Effects of Curcumin on Ileal Mucosal Morphology, Tight Junction Proteins, Inflammatory Cytokine Gene Expression, and Serum Immunoglobulin Levels in Early-Weaned Piglets

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Abstract

This study aimed to investigate the effects of curcumin on ileal mucosal morphology, tight junction protein and inflammatory cytokine gene expression, and serum immunoglobulin levels in early-weaned piglets. Fifty healthy 21-day-old weaned piglets (Duroc × Landrace × Large Yorkshire) with similar parity and body weight, half male and half female, were selected and randomly divided into 5 groups with 10 replicates per group and 1 pig per replicate. The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 50 mg/kg quinocetone and 200, 300, and 400 mg/kg curcumin, respectively. The preliminary feeding period lasted 7 days, with Escherichia coli inoculation on day 4; the formal experimental period lasted 21 days. The results showed: 1) Compared with the control group, the 300 and 400 mg/kg curcumin groups exhibited significantly increased ileal villus height and villus height/crypt depth ratio ($P<0.05$), significantly decreased villus width and crypt depth ($P<0.05$), significantly increased relative mRNA expression of ileal mucosal tight junction proteins occludin and zonula occludens-1 (ZO-1) ($P<0.05$), significantly decreased relative mRNA expression of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and Toll-like receptor 4 (TLR4) ($P<0.05$), and significantly increased relative mRNA expression of interleukin-10 (IL-10) and serum levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) ($P<0.05$); 2) The quinocetone group showed significantly lower ileal villus width and TLR4 mRNA relative expression than the control group ($P<0.05$), with no significant differences in other indices compared with the control group ($P>0.05$). These results demonstrate that supplementation with 300 or 400 mg/kg curcumin improved ileal mucosal epithelial morphology, enhanced intestinal mucosal barrier integrity, and increased piglet

immunity, with effects superior to those of quinocetone.

Full Text

Effects of Curcumin on Ileal Mucosal Morphology, Tight Junction Proteins, Inflammatory Cytokine Gene Expression, and Serum Immunoglobulin Levels in Early-Weaned Piglets

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Abstract: This experiment was conducted to investigate the effects of curcumin on ileal mucosal morphology, tight junction proteins, inflammatory cytokine gene expression, and serum immunoglobulin levels in early-weaned piglets. Fifty healthy weaned piglets at 21 days of age, with similar parity and body weight, of the "Duroc × Landrace × Large White" cross were selected, with males and females each accounting for 1/2. They were randomly divided into 5 groups, with 10 replicates per group and 1 pig per replicate. The control group was fed a basal diet, while the experimental groups were fed basal diets supplemented with 50 mg/kg quinocetone and 200, 300, or 400 mg/kg curcumin, respectively. The preliminary trial lasted 7 d, and on day 4 the piglets were inoculated with *Escherichia coli*; the formal trial lasted 21 d. The results showed that: 1) Compared with the control group, the 300 and 400 mg/kg curcumin groups had significantly increased ileal villus height and villus height/crypt depth ratio ($P<0.05$), significantly reduced villus width and crypt depth ($P<0.05$), significantly increased relative mRNA expression levels of the ileal mucosal tight junction proteins occludin and zonula occludens-1 (*ZO-1*) ($P<0.05$), significantly reduced relative mRNA expression levels of interleukin-1β (*IL-1β*), tumor necrosis factor-α (*TNF-α*), interleukin-6 (*IL-6*), and Toll-like receptor 4 (*TLR4*) ($P<0.05$), and significantly increased relative mRNA expression of interleukin-10 (*IL-10*) as well as serum immunoglobulin G (IgG) and immunoglobulin M (IgM) levels ($P<0.05$); 2) The quinocetone group had significantly lower ileal villus width and relative *TLR4* mRNA expression than the control group ($P<0.05$), while the remaining indices did not differ significantly from those of the control group ($P>0.05$). The results indicate that supplementation with 300 or 400 mg/kg curcumin can improve ileal mucosal epithelial morphology, enhance the integrity of the intestinal mucosal barrier, and improve piglet immunity, with effects superior to those of quinocetone.

Keywords: curcumin; weaned piglets; ileal mucosal morphology; tight junction

proteins; inflammatory cytokines; immunoglobulins

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In modern pig production, early weaning of piglets is one of the advanced technologies commonly adopted. However, during the weaning period, sudden physiological, nutritional, and environmental changes, weaning stress, and invasion by pathogenic microorganisms can all cause reduced feed intake, growth retardation and diarrhea, intestinal villus atrophy and crypt hyperplasia, decreased immune function, and other problems in weaned piglets, namely "early-weaning syndrome" [1]. The main reason for this consequence is that weaning stress damages the intestinal barrier, thereby leading to diarrhea. This not only significantly reduces the productive performance of piglets, but also seriously threatens their life and health, becoming an important factor restricting the development of the pig industry. Therefore,

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How to effectively reduce the occurrence of diarrhea after early weaning in piglets and enable piglets to pass smoothly through the weaning period has long been a research focus both in China and abroad.

The effects of feed antibiotics such as olaquindox and aureomycin in preventing pathogen infection and promoting animal growth have been recognized, but feed antibiotics have also brought a series of hazards to livestock production, such as bacterial drug resistance and antibiotic drug residues. Therefore, the development of safe, pollution-free, residue-free green feed additives to replace antibiotics has become a current hotspot and focus in the field of animal nutrition research. In recent years, research in China and abroad has made great progress in elucidating the mechanisms of intestinal mucosal barrier injury in early-weaned piglets, laying a foundation for developing new feed additives to prevent intestinal tract-related diseases. Curcumin is a natural plant extract that is abundant in China. It has broad pharmacological effects, including anti-inflammatory, antioxidant, anti-infective, antitumor, and lipid-lowering activities, and has very low toxic side effects[^2-^3], creating favorable conditions for its application in feed. Song Weibing[^4] reported that curcumin has

a protective effect on the permeability of the intestinal mucosa in rats with methotrexate (MTX)-induced enteritis and also has antioxidant effects. Wang Gai[^5] found that curcumin can ameliorate tight junction and intestinal mucosal barrier dysfunction mediated by hydrogen peroxide (H_2O_2), and reduce the expression of occludin and zonula occludens-1 (ZO-1). In addition, studies have found that curcumin protects neonatal rats against necrotizing enterocolitis by inhibiting the expression of the pro-inflammatory factors—tumor necrosis factor-α (*TNF-α*) and cyclooxygenase-2 (*COX-2*)—and increasing the expression of interleukin-10 (*IL-10*)[^6-^7]. Hou Hongtao[^8] reported that curcumin protects the small-intestinal mucosal barrier in rats by inhibiting the expression of nuclear transcription factor (*NF-κB*) and *TNF-α*, and reducing the expression of inflammatory factors *TNF-α* and interleukin-6 (*IL-6*) during obstructive jaundice.

At present, studies on the protective effects of curcumin on the intestinal mucosal barrier have mostly focused on the pharmacological basis of its anti-inflammatory effects in rats and mice. No relevant reports have yet been found on the protective effects of curcumin on the intestinal mucosal barrier of livestock and poultry or on its molecular mechanisms. In this study, early-weaned piglets were challenged with *Escherichia coli* to induce diarrhea, placing the experimental piglets in a diarrheal state and establishing a stress model. The effects of curcumin on ileal mucosal morphology, tight junction proteins, inflammatory factor gene expression, and serum immunoglobulin levels in weaned piglets were investigated, to explore the feasibility of applying curcumin as an antibiotic substitute in feed additives and to identify appropriate supplementation levels, thereby providing a scientific basis for the development and application of green feed additives for pigs.

1 Materials and Methods

1.1 Experimental Design

Fifty healthy 21-day-old weaned "Duroc × Landrace × Yorkshire" piglets of similar parity and body weight were selected, with males and females each accounting for one half. They were randomly divided into 5 groups, with 10 replicates per group and 1 pig per replicate. The trial included a control group (group A), an olaquindox group (group B), and 3 curcumin groups (groups C, D, and E), which were fed the basal diet, the basal diet supplemented with 50 mg/kg olaquindox, and diets supplemented with 200, 300, and 400 mg/kg curcumin, respectively. The composition and nutrient levels of the basal diet are shown in Table 1. The pretrial period was 7 d. On day 4, the piglets were inoculated with *Escherichia coli* (after the bacterial strain had been shaken and cultured in LB liquid medium for 12 h, its concentration was determined, and a total of *Escherichia coli* at (1 ×10^9) CFU per pig was administered orally twice per day, morning and evening); the formal trial period was 21 d.

Table 1 Composition and nutrient levels of the basal diet (DM basis) %

Items	Content
Ingredients	
Corn	57.90
Soybean meal	25.47
Fish meal	5.00
Whey powder	4.00
Cream powder	4.50
Limestone	0.30
CaH_2PO_4	1.20
Mould inhibitor	0.10
Acidifier	0.30
L-Lys · HCl	0.25
Choline chloride	0.10
DL-Met	0.05
NaCl	0.30
Trace mineral premix1)	0.50
Vitamin premix2)	0.03
Total	100.00
Nutrient levels3)	
DE/ (MJ/kg)	14.02
CP	20.05
Ca	0.62
AP	0.50
Lys	1.19
Met	0.36
Thr	0.78
Trp	0.20
Met+Cys	0.65

1) The trace mineral premix provided the following per kilogram of the feed:

diet: Cu 16.5 mg, Fe 100 mg, Mn 35 mg, Zn 100 mg, Se 0.3 mg, I 0.3 mg.

2) The vitamin premix provided the following per kilogram of diet: VA 11 000 IU, VD_3 1 500 IU, VE 16 IU, VK 1 mg, folic acid 0.3 mg, nicotinic acid 15 mg, pantothenic acid 10 mg, biotin 2.5 mg, VB_1 1 mg, VB_2 4.0 mg, VB_{12} 0.01 mg.

3) DE was a calculated value, while the others were measured values.

1.2 Experimental Materials

Curcumin was purchased from Sigma (USA), diclazuril from Dalian Ronghai Biotechnology Co., Ltd., and the experimental *Escherichia coli* was obtained from the China Veterinary Culture Collection Center. Eosin staining solution was purchased from Biyuntian Biotechnology Co., Ltd.; RNAiso Plus reagent kit, reverse transcription reagent kit, and real-time fluorescence quantitative

PCR reagent kit were purchased from TaKaRa; and the serum immunoglobulin assay kit was purchased from Nanjing Jiancheng Bioengineering Institute.

1.3 Sample Collection

At the end of the feeding trial, all experimental piglets were fasted and 10 mL of blood was collected from the anterior vena cava into vacuum blood collection tubes. The samples were left in an ice bath for 30 min, centrifuged at 3 000 r/min for 10 min, and stored in a −20 °C freezer for subsequent determination of serum biochemical indices.

Twelve hours before the end of the feeding trial, feeding of the piglets was stopped. After the trial ended, 6 piglets from each group were randomly selected and euthanized by jugular venesection. The abdominal cavity was opened and the ileum was removed. A 5-10 cm segment from the middle portion was taken, and the outer wall and contents were washed clean with ice-cold physiological saline. After blotting dry with filter paper, the intestine was cut open. Using slides treated with diethyl pyrocarbonate (DEPC), the intestinal mucosa was gently scraped, and approximately 0.5 g of mucosa was placed into a 2 mL centrifuge tube and stored at −80 °C for determination of the relative mRNA expression levels of claudin-1, occludin, *ZO-1*, interleukin-1β (*IL-1*β), *IL-6*, *IL-10*, *TNF-*α, and Toll-like receptor 4 (*TLR4*). Another approximately 5 cm ileal segment was taken; after its contents were washed away with physiological saline, it was placed into 4% paraformaldehyde fixative to fix the intestinal tissue.

1.4 Determination of Intestinal Mucosal Morphology

After the intestinal tissue was fixed with paraformaldehyde, it was trimmed, dehydrated, cleared, wax-infiltrated, embedded, and sectioned. The sections were stained with hematoxylin–eosin (HE). Five fields of view were selected from each section for photography, and in each photograph two villi that were longest, intact, and oriented evenly and straight were selected. Villus height, villus width, and crypt depth were analyzed, and the villus height/crypt depth (V/C) value was calculated.

1.5 Determination of the Relative Expression Levels of Tight Junction Protein and Inflammatory Factor Genes

1.5.1 Primer Design

The registered porcine housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and the gene sequences of porcine claudin-1, occludin, *ZO-1*, *IL-1*β, *IL-6*, *IL-10*, *TNF-*α, and *TLR4* mRNA were downloaded from NCBI. Primers were designed using Primer Premier 5.0 software. The primers were synthesized by Beijing Liuhe Huada Gene Technology Co., Ltd.; the primer sequences are shown in Table 2.

Table 2 Primer sequences for real-time fluorescence quantitative PCR

Table 2 Primer sequences for fluorescence-based quantitative real-time PCR

Genes	Accession number	Primer sequence (5′−3′)	Product size/bp
Glyceraldehyde-3-phosphate dehydrogenase *GAPDH*	AF017079	F:GAAGGTCGGAGTG:GAACGGATR:CATGGGTAGAATCATACTC	119
Occludin	NM_{001163647}.1	F:ATGCTTTCTCAGC:GCCAGCGTAR:AAGGTTCCATAGCCTCGGT	176
Zonula occludens protein 1 *ZO-1*	XM003353439.1	F:GAGGATGGTCAGA:GACCGTGGTR:GGAGGATGCTGTTGTCTC	169
Claudin-1	NM_{001161635}.1	F:GGACTAATAGCCA:ATCTTTGTR:CAGCCATCCGCATCTTCT	88
Interleukin-1β *IL-1β*	NM_{214055}.1	F:ACCTGGACCTTCC:GTTCTCR:GGATTCTTCATCGGCTTC	124
Tumor necrosis factor-α *TNF-α*	NM_{214022}.1	F:ACGCTCTTCTGCC:CTACTGCR:TCCCTCGGCTTTGACATT	62
Toll-like receptor 4 *TLR4*	NM_{001113039}.1	F:TGTGGCCATCGT:TTGCTAACR:GGGACACCACGACAATAACC	71
Interleukin-10 *IL-10*	NM_{214041}.1	F:CACTGCTCTATTG:GCCTGATCTTCCR:AAACTCTTCACTGGG	136
Interleukin-6 *IL-6*	NM_{214399}.1	F: CTTCAGTCCAGTCGC-CTTCTR:GCATTTTGTCTGAGGTGGCA	113

1.5.2 RNA extraction and cDNA synthesis

Total RNA from intestinal mucosal tissue was extracted using an RNAiso Plus reagent kit. RNA quality was assessed by denaturing agarose gel electrophoresis, and its concentration was determined with a nucleic acid/protein analyzer. cDNA synthesis was performed according to the reverse-transcription reagent kit instructions, and the cDNA obtained after reverse transcription was stored at −80 °C until use.

1.5.3 Real-time fluorescence quantitative PCR

Each sample was run in triplicate. The reaction system (20 μL) was as follows: SYBR® Premix Ex *Taq*^{TM} (2×), 10 μL; PCR Forward Primer (10 μmol/L), 0.4 μL; PCR Reverse Primer (10 μmol/L), 0.4 μL; cDNA, 2.0 μL;

ddH_2O, 7.2 μL; reaction program: 95 °C for 1 min, 95 °C for 5 s, 60 °C for 30 s, 45 cycles.

Ileal mucosal morphology images for groups A, B, C, D, and E

Figure 1: Ileal mucosal morphology images for groups A, B, C, D, and E

1.6 Serum immunoglobulin indices

Serum immunoglobulin G (IgG) and immunoglobulin M (IgM) levels were determined by immunoturbidimetry.

1.7 Data processing

SPSS 17.0 software was used for one-way analysis of variance (one-way ANOVA), and Duncan' s method was used for multiple comparisons. Differences were considered significant at $P<0.05$, and the results are expressed as "mean ± standard deviation."

The relative expression levels of target genes were calculated using the $-\Delta\Delta$CT method. $\Delta\Delta$CT is the relative value of ΔCT for each experimental group compared with the control group; the relative expression level of the target gene was expressed as $2^{-\Delta\Delta CT}$.

2 Results and analysis

2.1 Effects of curcumin on ileal mucosal morphology in weaned piglets

Observation of ileal tissue sections under a light microscope showed that in piglets of group A, the ileal villi were atrophied and shed, arranged irregularly, and exhibited breakage, twisting, and deformation; whereas compared with groups A, B, and C, groups D and E had intact intestinal mucosal structures, distinct layers, and ileal villi that were relatively orderly and densely arranged, with regular morphology (Fig. 1). As shown in Table 3, the ileal villus height and V/C value in groups D and E were significantly higher than those in groups A, B, and C ($P<0.05$), and group E was significantly higher than group D ($P<0.05$); the ileal villus width in groups B, D, and E was significantly decreased ($P<0.05$); the ileal crypt depth in groups D and E was significantly lower than that in groups A, B, and C ($P<0.05$); and there were no significant differences in villus height, crypt depth, or V/C value among groups A, B, and C ($P>0.05$).

A B C

D E

Figure 1. Ileal mucosal morphology in different groups (200×)

Fig.1 Ileal mucosal morphology in different groups (200×)

Table 3. Morphological analysis of ileum mucosa epithelium of weaning piglets

Items	A	B	C	D	E
Items	A	B	C	D	E
Villus height/μm	208.05$±27.12 ^a				
	206.95±22.37 ^a				
	212.18±25.27 ^a				
	222.19±28.67 ^b				
	238.61±26.90 ^c				
	$|Villus width/ m|$116.14±23.89 ^b				
	103.47±27.67 ^a				
	112.60±22.67 ^b				
	102.09±17.73 ^a				
	101.14±16.65 ^a				
	$|Crypt depth/ m|$155.70±32.44 ^b				
	148.03±28.27 ^b				
	145.11±29.32 ^b				
	132.41±28.58 ^a				
	133.06±25.54 ^a				
	$|V/C|$1.34±0.26 ^a				
	1.36±0.26 ^a				
	1.41±0.26 ^a				

In the same row, values with different small letter superscripts mean significant difference ($P<0.05$), while with the same or no letter superscripts mean no significant difference ($P>0.05$). The same as below.

2.2 Effects of curcumin on the expression of claudin-1, occludin and *ZO-1* mRNA in the ileal mucosa of weaned piglets

As shown in Table 4, there were no significant differences in claudin-1 mRNA expression among the groups ($P>0.05$); the relative expression levels of occludin and *ZO-1* mRNA in groups D and E were significantly higher than those in groups A and B ($P<0.05$), while there were no significant differences among groups A, B and C ($P>0.05$).

Table 4. Effects of curcumin on the relative expression levels of claudin-1, occludin and *ZO-1* mRNA in the ileum mucosa of weanling piglets

Items	A	B	C	D	E
Claudin	1.00$±0.18|1.09±0.14|1.14±0.29|1.31±0.25|1.42±0.43||*Occludin*|1.00±0.26 < *sup* > *a* < */sup* > |0.93±0.20 < *sup* > *a* < */sup* > |1.38±0.33 < *sup* > *ab* < */sup* > |1.77±0.47 < *sup* > *b* < */sup* > |1.86±0.35 < *sup* > *b* < */sup* > ||∗*ZO*−1∗ |1.00±0.32 < *sup* > *a* < */sup* > |1.02±0.25 < *sup* > *a* < */sup* > |1.18±0.30 < *sup* > *a* < */sup* > |1.73±0.28 < *sup* > *b* < */sup* > |1.90±$0.15b				

2.3 Effects of curcumin on the expression of inflammatory factors and *TLR4* mRNA in the ileal mucosa of weaned piglets

As shown in Table 5, compared with group A, the relative mRNA expression levels of *IL-1β*, *TNF-α*, and *IL-6* in the ileal mucosa of groups D and E were significantly decreased (P<0.05), whereas the relative mRNA expression level of *IL-10* was significantly increased (P<0.05). The differences among groups A, B, and C were not significant (P>0.05). The relative mRNA expression level of *TLR4* in groups B, D, and E was significantly lower than that in group A (P<0.05), whereas the difference between group C and group A was not significant (P>0.05).

Table 5 Effects of curcumin on the relative expression levels of inflammatory

cytokines and *TLR4* mRNA in the ileal mucosa of weaning piglets

Items	A	B	C	D	E

Items	A	B	C	D	E

Interleukin-1β *IL-1β*	1.00$±0.28 < *sup > b < /sup >* |0.85±0.23 < *sup > ab < /sup >* |0.76±0.22 < *sup > ab < /sup >* |0.54±0.18 < *sup > a < /sup >* |0.47±0.14 < *sup > a < /sup >*	1.00$±0.16 < *sup > b < /sup >* |1.08±0.23 < *sup > b < /sup >* |0.83±0.10 < *sup > ab < /sup >* |0.62±0.27 < *sup > a < /sup >* |0.57±0.15 < *sup > a < /sup >*			
Tumornecrosisfactor-α TNF-α		*Interleukin-6 − 6 ∗ IL − 6 ∗* |1.00±0.19 < *sup > b < /sup >* |0.83±0.14 < *sup > ab < /sup >* |0.82±0.27 < *sup > ab < /sup >* |0.64±0.15 < *sup > a < /sup >* |0.60±0.16 < *sup > a < /sup >*			
		Interleukin − 10 ∗ IL − 10 ∗ |1.00±0.21 < *sup > a < /sup >* |0.97±0.15 < *sup > a < /sup >* |1.26±0.25 < *sup > ab < /sup >* |1.68±0.31 < *sup > bc < /sup >* |1.81±0.28 < *sup > c < /sup >*			
		Toll − likereceptor4∗			

2.4 Effects of curcumin on serum immunoglobulin levels in weaned piglets

As shown in Table 6, the serum IgG and IgM levels in groups D and E were significantly higher than those in groups A, B, and C (P<0.05). The serum IgG level in group C was significantly higher than that in group A (P<0.05), whereas the IgM level did not differ significantly from that in group A (P>0.05). The serum IgG and IgM levels in group B were increased to varying degrees compared with group A, but the differences were not significant (P>0.05).

Table 6 Effects of curcumin on serum immunoglobulin levels of weaning piglets mg/mL

Items	A	B	C	D	E
Immunoglobulin G IgG	2.62±0.28 < *sup* > *a* < */sup* >				
	|2.89±0.19 < *sup* > *ab* < */sup* >				
	|3.13±0.22 < *sup* > *b* < */sup* >				
	|3.60±0.20 < *sup* > *c* < */sup* >				
	|3.91±0.35 < *sup* > *c* < */sup* >				
	||*ImmunoglobulinMIgM*|0.55±0.09 < *sup* > *a* < */sup* >				
	|0.60±0.03 < *sup* > *a* < */sup* >				
	|0.64±0.06 < *sup* > *a* < */sup* >				
	|0.87±0.04 < *sup* > *b* < */sup* >				
	|0.88±$0.06b				

3 Discussion

3.1 Effects of curcumin on ileal mucosal morphology in weaned piglets

The small intestine is the main site of nutrient absorption and transport, and its functional unit is the intestinal villus. Villus height, villus width, crypt depth, and V/C are important indicators that directly reflect the structural and functional integrity of the small-intestinal mucosa[^9]. Villus height reflects the absorptive capacity of the small intestine for nutrients; crypt depth reflects the maturation rate of epithelial cells in the small-intestinal mucosa; and V/C reflects the absorptive capacity of the small intestine, with a larger ratio indicating stronger absorptive capacity[^{10}]. Early weaning can markedly reduce villus height and increase crypt depth, thereby causing decreased digestive and absorptive capacity, diarrhea, and growth retardation[^{11}]. The results of this experiment showed that adding curcumin to the diet significantly increased ileal villus height and V/C values and reduced ileal villus width and crypt depth; among these, supplementation with 300 or 400 mg/kg curcumin was relatively more pronounced, and its effect was superior to that of quinocetone. This is consistent with results showing that curcumin promotes the growth performance of piglets[^{12}], indicating that curcumin can improve the epithelial morphology of the ileal mucosa, thereby enhancing the digestion and absorption of nutrients and exerting a growth-promoting effect on the body.

3.2 Effects of curcumin on tight-junction proteins and inflammatory cytokine gene expression in the ileal mucosa of weaned piglets

Tight junctions are an important component of the intestinal mucosal mechanical barrier and mainly include multiple proteins such as occludin, claudin, ZO-1, and junctional adhesion molecules[^{13}]. ZO-1 plays an important role in maintaining and regulating the integrity of tight-junction complexes. As one of the most important structural proteins in tight junctions, *occludin* can bind to proteins such as ZO-1 and together constitute the scaffold of tight junctions. Once *occludin* enters the tight junction, it reduces the permeability of the membrane to which it is connected, thereby protecting the intestinal mucosal barrier. *Claudin* also plays an important role in regulating cell junctions and adhesion. Disruption of tight junctions is often accompanied by changes in the expression and distribution of tight-junction proteins[^{14}]. Wang Gong[^5] found that curcumin can ameliorate tight-junction damage and intestinal mucosal barrier dysfunction mediated by H_2O_2, and upregulate the expression of occludin and ZO-1. The results of this experiment showed that adding 300 or 400 mg/kg curcumin to the diet significantly increased the relative mRNA expression of occludin and ZO-1 in the ileal mucosa, indicating that curcumin can regulate the expression of the tight-junction proteins occludin and ZO-1 in intestinal epithelial cells. This is consistent with the finding that curcumin significantly reduces diamine oxidase (DAO) activity and D-lactate content in the plasma of

piglets challenged with *Escherichia coli*[^{12}], further indicating that curcumin can alleviate damage to the intestinal mucosal barrier caused by *E. coli*, effectively promote the formation of intercellular tight-junction structures, thereby reducing intestinal mucosal permeability in early-weaned piglets and enhancing intestinal mucosal mechanical barrier function. This is similar to the research results of Wang Tao et al.[^{15}], who considered that curcumin can markedly improve the structural organization and degree of pathological injury of the small intestine in rats, reduce intestinal mucosal permeability, and maintain the integrity of intestinal mucosal mechanical barrier function.

3.3 Effects of curcumin on serum immunoglobulin levels in weaned piglets

Serum immunoglobulin concentration or titer is an important indicator reflecting the level of humoral immunity in the body. IgM is the main antibody in the primary immune response; IgG is the main antibody mediating the humoral immune response and plays an important role in defense mechanisms such as antibacterial and antiviral responses[^{16}]. This study showed that, as the supplemental concentration of curcumin increased, serum IgG and IgM levels tended to rise, and the 300 or 400 mg/kg curcumin groups were significantly higher than the control group and the quinocetone group, indicating that curcumin can enhance the immune function of weaned piglets.

The mucosal immune system of the digestive tract plays an important role in resisting invasion by pathogenic bacteria and maintaining the health of the body. Toll-like receptors (TLRs) are a family of pattern-recognition receptors related to immunity that have been discovered in recent years. As one of the major members of this family, TLR4 can recognize conserved components of specific microorganisms and activate the NF-κB and mitogen-activated protein kinase (MAPKs) signaling pathways, thereby inducing the expression of various cellular inflammatory factors[^{17}-18]. The cytokines *IL-1β*, *IL-6*, and *TNF-α*, as inflammatory factors, mainly enhance the body' s anti-infective capacity by promoting inflammatory responses[^{19}]. *IL-10* is an immunosuppressive cytokine secreted by lymphocytes such as T cells and B cells; its main functions are anti-infection and maintenance of intestinal immune homeostasis[^{20}]. At present, the anti-inflammatory effects of curcumin on the intestinal mucosa have been confirmed in many studies. Curcumin can inhibit the activities of NF-κB and p38, thereby reducing the expression of the inflammatory cytokines *TNF-α* and *IL-1β* in intestinal epithelial cells of rats with MTX-induced enteritis and increasing the expression of the anti-inflammatory factor *IL-10*[^{21}]. Hou Hongtao et al.[^8] found that curcumin reduced the expression of the inflammatory factors *TNF-α* and *IL-6* during obstructive jaundice by inhibiting the expression of *NF-κB* and *TNF-α*, thereby protecting the intestinal mucosal barrier of rats. In addition, Zeng et al.[^{22}] reported that curcumin exerted anti-inflammatory effects in rats with colitis by inhibiting the TLR4/NF-κB signaling pathway and the expression of interleukin-27 (*IL-27*) in intestinal epithelial cells. The

results of the present experiment showed that supplementation with 300 or 400 mg/kg curcumin significantly downregulated the mRNA expression of *IL-1β*, *IL-6*, *TNF-α*, and *TLR4* in the ileal mucosa of weaned piglets, and upregulated *IL-10* mRNA expression. This indicates that curcumin can improve the immune barrier function of the intestinal mucosa and alleviate the damage caused by *Escherichia coli* to the intestinal mucosa. It is therefore inferred that the protective effect of curcumin against the inflammatory response induced by *E. coli* challenge in the ileal epithelial cells of piglets may be exerted by inhibiting the NF-κB and MAPKs signaling pathways, reducing the expression of inflammatory factors and enhancing the expression of anti-inflammatory factors; the mechanism of action requires further verification.

4 Conclusion

Supplementation with 300 or 400 mg/kg curcumin significantly increased ileal villus height and the V/C ratio in weaned piglets, reduced ileal villus width and crypt depth, and enhanced the mRNA expression of occludin and *ZO-1* in the ileal mucosa. This indicates that curcumin can improve ileal mucosal epithelial morphology, promote the formation of intercellular tight-junction structures, and maintain the integrity of the mechanical barrier function of the intestinal mucosa.

Supplementation with 300 or 400 mg/kg curcumin significantly increased serum IgG and IgM levels in weaned piglets, downregulated the mRNA expression of the inflammatory factors *IL-1β*, *IL-6*, *TNF-α*, and *TLR4*, and upregulated the mRNA expression of the anti-inflammatory factor *IL-10*. It effectively stimulated nonspecific immunity in piglets, thereby enhancing the body' s immune capacity, with effects superior to those of quinocetone.

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Effects of Curcumin on Ileum Mucosal Morphology, Gene Expression of Tight Junction Proteins and Inflammatory Cytokines, and Serum Immunoglobulin Levels of Early Weaning Piglets

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Abstracts: This experiment was conducted to study the effects of curcumin on ileum mucosal morphology, gene expression of tight junction proteins and inflammatory cytokines, and serum immunoglobulin levels of early weaning piglets. Fifty Duroc×Landrace×Large White healthy piglets (male to female ratio was 1 1) with similar parity and body weight, weaned at 21 days of age, were randomly allocated into 5 groups with 10 replicates in each group and 1 pig in each replicate. The dietary treatments were the basal diet (control group), and the basal diet supplemented with 50 mg/kg quinocetone, or 200, 300 or 400 mg/kg curcumin (experimental groups). The preliminary trial lasted for 7 days and piglets were orally challenged with enterotoxigenic *Escherichia coli* on the fourth day. The experiment lasted for 21 days. The results showed as follows: 1) compared with the control group, supplementation with 300 or 400 mg/kg curcumin significantly improved villus height and villus height/crypt depth ratio (V/C)

(P<0.05), significantly reduced crypt depth and villus width (P<0.05), significantly increased the relative expression levels of occludin and zonula occluden-1 (*ZO-1*) mRNA (P<0.05), significantly decreased the relative expression levels of interleukin-1β (*IL-1β*), tumor necrosis factor-α (*TNF-α*), interleukin-6 (*IL-6*), and Toll-like receptor 4 (*TLR4*) mRNA (P<0.05), and significantly increased interleukin-10 (*IL-10*) mRNA relative expression level, serum immunoglobulin G (IgG) and immunoglobulin M (IgM) levels (P<0.05). 2) The villus width and *TLR4* mRNA relative expression level were significantly decreased by quinocetone supplementation (P<0.05), but the other indicators were not significantly affected compared with the control group (P>0.05). It is suggested that 300 or 400 mg/kg curcumin supplementation was more effective than quinocetone in improving ileum mucosal barrier integrity, morphology, and immune status of weaning piglets.

Key words: curcumin; weaning piglets; ileal mucosal morphology; tight junction protein;

inflammatory cytokine; immunoglobulin

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Note: Figure translations are in progress. See original paper for figures.