Effects of Qizha Oral Liquid Residue on Growth Performance and Intestinal Health in Weaned Piglets: Postprint

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Abstract

To investigate the feasibility of using Chinese herbal medicine residue as a feed ingredient or feed additive, this study utilized 120 weaned piglets at 21 days of age, randomly allocated into four groups: control, Qizha oral liquid residue, fermented Qizha oral liquid residue, and zinc oxide, to compare the effects of Qizha oral liquid residue before and after fermentation on growth performance, nutrient digestibility, intestinal mucosal morphology, and tight junction protein mRNA relative expression levels in weaned piglets. Each group comprised 5 replicates with 6 piglets per replicate. The experimental period lasted 28 days. The results showed: 1) During the entire experimental period (days 1–28), the feed-to-gain ratio of piglets in the fermented Qizha oral liquid residue group was significantly lower than that in the control and Qizha oral liquid residue groups ($P>0.05$), while the diarrhea rate of piglets in the zinc oxide group was significantly lower than that in all other groups ($P<0.05$). 2) On day 28, the digestibility of dry matter, crude fat, and crude protein in piglets from the fermented Qizha oral liquid residue group was significantly lower than that in the control group ($P<0.05$), crude fat digestibility was significantly lower than that in the Qizha oral liquid residue group ($P<0.05$), and crude protein digestibility was significantly lower than that in the zinc oxide group ($P<0.05$). 3) The villus height and villus height-to-crypt depth ratio in the jejunum and ileum of piglets in the fermented Qizha oral liquid residue group were greater than those in the Qizha oral liquid residue group ($P>0.05$), whereas villus width and crypt depth were smaller than those in the Qizha oral liquid residue group ($P>0.05$). 4) No significant differences were observed in the mRNA relative expression levels of occludin and tight junction-associated protein-1 in the jejunum, ileum, and colon among all experimental groups ($P>0.05$). In conclusion, compared with Qizha oral liquid residue, fermented Qizha oral liquid residue can improve

intestinal morphology in weaned piglets to a certain extent, while neither has a significant effect on tight junction protein mRNA relative expression levels.

Full Text

Abstract

To evaluate the possibility of herbal residues used as feedstuff or feed additive, a total of 120 piglets weaned at 21 days of age were randomly assigned to four groups, representing control group, Qi-Zha oral solution residues (QOR) group, fermented QOR group and zinc oxide group, the effects of QOR and fermented QOR on growth performance, nutrient digestibility, intestinal mucosal morphology and tight junction protein mRNA relative expression of weaned piglets were determined. Each group contained five replicates and each replicate contained six weaned piglets. The experiment lasted for 28 days. The results showed as follows: 1) During the whole experiment stage (day 1 to 28), the ratio of feed to gain (F/G) of piglets in fermented QOR group was lower than that in control group and QOR group ($P>0.05$); the diarrhea rate of piglets in zinc oxide group was significantly lower than that in other groups ($P<0.05$). 2) At day 28 of the experiment, the digestibility of dry matter (DM), ether extract (EE) and crude protein (CP) of piglets in fermented QOR group was significantly lower than that in control group ($P<0.05$), the EE digestibility was significantly lower than that in QOR group ($P<0.05$), and the CP digestibility was significantly lower than that in zinc oxide group ($P<0.05$). 3) The villus height and villus height to crypt depth (V/C) value in jejunum and ileum of piglets in fermented QOR group were higher than those in QOR group ($P>0.05$), and the crypt depth and villus width were lower than those in QOR group ($P>0.05$). 4) There were no significant differences on the mRNA relative expression of Occludin and zonula occluden 1 in jejunum, ileum and colon among all groups ($P>0.05$). In conclusion, compared with the QOR, the fermented QOR can improve the intestinal morphology of weaned piglets in a certain extent, there is no significant effect on the mRNA relative expression of tight junction protein when dietary supplementation with fermented QOR and QOR.

Keywords: Qi-Zha oral solution residues; fermentation; weaned piglets; growth performance; intestinal health

1. Materials and Methods

1.1 Fermentation of Qi-Zha Oral Solution Residues

The Qi-Zha oral solution residues (QOR) used in this experiment were derived from the residues of Qi-Zha oral solution (containing *Astragalus membranaceus*, *Atractylodes macrocephala*, *Poria cocos*, *Crataegus pinnatifida*, *Massa medicata fermentata*, and *Malt*). The primary active ingredients in QOR are *Astragalus* polysaccharides, *Atractylodes* polysaccharides, and *Poria* polysaccharides, with the polysaccharide content in QOR being 70%. The fermentation substrate

consisted of QOR (40%-60% of substrate weight) mixed with 0.4% compound probiotics (containing *Bacillus subtilis*, *Lactobacillus*, *Saccharomyces cerevisiae*, and *Candida utilis*, with viable bacteria count 2×10^1 CFU/g) and water at a 1:0.4 ratio. The mixture was fermented at 25°C for 25 days, with turning every 1-2 days, and then air-dried and stored for later use.

1.2 Experimental Design

A total of 120 weaned piglets (Duroc × Landrace × Yorkshire) with an initial body weight of approximately 6 kg were randomly divided into 4 groups with 5 replicates per group and 6 piglets per replicate. The experiment lasted for 28 days. The four dietary treatments were: 1) Control group: basal diet (commercial nursery piglet feed without antibiotics or growth promoters); 2) QOR group: basal diet + 5 kg/t QOR; 3) Fermented QOR group: basal diet + 5 kg/t fermented QOR; and 4) Zinc oxide group: basal diet + 3,000 mg/kg zinc oxide. The basal diet composition and nutrient levels are shown in Table 1. The experiment was conducted at the Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central China, Institute of Subtropical Agriculture, Chinese Academy of Sciences, from July 16 to August 14, 2015. All piglets were housed in semi-open pig pens with free access to feed and water.

1.3 Growth Performance Measurement

On days 1, 7, and 28 of the experiment, piglets were weighed after overnight fasting and feed intake was recorded to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G). From 09:00 to 16:00 daily, fecal consistency was scored using a 4-grade system: 1 = normal feces, 2 = soft feces, 3 = mild diarrhea, and 4 = severe diarrhea. The diarrhea rate was calculated as: (total number of diarrhea observations / total number of piglets × experimental days) × 100%.

1.4 Nutrient Content Analysis of Fermented QOR

Fermented QOR samples were collected and stored at -20°C. Nutrient contents including dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), and ash were analyzed according to the methods described by Zhang et al. [7]. Gross energy (GE) was measured using an oxygen bomb calorimeter.

1.5 Nutrient Digestibility Measurement

On days 1 and 21 of the experiment, 0.1% chromium oxide was added to the feed as an indigestible marker. Fecal samples were collected from each replicate on days 7 and 28, stored at -20°C, and analyzed for DM, CP, EE, and GE content using the methods described by Li et al. [8] to calculate nutrient digestibility.

1.6 Intestinal Morphology Analysis

At the end of the experiment, one piglet from each replicate (total 5 piglets per group) was euthanized. Approximately 2 cm segments of jejunum and ileum were collected, flushed with saline, fixed in 4% paraformaldehyde solution, and embedded in paraffin. Sections of 5 m thickness were stained with hematoxylin-eosin (HE) and examined under an OLYMPUS BX-51 microscope. Villus height (VH), villus width (VW), and crypt depth (CD) were measured, and the villus height to crypt depth ratio (V/C) was calculated.

1.7 Tight Junction Protein mRNA Expression Analysis

Jejunal, ileal, and colonic tissue samples were collected from euthanized piglets, immediately frozen in liquid nitrogen, and stored at -80°C. Total RNA was extracted using RNA Isolation Solvent and reverse-transcribed using PrimeScript RT reagent. Real-time quantitative PCR was performed using -actin as the reference gene to determine the relative mRNA expression levels of occludin and zonula occludens-1 (ZO-1). The primer sequences were: -actin (forward: CTGCGGCATCCACGAAACT, reverse: AGGGCCGTGATCTCCTTCTG); Occludin (forward: ATGCCTCCTCCCCTTTCG, reverse: CGCCCGTCGTGTAGTCTGTC); ZO-1 (forward: TACCCTGCGGCTGGAAGA, reverse: GGACGGGACCTGCTCATAACT). The PCR reaction mixture (10 L) contained 5 L Luminaris Color Higreen high ROX qPCR Master Mix (2×), 0.4 L each of forward and reverse primers, 2.2 L nuclease-free H2O, and 2 L cDNA template. The amplification program was: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

1.8 Statistical Analysis

Data were processed using Excel 2010 and analyzed using SPSS 17.0 software. One-way ANOVA was used for statistical analysis, and Duncan's multiple range test was used for pairwise comparisons. Results are expressed as "mean ± standard deviation". $P<0.05$ was considered statistically significant.

2. Results

2.1 Nutrient Content of QOR Before and After Fermentation

As shown in Table 2, the fermented QOR had higher DM, CP, ADF, ash, and GE contents compared with unfermented QOR by 0.84%, 10.70%, 3.57%, 1.73%, and 4.20%, respectively. In contrast, CF, NDF, and EE contents were lower in fermented QOR by 55.69%, 48.51%, and 32.45%, respectively.

2.2 Effects on Growth Performance and Diarrhea Rate

As shown in Table 3, there were no significant differences in ADFI and ADG among all groups during days 1-7, days 8-28, and the overall period ($P>0.05$). During days 1-7, the F/G of piglets in fermented QOR group was significantly

higher than that in the other three groups (P<0.05). During days 8-28 and the overall period, there were no significant differences in F/G among groups (P>0.05).

As shown in Table 4, the diarrhea rate of piglets in zinc oxide group was significantly lower than that in the other three groups during days 1-7, days 8-28, and the overall period (P<0.05). There were no significant differences in diarrhea rate among control, QOR, and fermented QOR groups (P>0.05).

2.3 Effects on Nutrient Digestibility

As shown in Table 5, there were no significant differences in DM, GE, CP, and EE digestibility among groups on day 7 (P>0.05). On day 28, DM digestibility in fermented QOR group was significantly lower than that in control group (P<0.05) but not significantly different from QOR and zinc oxide groups (P>0.05). EE digestibility in fermented QOR group was significantly lower than that in control and QOR groups (P<0.05) but not significantly different from zinc oxide group (P>0.05). CP digestibility in fermented QOR group was significantly lower than that in control and zinc oxide groups (P<0.05) but not significantly different from QOR group (P>0.05).

2.4 Effects on Intestinal Morphology

As shown in Table 6 and Figures 1-2, there were no significant differences in villus height among groups in jejunum and ileum (P>0.05). Villus width in jejunum was significantly lower in control group compared with QOR and zinc oxide groups (P<0.05), while fermented QOR group showed intermediate values. In ileum, villus width in fermented QOR group was significantly lower than that in zinc oxide group (P<0.05). Crypt depth in jejunum and ileum was not significantly different among groups (P>0.05). The V/C ratio in jejunum and ileum was not significantly different among groups (P>0.05).

2.5 Effects on Tight Junction Protein mRNA Expression

As shown in Table 7, there were no significant differences in occludin and ZO-1 mRNA relative expression levels in jejunum, ileum, and colon among all groups (P>0.05).

3. Discussion

3.1 Effects on Growth Performance and Diarrhea Rate

Weaning stress, environmental changes, and nutritional factors can significantly affect the growth performance of piglets, with diarrhea rate being a key indicator of intestinal health [9-10]. In this study, dietary supplementation with QOR and fermented QOR during the 28-day period showed no significant effects on growth performance, though fermented QOR reduced F/G by 3.9% compared with QOR. Previous studies have shown that fermented feed can improve ADFI

and ADG while reducing F/G and diarrhea incidence [11]. Other research has demonstrated that *Astragalus* polysaccharides can increase ADG and reduce diarrhea rate [12]. The lack of significant effects in this study may be attributed to the relatively low dosage of QOR (5 kg/t) and the short experimental period. Previous studies have shown that fermented Chinese herbal medicine can improve growth performance by enhancing nutrient digestibility and reducing pathogenic bacteria [13-14]. In this study, QOR and fermented QOR supplementation did not significantly reduce diarrhea rate, which may be related to the dosage and fermentation conditions. However, zinc oxide significantly reduced diarrhea rate, consistent with previous reports [15].

3.2 Effects on Nutrient Digestibility

The intestinal mucosa is the primary site for nutrient digestion and absorption. Weaning stress can damage intestinal structure and function, reducing nutrient digestibility [16-17]. In this study, fermented QOR supplementation reduced DM, GE, EE, and CP digestibility on day 28 compared with control and QOR groups. This may be because fermentation increased the content of indigestible fiber and reduced EE content. Previous studies have shown that Chinese herbal medicine can improve nutrient digestibility by enhancing digestive enzyme activity, reducing intestinal pH, and promoting beneficial microbiota [18]. However, excessive fiber can interfere with nutrient digestion and absorption [19-20]. In this study, the fermented QOR had higher ADF content, which may have negatively affected nutrient digestibility. The reduction in EE digestibility may be related to the decreased EE content in fermented QOR. The increased CP digestibility in QOR group may be attributed to the protein content of the herbal residues themselves.

3.3 Effects on Intestinal Morphology

Intestinal villus height, crypt depth, and V/C ratio are important indicators of intestinal health and function. Higher villus height and V/C ratio indicate better nutrient absorption capacity, while increased crypt depth suggests enhanced cell proliferation and intestinal repair [22-23]. This study showed that fermented QOR increased villus height and V/C ratio while reducing crypt depth and villus width in jejunum and ileum compared with QOR group, indicating that fermented QOR can improve intestinal morphology to some extent. This may be because fermentation produces beneficial metabolites such as organic acids and small peptides that promote intestinal development. Previous studies have shown that fermented Chinese herbal medicine can improve intestinal morphology by increasing villus height and reducing crypt depth [21]. However, the differences were not statistically significant, possibly due to the low dosage and short experimental period. Zinc oxide supplementation increased villus width, consistent with previous reports [24].

3.4 Effects on Tight Junction Protein mRNA Expression

Occludin and ZO-1 are important tight junction proteins that maintain intestinal barrier function [25]. This study found no significant effects of QOR or fermented QOR on occludin and ZO-1 mRNA expression in jejunum, ileum, and colon. Previous studies have shown that Chinese herbal medicine can upregulate tight junction protein expression and improve intestinal barrier function [26]. The lack of significant effects in this study may be due to the low dosage and short experimental period. Further research is needed to optimize the fermentation conditions and dosage of QOR to maximize its beneficial effects on intestinal health.

In conclusion, compared with unfermented QOR, fermented QOR can improve intestinal morphology of weaned piglets to some extent, but has no significant effect on tight junction protein mRNA expression. The optimal dosage and fermentation conditions of QOR require further investigation to enhance its efficacy as a feed additive for weaned piglets.

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