

Histological study of intestinal goblet cells, IgA, and CD3⁺ lymphocyte distribution in Huang-huai white goat

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Background: Ten healthy adult Huang-huai white goats were selected and sacrificed by jugular vein bleeding after anaesthesia to observe the distribution characteristics of the histological structure of the intestinal mucosa, goblet cells, IgA, and CD3⁺ lymphocytes.

Materials and methods: Three sections of the duodenum, the jejunum, and the ileum were immediately collected and fixed with 4% paraformaldehyde for 72 h to prepare tissue sections. After haematoxylin and eosin, periodic acid Schiff, and immunohistochemical staining was performed, the distribution characteristics of goblet cells, IgA-positive cells, and CD3⁺ lymphocytes were observed. Results showed high columnar epithelial cells in the duodenum and jejunum of Huang-huai white goat and low columnar epithelial cells in the ileum mucosa.

Results: Mucopolysaccharides secreted by intestinal goblet cells were mainly neutral, and the number of ileum goblet cells was significantly higher than that of the duodenum and the jejunum ($p < 0.05$). IgA-positive cells were distributed in the lamina propria of the duodenum, and the number of cells was significantly higher than that in the jejunum and the ileum ($p < 0.01$). The significant difference was found between the jejunum and the ileum ($p < 0.01$). The CD3⁺ cells in the intestinal mucosa were distributed in the lamina propria mucosae, and some of the positive cells in the jejunum were distributed between epithelial cells. CD3⁺ cells had the largest number in the jejunal lamina propria but had the lowest number in the ileum.

Conclusions: The jejunum was significantly higher than the duodenum ($p < 0.05$), and the ileum was much less than the jejunum ($p < 0.01$). (Folia Morphol 2020; 79, 2: 303–310)

Key words: goblet cells, IgA, CD3⁺, small intestine, Huang-huai white goat

INTRODUCTION

Huang-huai white goat is a special local breed in Anhui Province, China. It is characterised by its small size, strong physique, strong resistance to disease, and high reproduction rate. Studies on Huang-huai white goat have mainly focused on the genetic breeding of skin and its derivatives, but limited studies

on the digestive tract have been performed. As the main site of nutrient digestion and mucosal immune absorption, goblet cells are an important type of intestinal mucosal epithelial cells. Goblet cells originate from pluripotent stem cells at the base of the intestinal crypt [30], which can synthesize and secrete various factors, such as trefoil peptide and mucin,

jointly forming the intestinal mucus layer to protect epithelial cells [21]. The secreted mucus can promote food digestion, help the body absorb nutrients and resist pathogen invasion, and promote food swallowing through the mucus, which is of great significance [10, 14]. Studies have investigated the distribution of goblet cells in the small or large intestine of ostrich [5, 6], newborn goat [19], human [27], southern catfish [17], and other animals, but observations on the distribution of goblet cells in the small intestine of Huang-huai white goat have not been reported.

The IgA of small intestinal mucosal epithelium is mainly absorbed by microfold cells and presented to B lymphocytes, which are transformed into plasma cells for secretion. After polymeric IgA is secreted by IgA plasma cells in the small intestinal mucosa, it binds to the secretory component (SC) secreted by intestinal epithelial cells in the form of a covalent bond on the basophilic side of epithelial cells, which are carried into cells in an internalised manner to form a pinocytotic vesicle, transported within the vesicle to the apical epithelium, and released into the mucosal cavity through exocytosis in the form of an IgA-SC compound to form secretory IgA (SIgA). It is also a benign antibody [2, 9]. The first line of defence of intestinal mucosal immunity is mainly formed by SIgA. SIgA has the function of immune regulation, immune clearance, and intestinal microbial regulation [4, 8, 20] and plays an important role in resisting pathogen invasion and maintaining the body's health. CD3 is an important differentiation antigen on the surface of a T cell membrane and characteristic marker of mature T cells. CD3⁺ T lymphocytes possess important immune functions in small intestinal tissues. When T cell antigen receptors recognise and bind to antigens, CD3 can transmit signals to the cytoplasm of T cells to activate T cells, causing the specific proliferation of cytotoxic T cells and release of a variety of cytokines and directly or indirectly killing target cells [1, 24]. In this study, the histological characteristics of the small intestine of Huang-huai white goat and the distribution of goblet cell IgA and CD3⁺ T cells were observed to provide reference for the intestinal mucosal immunity of Huang-huai white goat.

MATERIALS AND METHODS

Experimental animals

Ten adult Huang-huai white goats either male or female were used in the experiment. They were given

general anaesthesia and sacrificed by venous bleeding. Then, three segments of the small intestine (duodenum, jejunum, and ileum) were quickly dissected, and each segment was about 1 cm long. They were subsequently fixed in 4% paraformaldehyde solution immediately for later use.

Preparation of paraffin tissue section

The fixed tissue samples of the small intestine were prepared into 0.5 cm intestinal segments, washed with water for 24 h, dehydrated with gradient alcohol, impregnated with transparent paraffin wax, and cut into 5 μ m continuous slices by using a rotary slicing machine. A water bath was used for section flattening, section dragging, and section baking for the later preparation.

Histochemical and immunohistochemical staining

Paraffin tissue sections were prepared, dewaxed, and dehydrated, and some sections were stained with periodic acid Schiff (PAS) to observe the distribution of goblet cells in the three small intestinal mucosa. The other tissue sections were then immersed and washed with double-distilled water and phosphate buffer saline (PBS) three times (5 min for each time), repaired with antigen, cooled under indoor temperature, sealed with hydrogen peroxide for 10 min, immersed, and washed with PBS. IgA polyclonal antibodies (rabbit anti-goat IgA H&L, HRP) and CD3 T cellular polyclonal antibodies were purchased from Sigma, incubated at 37°C for 2–3 h, immersed, and washed with PBS. Once diaminobenzidine colour development appeared, the antibodies were washed with tap water for 5 min, dehydrated with a haematoxylin complex, gradient alcohol, and xylene until they became transparent, and sealed with neutral gum.

Statistical analysis

Under 100 \times magnification, 10 tissue sections of the small intestine of Huang-huai white goats were randomly selected and 10 visual fields were randomly chosen for each section. Each slice was counted the number of cells of 5 complete views, the amounts of the goblet cells, IgA-positive cells, and CD3⁺ lymphocytes were determined using Image-Pro Plus 6.0. Data were statistically analysed through one-way ANOVA by using SPSS 20.0. Mean standard deviation was used to represent experimental data. $P < 0.05$ indicated a significant difference, whereas $p < 0.01$ denoted a very significant difference.

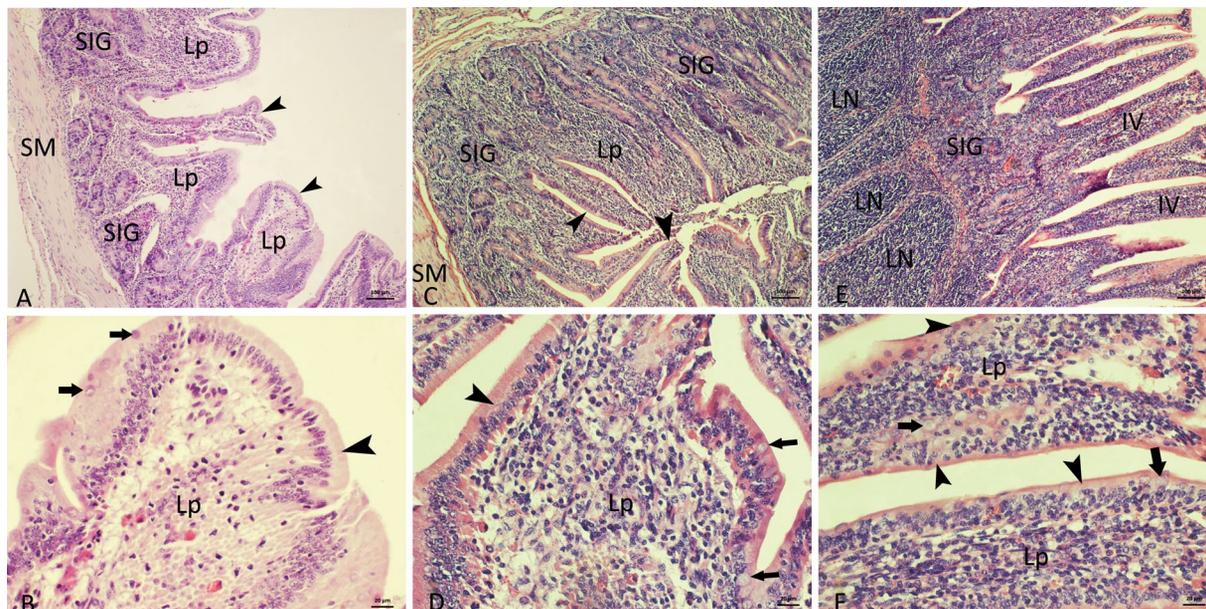


Figure 1. Basic histological characteristics of the intestinal mucosa of Huang-huai white goats (haematoxylin and eosin staining); **A, B.** The histological section of the duodenum; **C, D.** The histological section of the jejunum; **E, F.** The histological section of the jejunum ileum; IV — intestinal villus; LN — lymphoid nodule; Lp — lamina propria; SIG — small intestinal glands; SM — smooth muscle; arrow — goblet cells; arrowhead — epithelial cells.

RESULTS

Basic histological characteristics of the intestinal mucosa of Huang-huai white goats

In Figure 1, the small intestinal mucosa of Huang-huai white goats could be divided into three layers, namely, epithelial, lamina propria, and mucosal muscle. The duodenal villi of Huang-huai white goats were leaf shaped, whereas those of the jejunum were finger shaped and closely arranged. Epithelial cells were typically long columnar. The lamina propria of the mucosa contained more lymphocytes, and the jejunum had more goblet cells than the duodenum (Fig. 1A–D). The ileum villi of Huang-huai white goats were finger shaped, and high columnar epithelial cells changed to low columnar epithelial cells, and many goblet cells were found between them. The lamina propria contained more lymphocytes, the small intestinal glands were less than the jejunum and the duodenum, and the submucosa had typical aggregate lymphoid nodules (Fig. 1E, F).

Distribution of goblet cells in the small intestine mucosa of Huang-huai white goats

The small intestinal goblet cells of Huang-huai white goats are mainly distributed between mucosal epithelial cells and small intestinal gland epithelial cells (Fig. 2A–D). More goblet cells were found in the ileum mucosa than in the duodenum and the jeju-

num, and the goblet cells between the mucosal epithelial cells were significantly more than those in the duodenum and jejunum (Fig. 2E, F). After PAS staining was conducted, the goblet cells in the duodenum and jejunum showed co-existing purple- and blue-stained cells, indicating that the mucopolysaccharide secreted by goblet cells was neutral and acidic, and the number of the goblet cells secreting the mucopolysaccharide of both properties was the same (Fig. 2B, D). However, the goblet cells in the ileum mucosa of Huang-huai white goats had more purple stain, indicating that the mucopolysaccharide secreted by the goblet cells was neutral, and the goblet cells secreting neutral mucopolysaccharide were significantly more than the goblet cells secreting acid mucopolysaccharide (Fig. 2F). The percentage of goblet cells in every 100 cells in the three small intestinal tissue sections was statistically analysed using Image-Pro Plus 6.0 (Fig. 3). The number of ileum goblet cells was significantly higher than that of the duodenum and the jejunum ($p < 0.05$), but the difference between the duodenum and the jejunum was not significant ($p > 0.05$).

Distribution of IgA-positive cells in the small intestinal mucosa of Huang-huai white goats

The plasma cells of the small intestinal mucosa of Huang-huai white goats were mainly distributed

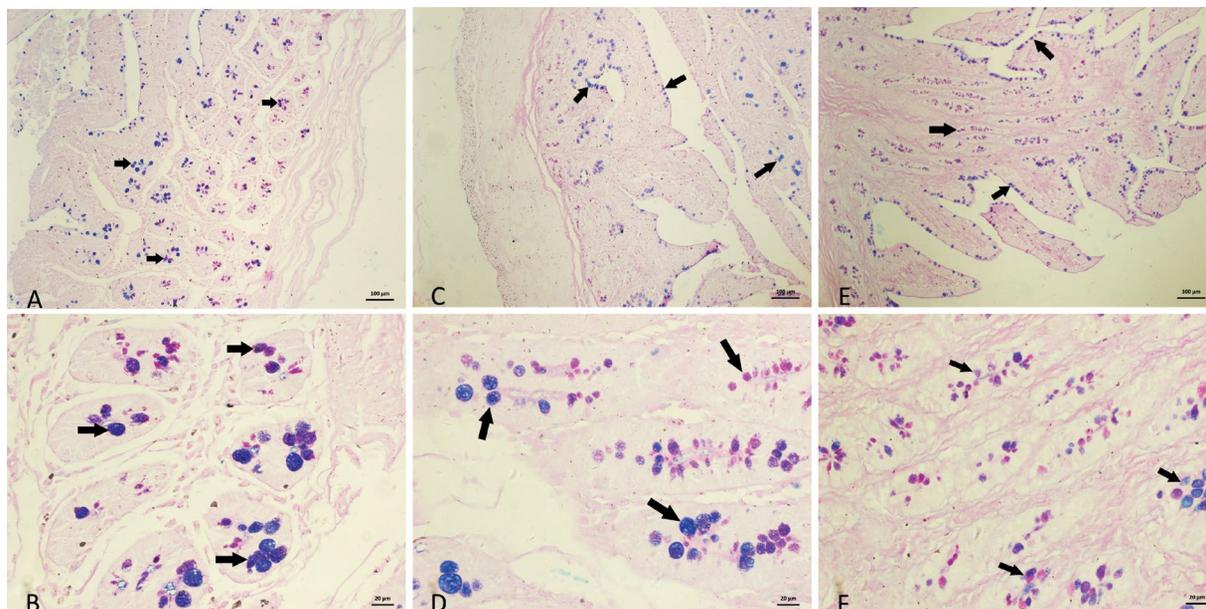


Figure 2. Distribution of goblet cells in the small intestine mucosa of Huang-huai white goats (AB-PBS); **A, B.** Goblet cell distribution and slime feature of duodenum; **C, D.** Goblet cell distribution and slime feature of jejunum; **E, F.** Goblet cell distribution and slime feature of ileum; arrow — goblet cells.

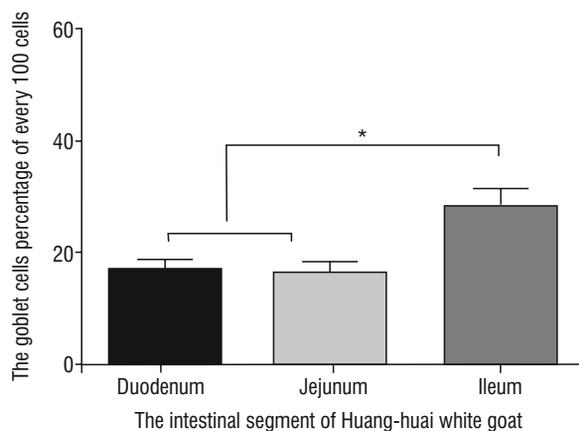


Figure 3. Comparison of goblet cells quantity in different intestinal segments of Huang-huai white goats; *Sgnificant difference.

in the lamina propria. Group distribution could be observed by haematoxylin and eosin staining. The cell body was strongly eosinophilic, and the nucleus was hyperchromatic, which was significantly skewed to the cell side. The distribution in the duodenum and the jejunum was high, whereas the distribution in the ileum was low (Fig. 4A–C). The results of IgA immunohistochemical staining showed that a large number of IgA-positive cells were distributed in the duodenal lamina of adult Huang-huai white goats, and the nuclei were round or oval and mostly inclined

to one side but were not distributed in the villous epithelial cells and intestinal glands of the small intestine (Fig. 5A, B). The IgA-positive cells were less in the jejunum than in the duodenum and were distributed in the lamina propria of the jejunum mucosa (Fig. 5C, D), and no IgA-positive cells were found between epithelial cells and intestinal glands. A few IgA-positive cells were found in the ileum and occasionally distributed in the lamina propria between the villi of the small intestine. The cells showed a positive reaction, and the nucleus was oriented to one side, but no IgA-positive cells were distributed in the aggregate lymph nodules (Fig. 5E, F). Statistical analysis revealed that the IgA-positive cells in the intestinal mucosa of adult Huang-huai white goats gradually decreased from the duodenum to the ileum, and the number of IgA-positive cells in the three duodenal jejunum and ileum was significantly different ($p < 0.01$; Fig. 6).

Distribution of CD3⁺ lymphocytes in the small intestinal mucosa of Huang-huai white goats

CD3⁺ lymphocyte immunohistochemical results showed that CD3⁺ lymphocytes were mainly distributed in the lamina propria of the small intestinal mucosa of Huang-huai white goats, whereas some rare positive cells in the jejunum were distributed between epithelial cells, and CD3 protein was distributed in

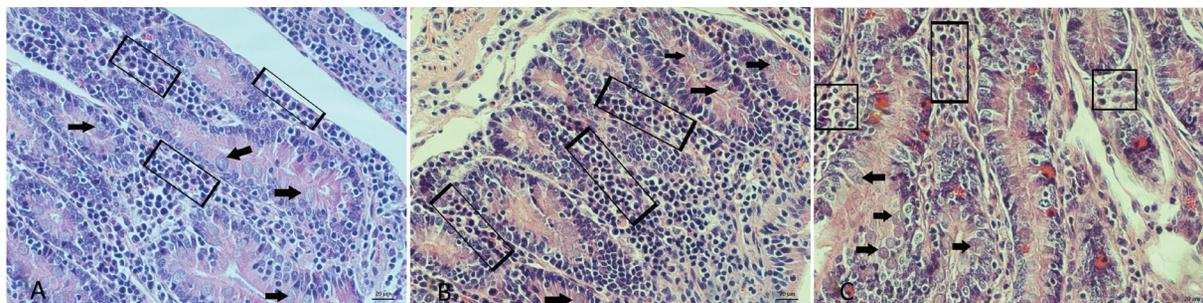


Figure 4. Tissue structure characteristics of plasma cells of small intestine of Huang-huai white goats (haematoxylin and eosin staining); **A.** Distribution of plasma cells in the duodenum; **B.** Distribution of plasma cells in the jejunum; **C.** Distribution of plasma cells in the ileum; arrow — goblet cells; rectangle box — plasma cells.

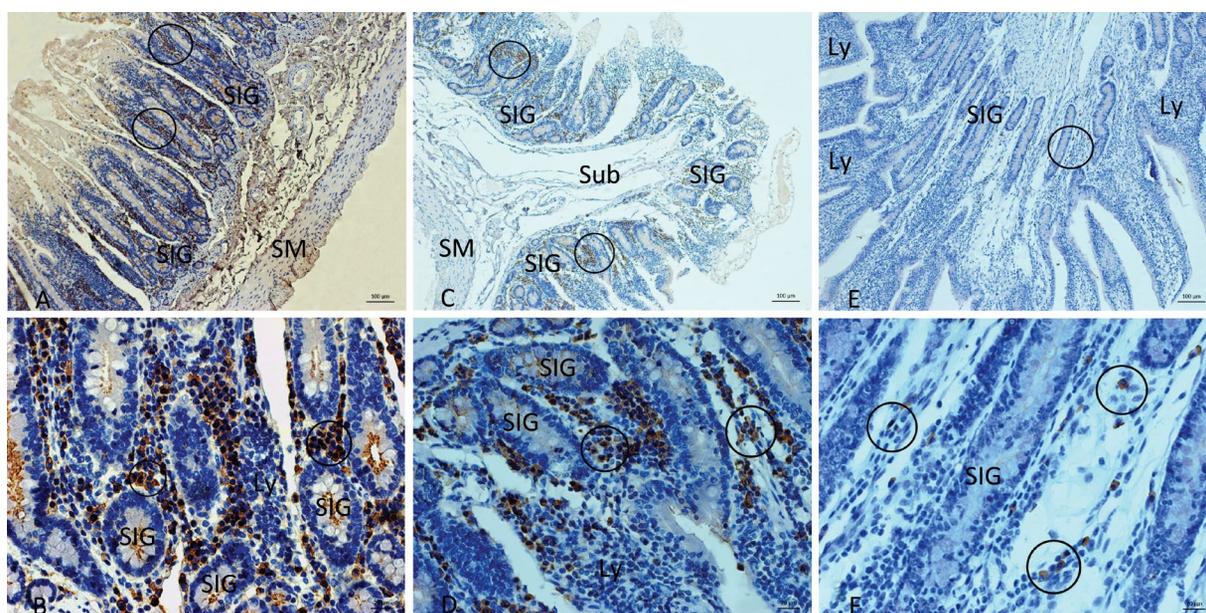


Figure 5. IgA positive cells distribution of small intestine of Huang-huai white goats (immune-histochemical staining); **A, B.** Distribution of IgA positive cells in the duodenum; **C, D.** Distribution of IgA positive cells in the jejunum; **E, F.** Distribution of IgA positive cells in the ileum; Ly — lymphocyte; SIG — small intestinal glands; SM — smooth muscle; Sub — submucosa; Circle — show IgA positive cells.

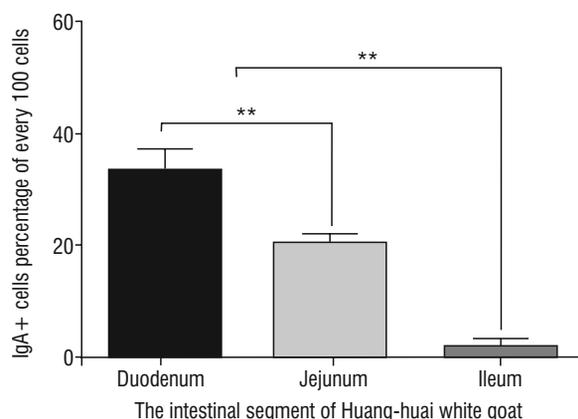


Figure 6. Comparison of IgA positive cells quantity in different intestinal segments of Huang-huai white goats; **Extremely significant difference.

the cytoplasm (Fig. 7). Statistical analysis indicated that among the duodenum, the jejunum, and the ileum, the jejunum mucosa had the largest number of CD3⁺ lymphocytes, whereas the ileum mucosa had the smallest number of CD3⁺ lymphocytes. The jejunum had a significant increase compared with the duodenum ($p < 0.05$), whereas the ileum had a significant decrease compared with the jejunum ($p < 0.01$; Fig. 8).

DISCUSSION

Intestinal goblet cells are mucus-secreting cells scattered in the intestinal mucosal epithelium, and their main functions are secreting mucus and mucin

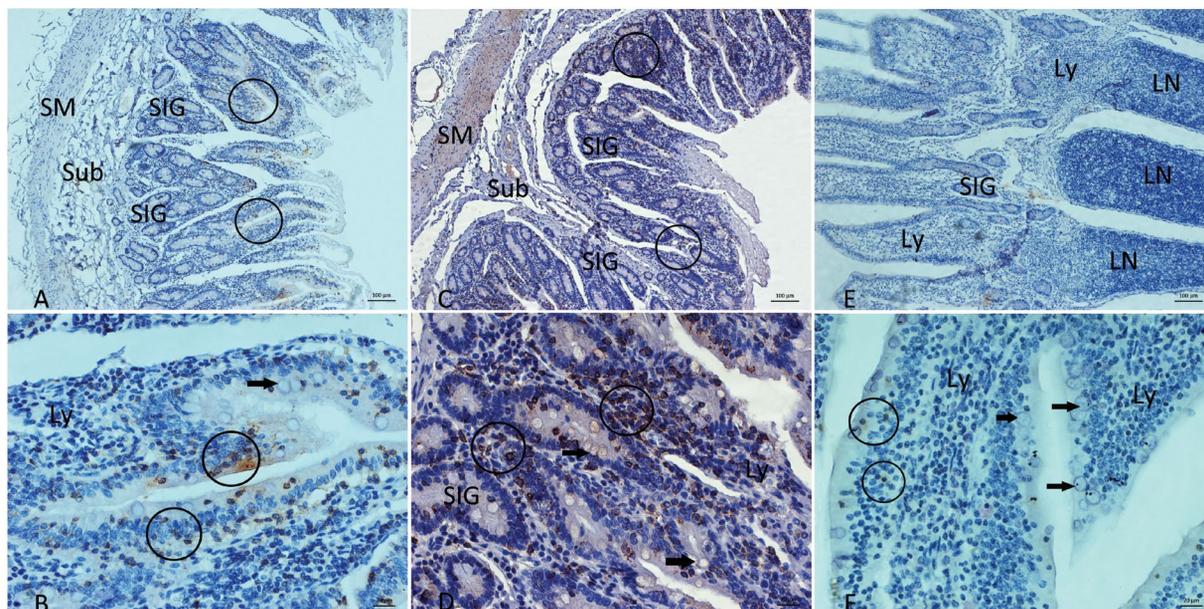


Figure 7. CD3⁺ cells distribution of small intestine of Huang-huai white goats (immune-histochemical staining);

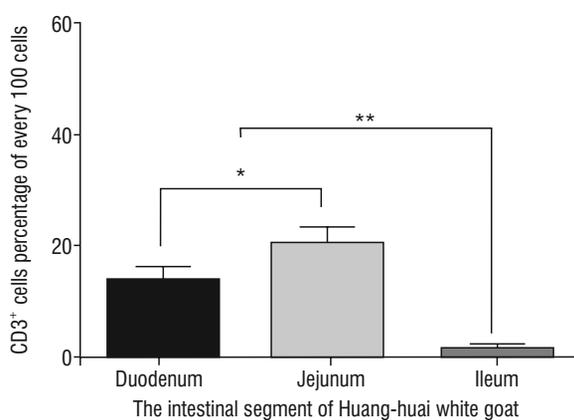


Figure 8. Comparison of CD3⁺ cells quantity in different intestinal segments of Huang-huai white goats; *Significant difference; **Extremely significant difference.

[11, 12], participating in intestinal antigen presentation [15, 23], facilitating immune regulation and intestinal injury surface reconstruction [13, 16, 18, 22, 25], and playing an important role in the inherent barrier of the intestinal tract. Our results indicated that the goblet cells in the ileum mucosa were significantly higher than those in the duodenum and jejunum in the small intestine of adult Huang-huai white goats probably because of the special physiological structure of the ileum. As the transition section between the small intestine and the large intestine, the ileum is extremely vulnerable to microbial invasion. Mucin

secreted by goblet cells in the ileum increases correspondingly and coats the ileum, thereby protecting the intestinal mucosa. Therefore, the secretion of more goblet cells in the ileum of adult Huang-huai white goats could compensate for the deficiency of the ileum mucosal barrier.

The lymphoid nodules in the intestinal mucosa aggregate lymph nodes, scattered lymphoid tissue, macrophage and plasma cells constitute the intestinal lymphoid tissue, which is also known as the intestinal associated lymphoid tissue (GALT). As the first line of defence of the immune system, GALT can secrete immunoglobulin when the intestinal mucosa is stimulated by antigens to resist microbial invasion [7]. Micropleated cells in the intestinal epithelium can engulf antigens and stimulate B lymphocytes to secrete IgA. IgA can remove antigens without activating the immune system. Therefore, the distribution and quantity of IgA can reflect the local cellular immune function of the small intestinal mucosa. The lamina propria of the small intestinal mucosa contains many plasma cells, which are activated B lymphocytes. Most of the plasma cells secrete IgA, whereas some of them secrete IgM, which can enter the intestinal cavity in the form of sIgA and bind to bacteria in the intestinal cavity so that they can adhere to the intestinal mucosa to prevent its displacement or directly neutralise its toxicity. Che and Yang [3] confirmed that IgA-positive

cells in the small intestine of piglets are mainly distributed in the mucosal epithelial lamina propria and distributed in the ileum collecting lymph nodes. Our experimental results showed that the cells of the small intestine of Huang-huai white goats that secreted IgA existed in the diffuse lymphoid tissue of the lamina propria. The nuclei of most positive cells were one sided, and the cytoplasm was eosinophilic. The duodenal mucosal lamina propria of Huang-huai white goats contained more IgA-positive cells, whereas the jejunum and ileum lamina had less IgA-positive cells probably because of the first contact of the duodenum with foreign antigens and immune responses; these findings were also probably related to duodenal jejunal antigens, bile salts, pancreatic secretions, and other local factors that stimulate the production and maturation of antibody-secreting cells [26]. However, IgA in the ileum of Huang-huai white goats was only distributed in the lamina propria of the small intestinal mucosa, and no positive cell distribution was observed in the aggregate lymph nodes, and these observations were inconsistent with the distribution of IgA secretory cells in the ileum of piglets [3].

CD3 is a common marker of all T lymphocytes. As an antigen receptor of T lymphocytes, CD3 is a signal transduction subunit, which can transfer an antigen stimulation signal received by a T cell receptor into cells, activate T lymphocytes, and play an essential role in T lymphocyte immunity [24, 28]. Stokes et al. [29] found that more than 90% of the intestinal epithelial lymphocytes are CD3 T lymphocytes, and about 65–80% of the lamina propria lymphocytes are CD3 T lymphocytes. Therefore, the number and distribution of CD3⁺ T lymphocytes in the small intestinal mucosa reflect the number and distribution of activated T lymphocytes to some extent and the local humoral immune function of the small intestinal mucosa. This experiment showed that CD3⁺ T lymphocytes in the small intestine of Huang-huai white goats were distributed in the mucosal layer of the duodenum, jejunum, and ileum, and the largest distribution was found in the jejunum, followed by the distribution in the duodenum. The smallest distribution was detected in the ileum, mainly in the lamina propria. However, CD3⁺ T lymphocytes distributed between mucosal epithelial cells were still rarely observed in the jejunum. The distribution characteristics of CD3⁺ T lymphocytes in the intestinal mucosa suggested that the humoral immunity of the intestinal mucosa of Huang-huai white goats mainly occurred in the jejunum.

CONCLUSIONS

From duodenum to jejunum, the epithelial cells change by high columnar to low columnar, goblet cells increased significantly, mucopolysaccharides secreted by intestinal goblet cells were mainly neutral, IgA positive cells significantly reduced, CD3⁺ cells of jejunum and duodenum were significantly higher than the ileum.

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