Improvement of growth performance and parameters of intestinal function in liquid fed early weanling pigs¹

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ABSTRACT: Liquid feeding, a widely used technique, has been applied as a feeding technique commonly in global swine production. The objective of this study was to evaluate the effects of liquid feeding on growth performance, nutrient digestibility, and intestinal barrier functions during the early weaning period in pigs. Three hundred and sixty 24-d-old weanling pigs (Duroc × Landrace × Yorkshire) with BW of 6.98 ± 0.15 kg were randomly assigned to a control diet (dry fed basal diet, **CON**) or as meal mixed with water in the ratio 1:4 (liquid fed basal diet, **LF**) with 6 replicates per treatment and 30 weanling pigs per replicate. The study lasted 7 d. On days 4 to 7, fresh fecal samples were collected to evaluate apparent total tract digestibility (**ATTD**) of nutrients. After 7 d, 2 weanling pigs per pen were euthanized and physiological samples were obtained. Results showed that LF increased ($P < 0.05$) ADG (281 g vs. 183 g), ADFI (374 g vs. 245 g),

and final BW (8.95 kg vs. 8.26 kg) compared with CON. Compared with CON, LF significantly decreased ($P < 0.05$) serum cortisol and D-lactate concentrations as well as the activity of diamine oxidase, enhanced ($P < 0.05$) the ATTD of ether extract and ash, increased ($P < 0.05$) the activities of amylase, lipase, and lactase in the jejunal mucosa. Furthermore, LF had higher ($P < 0.05$) villus height and villi height: crypt depth and increased ($P < 0.05$) mRNA expressions of insulin-like growth factors-1 receptor (*IGF-1R*), claudin-2 (*CLDN-2*), zonula occludens-1 (*ZO-1*), and zonula occludens-2 (*ZO-2*) in the jejunum. Moreover, LF had lower ($P < 0.05$) abundances of total bacteria and *Escherichia coli* and higher ($P < 0.05$) concentrations of acetic acid and butyric acid in cecal digesta. Altogether, the results indicated that liquid feeding not only promoted growth performance but also improved intestinal health by enhancing gut barrier functions in weanling pigs.

Key words: barrier function, growth performance, intestinal health, liquid feeding, weanling pigs

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J. Anim. Sci. 2019.97:2725–2738

doi: 10.1093/jas/skz134

INTRODUCTION

Weaning is abruptly stressful in weanling pigs' lives, resulting in low voluntary feed consumption, suboptimal growth rate, and higher morbidity and mortality (Madec et al., 1998; Lallès et al., 2004; Montagne et al., 2007). Weanling pigs maintaining or losing weight in the first week after weaning required an additional 10 d to reach market weight compared with pigs gaining 250 g/d in this period

¹This study was founded by the earmarked fund from the National Key Research and Development Program of China (2016YFD0501204) and the China Agriculture Research System (CARS-35) and the Science. There is no conflict of interest to disclose.

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Received November 12, 2018.

Accepted April 17, 2019.

(Tokach et al., 1992), which may result in serious economic losses to the pig industry. Moreover, lower feed intake (FI) in turn affects diverse aspects of structure and function in the intestine, including intestinal inflammation, villous atrophy, crypt hyperplasia, and lower activities of epithelial brush border enzyme (Pluske et al., 1997; Boudry et al., 2004; Lallès et al., 2007; Hu et al., 2012; McLamb et al., 2013). In addition, deteriorated intestinal barrier function may promote the translocation of bacteria and the entering of allergenic compounds from the gut into the body, resulting in growth retardation and severe diarrhea (Moeser et al., 2007; Smith et al., 2010; Wijtten et al., 2011). A sufficient FI after weaning might prevent the loss of the barrier function by providing a sufficient luminal nutrient supply (Spreeuwenberg et al., 2001; Wijtten et al., 2011). This indicates the importance of a sufficient luminal nutrient supply during the postweaning period to maintain the intestinal barrier functions. Therefore, nutrition and management in the first week after weaning are primarily aimed toward promoting weanling pigs FI.

Liquid feeding, a widely used technique that mixes water with feed at a constant ratio, is often applied in global swine production because of the allowance for a diversity of alternative feed ingredients and by-products (Tostenson et al., 2017). Furthermore, liquid feeding has been proven to exert a beneficial influence on the growth performance of pigs, including increases in body weight gain (BWG) and FI (Canibe and Jensen, 2003; Hurst et al., 2008; Price et al., 2013; Missotten et al., 2015) as a result of similar physical characteristics with sow milk (Deprez et al., 1987; Brooks et al., 2003). However, little information is available regarding the effects of liquid feeding on intestinal barrier function of pigs in the first week after weaning.

The present experiment was designed to evaluate the effects of liquid feeding on growth performance, nutrient digestibility, intestinal morphology, the expression levels of tight-junction proteins and intestinal development-related genes, gut microbiota, and microbial metabolites during the early weaning period in pigs.

MATERIALS AND METHODS

The experimental protocol used in the present experiment was reviewed and approved by the Animal Experimental Committee of Sichuan Agricultural University. This experiment was conducted at the Animal Experiment Center of Henan Sanli breeding Co., Ltd., Dengfeng, China.

Experimental Design and Animal Management

A total of three hundred and sixty 24-d-old pigs (Duroc × Landrace × Yorkshire, weaned at 24 ± 1 d) with BW of 6.98 ± 0.15 kg were used in a 7-d experiment. At the beginning of the experiment, weanling pigs were randomly assigned to 2 treatments with 6 replicate pens (15 males and 15 females per pen) on the basis of their initial BW and sex. The 2 treatment groups were CON (control, dry fed basal diet) and LF (liquid feeding, liquid fed basal diet).

The basal diet was formulated to meet or exceed the nutrient requirements recommended by the NRC (2012). Ingredients and compositions of the basal diet are presented in Table 1. Each pen (4.0×3.0 m) was equipped with a slatted plastic floor and 4 stainless-steel nipple drinkers. Water was provided ad libitum to weanling pigs. The LF diet was prepared by mixing the dry basal meal with water at a ratio of 4 L water per kg feed by an automatic liquid feeding device (HHIS-010, Henan Heshun Automation Equipment Co., Ltd., Dengfeng, China). The DM content of the liquid fed diets was averaged $18.5 \pm 0.3\%$ DM. The liquid feeding device comprised a trough (60×60 cm) and a circulation system which enabled the feed to be mixed with water in the trough. The quantity of feed in the trough was controlled by an infrared device which was used to sense the feed quantity. The CON was fed in the same feed troughs with the liquid feeding device off and the basal diet in the form of powder. Troughs were emptied daily and residual feed weighed back. Any fouled feed was removed, dried and weighed to estimate wastage. The wasted feed was also collected, dried and weighed to estimate wastage. Daily additions of dry feed to the trough were recorded. All weanling pigs were fed diets 6 times per day at 0800, 1100, 1400, 1700, 2000, and 2300 h for a 7-d period. All weanling pigs were housed in a temperature and relative humidity-controlled room with temperature maintained at 27 ± 1 °C, and relative humidity controlled at 55% to 65%. All weanling pigs were weighed at the beginning and the end of the experiment after 12 h of fasting, and FI per pen was recorded daily throughout the experiment to calculate ADG, ADFI, and G:F.

Diarrhea Rate

The occurrence of diarrhea was recorded every morning and evening from days 1 to 7 of the trial by the same person on 30 marked pigs per pen and

Table 1. Compositions and nutrient contents of the experimental diets (as-fed basis)

Item	Basal diet
Ingredient, %	
Corn	36.58
Full-fat soybean, extruded	5.00
Soybean meal, dehulled	5.00
Dried whey	15.00
Soybean protein concentrate	10.00
Fish meal	5.00
Spray-dried porcine plasma	6.00
Sucrose	3.00
Glucose	5.00
Coconut oil	5.00
L-Lysine HCl	0.40
D,L-Methionine	0.20
L-Threonine	0.13
Tryptophan	0.04
Choline chloride	0.18
Limestone	0.60
Dicalcium phosphate	0.20
Lactic acid	2.00
Antioxidant	0.02
Complex enzyme ¹	0.30
Chlortetracycline ²	0.05
Vitamin premix ³	0.10
Mineral premix ⁴	0.20
Calculated nutrient compositions	
DE, MJ/kg	15.24
CP, %	21.96
Ca, %	0.70
Total P, %	0.65
Available P, %	0.47
Sodium, %	0.55
Lys, %	1.60
Met, %	0.51
Met + Cys, %	0.91
Trp, %	0.30
Thr, %	0.96
Analyzed nutrient compositions	
GE, MJ/kg	17.07
CP, %	21.80
Crude fat, %	4.61
Crude ash, %	5.62
DM, %	91.40

¹Provided per gram of complex enzyme: xylanase, 3,500 units; β -mannanase, 50 units; protease, 3,000 units.

²ENDOX (Kemin industries Co., Ltd., Zhuhai, China). Butyl hydroxy anisid: 1.8%; Ethoxyquin: 2.7%.

³Provided per kilogram of complete diet: vitamin A, 15,000 IU; vitamin D₃, 1,000 IU; vitamin E, 25 IU; vitamin K₃, 5 mg; thiamin, 2 mg; riboflavin, 16 mg; vitamin B₆, 6 mg; vitamin B₁₂, 0.03 mg; nicotinic acid, 35 mg; calcium pantothenate, 25 mg; folic acid, 2.5 mg; biotin, 3.3 mg.

⁴Provided per kilogram of complete diet: Fe, 120 mg as ferrous sulfate; Zn, 120 mg as zinc sulfate; Cu, 20 mg as copper sulfate; Mn, 15 mg as manganese sulfate; I, 0.3 mg as potassium iodide; Se, 0.3 mg as sodium selenite.

based on the following: Scores were 0 = normal, firm feces; 1 = soft feces, possible slight diarrhea; 2 = formless, semifluid feces, moderate diarrhea; and 3 = very watery and frothy feces, severe diarrhea. Thus, an accumulative diarrhea score per treatment and day was calculated (Montagne et al., 2004). The occurrence of diarrhea was defined as a fecal score of 2 or 3 for consecutive morning and evening measurements. Diarrhea rate was calculated referring as follows: diarrhea score (%) = $A/(B \times 7 \text{ d}) \times 100$, in which A = total number of pigs per pen with diarrhea and B = number of pigs per pen.

Sample Collection

Samples of the dry basal diet were collected for chemical analysis. Fresh fecal samples were collected from approximately 15 pigs per pen immediately after defecation and then placed in individual plastic bags, from days 4 to 7 during the trial. After each collection of feces, 10 mL of a 10% H₂SO₄ solution was added to each 100 g of wet fecal sample for fixation of excreta nitrogen. All feed and fecal samples were stored at -20 °C until analysis.

On day 8, prior to the morning feeding and following overnight fasting, 2 weanling pigs (1 male and 1 female) with the average BW in each pen were chosen and bled. Blood samples were collected from the precaval vein into nonheparinized vacuum tubes. Briefly, after centrifugation (3,500 $\times$ g for 10 min at 4 °C), serum samples were collected and stored at -20 °C for serum parameters analysis. After bleeding, the same weanling pigs were euthanized with a lethal dose of sodium pentobarbital (200 mg/kg of BW) according to the previous study by Chen et al. (2013), then killed, and the abdomen was immediately unfolded for the collection of gut sections. The entire small intestine was removed and cut into 3 segments: duodenum, jejunum, and ileum according to the description presented in our previous study by Zheng et al. (2017). Subsequently, 20-cm proximal jejunum was cut and throw away to avoid confusion with duodenum. Then, approximately 2-cm segments of proximal jejunum were immediately isolated, gently washed with 0.9% physiological saline, and preserved in 10% formaldehyde-phosphate buffer for histological analysis. Next 20-cm segments of jejunum were emptied, carefully flushed with saline, and placed on an ice-cold surface. The mucosa of the jejunum was gently scraped with a glass slide and snap-frozen in liquid nitrogen and then stored at -80 °C for further analyses. In addition,

the digesta from middle cecum (10 cm) and middle colon (10 cm) was collected and stored at -80°C for measuring microbial quantity and microbial metabolites.

Measurement of Apparent Digestibility of Nutrients

Feces from 4 d of each pen were mixed thoroughly and dried at 65°C for 72 h, after which they were ground to pass through a 40-mesh screen. Apparent total tract digestibility (ATTD) of nutrients was measured using AIA as digestibility indicator. The AIA in diet and fecal samples were determined by a method described by Chinese National Standard (GB/T 23742, 2009). The AIA content of the basal diet averaged $0.20 \pm 0.002\%$ DM. After AIA analysis, all feed and fecal samples were analyzed for DM (method 930.15; AOAC, 1995), ash (method 923.03; AOAC, 1995), crude fat (method 920.39; AOAC, 1995), and CP (method 990.03; AOAC, 1995). Gross energy was determined using a specific adiabatic oxygen bomb calorimetry (Parr Instrument Co., Moline, IL). The ATTD was calculated using the following formula: $\text{ATTD} (\%) = \{1 - [(A_1 \times F_2)/(A_2 \times F_1)]\} \times 100$, in which A_1 = AIA content in diet (% DM), A_2 = AIA content in feces (% DM), F_1 = nutrient content in diet (% DM), and F_2 = nutrient content of feces (% DM).

Measurement of Enzyme Activities

Approximately 1 g of frozen jejunum mucosal samples were weighed and homogenized with 9 times the volume (wt/vol) of precooled physiological saline. The mixture was centrifuged at $4,000 \times g$ for 10 min at 4°C to collect the supernatant solution. The supernatant protein concentration was assayed using a protein quantification kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) as the protein standard. Subsequently, activities of trypsin, lipase, amylase, lactase, maltase, and sucrase in the supernatant solution were analyzed using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) combined with a UV-VIS Spectrophotometer (UV1100, MAPADA, Shanghai, China) according to the manufacturer's instructions.

Serum Physiochemical Parameters

Serum IGF-1, cortisol, diamine oxidase, and D-lactate levels were assayed using commercially

available porcine-specific ELISA kits (Beijing Chenglin Biotechnology Co., Ltd., Beijing, China) and an automatic biochemical instrument (Biochemical Analytical Instrument, Beckman CX4, Beckman Coulter Inc., Brea, CA). The minimum detectable levels were $0.1 \mu\text{g/L}$, $1.0 \mu\text{g/L}$, 1.0 pg/mL , and $10 \mu\text{g/L}$ for IGF-1, cortisol, diamine oxidase, and D-lactate, respectively. Moreover, the maximum CV was 10% for IGF-1, cortisol, diamine oxidase, and D-lactate. All measurements were conducted in triplicate at minimum according to the manufacturer's instructions.

Intestinal Morphology

The jejunum morphology was measured as described by the previous study by Pluske et al. (1996a). Briefly, the samples were fixated in neutral buffered formaldehyde, dehydrated, and embedded in paraffin wax before 4 transverse sections ($5 \mu\text{m}$) were cut, then installed on glass slides, and stained with eosin and hematoxylin. Villus height and crypt depth were determined with an Olympus CK 40 microscope (Olympus Optical Company, Shenzhen, China). The villus height was measured from the tip to the base, and the crypt depth was measured from the crypt-villus junction to the base. A minimum of 10 well-orientated villi and associated crypts from each intestinal segment were measured.

Total RNA Extraction and real-time quantitative PCR

Jejunum mucosal samples (approximately 0.1 g) were homogenized in 1 mL RNAiso Plus reagent (TaKaRa, Dalian, China), and total RNA was extracted according to the manufacturer's protocols. The concentration and quality of total RNA were assessed using a spectrophotometer (Beckman Coulter DU 800; Beckman Coulter Inc., Brea, CA), determining an optical density (OD)₂₆₀:OD₂₈₀ ratio ranging from 1.8 to 2.0 in all RNase-free water-treated RNA samples. Meanwhile, the integrity of RNA was checked by formaldehyde gel electrophoresis, and the 28S:18S ribosomal RNA band was determined as ≥ 1.8 , then the synthesis of the first strand of cDNA of each sample was obtained by reverse transcription using a PrimeScript reverse transcription reagent kit (TaKaRa) following the manufacturer's instructions.

Specific primers for the insulin-like growth factors 1 (IGF-1), insulin-like growth factors 1 receptor (IGF-1R), epidermal growth factor (EGF), glucagon-like peptide 2 (GLP-2), claudin

1 (*CLDN-1*), claudin-2 (*CLDN-2*), occludin (*OCN*), zonula occludens-1 (*ZO-1*), and zonula occludens-2 (*ZO-2*) were designed and purchased from Invitrogen (Shanghai, China), which are listed in Table 2. The real-time PCR reactions were performed on CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA), using SYBR Green PCR reagents (TaKaRa). A total volume of 10 µL PCR reaction system comprised 5 µL SYBR Green, 0.5 µL forward primer, 0.5 µL reverse primer, 1 µL cDNA, and 3 µL nuclease-free H₂O. The real-time PCR reactions were performed using the following cycle program: a pre-cycling stage at 95 °C for 30 s, and 40 cycles of denaturation at 95 °C for 10 s and annealing at annealing temperature for 25 s with a final extension at 72 °C for 5 min. A melting curve analysis was generated following each real-time quantitative PCR assay to check and verify the specificity and purity of all PCR products. The reference gene transcript (β -actin) was chosen as the reference gene to normalize cDNA loading. For calculation of the amplification efficiencies, a 10-fold serial dilution was used to generate standard curves for both targeted and reference genes, quantifying 6 concentrations. After verification that the primers amplified with an efficiency of approximately 100%, and the results were analyzed using the $2^{-\Delta\Delta C_t}$ method (Livak

and Schmittgen, 2001). Analysis of each standard and sample was run in triplicate simultaneously on the same PCR plate, and the average of each triplicate value expressed as numbers of copies was used for subsequent statistical analysis.

DNA Extraction and Quantification of Intestinal Microflora

Microbial genomic DNA was isolated from the digesta samples (approximately 0.2 g) using the E.Z.N.A stool DNA kit (Omega Bio-Tek, Doraville, GA) in accordance with the manufacturer's protocols. Primers and probes (Table 3) for total bacteria, *Escherichia coli*, *Lactobacillus*, *Bifidobacterium*, and *Bacillus* were obtained from the previous work by Fierer et al. (2005) and Qi et al. (2013), which were commercially synthesized by Invitrogen (Shanghai, China). Quantitative real-time PCR was performed with CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.). For determining total bacteria, each measurement was run in a volume of 25 µL with 1 µL forward primer, 1 µL reverse primer, 12.5 µL SYBR Premix EX Taq (TaKaRa), 1 µL template DNA, and 9.5 µL nuclease-free water. The thermal cycling conditions were an initial pre-denaturation step at 95 °C for 10 s, 40 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for

Table 2. Sequence of primers used for the real-time quantitative PCR analysis

Genes ¹	Primer sequences (5'–3') ²	Size (bp)	A_T ³ , °C	Accession number
<i>CLDN-1</i>	F: GCCACAGCAAGGTATGGTAAC R: AGTAGGGCACCTCCCAGAAG	140	60	NM_001258386.1
<i>CLDN-2</i>	F: GCATCATTTCTCCCTGTT R: TCTTGGCTTTGGGTGGTT	156	60	NM_001161638.1
<i>OCN</i>	F: CTACTCGTCCAACGGGAAAG R: ACGCCTCCAAGTTACCACTG	158	62	NM_001163647.2
<i>ZO-1</i>	F: CAGCCCCCGTACATGGAGA R: GCGCAGACGGTGTTTCATAGTT	114	60	XM_005659811.1
<i>ZO-2</i>	F: ATTCGGACCCATAGCAGACATAG R: GCGTCTCTTGGTTCTGTTTATAGC	90	60	NM_001206404.1
<i>IGF-1</i>	F: CTGAGGAGGCTGGAGATGTACT R: CCTGAACCTCCCTCTACTTGTGTTC	137	58.5	NM_001097417.1
<i>IGF-1R</i>	F: GGGATGACGAGAGACATCTATGAG R: GAAGGACCAGACTCAGACTGC	132	56.8	NM_214172.1
<i>EGF</i>	F: ATCTCAGGAATGGGAGTCAACC R: TCACTGGAGGATGGAATACAGC	165	60	NM_214020.1
<i>GLP-2</i>	F: ACTCACAGGGCACGTTTACCA R: AGGTCCCTTCAGCATGTCTCT	149	56	NM_005671883.1
β -Actin	F: TCTGGCACACACCTTCT R: TGATCTGGGTCATCTTCTCAC	114	57	DQ178122

¹*CLDN* = claudin, *OCN* = occludin, *ZO-1* = zonula occludens 1, *ZO-2* = zonula occludens 2, *IGF-1R* = IGF-1 receptor, *EGF* = epidermal growth factor, *GLP-2* = glucagon-like peptide 2.

²F = forward primer; R = reverse primer.

³ A_T = annealing temperature.

Table 3. Sequence of primers and probes used for the real-time PCR analysis of microbial populations

Primer	Nucleotide sequence (5'-3') ¹	Product size, bp	A_T ² , °C	Reference
Total bacteria	F: ACTCCTACGGGAGGCAGCAG R: ATTACCGCGGCTGCTGG	200	60	Han et al. (2012)
<i>Escherichia coli</i>	F: CATGCCGCGTGTATGAAGAA R: CGGGTAACGTCAATGAGCAAA P: AGGTATTAACTTTACTCCCTTCCTC	96	60	Qi et al. (2013)
<i>Lactobacillus</i>	F: ACTCCTACGGGAGGCAGCAG R: CAACAGTTACTCTGACACCCGTTCTTC P: AAGAAGGGTTTCGGCTCGTAAACTC-TGTT	126	60	Qi et al. (2013)
<i>Bifidobacterium</i>	F: CGCGTCCGGTGTGAAAG R: CTTCCCGATATCTACACATTCCA P: ATTCCACCGTTACACCGGGAA	121	60	Xiang et al. (2011)
<i>Bacillus</i>	F: GCAACGAGCGCAACCCTTGA R: TCATCCCCACCTTCCTCCGGT P: CGGTTTGTCACCGGCAGTCACCT	92	60	Qi (2011)

¹F = forward primer; R = reverse primer; P = probe.

² A_T = annealing temperature.

25 s, and extension at 72 °C for 60 s. For the quantification of *Lactobacillus*, *E. coli*, *Bifidobacterium*, and *Bacillus*, real-time PCR was conducted in a volume of 20 µL with 1 µL probe enhancer solution, 0.3 µL probe, 1 µL forward and 1 µL reverse primer, 8 µL RealMasterMix (Tiangen, Beijing, China), 1 µL template DNA, and 7.7 µL nuclease-free water. The PCR protocols involved 10 s at 95 °C and 50 cycles for 5 s at 95 °C, 25 s at annealing temperature, and 60 s at 72 °C. Copies per sample were calculated with the threshold cycle (C_T) values and standard curve from the previous work by Qi et al. (2013).

Microbial Metabolites Analysis

Approximately 0.7 g of digesta samples were used to determine the concentration of VFA by gas chromatography according to Chen et al. (2013). Briefly, the supernatants of digesta samples were centrifuged at $500 \times g$ for 10 min after adding 1:1 distilled water, 2 mL of supernatant was then transferred to a sterile tube and centrifuged at $12,000 \times g$ for 10 min, after which 1 mL of the supernatant was transferred to a new sterile tube to which 0.2 mL 25% metaphosphoric acid was added. This was left at room temperature for 30 min and then centrifuged at $12,000 \times g$ for 10 min. Five hundred microliters of supernatant was transferred to another sterile tube, to which 500 µL of methanol was added and the mixture was centrifuged at $12,000 \times g$ for 10 min. The supernatant was transferred to a sterile tube and was stored at -20 °C until ready for gas chromatography testing. The VFA (acetic acid, propionic

acid, and butyric acid) were separated and quantified in a gas chromatographic system (VARIAN CP-3800, Varian, Palo Alto, CA).

Statistical Analysis

Growth performance, nutrient digestibility, and diarrhea score data were analyzed by *t*-test using the statistical program of SAS (SAS Inst. Inc., Cary, NC) with pen as the experimental unit ($n = 6$). All other data were analyzed using the *t*-test of SAS (SAS Inst. Inc.) with average data of 2 sampled pigs per pen as the experimental unit ($n = 6$). The results were shown as mean and SEM. For significance determination, the α -level was set as 0.05. A probability level of $P \leq 0.05$ was considered significant, whereas $P < 0.10$ was considered a tendency.

RESULTS

Growth Performance and Diarrhea Score

No death occurred in neither CON nor LF pigs throughout the trial. Compared with CON group, weanling pigs in LF group had greater ($P < 0.05$) final BW, ADG, and ADFI, and tended ($P < 0.1$) to have a lower rate of diarrhea occurrence (Table 4). However, there was no significant difference in G:F between the 2 groups.

Physiochemical Parameters in Serum

Compared with CON group, LF significantly decreased ($P < 0.05$) cortisol concentration,

Table 4. Effects of liquid feeding on growth performance and diarrhea rate in weanling pigs¹

Item	CON ²	LF ³	SEM	P
Initial BW, kg	6.98	6.99	0.15	0.973
Final BW, kg	8.26 ^a	8.95 ^b	0.22	0.013
ADG, g	183 ^a	281 ^b	13	<0.001
ADFI, g	245 ^a	374 ^b	14	<0.001
G:F	0.75	0.75	0.07	0.999
Diarrhea rate ⁴ , %	5.40	4.80	0.30	0.073

¹Values means $n = 6$ for CON and LF groups.

²CON = basal diet with dry feeding.

³LF = basal diet with liquid feeding.

⁴Diarrhea score (%) = $A/(B \times 7 \text{ d}) \times 100$, where A = total number of piglets per pen with diarrhea and B = number of piglets per pen.

^{a,b}Means within a row lacking a common superscript differ at $P < 0.05$.

Table 5. Effects of liquid feeding on the serum parameters in 31-d-old weanling pigs¹

Item	CON ²	LF ³	SEM	P
IGF-1, µg/L	7.2	7.5	0.4	0.464
COR ⁴ , µg/L	103.5 ^a	92.2 ^b	5.2	0.048
DAO ⁵ , pg/mL	179.5 ^a	163.4 ^b	5.9	0.022
D-Lactate, µg/L	717.2 ^a	617.7 ^b	30.7	0.009

¹Values means $n = 6$ for CON and LF groups.

²CON = basal diet with dry feeding.

³LF = basal diet with liquid feeding.

⁴COR = cortisol.

⁵DAO = diamine oxidase.

^{a,b}Means within a row lacking a common superscript differ at $P < 0.05$.

D-lactate concentration, and the diamine oxidase activity in the serum of weanling pigs (Table 5). There were no significant differences in serum IGF-1 concentration between the 2 groups.

Nutrient Digestibility

As shown in Table 6, the ATTD of ether extract (EE) and ash in weanling pigs of LF group were greater than those of CON group ($P < 0.05$). However, no differences in ATTD of CP, DM, and GE were detected between the 2 groups.

Digestive Enzyme Activities

Compared with CON group, LF significantly increased ($P < 0.05$) the activities of lipase, amylase, and lactase in the jejunal mucosa of weanling pigs (Table 7). However, no differences in the activities of trypsin, maltase, and sucrase in the jejunal mucosa of weanling pigs were observed between the 2 groups.

Table 6. Effects of liquid feeding on the apparent total tract digestibility (ATTD) of nutrients in 31-d-old weanling pigs¹

Item, %	CON ²	LF ³	SEM	P
DM	85.74	85.61	0.60	0.835
CP	76.61	74.62	1.36	0.172
GE	84.43	84.43	0.68	0.999
Ether extract	72.45 ^a	77.54 ^b	1.56	0.015
Ash	65.24 ^a	66.88 ^b	0.67	0.033

¹Values means $n = 6$ for CON and LF groups. The samples were collected over 4 d from approximately 15 pigs per pen.

²CON = basal diet with dry feeding.

³LF = basal diet with liquid feeding.

^{a,b}Means within a row lacking a common superscript differ at $P < 0.05$.

Table 7. Effects of liquid feeding on the jejunum digestive enzyme activities in 31-d-old weanling pigs¹

Item	CON ²	LF ³	SEM	P
Trypsin, units/mgprot ⁴	1,111	1,087	93	0.794
Lipase, units/gprot ⁵	540 ^a	690 ^b	72	0.049
Amylase, units/mgprot	449 ^a	624 ^b	84	0.041
Lactase, units/mgprot	60 ^a	76 ^b	6	0.028
Maltase, units/mgprot	194	175	22	0.402
Sucrase, units/mgprot	67	70	10	0.777

¹Values means $n = 6$ for CON and LF groups.

²CON = basal diet with dry feeding.

³LF = basal diet with liquid feeding.

⁴mgprot = milligrams of protein.

⁵gprot = grams of protein.

^{a,b}Means within a row lacking a common superscript differ at $P < 0.05$.

Intestinal Morphology

The jejunal morphology is given in Table 8. The villus height and the ratio of villus height to crypt depth in the jejunum of weanling pigs in the LF group were greater ($P < 0.05$) than those of weanling pigs in the CON group. In addition, crypt depth in the jejunum of weanling pigs in the LF group tended to be lower ($P < 0.1$) than that of weanling pigs in the CON group.

Gene Expression of Tight-Junction Proteins and Intestinal Development-Related Genes

The mRNA expression levels of tight-junction proteins (*CLDN-1*, *CLDN-2*, *OCN*, *ZO-1*, and *ZO-2*) in the jejunum of weanling pigs are shown in Fig. 1. Compared with CON, LF upregulated ($P < 0.05$) *CLDN2*, *ZO-1*, and *ZO-2* mRNA levels and tended ($P < 0.1$) to increase *OCN* mRNA level in the jejunum of weanling pigs.

Table 8. Effects of liquid feeding on jejunal morphology in 31-d-old weanling pigs¹

Item	CON ²	LF ³	SEM	P
Villus height, μm	334.79 ^a	378.42 ^b	19.12	0.043
Crypt depth, μm	172.63	150.25	10.75	0.063
Villus:crypt ⁴	2.01 ^a	2.54 ^b	0.22	0.036

¹Values means $n = 6$ for CON and LF groups. The measurements were average of a minimum of 10 measures per sample.

²CON = basal diet with dry feeding.

³LF = basal diet with liquid feeding.

⁴Villus:crypt = villus height:crypt depth.

^{a,b}Means within a row lacking a common superscript differ at $P < 0.05$.

Figure 1. Effects of liquid feeding on mRNA level of jejunal barrier-related genes of 31-d-old weanling pigs. Each column represents the mean expression level with 6 independent replications. An asterisk above the bars indicate statistical significance ($P < 0.05$) of genes expression between the 2 treatments. CON = control diet; LF = liquid feeding. *CLDN-1* = claudin 1, *CLDN-2* = claudin-2, *OCLN* = occludin, *ZO-1* = zonula occludens 1, *ZO-2* = zonula occludens 2.

IGF-1R mRNA level in the jejunum of LF weanling pigs was significantly higher ($P < 0.05$) than that of CON weanling pigs (Fig. 2).

Intestinal Microbiota and Microbial Metabolites

The numbers of total bacteria and *E. coli* in cecal digesta of LF weanling pigs were lower ($P < 0.05$) than those of CON weanling pigs (Table 9).

Table 10 presents the differences in intestinal microbial metabolites between the 2 groups. The concentrations of acetic acid and butyric acid in cecal digesta of LF weanling pigs were greater ($P < 0.05$) than those of CON weanling pigs.

DISCUSSION

Growth Performance

Weaning imposes multiple stressors on weanling pigs that collectively reduce FI, growth, and health, particularly during the first week after

Figure 2. Effects of liquid feeding on mRNA level of jejunal development-related genes of 31-d-old weanling pigs. Each column represents the mean expression level with 6 independent replications. An asterisk above the bars indicate statistical significance ($P < 0.05$) of genes expression between the 2 treatments. CON = control diet; LF = liquid feeding. *IGF-1* = insulin-like growth factor-1, *IGF-1R* = IGF-1 receptor, *EGF* = epidermal growth factor, *GLP-2* = glucagon-like peptide 2.

Table 9. Effects of liquid feeding on the selected microbial populations (log cfu/g of wet digesta) in cecal and colonic digesta of 31-d-old weanling pigs, qPCR results¹

Item	CON ²	LF ³	SEM	P
Cecal digesta				
Total bacteria	11.05 ^a	10.58 ^b	0.19	0.033
<i>Lactobacillus</i>	7.19	7.17	0.22	0.948
<i>Bifidobacterium</i>	4.19	3.91	0.16	0.127
<i>Escherichia coli</i>	8.98 ^a	8.03 ^b	0.38	0.034
<i>Bacillus</i>	8.31	8.17	0.11	0.268
Colonic digesta				
Total bacteria	11.25	11.30	0.06	0.461
<i>Lactobacillus</i>	8.07	8.13	0.17	0.758
<i>Bifidobacterium</i>	4.29	4.52	0.27	0.812
<i>Escherichia coli</i>	8.85	8.79	0.16	0.695
<i>Bacillus</i>	9.03	8.99	0.50	0.936

¹Values means $n = 6$ for CON and LF groups.

²CON = basal diet with dry feeding.

³LF = basal diet with liquid feeding.

^{a,b}Means within a row lacking a common superscript differ at $P < 0.05$.

weaning (Campbell et al., 2013). Cortisol, a vital glucocorticoid secreted by the adrenal cortex under stress, plays a key role in estimating the severity of stress (Casal et al., 2017). Evidence for weaning stress in weanling pigs is that cortisol in the blood plasma increases after weaning (Van der Meulen et al., 2010). In this study, a lower cortisol level in weanling pigs fed liquid diets suggested that liquid feeding might alleviate weaning stress.

Sufficient FI during the immediate postweaning period is very important to the development of the small intestine and subsequent growth performance

Table 10. Effects of liquid feeding on the intestinal microbial metabolites ($\mu\text{mol/g}$ of wet digesta) in cecal and colonic digesta of 31-d-old weanling pigs¹

Item	CON ²	LF ³	SEM	P
Cecal digesta				
Acetic acid	35.2 ^a	41.5 ^b	2.5	0.028
Propionic acid	20.3	16.0	3.0	0.196
Butyric acid	7.6 ^a	11.3 ^b	1.7	0.039
Total VFA	63.1	68.8	6.2	0.374
Colonic digesta				
Acetic acid	43.4	41.9	6.9	0.801
Propionic acid	18.9	18.7	3.6	0.956
Butyric acid	9.4	9.1	2.1	0.864
Total VFA	71.7	69.6	10.6	0.826

¹Values means $n = 6$ for CON and LF groups.²CON = basal diet with dry feeding.³LF = basal diet with liquid feeding.^{a,b}Means within a row lacking a common superscript differ at $P < 0.05$.

(Zijlstra et al., 1996; McCracken et al., 1999). The key factor underlying the poor postweaning performance and intestinal barrier function damage is the immediate reduction in FI due to the abrupt transition from palatable liquid sow milk to less digestible dry starter diets (Le Dividich and Sève, 2000). As evidenced in the study reported here, liquid feeding during the early weaning period improves FI and BWG, which were in line with other reports in the literature (Kim et al., 2001; Price et al., 2013). The beneficial effects of liquid feeding on the FI and BWG of weanling pigs might be associated with provision of most of the pig's food and water requirements within a single source, thus eliminating the need to learn separate feeding and drinking behaviors (Meunier-Salaün et al., 2017). In addition, previous studies have indicated that management strategies that increased the water consumptions of young pigs resulted in improved growth (Gill et al., 1987) and prevented dehydration (Russell et al., 1996).

No effect on the ratio of feed to gain was observed in this experiment between weanling pigs fed the control and liquid diets. This is at variance with the findings in several other studies, where FI and growth rate have generally been improved by liquid feeding, but at the expense of some reduction in the ratio of feed to gain (Missotten et al., 2010; L'Anson et al., 2012). The poorer ratio of feed to gain of pigs fed liquid diets has often been attributed to wastage of feed and can be markedly affected by trough design (Lane, 2002). If trough design is poor, it can increase the amount of feed dribbled onto the floor during ingestion. During the present study, the quantity of feed each time was provided in a small amount while the frequency

of feeding was increased. Therefore, the wastage from the feeders was negligible, which were implemented to reduce wastage. These combined results suggested that liquid fed pigs surpassed dry fed pigs on animal performance during the early weaning period, suggesting that liquid feeding maybe a better feeding strategy for weanling pigs.

Nutrient Digestibility

Only limited research on the effects of liquid feeding on ATTD in weanling pigs has been published. Han et al. (2006) reported that liquid feeding improved digestibility of DM and CP at 30 d, but not at 10 d post-weaning. Lyberg et al. (2005) reported that liquid feeding growing-finishing pigs had greater phosphorus digestibility than that of dry fed pigs. However, L'Anson et al. (2013) reported that no differences in the ATTD in weanling pigs were observed between dry feeding and liquid feeding. In our study, liquid feeding had greater EE and ash digestibility compared with the CON group. The possible reason for the increasing of ATTD in LF owned to the increasing of enzyme activities in jejunal mucosa. Consistent with this, we found that pigs in LF had greater activities of lipase, amylase, and lactase in the jejunum.

The enzyme activities in digestive tract were considered as important factors that would influence intestinal health and nutrient digestibility (Yang et al., 2010). Van Dijk et al. (2002) found that weaning increased enterocyte mitotic activity at days 4 and 7 after weaning compared with unweaned pigs. There was no published research on the effects of liquid feed on digestive enzyme activities in weanling pigs. We suspected that this improvement in activities of digestive enzymes of pigs fed liquid diets was the postweaning high FI. Marion et al. (2003) and Huguet et al. (2006) showed that digestive enzyme development was related to FI and higher FI resulted in higher enzyme activities. In addition, increasing the liquid content of the diet may permit more effective permeation of the digesta by digestive enzymes inherent to the pigs, and enzymes produced by the gut microflora (Chost et al., 2004). Therefore, the increased digestive enzymes activities by liquid feeding may have contributed to the improvement of nutrient digestibility and reduced diarrhea incidence by enhancing pigs' digestive and absorptive function.

Intestinal Morphology

In our study, liquid feeding had greater villous length and the ratio of villi to crypt and tended to

lower crypt depth in the jejunum compared with the CON group. Similar effects of liquid feeding have been reported previously (Deprez et al., 1987; Han et al., 2006). As intestinal villus height is directly related to absorptive surface area of the total luminal villus, a reduction in villus could result in inadequate digestive enzyme development (Cera et al., 1988). Maintaining or increasing the villi height results in increasing digestive absorptive capacity of various nutrients (Caspary, 1992). Conversely, the deepening of crypts means the growth of small intestinal villus epithelial cells is slowed (Pluske et al., 1996b). A lower ratio of villus height to crypt depth is therefore associated with microbial challenges and antigenic components of the feed (Huang et al., 2012). The low FI observed at weaning coincides with a reduction in villus height and an increase in crypt depth (Pluske et al., 1996b). It was reported that postweaning high FI could improve the histology of small intestine in newly weaned pigs (Verdonk et al., 2001). Therefore, the better morphology of weanling pigs fed liquid diets might be attributed to greater FI during the early weaning period.

The level of *IGF-1R* in the intestinal epithelium is closely associated with the development of gastrointestinal tract (Rowland et al., 2011), which mediates intestinal villus enterocytes and mucosal surface area (Ginneken et al., 2007). In this study, liquid feeding upregulated the mRNA expression of *IGF-1R*. Sillence and Etherton (1987) showed that *IGF-1R* level was related to FI and higher FI resulted in higher *IGF-1R* level. The upregulated *IGF-1R* was consistent with better intestinal morphology in the present study.

Intestinal Barrier Function

The intestinal barrier is mainly formed by a layer of epithelial cells associated with tight junctions and is the primary digestive and absorptive site of nutrients. Therefore, the integrity of the intestinal barrier is fundamental to the proper functioning of the epithelial cells and to preventing the entry of pathogenic bacteria that cause inflammation (Smith et al., 2010; Wittish et al., 2014). However, stress associated with early weaning in pigs leads to impaired mucosal barrier function and increased intestinal permeability (Wijtten et al., 2011; Kim et al., 2012; Zhang et al., 2016).

Tight-junction proteins are the principal determinants of endothelial and epithelial paracellular barrier functions (Shen et al., 2011; Ren et al., 2014). The tight-junction proteins such as

CLDN-2, *OCN*, *ZO-1*, and *ZO-2* play a critical role in maintaining the intestinal barrier integrity, which efficiently prevent the paracellular diffusion of intestinal bacteria and other antigens across the epithelium (Ulluwishewa et al., 2011). Our present study showed that liquid feeding increased the mRNA expression of tight-junction proteins in weanling pigs during the early weaning period. These results were consistent with the previous study by Wijtten et al. (2011), which reported that adequate FI levels after weaning prevented the loss of the intestinal tight protein junctions.

The measurement of intestinal permeability, by testing the plasma concentrations of diamine oxidase activity and D-lactate, was a reliable, standard method to investigate the function of the intestinal mucosa barrier (Song et al., 2010; Zhao et al., 2011). When intestinal epithelial cells were injured, the adhesion of leukocytes and damage to intestinal endotheliocytes will increase the concentration of D-lactate and activity of diamine oxidase (Liu et al., 2007, 2012). In the present study, liquid feeding reduced intestinal permeability by lowering serum activity of diamine oxidase and the concentration of D-lactate during the early weaning period. These results were in line with the previous report by Spreuwenberg et al. (2001), which showed intestinal barrier permeability is compromised in weanling pigs with low FI at weaning and higher FI could improve the intestinal barrier permeability.

The improved intestinal mucosa permeability was associated with low diarrhea incidence, increased nutrient absorption, and less cost of immunity, thus resulted in improved postweaning growth rate (Moret and Prez-bosque, 2009; Zhang et al., 2015). Liquid feeding has been reported to decrease diarrhea incidence in weanling pigs and improve intestinal health (Brooks et al., 2003), which was consistent with our results. Therefore, upregulation of tight-junction proteins and improved intestinal mucosa permeability was associated with the decreased diarrhea rate and cortisol level, which subsequently improved BWG and intestinal health of weanling pigs.

Intestinal Microflora and Microbial Metabolites

Previous study has found that the intestine microbes are associated with nutrient digestion and absorption as well as gut health (Savage, 1986). The balances of beneficial bacteria (such as *Lactobacillus*, *Bifidobacterium*, and *Bacillus*) and harmful bacteria (such as pathogenic *E. coli*) in the gut are associated with the intestinal morphology

and diarrhea (Mikkelsen et al., 2003; Huang et al., 2004; Fairbrother et al., 2005; Hu et al., 2014). Furthermore, *E. coli* has been reported to destabilize and dissociate the *ZO-1*, *OCN*, and *CLDN-1* tight-junction complexes, subsequently deteriorated the intestinal barrier function (Muza-Moons et al., 2004), and caused gut health problems such as diarrhea. Our present study showed that liquid feeding decreased the population of *E. coli* in cecal digesta of weanling pigs during the early weaning period. The possible reason was that large amount of lactate (2%) was formulated in the basal diet and lactate in liquid diet was more efficient to lower the pH of diet than lactate in dry diet. Therefore, the low pH of the diet will assist in maintaining a low pH in the intestine and thereby assist in preventing the development of *E. coli* scours (Russell et al., 1996).

Our present study indicated that liquid feeding increased concentration of acetic acid and butyric acid in cecal digesta, which were consistent with the results of the population of *E. coli*. Acetic acid content is negatively correlated with the number of *E. coli* in the intestine, whereas butyric acid plays a role in maintaining the integrity of intestinal mucosa, barrier function, and inhibiting intestinal inflammation (Corrier et al., 1990). In addition, short chain fatty acids (SCFAs) can inhibit harmful bacteria through increasing intercellular acidity in harmful bacteria, destroying the balance of osmotic pressure in harmful bacteria, and thus play an important role in regulating microflora (Heinritz et al., 2016). Therefore, results from the current data indicate that the improved microflora and SCFAs induced by liquid feeding may contribute to a better intestinal environment, and thus improved intestinal health.

In summary, the results of the present study indicated that liquid feeding improved FI and thereby decreased indicators of weaning stress. This resulted in increased digestive enzyme activities and ATTD of nutrients and improved ADG. Furthermore, liquid feeding could improve intestinal health by improving intestinal morphology and barrier functions as well as microfloral composition. Therefore, our results suggested that liquid feeding could be a potential feeding pattern for enhancing the health and growth of weanling pigs during the early weaning period.

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