

Dietary Supplementary of Modified Si-jun-zi Granule Promotes Intestinal Development and Enhances Mucosal Immunity of Weaned Rex Rabbits

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Research

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Abstract

Background: Early-weaned in Rex rabbits and other animals is associated with the development and immunity of intestine. This article was aimed to investigate the potential benefits of a dietary supplementation, modified Si-jun-zi granule (M-SJZG), on enhancing immunity of small intestinal mucosa and promoting intestinal development of weaned Rex rabbits.

Results: In the present study, we found that dietary supplementation with 2% modified Si-jun-zi granule decreased the diarrhea frequency and mortality due to early-weaned, and significantly increased the average daily gain; the length and relative weight of intestine; the concentration of immunoglobulin in serum and SIgA in intestinal mucus. Additionally, the activity of lipase was raised by dietary Si-jun-zi granule addition. However the activities of ALT, ALP and AST in serum were obviously decreased. Compared with the un-supplementation control group, the ratio of villi length to crypt depth and the expression of tight junction protein(zonula occludens-1, Claudin-1 and Occludin) and Glucose transporter (re-combinant Sodium/Glucose co-transporter 1 and recombinant Glucose transporter 2) in jejunum were raised. In addition, Moreover early development of intestine and the injury of intestinal mucosa induced by early-weaning also could be improved.

Conclusions: This study indicated that modified Si-jun-zi granule has protective effects on diarrhea induced by early-weaning through enhancing immunity of small intestine mucosa and promoting intestinal development.

Introduction

In general, it will take three months for natural weaning in Rex rabbits breeding. Early weaning is a commonly used practice to ensure profit maximization by shorten dams reproductive cycle(Kumar et al., 2017), after which diarrhoea and even death is often happened to induce important economic losses(Richter et al., 2017; Yarnall and Thrusfield, 2017)due to underdevelopment of digestive system and relatively low immunity(Vicario et al., 2015), especially in which food can not satisfied the requirements of nutrition(Zhu et al., 2015). The intestinal development is closely related to the permeability and nutrition absorption(Wood et al., 2015). These functions can be estimated by intestinal tight junction protein (Zo-1, Claudin-1and Occludin) level and intestinal Glucose transporter (SGLT1 and GLUT2) level.

Antibiotic was used extensively for treating and preventing diarrhoea by effectively kill microorganisms(Szajewska et al., 2016). However, it not only kills pathogens, but also causes a certain degree of damage on probiotic group that is helpful to establish the immunity barrier for intestinal mucosa(Wischmeyer et al., 2016). In addition, antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes as the important factors for common's health has attracted more and more public attention. It has become the universal goal for breeding industry to reduce the antibiotic abuse. Therefore, traditional Chinese herbal compound with some advantages, natural, low toxicity and no

residue(Liu et al., 2015) have been widely concerned as the potential replacements to cure and prevent diarrhoea induced by early weaning.

In traditional Chinese medicine(TCM) theory, the deficiency of spleen-qi may exhibit symptoms, including poor appetite, dyspepsia, decreased immunity, and even gastrointestinal dysfunction, which are very close to diarrhoea induced by early weaning. Si-jun-zi decoction (SJZD) as traditional prescription for treating spleen-qi deficiency syndrome for thousands of years(Sun et al., 2016; Zheng et al., 2014). Modern medicine also demonstrated that SJZD can regulate intestinal immunity, improve gastrointestinal function and treat diarrhea induced by spleen-qi deficiency. Previous studies have showed that SJZD has various pharmacological activities which related to digestive system(Yu et al., 2014). For instance, the SJZD could prevent and treat the gastric mucosa injury, and inhibit the growth of gastric tumor induced by human sgc-7901 SP cells through promoting apoptosis in rats(Nie et al., 2019). JH Feng et al. has demonstrated that the SJZD can adjust the disorder of gastrointestinal electrical activity and motility of rats with spleen deficiency caused by reserpine(Feng et al., 2010). ZS Zhong et al. has identified that the SJZD could regulate the Cam-MLCK signal pathway, relieve the abnormal tension of gastric smooth muscle and promote gastrointestinal dynamics in rats with spleen deficiency(Zhong et al., 2018). Moreover, the SJZD can significantly reduce the diarrhea rate of piglets by enhancing the cellular and humoral immunity and alleviating weaning stress(Ren et al., 2016).ZH Huang et al. also proved that SJZD could enhance the immunity of small intestine mucosa and promote growing of piglets(Huang, Z. et al., 2017). In order to fit different clinical demands, SJZD was also been modified to owned wider pharmacological actions based on the original major formula and TCM theory,such as Jian-pi Mixture (SJZD + *Dioscorea oppositifolia* L.(Rhizome)) on Irritable bowel syndrome(Lian et al., 2020), SJZD combined with *Astragalus mongholicus* Bunge and *Dioscorea oppositifolia* L. to attenuates colon cancer liver metastasis by increasing macrophage cells(Zhou et al., 2019). However, the protective effect of modified Si-jun-zi granule that combines with *Astragalus mongholicus* Bunge, *Bupleurum chinense* DC, *Citrus × aurantium* L. and *Crataegus pinnatifida* Bunge(fruit) to impeded diarrhea induced by early-weaning in Rex rabbits has not been reported and need for further research. Both *Astragalus mongholicus* Bunge, *Bupleurum chinense* DC, *Citrus × aurantium* L. and *Crataegus pinnatifida* Bunge(fruit) were also cited in the TCM formula, which can be used to treat the diarrhea(Che et al., 2019) (Li et al., 2018), and were proved that could improve intestinal structure and immunity(Rahimi and Abdollahi, 2012) (Yu et al., 2018) (Tan et al., 2020).

Previous studies have shown that the protective effect of modified Si-jun-zi granule on diarrhea induced by early-weaning in Rex rabbits was confirmed by growth performance, diarrhea frequency and mortality, but the mechanisms are unclear(LIU et al., 2018). Then, it is necessary to study the potential mechanisms of the protective effect by histomorphology of small intestine, serum biochemical indices including total cholesterol (TC), glutamic pyruvic transaminase (ALT), glutamic oxalacetic transaminase (AST),alkaline phosphatase (ALP) and immunoglobulin (IgA, IgG and IgM) in serum, digestive enzyme activity (lipase, amylase and trypsin), secretory immunoglobulin A (SIgA) level in the intestinal mucus, intestinal tight junction protein (Zo-1, Claudin-1and Occludin) level and intestinal Glucose transporter (SGLT1 and

GLUT2) level. Our findings provide new theoretical basis of modified Si-jun-zi granule on enhancing intestinal development and immunity in early-weaned Rex rabbits as dietary supplementation.

Material And Methods

Modified Si-jun-zi granule

Modified Si-jun-zi granule (M-SJZG) is constituted of eight herbs, including *Codonopsis pilosula* (Franch.) Nannf, *Atractylodes Lancea* (Thunb.) DC, *Wolfiporia cocos*, *Glycyrrhiza uralensis* Fisch. ex DC (Preparata), *Astragalus mongholicus* Bunge, *Bupleurum chinense* DC, *Citrus × aurantium* L. and *Crataegus pinnatifida* Bunge (fruit) with a dosage ratio of 9:9:9:6:10:6:6:6, all of these were purchased from Sichuan c& Traditional Chinese Medicine CO., LTD. and accorded with Chinese veterinary drug code(the details are summarized in Supplementary Table 1). Herbs were mixed and wetted with distilled water (1:8, w/v) for 2 h. Then, the mixture was boiled for 3 h and filtered through multi-layer gauze. The filtrate was gathered and residues were decocted twice with water (1:6, w/v) for 3 h. At last, the combined filtrates was concentrated to 1.0 g/mL which measured by raw medicine. The decoction was mixed with starch (1:1, v/w). M-SJZG were prepared by wet granulation with proper concentration of alcohol as adhesive for convenience of daily administration.

The quality control of M-SJZG was analyzed using high-performance liquid chromatography (HPLC) with Corona Ultra detection(CAD) in Agilent HPLC system. M-SJZG (5 g) was dissolved in 10 mL Methanol solution (70%) and diluted 10 times. The solution centrifuged (1500 rpm, 15 min) and filtered (0.22 μm), and then, 20 μL was injected into an HPLC system for assay. The separation was performed using a reverse-phase column(Intersil ODS-3 5 μm, 4.6 mm × 250 mm I.D.) connected with a guard column (C18, 5 μm, 4.6 mm × 10 mm I.D.). The elution flow rate was 1.0 mL/min with a mobile phase gradient of A-B (A: H₂O/H₃PO₄ = 1000 mL/2 mL; B: CH₃CN), which was varied as follows: 0 min, 90% A, 10% B; 9 ~ 29 min, 90 ~ 85% A, 10 ~ 15% B; 29 ~ 37 min, 85 ~ 82% A, 15 ~ 18% B; 37 ~ 46 min, 82% A, 18% B; 46 ~ 51 min, 82 ~ 80% A, 18 ~ 20% B; 51 ~ 72 min, 80 ~ 72% A, 20 ~ 28% B; 72 ~ 82 min, 72% A, 28% B; 82 ~ 87 min, 72 ~ 70% A, 28 ~ 30% B; 87 ~ 92 min, 70 ~ 67% A, 30 ~ 33% B; 92 ~ 99 min, 67 ~ 50% A, 33 ~ 50% B; 99 ~ 110 min, 50% A, 50% B; 110 ~ 115 min, 50 ~ 47% A, 50 ~ 53% B; 115 ~ 118 min, 47 ~ 30% A, 53 ~ 70% B; 118 ~ 130 min, 30% A, 70% B; 0 min, 90% A, 10% B; 130 ~ 135 min, 30 ~ 90% A, 70 ~ 10% B; 135 ~ 140 min, 90% A, 10% B. The injection volume was 20 μL, and the UV detection wave length was set at 285 nm for saikoside A, liquiritin and hesperidin; 250 nm for glycyrrhizic acid. The fingerprint chromatogram of M-SJZG is shown in Supplementary Fig. 1.

Chemicals and reagents

Glutamine (Gln) was purchased from Huana Chemical Co., Ltd (china), HE stain (Hematoxylin and Eosin) were purchased from Thermo Fisher Co., Ltd (American), all Elisa kits was purchased from Nanjing Jiancheng Bioengineering Institute, FastKing RT kit was purchased from Tiangen Biotech (Beijing) CO., LTD, the others reagents was purchased from ChenDu Chron Chemicals Co., Ltd (china).

Animals

Two hundred and eighty Rex rabbits, approximately 45 days of age and 750-950g in weight, were obtained from the Rex rabbit institute, Sichuan Academy of Grassland Sciences. The animals were provided with basal diet and tap water at liberty and maintained in cages under controlled conditions ($23\pm 2^{\circ}\text{C}$, 12 h light/dark cycle). All experiments and procedures were carried out according to the Regulations of Experimental Animal Administration issued by the State Committee of Science and Technology of China. The composition and nutrient levels of the basal diet were listed in Table 1.

Growth performance, diarrhea frequency and mortality

One hundred and twenty Rex rabbits were randomly divided into four groups, including control group and M-SJZG (0.5%, 1%, 2%) groups. Except the control group, M-SJZG were administrated as dietary supplement for thirty consecutive days, to evaluate the protective effect by growth performance, diarrhea frequency (P_{diarrhea} was calculated as equation 1) and mortality.

$$P_{\text{diarrhea}} = \frac{\sum A}{\sum B} \times 100\% \quad (1)$$

Where A represents the the total times of diarrhea, and B represents the sum of survival days of each rabbit in the group.

Tissue collection and measurement

To revealed the protective mechanism of M-SJZG from intestinal development and immunity, the others one hundred and sixty rabbits were randomly divided into five groups, including control group, M-SJZG (0.5%, 1%, 2%) groups and Glutamine (Gln) (0.8%) group. M-SJZG (0.5%, 1%, 2%) or Gln (0.8%) were administrated as a dietary supplement for thirty consecutive days. In each group, the six rabbits were randomly selected, weighted and sacrificed on the 15th and 30th day after treatment, respectively. The intestinal length and relative weight were measured after washing by physiological saline. Then the duodenum, jejunum and ileum were collected and fixed with 4% paraformaldehyde, respectively.

Histomorphology

The histopathological changes of various intestinal segments were observed under a high-resolution microscope with photographic facility after being embedded in paraffin and made slides with HE staining. Image pro Plus was used to measure the length of villi and the depth of crypt, then the ratio was calculated as follows (Equation 2).

$$R_{(V/C)} = \frac{L_{\text{villi}}}{D_{\text{crypt}}} \quad (2)$$

Where L_{villi} and D_{crypts} stand for the length of villi and the depth of crypt, respectively.

ELISA analysis of serum biochemical indices and SIgA

The serum and intestinal mucus was collected after experimental period. Then the abundance of serum biochemical indices and SIgA on the intestinal mucus were assayed by ELISA kits according to the manufacturer's instructions.

Immunohistochemical analyses of SIgA

The abundance of SIgA was assessed for paraffin embedded slides after the sections were dewaxed. Endogen biotin and non-specific signals were blocked with appropriated reagents. Antigen retrieval was carried out in a microwave oven (two cycles for 5 min each at 780 W, in citrate buffer, pH 6.0, twice washed in PBS for 5 min each). Then, the treated slides were overnight incubated with primary antibodies at -4°C in a humidified chamber, washed in PBS, and visualized by biotinylated secondary antibodies followed by stained by DAB kit for 2 min, washed in distilled water. Finally, counterstained with Hematoxylin, dehydrated, transparentized and sealed. At least 10 fields of view from each sample were analyzed with the image analysis software for each protein of interest.

Zo-1, Claudin-1, Occludin, SGLT1 and GLUT2 level assay

Total RNA was isolated from samples of jejunum, ileum and colon with TRIZOL reagent (Tiangen Biotech, Beijing) and then treated with DNase I (Tiangen Biotech, Beijing) according to the manufacturer's instructions. Primers used in this study are presented in Table 2. GAPDH was used as an internal control to normalize expression of target gene transcripts. Real-time PCR was performed as previous studies described (Yin et al., 2015).

Statistical analysis

The one-way analysis of variance (ANOVA) was used to analyze experimental data by SPSS 25.0 software. All values were presented as the mean \pm SD, and $P < 0.05$ was considered statistically significant.

Results

Growth performance

As shown in Table. 3, the average daily food intake (ADFI) was not significantly affected compared with control group (Table. 3, $P > 0.05$). Moreover, the feed conversion ratio (FCR) was also significantly decreased after treatment (Table. 3, $P < 0.05$). However, significant improvement in the average daily gain (ADG) was observed after M-SJZG (1.0% and 2.0%) was administrated. These results indicated that M-SJZG had promoting effects on the growth of early-weaning rabbit.

Diarrhea frequency and mortality

The frequency of diarrhea was extremely significantly declined in all treatment groups compared to control group (Table. 4, $P < 0.01$), and the mortality were observably decreased after M-SJZG (1.0% and 2.0%) treatment compared with control group (Table 4, $P < 0.01$). Furthermore, the effect was dose-dependent. These results indicated that M-SJZG had protective effects on diarrhea induced by early-weaning.

Promotion effect of M-SJZG on the intestinal development in early-weaning Rex rabbits

The results showed that intestinal length was significantly increased by administrated M-SJZG (2%) compared to control group (Fig. 1A, $P < 0.05$) at 15d and 30d. Meanwhile, M-SJZG (0.5%, 1.0% and 2.0%) and Gln (0.8%) treatment could slightly increase the relative weight of intestine in early-weaning Rex rabbits (Fig. 1B, $P > 0.05$). Moreover, the ratio of villi length to crypt depth was remarkably risen in jejunum (Fig. 2B, $P < 0.05$) by administrated M-SJZG (2%) at 15d. However, this trend was not significant in duodenum and ileum between experimental period (Fig. 2A, 2C, $P > 0.05$). (HE staining results and data were listed in Supplementary Fig.2~4. and Supplementary Table.2~5.)

Effects of M-SJZG on serum biochemical indices

Compared with the control group, the concentration of IgA, IgG and IgM was significantly increased ($P < 0.05$) by dietary supplementation with 2% M-SJZG (Fig. 3A), but the activities of ALT and ALP in serum were obviously decreased (Fig. 3C). Moreover, Supplementation with 0.5, 1% or 2% M-SJZG significantly decreased ($P < 0.05$) the activity of serum AST (Fig. 3C). Additionally, the serum concentration of TC were significantly reduced ($P < 0.05$) (Fig. 3D).

Effect of M-SJZG on digestive enzyme activity

Supplementation with 0.5%, 1% or 2% M-SJZG significantly increased ($P < 0.05$) the activity of lipase, but not amylase and trypsin (Fig. 3B).

Promotion effect of M-SJZG on intestinal mucosal immunity in early-weaning Rex rabbits

As Elisa results shown in Fig. 4, the concentration of SIgA in intestinal mucus was significantly increased with M-SJZG (2.0%) treatment compared to control group ($P < 0.05$) in duodenum and jejunum at 15d and 30d. However, the increase of SIgA was not significant in ileum. In addition, the improvement effect was dosage dependence.

The immunohistochemical results of the express of SIgA was shown in Fig 5-7, the positive reaction mainly existed in cytoplasm. The average optical density of the express of SIgA in different groups was measured and shown in Fig 8. The expression of SIgA in jejunum was obviously up-regulation ($P < 0.05$) which soared 10.07% by high dosage compared with control group, and 8.80% by Gln treatment. The

slightly up-regulate ($P > 0.05$) also could be observed in the others treatment groups, including in duodenum and ileum, and shown dosage-dependence.

Intestinal tight junction protein and glucose transporter level

The results of real-time PCR (Fig. 9-13) showed that in response to M-SJZG or Gln treatment, the expressions of Zo-1, Claudin-1, Occludin, SGLT1 and GLUT2 in intestinal tissues from early weaning Rex rabbits were elevated to high levels. At the end of experimental period, high-dosage treatment significantly ($p < 0.05$) up-regulated the expression of all this proteins mainly in jejunum but SGLT1 in duodenum and ileum.

Discussion

Early-weaning commonly induced diarrhea in infant animals, even leading to death, often associated with important economic losses for breeding (Nabuurs, 1998). Studies reported that the diarrhea was induced by multiple reasons for early-weaning Rex rabbits (CHEN et al., 2017). On the one hand, the exterior which including infection by virus, bacteria and parasite or intoxication plays the important role for diarrhea (Hodges and Gill, 2010). On the other hand, inner reasons which include underdeveloped digestive system and insufficient nutrients could be the key factors (Keusch et al., 2016). The underdeveloped digestive system was concrete performed in two aspects: the physical and immune barrier, that leads the fragile intestinal track. Clinically studies showed that Gln, peptide growth factors and others, essential nutrients found in breast milk, helping digestive system development (Martin et al., 2016). Therefore, the early weaning reduced the intake of essential nutrition. Based on the discussion above, the promotion of intestinal development was not only beneficial in the establishment of barriers but also had the protective effect on diarrhea induced by early weaning.

SJZD, a Chinese herbal decoction consisting of *Codonopsis pilosula*, *Atractylodes lancea*, *Tuckahoe* and *Glycyrrhiza uralensis*, is widely used to nourish deficiency of spleen and stomach qi in China 800 years ago and is still widely used to improve immunity and digestive function disorders (Li et al., 2017). In recent decades, SJZD was modified to broaden the range of application. JingMing Chen et al. Combined SJZD with *Hedysarum polybotrys* and *Ligustrum lucidum*, to impede cachexia (Chen et al., 2019). Guihua Tian et al. researched the therapeutic effect of modified SJZD to chronic atrophic gastritis (Tian et al., 2019). In our study, the *Astragalus mongholicus Bunge*, *Bupleurum chinense*, *Citrus × aurantium* and *Crataegus pinnatifida Bunge* (fruit) were added to SJZD and prepared into granule as dietary supplement, for treating diarrhea induced by early weaning. Zhibo Guan et al. (Guan et al., 2018) has verified the effective constituents of Si-jun-zi decoction mainly included ginsenosides, flavonoids and polysaccharides with Fourier transform ion cyclotron resonance mass spectrometry. It has been proved that polysaccharides in SJZD has nourishment function and a variety of pharmacological effects (Huang, C. et al., 2017; Wang et al., 2016). Therefore, SJZD/M-SJZG could be the supplements for early weaning Rex rabbits growth and development, and help them to conform non-breast feeding.

Growth performance, diarrhea frequency and mortality were first assessed after early weaning and M-SJZG treatment. Our data showed that M-SJZG significantly improved growth performance, ADG and FCR. As we know, early weaning can affect the development of digestive system and induced some disorders, which can lead to diarrhea and even death. However, M-SJZG had noticeable effects on protection of it. This results indicated that M-SJZG might improve the survival rate from early weaning(Delgado et al., 2019). Previous studies have shown that dietary supplement such as Gln (positive group) could improve intestinal development and protect it from atrophy and injury under various stress conditions in young animals(Wang et al., 2015). The effects of M-SJZG were evaluated from the length and relative weight of intestine in early weaning Rex Rabbits that were always used to assess intestinal development level in previous studies(Fung et al., 2016). Intestine is one of the most important organs in the digestive system, whether its structure and function are normal or not can affect the digestion-absorption function. The length and relative weight of the intestine was found increased compared to control group after M-SJZG treatment. The ratio of villus height and crypt depth also as an important index, which in direct proportion to intestinal digestion and absorption capacity(Liu et al., 2019). The results shown that M-SJZG treatment has the dosage-dependence for the ratio increasing, and only high dose (2.0%) M-SJZG administrated has the similar effect to positive control (0.8% Gln).

A salient observation from the present study is M-SJZG supplementation reduced oxidative damage in the liver, as shown by oxidative damage and health markers reduction (including serum ALT, AST and ALP). Serum TC is mainly synthesized by the liver, as the structural component of cells, also can be converted into vitamin D3 to regulate calcium and phosphorus metabolism, promote intestinal calcium and phosphorus absorption and calcification. In present test, Serum TC reduction and the increased lipase activity marked the promotion of lipid metabolism and transformation. Therefore M-SJZG supplementation improved the growth performance can be partly attributed to the lipid metabolism. A series of serum immunoglobulin (IgA, IgG and IgM) are important indexes to measure the humoral immunity of the body. Serotype IgA is produced mainly by plasma cells in mesenteric lymphoid tissue. The concentration increase of immunoglobulin, especially serotype IgA, revealed the decrease of diarrhea frequency and mortality may be related to humoral and gut immunity.

The mucus of adjacent to the epithelial cells in the gastrointestinal tract constitutes an immunity barrier, thereby providing a layer to against infection, in which SIgA was the mainly antibody(Perez-Lopez et al., 2016). The early weaning induced diarrhea not only related to intestinal development but also to pathogenic bacterium infection. This study assessed the SIgA level in intestinal mucus by Elisa. The results illustrated that the content of SIgA was increased compared with control group through M-SJZG administrated, and it can be observed that the significantly promoting mainly focused on the first stage of administration (0 ~ 15d) which was lined with the tendency of $R_{(V/C)}$. This phenomenon could be attributed to the period of intestinal development and the function of SIgA(Gidenne and Fortun-Lamothe, 2002). Recently, G. P. Donaldson and his cooperators manifested gut microbiota utilize immunoglobulin A for mucosal colonization, which can improve intestinal adaptability to outside(Donaldson et al., 2018). Therefore, the SIgA for intestinal development was satisfied by production in the first stage of

administration (0 ~ 15d). Then, the expression of SIgA in intestine was estimated by immunohistochemical staining, and the result also demonstrated M-SJZG treatment can increase it, to a certain extent.

The epithelial surface of the gastrointestinal tract is also a physical barrier against the “outside”. The permeability and the absorption function were closely related to the intestinal tight junction proteins (Zo1, Claudin1, Occludin) and the glucose transporter (SGLT1 and GLUT2), respectively. The expression increase of all these proteins by M-SJZG treatment, was mainly reflected in jejunum which was corresponded with jejunum physiological function, digestion and absorption.

Based on the discussion above, the protective effects of M-SJZG on diarrhea induced by early-weaning in Rex rabbits could be attributed to the improvement of intestinal development and barriers establishment.

Conclusion

In this study, the protective effects of M-SJZG on diarrhea induced by early-weaning were examined in Rex rabbits. The main achievements of our study are as follows: (a) M-SJZG can promote the intestinal development and improve morphology and function. The optimal dose is 2% for early-weaned (45d) Rex rabbits as a dietary supplement. (b) The intestine was improved by dietary supplementation of 2% M-SJZG or 0.8% Gln. However, The 2% M-SJZG is better for enhancing intestinal glucose absorption. (c) Modern pharmacological thoughts and methods were combined with the ancient theories of traditional Chinese medicine to delve the protective mechanism of M-SJZG from the gene and protein levels, which laid a theoretical foundation for the clinical use of M-SJZG.

Abbreviations

ALT: glutamic pyruvic transaminase;

ALP: alkaline phosphatase

AST: glutamic oxalacetic transaminas;

Gln: Glutamine;

GLUT2: recombinant Glucose transporter 2;

IgA: immunoglobulin A;

IgG: immunoglobulin G;

IgM: immunoglobulin M;

M-SJZG: modified Si-jun-zi granule;

SJZD: Si-jun-zi decoction;

SIgA: secretory immunoglobulin A;

SGLT1: re-combinant Sodium/Glucose co-transporter 1;

TC: total cholesterol

TCM: traditional Chinese medicine

Zo-1: zonula occludens-1;

Declarations

Ethics approval and consent to participate

All animal procedures were in accordance with the national standard, Laboratory Animal-Requirements of Environment and Housing Facilities (GB14925-2001), the Sichuan Agricultural University Institutional Animal Care and Use Committee under permit number CSQ-2018203003.

Consent for publication

Not applicable.

Availability of data and material

The datasets produced and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors report no conflicts of interest.

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Author contributions

Shiqi Chen Methodology, Data Curation, Writing-Original draft preparation.

Ning Liu: Conceptualization, Investigation, Formal analysis.

Dongbo Li: Visualization, Validation.

Haibo Dou: Visualization.

Quanjin Liu: Validation.

Hanzhong Liu: Visualization.

Ping Wang: Supervision.

Zhiju Yu: Software.

Gang Shu, Juchun Lin: Supervision.

Wei Zhang, Huaqiao Tang: Software.

Guangneng Peng: Writing-Reviewing & Editing.

Kai Zhang: Writing-Review & Editing.

Bin Wen: Writing-Review & Editing.

Ling Zhao: Writing-Review & Editing.

Hualin Fu: Project administration, Funding acquisition, Writing-Reviewing & Editing

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Tables

Table 1.

The composition and nutrient levels of basal diet (air-dry basis) %

Items	Content	Nutrient Levels	Content
Ingredients		Digestible Energy (DE, MJ/kg)	10.21
Corn	20.0	Crude Protein (CP)	16.00
Bran	22.8	Crude Fibre (CF)	14.29
Peanut vine	40.1	EE	3.02
Soyben meal	14.6	Met+Cys	0.46
NaCl	0.5	Lys	0.56
CaHPO ₄	1.0	Ca	0.58
Premix	1.0	P	0.62
Total	100	I (mg/kg)	0.22

(1) The premix provided following per kilogram of diet: Lys 1.5 g; VA 8000 IU; VD 3 1000 IU; VE 50 mg; Fe 100 mg; Cu 50 mg; Mg 150 mg; Zn 50 mg; Mn 30 mg; Se 0.1 mg

(2) Nutirent levels were calculated values.

Table 2.
Primer sequences for qPCR

Gene	Geneback	Primer sequences (5'to3')		bp	Temperature°C
Claudin1	DQ_993356	F	GGAAGATGATGAGGAGCAA	77	59°C
		R	AGCCCAGCCAGTGAAAA		
Occludin	XM_008262318	F	CTTGCCTGGGACAGAACCTA	121	59°C
		R	AGCCATAACCGTAGCCGTAA		
Zo1	XM_008269782	F	GACTGATGCGAAGACGTTGA	117	59°C
		R	GCAGAATGGATGCTGTCAGA		
SGLT1	EU_414633	F	TGTTCCGCAGGGACACTAA	75	59°C
		R	GGGATCAGGACGTAAAGAGG		
GLUT2	XM_01734	F	AGGCACTGTCCACCACC	161	59°C
		R	GTCTCCAAGCCACCCAC		
GAPDH	NM_001082253	F	TGCCACCCACTCCTCTA	163	59°C
		R	AGTAAGAGCCCTCAAACCACCGG		

Table 3.
Effects of M-SJZG on growth performance of early-weaning Rex rabbits

index	control group	0.5%	1.0%	2.0%
ADFI/(g/d)	90.27±2.34	90.47±2.43	91.31±2.76	91.72±0.78
ADG/(g/d)	14.95±4.50	18.28±1.31	19.01±2.89*	19.29±3.27*
FCR	6.53±2.01	4.97±0.42*	4.89±0.69*	4.86±0.74*

*p < 0.05 vs the control group

Table 4.
Effects of M-SJZG on diarrhea rate and mortality rate of early-weaning Rex rabbits

index	control group	0.5%	1.0%	2.0%
Diarrhea rate/%	18.52±4.96	9.76±2.80**	4.79±2.16**	1.67±0.91**
Mortality/%	13.30±10.33	10.00±2.95	6.67±2.33**	1.90±0.50**

*p < 0.05 and **p < 0.01 compared with control group.

Figures

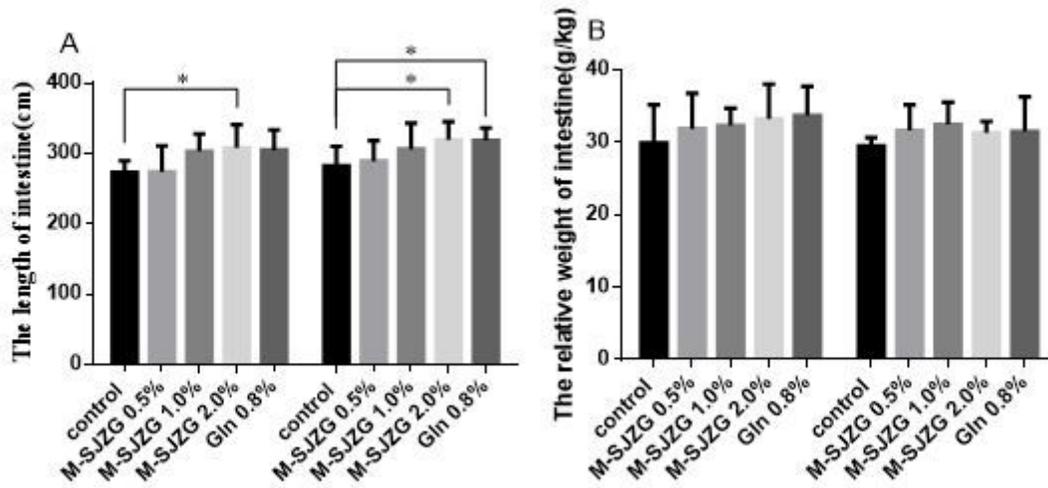


Figure 1

Effects of M-SJZG on intestinal length (A) and relative weight (B) in early-weaning Rex rabbits. Data are expressed as mean \pm SD. (n =6), *P<0.05.

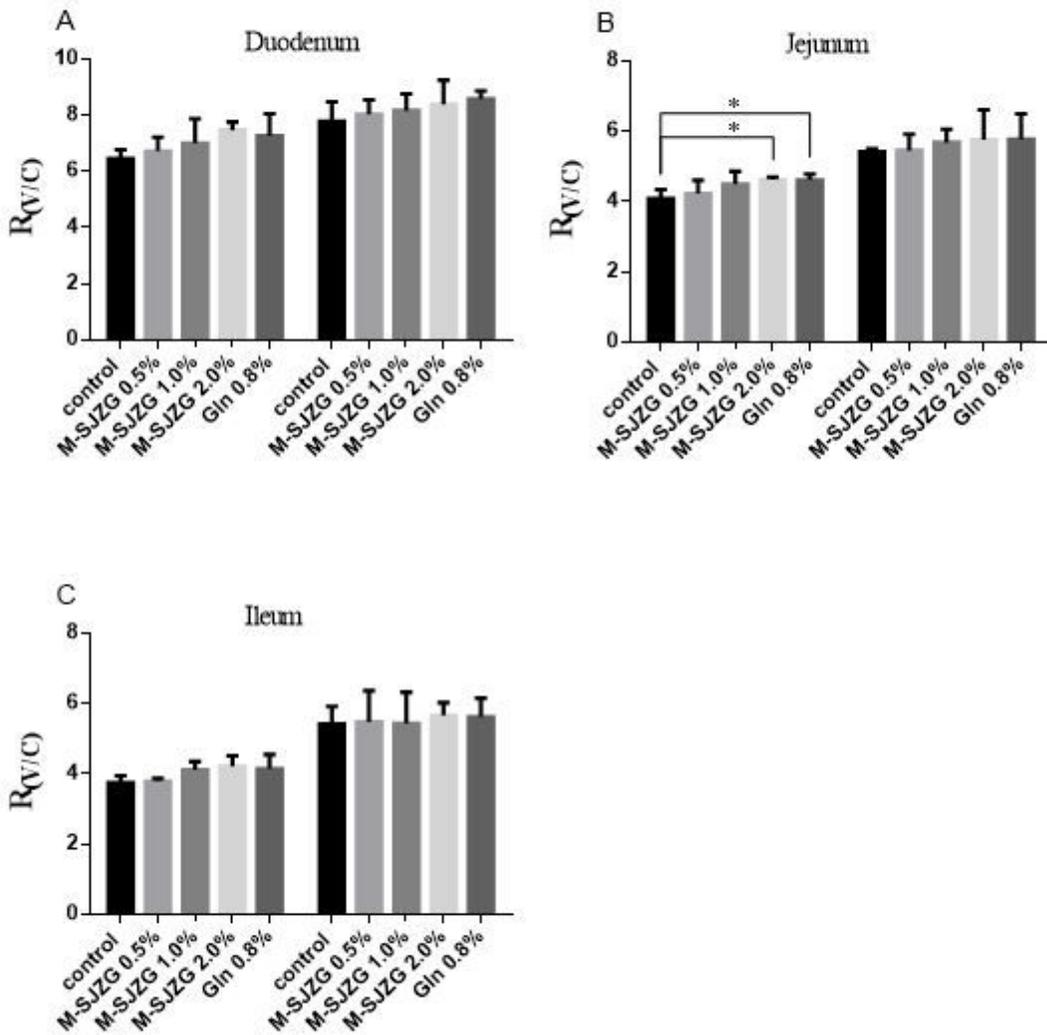


Figure 2

Effects of M-SJZG on the ratio of villi length to crypt depth in duodenum (A), jejunum (B) and ileum (C) in early-weaning Rex rabbits. Data are expressed as mean \pm SD. (n =6), *P<0.05.

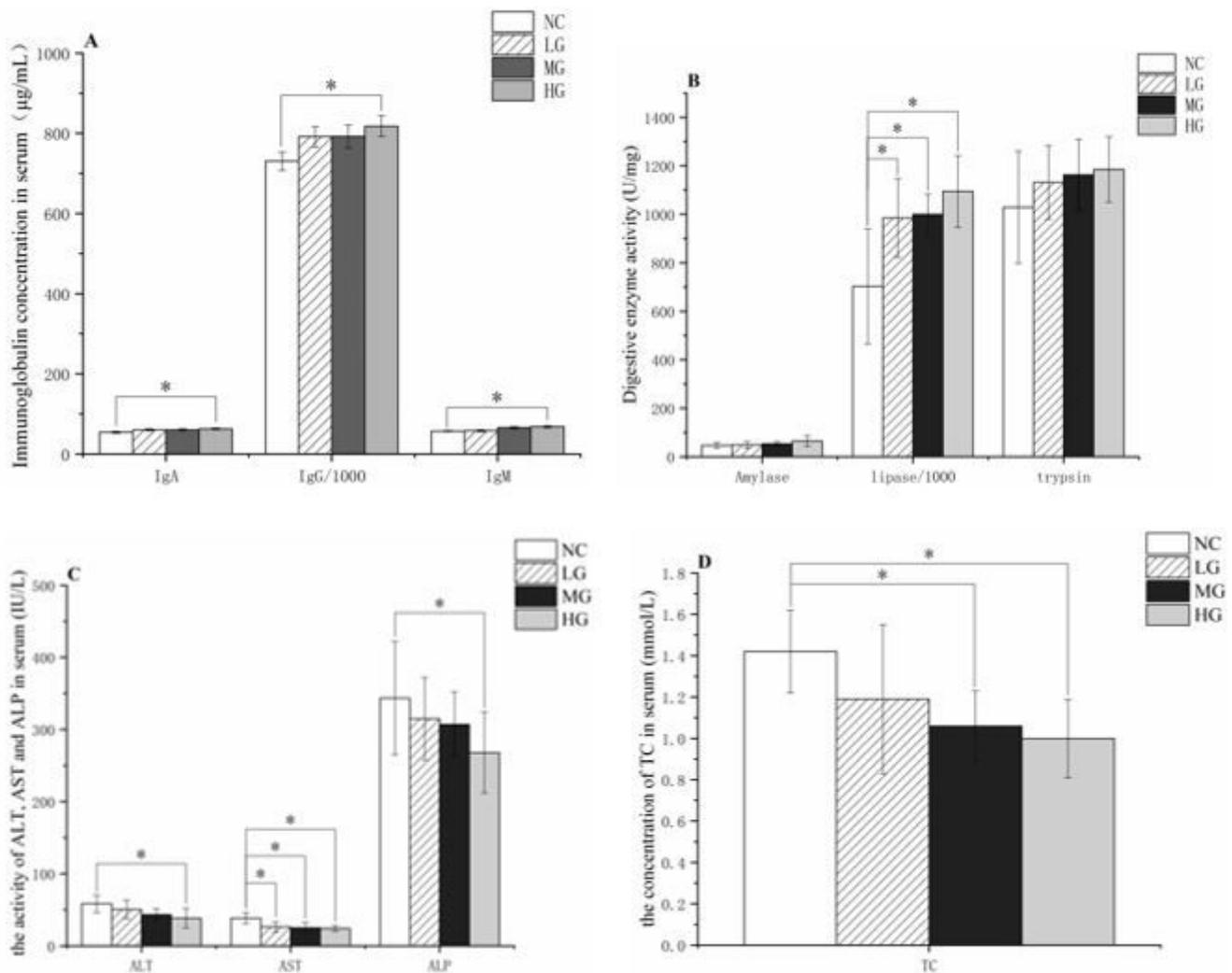


Figure 3

Serum biochemical indices after treatment, the concentration of immunoglobulin in the serum (A); the activity of digestive enzyme (B); the concentration of ALT, AST and ALP in the serum (C); the concentration of TC in the serum (D). * $p < 0.05$ compared with NC. NC, control group; LD, 0.5% M-SJZG group; MD, 1.0% M-SJZG group; HD, 2.0% M-SJZG group;

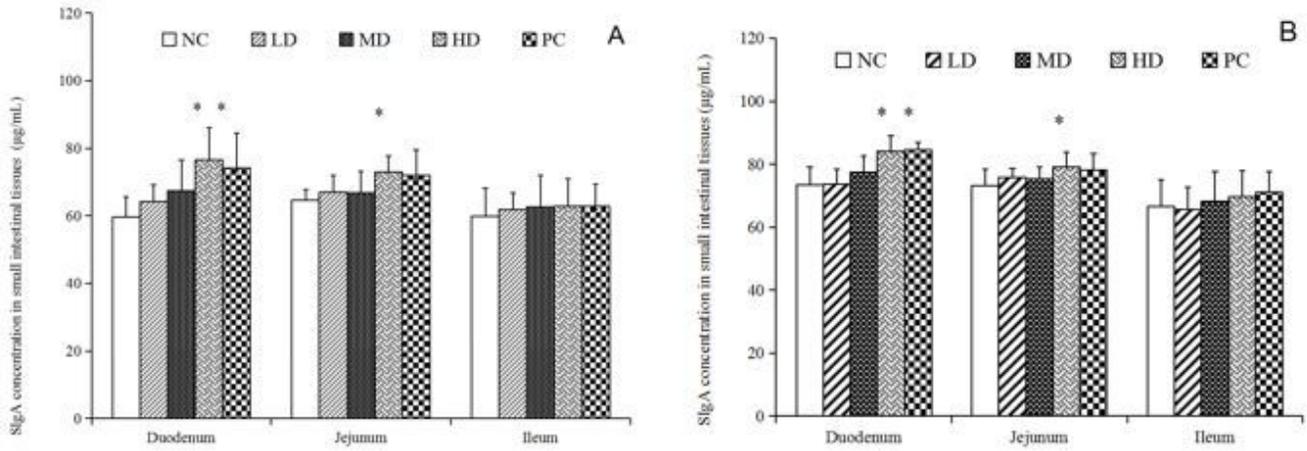


Figure 4

SIgA content in small intestinal tissues by Elisa, 0~15d (A) and 15~30d (B) (n=6), *p < 0.05 compared with NC. NC, control group; LD, 0.5% M-SJZG group; MD, 1.0% M-SJZG group; HD, 2.0% M-SJZG group; PC, positive, 0.8% Gln group.

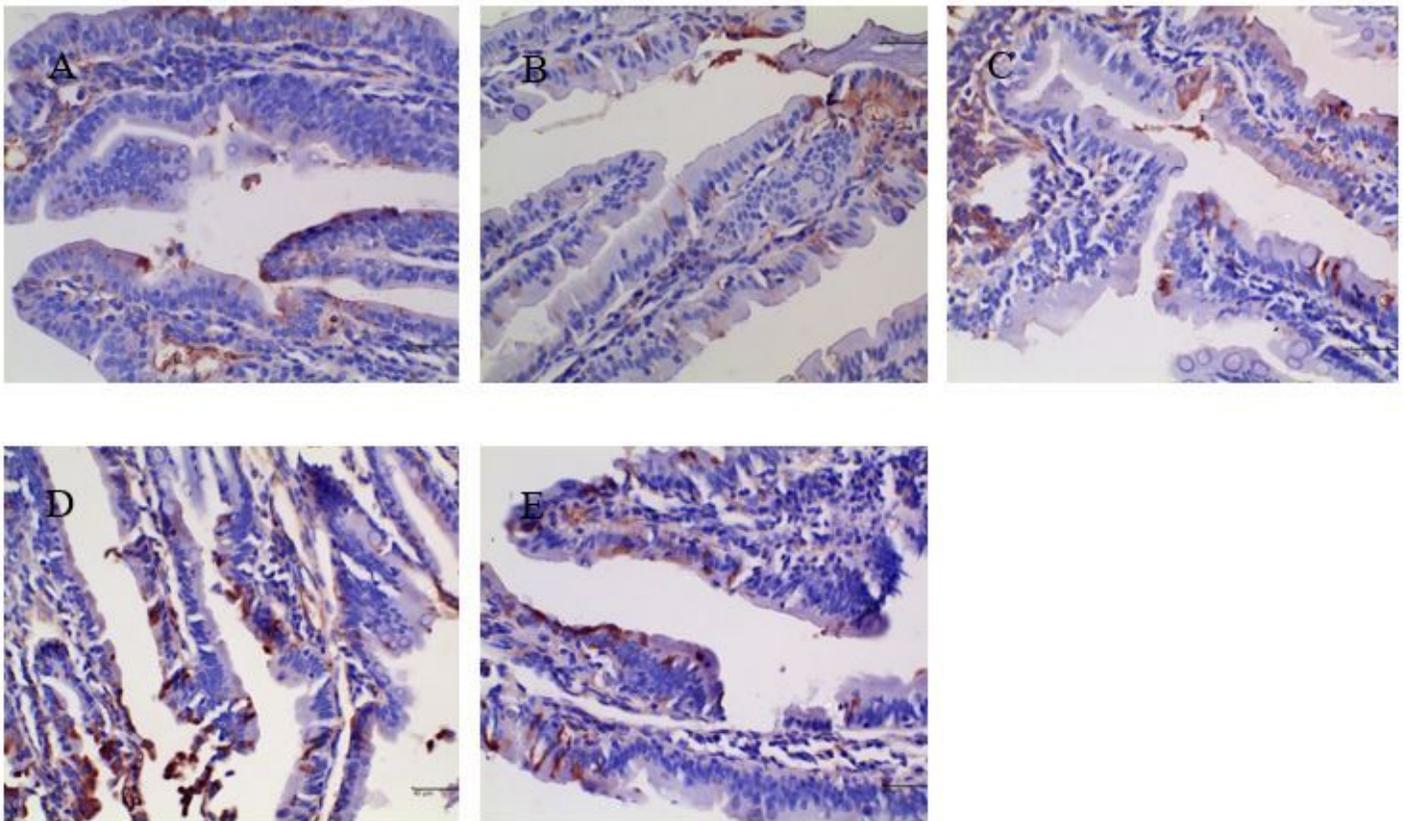


Figure 5

Immunohistochemical detection of SIgA of duodenum intestinal mucosa different groups 0~30d (×400). The negative and positive cells were blue and brown, respectively. The positive reaction was mainly operated in cytoplasm.

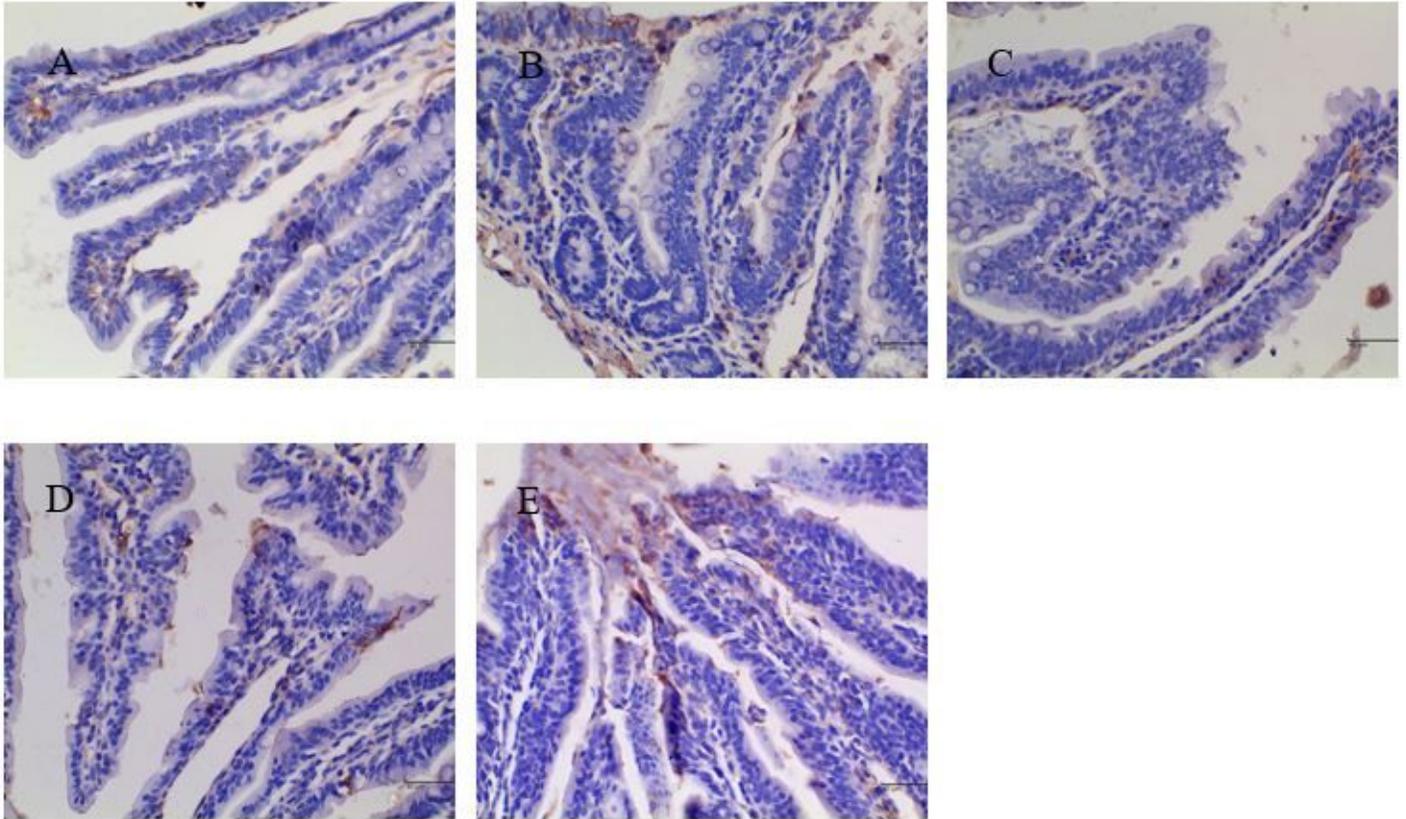


Figure 6

Immunohistochemical detection of SIgA of jejunum intestinal mucosa different groups (0~30d)(×400).

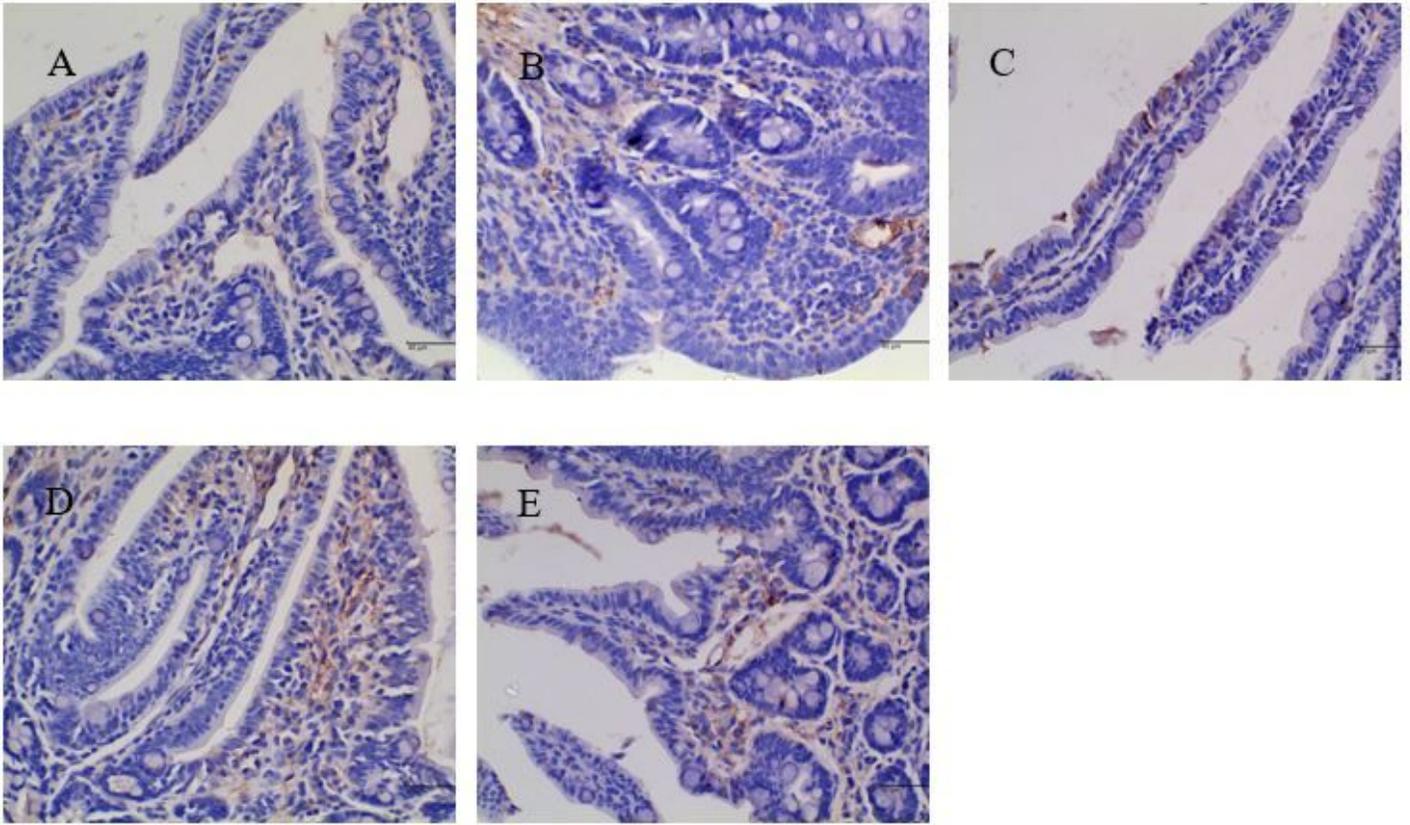


Figure 7

Immunohistochemical detection of SIgA of ileum intestinal mucosa different groups (0~30d)($\times 400$).

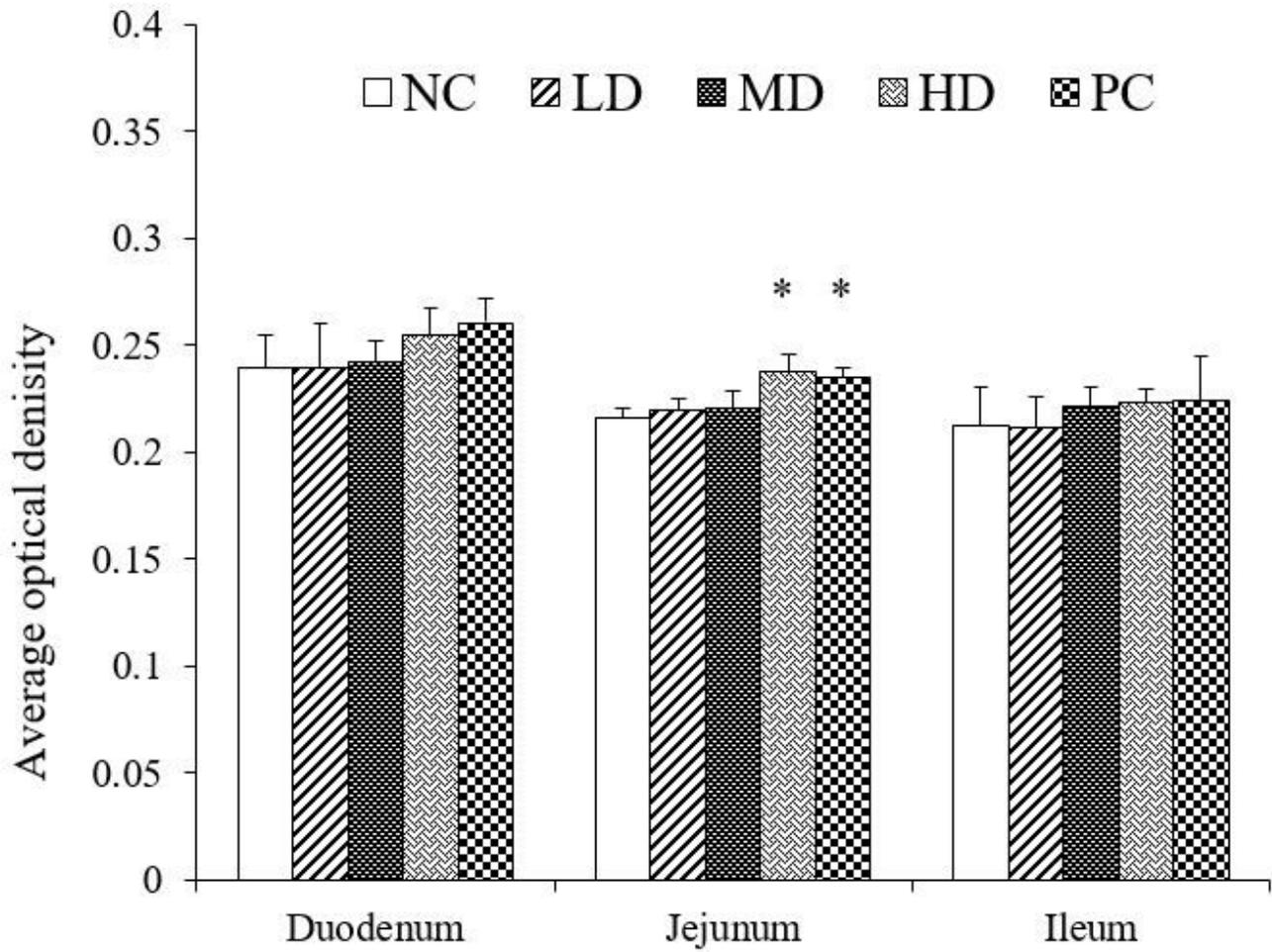


Figure 8

Average optical density of the express of SIgA in different groups on small intestinal mucosa (n=6). *p < 0.05 compared with NC. NC,control group; LD,0.5% M-SJZG group; MD, 1.0%M-SJZG group; HD, 2.0% M-SJZG group; PC, positive, 0.8% Gln group.

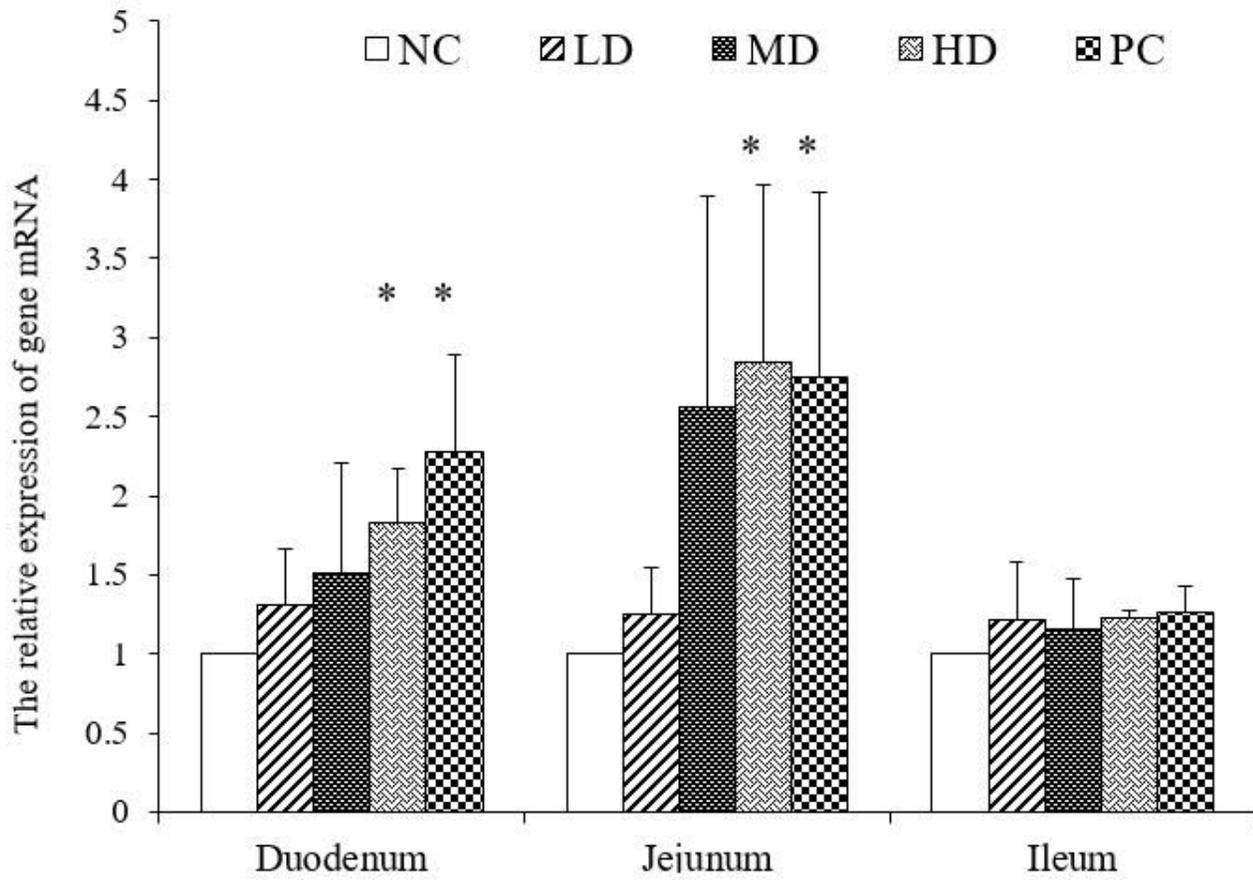


Figure 9

Effect of M-SJZG on gene expression of Zo1 in small intestinal mucosa of Rex Rabbits (n=6) *p < 0.05 compared with NC.

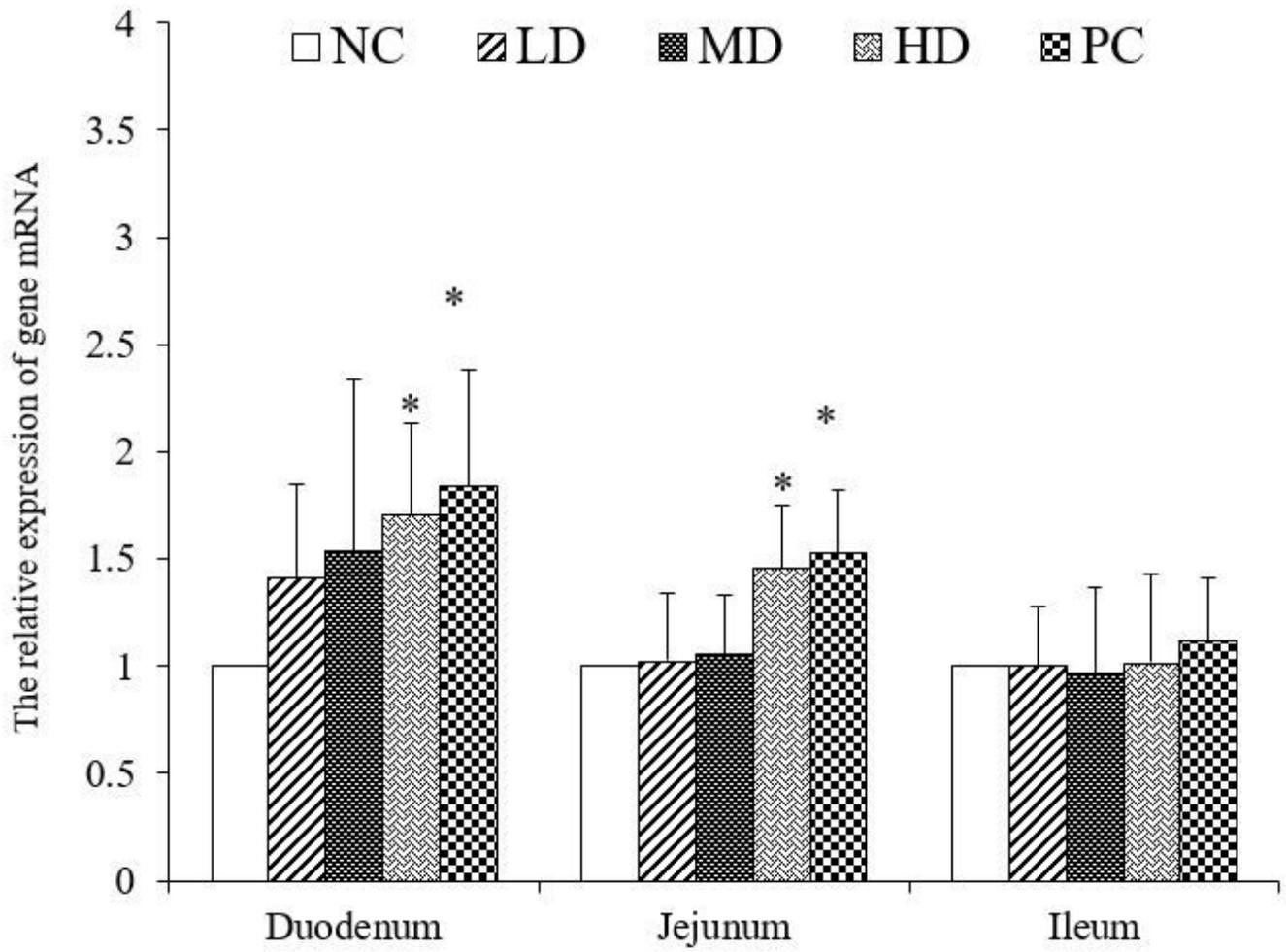


Figure 10

Effect of M-SJZG on gene expression of Claudin1 in small intestinal mucosa of Rex Rabbits (n=6) *p < 0.05 compared with NC.

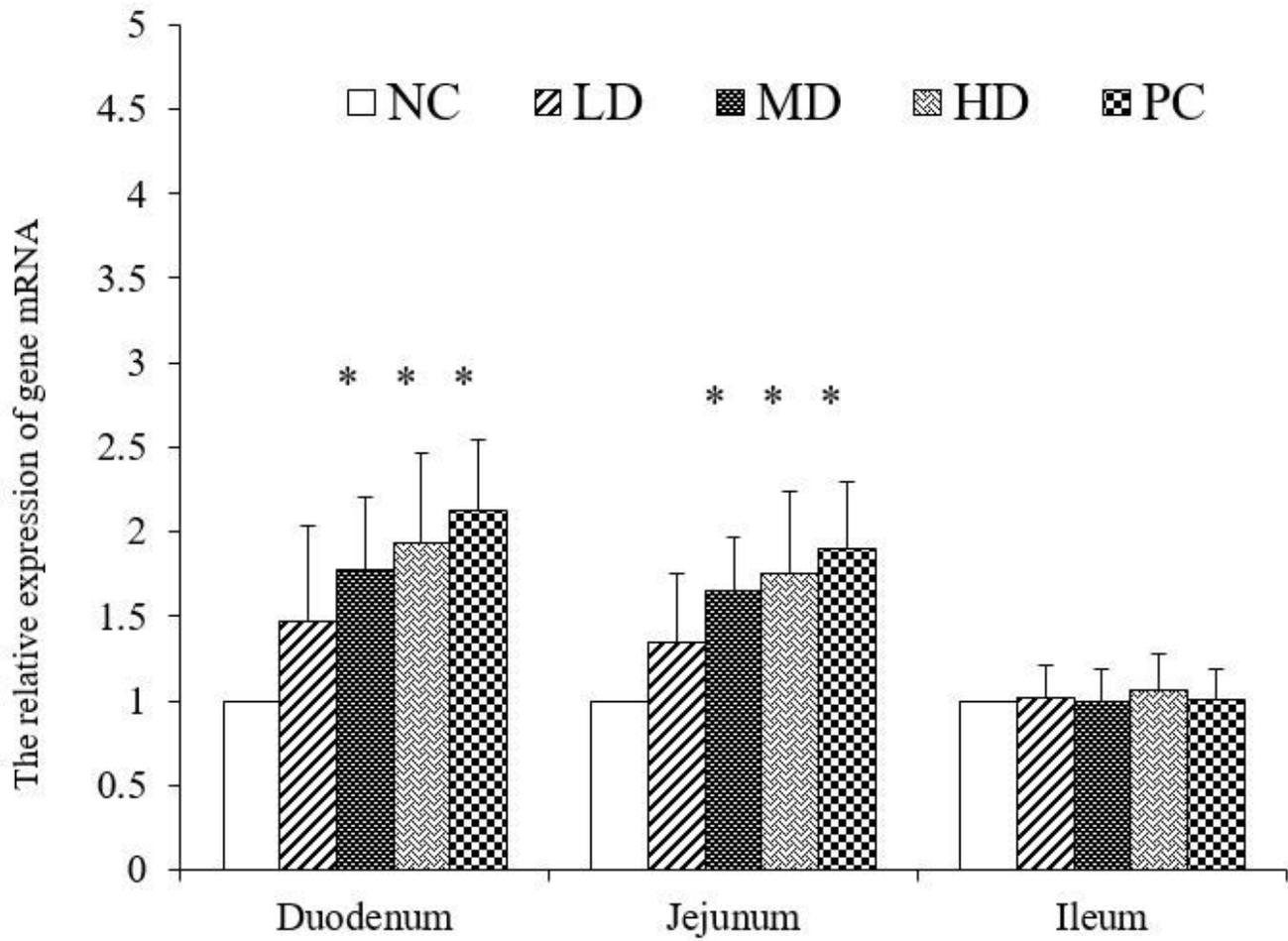


Figure 11

Effect of M-SJZG on gene expression of Occludin in small intestinal mucosa of Rex Rabbits (n=6) *p < 0.05 compared with NC.

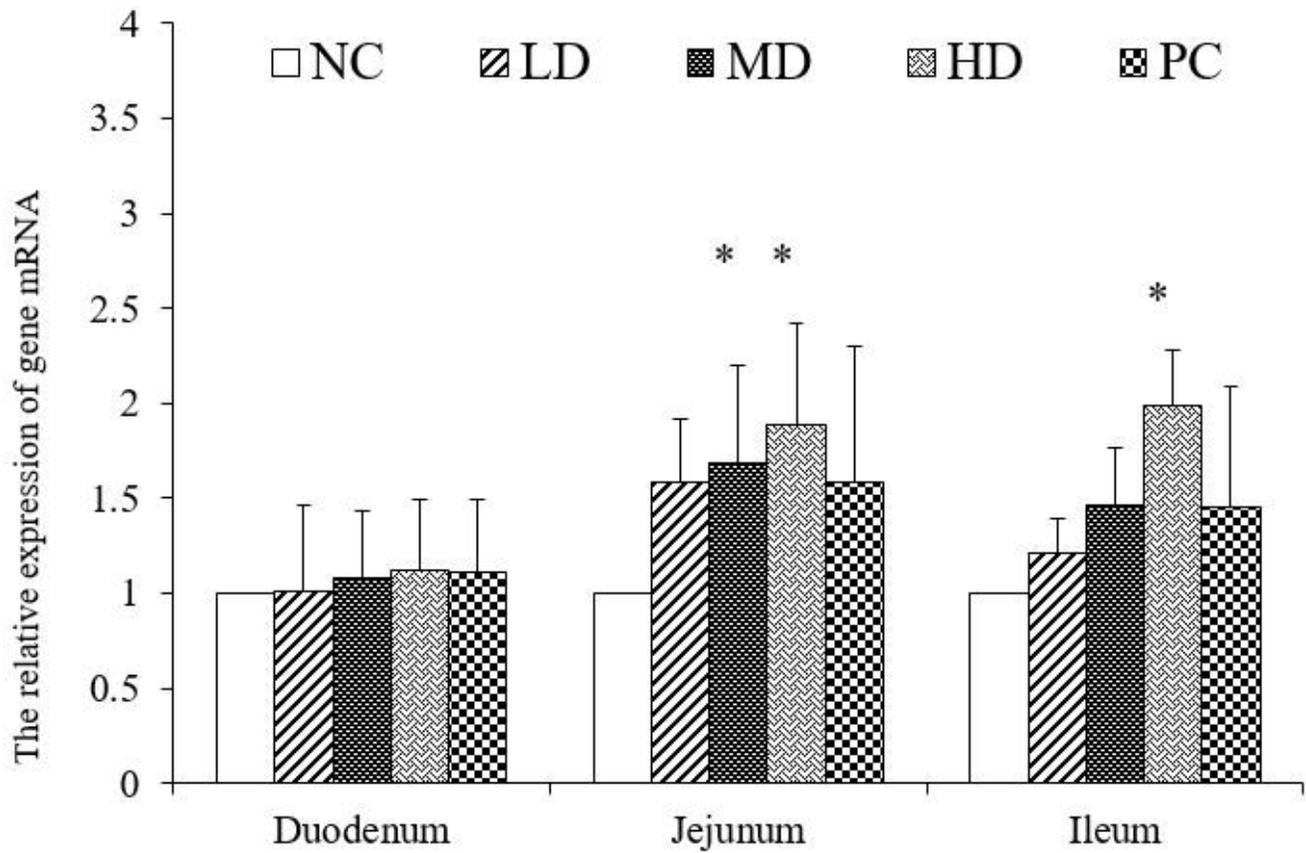


Figure 12

Effect of M-SJZG on gene expression of GLUT2 in small intestinal mucosa of Rex Rabbits (n=6) *p < 0.05 compared with NC.

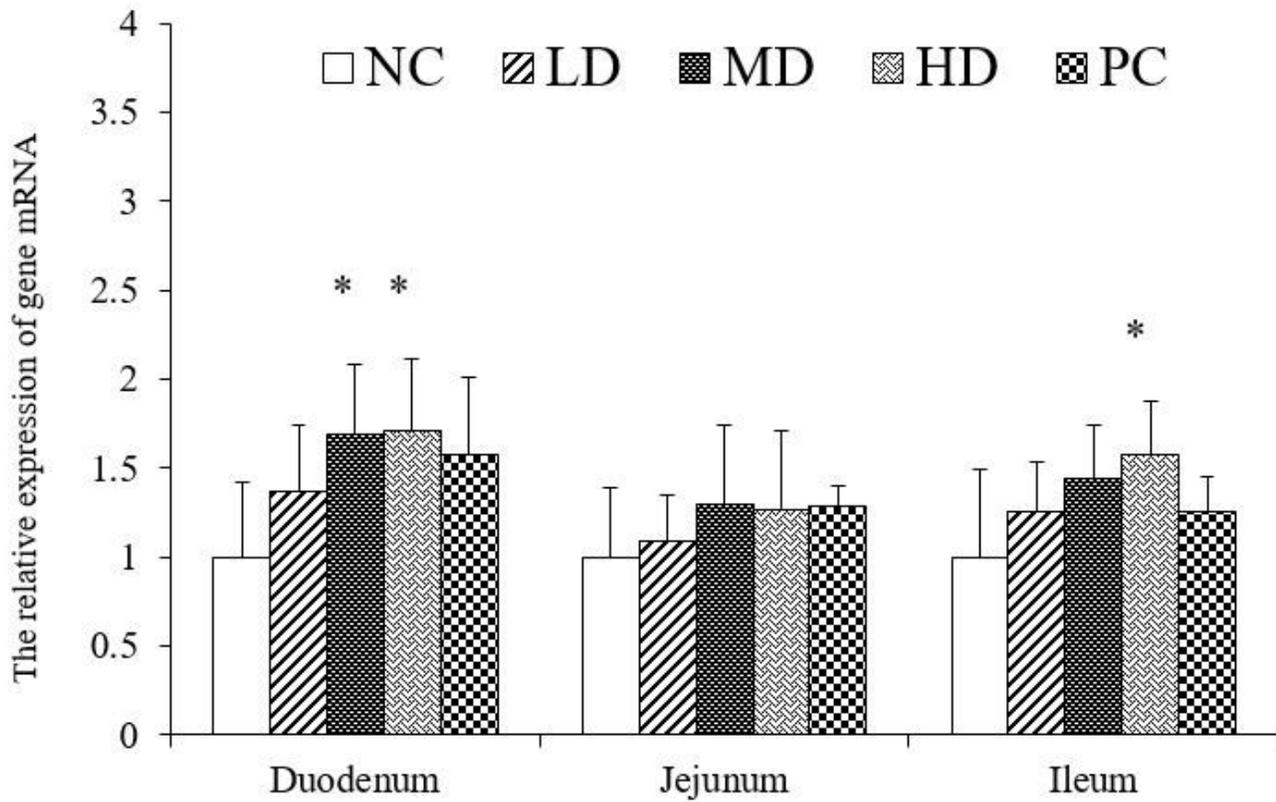


Figure 13

Effect of M-SJZG on gene expression of SGLT1 in small intestinal mucosa of Rex Rabbits (n=6) *p < 0.05 compared with NC.

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