Veterinary Microbiology 230 (2019) 187–194

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Lactobacillus johnsonii L531 reduces pathogen load and helps maintain short-chain fatty acid levels in the intestines of pigs challenged with *Salmonella enterica* Infantis

Ting He, Yao-Hong Zhu, Jiao Yu, Bing Xia, Xiao Liu, Gui-Yan Yang, Jin-Hui Su, Liang Guo, Meng-Ling Wang, Jiu-Feng Wang*

College of Veterinary Medicine, China Agricultural University, Beijing, 100193, China

ARTICLE INFO

Keywords:
Probiotic
Salmonella Infantis
Lactobacillus. johnsonii
Short-chain fatty acid
Pig

ABSTRACT

In the current study, we screened *Lactobacillus* strains isolated from the colon of clinically healthy weaned piglets for potential probiotic properties and isolated *Lactobacillus. johnsonii* L531, which produced high levels of beneficial metabolites (butyric, acetic, and lactic acid) *in vitro*. We also evaluated the efficacy of this metabolites-producing probiotic in treating *Salmonella*. Infantis infection. Oral administration of *L. johnsonii* L531 to newly weaned piglets significantly decreased levels of *Salmonella* colonization in colonic and jejunal contents, accelerated the clearance of *Salmonella* in feces after infection, and reduced *S*. Infantis translocation to the spleen. Pretreatment with SCFAs-promoting probiotic *L. johnsonii* L531 significantly ameliorated the depletion of SCFAs induced by *S*. Infantis infection and led to significantly greater weight gain and better feed conversion ratios compared to piglets challenged only with *S*. Infantis. These data provide further evidence that SCFAs-promoting probiotic *L. johnsonii* L531 treatment could be a suitable nonantibiotic alternative for controlling *Salmonella* infection and maintaining metabolic homeostasis, thereby enhancing the gut health of piglets during the critical weaning period.

1. Introduction

Weaning is one of the most critical phases of the pig's life cycle. In commercial pig production systems, this period is shortened from the natural weaning age of approximately 17 weeks to 3–4 weeks after birth (Gresse et al., 2017), which can profoundly impact piglet health and lead to decreased performance and sometimes mortality (Campbell et al., 2013). To reduce potential economic losses, antimicrobials have been used, but which has increased the risk of the emergence of multidrug-resistant bacteria (Barton, 2014). Alternatives to antibiotic use to maintain piglet health during the crucial transition period are therefore critically needed.

The piglet gut microbiome shows dramatic alterations in composition during the weaning period when the piglets diet changes from sow's milk to solid feed (Frese et al., 2015). Therefore, the dysbiotic or perturbed states of gut microbiota increase the susceptibility of piglets to enteric pathogens (e.g., *Salmonella*) (Gresse et al., 2017). *Salmonella* uses its virulence factors to trigger intestinal inflammation, which increases the luminal concentrations of terminal electron acceptors (e.g., host-derived nitrate and oxygen) (Rivera-Chávez et al., 2016; Litvak et al., 2018). These substances have the effect to deplete the short-chain fatty acids (SCFAs)-producing commensal bacteria with subsequently decreasing the concentrations of SCFAs and boosting the growth of *Salmonella* (Drumo et al., 2016; Byndloss and Baumler, 2018). Thus, intestinal infection with *Salmonella* results in inflammation-induced dysbiosis during the weaning transition in piglets which could drive a real vicious circle, further causing severe diarrhea (Gillis et al., 2018; Gresse et al., 2017).

Epidemiologic evidence shows that the proportion of *Salmonella enterica* subsp. *enterica* serovar Infantis (*S*. Infantis) in swine has increased over time (Yuan et al., 2018). Moreover, recent studies have shown that *S*. Infantis isolates display multidrug resistant, and thus pose a substantial public health threat (Borowiak et al., 2017; Tate et al., 2017). Our previous work showed that infection with *S*. Infantis induces intestinal inflammation and loss of microbial diversity in newly weaned piglets (Yang et al., 2017; Zhang et al., 2018). However, as most previous studies have involved only *S. enterica* serovar Typhimurium, the effects of *S*. Infantis infection on microbial metabolites such as SCFAs remain unknown.

The most abundant microbial metabolites produced in the gut are

* Corresponding author at: College of Veterinary Medicine, China Agricultural University, No. 2 Yuanmingyuan West Road, Beijing, 100193, China.
E-mail address: Jiufeng_wang@hotmail.com (J.-F. Wang).

https://doi.org/10.1016/j.vetmic.2019.02.003
Received 18 June 2018; Received in revised form 4 January 2019; Accepted 3 February 2019
0378-1135/

the SCFAs acetate, propionate, and butyrate, each of which acts as a microbial regulatory factor and major modulator of host-microbiome interactions, affecting gut homeostasis and defense against pathogen invasion (Byndloss and Baumler, 2018), immune system maturation (Rooks and Garrett, 2016) and regulation of inflammatory responses (Richards et al., 2016). Butyrate and propionate also down-regulate the expression of bacterial virulence genes such as *hilA* and *invF*, which are important transcriptional activators in *Salmonella* (Gantois et al., 2006; Boyen et al., 2008). Although our previous work demonstrated that probiotic supplementation is a viable microbiota-related strategy for preventing *S*. Infantis infection (Zhang et al., 2018), the efficacy of traditional microbiome-based treatments (single probiotics supplementation) can be limited by a variety of factors, such as the inability of exogenous probiotics to colonize the host by outcompeting an indigenous, pathogenic microbiome (Walter et al., 2018). Therefore, a novel microbiome-based metabolite treatment approach has been suggested, as metabolite-based treatment could overcome the above-mentioned limitation and enable an improved efficacy when coupled with traditional microbiome-based treatments such as probiotics (Suez and Elinav, 2017).

Lactobacillus can rapidly metabolize simple carbohydrates to lactic acid, acetate, or propionate and modulate the gut microbiota composition and/or metabolic output of the community (Derrien et al., 2015). Therefore, in this study, we screened *Lactobacillus* strains isolated from the colon of clinically healthy weaned piglets for potential probiotic properties and isolated *Lactobacillus johnsonii* L531, which exhibited the ability to produce higher levels of metabolites such as butyric, acetatic, and lactic acid *in vitro*. We also evaluated the efficacy of this potential probiotic in reducing *S*. Infantis colonization and fecal shedding in newly weaned piglets. We hypothesized that oral administration SCFAs-promoting probiotic *L. johnsonii* L531 would prevent the disruption of intestinal metabolic homeostasis induced by *S*. Infantis infection.

2. Materials and methods

2.1. Isolation and characterization of lactic acid–producing bacteria (LAB) in vitro

A total of 17 LAB strains were isolated from the colonic contents collected from 7 newly weaned piglets that remained clinically healthy.

All LAB isolates were stored at −80 °C in 25% glycerol and cultured in De Man, Rogosa, and Sharpe (MRS) broth and agar (Oxoid, Basingstoke, UK) at 37 °C under anaerobic conditions for 18–24 h. The species of the 17 isolates were determined by comparing the sequence of the 16S rRNA gene with sequences in the NCBI GenBank database using BLAST.

Initial screening of the isolates involved characterizing bacterial properties, which included tolerance to low pH and porcine bile salts and ability to adhere to epithelial cells *in vitro*. The ability of screened isolates to produce SCFAs and lactic acid *in vitro* was also investigated.

Tolerance to low pH was evaluated by incubating mid-log phase bacterial cultures and diluting bacterial cultures with 0.1 mol/l HCl-MRS broth (pH 2.0) to a final concentration of approximately 10^7 CFU/ml and anaerobically incubating the cultures at 37 °C for up to 120 min. The number of residual viable bacteria was determined at various time points (0, 30, 60, and 120 min).

Tolerance to bile salts was assessed by incubating 10^7 CFU/ml of each isolate in MRS containing various concentrations (0, 0.3%) of bile salts under anaerobic conditions at 37 °C. The number of residual viable bacteria was determined after 0, 2, and 4 h of incubation. Rates of bacterial survival in acid and bile tolerance assays were determined from triplicate analyses by appropriate serial dilutions and plating onto MRS agar. Plates were incubated anaerobically at 37 °C for 24 h, after which the number of CFU/ml was calculated.

Bacterial adhesion capacity was assessed using porcine jejunal epithelial J2 cells (IPEC-J2, ACC701, DSMZ). Confluent monolayers of IPEC-J2 cells (1×10^6 cells/well) were cultured in 6-well tissue culture plates as previously described (Zhang et al., 2018). Monolayers were infected in 2 ml of the cell culture medium lacking antibiotics and without heat-inactivated fetal calf serum but containing isolates at a multiplicity of infection (MOI) of 10 bacteria per epithelial cell. After 3 h of incubation at 37 °C and 5% CO_2, the IPEC-J2 cells were washed three times in phosphate-buffered saline (PBS) to remove non-adherent bacteria. The IPEC-J2 cells were then lysed by covering the monolayers with 200 µl of trypsin–EDTA (Gibco Life Technologies) for 10 min at 37 °C. Subsequently, 300 µl of cell culture medium was added in order to stop the reaction, and the number of viable adherent bacteria was determined by plating serial dilutions of the cell lysates on MRS agar plates. Adhesion was expressed as the percentage of adhered bacteria relative to the number of bacteria initially added. All experiments were performed in triplicate. Adhesion values were normalized against those of the positive control, *Lactobacillus rhamnosus* GG (LGG, ATCC 53103), a species known for good adhesion to IPEC-J2 cells.

Four isolates which exhibited high acid tolerance property and good adhesion capacity in *in vitro* assays were selected for further characterization. Concentrations of butyric, acetic, and lactic acids in bacterial culture supernatants were measured using ion chromatography (ICS 3000; Thermo, New York, NY), as previously described (Yu et al., 2017). Overnight cultures (24 h) of isolate strains were used, and MRS broth alone was used as a negative control. Each measurement was carried out in duplicate.

2.2. Ethics in animal experimentation

All animals were treated in strict accordance with the *Guidelines for Laboratory Animal Use and Care* from the Chinese Center for Disease Control and Prevention and the *Rules for Medical Laboratory Animals* (1998) from the Chinese Ministry of Health, under protocol CAU20161016-1, which was approved by the Animal Ethics Committee of China Agricultural University. All surgeries were performed under xylazine hydrochloride anesthesia, and every effort was made to minimize suffering.

2.3. Bacteria preparation and growth conditions

Based on the results of *in vitro* experiments, we chose *L. johnsonii* L531 as a putative probiotic strain for treating weaning piglets challenged in an *S*. Infantis model. A culture of *L. johnsonii* L531 was inoculated at a dilution of 1:100 in MRS broth and incubated at 37 °C for 18 h until reaching mid-log phase under microaerophilic conditions. Bacteria were pelleted by centrifugation at 3000 × *g* for 10 min at 4 °C, and then washed 3 times and resuspended in sterile physiologic saline. Bacteria were quantified by determining the number of CFUs after plating serial dilutions of bacterial suspensions onto MRS agar plates. The oral dose of *L. johnsonii* L531 was 10 ml of an approximately 10^9 CFU/ml suspension.

Salmonella enterica serovar Infantis strain CAU1508 used in the study was isolated in our laboratory from the intestinal contents of weaned piglets with diarrhea, carrying the virulence genes *sipA, sipB, sopA, sopB, sopD, sopE, sopE2, fljB, and sseI* (detected by real-time PCR), and harboring the pFPV-mCherry plasmid (Yang et al., 2017). The *S*. Infantis CAU1508 (mCherry) challenge strain was grown to mid-log phase in Luria-Bertani broth (Oxoid) at 37 °C with shaking. The *Salmonella* were then harvested by centrifugation at 3000 × *g* for 10 min at 4 °C, washed 3 times with sterile physiologic saline, and resuspended in normal saline. The inoculum of *S*. Infantis CAU1508 was 10 ml of an approximately 10^{11} CFU/ml suspension. The concentration of inoculum was determined by CFU counts after plating the serial dilutions of bacterial suspensions onto xylose lysine tergitol 4 (XLT4; Beijing Land Bridge Technology Co., Beijing, China) agar plates.

2.4. Animal experiments

A total of 18 healthy piglets (Landrace × Large White) of mixed gender selected from 8 different litters and weaned at 21 days of age were obtained from Beijing Hog Raising and Breeding Center. The average weight of the piglets was 5.84 ± 0.07 kg. Each animal was penned separately and provided *ad libitum* access to antibiotic-free feed and water from day 0 (when newly weaned and transported to the animal experimental facility of the College of Veterinary Medicine, China Agricultural University) until day 18 (when euthanized). Prior to initiation of the study, all piglets were determined to be free of *Salmonella* by analysis of feces and serum. Briefly, the fresh feces sample was added to buffered peptone water (Beijing Aoboxing Biotech Co., Beijing, China) and incubated at 37°C for 16–18 h with shaking. Then the pre-enrichment broth was transferred onto modified semi-solid Rappaport Vassiliads (MSRV; Beijing Land Bridge Technology Co., Beijing, China) agar and incubated for 24 h at 42 °C. After selective enrichment, an inoculating loop of white or gray colonies grown on MSRV was plated on XLT4 agar and incubated at 37 °C overnight. Bacterial colonies showing morphologic characteristics of *Salmonella* were then confirmed by PCR. Serum samples were determined by a porcine *Salmonella* Antibody ELISA kit (B, C1 and D combined) (Biocheck, London, UK) and performed as described by the manufacturer's instructions. All piglets were negative for *Salmonella* in microbiological and serological test.

On the day of weaning (day 0), piglets were assigned to 3 groups (n = 6 per group) with littermate, weight, and gender distributed evenly among the groups. The following three groups were: (1) control (CN) group (oral administration of sterile physiologic saline only, from day 1 to 8); (2) *S.* Infantis (SI) group (oral administration of sterile physiologic saline from day 1 to 7 and oral challenge with *S.* Infantis on day 8); and (3) *L. johnsonii* L531 + *S.* Infantis (LS) group (oral administration of *L. johnsonii* L531 from day 1 to 7 and oral challenge with *S.* Infantis on day 8). Groups were kept physically separated by a solid partition; there was no direct contact between animals from different groups.

At 9:00 AM on days 1–7, piglets in group LS were orally administered *L. johnsonii* L531 solution (1×10^9 CFU/ml, 10 ml/day) intragastrically; piglets in groups CN and SI were administered 10 ml of sterile physiologic saline daily. At 9:00 AM on day 8, piglets in groups LS and SI were orally challenged with *S.* Infantis culture (1×10^{11} CFU/ml, 10 ml/piglet) to induce diarrhea as described (Yang et al., 2017); piglets in group CN received 10 ml of sterile physiologic saline. On day 18 (10 days after *S.* Infantis challenge), all piglets in each of the 3 groups were sacrificed.

2.5. Samples and measurements

Each animal was examined for clinical signs of disease at regular intervals throughout the study. Body weight (BW) was recorded on days 1, 8, 15, and 18, and feed consumption was recorded daily. Average daily gain (ADG) and the ratio of gain to feed (G:F) were calculated.

To assess bacterial colonization, fresh fecal samples were collected prior to *S.* Infantis challenge (0 h) and at 6, 12, 24, 120, and 192 h post-challenge. Piglets were euthanized on day 10 post-infection, and the following samples were immediately collected for microbiological analysis: liver, spleen, and mesenteric lymph nodes; jejunum, cecum, proximal colon contents and mucosal tissues. The fresh jejunum, cecum, and proximal and distal colon contents were flash frozen in liquid nitrogen and stored at −80 °C for later SCFAs determination.

2.6. Bacterial enumeration

Bacteria were enumerated to assess fecal shedding and colonization with *S.* Infantis. Samples of 1 g of feces, organ tissue, intestinal contents, or mucosal tissues from each animal were homogenized in 9 ml of sterile saline, serially diluted, then plated on XLT4 agar plates for *Salmonella* culture and incubated for 24 h at 37 °C under aerobic conditions. Results are expressed as $\log_{10}$ CFU/g. All analyses were performed in triplicate.

2.7. SCFAs quantification

The concentrations of acetate, propionate, butyrate, and i-butyrate, i-valerate, valeric acid in the jejunum, cecum, proximal and distal colon contents were determined using gas chromatography (Agilent 7890 A, Agilent Technologies, Santa Clara, CA) according to previously described procedures (Shen et al., 2009).

2.8. Statistical analyses

Statistical analyses were conducted using SPSS 22.0 software (SPSS Inc., Chicago, IL). For data from intestinal content samples, the statistical model included the fixed effects of treatment, litter, gender, intestinal content or mucosal tissue, interactions between treatments and intestinal content or mucosal tissue, as well as random effects associated with individual pigs within a treatment. Differences in fecal shedding and organ colonization between the LS and SI groups were examined using the nonparametric Mann-Whitney test. The Chi-Square test (likelihood ratio) was used to analyze the frequencies of positive animals in the different range levels of *Salmonella* in intestinal segments. Differences in terms of metabolites produced *in vitro* (culture supernatant) and *in vivo* (intestinal contents) as parameters of growth performance were analyzed using one-way ANOVA. Differences between means were analyzed using Tukey's test. Spearman's test was used to assess correlations. A P-value of < 0.05 was considered statistically significant.

3. Results

3.1. In vitro probiotic characteristics of LAB

Large variations were noted in the survival capacity of the 17 LAB isolates at pH 2.0. The results indicated that acid resistance were strain-specific rather than a common property of the LAB species examined. Table 1 shows results for 11 LAB isolates that exhibited greater acid resistance over at least 3 time points. The remaining 6 isolates exhibited poor survival, with no viable bacteria detected after 30 min (data not shown). After 120 min at pH 2.0, strains *L. johnsonii* L531 and C21 exhibited high acid resistance (49.7 and 73.8% viability, respectively). Other isolates *L. reuteri* L25, *L. johnsonii* TL432, *L. reuteri* A4, and *L. johnsonii* L54 exhibited survival rates of only 6.98, 1.9, 0.05, and 0.0005%, respectively. Repeated tests showed that all LAB isolated were sensitive to both 2 and 4 h of exposure to 0.3% porcine bile salts, however.

Based on the results of acid resistance assays, isolates were screened for adhesion to porcine IPEC-J2 cells. Fig. 1A shows LAB isolates that exhibited greater adhesion to epithelial cells. Four isolates in particular (C21, L531, A4, L54) exhibited high numbers of bacteria adhering to IPEC-J2 cells after 3 h of incubation.

Metabolism of organic acids by *L. johnsonii* C21, L531, L54, and *L. reuteri* A4 was analyzed after culture for 24 h in MRS medium. Fig. 1B shows that in pure cultures, all of the tested isolates produced butyric acid, lactic acid, and acetic acid but not propionic acid. Among the 4 strains examined, *L. johnsonii* L531 produced the highest levels of butyric acid (18 μM), lactic acid (179 mM), and acetic acid (58 mM).

Based on the results of *in vitro* characterization of bacterial properties (particularly the ability to produce higher concentrations of SCFAs and lactic acid, which could confer added value as a putative probiotic), *L. johnsonii* L531 was selected as a SCFAs-promoting probiotic for further *in vivo* studies.

Table 1
Survival of part lactobacilli in low pH (2.0) (log_{10} CFU/ml).

Isolate Strain	Microorganism	0 min	30 min	60 min	120 min
L531	*Lactobacillus johnsonii*	6.938 ± 0.475[a]	7.208 ± 0.097[a]	6.900 ± 0.010[a]	6.634 ± 0.143[a]
C21	*L. johnsonii*	6.714 ± 0.227[a]	6.835 ± 0.317[a]	6.602 ± 0.049[a]	6.582 ± 0.185[a]
TL432	*L. johnsonii*	6.930 ± 0.079[a]	6.781 ± 0.100[a]	6.778 ± 0.079[a]	5.216 ± 0.110[b]
A4	*L. johnsonii*	6.855 ± 0.424[a]	3.434 ± 0.296[b]	3.485 ± 0.103[b]	3.580 ± 0.101[b]
L25	*Lactobacillus reuteri*	6.713 ± 0.145[a]	6.568 ± 0.035[a]	4.041 ± 0.098[b]	5.557 ± 0.142[c]
M46	*L. reuteri*	7.387 ± 0.324[a]	3.900 ± 0.014[b]	3.780 ± 0.058[b]	5.180 ± 0.248[c]
L54	*L. johnsonii*	7.830 ± 0.070[a]	4.683 ± 0.031[b]	3.815 ± 0.035[c]	2.500 ± 0.130[d]
M47	*L. johnsonii*	6.812 ± 0.080[a]	6.071 ± 0.304[a]	3.714 ± 0.278[b]	ND
M42	*L. johnsonii*	6.448 ± 0.017[a]	3.540 ± 0.060[b]	2.600 ± 0.092[c]	ND
TL431	*L. johnsonii*	6.700 ± 0.135[a]	2.470 ± 0.041[b]	2.500 ± 0.500[b]	ND
L41	*L. reuteri*	5.704 ± 0.245[a]	ND	1.570 ± 0.070[b]	0.901 ± 0.09[b]

ND, not determined.
Values expressed as Means ± SEM (n = 3).
[a−c] Means in the same row with different superscripts represent values statistically different from each other ($P < 0.05$).

3.2. Effect of L. Johnsonii L531 on piglet growth performance

The effects of the experimental treatments on ADG, G:F, and BW are summarized in Table 2. Oral challenge with *S.* Infantis led to a significant reduction in final ADG, G:F and BW, whereas administration of *L. johnsonii* L531 markedly enhanced growth performance and improved the ability to resist the harmful effects of *Salmonella* challenge.

Although both the LS and SI groups received the same challenge with *Salmonella*, the LS group exhibited significant positive effects post-challenge in terms of ADG, G:F, and BW in comparison with the SI group ($P < 0.001$, $P = 0.001$, and $P = 0.001$, respectively). In challenged piglets that received the probiotic treatment, these parameters were similar to the non-challenged CN group ($P = 0.944$, $P = 0.992$, and $P = 0.954$, respectively).

3.3. Effect of L. Johnsonii L531 on Salmonella populations

Differences in the level of *Salmonella* fecal shedding after *S.* Infantis challenge were observed (Fig. 2A). At 6 h after challenge, all piglets pretreated with *L. johnsonii* L531 began to excrete *Salmonella*, whereas half of the piglets in the SI group were negative or excreted fewer *Salmonella* in the feces. At 12 h, both the LS and SI groups had reached peak excretion. However, unlike the piglets in the SI group that continued shedding *Salmonella* steadily from 24 to 192 h, piglets pretreated with *L. johnsonii* L531 exhibited a sharp decline in shedding during this

Table 2
Dose effect of oral administration of *Lactobacillus johnsonii* L531 on the growth of postweaning piglets before and after *Salmonella* Infantis challenge.

Item[2]	Treatments[1]			SEM	*P*-value
	CN	SI	LS		
Phase 1 (days 1 to 7, pre-challenge)					
ADG, g/d	27.38	30.95	89.29	17.657	0.291
G:F, g/g	0.160	0.268	0.702	0.138	0.250
BW, kg **(day 1)**	5.96	5.76	5.80	0.075	0.541
Phase 2 (days 8 to 18, post-challenge)					
ADG, g/d	278.33[a]	43.33[b]	266.67[a]	29.662	0.000
G:F, g/g	0. 897[a]	0. 215[b]	0.879[a]	0.096	0.000
BW, kg **(day 18)**	8.93[a]	6.43[b]	9.09[a]	0.361	0.000

[a, b] Within a row, means without a common lowercase superscript differ ($P < 0.05$); Turkey's test.
[1] Treatments: **CN**, control group with sterile physiological saline; **SI**, received sterile physiological saline orally for phase 1 and followed by *Salmonella* Infantis (1.0×10^{11} CFU/ml, 10 ml) challenge; **LS**, pretreated with probiotic (1×10^{9} CFU/ml, 10 ml once daily) for phase 1 and followed by *Salmonella* Infantis (1.0×10^{11} CFU/ml, 10 ml) challenge. $n = 6$ per group.
[2] Item: ADG, average daily gain; G: F, gain to feed ratio; BW, body weight.

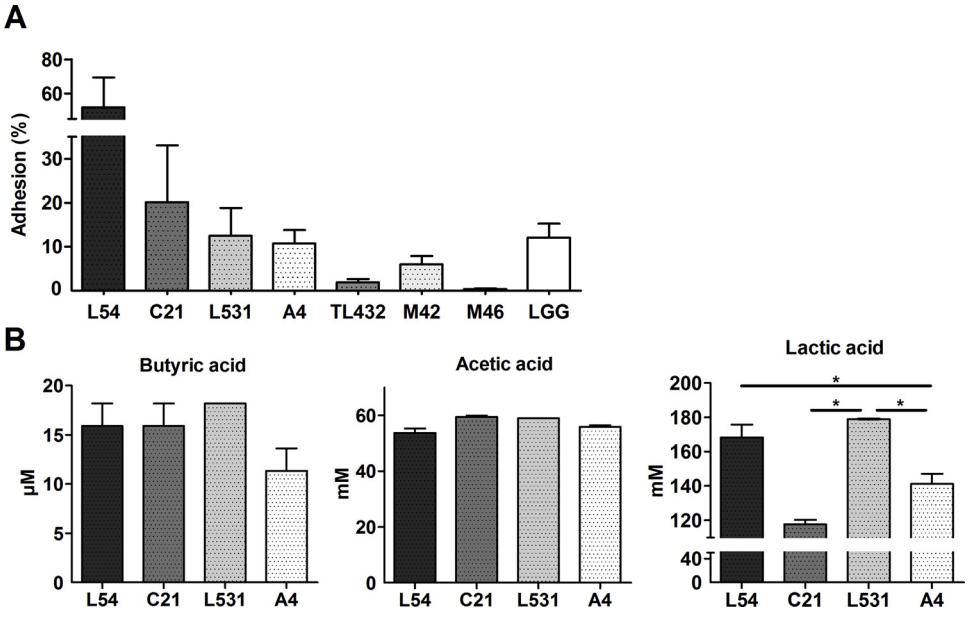

Fig. 1. Adhesion of selected LAB isolates to epithelial cells and organic acid metabolism *in vitro*. (A) Adhesion to IPEC-J2 cells after 3 h of incubation. Results were obtained from at least triplicate analyses. (B) Butyric, acetic, and lactic acid concentrations after 24 h of growth of selected isolates in MRS supplemented medium. The concentration in the control medium was subtracted from that of each experimental sample. Analyses were conducted in duplicate. Results are presented as the mean ± SEM. *$P <$ 0.05.

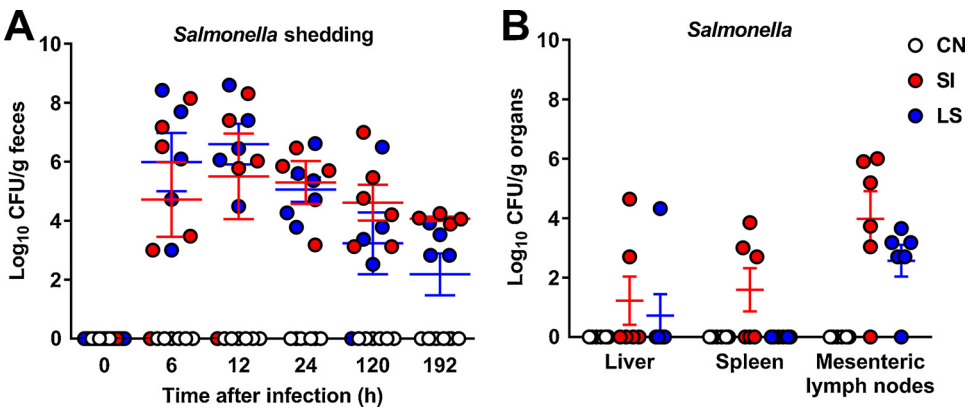

Fig. 2. Fecal shedding and bacterial invasion of systemic organs in weaned piglets after *Salmonella* Infantis challenge. (A) Determination of the number of *Salmonella* in fecal samples from indicated piglets. Fresh fecal samples from piglets of the indicated groups (n = 6 per group) were collected on days 8 (0, 6, 12, and 24 h after infection), 13, and 16 after weaning. (B) Bacterial invasion of systemic organs on day 18 (10 days after *S.* Infantis challenge). The number of *Salmonella* in fecal samples and colonizing the systemic organs after *S.* Infantis challenge was monitored using a culture-based enumeration assay. Treatments: CN, control group; SI, *Salmonella* Infantis challenge group; LS, pretreated with *Lactobacillus. johnsonii* L531 and *Salmonella* Infantis challenge group. For (A) and (B), each circle and square represent data for a single animal.

period, and some piglets were even negative for fecal shedding at 120 and 192 h. The number of *Salmonella* shed differed significantly between the LS and SI groups ($P = 0.018$) at 192 h after challenge.

Piglets were euthanized on day 18 (10 days after *S.* Infantis challenge) to assess pathogen colonization of the intestines and systemic organs. As shown in Fig. 3, *Salmonella* colonization occurred in different sites within the gastrointestinal tract (GIT). Both the intestinal contents and mucosal tissues were examined for colonization. In general, probiotic administration tended to reduce the number of piglets excreting large numbers of *Salmonella* and reduce the degree of pathogen colonization and expansion in the gut organs, as compared with the SI group. Moreover, the numbers of *Salmonella*-negative jejunal (Fig. 3A) and proximal colonic contents (Fig. 3E) were significantly higher in the LS group compared with the SI group ($P = 0.035$ and $P = 0.025$, respectively).

S. Infantis exhibited differential systemic organ colonization

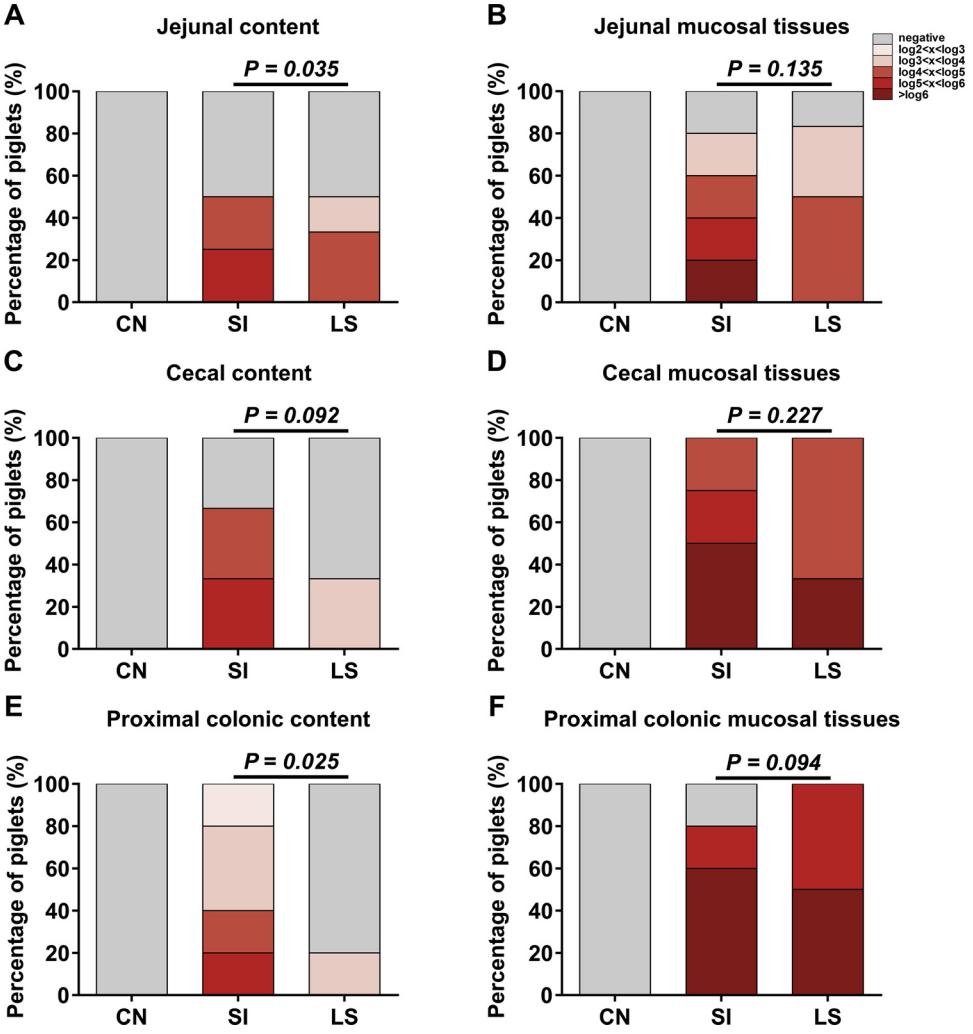

Fig. 3. *Salmonella* colonization of the mucosa and contents of different intestinal segments. The y-axis shows the percentage of piglets at various log CFU/g of sample categories. *Salmonella* burden was determined on day 18 (10 days after *S.* Infantis challenge) for contents of the jejunum (A), cecum (C), and proximal colon (E) and mucosal tissue of the jejunum (B), cecum (D), and proximal colon (F). Treatments: CN, control group; SI, *Salmonella* Infantis challenge group; LS, pretreated with *Lactobacillus. johnsonii* L531 and *Salmonella* Infantis challenge group. Differences between groups SI and LS were estimated using Chi-Square test (likelihood ratio) and considered significant at $P < 0.05$.

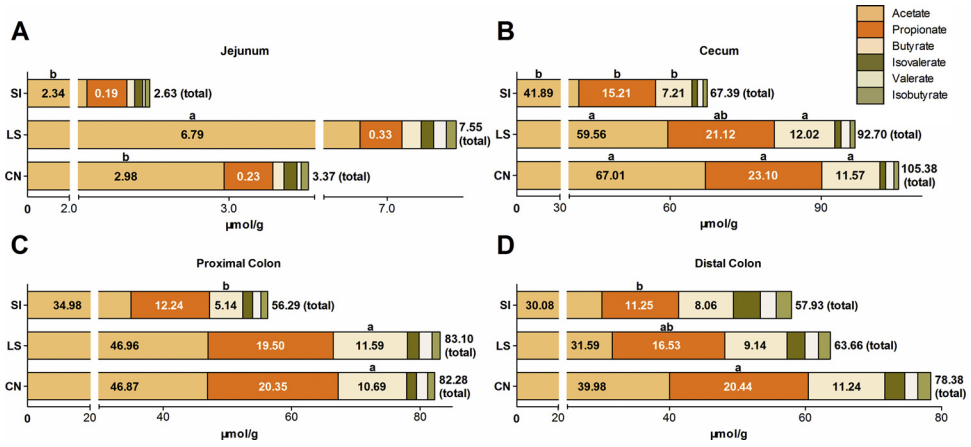

Fig. 4. Concentrations of SCFAs in different intestinal areas of weaned piglets as determined by gas chromatograph on day 18 (10 days after *S.* Infantis challenge). Bar-plot representing the median values of the concentrations of SCFAs in the jejunum (A), cecum (B), proximal colon (C), and distal colon (D). Treatments: CN, control group; SI, *Salmonella* Infantis challenge group; LS, pretreated with *Lactobacillus. johnsonii* L531 and *Salmonella* Infantis challenge group. [a,b]Different superscript letters indicate significant difference ($P < 0.05$).

efficiency between the LS and SI groups, as shown in Fig. 2B. In the SI group, all systemic organs were positive for *Salmonella* colonization, particularly the mesenteric lymph nodes. By contrast, compared with the SI group, administration of the probiotic *L. johnsonii* L531 protected piglets from *S.* Infantis invasion of the spleen ($P = 0.059$) and reduced the *Salmonella* burden in the liver and mesenteric lymph nodes.

3.4. Effect of L. Johnsonii L531 on SCFAs concentrations in different GIT locations

To determine changes in activity of the intestinal microbiota after *S.* Infantis challenge, concentrations of SCFAs in different areas of the GIT were analyzed using gas chromatography (Fig. 4).

In general, *S.* Infantis challenge caused a marked decline in the concentrations of acetate, propionate, butyrate, and total SCFAs in most of the intestinal sites examined. In the SI group, the cecum (Fig. 4B) was more severely affected, with significant decreases in the concentrations of acetate ($P = 0.002$), total SCFAs ($P = 0.007$), and propionate ($P = 0.035$) compared with the CN group. The propionate concentration in the distal colon (Fig. 4D) in the SI group also declined significantly ($P = 0.026$) in comparison with the CN group.

In contrast to the abovementioned results, the concentrations of SCFAs in the cecum and colon remained steady or displayed a tendency to decrease slightly compared with CN group. In piglets pretreated with SCFAs-promoting probiotic *L. johnsonii* L531, the concentrations and composition of SCFAs (especially butyrate) in the cecum and proximal colon were quite similar to those of the CN group. Without probiotic pretreatment, *S.* Infantis infection induced a significant decrease in the butyrate concentration in both the cecum ($P = 0.042$) and proximal colon ($P = 0.012$) compared with the LS group. Notably, the concentrations of acetate, propionate, and butyrate in the jejunum of piglets in the LS group were markedly increased (Fig. 4A) compared with the CN and SI groups, but only the change in acetate concentration was significant ($P < 0.05$).

3.5. Correlation analyses

The total amount of acetate produced in the jejunum was negatively correlated with *Salmonella* invasion in the spleen ($r = -0.560$; $P = 0.019$). Butyrate produced in the jejunum was negatively correlated with *Salmonella* colonization of the mesenteric lymph nodes ($r = -0.773$; $P = 0.005$) and presence in the cecum contents ($r = -0.679$; $P = 0.044$). The production of butyrate in the proximal colon was negatively correlated with expansion of *Salmonella* in the proximal colon contents ($r = -0.525$; $P = 0.044$).

It is worth noting that the *Salmonella* burden in the mesenteric lymph nodes was also strongly negatively correlated with the concentrations of various SCFAs in the distal colon (acetate, $r = -0.851$ [$P < 0.001$]; propionate, $r = -0.783$ [$P = 0.001$]; butyrate, $r = -0.620$ [$P = 0.014$]). In most of the corresponding intestinal areas, a positive correlation was also observed between i-butyrate, i-valerate, and valeric acid ($r = 0.703 \sim 0.968$; $P < 0.01$).

4. Discussion

The pathogenicity of *Salmonella* depends upon multiple factors, including pathogen characteristics (virulence genes, exposure dosage), the complex interplay between the pathogen and the intestinal microbiota, the host's ability to control the microbiota, and the levels of microbial metabolites generated in the gut (Bearson et al., 2013; Rivera-Chávez et al., 2016; Byndloss et al., 2017). Here, we found that oral inoculation of newly weaned piglets with *S. enterica* serovar Infantis causes fever, diarrhea, anorexia, depression, and poor growth performance. In the present study, we demonstrated that pretreatment with porcine-origin SCFAs-promoting probiotic *L. johnsonii* L531 represents a viable alternative to the use of antibiotics in weaned piglets because of the inhibitory effects on *Salmonella*, significant positive impacts on growth performance, and efficacy in maintaining intestinal metabolic homeostasis. Pretreatment with this potential probiotic significantly reduced levels of *Salmonella* colonization in colonic and jejunal contents, accelerated the clearance of *Salmonella* in the feces in infected piglets compared with piglets that were not pretreated. The poorer performance of piglets only challenged with *S.* Infantis in terms of ADG, G:F, and BW could be due to the rapid, high-level growth of the pathogen, as demonstrated by our microbiological data. By contrast, pretreatment with SCFAs-promoting probiotic *L. johnsonii* L531 significantly ameliorated the depletion of SCFAs induced by *S.* Infantis infection, confirming the hypothesis that probiotic pretreatment increases the resilience of the intestinal microbiota against *Salmonella* infection (Sommer et al., 2017). Considered collectively, these observations indicate that the consequences and physiologic responses in piglets challenged with *Salmonella* may be correlated and dependent on key bacterial metabolites (i.e., SCFAs).

Several researchers have described the microbiota of the large intestine as a 'microbial organ' (O'Hara and Shanahan, 2006), which functions to produce metabolites (e.g., SCFAs) at concentrations that promote health (Koh et al., 2016). Microbial organ dysfunction (e.g., *Salmonella* expansion) can lead in turn to deleterious changes in the microbial metabolite landscape (Byndloss and Baumler, 2018; Litvak et al., 2017). In the current study, *S.* Infantis infection reduced the levels of SCFAs in the gut, consistent with prior findings that *S.* Infantis colonization in piglets results in a loss of microbial diversity (Zhang et al., 2018) and fewer SCFAs-producing bacteria (Drumo et al., 2016). We showed here that *L. johnsonii* L531 may enhance the host's ability to control the microbial organ and contribute to amelioration of microbial organ dysfunction caused by *S.* Infantis infection.

One of the crucial features of the putative probiotic is its efficacy in maintaining levels of SCFAs produced by the microbiota, particularly the concentrate of butyrate in the cecum and colon. The SCFA acetate produced by intestinal bacteria helps reduce gut mucosal permeability (Fukuda et al., 2011). Microbiota-derived SCFAs promote the maturation and expansion of regulatory T (Treg) cells in the gut and exert profound anti-inflammatory effects (Atarashi et al., 2013). Microbiota-derived butyrate functions in coordination with Treg cells to activate epithelial cells via PPAR-γ signaling to drive the metabolism of colonocytes toward mitochondrial β-oxidation of butyrate and highs oxygen consumption. This energy metabolism is important for restricting oxygen availability in the intestinal lumen, which ensures the dominance of obligate anaerobic bacteria (Byndloss et al., 2017). The SCFAs-producing obligate anaerobic bacteria thereby could maintain gut homeostasis and prevent *Salmonella* expansion (Byndloss and Baumler, 2018; Litvak et al., 2018).

As aerotolerant anaerobes, *Lactobacillus* species could tolerate oxygen for short periods, contribute to the modification of the gut microbiota composition and thus SCFA production (Derrien et al., 2015). In addition, during the weaning transition the abundance of *L. johnsonii* declines (Pieper et al., 2008). Thus, by adding niche-stabilizing metabolites, pretreatment with an SCFAs-promoting probiotic could help overcome the colonization resistance of indigenous commensals and improve the long-term efficacy of exogenous probiotics (Suez and Elinav, 2017). Indeed, in piglets that no longer received *L. johnsonii* L531 after day 8 post-*Salmonella* challenge, metabolite production was still markedly affected at 10 days post-challenge. Therefore, it seems that the putative probiotic *L. johnsonii* L531 may be a viable antibiotic alternative with the ability to maintain SCFA production.

Pretreatment with *L. johnsonii* L531 effectively maintained SCFAs concentrations in the lower GIT. *Lactobacillus plantarum* supplemented also increased the level of butyrate in the colon of weaned piglets (Guerra-Ordaz et al., 2013). As probiotics have species/strain-specific characteristics in response to pathogen infections, pretreatment with *Bifidobacterium longum* subsp. *infantis* CECT 7210 did not affect the levels of butyrate and propionate in the colon of weaned piglets challenged with *Salmonella* (Barba-Vidal et al., 2017). The severity of *Salmonella* infection was negatively correlated with the levels of both total SCFAs and butyrate in the cecum (Borton et al., 2017). It is important to verify that SCFAs provided as supplements reach the site of action, while not being absorbed in the upper GIT and inducing supraphysiologic concentrations in the peripheral blood and organs that could cause unexpected physiologic responses (Suez and Elinav, 2017; Koh et al., 2016). Supplementation of tributyrin and lactitol did not alter in concentrations of SCFAs in the cecum of weaned piglets (Piva et al., 2008). Furthermore, tributyrin supplementation did not affect butyrate and propionate concentrations in the cecum of weaned piglets challenged with lipopolysaccharide, but maintained the cecal acetate fermentation (Gu et al., 2017). However, in mouse models, tributyrin supplementation could restore butyrate concentrations in the large intestine, maintain epithelial hypoxia and restrict *Salmonella* expansion (Rivera-Chávez et al., 2016; Byndloss et al., 2017). Although exogenous supplementation with coated butyric acid is a promising means of controlling *Salmonella* infections in weaned piglets, the effects of sodium butyrate depend not only on the dosage of supplementation but also on the period (before v. after weaning) of its oral administration (Boyen et al., 2008). A physiological dose of sodium butyrate supplementation could influence the intestinal anatomy and physiology in weaned piglets (Le Gall et al., 2009). Prebiotics have also shown promise in enhancing the production of SCFAs in the gut (Vermeulen et al., 2017), but the beneficial effects depend upon the structure and activity of the host microbiome (Suez and Elinav, 2017).

At low concentrations, both butyrate and propionate decrease the expression of virulence genes of *Salmonella* pathogenicity island 1 (SPI1), such as *sipA*, *sopB*, *sopD*, *sopE2*, *hilA*, *hilD*, and *invF* (Gantois et al., 2006; Vermeulen et al., 2017). As *S.* Infantis CAU1508 carries several virulence genes (*sipA*, *sopB*, *sopD* and *sopE2*), higher concentrations of propionate and butyrate might also be involved in reducing the invasive potential of *S.* Infantis. In contrast, a previous study showed that an increase in the concentration of acetate in the distal ileum has the opposite effect of butyrate and propionate, providing a signal for the expression of invasion genes in *Salmonella* (Lawhon et al., 2002). *L. johnsonii* L531 adhered well to porcine jejunal epithelial cells and produced higher amounts of metabolites *in vitro*, in agreement with the results of *in vivo* experiments. Considered in light of our results, these observations suggest that screening for probiotics exhibiting better adhesion to porcine colonic and cecal epithelial cells would be beneficial. Moreover, organic acids also increase acidity and lower the pH in the gut, creating environmental conditions unsuited to the survival of *Salmonella* (Ashayerizadeh et al., 2017).

In conclusion, the results of our study suggest that supplementation with the SCFAs-promoting probiotic *L. johnsonii* L531 is a viable metabolite-based alternative to classical probiotics for the control of *Salmonella* infections. SCFAs-promoting probiotic *L. johnsonii* L531 helps maintain metabolic homeostasis and enhances the host's ability to control the microbial organ and prevent its disruption by infecting *Salmonella*, thus providing significant benefits in terms of growth performance and health in piglets during the critical weaning period.

Conflict of interests

The authors declare that they have no competing interests.

Acknowledgements

This study was supported by the National Key R&D Program of China [grant number 2017YFD0502200], the National Natural Science Foundation of China [grant numbers 31672613, 31873034, and 31472242], and the Special Fund for Agro-Scientific Research in the Public Interest [China; grant number 201403054]. We would like to thank Dr. David Robinette for editing the manuscript.

References

Ashayerizadeh, A., Dastar, B., Shargh, M.S., Mahoonak, A.S., Zerehdaran, S., 2017. Fermented rapeseed meal is effective in controlling *Salmonella enterica* serovar Typhimurium infection and improving growth performance in broiler chicks. Vet. Microbiol. 201, 93–102.

Atarashi, K., Tanoue, T., Oshima, K., Suda, W., Nagano, Y., Nishikawa, H., Fukuda, S., Saito, T., Narushima, S., Hase, K., Kim, S., Fritz, J.V., Wilmes, P., Ueha, S., Matsushima, K., Ohno, H., Olle, B., Sakaguchi, S., Taniguchi, T., Morita, H., Hattori, M., Honda, K., 2013. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500, 232–236.

Barba-Vidal, E., Castillejos, L., Lopez-Colom, P., Rivero Urgell, M., Moreno Munoz, J.A., Martin-Orue, S.M., 2017. Evaluation of the Probiotic Strain Bifidobacterium longum subsp Infantis CECT 7210 Capacities to Improve Health Status and Fight Digestive Pathogens in a Piglet Model. Front. Microbiol. 8.

Barton, M.D., 2014. Impact of antibiotic use in the swine industry. Curr. Opin. Microbiol. 19, 9–15.

Bearson, S.M.D., Allen, H.K., Bearson, B.L., Looft, T., Brunelle, B.W., Kich, J.D., Tuggle, C.K., Bayles, D.O., Alt, D., Levine, U.Y., Stanton, T.B., 2013. Profiling the gastrointestinal microbiota in response to *Salmonella*: low versus high *Salmonella* shedding in the natural porcine host. Infect. Genet. Evol. 16, 330–340.

Borowiak, M., Szabo, I., Baumann, B., Junker, E., Hammerl, J.A., Kaesbohrer, A., Malorny, B., Fischer, J., 2017. VIM-1-producing *Salmonella* Infantis isolated from swine and minced pork meat in Germany. J. Antimicrob. Chemother. 72, 2131–2133.

Borton, M.A., Sabag-Daigle, A., Wu, J., Solden, L.M., O'Banion, B.S., Daly, R.A., Wolfe, R.A., Gonzalez, J.F., Wysocki, V.H., Ahmer, B.M.M., 2017. Chemical and pathogen-induced inflammation disrupt the murine intestinal microbiome. Microbiome 5, 47.

Boyen, F., Haesebrouck, F., Vanparys, A., Volf, J., Mahu, M., Van Immerseel, F., Rychlik, I., Dewulf, J., Ducatelle, R., Pasmans, F., 2008. Coated fatty acids alter virulence properties of *Salmonella* typhimurium and decrease intestinal colonization of pigs. Vet. Microbiol. 132, 319–327.

Byndloss, M.X., Baumler, A.J., 2018. The germ-organ theory of non-communicable diseases. Nat. Rev. Microbiol. 16, 103–110.

Byndloss, M.X., Olsan, E.E., Rivera-Chavez, F., Tiffany, C.R., Cevallos, S.A., Lokken, K.L., Torres, T.P., Byndloss, A.J., Faber, F., Gao, Y., Litvak, Y., Lopez, C.A., Xu, G., Napoli, E., Giulivi, C., Tsolis, R.M., Revzin, A., Lebrilla, C.B., Baumler, A.J., 2017. Microbiota-activated PPAR-gamma signaling inhibits dysbiotic Enterobacteriaceae expansion.

Science 357, 570–575.
Campbell, J.M., Crenshaw, J.D., Polo, J., 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4, 19.
Derrien, M., Vlieg, J.E.T., van, H., 2015. Fate, activity, and impact of ingested bacteria within the human gut microbiota. Trends Microbiol. 23, 354–366.
Drumo, R., Pesciaroli, M., Ruggeri, J., Tarantino, M., Chirullo, B., Pistoia, C., Petrucci, P., Martinelli, N., Moscati, L., Manuali, E., Pavone, S., Picciolini, M., Ammendola, S., Gabai, G., Battistoni, A., Pezzotti, G., Alborali, G.L., Napolioni, V., Pasquali, P., Magistrali, C.F., 2016. *Salmonella enterica* serovar typhimurium exploits inflammation to modify swine intestinal microbiota. Front. Cell. Infect. Microbiol. 5, 106.
Frese, S.A., Parker, K., Calvert, C.C., Mills, D.A., 2015. Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 3, 28.
Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., Tobe, T., Clarke, J.M., Topping, D.L., Suzuki, T., Taylor, T.D., Itoh, K., Kikuchi, J., Morita, H., Hattori, M., Ohno, H., 2011. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 469, 543–547.
Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Hautefort, I., Thompson, A., Hinton, J.C., Van Immerseel, F., 2006. Butyrate specifically down-regulates Salmonella pathogenicity island 1 gene expression. Appl. Environ. Microbiol. 72, 946–949.
Gillis, C.C., Hughes, E.R., Spiga, L., Winter, M.G., Zhu, W., de Carvalho, T.F., Chanin, R.B., Behrendt, C.L., Hooper, L.V., Santos, R.L., Winter, S.E., 2018. Dysbiosis-associated change in host metabolism generates lactate to support *Salmonella* growth. Cell Host Microbe 23, 54.
Gresse, R., Chaucheyras-Durand, F., Fleury, M.A., de Wiele, T., Forano, E., Blanquet-Diot, S., 2017. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol. 25, 851–873.
Gu, Y., Song, Y., Yin, H., Lin, S., Zhang, X., Che, L., Lin, Y., Xu, S., Feng, B., Wu, D., Fang, Z., 2017. Dietary supplementation with tributyrin prevented weaned pigs from growth retardation and lethal infection via modulation of inflammatory cytokines production, ileal FGF19 expression, and intestinal acetate fermentation. J. Anim. Sci. 95, 226–238.
Guerra-Ordaz, A.A., Molist, F., Hermes, R.G., de Segura, A., La Ragione, R.M., Woodward, M.J., Tchorzewska, M.A., Collins, J.W., Perez, J.F., Martin-Orue, S.M., 2013. Effect of inclusion of lactulose and *Lactobacillus plantarum* on the intestinal environment and performance of piglets at weaning. Anim. Feed Sci. Technol. 185, 160–168.
Koh, A., De Vadder, F., Kovatcheva-Datchary, P., Backhed, F., 2016. From dietary Fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 165, 1332–1345.
Lawhon, S.D., Maurer, R., Suyemoto, M., Altier, C., 2002. Intestinal short-chain fatty acids alter Salmonella typhimurium invasion gene expression and virulence through BarA/SirA. Mol. Microbiol. 46, 1451–1464.
Le Gall, M., Gallois, M., Seve, B., Louveau, I., Holst, J.J., Oswald, I.P., Lalles, J.-P., Guilloteau, P., 2009. Comparative effect of orally administered sodium butyrate before or after weaning on growth and several indices of gastrointestinal biology of piglets. Br. J. Nutr. 102, 1285–1296.
Litvak, Y., Byndloss, M.X., Tsolis, R.M., Baumler, A.J., 2017. Dysbiotic Proteobacteria expansion: a microbial signature of epithelial dysfunction. Curr. Opin. Microbiol. 39, 1–6.
Litvak, Y., Byndloss, M.X., Baumler, A.J., 2018. Colonocyte metabolism shapes the gut microbiota. Science 362 (80-), 1017.
O'Hara, A.M., Shanahan, F., 2006. The gut flora as a forgotten organ. EMBO Rep. 7, 688–693.
Pieper, R., Janczyk, P., Zeyner, A., Smidt, H., Guiard, V., Souffrant, W.B., 2008. Ecophysiology of the developing total bacterial and Lactobacillus communities in the terminal small intestine of weaning piglets. Microb. Ecol. 56, 474–483.
Piva, A., Grilli, E., Fabbri, L., Pizzamiglio, V., Gatta, P.P., Galvano, F., Bognanno, M., Fiorentini, L., Wolinski, J., Zabielski, R., Patterson, J.A., 2008. Intestinal metabolism of weaned piglets fed a typical United States or European diet with or without supplementation of tributyrin and lactitol. J. Anim. Sci. 86, 2952–2961.
Richards, J.L., Yap, Y.A., McLeod, K.H., Mackay, C.R., Marino, E., 2016. Dietary metabolites and the gut microbiota: an alternative approach to control inflammatory and autoimmune diseases. Clin. Transl. Immunol. 5, e82.
Rivera-Chávez, F., Zhang, L.F., Faber, F., Lopez, C.A., Byndloss, M.X., Olsan, E.E., Xu, G., Velazquez, E.M., Lebrilla, C.B., Winter, S.E., Baumler, A.J., 2016. Depletion of butyrate-producing Clostridia from the gut microbiota drives an aerobic luminal expansion of Salmonella. Cell Host Microbe 19, 443–454.
Rooks, M.G., Garrett, W.S., 2016. Gut microbiota, metabolites and host immunity. Nat. Rev. Immunol. 16, 341–352.
Shen, Y.B., Piao, X.S., Kim, S.W., Wang, L., Liu, P., Yoon, I., Zhen, Y.G., 2009. Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs. J. Anim. Sci. 87, 2614–2624.
Sommer, F., Anderson, J.M., Bharti, R., Raes, J., Rosenstiel, P., 2017. The resilience of the intestinal microbiota influences health and disease. Nat. Rev. Microbiol. 15, 630–638.
Suez, J., Elinav, E., 2017. The path towards microbiome-based metabolite treatment. Nat. Microbiol. 2, 17075.
Tate, H., Folster, J.P., Hsu, C.-H., Chen, J., Hoffmann, M., Li, C., Morales, C., Tyson, G.H., Mukherjee, S., Brown, A.C., Green, A., Wilson, W., Dessai, U., Abbott, J., Joseph, L., Haro, J., Ayers, S., McDermott, P.F., Zhao, S., 2017. Comparative analysis of extended-spectrum-beta-Lactamase CTX-M-65-Producing *Salmonella enterica* serovar infantis isolates from humans, food animals, and retail chickens in the United States. Antimicrob. Agents Chemother. 61, e00488–17.
Vermeulen, K., Verspreet, J., Courtin, C.M., Haesebrouck, F., Ducatelle, R., Van Immerseel, F., 2017. Reduced particle size wheat bran is butyrogenic and lowers *Salmonella* colonization, when added to poultry feed. Vet. Microbiol. 198, 64–71.
Walter, J., Maldonado-Gomez, M.X., Martinez, I., 2018. To engraft or not to engraft: an ecological framework for gut microbiome modulation with live microbes. Curr. Opin. Biotechnol. 49, 129–139.
Yang, G.Y., Yu, J., Su, J.H., Jiao, L.G., Liu, X., Zhu, Y.H., 2017. Oral administration of *Lactobacillus rhamnosus* GG ameliorates *Salmonella* Infantis-Induced inflammation in a pig model via activation of the IL-22BP/IL-22/STAT3 pathway. Front. Cell. Infect. Microbiol. 7, 323.
Yu, H.T., Ding, X.L., Li, N., Zhang, X.Y., Zeng, X.F., Wang, S., Liu, H.B., Wang, Y.M., Jia, H.M., Qiao, S.Y., 2017. Dietary supplemented antimicrobial peptide microcin J25 improves the growth performance, apparent total tract digestibility, fecal microbiota, and intestinal barrier function of weaned pigs. J. Anim. Sci. 95, 5064–5076.
Yuan, C., Krull, A., Wang, C., Erdman, M., Fedorka-Cray, P.J., Logue, C.M., O'Connor, A.M., 2018. Changes in the prevalence of *Salmonella* serovars associated swine production and correlations of avian, bovine and swine-associated serovars with human-associated serovars in the United States (1997–2015). Zoonoses Public Health 0, 1–14.
Zhang, W., Zhu, Y.H., Yang, G.Y., Liu, X., Xia, B., Hu, X., Su, J.H., Wang, J.F., 2018. *Lactobacillus rhamnosus* GG affects microbiots and suppresses autophagy in the intestines of pigs challenged with *Salmonella* infantis. Front. Microbiol. 8, 2705.