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Probiotic administration aggravates dextran sulfate sodium salt-induced inflammation and intestinal epithelium disruption in weaned pig

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Abstract

Background *A. muciniphila* (AKK) has attracted extensive research interest as a potential next-generation probiotics, but its role in intestinal pathology is remains unclear. Herein, this study was conducted to investigate the effects of *A. muciniphila* DSM 22,959 on growth performance, intestinal barrier function, microecology and inflammatory response of weaned piglets stimulated by dextran sulfate sodium salt (DSS).

Method Twenty-four Duroc × Landrace × Yorkshire (DLY) weaned piglets used for a 2 × 2 factorial arrangement of treatments were divided into four groups with six piglets in each group. From 1 to 15 d, the CA and DA groups were orally fed with 1.0×10^{11} colony-forming units *A. muciniphila* per day, while the CON and DCON groups were received gastric infusion of anaerobic sterile saline per day. The pigs were orally challenged (DCON, DA) or not (CON, CA) with DSS from day 9 to the end of the experiment and slaughtered on day 16.

Results Presence of *A. muciniphila* in DSS-challenged weaned pigs resulted in numerically increased diarrhea rate, blood neutrophilic granulocyte, serum C-reactive protein and immunoglobulin M levels, and numerically reduced final weight, average daily feed intake and average daily gain. The decrease in intestinal villus height, villous height: crypt depth ratio and digestibility was accompanied by lower expression of *ZO1*, *ZO2*, *Claudin1*, *DMT1*, *CAT1*, *SGLT1* and *PBD114* genes, as well as decreased enzyme activities of intestinal alkaline phosphatase, lactase, sucrase and maltase of piglets in DA group compared to piglets in DCON group. The abundance of *Bifdobacterium*, *Lactobacillus*, *A. muciniphila*, *Ruminococcus gnavus* was numerically higher in digesta of pigs in DA group than those in DCON group. The inflammatory responses of piglets were dramatically changed by the simultaneous presence of *A. muciniphila* and DSS: expression level of *IL17A*, *IL17F*, *IL23*, *RORγt*, *Stat3* was elevated in DA pigs compared to the other pig groups.

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Conclusions Our result showed that the oral *A. muciniphila* aggravates DSS-induced health damage of weaned piglet, which may attribute to the deteriorating intestinal morphology, dysbiosis of microbiota and inflammatory response disorders.

Keywords *A. muciniphila*, Weaned pigs, Growth performance, Intestinal morphology, Microbiota, Inflammatory response

Background

Globally, the growing consumption of over-the-counter probiotics is driving the formation of a multi-billion-dollar industry chain [1]. However, a double-blind, randomized, placebo-controlled clinical trial proved that in patients diagnosed with acute pancreatitis, oral administration of six different strains of freeze-dried, viable probiotics worsened the condition [2], which reminds us to be very careful about defining strain knowledge as probiotics. From early tests to clinical applications, in order to assess the physiological effects of bacterial strains and their potentially practical relevance, many properties should be documented including bacterial gut colonization ability, strain level activity, interactions with the native microbiome, safety and impacts on the host, and so on [1]. Over the past two decades, multiple reports in in vitro studies, laboratory animal models, and clinical trials have shown numerous and strong association between oral consumption of certain bacterial strains and promoting growth and health [3, 4]. Among the different potential candidates observed, *Akkermansia muciniphila* (*A. muciniphila*) has attracted extensive research interest as a recurring potential next-generation probiotics.

A. muciniphila, a Gram-negative anaerobic bacterium from *Verrucomicrobia* phylum, has been present in the multiple mammals gut intestinal mucosa since early life, accounting about 1–3% of the intestinal microbial composition. This bacterium can strongly attach to intestinal epithelial cells by secreting mucolytic enzymes (e.g., α-L fucosidase, α- and β-D-galactosidase) which degrade host mucus as a sole source of carbon and nitrogen [5–8]. Since its discovery, numerous studies have highlighted that *A. muciniphila*, with its ability to target glucose homeostasis [5], metabolic disorders [6], as well as intestinal inflammation [7], is one of the most promising probiotics. The deficiency of *A. muciniphila* also has been strong clinical links to multiple diseases conditions such as diabetes, autism, obesity, hypertension, sclerosis, or even cancer [8]. In conflict with the beneficial effects of this bacterium, several evidence show that *A. muciniphila* may harm host health. It was reported that *A. muciniphila* can assist adherent-invasive *Escherichia coli* in eroding epithelial cells in mice fed with tryptophane-deprived diet [9]. Furthermore, Ganesh et al. [10] also found that an increased abundance of *A. muciniphila* in the intestine of *S. typhimurium*-infected mice. Correspondingly, although the efficacy and safety of pasteurized *A. muciniphila* was confirmed in a human proof-of-concept study in 2021 [11], its use as a living bacteria supplement is still being studied.

Studies investigating the role of *A. muciniphila* in intestinal pathology provides a novel insight. For instance, it has been indicated that administration of fiber-deprived diet to specific-pathogen-free mice causes intestinal barrier dysfunction, which was speculated to be correlated with an increased abundance of *A. muciniphila* that has the ability to degrade mucus [12]. In *IL33*$^{-/-}$ mice, *A. muciniphila* has also been reported to cause an increased intestinal susceptibility to colitis caused by DSS [13], which substantially increase the risk of the onset and formation of colorectal cancer (CRC) [14]. To date, although emerging evidence emphasized the potential of *A. muciniphila* as a pathogen, its involvement under intestinal pathology affecting host health is just beginning to be explored. Intriguingly, a study confirmed that the genomic diversity of *A. muciniphila* isolated from human intestinal mucosa is higher than that isolated from mice [15]. Indeed, Saarela et al. [16] reviewed species-specificity of the probiotic, and proposed the idea that probiotic strains may perform better in environmental conditions similar to those in which they were originally isolated. Compared to rodent models (e.g., *Mus musculus*, *Rattus norvegicus*), the pig (Sus scrofa) is considered a superior model for simulating human anatomy, physiology as well as pathophysiological responses [17]. Therefore, an experimental inflammatory injury model in piglets was established to assess the effects of a poorly defined potential probiotic isolated from humans, *A. muciniphila* DSM 22,959, on health damage caused by DSS. Also, the conceivable outcomes of *A. muciniphila*, focusing on intestinal barrier function, microecology and inflammatory response were also investigated.

Results

Growth performance, organ index and serum biochemical parameters

Growth performance and diarrhea rate were determined for the period prior to DSS challenge (days 0 to 8) and after challenged with DSS (days 9 to 15) and are presented in Tables 1 and 2, respectively. Prior to DSS challenge, there were no significant differences in growth performance and diarrhea rate among groups due to intragastric administration of *A. muciniphila* (AKK). In the following week after the challenge with DSS, final

Table 1 Effect of *A. muciniphila* supplementation on growth performance and diarrhea rate in weaned pigs before DSS challenge[1]

Items	Treatments[2]				SEM	*P*-value
	CON	CA	DCON	DA		
0–8 day						
Initial weight, kg	7.89	7.78	7.48	7.51	0.35	0.58
Final weight, kg	9.95	9.62	9.69	9.33	0.52	0.74
ADFI, g/d	345.2	305.6	323.8	298.6	25.77	0.30
ADG, g/d	257.3	229.2	277.1	228.1	48.55	0.70
F: G	1.38	1.42	1.26	1.55	0.25	0.73
Diarrhea rate, %	35.19	33.33	37.04	22.22	14.3	0.73

ADFI average daily feed intake, *ADG* average daily gain, *F: G* Feed: Gain ratio

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

[a, b, c] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

Table 2 Effect of *A. muciniphila* supplementation on growth performance and diarrhea rate in weaned pigs upon DSS challenge[1]

Items	Treatments[2]				SEM	*P*-value		
	CON	CA	DCON	DA		AKK	DSS	Interaction
9–15 day								
Initial weight, kg	10.19	9.71	9.87	9.4	0.62	0.29	0.48	0.99
Final weight, kg	12.98[a]	12.81[ab]	10.34[bc]	9.37[c]	0.93	0.40	< 0.01	0.55
ADFI, g/d	505.7[ab]	515.3[a]	364[ab]	268.3[b]	88.80	0.49	< 0.01	0.40
ADG, g/d	398.6[a]	442.9[a]	67.14[b]	-4.286[b]	80.96	0.82	< 0.01	0.33
F: G	-	-	-	-	-	-	-	-
Diarrhea rate, %	17.50[b]	7.50[b]	25.00[ab]	42.50[a]	8.00	0.51	< 0.01	0.02

ADFI average daily feed intake, *ADG* average daily gain, *F: G* Feed: Gain ratio

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

[a, b, c] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

Table 3 Effects of *A. muciniphila* supplementation on organ index in weaned pigs upon DSS challenge[1]

Items	Treatments[2]				SEM	*P*-value		
	CON	CA	DCON	DA		AKK	DSS	Interaction
Heart index, %	0.53	0.51	0.82	0.63	0.13	0.31	0.04	0.43
Liver index, %	2.53	2.68	2.90	2.53	0.24	0.54	0.51	0.15
Spleen index, %	0.21	0.21	0.30	0.25	0.04	0.36	0.02	0.45
Lung index, %	1.61	1.83	2.32	2.67	0.42	0.35	0.02	0.83
Kidney index, %	0.48[b]	0.52[ab]	0.59[a]	0.58[ab]	0.03	0.64	< 0.01	0.39

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

[a, b] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

weight, average daily feed intake (ADFI), and average daily gain (ADG) were decreased in challenged groups compared to those in non-challenged groups ($P < 0.05$). However, no statistically significant interaction between AKK and DSS challenge was observed for final weight, ADFI and ADG. Compared with the DCON group, DA group numerically reduced the final weight, ADFI, ADG. Although DCON group had no differences of diarrhea rate than that of CON group post challenge, the intragastric administration of *A. muciniphila* resulted out increased diarrhea rate was seen ($P < 0.05$), and there was an interaction between AKK and DSS challenge ($P = 0.02$).

The effects of the intragastric administration of *A. muciniphila* on the organ index and serum biochemical parameters are shown in Tables 3 and 4, respectively. DSS challenge resulted in higher indexes of heart, spleen, lung and kidney in challenged groups compared to those in non-challenged groups ($P < 0.05$). In contrast, the indexes of the organs in the DCON group were not different from those in the DA group. As for the serum biochemical parameters, DSS challenge resulted in a

Table 4 Effects of *A. muciniphila* supplementation on serum biochemical parameters and immunoglobulin in weaned pigs upon DSS challenge[1]

Items	Treatments[2]				SEM	*P*-value		
	CON	CA	DCON	DA		AKK	DSS	Interaction
ALT, U/L	90.15	76.04	69.79	68.55	12.58	0.40	0.14	0.48
AST, U/L	46.79	40.78	31.91	55.33	12.02	0.32	0.99	0.10
ALP, U/L	205.40	225.80	179.60	128.00	45.07	0.73	0.05	0.34
UREA, mmol/L	2.07	2.77	2.44	2.93	0.89	0.36	0.68	0.87
CREA, μmol/L	66.01	69.81	69.33	65.86	5.82	0.97	0.94	0.39
CRP, mg/L	8.36^{b}	10.29^{ab}	11.32^{ab}	16.57^{a}	2.52	0.06	0.02	0.37

ALT glutamic pyruvic transaminase, *AST* glutamic oxaloacetic transaminase, *ALP* alkaline phosphatase, *CREA* creatinine, *CRP* C-reactive protein

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

[a, b] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

Table 5 Effects of *A. muciniphila* supplementation on intestinal morphology in weaned pigs upon DSS challenge[1]

Items	Treatments[2]				SEM	*P*-value		
	CON	CA	DCON	DA		AKK	DSS	Interaction
Duodenum								
Villus height, μm	400.50^{a}	379.70^{a}	339.3^{b}	266.79^{c}	18.06	< 0.01	< 0.01	0.05
Crypt depth, μm	177.40	172.70	189.90	173.70	9.33	0.13	0.32	0.39
VCR	2.26^{a}	2.21^{a}	1.79^{b}	1.55^{c}	0.05	< 0.01	< 0.01	0.01
Jejunum								
Villus height, μm	350.80^{a}	356.50^{a}	298.30^{b}	258.90^{b}	15.31	0.14	< 0.01	0.05
Crypt depth, μm	161.20	150.90	166.30	147.60	6.92	< 0.01	0.85	0.40
VCR	2.18a	2.26a	1.79b	1.76b	0.05	0.552	< 0.01	0.10
Ileum								
Villus height, μm	318.40^{a}	312.30^{a}	278.90^{ab}	218.60^{c}	16.38	0.01	< 0.01	0.03
Crypt depth, μm	143.10	144.20	155.20	130.70	9.42	0.10	0.92	0.07
VCR	2.23^{a}	2.18^{a}	1.81^{b}	1.70^{c}	0.04	< 0.01	< 0.01	0.36

VCR Villous height: crypt depth ratio

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

[a, b] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

significantly lower concentration of alkaline phosphatase (ALP) ($P = 0.05$) and higher concentration of C-reactive protein (CRP) ($P = 0.02$) for challenged groups compared to non-challenged groups. Although not different among the treatments, the concentration of CRP was numerically elevated in DA group than those in DCON group.

Intestinal morphology, nutrients digestibility and mucosal enzyme activity

Intestinal morphology data are presented in Table 5 and stained transverse sections of intestinal tissue in Fig. 1. The DSS challenge decreased the villus height and villous height: crypt depth ratio (VCR) of duodenum, jejunum and ileum for challenged groups compared to non-challenged groups ($P < 0.05$). Intragastric administration of *A. muciniphila* resulted in a significantly decreased villus height and VCR of duodenum and ileum for gavage groups compared to un-gavage groups ($P < 0.05$). Duodenal and ileal villus height and VCR were lower in the DA group than in the DCON group, and there was an interaction between AKK and DSS challenge for duodenal VCR ($P = 0.01$) and ileal villus height ($P = 0.03$).

The effects of the intragastric administration of *A. muciniphila* on the nutrient digestibility and intestinal mucosal enzyme activities were presented in Tables 6 and 7, respectively. Subsequent to DSS challenge, DA group numerically decreased the digestibility of DM, GE, Ash and CP than that of the DCON group. In addition, there was an interaction between AKK and DSS challenge for the digestibility of DM ($P < 0.01$). Although the digestibility of EE was not significantly affected by AKK or DSS, whereas there was an interaction between AKK and DSS challenge ($P = 0.04$). In the small intestine, the DSS challenge decreased the enzyme activities for challenged groups compared to non-challenged groups ($P < 0.05$). An interaction between AKK and DSS challenge was observed for duodenal IAP ($P = 0.04$), duodenal lactase ($P = 0.04$) and jejunal lactase ($P = 0.04$). The duodenal IAP

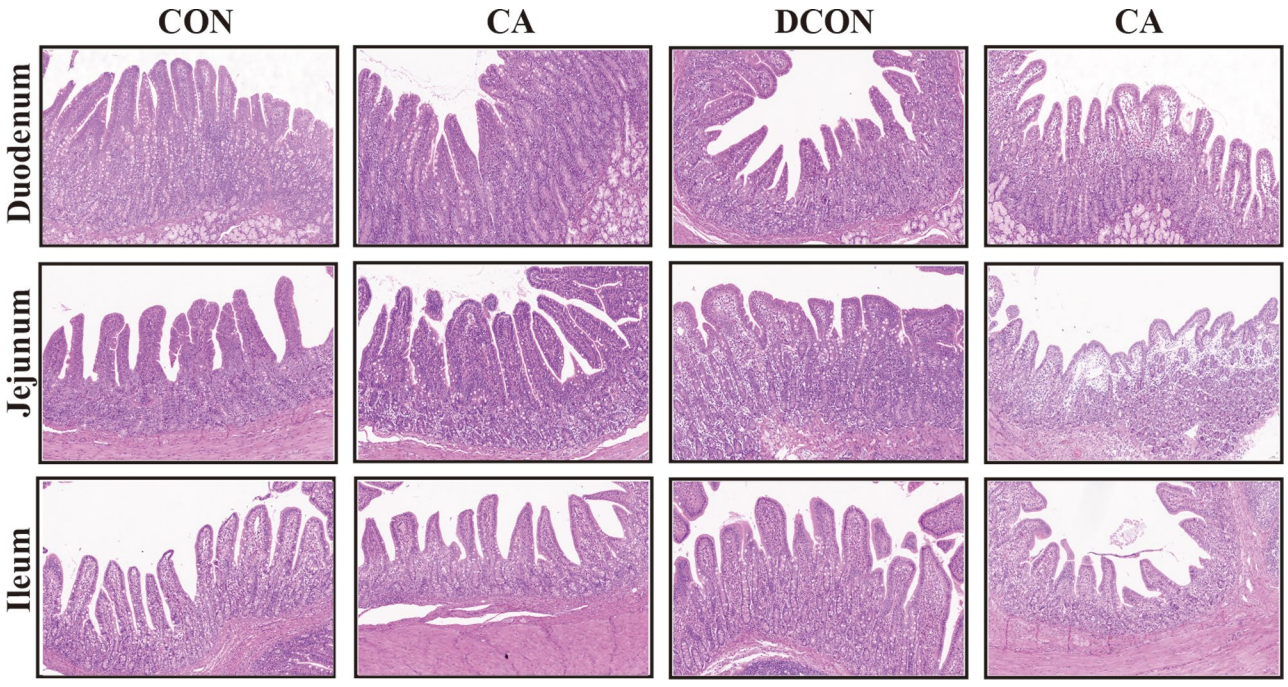

Fig. 1 Dextran sulfate sodium (DSS)-induced colitis was aggravated by oral administration of *A. muciniphila* to piglets (H&E, × 200). CON, pigs were fed with a basal diet; CA, pigs were gavage every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

Table 6 Effects of *A. muciniphila* supplementation on apparent total tract nutrient digestibility in weaned pigs upon DSS challenge[1]

Items	Treatments[2]				SEM	*P*-value		
	CON	CA	DCON	DA		AKK	DSS	Interaction
DM, %	82.03[a]	84.11[a]	81.74[ab]	79.01[b]	0.97	0.64	< 0.01	< 0.01
GE, %	82.8[ab]	84.96[a]	81.13[ab]	79.6[b]	1.51	0.77	< 0.01	0.10
Ash, %	34.52[a]	35.81[a]	29.46[ab]	22.2[b]	3.52	0.25	< 0.01	0.11
CP, %	75.70[ab]	78.33[a]	75.30[ab]	68.60[b]	3.33	0.40	< 0.01	0.07
EE, %	68.51	72.15	70.2	58.64	4.87	0.27	0.11	0.04

DM dry matter, *CP* crude protein, *EE* ether extract

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

[a, b] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

and lactase enzyme activities as well as jejunal lactase, sucrase and maltase enzyme activities were lower in DA group than those in the DCON group ($P < 0.05$).

Microbial populations and fermentation products

The effects of the intragastric administration of *A. muciniphila* on number of specific microbial groups are shown in Table 8 and Supplementary Fig. 1. In the cecum, piglets in the challenged groups had lower abundance of *Bacillus* ($P = 0.02$), *Bifdobacterium* ($P < 0.01$), *Lactobacillus* ($P < 0.01$) and *Mucispirillum* ($P < 0.01$) and higher abundance of *A. muciniphila* ($P = 0.02$) than that in the non-challenged groups. Compared with the no-gavage groups, piglets in *A. muciniphila* gavage groups had a significantly higher abundance of total bacteria ($P = 0.04$), *Clostridium* ($P = 0.04$) and *R. gnavus* ($P < 0.01$) and tended to increase the abundance of *A. muciniphila* ($P = 0.07$) and *R. torques* ($P = 0.09$), whereas there was no interaction between AKK and DSS challenge. In the colon, piglets in the challenged groups had lower abundance of *Bacillus* ($P < 0.01$), *Bifdobacterium* ($P = 0.02$), *Lactobacillus* ($P < 0.01$), *Clostridium* ($P < 0.01$) and *Mucispirillum* ($P < 0.01$) and higher abundance of *A. muciniphila* ($P = 0.05$) than that in the non-challenged groups. Compared with the no-gavage groups, piglets in *A. muciniphila* gavage groups had a higher abundance of *Clostridium* ($P = 0.05$) and *R. gnavus* ($P < 0.01$) and tended to increase the abundance of *A. muciniphila* ($P = 0.07$) and *Escherichia coli* ($P = 0.06$). The abundance of *Clostridium* in the colon was affected by the AKK × DSS

Table 7 Effects of *A. muciniphila* supplementation on intestinal mucosal enzyme activity in weaned pigs upon DSS challenge[1]

Items	Treatments[2]				SEM	*P*-value		
	CON	CA	DCON	DA		AKK	DSS	Interaction
Duodenum								
IAP, U/g prot	44.95^{a}	40.82^{a}	33.11^{b}	20.50^{c}	3.51	< 0.01	< 0.01	0.04
Lactase, U/mg prot	145.50^{a}	142.80^{a}	111.20^{b}	78.09^{c}	10.48	0.02	< 0.01	0.04
Sucrase, U/mg prot	171.50^{a}	143.60^{ab}	129.70^{ab}	104.40^{b}	17.99	0.05	< 0.01	0.92
Maltase, U/mg prot	141.30^{a}	130.00^{ab}	106.50^{bc}	100.70^{c}	9.15	0.20	< 0.01	0.67
Jejunum								
IAP, U/g prot	64.31^{a}	52.05^{ab}	39.55^{bc}	32.83^{c}	5.86	0.02	< 0.01	0.46
Lactase, U/mg prot	275.40^{a}	268.50^{a}	178.70^{b}	103.80^{c}	25.77	0.02	< 0.01	0.04
Sucrase, U/mg prot	256.90^{a}	187.50^{ab}	148.10^{b}	56.18^{c}	30.91	< 0.01	< 0.01	0.61
Maltase, U/mg prot	425.80^{a}	355.60^{ab}	309.40^{bc}	228.20^{d}	26.54	< 0.01	< 0.01	0.77
Ileum								
IAP, U/g prot	47.96^{a}	48.05^{a}	46.05^{ab}	34.48^{b}	4.54	0.09	0.03	0.09
Lactase, U/mg prot	169.60	174.50	170.30	152.70	16.00	0.33	0.37	0.58
Sucrase, U/mg prot	150.10^{a}	126.40^{ab}	125.80^{ab}	86.36^{b}	19.92	0.04	0.04	0.58
Maltase, U/mg prot	139.30^{a}	129.90^{ab}	104.00^{ab}	93.07^{b}	14.48	0.34	< 0.01	0.94

IAP intestinal alkaline phosphatase

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2 a, b] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

[3] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

Table 8 Effects of *A. muciniphila* supplementation on specific microbial populations in the cecum and colon of weaned pigs upon DSS challenge, $\log_{10}$(copies/g)[1]

Items	Treatments[2]				SEM	*P*-value		
	CON	CA	DCON	DA		AKK	DSS	Interaction
Cecum								
Total bacteria	13.57	13.76	13.23	13.81	0.24	0.04	0.40	0.27
Bacillus	7.38	9.53	3.41	3.74	2.56	0.50	0.02	0.62
Bifidobacterium	7.53^{ab}	8.23^{a}	2.26^{ab}	3.97^{b}	2.06	0.42	< 0.01	0.73
Lactobacillus	7.88^{ab}	10.32^{a}	1.189^{c}	3.21^{bc}	2.15	0.16	< 0.01	0.89
Escherichia coli	5.31	7.30	2.21	4.93	2.02	0.12	0.07	0.81
Clostridium	6.60	8.63	4.065	7.78	1.78	0.04	0.20	0.51
A. muciniphila	5.21^{b}	7.58^{ab}	8.01^{ab}	8.84^{a}	1.16	0.07	0.02	0.36
Mucispirillum	7.47	7.28	5.70	5.92	0.74	0.98	< 0.01	0.71
R. gnavus	7.94^{a}	10.10^{a}	8.56^{ab}	10.02^{a}	0.72	< 0.01	0.60	0.50
R. torques	2.56	5.01	5.18	6.98	1.68	0.09	0.07	0.79
Colon								
Total bacteria	14.03	14.19	13.91	14.06	0.27	0.43	0.52	0.99
Bacillus	7.71	9.32	3.08	3.44	2.43	0.58	< 0.01	0.72
Bifidobacterium	6.53	8.07	2.31	3.94	2.39	0.36	0.02	0.98
Lactobacillus	8.18^{ab}	10.18^{a}	1.13^{b}	3.56^{bc}	2.27	0.19	< 0.01	0.90
Escherichia coli	5.89	8.16	3.12	6.93	2.15	0.06	0.21	0.62
Clostridium	7.53^{ab}	9.83^{a}	2.29^{b}	6.18^{ab}	2.06	0.05	< 0.01	0.59
A. muciniphila	6.55^{b}	6.43^{b}	6.48^{b}	8.57^{a}	0.70	0.07	0.05	0.04
Mucispirillum	6.35	5.86	3.95	5.21	1.04	0.61	0.05	0.25
R. gnavus	8.57^{b}	10.90^{a}	8.89^{ab}	10.13^{ab}	0.76	< 0.01	0.68	0.32
R. torques	4.00	6.96	3.20	4.32	1.97	0.16	0.24	0.52

R. gnavus Ruminococcus gnavus, R. torques Ruminococcus torques

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL A. muciniphila (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL A. muciniphila (DSM 22959) and challenged by DSS

[a, b] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

interaction ($P = 0.04$), whereas other Microbial populations were not affected by the interaction between AKK and DSS challenge.

The effects of the intragastric administration of *A. muciniphila* on the concentration of SCFAs in digesta samples are presented in Table 9. Following DSS challenge, piglets in challenged groups had lower ($P < 0.05$) concentration of cecal acetic acid, propanoic acid and butyric acid and tended to decrease the concentration of colonic propanoic acid ($P = 0.06$) and butyric acid ($P = 0.09$) compared to non-challenged groups. In addition, piglets in *A. muciniphila* gavage groups tended to increase the concentration of cecal isobutyric acid ($P = 0.08$) and isovaleric acid ($P = 0.06$) and colonic isobutyric acid ($P = 0.06$) when compared to those in un-gavage groups. However, these intestinal SCFAs were not affected by the interaction between AKK and DSS challenge.

Hematology and plasma immunoglobulin concentrations

The effects of the intragastric administration of *A. muciniphila* on the composition of peripheral blood lymphocyte percentages and plasma immunoglobulin concentrations were presented in Table 10. DSS challenge elevated the percentage of neutrophils and basophilic granulocytes in challenged groups compared to non-challenged groups ($P < 0.05$). Piglets in gavage *A. muciniphila* groups had lower number of red blood cell, blood platelet and plateletcrit and higher percentage of neutrophils for gavage groups compared to the un-gavage groups ($P < 0.05$). The percentage of neutrophils were elevated ($P < 0.05$) in the DA group than those in the DCON group, whereas there was no significant interaction between AKK and DSS challenge ($P = 0.10$). As for the immunoglobulins, DSS challenge resulted in a significantly higher concentration of IgG ($P = 0.05$) and IgM ($P < 0.01$) for challenged groups compared to non-challenged groups. There was no significant difference was observed between the *A. muciniphila* gavage groups, whereas the concentration of IgM was numerically elevated in DA group than that in DCON group.

Gene expression profiles

The effects of the intragastric administration of *A. muciniphila* on the expression levels of a panel of selected intestinal barrier-related genes are shown in Fig. 2. Following DSS challenge, the jejunal *Muc2*, *Zo2* and *Claudin1* mRNA abundance and ileal *Zo2* and *Occludin1* mRNA abundance for challenged groups were significantly down-regulated compared to non-challenged groups ($P < 0.05$). In addition, piglets in *A. muciniphila* gavage groups had higher duodenal *Muc1* ($P < 0.01$) and *Zo2* ($P = 0.05$) mRNA abundance and lower jejunal *Zo1* ($P = 0.03$), *Zo2* ($P < 0.01$) and *Claudin1* ($P < 0.01$) mRNA abundance when compared to those in un-gavage groups. Compared with the DCON group, piglets in DA group had a numerically lower RNA abundance of *Zo1*, *Zo2* and *Claudin*.

The effects of the intragastric administration of *A. muciniphila* on the expression levels of a panel of selected nutrient transporters-related genes are shown in Fig. 3. The piglets in DSS challenged groups had lower jejunal *DMT1*, *CAT1* and *ZnT1* mRNA abundance and ileal *ZnT1* mRNA abundance when compared to those in

Table 9 Effects of *A. muciniphila* supplementation on concentrations of SCFAs in the cecal and colonic digesta of weaned pigs upon DSS challenge[1]

Items	Treatments[2]				SEM	*P*-value		
	CON	CA	DCON	DA		AKK	DSS	Interaction
Cecum								
Acetic acid	529.30^{a}	491.10^{ab}	324.10^{b}	352.00^{ab}	67.27	0.91	< 0.01	0.50
Propanoic acid	266.00^{a}	229.80^{ab}	147.80^{ab}	124.60^{b}	42.08	0.33	< 0.01	0.83
Isobutyric acid	4.92	8.89	5.29	8.59	2.74	0.08	0.99	0.86
Butyric acid	125.00^{a}	97.52^{ab}	62.72^{ab}	27.68^{b}	26.90	0.12	< 0.01	0.84
Isovaleric acid	1.94	9.04	1.93	8.64	4.78	0.06	0.95	0.95
Valeric acid	40.45	24.52	26.49	13.79	12.07	0.11	0.17	0.85
Colon								
Acetic acid	475.50	428.40	330.90	388.00	74.50	0.93	0.10	0.34
Propanoic acid	220.80	194.30	145.90	151.60	41.97	0.73	0.06	0.59
Isobutyric acid	9.61	13.75	9.99	15.42	3.39	0.06	0.67	0.79
Butyric acid	123.60	103.50	78.32	69.69	31.50	0.53	0.09	0.80
Isovaleric acid	11.00	19.55	13.37	18.88	6.47	0.13	0.85	0.74
Valeric acid	42.12	28.04	31.98	24.85	14.83	0.33	0.53	0.74

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

[a, b] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

Table 10 Effects of *A. muciniphila* supplementation on hematology and plasma immunoglobulin concentrations in weaned pigs upon DSS challenge[1]

Items	Treatments[2]				SEM	*P*-value		
	CON	CA	DCON	DA		AKK	DSS	Interaction
WBC, 10^9/L	34.86	29.62	29.34	32.30	5.07	0.75	0.70	0.27
Neut, %	28.43^{b}	35.33^{ab}	31.78^{b}	60.00^{a}	8.45	0.01	0.04	0.10
Lymph, %	61.90	56.13	50.74	45.50	11.43	0.49	0.19	0.97
Mono, %	1.56	6.975	2.72	6.62	4.83	0.18	0.91	0.82
Eos, %	0.88	0.70	1.68	1.66	0.90	0.88	0.20	0.90
Baso, %	1.12^{ab}	0.87^{b}	1.08^{ab}	1.34^{a}	0.13	0.93	0.03	0.01
Neut, 10^9/L	9.00	10.28	9.32	18.93	4.63	0.11	0.18	0.21
Lymph, 10^9/L	21.44	16.58	18.36	13.72	3.82	0.09	0.28	0.97
Mono, 10^9/L	0.53	2.19	0.78	1.91	1.27	0.15	0.99	0.78
Eos, 10^9/L	0.30	0.19	0.51	0.53	0.28	0.81	0.18	0.75
Baso, 10^9/L	0.39	0.28	0.57	0.34	0.12	0.51	0.20	0.06
RBC, 10^9/L	6.67	5.76	6.53	6.07	0.44	0.04	0.77	0.48
HGB, g/L	99.40	89.00	96.40	91.00	6.94	0.12	0.92	0.61
HCT, %	35.04	30.95	33.30	30.14	2.68	0.07	0.50	0.80
MCV, fL	52.80	53.50	51.00	50.00	2.43	0.93	0.13	0.62
MCHC, g/L	284.40	287.50	290.40	307.50	9.20	0.14	0.07	0.30
MCH, pg	15.20	15.25	14.60	15.20	0.64	0.47	0.47	0.54
RDW-SD, fL	50.00	48.00	46.20	45.40	3.88	0.61	0.25	0.83
RDW-CV, %	26.40	24.75	25.40	25.20	2.31	0.57	0.86	0.65
PLT, 10^9/L	595.60^{a}	451.30^{ab}	579.6^{ab}	388.30^{b}	67.35	< 0.01	0.42	0.63
PCT, %	0.54	0.43	0.50	0.36	0.05	< 0.01	0.18	0.74
MPV, fL	9.06	9.55	8.64	8.78	0.40	0.29	0.06	0.55
PDW, %	15.00	14.75	14.80	15.20	0.25	0.69	0.50	0.10
IMG, %	0.68	0.87	1.08	1.04	0.20	0.78	0.32	0.68
IgG, g/L	3.13	3.34	3.92	3.93	0.46	0.75	0.05	0.76
IgM, g/L	0.44^{b}	0.59^{ab}	0.73^{ab}	0.78^{a}	0.11	0.22	< 0.01	0.52

WBC white blood cell, *RBC* red blood cell, *HGB* hemoglobin, *HCT* hematocrit, *MCV* mean corpuscular volume, *MCHC* mean corpuscular hemoglobin concentration, *RDW-SD* red cell distribution width standard deviation, *RDW-CV* red Blood Cell Distribution Width Coefficient of Variation, *PLT* blood platelet count, *PCT* Plateletcrit, *MPV* mean platelet volume, *PDW* platelet volume distribution width, *IMG* immature Granulocytes, *IgG* immunoglobulin G, *IgM* immunoglobulin M

[1] Mean and total SEM are list in Separate columns, $n = 6$

[2] CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS

[a, b] mean values within a row with unlike superscript letters were significantly different, $P < 0.05$

non-challenged groups ($P < 0.05$). The selected nutrient transporters-related genes expression was not affected by intragastric administration of *A. muciniphila*, whereas there was an interaction between AKK and DSS challenge for the gene expression of *SGLT1* ($P = 0.03$) in duodenal mucosal tissue. In contrasted to the DCON group, piglets in DA group had a numerically lower RNA abundance of *DMT1*, *CAT1* and *SGLT1*.

The effects of the intragastric administration of *A. muciniphila* on the expression levels of a panel of selected intestinal β-defensin-related genes are shown in Fig. 4. The piglets in DSS challenged groups had reduced the duodenal *PBD129* mRNA abundance, jejunal *PBD3*, *PBD114* and *PBD129* mRNA abundance and ileal *PBD3* mRNA abundance when compared to those in non-challenged groups ($P < 0.05$). Jejunal *PBD3* mRNA abundance was affected by AKK ($P = 0.01$) and AKK × DSS interaction ($P = 0.02$). Although the duodenal and ileal *PBD114* mRNA abundance was not significantly affected by AKK or DSS, whereas there was an interaction between AKK and DSS challenge ($P = 0.02$). In contrasted to the DCON group, piglets in DA group had a numerically lower RNA abundance of *PBD114*.

The effects of the intragastric administration of *A. muciniphila* on the expression levels of a panel of selected intestinal inflammatory factor-related genes are shown in Fig. 5. The piglets in DSS challenged groups had higher jejunal *IL1-β*, *IFN-γ*, *IL23* and *IL17F* mRNA abundance and ileal *RORγt* mRNA bundance when compared to those in non-challenged groups ($P < 0.05$). The intestinal *IL17A*, *IL17F* and *RORγt* mRNA abundance was affected by the AKK × DSS interaction ($P < 0.05$). Furthermore, the intestinal *IL23*, *IL17A*, *IL17F* and *RORγt* mRNA abundance were higher in DA group than those in the DCON group ($P < 0.05$).

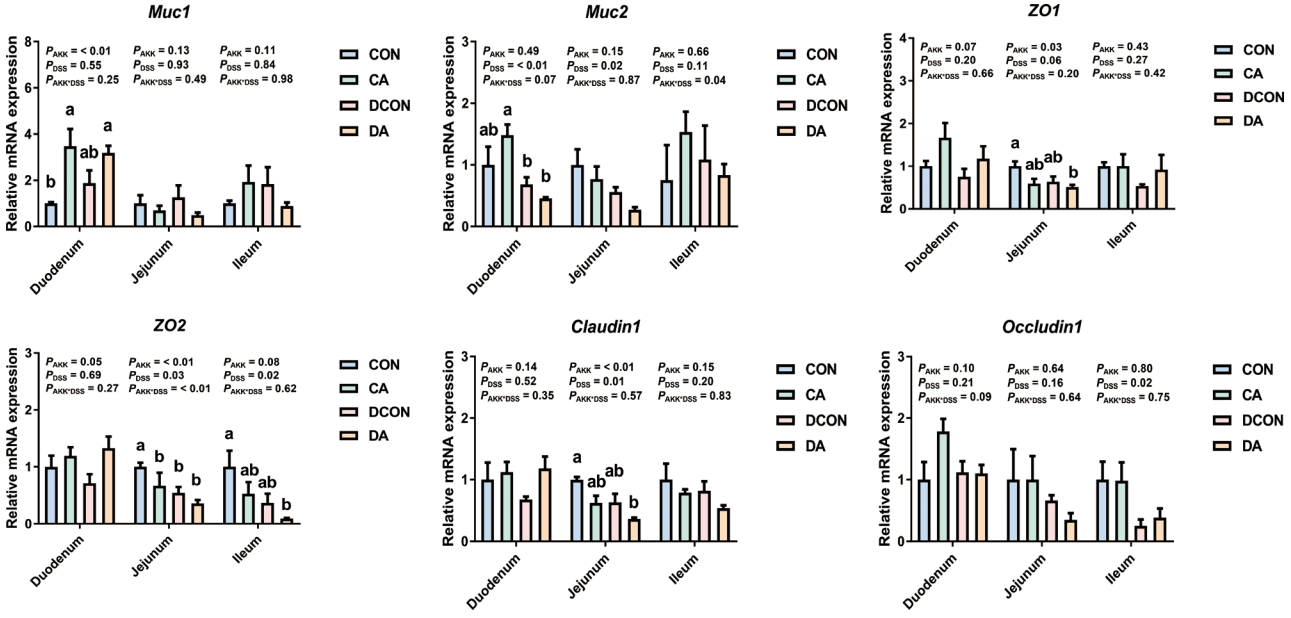

Fig. 2 Administering *A. muciniphila* to dextran sulfate sodium (DSS)-treated piglets impaired intestinal barrier function. Values are means, with their standard errors represented by vertical bars ($n=6$). Bars that do not share the same superscripts are significantly different ($P<0.05$). CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS. *MUC1* Mucin 1, *MUC2* Mucin 2, *ZO1* zonula occludens-1, *ZO2* zonula occludens-2

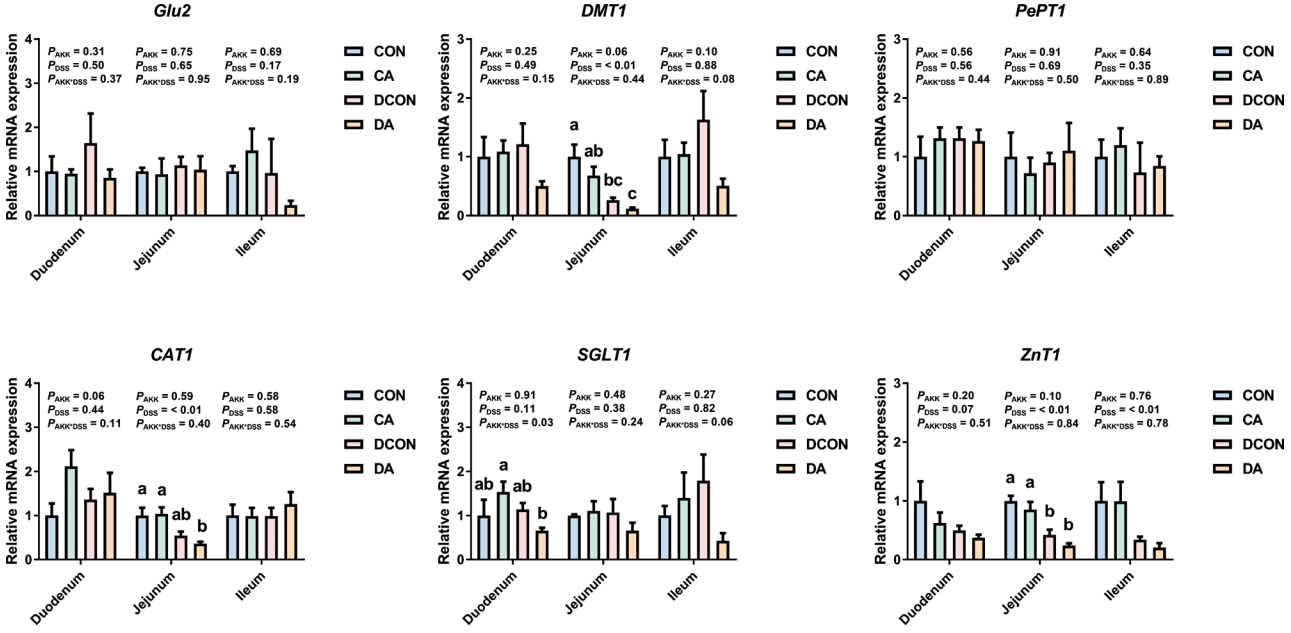

Fig. 3 Administering *A. muciniphila* to dextran sulfate sodium (DSS)-treated piglets impaired intestinal nutrient transporter function. Values are means, with their standard errors represented by vertical bars ($n=6$). Bars that do not share the same superscripts are significantly different ($P<0.05$). CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS. *SGLT1* sodium-glucose cotransporter-1, *GLUT2* glucose transporter 2, *DMT1* divalent metal ion transporter 1, *ZnT1* zinc transporter 1, *CAT-1* cationic amino acid transporter 1, *PePT1* solute carrier family 15 Member 1

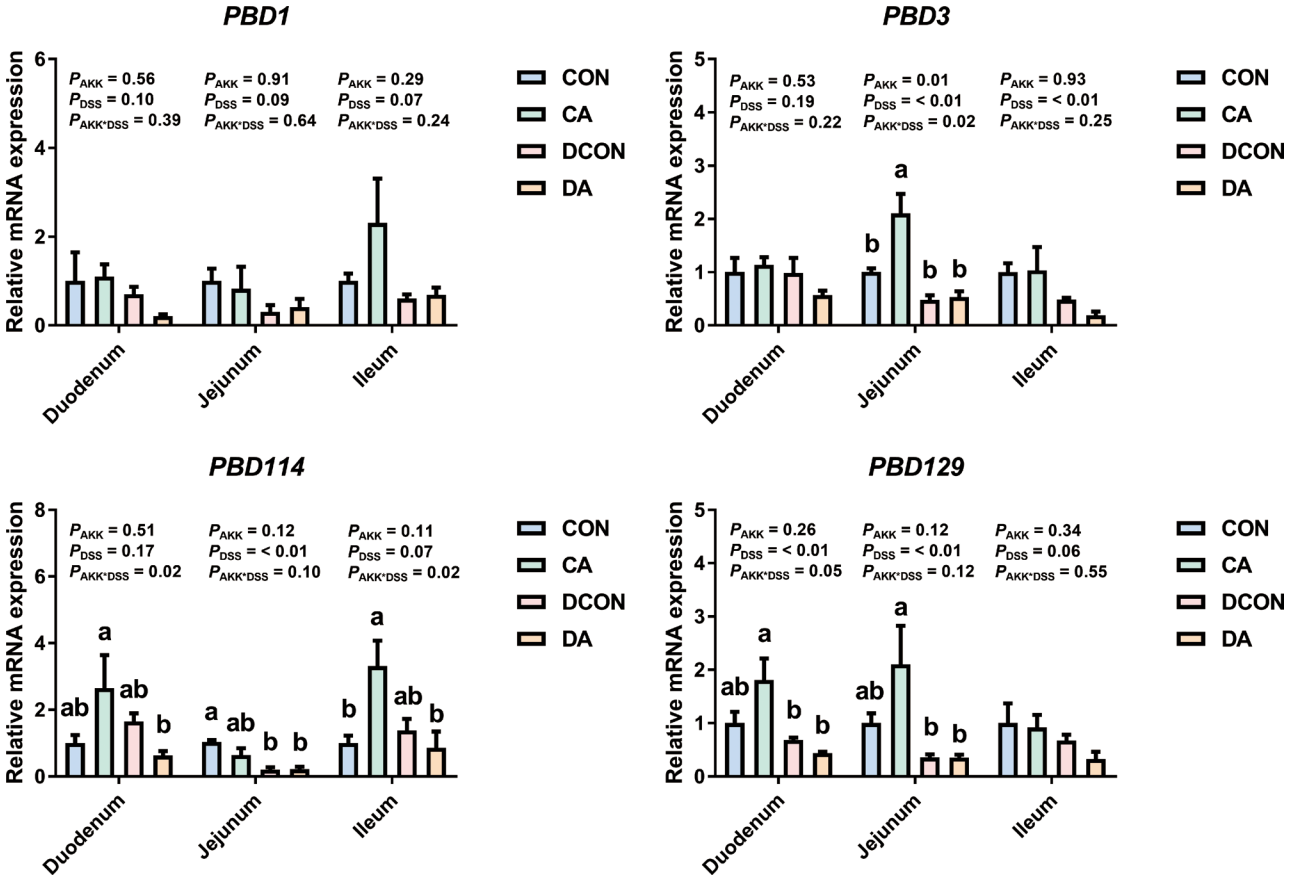

Fig. 4 Administering *A. muciniphila* to dextran sulfate sodium (DSS)-treated piglets impaired intestinal endogenous antimicrobial ability. Values are means, with their standard errors represented by vertical bars ($n = 6$). Bars that do not share the same superscripts are significantly different ($P < 0.05$). CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS. *PBD1* porcine β-defensin 1, *PBD3* porcine β-defensin 3, *PBD114* porcine β-defensin 114, *PBD129* porcine β-defensin 129

Discussion

As a potential probiotic, *A. muciniphila* has attracted considerable research interest. The results of studies about *A. muciniphila* are contradictory: on the one hand, there is a growing trend of researches suggesting the beneficial effects of *A. muciniphila* or its related outer-membrane components (e.g., Amuc_1100) on metabolic homeostasis and intestinal health [5–8], on the other hand it was also reported that *A. muciniphila* showed a stronger ability to increase intestinal inflammation in murine [9, 10]. Therefore, the present study described the responses of growth performance as well as intestinal function, microecology and immunity property to *A. muciniphila* orally in a DSS-challenged porcine model. This work aims to shed light on, under which circumstances, a mucin-degrading commensal bacterium can exert such negative effects on host health.

Studies reported that oral *A. muciniphila* improved the growth performance in mice challenged by DSS [18, 19]. In case of weaned pigs, oral administration *A. muciniphila* could resist the infection of enterotoxigenic *Escherichia coli* (ETEC), thereby improving the growth performance of animal to a certain extent [20]. On the contrary, we showed that *A. muciniphila* orally aggravated the reduction in final weight, ADFI and ADG, and increased diarrhea rates for pigs receiving DSS challenge, while pigs in CA group did not differ in growth performance or diarrhea rates from the CON group. Similar with a previous study in rat [21], our data indicated that subjects challenged with DSS were affected due to the greater amount of stress caused by the *A. muciniphila*. The different results of the effects of *A. muciniphila* on animal growth performance may be ascribed to several factors, including the different animal models used, distinct experimental stimulants, and even differing strains or dosages of *A. muciniphila*.

Organ size is usually expressed as a ratio of organ weight to body weight in the toxicological bioassays, which is an important indicator to reflect whether the organ has edema, congestion, atrophy, hypertrophy, or even hyperplasia [22]. Specifically, significant changes in the size of organs responsible for metabolism, such as

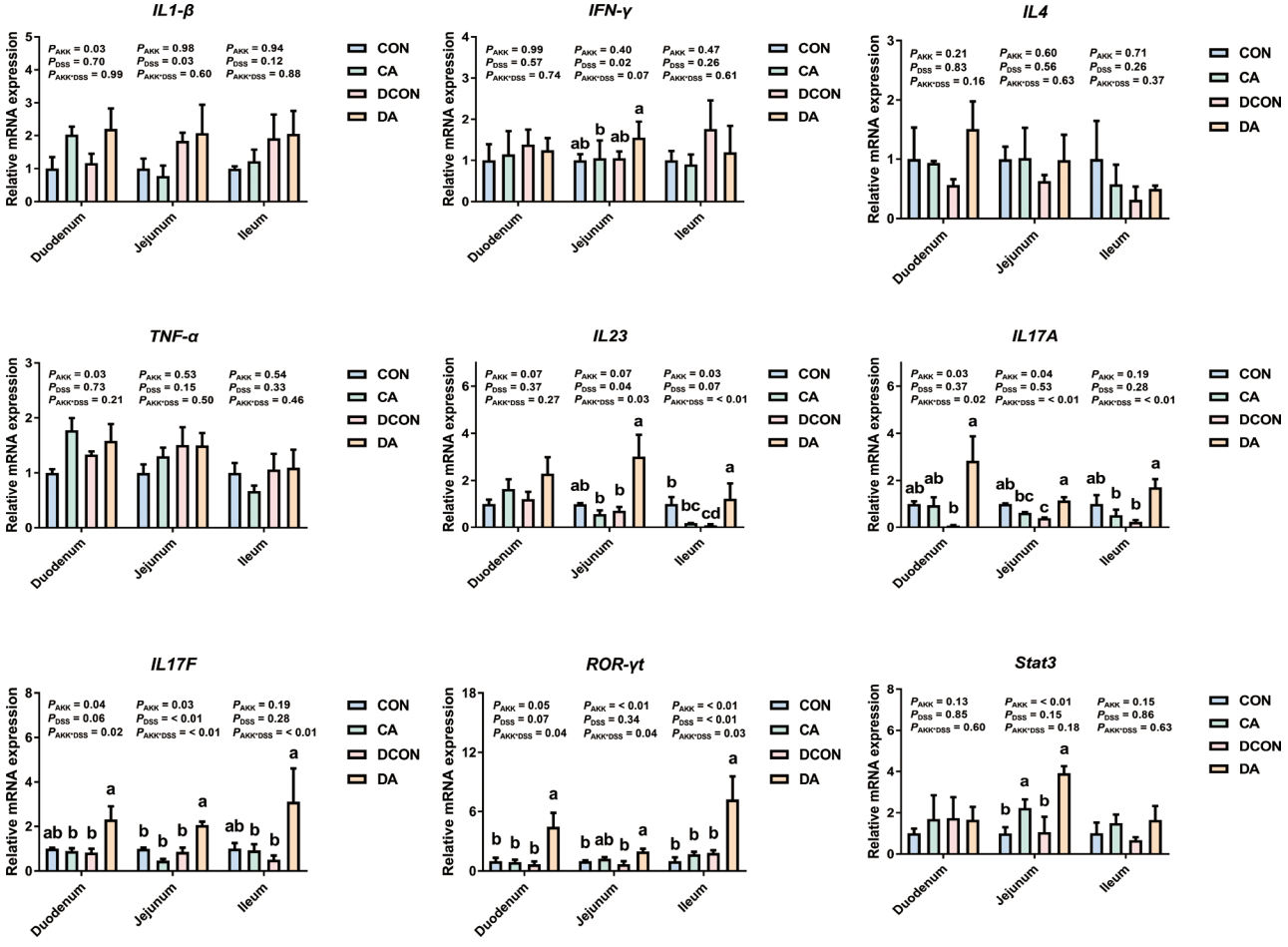

Fig. 5 Administering *A. muciniphila* to dextran sulfate sodium (DSS)-treated piglets aggravated intestinal inflammation. Values are means, with their standard errors represented by vertical bars ($n=6$). Bars that do not share the same superscripts are significantly different ($P<0.05$). CON, pigs were fed with a basal diet; CA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959); DCON, pigs were fed with a basal diet and challenged by DSS; DA, pigs were gavaged every day with 1×10^{11} CFU/10 mL *A. muciniphila* (DSM 22959) and challenged by DSS. *IL1β* interleukin-1β, *IL4* interleukin-4, *IL6* interleukin-6, *IL10* interleukin-10, *IL17A* interleukin-17 A, *IL17F* interleukin-17 F, *IL23* interleukin-23, *TNFα* tumor necrosis factor-α, *IFNγ* interferon-gamma, *RORγt* retinoic acid receptor-related orphan receptor gamma t, *STAT3* signal transducer and activator of transcription 3

liver and kidney, can lead to growth retardation, while changes in the spleen, an organ closely related to immune function, can damage the immunity of the body [23, 24]. In this study, while *A. muciniphila* orally had no impact on increased relative organ weight of the heart, liver, spleen, lung, and kidney for pigs receiving DSS challenge, it did increase the serum concentration of CRP, which has been widely used as a hallmark of organ functional damage [25]. This may be due to the role of CRP in binding to phosphocholine on microbes and clearing necrotic and apoptotic cells, so it is increased in response to bacterial infections.

The intestinal morphology and structure can partly reflect the health of intestinal tract [26]. Our findings revealed that *A. muciniphila* orally can reduce villus height and VCR of piglets subjected to DSS. As a matter of fact, *A. muciniphila* aggravated abnormal intestinal morphology during the challenged period, mainly manifested by villi atrophy and crypt hyperplasia. The expression levels of mucoproteins (e.g., mucins) and tight junction related proteins (e.g., occludin, claudins and zonula occludens) are key indicators of intestinal epithelial barrier stability [27]. Our research findings clearly indicated that *A. muciniphila* orally effectively increased the mRNA level of *Muc1*. In line with this, Bhanu Priya et al. [10] also found that *A. muciniphila* can stimulate the mRNA expression levels of *Muc2*, which is coincide with an increase in the numbers of goblet cells that fill the mucus. However, *A. muciniphila* orally leads to significant reductions in mRNA levels of *ZO-1*, *ZO-2*, and *Claudin-1* in the intestine for pigs receiving DSS challenge. The morphological and structural integrity of the gut are essential for the digestion, absorption, and transport of nutrients [26]. This study further showed that *A. muciniphila* orally aggravated the reduction in the ATTD of DM, GE and CP for pigs receiving DSS challenge. In

animals, several endogenous intestinal apical hydrolase activities (e.g., sucrase, maltase, and lactase) and intestinal alkaline phosphatase (IAP) are involved in carbohydrate and lipid digestion, respectively [28]. In this study, *A. muciniphila* orally not only down-regulated the activities of IAP and lactase in duodenum of DSS-challenged pigs but also down-regulated the activities of lactase, sucrase and maltase in the jejunum. On top of this, it was observed that *A. muciniphila* orally leads to significant reductions in mRNA levels of *DMT1*, *CAT1* and *SGLT1* in the intestine for pigs receiving DSS challenge. These outcomes collectively point out that *A. muciniphila*, in some way, negatively interacted with some aspects of the intestinal health.

Recently, growing body of evidence suggested that dysbiosis of the gut microbiota is known to occur in impaired gut, which poses a huge challenge for the colonization of specific beneficial microbes in the gut [29]. Within this work, qPCR results showed an increased abundance of *A. muciniphila* in digesta not only in the DA group, but also in the ECON group, which was in accordance with a previous study [21]. Studies reported that administration of *A. muciniphila* orally could alleviate dysbiosis of the gut microbiota and subsequently improve intestinal health in murine [7]. Remarkably, *A. muciniphila* orally did increase the abundance of common probiotics such as *Bifidobacterium* and *Lactobacillus*, which were in consistent with findings in mice [30, 31]. It should be noted that *Lactobacillus reuteri*, one of the common *Lactobacillus* bacteria, can be detrimental in a gnotobiotic setting model of mice with TLR7-dependent lupus [32]. On top of this, we pinpointed that the abundance of some mucolytic bacteria, including *R. torques* and *R.gnavus*, were increased post administration of *A. muciniphila* orally. Although *Ruminococcus spp.* described in patients with inflammatory bowel disease was found to play a pathogenic role by increasing serotonin biosynthesis [33], further studies are needed to describe the cooperative mechanism between mucolytic bacteria represented by *Ruminococcus spp.* and *A. muciniphila* in affecting intestinal barrier integrity. It is also known that *A. muciniphila* could yield short-chain fatty acid (SCFAs) via its mucosal foraging activity, which play a crucial role in promoting microecosystem homeostasis in the gut [7]. In this study, we found that *A. muciniphila* orally had no influence the concentration of SCFAs in digesta; therefore, other outcomes such as host defense peptides levels were investigated to understand the changes in intestinal microecology.

The host is able to affect the microecology of the gut microbiota dwelling in the intestinal tract through multiple mechanisms, including the secretion of host defense peptides (HDPs) and immunoglobulins [34]. It was reported that both live and pasteurized *A. muciniphila* resulted out an increased the secretion of intestinal antibacterial peptide RegIII lectins (e.g., Reg3β and Reg3γ) in mice against *Salmonella* infection [35]. Similarly, this study demonstrated that increased expression of porcine β-defensins in the absence of DSS challenge is dependent of the *A. muciniphila* orally. In contrast, *A. muciniphila* orally aggravated the reduction in the intestinal mRNA level of *PBD114* for pigs receiving DSS challenge. In addition, the levels of blood neutrophilic granulocyte and serum IgM was increased in administration of *A. muciniphila* orally pigs post DSS challenge, implying an imbalance of intestinal microbiota triggers multiple immune disorders. Hence, a more complex mechanism should be also investigated in relationship to the reported properties of *A. muciniphila*. In particular, an in vitro study has reported the pro-inflammatory potential of *A. muciniphila*, confirming its ability to upregulating the expression of costimulatory molecules surface markers such as MHC-II, CD80 and CD40, but not *ARG1* or CD206 in RAW 264.7 macrophages [36].

The microbiota has mutualistic interactions with the host immune system in the mucus layer, which play a crucial role in the maintenance of the balance between Th17 and Treg cell [37]. Th17 cell trigger the intestinal inflammation through secretion of proinflammatory cytokines such as *IL-17 A*, *IL-17 F*, and *IL-21*, while regulatory T (T_{reg}) cell generate suppressive cytokines (e.g., IL-10 and TGF-β) to maintain intestinal homeostasis [38]. It was initially discovered that *A. muciniphila* contributes to the expansion of T_{reg} cell in mice with multiple sclerosis, thereby elevating expression of IL-10, and also limits the transcription of inflammatory cytokines such as IL-1β and IL-6 [39], likely by the increased production of SCFAs and enhanced expression levels of G protein-coupled receptors (e.g., GPR41 and GPR43) in the intestine [40]. Here, our work has indicated that *A. muciniphila* orally had no impact on the mRNA level of *IL10*, *IL-4*, *IFN-γ*, *IL1β*, *TNF-α*, *Foxp3*, but increased the mRNA level of *IL17A*, *IL17F*, *IL-23*, *RORγt* and *Stat3* in the intestine for pigs receiving DSS challenge, which is in line with previous data in mice [10, 41]. A recent work in mice under conditions of homeostasis has revealed that colonization of the gut by *A. muciniphila* induces a variety of antigen-specific T cell responses, while some of which employ markers consistent with pro-inflammatory T cells (e.g., $\gamma\delta T_H1$, $\gamma\delta T_H17$) [42]. Similarly, our study supports the hypothesis that *A. muciniphila*-specific immune responses are intestinal context and physiological state dependent.

Conclusions

In summary, our results showed that administration of *A. muciniphila* orally impairs the health status and intestinal homeostasis, and aggravates inflammatory symptoms

of DSS-challenged weaned pigs. More importantly, our study supports the emerging evidence that intestinal context is critical in driving a symbiotic bacterium to change their role and turn into harmful bacterium [9, 10, 13, 21]. Because the mechanisms of IL17-class cytokines caused by the synergistic effect of *A. muciniphila* and DSS are still not fully understood, future research will identify how the immune subsets respond to the colonization of *A. muciniphila* during the inflammatory conditions, which may open new avenues for developing protective strategies to maintain intestinal health and well-being.

Materials and methods

Bacterial strains

The homo sapiens *A. muciniphila* (DSM 22959), kindly provided by the Professor Li Liu from Nanjing Agricultural university, was cultured anaerobically in a brain heart infusion medium (BHI) at 37℃ as previously described [43]. The preparation process of *A. muciniphila* used in animal trial as follows: Briefly, *A. muciniphila* was grown to mid-log phase in fresh BHI medium (pH 7.4 ± 0.2) at 37 °C with shaking. The medium containing *A. muciniphila* (10^8 copies/mL) was collected in a 50 mL centrifuge rube, and centrifuged at 10,000 × g for 5 min to remove media. Subsequently, the pellet containing *A. muciniphila* cells were rinsed with 0.01 M phosphate-buffered saline (PBS) twice, and then suspended into 0.5 mL BHI medium to obtain the concentrated concentration of *A. muciniphila* (1.0×10^{10} copies/mL). During the trial, we administered 1.0×10^{11} copies/pig per day. Before administration, *A. muciniphila* were diluted with heated BHI medium (37℃) to a concentration of 1.0×10^{11} copies/10 mL.

Animal and experimental procedure

The experiment was conducted using 24 healthy DLY (Duroc × Landrace × Yorkshire) castrated and weaned piglets (initial body weight of 7.66 ± 0.12 kg) at 28 d of age. The piglets were housed in weaning rooms where the temperature and humidity were controlled at 26 °C ± 1.5 °C and 65% ± 5%, respectively. The piglets individually penned in a metabolism cage (0.7 m × 1.5 m × 1.5 m) were provided with fresh feed and water *ad libitum* throughout the trial. The piglets were used for a 2 × 2 factorial completely randomized design, and were randomly allocated to four groups of six subjects each, balanced for litter and body weight (BW). The adaptation period was 5 days, and the trial period was 16 days in 2 stages, consisting of a gavage stage (d 0 to 8; at 34–42 d of age) and a challenge period (d 9 to 15; 43–49 d of age). For the entire experimental period, all piglets received a same stand basal diet (Supplementary Table 1), which was formulated to meet or exceed National Research Council 2012 (NRC) swine nutrient requirements. The piglets were weighed on day 0, 8, 9 and 15 after 12-h fasting in the morning at 8:30, meanwhile the daily feed intake and daily waste feed were carefully recorded. Average daily gain (ADG), average daily feed intake (ADFI), and the feed to gain ratio (F/G) were calculated based on recorded data.

The present study was carried out in compliance with the guidelines for the care and use of laboratory animals (2017 Revision) formulated by the State Council of China. All animal procedure were approved by the Institutional Animal Ethics Committee of Sichuan Agricultural University, Chengdu, China (approval no. SICAU-2023-10). During the trial, all piglets received 10 ml of heated BHI medium every other day with or without the prefixed doses of *A. muciniphila* (1.0×10^{10} copies/mL) directly per os. At 9 d, the piglets in DCON and DA groups were given an oral challenge with a 50 mL sterile saline containing 6% dextran sulphate sodium (DSS, MP Biomedicals, USA) with molecular weight 36,000-50000 for 7 days (once a day), while piglets in CON and CA groups were oral administered equivalent amount of vehicle (i.e., saline) not containing DSS. Clinical signs of disease were observed throughout the challenge period. Meanwhile, each pig's diarrhea score was recorded for on a daily basis post challenge. The score ranged from 0 to 4, with 0∽1 representing normal fences, 2 representing moist feces, 3 representing mild diarrhea, 4 representing severe diarrhea and 5 representing watery diarrhea. The frequency of diarrhea was defined as maintaining fecal scores of 2 or greater for two consecutive days. On day 8, piglets were anesthetized with an intravenous injection of sodium pentobarbital (200 mg/kg BW) for samples collection. Subsequently, blood was collected into a 10 ml blood-collecting vessel via jugular puncture for serum and plasma, and the collection vessels of the latter containing EDTA. The 5 cm segments of mid-duodenum, mid-jejunum and mid-ileum were collected, immobilized in 4% paraformaldehyde solution for follow-up histological observation. Mucosa samples of intestinal tissue were obtained, snap-frozen by liquid nitrogen, and stored at − 80 °C for isolation of RNA and analysis of gene expression as well as related enzyme activities. Meanwhile, colonic and cecal digesta were snap-frozen and stored at − 80 °C for isolation of microbial DNA and analysis of microbial abundance and the concentration of short-chain fatty acid (SCFAs).

Hematology and serum parameter

The total white blood cell (WBC) counts and differentials of blood were measured by using the Exigo Veterinary Hematology System (Boule Diagnostics, Spånga, Sweden) approximately 3 h post slaughter. Blood samples without EDTA as additive were centrifuged at 3500 × g for 20 min, after which the serum was obtained and

stored at − 20 °C for analysis of related parameter. The serum samples were analyzed by an automatic biochemical analyzer (Beckman Coulter Inc., Brea, CA, USA) following the International Federation of Clinical Chemistry methods to provide quantitative determinations for glutamic pyruvic transaminase (ALT), glutamic oxaloacetic transaminase (AST), alkaline phosphatase (ALP), creatinine (CREA), urea (UREA), C-reactive protein (CRP), immunoglobulin G (IgG) and immunoglobulin M (IgM).

Intestinal morphology

Intestinal segment about 3 cm in length, collected from midsection of duodenum, jejunum and ileum, were fixed in 4% paraformaldehyde solution for 24 h, and then excised, dehydrated and embedded in paraffin. Afterwards, cross-sections of 3 μm sections were cut from paraffin-coated intestinal samples with HM 360 Microtome and transferred to 70% ethanol solution for dehydration before staining with hematoxylin and eosin. An Olympus CK40 inverted phase-contrast microscopy was used for the observation of section at a 200 × magnification. For each section, twenty well-orientated and intact villi and their associated crypts were measured with Image-Pro Plus software (MD, US) based on a previous study [44] for the evaluation of intestinal morphology.

Apparent total tract digestibility

The fresh fecal samples were collected from pigs in each group over 3 consecutive days (days 13 to 15). Each fecal samples (100 g) were mixed with 10 mL of a 10% H_2SO_4 solution in a sealed plastic bag, and subsequently stored at − 20 °C until further analysis. Fecal samples were thawed, homogenized thoroughly, dried at 70 °C for 48 h, and then crushed through a 1-mm screen. Proximate analysis including dry matter (DM), crude protein (CP), ether extract (EE), ash, and gross energy (GE) were performed on feces as previously described by the AOAC international [45]. All samples were analyzed for dry matter (DM, Method 930.15; AOAC), crude protein (CP, Method 930.15; AOAC), crude fat (EE, Method 920.39; AOAC), and crude ash (Ash, Method 942.05; AOAC), and gross energy (GE) using an automatic isoperibol oxygen bomb calorimetry (Parr Instrument Company, USA). Chromic oxide in diets and fecal samples was measured according to the methodology outlined given by Fenton (1979) [46].

Intestinal enzyme activities

The enzyme activities of lactase, sucrase, alkaline phosphatase and maltase in intestinal mucosa were determined using the Nanjing Jiancheng Bioengineering assay kit combined with SpectraMax M2 spectrophotometer. Briefly, the mucosa isolated from duodenum, jejunum and ileum were homogenized in a precooled 0.9% saline (1:9, wt/vol). Afterwards, the homogenate was centrifuged at 3,500 × g, 4 °C for 15 min, after which the supernatant samples used for mensuration of enzyme activities were obtained. The enzyme activities determined twice by a spectrophotometer, and one enzyme activity unit was defined as the amount of substrate hydrolysis of 1 mol per minute per milligram of protein tissue homogenate at 37 °C and pH = 6.0.

Intestinal microbiological analysis

The Omega Bio-Tek Stool DNA Kit (Doraville, CA, US) was used to extract microbial genomic DNA from the digesta samples as stated in the manufacturer's protocol. The concentration and purity of microbial genomic DNA was measured with a Nanodrop P330 (Implen). Subsequently, microbial genomic DNA was amplified by quantification real-time PCR on a CFX96 Real-Time PCR system (Bio-Rad Laboratories, Inc., Hercules, CA). For quantitative of total bacteria, *Clostridium*, *A. muciniphila*, *Mucispirillum*, *R. gnavus* and *R. torques*, RT-qPCR was conducted using the SYBR Green as the fluorescent dye. Reaction was carried out at a volume of 25 μL: 12.5 μL SYBR Premix Ex Taq (2×), 1 μL (100 nmol/L) for each primer, 1 μL 50 × ROX reference dye *3, 7.5 μL ddH_2O, and 2 μL template DNA. The related standard curves generated by running real time q-PCR on continuous dilution of templates with known concentration, can be used to estimate absolute quantification from the number of gene copies (Supplementary Fig. 1). Templates used for construction of standard curves were extracted from 1.5% agarose gels with AxyPrep DNA Gel Extraction Kit (Biosharp, China). For quantitative of *Lactobacillus*, *E. coli*, *Bacillus*, and *Bifidobacterium*, which was performed in duplicate by using the SuperReal PreMix (Probe) kit (Tiangen Biotech Co., Ltd., Beijing, China). Reaction was carried out at a volume of 25 μL: 12.5 μL Super Real PreMix (2×), 1 μL of each primer (100 nmol/L), 1 μL probe (100 nmol/L), 1 μL 50 × ROX Reference Dye*3, 6.5 μL of ddH_2O, and 2 μL template DNA. The standard curves of these microorganisms were provided by a previous study [44]. All thermal cycling conditions are as follows: an initial enzyme is activated and denatured at 95 °C for 15 min, follow by 40 cycles of 95 °C for 3 s and 60 °C for 1 min, and the RT-qPCR products are dissociated at 60–95 °C with an increase of 0.5 °C every 1 s. All the primers and probes were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd., and corresponding references are provided in Supplementary Table 2.

Analysis of SCFAs

The concentration of SCFAs in digesta were determined on a gas chromatographic following the instructions described by Cummings et al. [47]. Briefly, thawed digesta (0.5 g) was homogenized in an Eppendorf tube with 2 mL

of Milli-Q H_2O. Afterwards, the mixture was centrifuged at 12,000 × g for 15 min to obtain the supernatant. The 2 mL suspension liquid was combined in a 9:1 ratio with 0.2 mL 25% metaphosphoric acid. After being placed at 4 °C for 30 min, the mixed liquid was centrifuged at 12,000 × g again for 15 min. Finally, supernatant was filtered by 0.45-mm filter membrane to obtain the injection samples (1 μL) for testing. The gas chromatographic system (Agilent Technologies, Santa Clara, CA, USA) equipped with a polyethene glycol packed column (30 m × 0.32 mm internal diameter and 0.25 μm film thickness) was used. The SCFAs levels in digesta were calculated based on the corresponding internal standard.

Gene expression analysis

Total RNA from each snap-frozen tissue (approximately 0.1 g) isolated from duodenum, jejunum, and ileum were prepared with the RNAiso Plus reagent (TaKaRa, Dalian, China). In order to mitigate the interference of DSS contamination in mucosal tissues, RNA precipitation from each sample was purified with lithium chloride (LiCl) as previously described by Sommer et al. [48]. The RNA from the purification treatment was quickly measured using the Nanodrop P330 (Implen), of which OD_{260}:OD_{280} ratios ranged from 1.8 to 2.0 were regarded as suitable for further analysis. Subsequently, total RNA (1.0 μg) reverse transcription into complementary DNA (cDNA) using a FastQuant RT kit as stated in the manufacturer's instructions. All primers sequences designed with Primer 5.0 software were presented in Supplementary Table 3. Quantification real-time PCR reaction was performed using 5 μL SYBR Green ReadyMix (1×), 0.2 μL ROX Reference Dye II (50×), 0.4 μL of each primer, 3 μL double-distilled H_2O and 1 μL complementary DNA in a total of 10 μL reaction volume on a CFX96 Real-Time PCR Detection System. The procedures used in RT-qPCR involve several steps: The initial pre-denaturation procedure started at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 34 s. The relative expression of each target gene mRNA was standardized by the housekeeping gene GAPDH mRNA abundance, which were calculated according to the $2^{-\Delta\Delta Ct}$ method [49].

Statistical analysis

All data were showed as means ± standard error of mean (SEM) and analyzed using SAS statistical software (version 9.4; SAS Inst. Inc., USA). Growth performance and diarrhea score of piglets before DSS challenge was analyzed with a one-way ANOVA. Post the challenge stage, the data were analyzed by the 2-way ANOVA test followed by Tukey's multiple-range comparisons. The statistical model $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i \times \beta_j + \varepsilon_{ijk}$, where μ was the mean, α_i was the effect of administration of *A. muciniphila*, β_j was the effect of DSS challenge, $\alpha_i \times \beta_j$ was the interaction between administration of *A. muciniphila* and DSS challenge, and ε_{ijk} was the residual effect. For analysis of intestinal microbiota, relative abundance levels were transformed (log10) before the statistical analysis. Significant differences were set at $P \le 0.05$, whereas $0.05 < P < 0.10$ indicating a trend.

Abbreviations

ADFI	Average daily feed intake
ADG	Average daily gain
F-G	Feed-Gain ratio
ALT	Glutamic pyruvic transaminase
AST	Glutamic oxaloacetic transaminase
ALP	Alkaline phosphatase
CREA	Creatinine
CRP	C-reactive protein
VCR	Villous height: crypt depth ratio
DM	Dry matter
CP	Crude protein
EE	Ether extract
IAP	Intestinal alkaline phosphatase
WBC	White blood cell
RBC	Red blood cell
HGB	Hemoglobin
HCT	Hematocrit
MCV	Mean corpuscular volume
MCHC	Mean corpuscular hemoglobin concentration
RDW-SD	Red cell distribution width standard deviation
RDW-CV	Red Blood Cell Distribution Width Coefficient of Variation
PLT	Blood platelet count
PCT	Plateletcrit
MPV	Mean platelet volume
PDW	Platelet volume distribution width
IMG	Immature Granulocytes
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MUC1	Mucin 1
MUC2	Mucin 2
ZO1	Zonula occludens-1
ZO2	Zonula occludens-2
SGLT1	Sodium-glucose cotransporter-1
GLUT2	Glucose transporter 2
DMT1	Divalent metal ion transporter 1
ZnT1	Zinc transporter 1
CAT-1	Cationic amino acid transporter 1
PePT1	Solute carrier family 15 Member 1
PBD1	Porcine β-defensin 1
PBD3	Porcine β-defensin 3
PBD114	Porcine β-defensin 114
PBD129	Porcine β-defensin 129
IL1β	Interleukin-1β
IL4	Interleukin-4
IL6	Interleukin-6
IL10	Interleukin-10
IL17A	Interleukin-17 A
IL17F	Interleukin-17 F
IL23	Interleukin-23
TNFα	Tumor necrosis factor-α
IFNγ	Interferon-gamma
RORγt	Retinoic acid receptor-related orphan receptor gamma t
STAT3	Signal transducer and activator of transcription 3

Supplementary Information

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Supplementary Material 1

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Author contributions
J. H. and L.L. conceived the experiment. K.X., W.C. and L.L carried out the experiment. W.C. and L.L. performed statistical analysis. K.X. wrote the main manuscript original text. B.Y., Z.H., X.M., J.Y., P.Z., H.Y. and H.L. contributed to the final version of the manuscript. All the authors read and approved the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All animal procedure were approved by the Committee on Animal Care Advisory of Sichuan Agricultural University, Chengdu, China (approval no. SICAU-2023-10). The present research was carried out in compliance with the guidelines for the care and use of laboratory animals (2017 Revision) formulated by the State Council of China.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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