Effect of dietary licorice flavonoids powder on performance, intestinal immunity and health of weaned piglets

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Abstract

Licorice flavonoids, a bioactive substance derived from glycyrrhiza, have been reported for many pharmacological properties and are beneficial to animal health. This study aimed to explore the effects of licorice flavonoids powder (LFP) on growth performance and intestinal health of piglets. A total of 96 weaned piglets were randomly assigned into 4 treatments and supplemented with 0, 50, 150 and 250 mg/kg LFP for 5 weeks. Dietary LFP supplementation trended to increase (P = 0.068) average daily gain (ADG) and reduce (P = 0.089) the feed intake/body gain (F/G) of piglets than that of the control group during 15-35 d; and concentrations of LFP supplementation reduced (P < 0.01) diarrhea index during14-35 d and 0-35 d. Piglets fed on diets supplied with LFP had a lower (P < 0.05) pH in cecum and colon. Dietary LFP supplementation increased (P < 0.01) the villi height and the ratio of villi height/crypt depth in duodenum, and reduced (P < 0.05) crypt depth in duodenum. Compared with the control group, 250 mg/kg LFP supplementation up-regulate (P < 0.05) the mRNA level of *OCLN* in ileum. Meanwhile, dietary LFP supplementation down-regulated (P < 0.05) mRNA abundance of *IL-1β*, *IL-8* and *INOS* in duodenum. 150 mg/kg LFP supplementation down-regulated (P < 0.05) mRNA abundance of *IL-1β* and 250 mg/kg LFP up-regulated (P < 0.05) the expression of *IL-10* in ileum.

In summary, dietary LFP supplementation has a trend to improve the performance of weaning piglets, those improvements are accompanied by reduction in diarrhea, enhancement of intestinal morphological structure, barrier function, immune function, and development. In general, 150 mg/kg LFP supplementation is more effective.

Keywords: licorice flavonoids powder, performance, intestinal immunity, barrier function, piglets

1. INTRODUCTION

Weaning is often related varying intestinal disorders in piglets including low feed intake and depressed growth rate (Pluske *et al.*,1997). During the post-weaning period, significant changes appear in the structure and function of the small gut. All these changes can lead to diarrhea, a reduced growth rate, and in some cases even death in weaned pigs (Hedemann *et al.*,2003; Peace *et al.*,2011; Spreeuwenberg *et al.*,2001; Hu *et al.*,2013). For example, it destroys the epithelial barrier function, up-regulates the expression of pro-inflammatory cytokines, and reduces the expression of tight junction proteins. Previously, antibiotics were widely used to avoid the negative effects of early weaning stress and served as growth promoters in diets. But, misuse of antibiotics has caused bacterial resistance and antibiotics-residue in humans and livestock (Rossolini *et al.*,2014; Sajid *et al.*,2016; Campagnolo *et al.*,2002). Since July 1, 2020, the addition of antibiotics in feed has been completely banned in China, that stimulate to look for alternatives to antibiotics. Herbal and phytogenic products have received increasing attention in the matter of improving performance and health of animals in China.

Licorice, a traditional herbal medicine and stemming from ancient Chinese practices, has been regularly used to suppress inflammation (Mamedov *et al.*,2019). Licorice flavonoids powder (LFP) contains various bioactive compounds including flavonoids and glycyrrhizin that are reported for many pharmacological properties such as anti-microbial (Ahn *et al.*,2012; Long *et al.*,2013), antitumor (Wang *et al.*,2013; Li *et al.*,2014), anti-inflammatory (Wu *et al.*,2011), antiviral (Yeh *et al.*,2013) and immuno-modulator (Hong *et al.*,2009; Li *et al.*,2012). Hence, the significant biological activities of licorice extract render it a potential

alternative for in-feed antibiotics. Licorice improves the microscopic structure of the colonic mucosa in acetic acid-induced ulcerative colitis in rats and down-regulated expression of *TNF-α*, *IL-6* and *IL-1β* (Samadnejad *et al.*,2012). Supplementation with 1% licorice extracts to the diet have shown to increase the body weight (BW) and feed intake/body gain (F/G) of broilers (Appusamy *et al.*,2014). However, the application of LFP in livestock are limited and whether it could promote intestinal health and growth performance of weaned piglets remain unclear. Therefore, the present project is aimed at investigating the potential impact of LFP on growth performance, intestinal mucosal immunity, small intestinal microarchitecture along with expressions of tight junction-related genes, development-related genes and inflammation-related genes in intestinal tissues in weaned piglets.

2. MATERIALS AND METHODS

2.1 Animals, diet and experimental design

A total of 96 weaned piglets (Duroc × Landrace × Yorkshire, DLY) with average BW of 8.05 ± 0.23 kg were randomly divided into 4 dietary treatments with 6 replicates and 4 pigs per pen in an experimental unit. Piglets were fed on a corn-soybean meal-basal diet (BD) supplemented with 0, 50, 150 or 250 mg/kg LFP. The BD (Supplementary Table 1) was formulated to meet the nutrient requirements of piglets according to the NRC (2012), which was matched the formulation used in our laboratory (Chen *et al.*,2018), and provided in powder form. Licorice flavonoids powder was derived from the residue after water extraction from *Glycyrrhiza uralensis Fisch.*, *Glycyrrhiza inflata Bat.* and *Glycyrrhiza glabra L.* and obtained from glycyrrhizae residue followed by alcohol extraction, alkali solution, acid precipitation, vacuum distillation and drying. It had good water solubility and contained 60%

flavonoid and 10% glycyrrhizin and which was supplied by Kaimeijia Biotechnology Company of China. The piglets were housed in a floor pen with room temperature maintained at 28 ± 1°C and had free access to feeds and water. The trial lasted for 5 weeks. The diarrhea scoring criteria was the same as described previously (Che *et al.*,2019). Scores of pigs had been recorded following observations of per piglet and signs of stool consistency in the pen at 8:00 and 16:00 every day. Diarrhea score was based on the following index that 0, normal (feces firm and well formed); 1, soft consistency (feces soft and formed); 2, mild diarrhea (fluid feces, usually yellowish); and 3, severe diarrhea (feces-watery and projectile), and diarrhea index = diarrhea scores/total of trial piglets/days. Individual BW of pigs and feed intakes of per pen were measured weekly to obtain the average weight of per pen pigs to determine average daily gain (ADG), average daily feed intake (ADFI) and F/G.

2.2 Sample collection

At the end of the experiment, all piglets were fasted for 8 hours and weighted, and then 6 piglets per group with average body weight were euthanized by injecting pentobarbital sodium, and abdomen was opened to remove duodenum, ileum and jejunum, emptied and sampled immediately. The middle sections of duodenum, ileum and jejunum were collected, some fixed in 4% paraformaldehyde solution for intestinal histology analysis, and some snap-frozen in liquid nitrogen, and stored at -80 °C until use.

2.3 Histomorphometric measurements

Duodenual, ileal and jejunal samples were preserved in 4% paraformaldehyde solution. Afterward, the intestinal samples of 3 pigs from each group were dehydrated and infiltrated with paraffin wax. The samples were sectioned at 5 μm thickness and stained with

hematoxylin and eosin (HE). Crypt depth and villus height were measured at 10× magnification using digital trinocular microscope camera microscope (BA400 Digital, Motic, Fujian, China) and Image-Pro plus 6.0 (Media Cybernetics, Maryland, USA) in at least 10 well-oriented villus and crypt columns. The ratio of villus height/crypt depth (VH/CD) used to be calculated.

2.4 The pH of gastrointestinal contents and secretory immunoglobulin A in intestinal mucosal

The pH value of the stomach and intestinal contents was detected by a portable pH meter (PHS-3C, Lei-Ci, Shanghai, China). All the digesta of a single segment (the stomach and three intestinal section) were pooled and homogenized by glass rod, then pH was determined. The average pH of each digesta sample was based on three recording.

Approximately 1.0 g of mucosal scrapings was homogenized in 9 mL PBS. After centrifugation at 3000×g for 15 min at 4 °C, and the supernatant was applied for the measurement of secretory IgA using the ELSIA kit (No. MM-3623401, MeiMian, Jiangsu, China). Concentrations of protein had been decided with the bicinchoninic acid (BCA) protein assay kit (No. D1001-A, MeiMian, Jiangsu, China). For each measurement, the compared samples were run on the same plate and each measurement was performed in triplicate.

2.5 Q-PCR analyses

Total RNA of intestinal mucous sample was extracted by using Trizol (TaKaRa, Dalian, China). After assessing RNA quantity and quality, the cDNA was synthesized by using PrimeScript RT reagent kit (No. RR047A, TaKaRa, Dalian, China). Q-PCR was performed

on QuantStudio 5 Flex system (Applied Biosystems, Foster, USA) using the SYBR kit (No. RR820A, TaKaRa, Dalian, China). The primers (Supplementary Table 2) of 3 tight junction-related genes, 9 inflammation-related genes, 3 intestinal development-related genes, and 2 house-keeping genes (*GAPDH* and *β-ACTIN*) were designed using Primer Express 3.0 (Applied Biosystems, Foster, USA). The relative mRNA level was quantified as earlier described the use of $2^{-\Delta\Delta Ct}$ method (Xu *et al.*,2017).

2.6 Statistical analysis

Statistical analysis was performed using SAS 9.4 software. The statistical significance of difference between groups was evaluated using one-way analysis of variance (ANOVA), followed by Tukey's test for multiple comparisons. The linear and quadratic relationship between dose of LFP supplementation and growth performance, diarrhea index, the pH of gastric and intestinal contents, intestinal mucosal SIgA, intestine morphology, mRNA abundances of tight junction-related genes, intestinal developmental-related genes and inflammation-related genes were checked using the CONTRAST statement of PROC GLM of SAS 9.4 (SAS institute Inc., Cary, NC, USA), respectively. Data are presented as means with their standard error of the mean (SEM); significance was declared at $P < 0.05$, and a tendency was considered when $0.05 \leq P < 0.10$.

3. RESULTS

3.1 Effect of dietary LFP supplementation on performance

Data of the growth performance (Table 1) shows that dietary LFP supplementation has some improvement on the growth performance. Compared with the control group, dietary LFP supplementation had a trend to increase ($P = 0.068$) ADG and reduce ($P = 0.089$) the

F/G of piglets during 14-35 d. Dietary LFP supplementation quadratically (P = 0.028) reduced the F/G during the whole period.

During the late and whole period, the addition of LFP significantly reduced (P < 0.05) the diarrhea index, but no differences were observed among the groups of 50, 150 and 250 mg/kg (Table 2).

3.2 Effect of dietary LFP supplementation on the pH of gastrointestinal contents and intestinal mucosa SIgA

The pH of gastrointestinal contents is presented in Table 3. Dietary LFP supplementation significantly reduced (P < 0.05) pH in colon. Compared with the 0 mg/kg group, 150 and 250 mg/kg LFP supplementation significantly decreased (P < 0.05) the pH in cecum, respectively. Dietary LFP supplementation did not affect the pH in the stomach among the 4 treatments.

The effect of LFP supplementation on intestinal mucosa SIgA in the duodenum, ileum and jejunum of piglets is showed in Table 3. Accordingly, there were no differences in SIgA of the duodenum, ileum and jejunum response to dietary LFP treatment.

3.3 Effect of dietary LFP supplementation on intestinal histomorphology

Licorice flavonoids powder supplementation exhibits an impact on intestinal morphology (Table 4). Dietary LFP supplementation significantly increased the villus height and VH/CD, and decreased (P < 0.05) crypt depth of duodenum in piglets. Compared with the control group, dietary 250 mg/kg LFP supplementation increased (P < 0.05) the villus height of ileum, and 150 mg/kg LFP inclusion decreased (P < 0.05) crypt depth and trended to increase (P=0.087) VH/CD in ileum.

3.4 Effect of dietary LFP supplementation on mRNA abundances of tight junction-related

genes

Effects of LFP supplementation on the expression of 3 tight junction-related genes in small intestinal mucosal tissue were also investigated (Fig.1). Relative to the 0 mg/kg LFP group, LFP supplementation trended to up-regulate (P =0.074) the mRNA level of *OCLN* in duodenum (Fig.1A) and 250 mg/kg LFP supplementation up-regulate ($P < 0.05$) the mRNA level of *OCLN* in ileum (Fig.1C). In addition, 50 mg/kg LFP supplementation up-regulated ($P < 0.05$) the mRNA levels of *TJP-1* and trended to up-regulate (P =0.072) the mRNA level of *TJP-2* in ileum (Fig.1C).

3.5 Effect of dietary LFP supplementation on mRNA abundances of intestinal developmental-related genes

We further investigated the effect of LFP supplementation on expression of 3 intestinal developmental-related genes (Fig.2). There were no differences on expression of 3 intestinal developmental-related genes of the duodenum, ileum and jejunum in response to levels of dietary LFP treatment.

3.6 Effect of dietary LFP supplementation on mRNA abundances of inflammation-related genes

We explored mRNA abundance of 9 inflammation-related genes in small intestinal mucosal tissue of piglets (Fig.3). All LFP supplementation down-regulated ($P < 0.05$) the mRNA levels of 2 inflammation-related genes (*IL-1β*, *IL-8*) in the duodenum (Fig.3A), 150 and 250 mg/kg LFP group down-regulated ($P < 0.05$) the mRNA levels of *INOS* in the duodenum (Fig.3A). LFP supplementation trended to down-regulate (P =0.065) the mRNA levels of *ICAM-1* in duodenum (Fig.3A). LFP inclusion trended to down-regulate (P =0.099)

194 the mRNA levels of *IL-8* and 50 mg/kg dietary LFP inclusion down-regulated ($P < 0.05$) the
195 mRNA levels of *IL-2* in jejunum (Fig.3B). 150 mg/kg LFP supplementation down-regulated
196 ($P < 0.05$) expression of *IL-1β* in the ileum and 150 mg/kg LFP group exhibited a lowest
197 mRNA abundance (Fig.3C). Inclusion of 250 mg/kg LFP up-regulated ($P < 0.05$) the
198 expression of *IL-10* in ileum compared with the control group (Fig.3C).

199 **4. DISCUSSION**

200 Supplementation with 1% licorice extract is reported to improve the BW and F/G of
201 broilers (Appusamy *et al.*,2014). In the present study, dietary LFP supplementation improved
202 ADG and F/G (Table 1), and reduced diarrhea index (Table 2). Licorice flavonoids powder
203 contains many bio-active compounds (flavonoids and glycyrrhizin), and have many
204 pharmacological properties. Flavonoids with antimicrobial and anti-inflammatory activities
205 improves the digestion and absorption of feed nutrients by inhibiting the competition between
206 harmful bacteria and nutrients in animals (Pastorino *et al.*,2018). Previously, studies reveal
207 that piglets supplied with dietary flavonoids exhibited better growth performance in normal
208 (Cui *et al.*,2019) or stressed condition (Zhu *et al.*,2015). Therefore, the beneficial effect of
209 dietary LFP supplementation on growth performance of weaned piglets may partly be
210 attributed to its antimicrobial and anti-inflammatory activities. Furthermore, LFP contain 10%
211 glycyrrhizin, which is 50 times sweeter than regenerated sugar (Isbrucker *et al.*,2006), and
212 this may be well explained that 50 and 150 mg/kg LFP supplementation improved the feed
213 intake of piglets (Table 1). Furthermore, glycyrrhizin belongs to organic acid, thus may
214 contribute to an acidic environment in the gastrointestinal tract. The pH of intestinal content is
215 an indicator of intestinal well-being and microbial activity (Nyachoti *et al.*,2006). The acidic

environment is an ideal condition for the nutrient digestion and absorption of nutrients in the gut of animal, it can also promote beneficial bacteria and inhibits the pathogenic bacteria. In the present study, levels of LFP supplementation (50-150 mg/kg) decreased pH in jejunum, cecum and colon of piglets (Table 3), suggested LFP enhanced the gastrointestinal health status. Licorice flavonoids powder also exhibited a tendency to decrease stomach pH, while the low level of pH in the stomach, in turn, increased digestion of certain nutrients, thus promoted to increase villus height in the intestine of weaned piglets (Xiong *et al.*,2015).

The integrity of the intestinal mucosa is a prerequisite for the digestion and absorption of nutrients, which is closely related to the growth of piglets. The atrophy of villi is caused by either a reduced crypt cell renewal rate or increased villous epithelial cell loss rate in piglets during the weaning period (Campbell *et al.*,2013). The increase of villi height and VH/CD indicates improved digestion and absorption of nutrients. Furthermore, a lower VH/CD is related to microbial challenges and antigenic components in the feed (Huang *et al.*,2012). The present results showed that LFP supplementation elevated duodenal and ileum mucosal integrity by improving duodenal and ileal villus height and the rate of VH/CD and reducing their crypt depth (Table 4). The results are similar to these previous studies that licorice extract improves the microstructure of colonic mucosa in piglets suffered from acetic acid induced ulcerative colitis in rats (Samadnejad *et al.*,2012) and licorice aqueous extract exert its action on stimulating the growth of intestinal crypt cells in polyamine-depleted IEC-6 cells model (He *et al.*,2012).

Intestinal morphology reflects the health condition of the gut, changes in morphology may affect the performance of piglets (Li *et al.*,1990). Licorice flavonoids powder improved

238 the morphology of small intestine, especially in duodenum. The reason possibly is that LFP
239 were decomposed into different products in different location of the gastrointestinal intestinal
240 tract, such as glycyrrhizic acid and glycyrrhetinic acid, then they be absorbed to improve the
241 morphology. Duodenum may be the main absorption sites of LFP, but only some weak effects
242 in jejunum and ileum.

243 To explore the effect of LFP on intestinal health of weaned piglets, we further
244 investigated the expression of intestinal barrier genes and developmental-rated genes in the
245 gut. Tight junction proteins (TJP-1, TJP-2, and OCLN) play a critical role in the integrity of
246 the intestinal barrier by closing the paracellular space between epithelial cells, thereby
247 preventing the translocation of intestinal bacteria and movements of various across
248 paracellular pathway (Ulluwishewa *et al.*,2011). In this study, LFP supplementation increased
249 the mRNA levels of *OCLN*, *TJP-1* and *TJP-2* in small intestine tissues of weaned piglets
250 (Fig.1), indicating improvement of the intestinal barrier integrity. Previous studies revealed
251 that different sources of flavonoid compounds alleviate distress induced intestinal epithelial
252 cell injury and restore the expression of tight junction protein TJP-1 and OCLN (Watson *et*
253 *al.*,2004; Wang *et al.*,2016; Carrasco-Pozo *et al.*,2013). Licorice flavonoids powder has more
254 than 300 phenols and flavonoids belong to phenols, while propolis polyphenols is found to
255 enhance the expressions of TJP-1 and OCLN in intestinal monolayer epithelial cells, and
256 enhance the function of intestinal barrier signaling pathways (Wang *et al.*,2016). Among the 3
257 developmental-rated genes, IGF-1 decreased the intestinal permeability of rats and enhanced
258 the barrier function of intestinal epithelial cells (Lorenzo-Zuniga *et al.*,2006). GLP-2,
259 mediated by GLP-2R, stimulate the proliferation of epithelial cells, promote the increase of

villi height and crypt depth in the small intestine, and has been shown to affect the regulation of gastric drainage and intestinal absorption in relation to growth (Kato *et al.*,1999; Burrin *et al.*,2000). Although there were not statistically differences, piglets received LFP exhibited higher mRNA profiles of 3 intestinal developmental-related genes in duodenum, jejunum and ileum in response to dietary LFP in this study (Fig.2). The stimulation expression of GLP-2 and IGF-1 in the intestine improves the proliferation of intestinal epithelial cells, and increases the function of intestinal barrier (Petersen *et al.*,2001). These trial results of intestinal barrier genes revealed that LFP had a beneficial effect on the intestinal health of weaned piglets. The beneficial effect of the intestinal barrier may be correlated with flavonoids, the main active ingredient of the LFP.

Intestine is the major immune organ in animals. SIgA plays a crucial role in preventing pathogen adhesion to host cells in defense of mucosal epithelia, thus blocking transmission and further infection (Corthesy *et al.*,2010; Ren *et al.*). Intestinal SIgA content was used as an indicator to evaluate intestinal mucosal immunity (Zhang *et al.*,2007). In this study, compared with the control group, piglets received LFP exhibited higher SIgA values (Table 3). We further investigated the effect of LFP on the expression of 9 inflammation-related genes in the guts of piglets. Licorice flavonoids powder supplementation down-regulated the expression of *IL-1β* in the duodenal mucosa and ileum mucosa, *IL-2* in jejunum mucosa and *IL-8* in the duodenum (Fig.3). Pro-inflammatory cytokines (IL-2 and IL-1β) have been proven to mediate host inflammatory responses and prevent susceptibility to infection (Al-Sadi *et al.*,2007). IL-8 is a potent pro-inflammatory chemokine produced by a variety of cell types and plays an important role in the activation of neutrophils and recruitment during inflammation

(Hammond *et al.*,1995). Furthermore, levels of LFP supplementation trended to down-regulated the mRNA levels of *ICAM-1*, 150 and 250 mg/kg LFP down-regulated *INOS* in duodenum (Fig.3A). INOS is activated by immune-stimulating cytokines or pathogens by activation of inducible nuclear factors to produce high concentrations of NO (Aktan *et al.*,2004). ICAM-1 promotes the firm adherence of leukocytes to endothelial cells, and MCP-1 is involved in leukocyte rolling and endothelial cell migration (Tanaka *et al.*,2011; Liang *et al.*,2009). A previous study indicates that a valuable effect of licorice extract against acetic acid-induced ulcerative colitis is attributed to its anti-inflammatory properties (Samadnejad *et al.*,2012). In a mouse model, LFP decreases mRNA abundance of *IL-1* and *INOS*, and inhibits skin swelling and inflammation (Cho *et al.*,2010). As an important anti-inflammatory factor, IL-10 plays an important role in maintaining intestinal mucosal immune homeostasis (Sanjabi *et al.*,2009). In this study, 250 mg/kg LFP increased the mRNA abundance of *IL-10* in ileum (Fig.3C). The results showed that moderate addition of LFP contributes to the health immunity status in the gut of weaned piglets, and further supported that LFP supplementation improved the health and function of intestine in weaned piglets, which in turn improved the growth performance.

In conclusion, dietary LFP supplementation exhibits beneficial effect on piglets after weaning and reduces diarrhea, the effects are associated with improvement of the gut barrier, development and integrity, and enhancement of the immune and health status in the small intestine. In general, 150 mg/kg LFP is more effective. The results provide information for the application of LFP in diets of weaned piglets.

ANIMAL WELFARE STATEMENT

The weaned piglets' experiment is followed the actual law of animal protection and was approved by the Animal Care and Use Committee of the Sichuan Agricultural University (Ethic Approval Code: SCAUAC201811-1).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest, financial or otherwise.

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Figure legends:

Fig.1 Effect of dietary licorice flavonoids powder (LFP) supplementation on mRNA abundances of intestinal tight junction-related genes in piglets. A: duodenum; B: jejunum; C: ileum. Mean values (n = 6) with different letters were significantly different ($P < 0.05$).

Fig.2 Effect of dietary licorice flavonoids powder (LFP) supplementation on mRNA abundances of intestinal development-related genes in piglets. A: duodenum; B: jejunum; C: ileum. Mean values (n = 6) with different letters were significantly different ($P < 0.05$).

Fig.3 Effect of dietary licorice flavonoids powder (LFP) supplementation on mRNA abundances of intestinal inflammation-related genes in piglets. A: duodenum; B: jejunum; C: ileum. Mean values (n = 6) with different letters were significantly different ($P < 0.05$).

Table 1. Effect of dietary licorice flavonoids powder (LFP) supplementation on growth performance of piglets.

Items	Dietary treatments				SEM	*P*-value		
	0 mg/kg LFP	50 mg/kg LFP	150 mg/kg LFP	250 mg/kg LFP		ANOVA	Linear	Quadratic
BW (Kg)								
0 d	8.04	8.08	8.05	8.05	0.05	0.994	0.814	0.927
14 d	11.43	11.70	11.67	11.46	0.11	0.259	0.112	0.265
35 d	18.76	19.09	19.85	19.08	0.24	0.193	0.135	0.144
ADG (g/d)								
0-14 d	234.08	256.90	257.59	241.92	6.50	0.525	0.382	0.750
15-35 d	334.97	351.91	385.09	354.00	6.09	0.068	0.100	0.055
0-35 d	299.92	313.90	334.09	309.17	7.78	0.246	0.750	0.227
ADFI (g/d)								
0-14 d	449.58	455.29	458.34	441.08	7.59	0.878	0.998	0.866
15-35 d	732.75	763.92	800.89	706.80	14.45	0.107	0.996	0.839
0-35 d	619.49	640.47	663.87	600.51	10.29	0.149	0.996	0.910
F/G								
0-14 d	1.93	1.67	1.78	1.82	0.03	0.661	0.499	0.281
15-35 d	2.14	2.18	2.08	2.00	0.03	0.089	0.854	0.024
0-35 d	2.07	2.05	1.99	1.95	0.02	0.133	0.644	0.028

BW, body weight; ADG: average daily gain; ADFI: average daily feed intake; F/G: feed intake/body gain. Mean values with different letters are significantly different ($P < 0.05$). SEM, total standard error of means (n = 6).

Table 2. Effect of dietary licorice flavonoids powder (LFP) supplementation on diarrhea index of piglets.

Items	Dietary treatments				SEM	*P*-value		
	0 mg/kg LFP	50 mg/kg LFP	150 mg/kg LFP	250 mg/kg LFP		ANOVA	Linear	Quadratic
0-14 d	0.14	0.11	0.07	0.07	0.02	0.259	0.522	0.063
14-35 d	0.16^{b}	0.03^{a}	0.04^{a}	0.04^{a}	0.01	0.001	0.001	0.017
0-35 d	0.07^{b}	0.03^{a}	0.03^{a}	0.02^{a}	0.01	0.008	0.012	0.013

Mean values with different letters are significantly different ($P < 0.05$). SEM, total standard error of means (n = 6).

Table 3. Effect of dietary licorice flavonoids powder (LFP) supplementation on pH of gastric and intestinal contents and intestinal mucosal SIgA of piglets

Items	Dietary treatments				SEM	*P*-value		
	0 mg/kg LFP	50 mg/kg LFP	150 mg/kg LFP	250 mg/kg LFP		ANOVA	Linear	Quadratic
pH								
Stomach	3.16	2.39	2.51	2.76	0.21	0.972	0.727	0.856
Jejunum	7.13	6.54	6.70	6.64	0.10	0.134	0.035	0.359
Cecum	6.28^{b}	5.87^{ab}	5.80^{a}	5.63^{a}	0.08	0.026	0.037	0.019
Colon	6.46^{b}	5.75^{a}	5.96^{a}	5.76^{a}	0.08	$P < 0.001$	$P < 0.001$	0.026
SIgA								
Duodenum	12.58	13.97	13.57	13.11	0.49	0.140	0.251	0.283
Jejunum	11.51	12.91	13.57	13.13	0.55	0.345	0.131	0.300
Ileum	12.55	14.08	13.85	13.75	0.55	0.783	0.388	0.674

Mean values with different letters are significantly different ($P < 0.05$). SEM, total standard error of means (n = 6).

Table 4. Effect of dietary licorice flavonoids powder (LFP) supplementation on small intestine morphology of piglets.

Items	Dietary treatments				SEM	*P*-value		
	0 mg/kg LFP	50 mg/kg LFP	150 mg/kg LFP	250 mg/kg LFP		ANOVA	Linear	Quadratic
Duodenum								
villus height (μm)	319.93^{a}	410.65^{b}	427.29^{bc}	456.54^{c}	8.87	$P < 0.001$	$P < 0.001$	$P < 0.001$
crypt depth (μm)	417.23^{b}	328.57^{a}	354.08^{a}	346.67^{a}	7.45	$P < 0.001$	$P < 0.001$	0.104
VH/CD	0.81^{a}	1.30^{b}	1.24^{b}	1.42^{b}	0.04	$P < 0.001$	$P < 0.001$	$P < 0.001$
Jejunum								
villus height (μm)	333.75	314.84	318.59	353.52	6.41	0.064	0.250	0.298
crypt depth (μm)	233.84	235.59	229.35	231.58	5.14	0.102	0.021	0.344
VH/CD	1.46	1.47	1.43	1.55	0.04	0.148	0.091	0.143
Ileum								
villus height (μm)	275.55^{a}	287.27^{ab}	277.18^{a}	308.08^{b}	4.81	0.012	0.054	0.023
crypt depth (μm)	203.42^{b}	202.59^{b}	169.98^{a}	191.63^{b}	8.21	0.041	0.849	0.074
VH/CD	1.31	1.37	1.55	1.49	0.04	0.087	0.961	0.014

Mean values with different letters are significantly different ($P < 0.05$). SEM, total standard error of means (n = 3).