of each organ was recorded. Approximately, 30 mL of digesta samples from the stomach, medial ileum (30 cm), medial cecum (30 cm), and medial colon (30 cm) was collected for the pH analysis using a digital pH meter (Fisher Scientific, Sunnyvale, USA).

Intestinal histology and morphology analysis

The cross sections of the cecum and colon samples preserved in formaldehyde and glutaraldehyde mixing fixative were prepared using standard paraffin embedding techniques. The

Table 1. Ingredient and chemical composition of piglet diets, as-fed basis

Ingredients	%
Corn	39.7
Extruded corn	20.0
Soybean meal, 44% crude protein	9.0
Fish meal, 63% crude protein	7.0
Spray-dried porcine plasma	5.0
Whey powder	9.0
Glucose	3.0
Soybean oil	3.8
Limestone	1.05
Choline chloride	0.1
Antioxidants premix ¹	0.05
Citric acid	0.5
NaCl	0.1
Mineral premix ²	0.15
L-Lys HCl	0.45
DL-Met	0.20
L-Thr	0.14
L-Trp	0.02
Vitamin premix ³	0.74
Total	100
Metabolizable energy, kcal/kg	3,393
Crude protein, %	18.50
Calcium, %	0.80
Phosphorus, %	0.38
Folic acid, mg/kg	0.20
Lys, %	1.37
Met + Cys, %	0.75
Thr, %	0.81
Trp, %	0.22

¹Antioxidants premix per kilogram of feed: 50 mg ethoxyquin.

²Mineral premix per kilogram of feed: 150 mg Fe (FeSO₄), 100 mg Zn (ZnSO₄), 30 mg Mn (MnSO₄), 25 mg Cu (CuSO₄), 0.5 mg I (KIO₃), 0.3 mg Co (CoSO₄), and 0.3 mg Se (Na₂SeO₃).

³Vitamin premix supplied per kilogram of feed: 2,200 IU vitamin A, 220 IU vitamin D₃, 0.5 mg vitamin K₃, 0.0175 mg vitamin B₁₂, 3.5 mg riboflavin, 30 mg niacin, 10 mg D-pantothenic acid, 0.05 mg biotin, 0.3 mg thiamine, and 7 mg pyridoxine.

samples were sectioned at 5 μm thickness and were stained with hematoxylin and eosin. The crypt depth (CD) was measured under a microscope with 40× combined magnification using an image processing and analysis system (version 1; Leica Imaging Systems Ltd, Cambridge, UK). At least, 10 well-oriented intact villi and the associated CD of each section were measured in each piglet.

Determination of SCFAs

The amount of SCFAs in the cecum and colon digesta was measured using gas chromatography (GC) according to the method of Alfa et al. (2018). Briefly, digesta samples were homogenized, and 1 g of them was diluted with ddH₂O. After mechanical shaking and centrifuging at 19,152 × g for 15 min, a mixture of supernatant fluid and 25% metaphosphoric acid solution (9:1, v/v) was allowed to stand overnight. Afterward, the solution was centrifuged and filtered through a membrane filter (pore size 0.45 μm). The SCFAs concentrations of samples on a DB-FFAP column (30 m long × 250 μm diameter × 0.25 μm film thickness) were separated by using an Agilent Technologies 7890B GC System with flame ionization detector (FID). Nitrogen was used as the carrier gas with a flow rate of 0.8 mL/min. An FID was used to determine acetate, propionate, butyrate,

isobutyrate, valerate, and isovalerate concentrations. The temperatures of the detector and injector temperatures were 280 and 250 °C, respectively.

16S rRNA high-throughput sequencing

The cecum contents from piglets in the control group (without folate supplementation) and the group receiving 9 mg/kg feed of folate (*n* = 7 per treatment) were collected for microbiota diversity analysis, as described previously (Zou et al., 2019). The total bacterial DNA was extracted from the cecum content (approximately 0.25 g) using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Bacterial community diversity and composition in each cecum sample were determined by 16S ribosomal DNA (rDNA) high-throughput sequencing. The V4 hypervariable region of 16S rRNA gene was PCR amplified using 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGG GTWTCTAAT-3') primers with barcodes. Paired-end sequencing was performed on a ThermoFisher IonS5XL platform (Novogene, Beijing, China).

Bioinformatics analysis of sequencing data

Raw 16S data sequences were obtained, before being screened and assembled using the QIIME (v1.9.1) software packages. UPARSE (v7.0.1001) was used to analyze these clean reads and determine operational taxonomic units (OTUs). Subsequently, high-quality sequences were aligned against the SILVA (<http://www.arb-silva.de/>) and clustered into OTUs at a 97% similarity level using the MUSCLE (v3.8.31) algorithm.

Functional metagenomes of all samples were predicted via PICRUST. OTUs were normalized by copy number, and the gene categories were predicted at level 3 functional difference (Kyoto Encyclopedia of Genes and Genomes [KEGG] Orthology) abundances using the STAMP (V2.1.3) software package.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 statistical package (SPSS Inc., Chicago, IL, USA). Alpha and beta diversities were analyzed using QIIME (v1.9.1). R package software (V2.15.3, R Core Team, Auckland, New Zealand) was used to perform for the statistical analyses of microbial community and create visualized diagrams. Data for microbial metabolic functions were analyzed by Student's t-test. A value of *P* < 0.05 was taken to indicate statistical significance. Tendencies were reported when *P* < 0.1.

Results

Effect of folate supplementation on organ weights and intestinal morphology

The effect of folate supplementation on organ weights is provided in Table 2. The relative weights of the liver, spleen, and kidney were increased by folate supplementation. The liver and spleen of piglets fed 9 and 18 mg/kg folate had higher (*P* < 0.05) relative weight than the control group. In addition, the relative weight of the kidney in piglets fed diets containing 18 mg/kg folate was significantly increased (*P* < 0.05) compared with the control group. However, there were no differences in the relative length of the small intestine (m/kg BW) among the four treatments.

Table 2. Effect of different folate levels on organ index of piglets¹

Items	Supplement folate level, mg/kg				SEM	P-value
	0	3	9	18		
Liver, g/kg BW	20.71 ^a	23.42 ^{ab}	24.86 ^b	26.14 ^b	0.72	0.041
Spleen, g/kg BW	1.59 ^a	2.03 ^{ab}	2.25 ^b	2.22 ^b	0.09	0.024
Kidney, g/kg BW	5.32 ^a	5.78 ^a	5.94 ^{ab}	6.94 ^b	0.21	0.033
Length of the small intestine, m/kg BW	1.17	1.25	1.33	1.24	0.03	0.362

¹n = 7.^{a,b}Values in the same row with different superscript letters are significantly different ($P < 0.05$).

The effect of folate supplementation on the intestinal histology analysis of piglets and on the morphology of cecum and colon is illustrated in [Figure 1](#) and [Table 3](#), respectively. Piglets fed with 9 mg/kg folate had greater ($P < 0.05$) cecum CD than piglets in the control group. However, there were no differences in colonic CD among the four treatments.

Effect of folate supplementation on digesta pH

The effect of folate supplementation on the gastrointestinal digesta pH is presented in [Table 4](#). The stomach pH was higher ($P < 0.05$) for diets containing folate (3, 9, and 18 mg/kg, respectively) compared with the control group. Additionally, a tendency ($P = 0.088$) for lower cecum pH in diets containing 9 mg/kg folate was observed. However, there were no differences in the ileum and colon pH compared with the control group.

Effect of folate supplementation on the SCFAs concentration

Different folate supplementation greatly influences the amount of SCFAs in the cecum and colon of weaned piglets ([Table 5](#)). Compared with the control group, diets containing folate (9 and 18 mg/kg) can significantly increase ($P < 0.05$) the acetic acid (AA) content in the cecum, and the amounts of valeric acid (VA) significantly increased ($P < 0.05$) in the colon in diets containing folate (18 mg/kg). Furthermore, the amounts of isobutyric acid (IBA), butyric acid (BA), isovaleric acid (IVA), and AA: propionic acid ratio in the colon in diets containing folate (18 mg/kg) have increased trend compared with the control group ($P < 0.1$). There were no significant differences observed in the total amounts of SCFAs in the cecum and colon of piglets ($P > 0.05$).

Microbial assessment in cecum contents

Our results showed that diet supplementation of 9 mg/kg folic acid has a positive effect on intestinal morphology, growth performance, and suppressing diarrhea (data not shown) of weaned piglets. Therefore, we used the cecum content of the 9 mg/kg folate group for 16s rRNA high-throughput sequencing. There were no significant differences between treatments for the indices of diversity, including observed_species, Shannon, Simpson, Chao1, abundance-based coverage estimator (ACE), and phylogenetic diversity (PD)_whole_tree indices, of cecal microbiota (data not shown). In addition, based on an unweighted pair-group method with arithmetic mean analysis, Firmicutes, Bacteroidetes, and Spirochaetes were found to be the dominant bacteria in the cecal microbiota ([Figure 2A](#)). At the genus level, there was a higher ($P < 0.05$) relative abundance of *Lactobacillus reuteri*, *Lactobacillus salivarius*, and *Lactobacillus mucosae* in the cecum when piglets were supplemented with 9 mg/kg folate ([Figure 2B](#)).

Effect of folate on metabolic function of microbiota

A principal coordinate analysis of functional profiles showed that there was clear clustering between the control group and folate supplementation group based on the KEGG annotation ([Figure 3A](#)). Compared with the control group, tyrosine metabolism, polycyclic aromatic hydrocarbon degradation, thiamine metabolism, ubiquitin system, retinol metabolism, drug metabolism-cytochrome P450, and sulfur relay system were significantly increased ($P < 0.05$) when piglets were supplemented with 9 mg/kg folate ([Figure 3B](#)).

Discussion

Early weaning of piglets is often accompanied by severe growth inhibition and diarrhea, causing huge economic losses to commercial swine production. In the present study, we provide evidence that folate, when supplemented at the early weaning process, had a positive effect on organ index, digesta pH, SCFAs concentration, and intestinal microbiota of piglets.

The organ index can reflect the strength of organ function to a certain extent. The liver, as an organ with the most vigorous metabolism and the largest digestive gland in the digestive system, has the functions of storing glycogen, secreting bile, and synthesizing secreted proteins. The enhanced dietary folate intake may protect against the accumulation of the mtDNA deletion in the liver of chemotherapeutic drug-treated rats, which indicated that folate is an important nutrient in the maintenance of mitochondrial function ([Branda et al., 2002](#)). The kidneys play an important role in excreting metabolites, maintaining fluid balance and acid-base balance in the body. The spleen contains about 40% T cells and 60% B cells, which play a key role in humoral immunity. The increase in the weight of the immune organs is associated with rapid growth and development ([Mejicanos et al., 2020](#)). A series of studies have confirmed that vitamins can increase the weight of immune organs, promote the development of immune organs, and then improve the body's immunity ([Partearroyo et al., 2013](#); [Pan et al., 2017](#); [Sun, 2018](#)). But up to now, there is no report on the direct effect of folate on the weight and index of weaned piglet organs. Our results show that the diet supplementation with 9 mg/kg folate can significantly increase the liver, kidney, and spleen weight of weaned piglets, suggesting that folate can improve the metabolism and immune function of weaning-stressed piglets by promoting the development of organs index.

The properties of intestinal folate absorption were documented decades ago. Folates are absorbed primarily in the duodenum and jejunum within the acid microenvironment at the cell surface ([Qiu et al., 2006, 2007](#)). The colon is a rich breeding ground for bacteria that synthesize folates and hence are a potential endogenous source of folates ([Maynard](#)

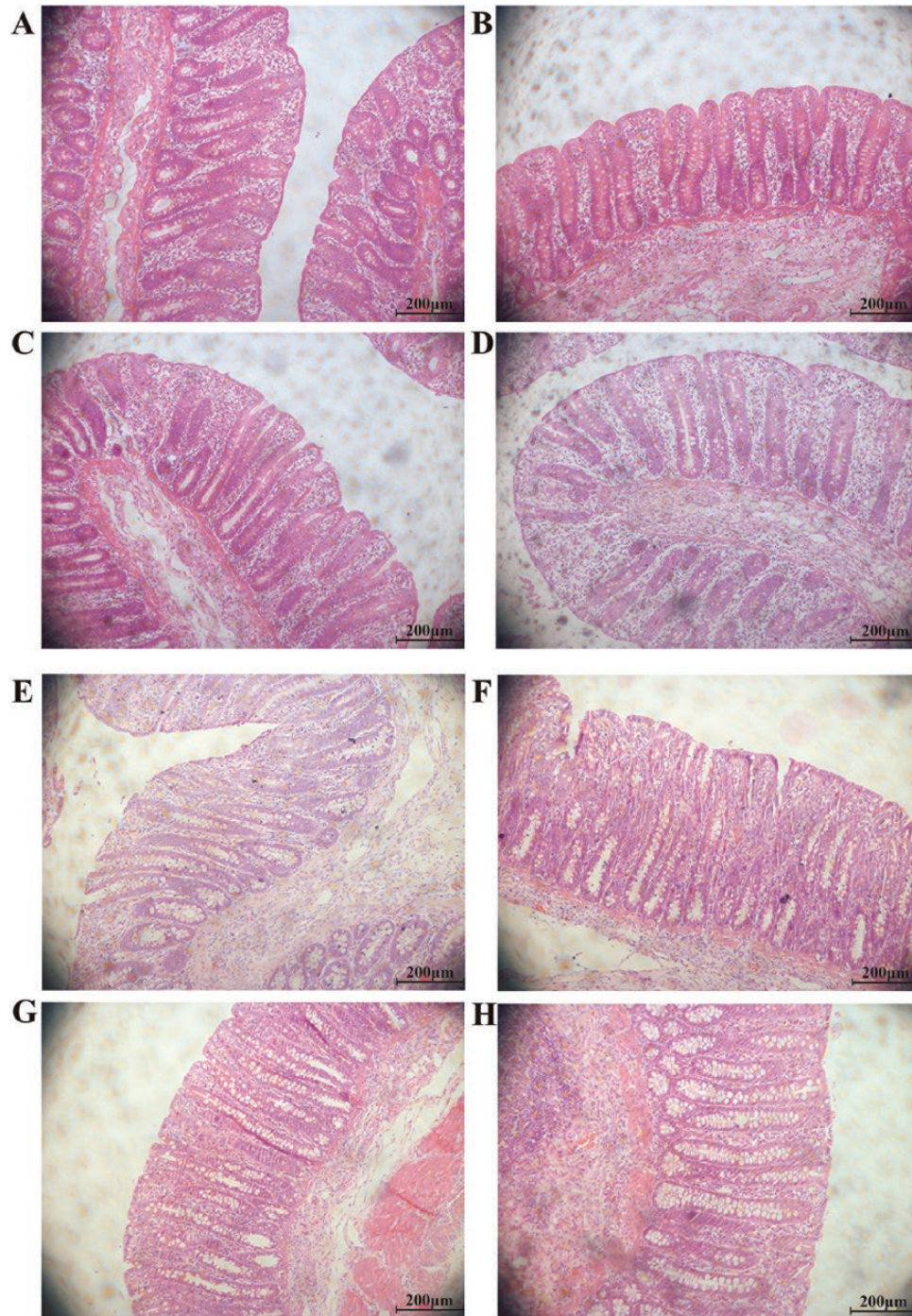


Figure 1. Intestinal histology analysis of weanling piglets fed different dietary concentrations of folate (magnification 200×). (A) cecum 0 mg/kg folate, (B) cecum 3 mg/kg folate, (C) cecum 9 mg/kg folate, (D) cecum 18 mg/kg folate, (E) colon 0 mg/kg folate, (F) colon 3 mg/kg folate, (G) colon 9 mg/kg folate, and (H) colon 18 mg/kg folate.

Table 3. Effects of different folate levels on the morphology of the cecum and colon¹

Items	Supplement folate level, mg/kg				SEM	P-value
	0	3	9	18		
Cecum CD, μm	410.71 ^a	431.00 ^{ab}	478.14 ^b	454.43 ^{ab}	10.12	0.094
Colon CD, μm	339.14	312.86	341.86	329.00	6.15	0.346

¹n = 7.

^{a,b}Values in the same row with different superscript letters are significantly different ($P < 0.05$).

Table 4. Effects of different folate levels on the pH value of chyme in different digestive tracts of piglets¹

Items	Supplement folate level, mg/kg				SEM	P-value
	0	3	9	18		
Stomach pH	3.20 ^a	5.16 ^b	4.95 ^b	5.60 ^b	0.31	0.025
Ileum pH	6.86	7.29	7.52	7.02	0.11	0.165
Cecum pH	6.32 ^a	6.17 ^a	5.70 ^b	5.84 ^{ab}	0.10	0.088
Colon pH	6.64	6.42	6.51	6.43	0.07	0.684

¹n = 7.^{a,b}Values in the same row with different superscript letters are significantly different ($P < 0.05$).**Table 5.** SCFAs concentration (mg/g fresh content) in the cecum and colon of weanling piglets fed different dietary concentrations of folate¹

Items		Supplement folate level, mg/kg				SEM	P-value
		0	3	9	18		
Cecum	AA	3.45 ^a	4.37 ^{ab}	5.32 ^b	4.82 ^b	0.24	0.03
	Propionic acid	2.32	2.61	3.00	3.10	0.18	0.38
	IBA	0.08	0.10	0.10	0.10	0.01	0.43
	BA	0.83	0.93	1.14	1.09	0.10	0.67
	IVA	0.08	0.11	0.11	0.12	0.01	0.50
	VA	0.13	0.16	0.17	0.23	0.02	0.22
	Acetic: propionic acid ratio	1.70	1.77	1.81	1.56	0.07	0.68
Colon	Total SCFAs	6.89	8.28	9.84	9.46	0.49	0.12
	AA	3.15	2.55	4.08	4.01	0.27	0.14
	Propionic acid	1.72	2.47	2.01	2.36	0.15	0.29
	IBA	0.13 ^b	0.20 ^{ab}	0.23 ^a	0.24 ^a	0.02	0.06
	BA	0.74 ^b	1.22 ^{ab}	1.24 ^{ab}	1.47 ^b	0.11	0.10
	IVA	0.22 ^b	0.32 ^{ab}	0.43 ^a	0.41 ^a	0.03	0.06
	VA	0.16 ^b	0.27 ^{ab}	0.33 ^{ab}	0.43 ^a	0.04	0.04
	Acetic: propionic acid ratio	1.91 ^a	1.27 ^b	2.07 ^a	1.73 ^{ab}	0.11	0.07
	Total SCFAs	6.12 ^b	7.03 ^{ab}	8.33 ^{ab}	8.92 ^a	0.46	0.12

¹n = 7.^{a,b}Values in the same row with different superscript letters are significantly different ($P < 0.05$).

et al., 2018), whereas the rate of folate net absorption from the colon was estimated to be approximately one-tenth rate of absorption from the small intestine. The mechanism of folates absorption is across the apical brush-border membrane of the proximal small intestine by the proton-coupled folate transporter (Visentin et al., 2014). The animal's gastrointestinal tract is not only an important organ for the nutrient digestion and absorption but also the largest immune organ (Zou et al., 2017). The proper acidity required to maintain the normal physiological functions of the digestive system of the weaned piglet is an important measure to ensure the health of the piglet. The pH of the gastrointestinal tract of weaned piglets is increased, which is easy to cause the rapid proliferation of *Escherichia coli* and cause intestinal diseases (Xiong et al., 2019). Our results have shown that diet supplementation with 9 mg/kg folate can decrease the pH value of the cecum ($P = 0.088$). However, it is worth noting that folate treatment caused a significant increase ($P < 0.05$) in the stomach pH value of weaned piglets. The possible reason was that piglets have increased feed intake and did not have strict fasting before slaughter, resulting in slow emptying of a gastric chyme. The cecum pH value of weaned piglets may be related to the promotion of the growth of cecal *Lactobacillus* by folate. Furthermore, the lactic acid produced by the metabolism of *Lactobacilli* can reduce the pH of the cecal content (Li et al., 2020).

It is well known that SCFAs play an important role in maintaining the morphology and function of intestinal epithelial cells, gut microbiota balance, and intestinal immune response (Liu et al., 2018). It is well known that the main way for animals to obtain SCFAs is through the fermentation of hindgut microbiota. On the other hand, the released SCFAs could be metabolized by the intestinal microorganisms as a carbon source and converted into other SCFAs. For example, AA could be converted into BA, propionic acid could be converted into AA, and BA could be converted into propionic acid and AA (Barcenilla et al., 2000; Duncan Sylvania et al., 2004). SCFAs can provide 60% to 70% of the energy of colonic epithelium. Generally, the intestinal tract uses BA first, followed by propionic acid and AA. Shibata et al. (2017) reported that SCFAs can inhibit the colonization and growth of some pathogens (*Salmonella* and *E.coli*) by reducing the pH value of the large intestine. Hung and Suzuki (2018) found that SCFAs, such as acetate, propionate, and butyrate, transported by monocarboxylate transporter (MCT)-1 suppress TNF- α -induced inflammatory signaling in Caco-2 cells and mouse colons. In addition, SCFAs act as specific G protein-coupled receptor signaling molecules and are involved in the regulation of host energy (glucose and lipid) metabolism (den Besten et al., 2013). The findings in the present study indicated that folate treatment could generate more SCFAs (AA, IBA, BA, IVA, and VA) and promote the intestinal health

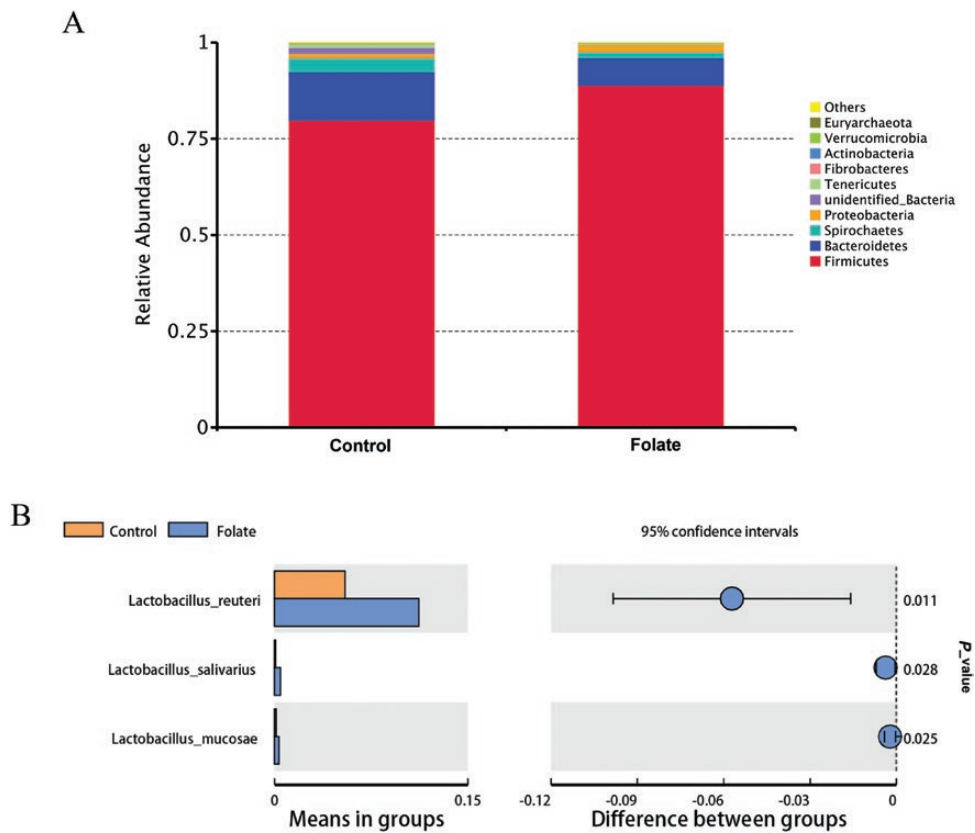


Figure 2. Microbial communities in the cecum of weaned piglets between control and folate-supplemented groups after 14-d of feeding. (A) Top 10 cecum bacterial communities at the genus level in weaned piglets and (B) significantly different in weaned piglets. $n = 7$.

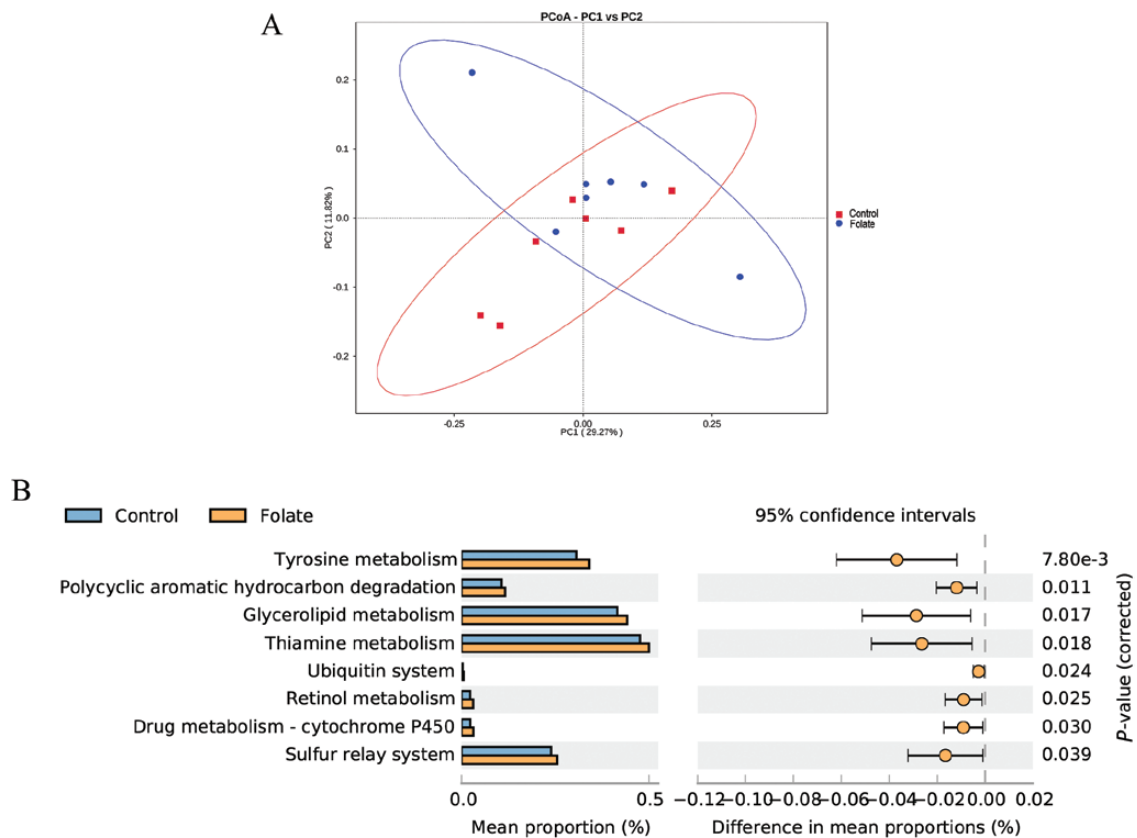


Figure 3. Analysis of functional differences in the cecal microbiota of weaned piglets between control and folate-supplemented groups after 14-d of feeding. (A) Principal components analysis (PCoA) plot of functional profiles among groups and (B) t-test bar plot of significantly different KEGG signal pathways. $n = 7$.

of the weaned piglets, which are closely correlated with the decreased cecal pH of weaned piglets in the folate treatment. It is worth noting that the dietary supplementation of 3 mg/kg folate caused a decrease in the concentration of AA in the colon ($P > 0.05$), due to which AA may be converted into BA by intestinal microbiota.

The previous studies show that ruminal total volatile fatty acids concentration, cellulolytic bacteria abundance, and enzyme activity increased with folate supplementation in steers (Wang et al., 2016, 2020a). Zhu et al. (2019) reported that vitamin B12 can be used as a growth substrate for beneficial bacteria to promote the growth of *Lactobacillus* so as to competitively inhibit the proliferation of *E. coli*, thereby improving the structure of the piglet's intestinal microflora and promoting the intestinal microecological balance. Zhang et al. (2020) found that the intestinal dysbacteriosis was significantly correlated with folate deficiency in a folate-deficient rat model, and supplementation of folic acid bacteria can restore the destruction of the intestinal microbiota and restore serum folate and homocysteine to normal levels. In the present study, we employed 16S rRNA sequencing to investigate the effects of folate on the richness and diversity of cecal microbiota and found that folate had positive influences on *Lactobacillus* of the cecum segment. Most of the *Lactobacillus* that produce folate belong to the *Bifidobacterium*, *Streptococcus*, *Lactobacillus*, and *Lactococcus* genus. Among them, the relative production of folic acid of *Bifidobacterium* and *Streptococcus* is lower than that of *Lactobacillus* and *Lactococcus* genus. Therefore, our results show that the addition of folate to the diet can alleviate the decrease in the number of intestinal lactobacilli in piglets caused by waned stress.

Conclusions

The results of the present study indicated that diet supplementation with folate can improve the weight of the liver, kidney, and spleen in piglets at the weaning process. Piglets supplemented with 9 mg/kg folate can increase cecum CD and decrease the cecum pH compared with the control group. Further investigation indicated that there were close interactions between cecum pH value and SCFAs. In addition, diet supplementation with 9 mg/kg folate had greater proportions of beneficial bacteria (*Lactobacillus*) in piglet cecum. These findings will provide valuable guidance for the regulation of organ index, digesta pH, intestinal microbiota, and SCFAs concentration through folate to improve weaned piglet health and productivity.

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Author Contributions

L.W., J.L., Y.H., and Y.Y. designed the study. L.W. and Y.H. carried out the animal trials and sample analysis. W.L., L.Z., J.L., H.Y., and Y.Y. wrote and revised the manuscript.

Conflict of interest statement

The authors have no real or perceived conflicts of interest.

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