

# Embelin alleviates weaned piglets intestinal inflammation and barrier dysfunction via PCAF/NF- $\kappa$ B signaling pathway in intestinal epithelial cells.

**Weilei Yao**

Huazhong Agriculture University: Huazhong Agricultural University

**Tongxin Wang**

Huazhong Agriculture University: Huazhong Agricultural University

**Lu Huang**

Huazhong Agriculture University: Huazhong Agricultural University

**Zhengxi Bao**

Huazhong Agriculture University: Huazhong Agricultural University

**Shu Wen**

Huazhong Agriculture University: Huazhong Agricultural University

**Feiruo Huang** (✉ [huangfeiruo@mail.hzau.edu.cn](mailto:huangfeiruo@mail.hzau.edu.cn))

Huazhong Agriculture University <https://orcid.org/0000-0001-5404-8091>

---

## Research Article

**Keywords:** Intestinal barrier, Inflammation, PCAF, acetylation, Weaned pig

**Posted Date:** May 17th, 2022

**DOI:** <https://doi.org/10.21203/rs.3.rs-1643633/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

# Abstract

**Background:** Intestinal barrier plays key roles in maintaining intestinal homeostasis. Inflammation damage can severely destroy the intestinal integrity of mammals. This study was conducted to investigate the protective effects of embelin and its molecular mechanisms on intestinal inflammation in a porcine model. One hundred sixty 21 day-old castrated weaned pigs (Duroc×Landrace×Yorkshire, average initial body weight was  $7.05 \pm 0.28$  kg, equal numbers of castrated males and females) were allotted to four groups and fed with a basal diet or a basal diet containing 200, 400, or 600 mg embelin/kg for 28 days, the growth performance, intestinal inflammatory cytokines, morphology of jejunum and ileum, tight junctions in the intestinal mucosa of piglets were tested. IPEC-1 cells with overexpression of P300/CBP associating factor (PCAF) were treated with embelin, the activity of PCAF and acetylation of nuclear factor- $\kappa$ B (NF- $\kappa$ B) were analyzed to determine the effect of embelin on PCAF/NF- $\kappa$ B pathway in vitro.

**Results:** The results showed that embelin decreased ( $P < 0.05$ ) serum D-lactate and diamine oxidase (DAO) levels, and enhanced the expression of ZO-1, Occludin and claudin-1 protein in jejunum and ileum. Moreover, the expression levels of critical inflammation molecules (interleukin-1 $\beta$ , interleukin-6, tumor necrosis factor- $\alpha$ , and NF- $\kappa$ B) were down-regulated ( $P < 0.05$ ) by embelin in jejunal and ileal mucosa. Meanwhile, the activity of PCAF were down-regulated ( $P < 0.05$ ) by embelin. Importantly, transfection of PCAF siRNAs to IPEC-1 cell decreased NF- $\kappa$ B activities; embelin treatment downregulated ( $P < 0.05$ ) the acetylation and activities of NF- $\kappa$ B by 31.7–74.6% in IPEC-1 cells with overexpression of PCAF.

**Conclusions:** These results suggested that embelin ameliorates intestinal inflammation in weaned pigs, which might be mediated by suppressing the PCAF/NF- $\kappa$ B signaling pathway.

## 1. Introduction

Breast feeding and weaning are important physiologically significant luminal events that influence the growth of the small intestine in humans [1]. Previous study indicated that breast fed infants have smaller villi and crypts than bottle fed infants, suggesting that crypt fission may be the predominant mechanism of epithelial growth during milk feeding whereas crypt hyperplasia predominates later during weaning (2–6 months of age). As known that weaning process is commonly associated with intestinal inflammation and barrier dysfunction, and this phenomenon is particularly evident in piglets, which are responsible for the stunted growth and diarrhoea observed in the first 2 weeks after weaning [2, 3], therefore, weaned piglets are regarded as an important laboratory animal for studying the intestinal health of infants. Intestinal barrier is mainly formed by a single layer of enterocytes and intercellular tight junctions of enterocytes [4]. It serves as a selective permeable membrane which not only permits the absorption of nutrients from the intestinal lumen into circulation but also provides an innate defense barrier to resist the passage of luminal pathogens and toxins into systemic circulation. But many stimuli factors including infection, inflammation can damage intestinal barrier function by inducing overproduction of pro-

inflammatory cytokines [5, 6]. Therefore, inhibition of intestinal inflammation during the weaning stage may play an important role in alleviating intestinal damage in infants.

The transition of weaned piglets from breast milk to solid feed will cause intestinal inflammation, and then lead to diarrhea, growth performance decline, and even death of piglets, and finally bring huge economic losses to animal husbandry [7]. Therefore, the use of active ingredients in weaned piglets can alleviate of intestinal inflammation, prevent or slow down diarrhea, improve growth performance and promote the development of antibiotics alternative feed additives. Numerous studies showed that the expression of inflammatory factor signaling pathways was elevated in the intestine of weaned piglets [8, 9]. Among them, the inhibition of the activity of NF- $\kappa$ B, the initiator of the inflammatory pathway, is considered to be the most effective way to alleviate intestinal inflammation [10]. Moreover, PCAF has been reported to promote the inflammatory signaling pathways by acetylating the NF-Kb [11]. Many studies have pointed out that plants and their extracts play an important role in promoting animal growth, enhancing immunity and maintaining animal health [12–14]. As another excellent anti-inflammatory plant extract, embelin, the major component of *Embelia ribes* Burm plant (specifically, its fruit), has been considered to be an acetylation inhibitor of PCAF [15]. Due to these efficacies, embelin may be beneficial in improving the intestinal inflammation, growth performance, and diarrhea of weaning pigs.

Currently, most of the studies of embelin have focused on the anti-inflammatory and antioxidant activities *in vitro* studies or some *in vivo* studies [16, 17]. It has long been reported that weaning stress can damage the structure and function of the gut of an animal [18]. However, the beneficial effects of embelin on the alleviation of the intestinal inflammation and gut barrier as well as the improvement of diarrhea in weaned pigs have not been reported. The purpose of this study was to investigate the effects of embelin on the diarrhea, intestinal barrier function and inflammation of weaned piglets, thus providing a theoretical basis and experimental evidence for understanding the biological effects of embelin and its potential as a functional feed additive.

## 2. Material And Methods

### 2.1 Animals and experimental design

All animal protocols used in this study were in accordance with the Guidelines for the Care and Use of Animals for Research and Teaching and approved by the Animal Care and Use Committee of Huazhong Agricultural University.

A total of 160 weaned pigs (Duroc×Landrace×Yorkshire) were randomly assigned to 4 dietary treatments with 4 replicate pens per treatment. Their initial body weight (BW) was  $7.05 \pm 0.28$  kg. Control pigs (CON) were fed a basal diet, and the other animals were fed a basal diet supplemented with 200mg/kg, 400mg or 600mg/kg embelin (E200, E400, E600) for 2 weeks. The median lethal dose of embelin is reported to be 2000 mg/kg body weight in mice and rats without any mortality or adverse effects indicating its safety [19]. The basal diets were formulated to meet the NRC (2012) specifications for weaned pigs [20]. And the

ingredients and composition of the basal diet are listed in Supplemental Table 1. The embelin was purchased from Xin Lu Biotechnology Company (Xi'an, China), and extracted from dried fruit rind of *Embelia ribes* with a purity of 98.1%, as measured by HPLC. All pigs were allowed ad libitum access to feed and water throughout the experimental period. The animals were kept in a temperature-controlled environment at 24 ~ 25°C and alternating light and dark cycles with 12h intervals. All experimental pigs were healthy during the feeding period. Weights were obtained on every pig and feed disappearance was recorded on d 0, 7, 14 and prior to slaughter to calculate average daily feed intake (ADG), average daily gain (ADFI), and feed/gain ratio (F/G).

## 2.2 Sample collections

At the end of the trial, blood samples were collected by venepuncture at 08.00hours after 12h of fasting. Then, the samples were centrifuged at 3500g at 4°C for 10min. After centrifugation, the serum samples were collected and frozen at - 20°C until analysed. After blood collection, pigs were euthanised with an intravenous injection of chlorpromazine hydrochloride (3mg/kg body weight) and then slaughtered by exsanguination protocols. Immediately, a piece (2cm-length) of the middle jejunum and distal ileum (2cm-length) were collected, rinsed with cold saline (NaCl 9 g/L, 4°C), and fixed in paraformaldehyde. Other segments of the middle jejunum and distal ileum (5 cm-length) of the piglets were opened and thoroughly rinsed with sterile normal saline, and then the mucosa was collected by scraping with glass slides and immediately snap- frozen in liquid nitrogen and stored at - 80°C. Fecal scores were monitored during the feeding trial and quantified using a scale ranging from 0 to 3 with 0 = normally shaped feces, 1 = shapeless (loose) feces, 2 = thick, liquid (soft) feces, and 3 = thin, liquid feces and watery diarrhea. A piglet with a score greater than 1 was regarded as having diarrhea. The incidence of diarrhea (%) was calculated as a percentage of the number of diarrheal piglets during the period divided by the total number of piglets.

## 2.3 Intestinal cytokines determined by ELISA

After grinding in liquid nitrogen, total protein was extracted from the jejunal and ileal mucosa samples with lysis buffer (KeyGEN, Nanjing, China), followed by clarification by centrifugation (3500×g for 10 min at 4°C). The concentrations of IL-1β, IL-6, IL-10, and tumor necrosis factor-α (TNF-α) were determined by using ELISA kits (Nanjing Jiancheng Bioengineering company, Jiangsu, China).

## 2.4 Analysis of intestinal morphology and permeability

Histological examination of the intestinal morphology was conducted [17]. Briefly, after a 24h fixing, the intestinal segments were dehydrated in ethanol concentration gradient and cleared up with xylene, embedded in paraffin, cut incross sections with a thickness of approximately 5μm with a microtome and then stained with haematoxylin and eosin. Villous height and crypt depth of two intestinal segments were measured and villous height/crypt depth ratio (VCR) was calculated. Immunofluorescence analysis. Intestinal mucosa permeability is the principal basis for the diagnosis of the intestinal barrier dysfunction [21]. Serum diamine oxidase (DAO) and D-lactate and endothelin - 1 (ET-1) and nitric oxide (NO) can be used as indicators of intestinal integrity of the gastrointestinal tract [22]. The contents of DAO and D-

lactate and ET-1 and NO of serum were measured using an instrument (Biochemical Analytical Instrument, Beckman CX4, Beckman Coulter Inc., Brea, CA).

## 2.5 Cell culture and treatment

Intestinal porcine epithelial cells (IPEC-1) were isolated from the jejunum of unsuckled newborn pigs, as previously described [23]. The cells were grown in serial passage in uncoated plastic cell culture flasks (75 cm<sup>2</sup>) with a vent cap in DMEM-F12 containing 17.5 mM D-glucose, 15 mM HEPES (pH 7.4), 5% FBS, epidermal growth factor (5 µg/L), insulin (5 µg/ml), transferrin (5 µg/ml), selenium (5 ng/ml), penicillin (50 µg/ml), streptomycin (4 µg/ml) and 0.25 µg/ml amphotericin B (Fungizone). Medium was changed every 2 days. All cell cultures were carried out at 37°C in a 5% CO<sub>2</sub> incubator. At confluence, cells were passaged using trypsinization [24]. Cells in 6-well plates were pre-treated with embelin (2.5 and 5 µM) for 2 h and incubated further for 48h. Besides, cells were transfected by Lipofectamine 2000 (Invitrogen) with a mixture of small interfering RNA (siRNA) directed isolation of cells and cell culture towards PCAF (Qiagen) for 4h (5'-GCAGATACCAAA-CAAGTTT-3'). To confirm PCAF knock-down, Western blot was used to analyse PCAF expression.

## 2.6 Total RNA isolation and reverse transcription

Total RNA was isolated from frozen jejunal or ileal samples using TRIzol (Takara Biotechnology Co. Ltd). All the procedures were guided by the manufacturer's manual. Briefly, 100mg tissues were put into a mortar and grinded with 1ml TRIzol reagent. The proteins in the grinded samples were precipitated by chloroform. After centrifugation, the supernatant was transferred into a new tube and isopropanol was added and mixed for 10min. The total RNA has settled by centrifugation. The integrity of RNA was checked by electrophoresis on a 1.5% agarose gel, and the concentration and quality were verified by UV spectrophotometry using a NanoDrop 2000 (Thermo Fisher Scientific, Inc.). After RNA isolation, 1 µg of total RNA was reverse-transcribed into cDNA using a PrimeScript™ RT reagent reverse-transcribed into cDNA using a PrimeScript™ RT reagent kit with cDNA Eraser (Takara Biotechnology Co. Ltd). The following conditions were used: 42°C for 2min, then 37°C for 15min, followed by 85°C for 5s.

Real-time quantitative PCR was performed in an Option Monitor 3 Real-Time PCR Detection System (Bio-Rad) using the SYBR Green Supermix (TaKaRa). Expression levels of β-actin (housekeeper genes), TNF-α, IL-6, IL-10, IL-1β, ZO-1, Occludin and Claudin-1 in the small intestinal were analysed using SYBR Premix Ex Taq II (Tli RNaseH Plus) reagents (TakaRa) and the QuanStudio 6 Flex Real-Time PCR detection system (Applied Biosystems). All primers were commercially synthesised and purified by Sangon Biotech Co. Ltd and are shown in Supplemental Table 2. The reaction was performed in a volume of 10 µl consisting of 5 µl of SYBR Premix ExTaq (2×), 1 µl of reverse primers, 1 µl of forward primers, 2 µl of doubled-distilled water and 1 µl of cDNA template. Cycling conditions were as follows: 5°C for 30s, followed by forty cycles at 95°C for 5s, 60°C for 34s, under melt curve conditions at 95°C for 15s, 60°C for 1min and then 95°C for 15s (temperature change velocity 0.5°C/s). The target gene mRNA expression level was calculated using the 2<sup>-ΔΔCt</sup> method [25]. Each sample was repeated in triplicate.

## 2.7 Western blotting and immunoprecipitation

Western blot analyses were performed as described previously [26]. For the analyses, the major antibodies, including ZO-1, Occludin, Claudin-1, NFκB and β-actin (ACTB), are submitted in online Supplementary Table 3. The mucosal scrapings were homogenized on ice in RIPA lysis buffer (Upstate; Temecula, CA) containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO) and phosphatase inhibitor cocktail 1 (Sigma-Aldrich, St. Louis, MO). After centrifugation at 4°C and 14,000×g, the supernatants were collected for the assay. Detection of concentrations of protein in the ileum was performed by the bicinchoninic acid assay. Western blotting was performed for acetylation detection. For blocking, 50m M Tris (pH 7.5) with 1% peptone (AMRESCO) and 10% (v/v) Tween-20 were used. For the primary and secondary antibodies, 50m M Tris (pH 7.5) with 0.1% peptone was prepared [27].

## 2.8 Statistical analysis

All the results from experiment were analyzed by using the one-way ANOVA, performed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, US). Replicate (n = 10) served as the experimental unit for animal experiment, and replicate (n = 3) served as the experimental unit for cell experiment. Data were analyzed using the general linear model (GLM) with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The results in the tables are shown with the means ± SEM, and other figure results are presented with means ± SD. Means are considered significantly different at  $P < 0.05$ .

## 3. Results

### 3.1 Growth Performance of Weaned Piglets

The effects of embelin on the growth performance of weaned piglets were shown in Table 1. Piglets dietary administered embelin increased the final weight and ADG ( $P < 0.05$ ), and reduced the feeding efficiency ( $P < 0.05$ ), and had no significant effect on ADFI ( $P = 0.49$ ). In addition, dietary embelin significantly decreased the diarrhea rate compared with that in control group ( $P < 0.05$ ). Piglets dietary administered embelin reduced the diarrhea rate as much as 45%. The F/G and diarrhea rates in the E400 and E600 embelin groups were significantly lower than those in the E200 group.

Table 1  
Effects of embelin on growth performance and diarrhea rate of weaned piglets<sup>1</sup>

Item	CON	E200	E400	E600	P Value
Initial weight,kg	6.78 ± 0.23	6.76 ± 0.18	6.77 ± 0.19	6.74 ± 0.21	0.36
Final weight,kg	12.9 ± 1.16 <sup>b</sup>	13.3 ± 1.45 <sup>b</sup>	13.8 ± 1.08 <sup>a</sup>	13.9 ± 1.13 <sup>a</sup>	< 0.05
ADG/(g/d)	219 ± 12.4 <sup>b</sup>	234 ± 14.3 <sup>b</sup>	251 ± 12.2 <sup>a</sup>	257 ± 13.5 <sup>a</sup>	< 0.05
ADFI/(g/d)	410 ± 9.06	418 ± 11.3	414 ± 10.5	418 ± 9.78	0.49
F/G	1.83 ± 0.09 <sup>a</sup>	1.78 ± 0.11 <sup>b</sup>	1.65 ± 0.08 <sup>c</sup>	1.62 ± 0.09 <sup>c</sup>	< 0.05
Diarrhea incidence	9.56 ± 1.02 <sup>a</sup>	7.14 ± 0.09 <sup>b</sup>	6.49 ± 1.01 <sup>c</sup>	5.24 ± 1.01 <sup>c</sup>	< 0.05

<sup>1</sup>Values are means ± SEMs, n = 10/group. Labeled means in a row without a common superscript letter differ, *P* < 0.05. ADFI: Average daily feed intake; ADG: Average daily gain; F/G: gain/feed for feed efficiency.

## 3.2 Intestinal inflammatory cytokines of Weaned Piglets

As shown in Fig. 1, dietary supplementation of embelin increased expression of jejunum and ileum IL-10 (*P* < 0.05, Fig. 1A, F), where E400 and E600 embelin groups were significantly higher relative to the control and E200 groups. Piglets dietary administered embelin had significant reduced the expression IL-6, IL-1β, TNF-α in jejunum and ileum (*P* < 0.05, Fig. 1B-D, G-I). Relative to the control group, E400 and E600 embelin group significantly reduced jejunum and ileum NF-κB expression (*P* < 0.05, Fig. 1E, J).

## 3.3 Morphology and permeability of jejunum and ileum in Weaned Piglets

As shown in Table 2, diets supplemented with E400 and E600 of embelin significantly increased the villus height in the jejunum (*P* < 0.05). Although there was no significant effect on the villus height in ileum (*P* = 0.46). All three dose addition groups had no significant effect on jejunal (*P* = 0.35) and ileal crypt depths (*P* = 0.38). Both E400 and E600 embelin significantly improved the villus height to crypt depth ratios relative to the control and E200 embelin groups (*P* < 0.05). For intestinal permeability (Table 3), compared to the control group, dietary embelin significantly decreased the diamine oxidase (DAO) and D-lactate contents (*P* < 0.05). The DAO and D-lactate contents in the groups treated with E200, E400 and E600 embelin were significantly lower than those in the control groups (*P* < 0.05). The addition of embelin had no significant effect on the content of both ET-1 (*P* = 0.37) and NO (*P* = 0.45).

Table 2  
Small intestinal morphology of weaned piglets fed embelin<sup>1</sup>

Item	CON	E200	E400	E600	P Value
Villus height(μm)					
Jejunum	311 ± 7.56 <sup>b</sup>	322 ± 5.26 <sup>b</sup>	358 ± 6.32 <sup>a</sup>	360 ± 8.42 <sup>a</sup>	< 0.05
Ileum	295 ± 9.45	306 ± 6.39	318 ± 7.86	314 ± 9.45	0.46
Crypt depth(μm)					
Jejunum	220 ± 7.56	215 ± 6.28	203 ± 8.56	208 ± 7.43	0.35
Ileum	224 ± 6.39	220 ± 7.59	212 ± 8.46	208 ± 9.25	0.38
Villus height: crypt depth					
Jejunum	1.41 ± 0.16 <sup>b</sup>	1.49 ± 0.12 <sup>b</sup>	1.76 ± 0.09 <sup>a</sup>	1.73 ± 0.11 <sup>a</sup>	< 0.05
Ileum	1.32 ± 0.14 <sup>b</sup>	1.39 ± 0.12 <sup>b</sup>	1.50 ± 0.07 <sup>a</sup>	1.51 ± 0.08 <sup>a</sup>	< 0.05
<sup>1</sup> Values are means ± SEMs, n = 10/group. Labeled means in a row without a common superscript letter differ, <i>P</i> < 0.05.					

Table 3  
Effects of embelin on intestinal permeability in piglets<sup>1</sup>

Item	CON	E200	E400	E600	P Value
DAO, ng/mL	25.3 ± 3.24 <sup>a</sup>	11.3 ± 2.27 <sup>b</sup>	10.6 ± 1.56 <sup>b</sup>	9.51 ± 1.84 <sup>b</sup>	< 0.05
D-Lactate, μmol/mL	45.9 ± 3.16 <sup>a</sup>	35.6 ± 3.04 <sup>b</sup>	32.5 ± 2.96 <sup>b</sup>	21.9 ± 2.17 <sup>c</sup>	< 0.05
ET-1, ng/L	141 ± 10.6	136 ± 8.15	132 ± 8.96	128 ± 9.47	0.37
NO, μmol/L	60.3 ± 3.16	58.4 ± 2.05	57.6 ± 2.28	54.4 ± 2.37	0.45
<sup>1</sup> Values are means ± SEMs, n = 10/group. Labeled means in a row without a common superscript letter differ, <i>P</i> < 0.05. DAO: Diamine oxidase; ET-1: Endothelin-1;					

### 3.4 Tight junctions in the intestinal mucosa of Weaned Piglets

As shown in Fig. 2, for tight junction-related components, compared with the control group, dietary embelin significantly increased (*P* < 0.05) ZO-1 and claudin-1 protein expression in both the jejunal and ileal mucosa and occludin in the ileum (Fig. 2A-B, E-F). There was no significant effect for jejunal occludin protein levels (*P* > 0.05, Fig. 2C). The expression of ZO-1 in both jejunal and ileal mucosa of the E400 and E600 embelin groups was significantly higher than those in the control and E200 groups (*P* <

0.05). In addition, the expression of jejunal claudin-1 and ileal occludin of E600 embelin groups was significantly higher than that in the other groups ( $P < 0.05$ ).

### 3.5 Dietary embelin decreases the PCAF activity and acetylation of NF- $\kappa$ B and in weaned piglets

Addition of E600 embelin significantly reduced PCAF and NF- $\kappa$ B activity in the jejunum compare to the other three groups ( $P < 0.05$ , Fig. 3A-B, D-E). The activity of PCAF and NF- $\kappa$ B in the ileum of E400 and E600 embelin groups was significantly lower than that in the control and E200 embelin groups ( $P < 0.05$ ). The acetylation level of NF- $\kappa$ B in the E400 and E600 embelin groups was significantly lower ( $P < 0.05$ ) than those of the E200 embelin group (Fig. 3C). Compared with the control group, all dosages of the embelin groups significantly reduced ( $P < 0.05$ ) the NF- $\kappa$ B acetylation levels in ileum (Fig. 3F).

### 3.6 Embelin inhibits the PCAF-dependent acetylation of NF- $\kappa$ B in IPEC-1 cell

All three doses of embelin in IPEC-1 cell significantly reduced PCAF and NF- $\kappa$ B activity (Fig. 4A, B,  $P < 0.05$ ). IPEC-1 cell were transfected with the indicated K (lysine) acetyltransferase (KAT) constructs in combination with expression vectors containing PCAF. As expected, PCAF expression was increased ( $P < 0.05$ ) by 65%, while transfection with siRNAs targeting PCAF in IPEC-1 cell decreased the protein expression by  $\sim 75\%$  ( $P < 0.05$ , Fig. 4C). Both interference with PCAF and addition of embelin significantly reduced NF- $\kappa$ B activity ( $P < 0.05$ ). Overexpression of PCAF significantly increased NF- $\kappa$ B activity, accompanied by increased acetylation levels of NF- $\kappa$ B ( $P < 0.05$ ). Interestingly, only upon stimulation with embelin could we detect a decrease ( $P < 0.05$ ) in the levels of activity and acetylation of NF- $\kappa$ B in IPEC-1 cell overexpressing PCAF (Fig. 4D, E).

## 4. Discussion

Weaning is a major contributor to intestinal epithelial barrier dysfunction, characterized by increased intestinal permeability, leading to the invasion of intestinal pathogens and then triggering intestinal inflammation [28, 29]. Therefore, effective strategy is urgently needed in this critical phase especially in piglets and infants of human beings. Embelin, one of the naturally-occurring benzoquinone compound, possesses various biological effects and has attracted considerable attention as a functional ingredient to alleviate inflammation in animals and human beings. Recently, numerous studies have focused on improving the gut health of animals through dietary supplementation with antioxidative and antibacterial nutrients, such as resveratrol, oregano oil, and selenium [30–32]. Previous studies *in vitro* showed that embelin has potential anti-inflammatory effects, furthermore, embelin is regarded as an extremely potent natural inhibitor of p300/CBP - associating factor (PCAF), which has been observed to activate the inflammatory pathway through acetylation of the NF- $\kappa$ B. Therefore, embelin may have a predictable potential ability to attenuate the intestinal inflammation of weaned animals, especially in piglets, which

has been proved to be a suitable animal model for human infants [33]. However, the effect and dose effect of dietary embelin on growth performance, diarrhea, intestinal barrier function and inflammation in weaned piglets are poorly understood. In this work, we demonstrated for the first time that as a natural ingredients, embelin efficiently improved growth performance and diarrhea, ameliorated intestinal barrier dysfunction and inflammation of weaning piglets.

First, the effect of dietary embelin on growth performance and diarrhea was measured. The median lethal dose of embelin is reported to be 2000 mg/kg body weight in mice and rats without any mortality or adverse effects indicating its safety [19]. So we chose 200, 400 and 600 mg/kg embelin as the additive dosage. At the beginning of the study, we collected blood samples and verified that there were no differences between piglets and that they were healthy at the beginning of the study. However, after a 28-day period trial, the control group had a higher incidence of diarrhea, which may be related to weaning events. Dietary embelin increased ADG and decreased F/G compared to the control group, indicating improved growth performance. Moreover, dietary embelin significantly reduced the rate of diarrhea in weaned piglets. Previous studies have shown that body weight gain and nutrient absorption as well as diarrhea rates in piglets are related to oxidative stability, intestinal morphology and intestinal inflammation [34], then we tested the intestinal barrier function of weaned piglets after treating with embelin.

The integrity of the intestinal epithelial barrier acts a key role in the digestion and absorption of nutrients and the inhibition of invasion by pathogenic bacteria [35, 36]. However, numerous mammalian studies have shown that weaning stress can severely disrupt small intestinal barrier function and exacerbate intestinal inflammation within a short period of time after weaning [37–39]. It is well known that intestinal permeability is usually assessed by some blood indicators. D-lactic acid is a specific end product of intestinal bacteria and is released into the blood when the intestinal mucosa is damaged. DAO is an intracellular enzyme synthesized mainly by mammalian intestinal epithelial cells and is mainly found in the cytoplasm. Once the intestinal epithelial barrier is damaged, intestinal DAO is released into the circulation [40]. Therefore, blood D-lactic acid concentration and DAO activity can be used as circulating markers to monitor the extent of intestinal barrier damage. In the present study, the addition of embelin to the diet reduced serum D-lactic acid concentration and DAO activity in weaned piglets, suggesting that embelin has a beneficial effect on reducing intestinal permeability in weaned piglets. Furthermore, tight junctions between intestinal epithelial cells maintain and regulate the intestinal epithelial barrier [41]. The formation of tight junctions requires several unique proteins, such as the claudin family, occludin, and intracellular linker proteins, such as ZOs, which anchor directly or indirectly to the actin-based cytoskeleton and then form a selective permeability barrier [42]. Thus, the expression level and distribution of tight junction proteins are closely related to intestinal barrier function. However, weaning stress can disrupt intestinal tight junctions by down-regulating the expression of intestinal barrier-related genes in pigs [43, 44]. The results of this study showed that the addition of embelin to the diet significantly upregulated the expression of claudin-1, occludin and ZO-1 in the small intestine of weaned piglets. Thus, embelin can improve intestinal barrier function by maintaining the expression of

tight junction proteins, which may be partly responsible for embelin's ability to reduce intestinal permeability and inflammatory response to weaning stress injury in piglets.

Accumulating evidence has confirmed that inflammation is an important inducing factor in intestinal barrier disruption [45, 46]. Some cytokines are thought to be critical in the predisposition to and exacerbation of some gastrointestinal dysfunctions. NF- $\kappa$ B is a central mediator of intestinal inflammatory diseases [47] and has been shown to play a role in the control of intestinal permeability [48]. Moreover, pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6 have been shown to exert negative roles in maintaining the tight junction and cytoskeleton structure and function. In the present study, embelin feed inhibited NF- $\kappa$ B activity and the pro-inflammatory factors TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, which is consistent with previous studies. Evidence to date demonstrates that the functions of NF- $\kappa$ B are modulated by diverse post-transcriptional modifications. Among these modifications, acetylation has been shown to enhance the nuclear localization of NF- $\kappa$ B and lead to the transcription of NF- $\kappa$ B target genes [49]. NF- $\kappa$ B is mainly acetylated by PCAF. PCAF acetylates multiple lysine residues of NF- $\kappa$ B, including Lys-122, -123, -218, -221 and -310, activating its transcriptional activity [50]. In the present study, dietary supplementation with embelin at 3 dosages decreased PCAF activity, suggesting that embelin may attenuate NF- $\kappa$ B activity by inhibiting the acetylation activity of PCAF.

To further explore the molecular mechanism of embelin in attenuating intestinal inflammatory response, we investigated the effects of embelin on the activation of PCAF/NF- $\kappa$ B signaling pathway which has been considered as a key inducer of inflammation. It was reported that embelin rapidly inactivates PCAF activity and has a strong apoptosis-inducing effect on leukemia cell lines and on prostate and pancreatic cancer cells through the inhibition of DNA binding [51]. In our study, embelin decreased PCAF activity and NF- $\kappa$ B acetylation suggesting that embelin-induced decreased PCAF activity may reduce inflammatory cytokines in weaned piglets. Furthermore, the upregulation of PCAF and NF- $\kappa$ B in weaned piglets was reversed by embelin treatment, so we propose that the PCAF-NF- $\kappa$ B pathway may be involved in the embelin-mediated alleviation the inflammatory response after weaning, siRNAs inhibits PCAF in cultured IPEC-1 cell, indicating that nonembelin-mediated inhibition of PCAF has similar effects. Activated PCAF can stimulate the activation of NF- $\kappa$ B signal pathway and then increase the expression of various inflammatory cytokines' genes, including IL-6, TNF- $\alpha$  and IL-1 $\beta$ . Therefore, reducing the inflammatory response mediated by the PCAF/NF- $\kappa$ B signaling pathway may play a protective effect in the intestine of weaned pigs.

In conclusion, the present study indicated for the first time that dietary embelin supplementation exerts beneficial effects on ameliorating the intestinal inflammation in weaned pigs. The mechanisms of action might be closely related to improving tight junction protein expression, suppressing intestinal inflammatory mediators released, alleviating intestinal inflammation via downregulation of acetylation of NF- $\kappa$ B by PCAF.

Nevertheless, there were some limitations in this study. In this experiment, due to the imperfect experimental plan, the optimal concentration of embelin in the piglet diet was not further clarified. In

addition, there may be other potential mechanisms that have not been explored in this study. For example, embelin itself has a certain antibacterial effect, which may be an important factor in improving the gut microbiota and intestinal health. Future experiments could focus on evaluating the anti-inflammatory and antibacterial properties of embelin, and further clarification of its role in piglet diet production and human infant health.

## Abbreviations

ADFI

Average daily feed intake

ADG

Average daily gain

DAO

Diamine oxidase

ET-1

Endothelin-1

F/G

Feed/gain ratio

IL-1 $\beta$

interleukin-1 $\beta$

IL-6

interleukin-6

IL-10

interleukin-10

NF- $\kappa$ B

nuclear factor- $\kappa$ B

TNF- $\alpha$

tumor necrosis factor- $\alpha$

ZO1

Zonula occludens protein

PCAF, P300/CBP associating factor.

## Declarations

### **Ethics approval and consent to participate:**

All animal protocols used in this study were in accordance with the Guidelines for the Care and Use of Animals for Research and Teaching and approved by the Animal Care and Use Committee of Huazhong Agricultural University.

### **Consent for publication**

All authors read and approved the final manuscript.

### **Availability of data and material**

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

WY, TW, LH, ZB, SW, and FH, no conflicts of interest

### **Funding**

The research is supported by the National Natural Science Foundation of China (Grant no. 32072742), National Key Research and Development Program (Grant no. 2021YFD1300300) and Hubei Provincial Natural Science Foundation of China (Grant no. 2018CFA071).

### **Authors' contributions**

W.Y., T.W., and F.H. designed the experiment; Z.B., and S.W. conducted the experiment. W.Y., and L.H. analyzed the data; W.Y. wrote the manuscript.

### **Acknowledgements**

We sincerely thank each of the authors for their contributions during the conduct of the experiments and for the facilities provided by the experimental platform at the College of Animal Science and Technology, Huazhong Agricultural University.

## **References**

1. Cummins AG, Thompson FM. Effect of breast milk and weaning on epithelial growth of the small intestine in humans. *Gut*. 2002;51(5):748–54. <http://dx.doi.org/10.1136/gut.51.5.748>.
2. Zeng Y, Wang Z, Zou T, Chen J, Li G, Zheng L, Li S, You J. Bacteriophage as an Alternative to Antibiotics Promotes Growth Performance by Regulating Intestinal Inflammation, Intestinal Barrier Function and Gut Microbiota in Weaned Piglets. *Front Vet Sci*. 2021;8:623899. <http://dx.doi.org/10.3389/fvets.2021.623899>.
3. Zou TD, Deng CX, Wang ZR, Ye YL, You JM. Dietary alanyl-glutamine improves growth performance of weaned piglets through maintaining intestinal morphology and digestion-absorption function. *Animal*. 2019;13(9):1826–33. <http://dx.doi.org/10.1017/s1751731119000223>.
4. Dokladny K, Zuhl MN, Moseley PL. Intestinal epithelial barrier function and tight junction proteins with heat and exercise. *J Appl Physiol* (1985) 2016, 120 (6), 692–701.

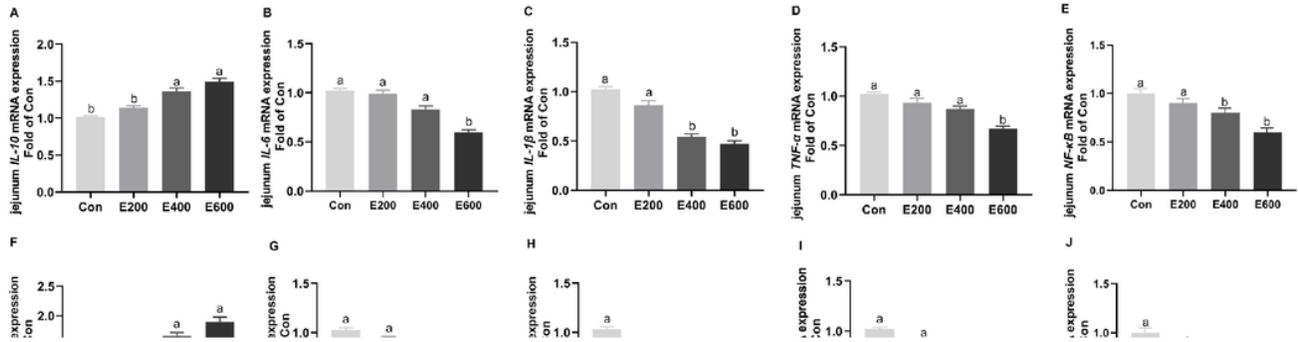
- <http://dx.doi.org/10.1152/japplphysiol.00536.2015>.
5. Oz HS, Chen TS, Nagasawa H. Comparative efficacies of 2 cysteine prodrugs and a glutathione delivery agent in a colitis model. *Transl Res*. 2007;150(2):122–9. <http://dx.doi.org/10.1016/j.trsl.2006.12.010>.
  6. Oz HS, Chen TS, McClain CJ, de Villiers WJ. Antioxidants as novel therapy in a murine model of colitis. *J Nutr Biochem*. 2005;16(5):297–304. <http://dx.doi.org/10.1016/j.jnutbio.2004.09.007>.
  7. Rhouma M, Fairbrother JM, Beaudry F, Letellier A. Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. *Acta Vet Scand*. 2017;59(1):31. <http://dx.doi.org/10.1186/s13028-017-0299-7>.
  8. Liao P, Li Y, Li M, Chen X, Yuan D, Tang M, Xu K. Baicalin alleviates deoxynivalenol-induced intestinal inflammation and oxidative stress damage by inhibiting NF- $\kappa$ B and increasing mTOR signaling pathways in piglets. *Food Chem Toxicol*. 2020;140:111326. <http://dx.doi.org/10.1016/j.fct.2020.111326>.
  9. Song ZH, Xiao K, Ke YL, Jiao le F, Hu CH. Zinc oxide influences mitogen-activated protein kinase and TGF- $\beta$ 1 signaling pathways, and enhances intestinal barrier integrity in weaned pigs. *Innate Immun*. 2015;21(4):341–8. <http://dx.doi.org/10.1177/1753425914536450>.
  10. Neurath MF, Becker C, Barbulescu K. Role of NF-kappaB in immune and inflammatory responses in the gut. *Gut*. 1998;43(6):856–60. <http://dx.doi.org/10.1136/gut.43.6.856>.
  11. Park SY, Lee YH, Seong AR, Lee J, Jun W, Yoon HG. Selective inhibition of PCAF suppresses microglial-mediated  $\beta$ -amyloid neurotoxicity. *Int J Mol Med*. 2013;32(2):469–75. <http://dx.doi.org/10.3892/ijmm.2013.1407>.
  12. Lillehoj H, Liu Y, Calsamiglia S, Fernandez-Miyakawa ME, Chi F, Cravens RL, Oh S, Gay CG. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. *Vet Res*. 2018;49(1):76. <http://dx.doi.org/10.1186/s13567-018-0562-6>.
  13. Callaway TR, Lillehoj H, Chuanchuen R, Gay CG Alternatives to Antibiotics: A Symposium on the Challenges and Solutions for Animal Health and Production. *Antibiotics (Basel)* 2021, 10 (5). <http://dx.doi.org/10.3390/antibiotics10050471>.
  14. Yadav A, Kolluri G, M G, Karthik K, Malik Y, Dhama K. Exploring alternatives to antibiotics as health promoting agents in poultry- a review. *J Experimental Biology Agricultural Sci*. 2016;4:368–83. [http://dx.doi.org/10.18006/2016.4\(3S\).368.383](http://dx.doi.org/10.18006/2016.4(3S).368.383).
  15. Ko JH, Lee SG, Yang WM, Um JY, Sethi G, Mishra S, Shanmugam MK, Ahn KS. The Application of Embelin for Cancer Prevention and Therapy. *Molecules* 2018, 23 (3). <http://dx.doi.org/10.3390/molecules23030621>.
  16. Schaible AM, Traber H, Temml V, Noha SM, Filosa R, Peduto A, Weinigel C, Barz D, Schuster D, Werz O. Potent inhibition of human 5-lipoxygenase and microsomal prostaglandin E $\square$  synthase-1 by the anti-carcinogenic and anti-inflammatory agent embelin. *Biochem Pharmacol*. 2013;86(4):476–86. <http://dx.doi.org/10.1016/j.bcp.2013.04.015>.

17. Wang H, Zhang H, Wang Y, Yang L, Wang D. Embelin can protect mice from thioacetamide-induced acute liver injury. *Biomed Pharmacother.* 2019;118:109360. <http://dx.doi.org/10.1016/j.biopha.2019.109360>.
18. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. *J Anim Sci Biotechnol.* 2013;4(1):19. <http://dx.doi.org/10.1186/2049-1891-4-19>.
19. Mahendran S, Badami S, Maithili VWITHDRAWN. Evaluation of antidiabetic effect of embelin from *Embelia ribes* in alloxan induced diabetes in rats. *Biomed Pharmacother.* 2010. <http://dx.doi.org/10.1016/j.biopha.2010.08.003>.
20. Council NR Nutrient Requirements of Swine: Eleventh Revised Edition; The National Academies Press, 2012. <http://dx.doi.org/doi:10.17226/13298>.
21. Qin L, Ji W, Wang J, Li B, Hu J, Wu X. Effects of dietary supplementation with yeast glycoprotein on growth performance, intestinal mucosal morphology, immune response and colonic microbiota in weaned piglets. *Food Funct.* 2019;10(5):2359–71. <http://dx.doi.org/10.1039/c8fo02327a>.
22. Wu M, Xiao H, Ren W, Yin J, Tan B, Liu G, Li L, Nyachoti CM, Xiong X, Wu G. Therapeutic effects of glutamic acid in piglets challenged with deoxynivalenol. *PLoS ONE.* 2014;9(7):e100591. <http://dx.doi.org/10.1371/journal.pone.0100591>.
23. Wang T, Yao W, Li J, Shao Y, He Q, Xia J, Huang F. Dietary garcinol supplementation improves diarrhea and intestinal barrier function associated with its modulation of gut microbiota in weaned piglets. *J Anim Sci Biotechnol.* 2020;11:12. <http://dx.doi.org/10.1186/s40104-020-0426-6>.
24. Dekaney CM, Wu G, Yin YL, Jaeger LA. Regulation of ornithine aminotransferase gene expression and activity by all-transretinoic acid in Caco-2 intestinal epithelial cells. *J Nutr Biochem.* 2008;19(10):674–81. <http://dx.doi.org/10.1016/j.jnutbio.2007.09.002>.
25. Yao W, Xia J, Wang T, Li J, Huang L, Huang F. Garcinol promotes hepatic gluconeogenesis by inhibiting P300/CBP-associated factor in late-pregnant sows. *Br J Nutr.* 2021;126(1):1–8. <http://dx.doi.org/10.1017/s000711452000375x>.
26. Yan X, Pepper MP, Vatamaniuk MZ, Roneker CA, Li L, Lei XG. Dietary selenium deficiency partially rescues type 2 diabetes-like phenotypes of glutathione peroxidase-1-overexpressing male mice. *J Nutr.* 2012;142(11):1975–82. <http://dx.doi.org/10.3945/jn.112.164764>.
27. Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T, Yao J, Zhou L, Zeng Y, Li H, et al. Regulation of cellular metabolism by protein lysine acetylation. *Science.* 2010;327(5968):1000–4. <http://dx.doi.org/10.1126/science.1179689>.
28. Chen J, Yu B, Chen D, Huang Z, Mao X, Zheng P, Yu J, Luo J, He J. Chlorogenic acid improves intestinal barrier functions by suppressing mucosa inflammation and improving antioxidant capacity in weaned pigs. *J Nutr Biochem.* 2018;59:84–92. <http://dx.doi.org/10.1016/j.jnutbio.2018.06.005>.
29. Smith F, Clark JE, Overman BL, Tozel CC, Huang JH, Rivier JE, Bliklager AT, Moeser AJ. Early weaning stress impairs development of mucosal barrier function in the porcine intestine. *Am J Physiol Gastrointest Liver Physiol.* 2010;298(3):G352–63. <http://dx.doi.org/10.1152/ajpgi.00081.2009>.

30. Meng Q, Sun S, Luo Z, Shi B, Shan A, Cheng B. Maternal dietary resveratrol alleviates weaning-associated diarrhea and intestinal inflammation in pig offspring by changing intestinal gene expression and microbiota. *Food Funct.* 2019;10(9):5626–43. <http://dx.doi.org/10.1039/c9fo00637k>.
31. Dall'Aglio C, Mercati F, Faeti V, Acuti G, Trabalza Marinucci M, De Felice E, Tardella FM, Franciosini MP, Casagrande Proietti P, Catorci D, et al. Immuno- and glyco-histochemistry as a tool to evaluate the oregano supplemented feed effects in pig gut. *Eur J Histochem* 2020, 64 (1). <http://dx.doi.org/10.4081/ejh.2020.3110>.
32. Liu L, Wu C, Chen D, Yu B, Huang Z, Luo Y, Zheng P, Mao X, Yu J, Luo J, et al. Selenium-Enriched Yeast Alleviates Oxidative Stress-Induced Intestinal Mucosa Disruption in Weaned Pigs. *Oxid Med Cell Longev* 2020, 2020, 5490743. <http://dx.doi.org/10.1155/2020/5490743>.
33. Caballero Valcárcel AM, Martínez Graciá C, Martínez Miró S, Madrid Sánchez J, González Bermúdez CA, Domenech Asensi G, López Nicolás R, Santaella Pascual M. Iron bioavailability of four iron sources used to fortify infant cereals, using anemic weaning pigs as a model. *Eur J Nutr.* 2019;58(5):1911–22. <http://dx.doi.org/10.1007/s00394-018-1742-x>.
34. Sies H, Stahl W, Sevanian A. Nutritional, dietary and postprandial oxidative stress. *J Nutr.* 2005;135(5):969–72. <http://dx.doi.org/10.1093/jn/135.5.969>.
35. Magalhaes JG, Tattoli I, Girardin SE. The intestinal epithelial barrier: how to distinguish between the microbial flora and pathogens. *Semin Immunol.* 2007;19(2):106–15. <http://dx.doi.org/10.1016/j.smim.2006.12.006>.
36. Lee B, Moon KM, Kim CY Tight Junction in the Intestinal Epithelium: Its Association with Diseases and Regulation by Phytochemicals. *J Immunol Res* 2018, 2018, 2645465. <http://dx.doi.org/10.1155/2018/2645465>.
37. Wan J, Zhang J, Chen D, Yu B, Mao X, Zheng P, Yu J, Luo J, He J. Alginate oligosaccharide-induced intestinal morphology, barrier function and epithelium apoptosis modifications have beneficial effects on the growth performance of weaned pigs. *J Anim Sci Biotechnol.* 2018;9:58. <http://dx.doi.org/10.1186/s40104-018-0273-x>.
38. Xiao K, Song ZH, Jiao LF, Ke YL, Hu CH. Developmental changes of TGF- $\beta$ 1 and Smads signaling pathway in intestinal adaption of weaned pigs. *PLoS ONE.* 2014;9(8):e104589. <http://dx.doi.org/10.1371/journal.pone.0104589>.
39. Feng J, Wang L, Xie Y, Chen Y, Yi H, He D. Effects of antimicrobial peptide cathelicidin-BF on diarrhea controlling, immune responses, intestinal inflammation and intestinal barrier function in piglets with postweaning diarrhea. *Int Immunopharmacol.* 2020;85:106658. <http://dx.doi.org/10.1016/j.intimp.2020.106658>.
40. Bischoff SC, Barbara G, Buurman W, Ockhuizen T, Schulzke JD, Serino M, Tilg H, Watson A, Wells JM. Intestinal permeability—a new target for disease prevention and therapy. *BMC Gastroenterol.* 2014;14:189. <http://dx.doi.org/10.1186/s12876-014-0189-7>.

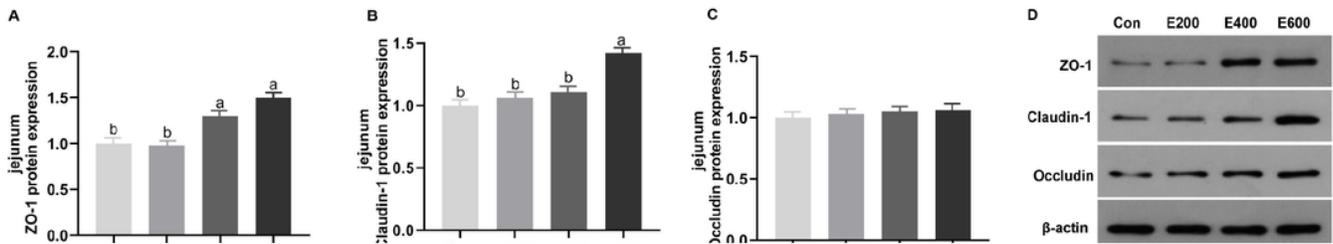
41. Shen L. Tight junctions on the move: molecular mechanisms for epithelial barrier regulation. *Ann N Y Acad Sci.* 2012;1258:9–18. <http://dx.doi.org/10.1111/j.1749-6632.2012.06613.x>.
42. Gao Y, Mruk DD, Lui WY, Lee WM, Cheng CY. F5-peptide induces aspermatogenesis by disrupting organization of actin- and microtubule-based cytoskeletons in the testis. *Oncotarget.* 2016;7(39):64203–20. <http://dx.doi.org/10.18632/oncotarget.11887>.
43. Li C, Wang W, Liu T, Zhang Q, Wang G, Li F, Li F, Yue X, Li T. Effect of Early Weaning on the Intestinal Microbiota and Expression of Genes Related to Barrier Function in Lambs. *Front Microbiol.* 2018;9:1431. <http://dx.doi.org/10.3389/fmicb.2018.01431>.
44. Chen H, Mao X, He J, Yu B, Huang Z, Yu J, Zheng P, Chen D. Dietary fibre affects intestinal mucosal barrier function and regulates intestinal bacteria in weaning piglets. *Br J Nutr.* 2013;110(10):1837–48. <http://dx.doi.org/10.1017/s0007114513001293>.
45. Salim SY, Söderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflamm Bowel Dis.* 2011;17(1):362–81. <http://dx.doi.org/10.1002/ibd.21403>.
46. Savidge TC, Newman P, Pothoulakis C, Ruhl A, Neunlist M, Bourreille A, Hurst R, Sofroniew MV. Enteric glia regulate intestinal barrier function and inflammation via release of S-nitrosoglutathione. *Gastroenterology* 2007, 132 (4), 1344–1358. <http://dx.doi.org/10.1053/j.gastro.2007.01.051>.
47. Liu T, Zhang L, Joo D, Sun S-C. NF- $\kappa$ B signaling in inflammation. *Signal Transduct Target Therapy.* 2017;2(1):17023. <http://dx.doi.org/10.1038/sigtrans.2017.23>.
48. Wang L, Walia B, Evans J, Gewirtz AT, Merlin D, Sitaraman SV. IL-6 induces NF-kappa B activation in the intestinal epithelia. *J Immunol.* 2003;171(6):3194–201. <http://dx.doi.org/10.4049/jimmunol.171.6.3194>.
49. Chen LF, Mu Y, Greene WC. Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. *Embo j.* 2002;21(23):6539–48. <http://dx.doi.org/10.1093/emboj/cdf660>.
50. Chen L, Fischle W, Verdin E, Greene WC. Duration of nuclear NF-kappaB action regulated by reversible acetylation. *Science.* 2001;293(5535):1653–7. <http://dx.doi.org/10.1126/science.1062374>.
51. Roy SK, Srivastava RK, Shankar S. Inhibition of PI3K/AKT and MAPK/ERK pathways causes activation of FOXO transcription factor, leading to cell cycle arrest and apoptosis in pancreatic cancer. *J Mol Signal.* 2010;5:10. <http://dx.doi.org/10.1186/1750-2187-5-10>.

## Figures



**Figure 1**

Effects of dietary embelin on the IL-10 (A, F), IL-6(B, G), IL-1 $\beta$  (C, H),TNF- $\alpha$  (D, I) and NF- $\kappa$ B (E, J) in jejunum and ileum of weaned piglets. Data are shown as the mean  $\pm$  SDs, n = 10/group. Means without a common letter differ,  $P < 0.05$ . IL-1 $\beta$ : interleukin-1 $\beta$ ; IL-6: interleukin-6; IL-10; NF- $\kappa$ B: nuclear factor- $\kappa$ B; TNF- $\alpha$ : tumor necrosis factor- $\alpha$ ;

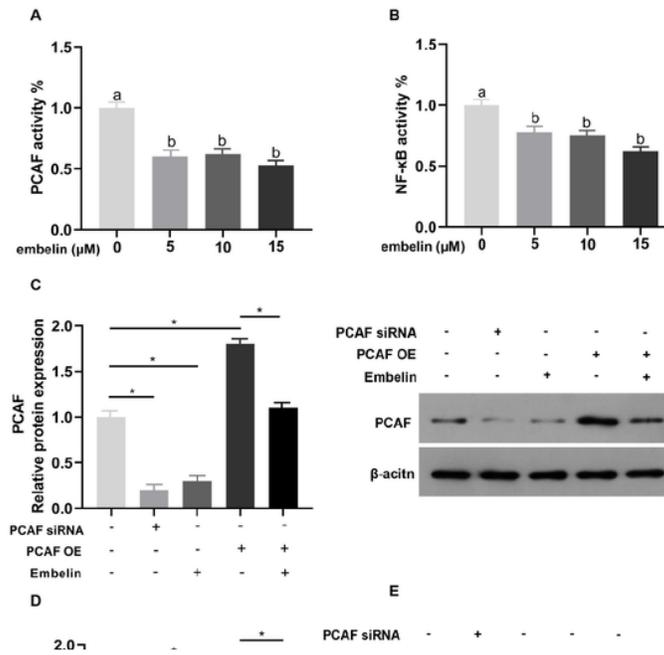


## Figure 2

Effects of dietary embelin on the expression of ZO-1 (A, E), Claudin-1 (B, F) and Occludin (C, G) in jejunum and ileum of weaned piglets. The protein expression in the intestine tissues in each group was determined by Western blotting (D, H), and each blot represents at least three independent samples. Data are shown as the mean  $\pm$  SDs,  $n = 10/\text{group}$ . Means for a given protein without a common letter differ,  $P < 0.05$ .

## Figure 3

Effects of dietary embelin on the PCAF (A, D) and NF- $\kappa$ B (B, E) activity, acetylation level of NF- $\kappa$ B (C, F) in jejunum and ileum of weaned piglets. Data are shown as the mean  $\pm$  SDs,  $n = 5-10/\text{group}$ . Means for a given protein without a common letter differ,  $P < 0.05$ . NF- $\kappa$ B: nuclear factor- $\kappa$ B; PCAF, P300/CBP associating factor.



**Figure 4**

Effects and mechanisms of dietary embelin on the the activity of PCAF (A) and NF-κB (B, D), protein expression of PCAF (C), acetylation level of NF-κB (E) in IPEC-1 cells. Data are shown as the mean ± SDs, n = 10/group. Means for a given protein without a common letter differ,  $P < 0.05$ ; \* $P < 0.05$ . NF-κB: nuclear factor-κB; PCAF, P300/CBP associating factor.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryMaterials.docx](#)