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Original Research Article

Indole-3-propionic acid enhances growth performance and reduces diarrhea via modulating redox status and intestinal inflammation in weaned piglets

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ABSTRACT

Indole-3-propionic acid (IPA) has anti-inflammatory properties, which can be beneficial for weaned piglets with underdeveloped immune systems. The study explores the impact of IPA supplementation on growth performance, oxidative stress, and inflammation response in weaned piglets. In Exp. 1, 90 weaned piglets were divided into six groups (5 replicates per group, 3 pigs per replicate), with each group receiving a basal diet with varying amounts of IPA (0, 50, 100, 200, 400, or 600 mg/kg) for 42 d. Piglets fed the diets with 50, 100, and 200 mg/kg of IPA exhibited reduced feed conversion ratios (F:G) compared to the control piglets ($P = 0.035$). Notably, 50 and 100 mg/kg IPA treatments significantly reduced diarrhea incidence and serum interleukin (IL)-6 content ($P < 0.05$). Conversely, a high dosage of 600 mg/kg IPA led to increased serum contents of tumor necrosis factor (TNF)-α, and IL-6 ($P < 0.05$). Optimal antioxidant benefits were observed at 100 mg/kg IPA supplementation, which significantly reduced malondialdehyde levels while enhancing serum total antioxidant capacity and total superoxide dismutase activity on d 14 ($P < 0.05$). Exp. 2 investigated the effects of IPA on lipopolysaccharide (LPS) challenge in weaned piglets. The study consisted of 32 weaned piglets allocated into 4 groups, with 8 replicates per group and 1 piglet per replicate: a control group, a LPS challenge group, a LPS challenge group supplemented with 100 mg/kg IPA, and a group supplemented with 100 mg/kg IPA alone. Upon administration of LPS or saline injection, the results indicated that dietary IPA supplementation in challenged piglets enhanced villus height: crypt depth, modulated *IL-8* and *IL-22* mRNA relative expression, and increased the tight junction protein claudin-1 mRNA relative expression in the intestinal mucosa ($P < 0.05$). These findings suggest that dietary supplementation of IPA at specific concentrations significantly improves growth performance, reduces diarrhea incidence, and mitigates inflammation and oxidative stress in weaned piglets. It may be concluded that incorporating IPA into the diet of weaned piglets can effectively improve their health and development.

1. Introduction

Piglets experience a variety of stressors during the weaning process, including nutritional, psychological, and environmental factors. Stress increases free radical production within the body, in which could lead to cellular toxicity (Moeser et al., 2017). Furthermore, the weakened immune system at weaning contributes to an imbalance in intestinal microecology and impaired intestinal barrier function. As a result, pathogenic microorganisms can easily penetrate the compromised intestinal barrier (Moeser et al., 2017),

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potentially resulting in reduced growth, increased mortality, and elevated economic losses (Rhouma et al., 2017). Antibiotics have been used to prevent and treat diarrhea in weaned pigs as feed additives. But overuse of antibiotics has exacerbated antibiotic resistance, which has become a serious community health issue. Consequently, finding safe and efficacious substitutes for antibiotics in feed additives has become a major industry and academic focus.

Indole-3-propionic acid (IPA) is a compound that originates from gut bacteria metabolizing tryptophan (Dodd et al., 2017; Owumi et al., 2022). IPA is an agonist of the pregnane X receptor (PXR) (Zhao et al., 2022) and it can strengthen the intestinal barrier by directly binding to PXR, thereby maintaining the homeostasis of intestinal epithelial cells, and reducing plasma endotoxin levels (Garg et al., 2016). Furthermore, through its activation of PXR, IPA can increase the tight-junction protein expression and suppress the nuclear factor-kappa B (NF-κB) activation (Venkatesh et al., 2014). Structurally akin to melatonin, IPA is adept at neutralizing hydroxyl radicals, contributing to its efficacy as an antioxidant (Karbownik et al., 2001). It also synergizes with the innate antioxidant systems of cells (Poeggeler et al., 1999), and does not transform into a pro-oxidative reactive intermediate (Chyan et al., 1999, Karbownik et al., 2001), marking it as an important antioxidant choice. These findings suggest that IPA holds promise in mitigating weaning stress in piglets by modulating intestinal homeostasis and reducing the impacts of inflammatory and oxidative stress. However, more research is needed as studies specifically exploring the impacts of IPA on weaned piglets are currently insufficient.

Given that IPA is manufactured solely through symbiotic gut microbiota, and considering the underdeveloped state of intestinal microbes in weaned piglets, it is plausible that young piglets may not naturally synthesize sufficient amounts of IPA on their own (Dodd et al., 2017). Hence, this study investigated the potential of dietary IPA supplementation in reducing diarrhea in piglets through the modulation of inflammation.

2. Materials and methods

2.1. Animal ethics statements

This study was approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences (IFR-CAAS-20230901).

2.2. Experimental protocol

Two experiments were conducted to assess the impact of dietary supplementation of IPA on weaned piglets. In Exp. 1, 90 weaned piglets (28 ± 2 days of age; body weight [BW] = 8.61 ± 0.71 kg; Duroc × Landrace × Large White) were distributed into six diets in a randomized complete block design with 5 replicates (pens) per treatment and 3 piglets per pen ($n = 5$). The control group was fed a corn-soybean meal diet, for the other five treatments, IPA dosages of 50, 100, 200, 400, and 600 mg/kg were added to the basal diet, respectively. A total of 30 piglets were selected (one per pen) based on their weight closest to the mean, for serum sampling on d 14 and 42 of the trial. The blood samples were allowed to equilibrate to room temperature, then gently tilted for 1 h before being centrifuged at 3000 × *g* for 10 min at 4 °C to separate the supernatants. The resulting serum was stored at −20 °C until further analysis.

In Exp. 2, 32 weaned piglets (26 ± 2 days of age; BW = 7.93 ± 0.21 kg; Duroc × Landrace × Large White) were selected. The piglets were stochastically divided into 4 groups with a 2 × 2 factorial design with 8 replicates per treatment and 1 pig per replicate (pen) ($n = 8$). For each treatment, there were 4 barrows and 4 gilts. The 4 groups were: the control group; the lipopolysaccharide (LPS) challenge group; the LPS challenge + IPA (100 mg/kg) group; the IPA (100 mg/kg) group. On d 14, all the pigs in the LPS challenge group and LPS challenge + IPA (100 mg/kg) group were administrated intraperitoneally with LPS (Sigma Chemical Co., St. Louis, MO) *Escherichia coli* (serotype O55: B5) at a level of 50 μg/kg BW. Other piglets were administrated intraperitoneally with sterile saline as a sham treatment. LPS was diluted with sterile saline (1 mg/mL). Three hours after being injected with LPS or saline, electrical stunners were used to stun all pigs (220 V). Following slaughter, tissue samples from the middle of the duodenum, jejunum, and ileum were taken and subsequently fixed in a 4% formaldehyde-phosphate buffer for preservation. Samples measuring approximately 2 cm were then stored at 4 °C for microscopic analysis. Additionally, mucosa of the jejunum and ileum was frozen in liquid nitrogen and stored at −80 °C. The diet provided to weaned piglets surpassed the dietary guidelines outlined by the NRC (2012) as shown in Table 1. The IPA (purity ≥99%) was chemically synthesized and purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Briefly, values of metabolizable energy (ME) and amino acid nutrient levels were calculated with reference to the NRC (2012). An analysis of the diet's nutritional composition was conducted in accordance with AOAC (2012). Specifically, N (as determined by method 990.03) was measured using an automated Kjeldahl nitrogen analyzer and the crude protein value was obtained by N × 6.25. According to the standards GB/T 6436-2018 and GB/T 6437-2018, the concentrations of calcium and phosphorus in the feed were measured using the ammonium metavanadate colorimetric and ethylene diamine tetraacetic acid (EDTA) methods.

Table 1
Composition and nutrient levels of diets (as-fed basis, %).

Item	Pre-starter (d 0–14)	Starter (d 15–42)
Ingredients		
Corn	16.45	21.17
Extruded corn	32.00	40.00
Soybean meal	14.00	17.50
Extruded soybean	11.50	6.00
Fish meal	5.60	3.00
Whey	15.00	5.00
Soybean oil	1.00	1.20
Wheat bran		1.50
Dicalcium phosphate	0.40	0.60
Limestone	0.75	0.90
Salt	0.30	0.30
Choline chloride (60%)	0.05	0.05
L-Lysine HCl	1.20	1.08
DL-Methionine	0.09	0.08
Threonine	0.27	0.24
Tryptophan	0.02	0.01
Phytase	0.02	0.02
Acidifier	0.35	0.35
Vitamin and mineral premix[1]	1.00	1.00
Total	100.00	100.00
Analyzed nutrient levels		
Crude protein	19.14	18.02
Calcium	0.78	0.64
Phosphorus	0.73	0.59
Calculated nutrient levels		
ME, kcal/kg	3400	3350
Lysine	1.30	1.15
Methionine	0.38	0.34
Threonine	0.76	0.68
Tryptophan	0.21	0.19

[1] Premix supplied per kilogram of diets: vitamin A, 35.2 mg; vitamin D_3, 7.68 mg; vitamin E, 128 mg; vitamin K_3, 8.16 mg; vitamin B_1, 4 mg; vitamin B_2, 12 mg; vitamin B_6, 8.32 mg; vitamin B_{12}, 4.8 mg; niacin, 38.4 mg; calcium pantothenate, 25 mg; folic acid, 1.68 mg; biotin, 0.16 mg; iron ($FeSO_4 \bullet H_2O$), 171 mg; manganese ($MnSO_4 \bullet H_2O$), 42.31 mg; copper ($CuSO_4 \bullet 5H_2O$), 125 mg; selenium (Na_2SeO_3), 0.19 mg; cobalt ($CoCl_2$), 0.19 mg; iodine ($Ca(IO_3)_2$), 0.54 mg.

2.3. Diarrhea incidence and growth performance

In Exp. 1, the weight of each pig was measured on d 0, 14, 28, and 42; in Exp. 2, the weight of each pig was measured on d 0 and 14. During these days, feed disappearance was recorded. Feed measurements and BW were used to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (F:G). Based on the following scale, diarrhea scores were recorded daily by the same person: 3 = watery feces, 2 = sloppy feces, and 1 = well-formed feces (Marquardt et al., 1999). A score of 3 indicates diarrhea in piglets. The diarrhea incidence of weaned piglets was determined using the following formula (Ou et al., 2007):

Diarrhea incidence (%) = [(number of days of diarrhea × number of weaned piglets with diarrhea)/(number of days of the experiment × total number of weaned piglets)] × 100.

2.4. Assay of serum cytokines

To determine cytokine levels in serum, assay kits ranging from immunoglobulin (Ig) A, interleukin (IL)-6, IL-10, and tumor necrosis factor (TNF)-α were used, as instructed by the manufacturer. The assay kits were purchased from the Shanghai Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China).

2.5. Serum antioxidant indices

Serum levels of malondialdehyde (MDA), glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD), catalase (CAT), and total antioxidant capacity (T-AOC) were determined using established assay kits (Nanjing Jiancheng Bioengineering institute, Nanjing, china).

2.6. Intestinal morphology

The crypt depth (CD) and villus height (VH) of the duodenum, jejunum, and ileum were quantified. A 4% paraformaldehyde solution was applied to the intestinal samples, after which the intestinal samples were embedded in paraffin wax for 24 h. Following this, the sections were sliced at a thickness of 5 mm and stained with hematoxylin and eosin. Ten intact villus-crypt structures were observed under a microscope, with the VH and CD measurements conducted using the Image Pro-Plus 6.0 Software Analysis System (Media Cybernetics, Singapore). The villus height: crypt depth (VCR) was then determined.

2.7. RNA extraction and real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

TRIzol reagent was employed for RNA extraction from the jejunal and ileal mucosa in adherence to the manufacturer's guidelines (Thermo Fisher Scientific, MA, USA). Following reverse transcription, the TransScript First-Strand cDNA Synthesis Kit was utilized to synthesize cDNA from total RNA (TransGen Biotech, Beijing, China). The reaction solution was prepared per the instructions provided by the manufacturer of SYBR Green reagent (Thermo Fisher Scientific, MA, USA), followed by PCR amplification using the real-time fluorescence quantitative PCR system (Bio-Rad, Hercules, CA, USA). The primer sequences for each gene are provided in Table 2, with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) serving as an internal reference. Relative fold changes in gene expression were calculated using the cycle threshold (Ct) method, as described by Cai et al. (2024).

Table 2
Primer sequences used for real-time quantitative reverse transcriptase polymerase chain reaction.

Gene	Accession number	Primer sequence (5′ to 3′)	Size, bp
GAPDH	NM_001206359.1	F: GCTTGTCATCAATGGAAAGG R: CATACGTAGCACCAGCATCA	86
IL-6	NM_214399.1	F: AATGTCGAGGCTGTGCAGATT R: TGGTGGCTTTGTCTGGATTCT	82
IL-8	NM_213867.1	F: CCGTGTCAACATGACTTCCAA R: GCCTCACAGAGAGCTGCAGAA	75
IL-10	NM_214041.1	F: GACGATGAAGATGAGGAAGA R: AGGTTTTTCTTTGGTTTCCC	54
IL-22	KX588234.1	F: TGGCCCTGAAGAATGAATGC R: CTGTGAAGTTTGGCTTCCCAT	139
ZO-1	CV870309	F: CGATCACTCCAGCATACAAT R: CACTTGGCAGAAGATTGTGA	111
Occludin	NM_001163647.2	F: TCAGGTGCACCCTCCAGATT R: TCAGGTGCACCCTCCAGATT	112
Claudin-1	NM_001244539.1	F: CCTCAATACAGGAGGGAAGC R: CTCTCCCCACATTCGAGATGATT	76

GAPDH = glyceraldehyde-3-phosphate dehydrogenase; *IL* =interleukin; *ZO-1* = zonula occludens-1.

2.8. Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) using the GLM procedure in SAS 9.2 (SAS Inst. Inc., Cary, NY, USA). In Exp. 1, one-way ANOVA was employed for statistical analysis, with orthogonal polynomial comparison tests utilized to assess the impacts of varying doses of IPA supplementation. Linear and quadratic relationships were also established. The statistical model used was as follows:

$$Y_{ij} = \mu + T_i + P_j + \varepsilon_{ij}$$

where Y_{ij} is the observation of dependent variables; μ is the overall mean; T_i is the fixed treatment effect; P_j is the random piglet effect, and ε_{ij} is the residual error for the observation.

In Exp. 2, a completely randomized design with a 2 × 2 factorial treatment arrangement was employed, with the statistical model incorporating the effects of LPS, IPA, and their interactions. The statistical model used was as follows:

$$Y_{ijk} = \mu + L_i + I_j + (L \times I)_{ij} + P_k + \varepsilon_{ijk}$$

where Y_{ijk} is the observation of dependent variables; μ is the overall mean; L_i is the fixed treatment effect of LPS; I_j is the fixed treatment effect of IPA; $(L \times I)_{ij}$ is the interaction effect between LPS and IPA; P_k is the random piglet effect, and ε_{ijk} is the residual error for the observation.

Multiple comparisons were performed using Tukey's honest significance difference test in cases where the main effect or interaction reached statistical significance. The experimental unit for growth performance was represented by pens, while individual piglets served as the experimental unit for analyzing serum cytokines, serum antioxidant indices, intestinal morphology, and gene expression. Furthermore, the Chi-square test was utilized to assess the incidence of diarrhea in piglets. The difference was considered significant when $P < 0.05$.

3. Results

3.1. Diarrhea incidence and growth performance

In Exp. 1, a comparison of the growth performance of piglets treated with different doses of IPA is shown in Table 3. During d 29

Table 3
Effects of indole-3-propionic acid (IPA) on the growth performance of weaned piglets (Exp.1) ($n = 5$).

Item	IPA level, mg/kg diet						SEM	*P*-value		
	0	50	100	200	400	600		ANOVA	Linear	Quadratic
d 0 BW, kg	8.61	8.61	8.59	8.61	8.64	8.61	0.713	1.000	0.987	1.000
d 14 BW, kg	11.91	11.16	12.12	11.70	11.92	11.85	0.825	0.988	0.842	0.775
d 28 BW, kg	17.21	16.72	18.14	17.23	17.35	17.72	1.189	0.967	0.690	0.899
d 42 BW, kg	23.38	25.61	25.79	25.50	25.25	25.75	1.871	0.170	0.551	0.767
d 0–14										
ADG, g/d	223	242	252	234	241	238	19.8	0.892	0.743	0.144
ADFI, g/d	377	363	386	380	392	363	30.4	0.983	0.963	0.311
F: G	1.69	1.51	1.56	1.62	1.62	1.54	0.096	0.595	0.542	0.361
d 15–28										
ADG, g/d	378	397	430	395	388	420	34.5	0.727	0.624	0.575
ADFI, g/d	687	709	721	722	741	743	57.4	0.955	0.683	0.868
F: G	1.80	1.79	1.73	1.82	1.93	1.73	0.075	0.064	0.991	0.649
d 29–42										
ADG, g/d	441^{b}	637^{a}	606^{a}	591^{a}	564^{a}	573^{a}	53.2	0.002	0.466	0.199
ADFI, g/d	928	1023	989	959	995	1059	104.2	0.654	0.620	0.876
F:G	2.10^{a}	1.68^{b}	1.63^{b}	1.62^{b}	1.77^{ab}	1.87^{ab}	0.171	0.014	0.161	0.299
d 0–42										
ADG, g/d	352^{b}	405^{a}	410^{a}	402^{a}	395^{a}	408^{a}	35.9	0.022	0.420	0.674
ADFI, g/d	664	702	698	682	709	715	56.7	0.771	0.653	0.812
F:G	1.88^{a}	1.74^{b}	1.70^{b}	1.70^{b}	1.80^{ab}	1.77^{ab}	0.077	0.035	0.296	0.182

ADG = average daily gain; ADFI = average daily feed intake; F:G = feed conversion ratio.
[a,b] Means listed in the same row with different letter superscripts are significantly different ($P < 0.05$).

to 42, piglets fed IPA-supplemented diets had a lower F:G and a higher ADG ($P < 0.05$) than the control piglets. From d 0 to 42, the IPA-supplemented diets for piglets increased their ADG compared with the control treatment ($P = 0.022$). Additionally, the piglets fed diets supplemented with 50, 100, and 200 mg/kg of IPA had a lower F:G compared to those fed the control diet ($P = 0.035$).

Table 4 demonstrates the effects of dietary supplementation with different doses of IPA on the incidence of diarrhea in weaned piglets. Weaned piglets receiving 50 and 100 mg/kg of IPA exhibited reduced incidences of diarrhea on d 0 to 14, 15 to 28, and 0 to 42 of the study, in comparison to the control ($P < 0.05$). In addition, administration of 400 mg/kg of IPA led to a decrease in the occurrence of diarrhea during d 15 to 28 and d 0 to 42 of the trial, in comparison to the control group ($P < 0.05$). There was no statistically significant difference in the incidence of diarrhea among weaned piglets consuming diets supplemented with 200 and 600 mg/kg of IPA, as compared to the control group ($P > 0.05$).

In Exp. 2, the impact of IPA and LPS challenge on the growth performance of weaned piglets are detailed in Table S1. No statistically significant differences were observed in ADFI, ADG, F:G, and incidence of diarrhea between the treatment groups ($P > 0.05$).

3.2. Inflammatory factors

Table 5 illustrates the impact of varying doses of IPA on serum inflammatory factors in weaned piglets in Exp. 1. Piglets administered a diet containing either 50 or 100 mg/kg of IPA on d 14 exhibited markedly reduced serum level of IL-6 compared to the group of control ($P < 0.001$). Conversely, piglets consuming a diet supplemented with 600 mg/kg of IPA demonstrated significantly elevated levels of serum TNF-α, IL-6, and IL-10 compared to the group of control ($P < 0.05$). Furthermore, on d 42, piglets fed diets enriched with 50 and 100 mg/kg of IPA displayed notably lower serum IL-6 levels than the group control ($P < 0.001$). Piglets supplemented with 600 mg/kg IPA exhibited significantly elevated levels of serum IL-6 and TNF-α levels compared to the group of control ($P < 0.05$). No statistically significant differences were observed in IgA content between the treatment groups ($P > 0.05$).

Table 4
Effects of indole-3-propionic acid (IPA) on the diarrhea incidence (%) of weaned piglets (Exp. 1) ($n = 5$).

Item	IPA level, mg/kg diet						SEM	*P*-value[1]
	0	50	100	200	400	600		
d 0–14	7.50^{a}	2.20^{bc}	0.43^{c}	4.17^{ab}	3.39^{ab}	5.00^{ab}	0.880	<0.001
d 15–28	7.14^{ab}	0.51^{d}	3.81^{c}	6.25^{bc}	2.86^{cd}	12.95^{a}	1.240	<0.001
d 29–42	0.45^{cd}	1.53^{b}	0.95^{bc}	0.89^{bc}	0.00^{d}	4.02^{a}	0.420	<0.001
d 0–42	5.09^{ab}	1.45^{d}	1.68^{d}	3.78^{bc}	2.13^{cd}	7.27^{a}	0.910	<0.001

[a-d] Means listed in the same row with different letter superscripts are significantly different ($P < 0.05$).
[1] The Chi-square test was utilized to assess the incidence of diarrhea in piglets.

3.3. Redox status

Table 6 illustrates the impact on serum redox status of weaned piglets of varying doses of IPA. Specifically, on d 14 of supplementation, piglets administered 100 mg/kg IPA exhibited notable increases in serum T-AOC and T-SOD ($P < 0.05$) levels compared to the group control, while MDA levels were significantly lower than the control group ($P = 0.004$). Conversely, piglets supplemented with 600 mg/kg IPA demonstrated significantly lower serum T-SOD levels than the control group ($P < 0.001$), with a concomitant increase in MDA content ($P = 0.004$).

3.4. Intestinal morphology

Table 7 and Fig. S1 present the impact of dietary IPA and LPS challenges on intestinal morphology in piglets. In comparison to the LPS + IPA group and LPS group, the IPA group had a significantly increased VH in the duodenum ($P < 0.001$). In the duodenum, a significant increase in VCR was observed in the IPA group compared to the other groups ($P < 0.001$). The VCR in the jejunum was significantly wider in the IPA and control groups than in the LPS and LPS + IPA groups ($P = 0.004$). There was a significant reduction in VH and VCR in the ileum of the LPS group compared to both the control and IPA groups ($P < 0.05$). In contrast, the LPS + IPA group exhibited significantly elevated VH and VCR values in comparison to the LPS group ($P < 0.05$). Although no significant disparity was observed between the control and IPA groups ($P > 0.05$), the VH and

Table 5
Effects of indole-3-propionic acid (IPA) on the serum inflammatory factors of weaned piglets (Exp. 1) ($n = 5$).

Item	IPA level, mg/kg diet						SEM	*P*-value		
	0	50	100	200	400	600		ANOVA	Linear	Quadratic
d 14										
TNF-α, pg/mL	30.49^{c}	30.93^{c}	30.89^{c}	32.02^{bc}	34.72^{ab}	37.01^{a}	1.433	<0.001	0.005	0.002
IL-6, pg/mL	131.04^{c}	115.96^{c}	117.18^{c}	129.68^{c}	132.82^{c}	150.39^{a}	6.962	<0.001	0.004	<0.001
IL-10, pg/mL	26.89^{c}	27.24^{c}	28.22^{c}	27.27^{c}	29.54^{c}	33.14^{a}	1.471	0.010	0.008	0.003
IgA, μg/mL	150.97	148.55	141.10	157.59	159.24	157.17	5.638	0.173	0.092	0.187
d 42										
TNF-α, pg/mL	31.92^{c}	30.80^{c}	31.79^{c}	31.83^{c}	33.50^{c}	39.34^{a}	1.654	<0.001	<0.001	0.002
IL-6, pg/mL	128.61^{c}	117.18^{c}	119.90^{c}	131.46^{c}	133.04^{c}	151.39^{a}	6.545	<0.001	<0.001	<0.001
IL-10, pg/mL	27.86	28.00	28.77	26.46	27.56	30.08	1.201	0.418	0.465	0.381
IgA, μg/mL	144.62	143.52	147.31	143.03	147.86	164.55	5.914	0.076	0.026	0.015

TNF-α = tumor necrosis factor α; IL-6 = interleukin-6; IL-10 = interleukin-10; IgA = immunoglobulin A.
[a-c]Means listed in the same row with different letter superscripts are significantly different ($P < 0.05$).

Table 6
Effects of indole-3-propionic acid (IPA) on serum antioxidant capacity of weaned piglets (Exp. 1) ($n = 5$).

Items	IPA level, mg/kg diet						SEM	*P*-value		
	0	50	100	200	400	600		ANOVA	Linear	Quadratic
d 14										
T-SOD, U/mL	44.72^{bc}	45.81^{c}	49.70^{a}	42.65^{bc}	41.47^{cd}	38.56^{d}	1.997	<0.001	0.007	<0.001
GSH-Px, U/mL	279.35	295.26	272.09	303.90	258.14	258.42	20.501	0.546	0.291	0.389
CAT, U/mL	4.52	5.22	6.13	4.17	5.99	5.18	0.858	0.569	0.619	0.790
T-AOC, μmol trolox/mL	0.66^{c}	0.70^{c}	0.80^{a}	0.69^{c}	0.68^{c}	0.65^{c}	0.036	0.034	0.492	0.036
MDA, nmol/mL	10.23^{c}	5.97^{bc}	3.41^{c}	6.95^{c}	6.49^{c}	19.18^{a}	3.061	0.004	0.115	<0.001
d 42										
T-SOD, U/mL	41.91^{ab}	44.82^{a}	44.37^{a}	43.93^{a}	37.48^{c}	41.47^{ab}	1.787	0.022	0.099	0.133
GSH-Px, U/mL	300.54	298.39	283.41	303.29	306.65	331.11	18.879	0.674	0.217	0.231
CAT, U/mL	5.06	5.65	4.94	5.04	4.76	4.34	0.593	0.795	0.222	0.400
T-AOC, μmol trolox/mL	0.62	0.67	0.69	0.67	0.62	0.69	0.026	0.202	0.330	0.481
MDA, nmol/mL	1.57	1.84	1.97	2.16	2.56	1.97	0.869	0.986	0.561	0.784

T-SOD = total superoxide dismutase; GSH-Px = glutathione peroxidase; CAT = catalase; T-AOC = total antioxidant capacity; MDA = malondialdehyde.
[a-d]Means listed in the same row with different letter superscripts are significantly different ($P < 0.05$).

Table 7
Effects of indole-3-propionic acid (IPA) on intestinal morphology of weaned piglets under lipopolysaccharide (LPS) challenge (Exp. 2) ($n = 8$)[1].

Item	**CTRL**	**LPS**	**LPS + IPA**	**IPA**	**SEM**	***P*-value**		
						IPA	**LPS**	**Interaction**
Duodenum								
Villus height, μm	437^{ab}	386^{c}	384^{c}	503^{a}	35.5	0.368	0.027	0.351
Crypt depth, μm	275	251	249	227	18.5	0.234	0.991	0.270
Villus height:crypt depth	1.62^{c}	1.54^{c}	1.54^{c}	2.24^{a}	0.139	<0.001	<0.001	0.003
Jejunum								
Villus height, μm	358	304	304	361	28.8	0.965	0.081	0.952
Crypt depth, μm	177	193	177	157	14.3	0.257	0.244	0.909
Villus height:crypt depth	2.05^{a}	1.62^{c}	1.71^{c}	2.28^{a}	0.138	0.158	0.004	0.541
Ileum								
Villus height, μm	298^{ab}	237^{c}	268^{c}	325^{a}	19.2	0.107	0.006	0.921
Crypt depth, μm	157	173	157	155	8.0	0.528	0.132	0.195
Villus height:crypt depth	1.92^{ab}	1.36^{c}	1.71^{c}	2.10^{a}	0.121	0.010	<0.001	0.108

[a-c]Means listed in the same row with different superscripts are significantly different ($P < 0.05$).

VCR values of the control group were found to be lower than those of the LPS + IPA group ($P < 0.05$).

3.5. Gene expression of inflammatory factors in the jejunal and ileal mucosa

The results are presented in Fig. 1 and Fig. S2 demonstrates that dietary challenges with IPA and LPS led to the upregulation of inflammatory factor gene expression in the jejunum and ileum. Specifically, the LPS group *IL-6* and *IL*-22 mRNA relative expression significantly increased compared to the control group ($P < 0.05$), while *IL-8* and *IL*-10 mRNA relative expression remained unaffected in the jejunum ($P > 0.05$). Additionally, *IL*-22 mRNA relative expression was significantly lower in the LPS + IPA group than in the LPS group ($P = 0.044$). There was a significant increase in *IL*-10 mRNA relative expression in the LPS + IPA and IPA groups compared to the LPS group ($P = 0.020$). There was a notable increase in *IL-6*, *IL-8*, and *IL*-22 mRNA relative expression in the ileum of the LPS group when compared to the control group ($P < 0.05$), with no significant alteration in *IL*-10 mRNA relative expression ($P > 0.05$). The LPS + IPA group showed a significant decrease in *IL-8* and *IL*-22 mRNA relative expression compared to the LPS group

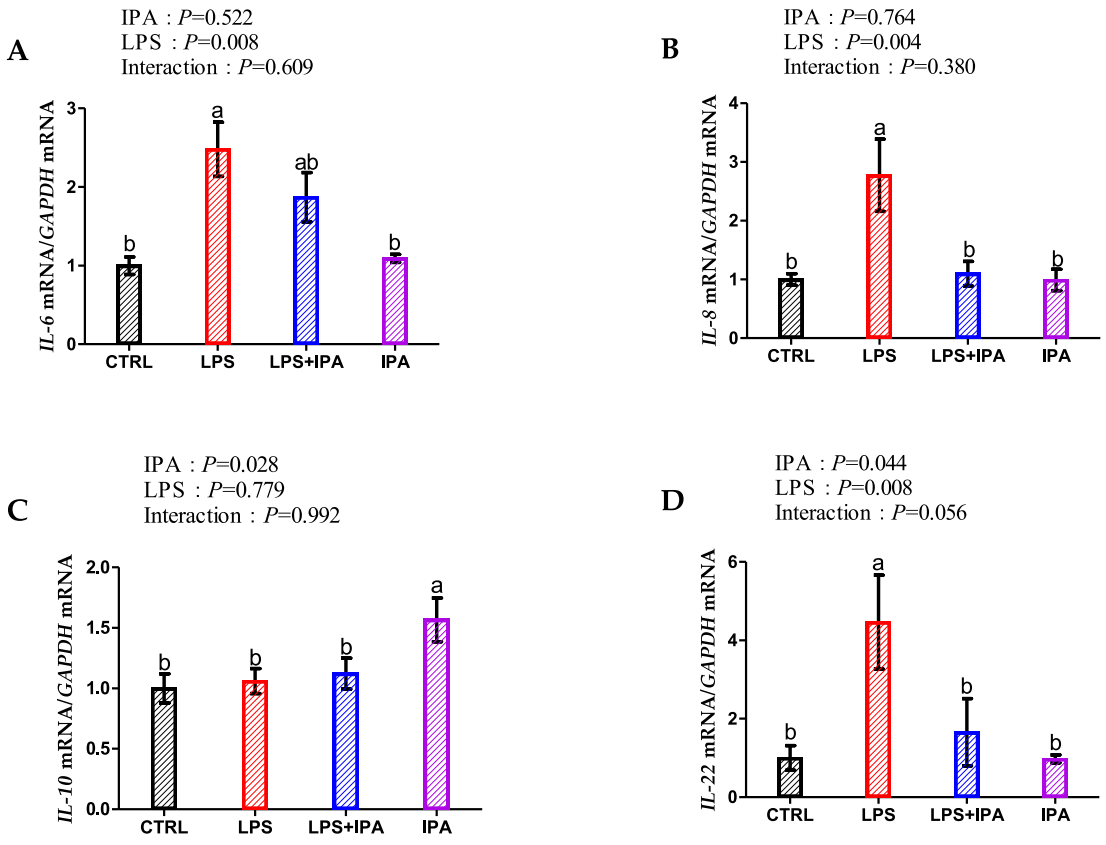

Fig. 1. Effects of indole-3-propionic acid (IPA) on the mRNA relative expression of genes related to inflammatory factors in the ileum mucosa of weaned piglets under lipopolysaccharide (LPS) challenge (Exp. 2) ($n = 8$). (A) The mRNA relative expression of *IL-6*. (B) The mRNA relative expression of *IL-8*. (C) The mRNA relative expression of *IL-10*. (D) The mRNA relative expression of *IL-22*. *IL-6* = interleukin-6; *IL-8* = interleukin-8; *IL-10* = interleukin-10; *IL-22* = interleukin-22; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase. CTRL: control group; LPS: lipopolysaccharide group; LPS + IPA: lipopolysaccharide + indole-3-propionic acid (100 mg/kg) group; IPA: indole-3-propionic acid (100 mg/kg) group. [a,b] Values without common letters differ significantly ($P < 0.05$). Data are expressed as mean ± standard error.

($P < 0.05$). Notably, the IPA group exhibited significantly higher *IL-10* mRNA relative expression levels compared to the LPS group ($P = 0.028$).

3.6. Gene expression of tight junctions in the jejunal and ileal mucosa

Figure 2 and Fig. S3 illustrate the impact of dietary IPA and LPS on gene expression of tight junctions in the jejunum and ileum. There was no statistically significant difference in tight junction gene expression in the jejunum ($P > 0.05$). However, the mRNA relative expression of claudin-1 was significantly higher in the LPS + IPA and IPA groups compared to the control and LPS groups in the ileum ($P = 0.039$).

4. Discussion

Piglets commonly experience diarrhea following weaning as a result of the transition from milk to solid feed containing anti-nutritional components, separation from the sows, and a change in the surroundings. The transition hinders nutrient digestion and absorption of nutrients as well as the development of piglets (Kraehenbuhl et al., 1997; Smith et al., 2010). Our study found that the administration of 50 and 100 mg/kg of IPA decreased the diarrhea incidence and improved the growth performance of pigs, representing the first documented instance of IPA's beneficial effects on the health of pigs. Moreover, we employed LPS to simulate weaning stress, noting that the LPS challenge diminished intestinal VH and VCR in weaned piglets, indicative of LPS-induced intestinal damage. However, IPA supplementation mitigated these detrimental effects of LPS. Weaned piglets' intestinal morphology is an important indicator of their health (Liu et al., 2012). Previous research has linked the blood concentration of IPA directly to the prevalence of intestinal disorders, such as colitis, where IPA levels rise as intestinal health improves. Thus, IPA concentration may serve as a biomarker for intestinal diseases (Pavlova et al., 2017; Menni et al., 2019). Taken together, our findings and those of previous studies suggest that IPA contributes positively to intestinal health.

Excessive free radicals can cause oxidative stress in tissues and organs, subsequently causing diarrhea. In our experiment, we demonstrated that 100 mg/kg of IPA possesses significant antioxidant properties, evidenced by its ability to enhance the activities of endogenous antioxidant enzymes, diminish the oxidative metabolite MDA, and boost the T-AOC in weaned piglets. Indeed, IPA shares a structural resemblance with melatonin, enabling it to effectively neutralize free radicals (Karbownik et al., 2001). IPA stands out as an ideal antioxidant due to its synergistic action with intracellular glutathione and its stability, preventing conversion into pro-oxidative intermediates (Poeggeler et al., 1999; Chyan et al., 1999). IPA has been shown to mitigate neuronal damage resulting from ischemia and hypoxia (Hwang et al., 2009), and in the thyroid and kidneys, it can reduce lipid peroxidation triggered by $KBrO_3$ (Karbownik et al., 2005). In the liver, pre-treatment with IPA has been found to alleviate functional damage caused by ischemia (Lefler et al., 2002). However, 600 mg/kg IPA yielded

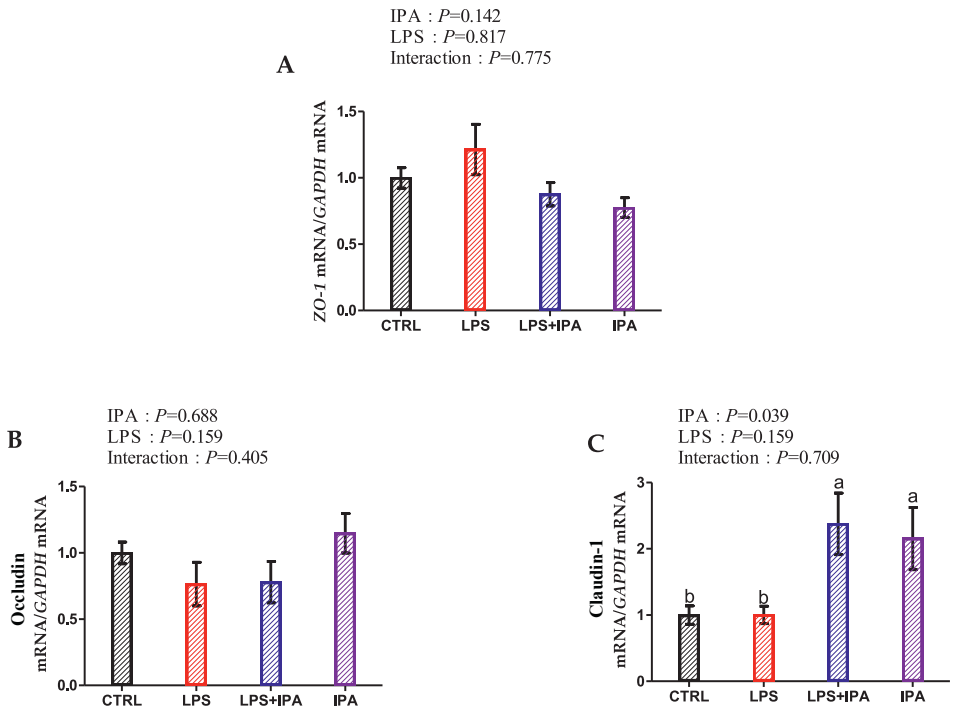

Fig. 2. Effects of indole-3-propionic acid (IPA) on the mRNA relative expression of genes related to tight junctions in the ileum mucosa of weaned piglets under lipopolysaccharide (LPS) challenge (Exp. 2). (A) The mRNA relative expression of *ZO-1*. (B) The mRNA expression of occludin. (C) The mRNA relative expression of claudin-1. *ZO-1* = zonula occludens-1; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase. CTRL: control group; LPS: lipopolysaccharide group; LPS + IPA: lipopolysaccharide + indole-3-propionic acid (100 mg/kg) group; IPA: indole-3-propionic acid (100 mg/kg) group. [a,b] Values without common letters differ significantly ($P < 0.05$). Data are expressed as mean ± standard error.

contrary results. It has been noted that IPA can lead to a reduction in breast cancer incidences through the mechanisms of inducing oxidative stress and activating the aryl hydrocarbon receptor (AHR) and PXR receptors (Sari et al., 2020). This indicates that IPA's impact on oxidative stress varies depending on the context. The cause of oxidative stress in weaned piglets induced by the addition of high doses of IPA requires further investigation.

Inflammation plays a pivotal role in diarrhea among weaned piglets. Intestinal epithelium absorbs IPA into the bloodstream, preventing inflammation in several tissues (Roager and Licht, 2018). In the kidneys, IPA helps mitigate injury by suppressing the expression of inflammation genes in renal tubules (Karbownik et al., 2006). Furthermore, IPA has been shown to decrease the release and expression of TNF-α, IL-1β, and IL-6 in the liver (Zhao et al., 2019). In this research, the inclusion of 50 and 100 mg/kg IPA was observed to lower serum IL-6 levels in weaned piglets, suggesting that the exogenous supplementation of IPA enhances the health status of piglets and alleviates weaning stress. However, piglets fed a diet containing 600 mg/kg of IPA demonstrated significantly higher serum levels of TNF-α, IL-6, and IL-10. This suggests that elevated doses of IPA might trigger pro-inflammatory responses. Further research is required to understand the underlying mechanisms of these results. A major component of the *E. coli* cell wall is LPS, which can recognize cell membrane receptors to activate the NF-κB pathway and secrete inflammatory mediators (Lai et al., 2017). The inflammatory mediators generated by the LPS challenge can recognize membrane receptors and further enhance inflammatory signals, causing immune cells to aggregate in tissue and causing tissue damage (Li et al., 2018). We further employed LPS to stimulate intestinal inflammation to mimic the weaning stress of piglets. In the small intestine of piglets, the mRNA relative expression of *IL-6*, *IL-8*, and *IL-22* increased after the LPS challenge in the present study. Based on these results, it was concluded that LPS caused intestinal mucosal inflammation. However, we also showed that the dietary addition of IPA can effectively reduce the increased mRNA relative expression of *IL-8* and *IL-22* induced by LPS, indicating that IPA can alleviate the intestinal inflammation induced by LPS. Moreover, the claudin-1 mRNA relative expression in the gut is increased, suggesting that IPA helps improve intestinal integrity and barrier function, and reduces tissue damage. This is the first report demonstrating that IPA positively affects intestinal immunity in pigs. Other studies have also demonstrated that IPA can mitigate intestinal inflammation induced by LPS or dextran sodium sulfate (Venkatesh et al., 2014; Fu et al., 2022). The anti-inflammatory effects of IPA have been attributed to its regulation of the NF-κB signaling pathway, leading to the inhibition of NF-κB activation and subsequent reduction in the expression of inflammatory markers, thereby mitigating inflammatory damage (Zhou et al., 2006). Recently, it has been discovered that IPA also modulates the expression of inflammatory factors by targeting the protein kinase B/phosphoinositide 3-kinase/mammalian target of rapamycin signaling pathway (Li et al., 2021). Additional research is required to ascertain if IPA's anti-inflammatory actions in weaned piglets are facilitated via these signaling pathways.

5. Conclusions

Our findings suggest that incorporating IPA into the diet of piglets improves growth performance and reduces diarrhea incidences. The protective effects of IPA on barrier function and intestinal integrity may be due to a decrease in inflammatory responses and oxidative stress. These outcomes support IPA as a promising dietary supplement for reducing weaning stress in piglets, with a recommended supplemental dosage of 100 mg/kg.

Credit author contribution statement

Dongxu Ming: Formal analysis, Writing – original draft, Writing – review & editing. **Xincong Xu:** Data curation. **Xianren Jiang:** Investigation. **Yanpin Li:** Methodology. **Wenjuan Sun:** Project administration. **Jiangbo Xiang:** Resources. **Mingyuan Huang:** Resources. **Yu Pi:** Conceptualization, Validation. **Xilong Li:** Funding acquisition, Supervision.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.08.004.

References

Association of Official Agricultural Chemists (AOAC): Arlington. Official methods of analysis. 19th ed. 2012. VA, USA.
Cai L, Ming DX, Chen WN, Zhao Y, Li YP, Sun WJ, et al. Silybin alleviated hepatic injury by regulating redoxbalance, inflammatory response, and mitochondrial function in weaned piglets under paraquat-induced oxidative stress. Antioxidants 2024;13:324.
Chyan YJ, Poeggeler B, Omar RA, Chain DG, Frangione B, Ghiso J, et al. Potent neuroprotective properties against the Alzheimer beta-amyloid by an endogenous melatonin-related indole structure, indole-3-propionic acid. J Biol Chem 1999;274(31):21937–42.
Dodd D, Spitzer MH, Van Treuren W, Merrill BD, Hryckowian AJ, Higginbottom SK, et al. A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. Nature 2017;551(7682):648–52.
Fu YW, Gao H, Hou XH, Chen Y, Xu K. Pretreatment with IPA ameliorates colitis in mice: colon transcriptome and fecal 16S amplicon profiling. Front Immunol 2022;13(9):1014881.
Garg A, Zhao A, Erickson SL, Mukherjee S, Lau AJ, Alston L, et al. Pregnane X receptor activation attenuates inflammation-associated intestinal epithelial barrier dysfunction by inhibiting cytokine-induced myosin light-chain kinase expression and c-Jun N-Terminal Kinase 1/2 activation. J Pharmacol Exp Therapeut 2016;359(1):91–101.
Hwang IK, Yoo KY, Li H, Park OK, Lee CH, Choi JH, et al. Indole-3-propionic acid attenuates neuronal damage and oxidative stress in the ischemic hippocampus. J Neurosci Res 2009;87(9):2126–37.
Karbownik M, Garcia JJ, Lewinski A, Reiter RJ. Carcinogen-induced, free radical-mediated reduction in microsomal membrane fluidity: reversal by indole-3-propionic acid. J Bioenerg Biomembr 2001a;33(1):73–8.
Karbownik M, Reiter RJ, Garcia JJ, Cabrera J, Burkhardt S, Osuna C, et al. Indole-3-propionic acid, a melatonin-related molecule, protects hepatic microsomal membranes from iron-induced oxidative damage: relevance to cancer reduction. J Cell Biochem 2001b;81(3):507–13.
Karbownik M, Stasiak M, Zasada K, Zygmunt A, Lewinski A. Comparison of potential protective effects of melatonin, indole-3-propionic acid, and propylthiouracil against lipid peroxidation caused by potassium bromate in the thyroid gland. J Cell Biochem 2005;95(1):131–8.
Karbownik M, Stasiak M, Zygmunt A, Zasada K, Lewinski A. Protective effects of melatonin and indole-3-propionic acid against lipid peroxidation, caused by potassium bromate in the rat kidney. Cell Biochem Funct 2006;24(6):483–9.
Kraehenbuhl JP, Pringault E, Neutra MR. Review article: intestinal epithelia and barrier functions. Aliment Pharmacol Ther 1997;11(Suppl 3):3–8.
Lai JL, Liu YH, Liu C, Qi MP, Liu RN, Zhu XF, et al. Indirubin inhibits LPS-induced inflammation via TLR4 abrogation mediated by the NF-kB and MAPK signaling pathways. Inflammation 2017;40(1):1–12.
Lefler SR, St PS, Rodriguez-Davalos MI, Perumean JC, Spence NA, Moss AA, et al. Amelioration of hepatic ischemia/reperfusion injury using indolepropionic acid. Transplant Proc 2002;34(8):3065–8.
Li JJ, Zhang L, Wu T, Li YF, Zhou XJ, Ruan Z. Indole-3-propionic acid improved the intestinal barrier by enhancing epithelial barrier and mucus barrier. J Agric Food Chem 2021;69(5):1487–95.
Li X, Peng H, Wu JX, Xu YC. Brain natriuretic peptide-regulated expression of inflammatory cytokines in lipopolysaccharide (LPS)-activated macrophages via nf-kappab and mitogen activated protein kinase (MAPK) pathways. Med Sci Mon Int Med J Exp Clin Res 2018;24:3119–26.
Liu Y, Chen F, Odle J, Lin X, Jacobi SK, Zhu H, et al. Fish oil enhances intestinal integrity and inhibits TLR4 and NOD2 signaling pathways in weaned pigs after LPS challenge. J Nutr 2012;142(11):2017–24.
Marquardt RR, Jin LZ, Kim JW, Fang L, Frohlich AA, Baidoo SK. Passive protective effect of egg-yolk antibodies against enterotoxigenic *Escherichia coli* K88+ infection in neonatal and early-weaned piglets. FEMS Immunol Med Microbiol 1999;23(4). 283-28.
Menni C, Hernandez MM, Vital M, Mohney RP, Spector TD, Valdes AM. Circulating levels of the anti-oxidant indoleproprionic acid are associated with higher gut microbiome diversity. Gut Microb 2019;10(6):688–95.
Moeser AJ, Pohl CS, Rajput M. Weaning stress and gastrointestinal barrier development: implications for lifelong gut health in pigs. Anim Nutr 2017;3(4):313–21.
National Research Council (NRC). Nutrient requirements of swine. 11th ed. Washington, DC, USA: National Academic Press; 2012.
Ou DY, Li DF, Cao YH, Li XL, Yin JD, Qiao SY, et al. Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. J Nutr Biochem 2007;18(12):820–6.
Owumi SE, Adedara IA, Oyelere AK. Indole-3-propionic acid mitigates chlorpyrifos-mediated neurotoxicity by modulating cholinergic and redox-regulatory systems, inflammatory stress, apoptotic responses and DNA damage in rats. Environ Toxicol Pharmacol 2022;89(1):103786.
Pavlova T, Vidova V, Bienertova-Vasku J, Janku P, Almasi M, Klanova J, et al. Urinary intermediates of tryptophan as indicators of the gut microbial metabolism. Anal Chim Acta 2017;987(9):72–80.
Poeggeler B, Pappolla MA, Hardeland R, Rassoulpour A, Hodgkins PS, Guidetti P, et al. Indole-3-propionate: a potent hydroxyl radical scavenger in rat brain. Brain Res 1999;815(2):382–8.
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.
Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. Nat Commun 2018;9(1):3294.
Sari Z, Miko E, Kovacs T, Janko L, Csonka T, Lente G, et al. Indolepropionic acid, a metabolite of the microbiome, has cytostatic properties in breast cancer by activating ahr and pxr receptors and inducing oxidative stress. Cancers 2020;12(9):2411.
Smith F, Clark JE, Overman BL, Tozel CC, Huang JH, Rivier JE, et al. Early weaning stress impairs development of mucosal barrier function in the porcine intestine. Am J Physiol Gastrointest Liver Physiol 2010;298(3):G352–63.
Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, et al. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. Immunity 2014;41(2):296–310.
Zhao QL, Chen TT, Ni C, Hu YY, Nan Y, Lin W, et al. Indole-3-propionic acid attenuates HI-related blood-brain barrier injury in neonatal rats by modulating the PXR signaling pathway. ACS Chem Neurosci 2022;13(19):2897–912.
Zhao ZH, Xin FZ, Xue Y, Hu Z, Han Y, Ma F, et al. Indole-3-propionic acid inhibits gut dysbiosis and endotoxin leakage to attenuate steatohepatitis in rats. Exp Mol Med 2019;51(9):1–14.
Zhou C, Tabb MM, Nelson EL, Grun F, Verma S, Sadatrafiei A, et al. Mutual repression between steroid and xenobiotic receptor and NF-kappaB signalling pathways links xenobiotic metabolism and inflammation. J Clin Invest 2006;116(8):2280–9.