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Histomorphometric Adaptation of Yak (*Bos grunniens*) Abomasum to the Qinghai-Tibetan Plateau Environment

Adaptación Histomorfométrica del Abomaso de Yak (*Bos grunniens*) al Medio Ambiente de la Meseta Tibetana Qinghai

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SUMMARY: Six abomasums of yaks (*Bos grunniens*) were studied with gross dissection and histological methods. It was found that the mucosa of the yak abomasum was covered with simple columnar epithelium. There were lots of spiral folds (≥10) in the fundic glandular area. The developed membrane of lamina propria was occupied by high density glands. According to the morphological characteristics of the glands, the abomasum was divided into the cardiac, gastric and pyloric glands. Cardiac glands were curved tubular glands with the intumescent bottom and small glandular cavity. Fundic glands were simple tubular glands or branched tubular glands, where the chief, parietal and mucous neck cells can be observed clearly. Pyloric glands were curled tubular glands, the closer to the deep of the lamina propria, the more obvious the glands curl. Staining of glycoconjugate revealed that the mucosal epithelium of the cardiac gastric and pyloric glands and gastric pits epithelium mainly secreted neutral glycoconjugate, but other portions of cardiac and gastric glands secreted mixed and acid glycoconjugate respectively. By Gordon-Sweet's reticular fiber staining, it was found that the mucous neck cells possessed the characteristic of argyrophilic phenomenon. There was a large number of argyrophilic granules in the supranuclear cytoplasm in contrast with the chief cells. Furthermore, there were isolated lymphoid nodules and diffuse lymphoid tissue in the abomasum glands, especially in corpus abomasi. Grimelius silver staining showed that the argyrophil cells were located in the glandular epithelium and lamina propria of glands, which can also be observed in connective tissue. These endocrine cells dispersed individually in epithelial cells, occasionally in 3–5 cell groups. Therefore, the yaks were grazed throughout the year on diverse natural grassland and had evolved morphological characteristics of the abomasum enabling them to consume a wide variety of plant species, thereby better adapting them to harsh plateau environment.

KEY WORDS: Yak; Abomasum; Histomorphometric adaptation; Qinghai-Tibetan Plateau.

INTRODUCTION

The yak (*Bos grunniens*), as a year-round grazing animal, is one of the unique livestocks in the world, living in the Qinghai-Tibetan Plateau. The various rangelands of the plateau are characterized by their high altitude, short growing season (from June to September) and great seasonal variation in feed supply (Long *et al*., 2004, 2005). To adapt to the harsh conditions, the yaks have acquired many special morphological features in the long-term natural selection. Many studies showed that the location of the cranial cervical ganglion in the yaks corresponded with that of other domestic animals, whereas its shape was different (Ozgel *et al*., 2009; Kabak, 2007; Shao *et al*., 2007). Shao (2010) reported that the lingual prominence of yak tongue was greater and more developed than in cattle (Bos taurus).

The yak stomach consists of four parts, each with its own morphological particularities, including rumen, reticulum and omasum, known as forestomach (Hofmann, 1973). Although there has been considerable research into the organization of the stomach in cattle (Vivo *et al*., 1990), sheep (Wardrop, 1961; Franco *et al*., 1992, 1993a, 1993b, 1993c; Redondo *et al*., 1997; Regodon *et al*., 1996), deer (Franco *et al*., 2004a, 2004b, 2011, 2012; Redondo *et al*., 2005; 2011; Masot *et al*., 2007a, 2007b), and goat (Molinari & Jorquera, 1988; Ramkirshna & Tiwari, 1979; Nwaogu & Ezseaor, 2008; El-Gendy & Derbalah, 2010; Garcia *et al*., 2012), it is that no reports describing the stomach in the yak have been published previously, the aim of the present study was to describe the morphological and histological features

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of the yak abomasum and to provide a morphological basis for further research on adaption for the balance of whole-body energy homeostasis in poor grazing in the Qinghai-TibetanPlateau, China.

MATERIAL AND METHOD

Animals. All research protocols used in the current experiment were approved by Animal Ethics Committee of the Qinghai province, China.

The stomachs of 6 healthy yak (4 castrated, 2 males 3-6-yr-old) with a mean BW of 255 ±20 kg were collected immediately after being slaughtered from Datong slaughter house, Datong county (> 3,000 m above sea level), Qinghai, China. Four parts of the stomachs were fixed with 10% Paraformaldehyde solution (PH 7.4) after observation of gross anatomy.

Sampling and methods. Once the abomasum had been separated, small pieces of tissue were dissected for analysis. Tissues for histological examination were fixed in 10% buffered formaldehyde for 24 hours, after the specimens were dehydrated through a graded series of alcohol, cleared and embedded in paraffin wax, and sectioned at 5-7 mm. Sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS) alcian blue (AB), AB pH 2.5, AB pH 2.5- PAS (Pearse *et al*., 1985; Grimelius, 1968), which displayed the fanclus abomasi, corpus abomasi and pars pylorica, respectively. The slides were observed using bright microscopy (BH-2, Olympus, Nagano, Japan).

Statistical analysis. The results were shown as mean ± SE. A post-hoc (Tukey) analysis was carried out to test for significant differences in amounts of argyrophilic cells between different positions of abomasum. A value of $P<0.05$ was considered significant.

RESULTS

The cardiac glands area (fanclus abomasi). The surface cells of the mucosa were simple columnar epithelium cells with oval nuclei located in the basal, which was stained lightly in the cytoplasm. A large amount of mucinogen granules in the supranuclear region of cytoplasm was shown by a strong positive reaction to PAS. The gastric pit epithelium was also a simple columnar epithelium which was shallower than the surface mucous cells. The top of cells was filled with lightly stained mucus, the nuclear around noted darkly stained cytoplasm, which showed eosinophilic granules. In addition, the nuclei was often pressed against the basal cell when much of mucus existed. However, the basal region of cytoplasm with weakly basophilia was rather small (Fig. 1A), and showed positive reaction to PAS.

The lamina propria was the thicker, in contrast to the vela abomasica. Being rich in collagen fibers and reticular fibers, the latter constituted stent for the lamina propria. And a large number of closely arranged glands were located here. The shallow and deep gland cells of the lamina propria had two types in shape. The former showed the branched tubular glands arranged sparsely, with the shorter duct and smaller lumen. The bottom of duct was enlarged, and the more toward the lamina propria deeply, the more obvious the phenomenon of enlargement was. Depending on the composition of cells, the glands can be divided into serous and mucous glands, this part was classed as mucous gland. The mucous cells emerged in the shape of tall columnar or cone with oval nuclei located in the basal region, and with abundant of cytoplasm stained lightly. Occasionally, the apoptosis cells were observed with shrinkage and chromatin condensation in the mucous glands. A small amount of parietal and endocrine cells distributed among mucous cells. However, the deep gland cells of the lamina propria curled up into gathered package tightly (Fig. 1B), and gland cells consisted of parietal cells and a small amount of chief cells. The larger parietal cells, with small and round nuclei and eosinophilic cytoplasm, dispersed in the mucous cells. The chief cells, with strong basophilic cytoplasm and round nuclei located in the basal region, were tapered or cylindrical in shape. More parietal cells were on the body of cardia glands. moreover, more chief cells at the bottom. Further, fibroblast, plasma cells and lymphocytes also scatter in the lamina propria. A small amount of smooth muscle fibers from mucosa muscularis extended the deep of the lamina propria. The mucosa muscularis was more significant than the vela abomasica. The submucosa was composed of the loose connective tissue, which was rich in arteries, veins and lymphatic vessels.

The muscularis included two layers of smooth muscle, the inner and the outer. The inner ring was about 410.9–676.8 mm in the thickness, of which the muscle bundle was the more coarser than the outer. While the outside longitudinal muscle was only about 175.8–277.0 mm (Fig. 1C).

The adventitia (serosa) was composed of loose connective tissue, with small collagen fibers, less blood vessels and a small amount of fat cells.

The gastric glands area (corpus abomasi). The mucosa formed many folds lined with simple columnar epithelium. There were oval nuclei located in the basal region and

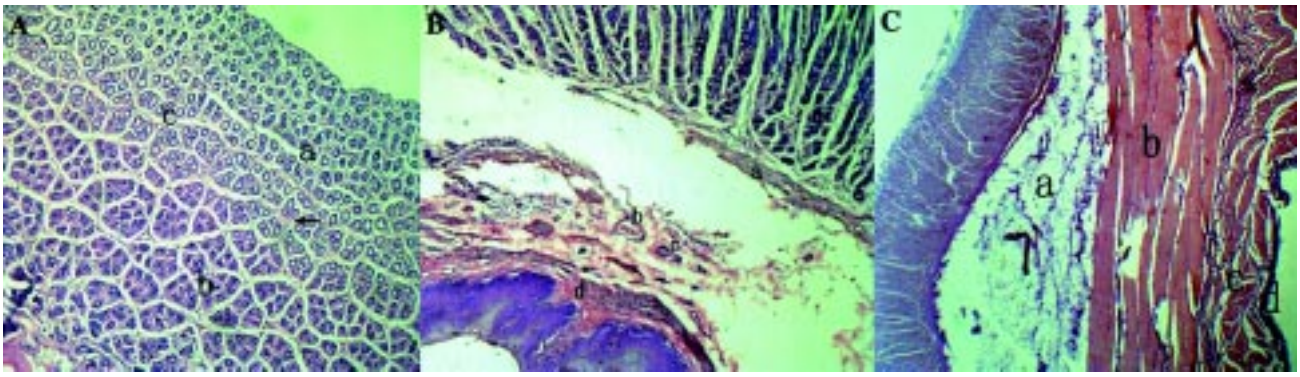

Fig. 1. Light microscopic view of the fanclus abomasi. (A) Gastric pit (a) and epithelium (arrow) of fanclus abomasi. Stained with PAS X400. (B) Upper (a) and lower (b) cardiac gland, reticular fiber (c) and blood vessel (d) in lamina propria of fanclus abomasi. HE X100. (C) Elastic fiber (a) in lamina propria, inner (b), outer (c) muscular layer and chorion (d) of fanclus abomasi. Stained with elastic fiber X 40.

mucinogen granules at the top of cells with stained lightly or not. Gastric pits arranged densely, which were more deeper than cardia gland. Mucus at the top of gastric pit cells was stained lightly, where the oval nuclei often was squeezed into platykurtic in shape, and showed positive reaction to sugar conjugate (Fig. 2A).

The lamina propria was quite thick, filled with gastric glands. It contained two types of long and straight glands, a single tubular and branch tubular glands arranged densely. And the glands were companied with lots of capillaries (Fig. 2B). Reticular fibers constituted the gland bracket in the lamina propria, and were often distributed along the basement of membrane (Fig. 2C), where many small arteries were found as well. And compared with reticular fibers, there were less collagen and elastic fibers, where mast cells and lymphocytes were able to be observed (Fig. 2D).

The gland tube of the gastric glands had small cavity and was very thin and long, which arranged tightly. The closer to the cardia, the shorter the tube was (Fig. 2E). The results of conventional staining and special staining indicated that the yak gastric glands were also composed of mucous neck, parietal, chief and endocrine cells.

The mucous neck cells distributed in the neck of glands. Due to the extrusion of the parietal cells, they were usually irregular in shape, with oval or flat round nuclei located in the base of cell. Gordon-Sweet's staining showed that the cytoplasm of mucous neck cells were filled with argyrophilic granules (Fig. 2F).

The parietal cells, with large volume, were oval or round. Abundant cytoplasm, which was stained strongly to HE, was filled with dense eosinophilic granules. They had two small and round nucleus located in the center of the cells generally, but three occasionally. There were more parietal cells in the body and the bottom of gland. The former was the most dense, lined up outside of the main and mucous neck cells. A portion of some glands was mainly covered with parietal cells, which decreased gradually at the bottom, Whereas, it significantly reduced in the blind end. Approaching isthmus in the neck of the gland, a small amount of parietal cells on the glandular epithelium was able to be observed.

The chief cells mainly distributed at the body and the bottom of gland, with low columnar or cubic in shape, which located between the parietal cells. There were spherical or oval nuclei located in the base of the cells. Visible basophilic granules to HE presented in the cytoplasm (Fig. 3A).

A few of the endocrine cells distributed in the gastric glands, with round, conical or triangular in shape, which were mainly embedded in the chief cells. The cytoplasm of the endocrine cells were filled with tiny argyrophilic granules so that we could observe at the neck and body of glands with Gordon-Sweet's staining.

The muscularis mucosa, with only continuous layer, was very thin.

The submucosa was composed of loose connective tissue with varying thickness. There were lots of veins, arteries, fibroblasts, mast cells, macrophages and lymphocytes, and with the coarser diameter of vein and artery and the finer collagen fibers. Nearby the muscularis, the fat cells were observed as well.

The muscularis was divided into two layers containing the inner ring muscle and outer longitudinal muscle. The thickness of the inner smooth muscle was about four times larger than the outer longitudinal muscle. Besides,

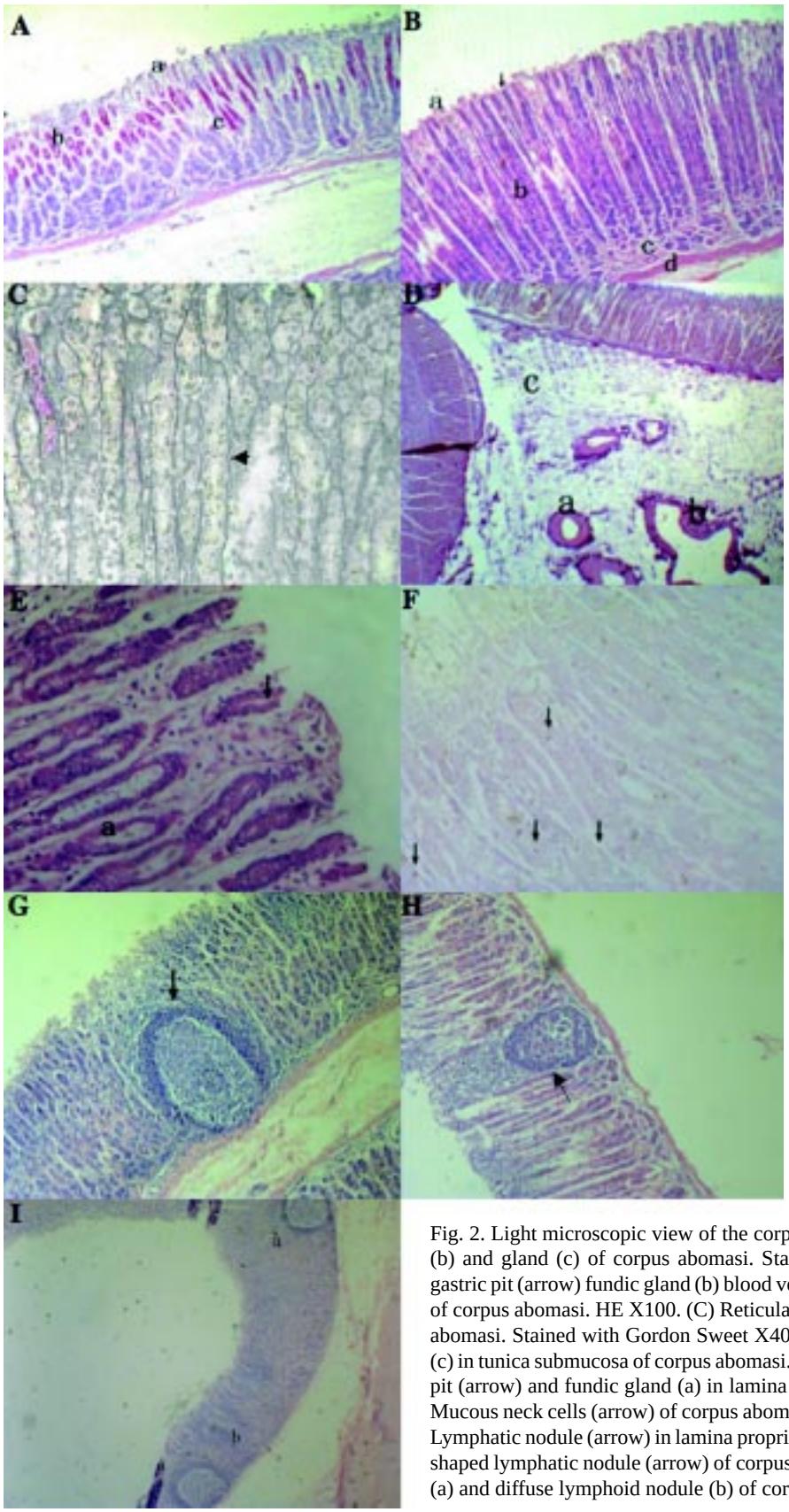

Fig. 2. Light microscopic view of the corpus abomasi. (A) Epithelium (a) gastric pit (b) and gland (c) of corpus abomasi. Stained with PAS X100. (B) Epithelium (a) gastric pit (arrow) fundic gland (b) blood vessel (c) and lamina muscularis mucosa (d) of corpus abomasi. HE X100. (C) Reticular fiber (arrow) in lamina propria of corpus abomasi. Stained with Gordon Sweet X400. (D) Aorta (a), vein (b) and elastic fiber (c) in tunica submucosa of corpus abomasi. Stained with elastic fiber X40. (E) Gastric pit (arrow) and fundic gland (a) in lamina propria of corpus abomasi. HE X400. (F) Mucous neck cells (arrow) of corpus abomasi. Stained with Gordon Sweet X400. (G) Lymphatic nodule (arrow) in lamina propria of corpus abomasi. HE X100. (H) Bottle-shaped lymphatic nodule (arrow) of corpus abomasi. HE X100. (I) Lymphatic nodule (a) and diffuse lymphoid nodule (b) of corpus abomasi. HE X 100.

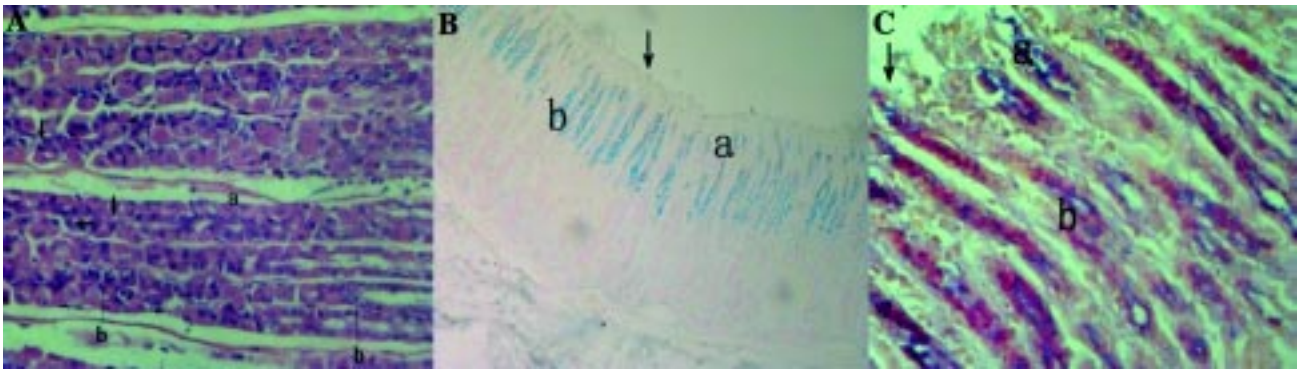

Fig. 3. Light microscopic view of the fundic glandular region. (A) Chief cells (arrow) parietal cells (a) and mucous neck cells (b) of fundic glandular region. HE X400. (B) Epithelium (arrow), gastric pit (a) and fundic gland (b) in fundic glandular region. Stained with AB-PAS X400. (C) Epithelium (arrow), gastric pit (a) and fundic gland (b) in fundic glandular region. Stained with AB X100.

the inner muscle fascicle was denser, while the outer one was looser.

The adventitia (serosa) was composed of thinner loose connective tissue, in which there were fewer small veins, capillaries, lymphatic capillaries and fat cells and more lymphocytes, plasma cells, and fibroblasts.

The pyloric glands area (pars pylorica). The thickness of mucosal layer in pyloric glands were similar as in cardiac glands, which were thicker (367.2–461.5 mm) than in gastric glands. There were intensive gastric pits with deep pleated mucosa (Fig. 4A). Mucosal epithelium was a simple columnar epithelium (Fig. 4B), which packed tightly, with oval nuclei located at the base of the cell. Both the basal and supranuclear region had small amount of cytoplasm, which were basophilic and eosinophilic, respectively. There was lightly stained or non-colored mucus at the top of cell. The gastric pits were very deep, with the lower epithelial cells, which also showed a strong positive reaction to PAS.

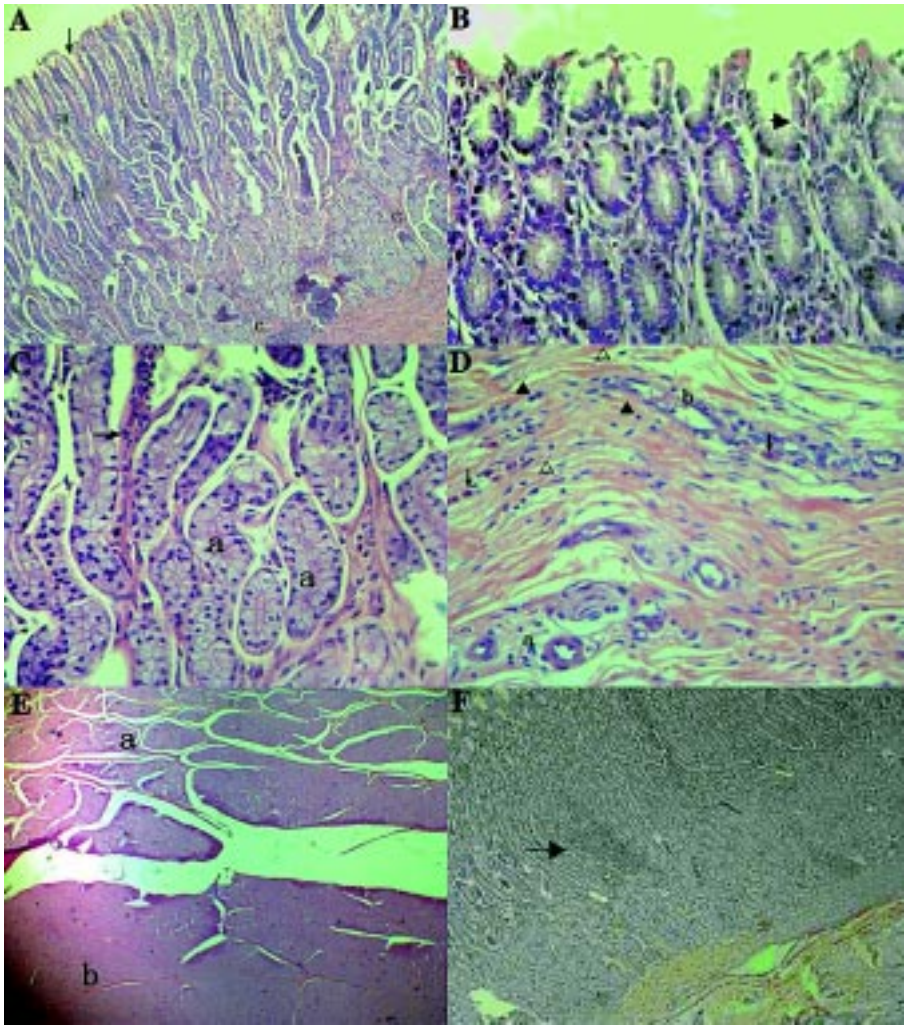

Fig. 4. Light microscopic view of the pars pylorica. (A) Gastric pit (arrow) lymphocyte (a) pyloric gland (b) and muscular mucosa (c) of pars pylorica. HE X100. (B) Simple columnar epithelium (arrow) of pars pylorica. HE X400. (C) Glandulae mucosa (a) and collagen fiber (arrow) in base lamina propria of pars pylorica. HE X400. (D) Macrophage cell (arrow), fibroblast (Δ), plasma cell (▲), aorta (a) and vein (b) in tunica submucosa of pars pylorica. HE X400. (E) Inner (a) and outer (b) muscular layer of pars pylorica. HE X40. (F) Diffuse lymphoid tissue (arrow) of pars pylorica. HE X100.

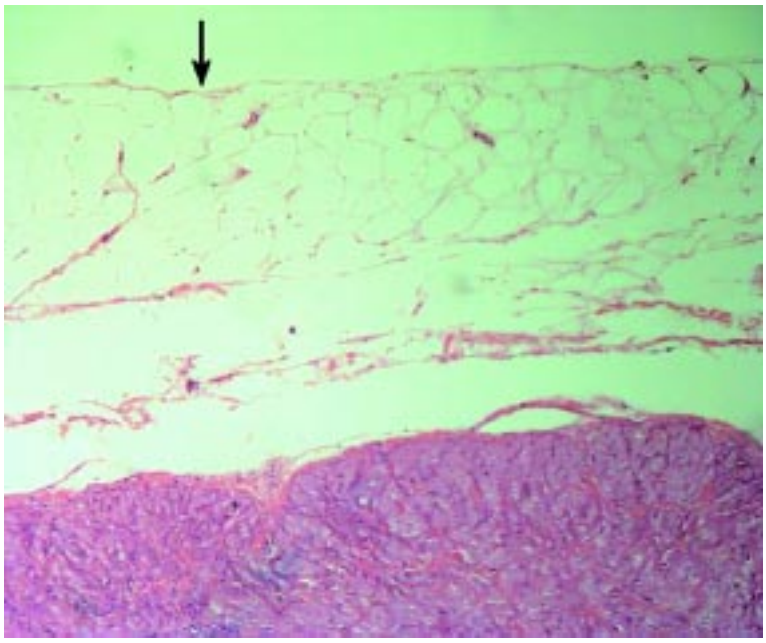

Fig. 5. Adipose tissue (arrow) in tunica serosa of adventitia. HE X100.

The lamina propria were rich in pyloric glands. There were a small amount of tubular glands in the shallow layer, where connective tissue was very abundant. However, due to a large number of branched tubular glands in the deep layer, the component of connective tissue was relatively small. The pyloric glands were divided into multi-branched tubular gland and curled tubular gland, but was usually shorter. The morphology of the glands gradually changed with undulating folds, the closer to the depth of lamina propria, the more obvious the glands curl. The glandular epithelium was a simple columnar epithelium, with spherical or flat nuclei located in the base of the cell, which was more dwarf than gastric pit. Meanwhile, nucleolus could be observed in the closely arranged nucleus. The supranuclear region was filled with small eosinophilic granules within mucus, which stained lightly to HE. Like the mucous neck and duodenal cells, the most cytoplasm in the pyloric gland epithelium in the deep layer is pale, with round nuclei located in the base of the cell (Fig. 4C). In addition, less parietal cells, more capillaries and argyrophil cells could also be observed, especially lymph nodes. The bracket of glands was constituted with collagen fibers and reticular fibers. Due to muscularis mucosa embedding into the lamina propria, we could see more smooth muscle fibers at the bottom of glands in the lamina propria.

The muscularis mucosa was a thin layer, in which there were small arteries and veins.

The submucosa was composed of thin loose connective tissue (Fig. 5D), which had many varieties of cells, such as fibroblasts, macrophages, lymphocytes and plasma cells. In addition, abundant of small veins, very thin arteries and capillaries were observed, and small lymphatic vessels occasionally.

The muscularis was more muscular than the pyloric glands, which composed of the outer and inner layers of muscle (Fig. 4E). The muscle bundles of inner layer were less loose and thin than the outer layer. Meanwhile, two layers of muscle bundles were separated by connective tissue.

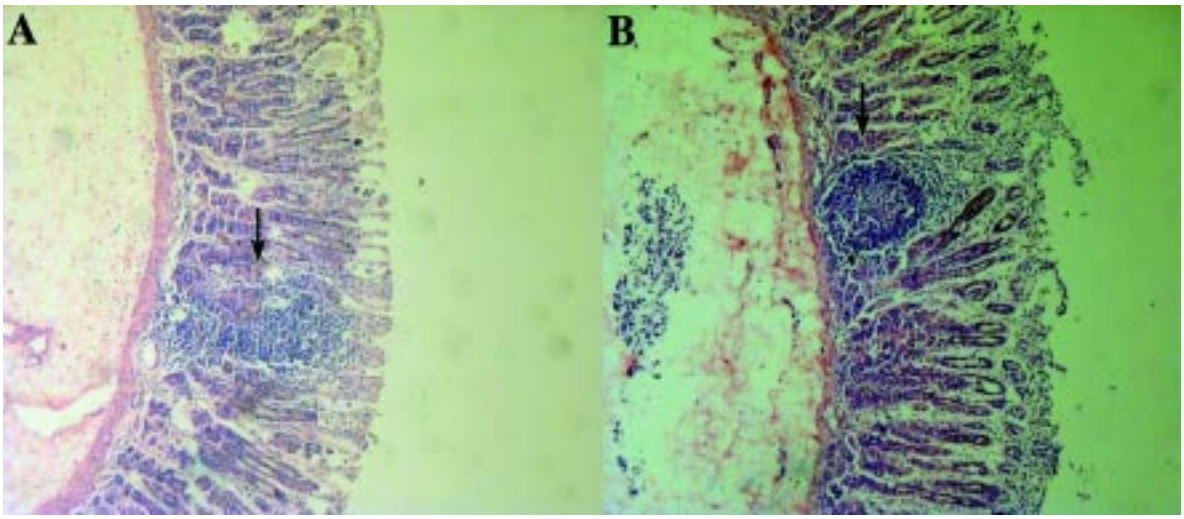

Fig. 6. (A) Diffuse lymphoid tissue (arrow) in lamina propria of fanclus abomasi. HE X100. (B) Solitary lymphoid nodule (arrow) in lamina propria of fanclus abomasi. HE X100.

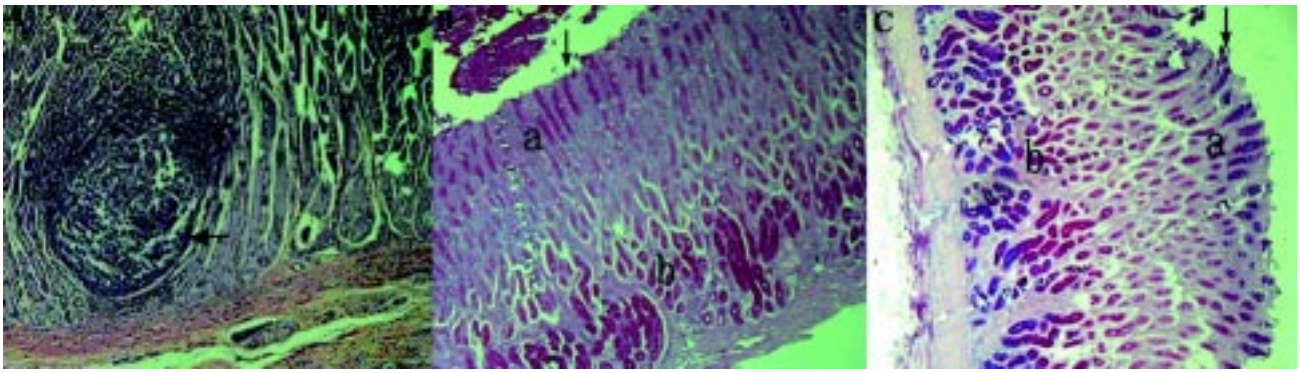

Fig. 7. Light microscopic view of the pyloric gland region. (A) Solitary lymphoid nodule (arrow) of pyloric gland region. HE X 100. (B) Epithelium (arrow), gastric pit (a) and pyloric gland (b) in pyloric gland region. Stained with PAS X 100. (C) Epithelium (arrow), gastric pit (a) and pyloric gland (b) in pyloric gland region. Stained with AB-PAS X 100.

The serosa composed of loose connective tissue, 550.2–595.7mm in thickness, which contains a lot of fat cells toward muscular layer (Fig. 5).

Distribution and composition of the mucosa-associated lymphoid tissue. The distribution and composition of mucosa-associated lymphoid tissue was basically same as in the fanclus abomasi, the corpus abomasi and pars pylorica, which was concentrated in the lamina propria and had two forms, isolate lymphoid nodule and diffuse lymphoid tissue. The former mainly distributed in the fanclus abomasi, the corpus abomasi and pars pylorica, especially more to the corpus abomasi. Further, it generally located in the deep layers of the lamina propria, occasionally, some lymphoid nodules occupying the entire lamina propria. The structure of isolate lymphoid nodule was basically same as the lymphoid nodule of lymph node, which were mainly constituted by large and medium-sized lymphocytes and peripheral lymphocytes, sometime, mast cells visibly. In addition, both had germinal center, but not more developed, which was stained lightly.

There were two forms of diffuse lymphoid tissue, the loose and dense lymphoid tissues. The lymphocytes sparsely arranged and scattered in the loose. The arrangement density of lymphocytes in the superficial lamina propria was greater than the deep layer. Reticular fibers formed the stent, where small lymphocytes were even more, following the plasma cells with varied shapes and typical structures. The dense lymphoid tissue, where irregular lymphocytes gathered together intensively, had no germinal centers. There was obvious boundary between dense lymphoid tissue and the surrounding tissue, because the former mainly constituted of small lymphocytes. Comparing with isolate lymphoid nodules, the difference in dense lymphoid tissue was except no germinal centers, the distribution of reticular fibers was also different. Reticular fibers in the lymphoid nodules mainly distributed in the periphery, as well as fewer germinal centers, while the distribution of reticular fibers in the dense lymphoid tissue was uniform.

Few lymphocytes presented in the mucosal epithelium in the fanclus abomasi, corpus abomasi and pars pylorica. In the fanclus abomasi, there were diffuse lymphoid tissues and isolate lymphoid nodules in the lamina propria. The former composed of dense lymphoid tissues and even more developed (Fig. 6A and B). In the corpus abomasi, there were large number of lymphoid nodules distributing intensively in the lamina propria, especially in the deep of lamina propria nearby the muscularis mucosa (Fig. 3G, H and I). There were diffuse lymphoid tissue-dense lymphoid tissue in the pars pylorica. Moreover, lymphoid nodules can be observed in the shallow and deep of lamina propria (Figs. 4F and 7A).

The results of glycoconjugates staining in glandular mucosa

Cardiac glandular area. The result of PAS reaction in cardiac glandular area of the abomasum (Table I) was that the surface of mucosal epithelium showed a positive reaction to PAS. All gastric pit epithelium cells reacted strongly with PAS, filled with red granules in the supranuclear cytoplasm. A small amount of positive granules at the top of gastric pit epithelium cells can also be found. There was a narrow negative area between the gastric pits and glands. The reactions to PAS was positive at the neck and bottom of glands epithelium, but the body of glands epithelium negative.

The AB staining results showed that the mucosal epithelial exhibited negative reaction, while the gastric pits

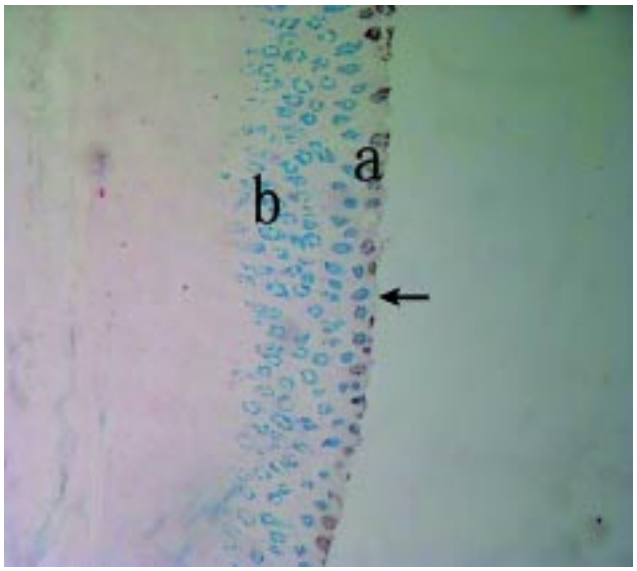

Fig. 8. Epithelium (arrow) gastric pit (a) and cardiac gland (b) in cardiac glandular area. Stained with AB X 100

showed positive reaction. There were blue particles distributing densely in the supranuclear region and a small amount of blue particles in the top region, with nucleus stained red. As same as PAS reaction, a narrow negative area was revealed between the gastric pits and glands. The neck of glands showed a strong positive reaction to AB, then reactions to AB were weakly positive at the bottom, but glands at the bottom of the lamina propria was negative (Fig. 8).

The AB-PAS staining results showed that the mucosal epithelial was stained blue. The upper and lower gastric pit epithelium was purple and red, respectively. The entire glands were purple, including its isthmus, neck, body and bottom. Moreover, the surface of mucosal epithelial and gastric pit epithelium mainly secreted neutral glycoconjugates, but glands secreted mixed glycoconjugates.

Fundic glandular area. As we know from Table I that the reactions to PAS were positive in the mucosal epithelial and gastric pit in the fundic glandular area, and more strongly positive in the gastric pit. The body of gland epithelium showed a strong positive reaction to PAS, while its bottom a negative reaction.

The AB staining showed that the mucosal epithelial was not stained. All gastric pit epithelium cells showed a positive reaction to AB, which there were nuclei stained weakly red and blue granules in the cytoplasm. The isthmus of gland epithelium exhibited a slightly positive reaction to AB, and the neck and body of gland epithelium reacted strongly with AB, but the bottom showed a negative reaction to AB (Fig. 3B).

The AB-PAS staining showed that the mucosal epithelial was stained lightly red. The gastric pit epithelium was red, including litter cells with visible purple granules. The isthmus and body of glands both were red, and the neck of glands were blue, accompanying with some visible purple granulosa cells. But the bottom of glands exhibited a negative reaction (Fig. 3C). Moreover, the surface of mucosal and gastric pit epitheliums mainly secreted neutral glycoconjugates, while the glands secreted acidic glycoconjugates.

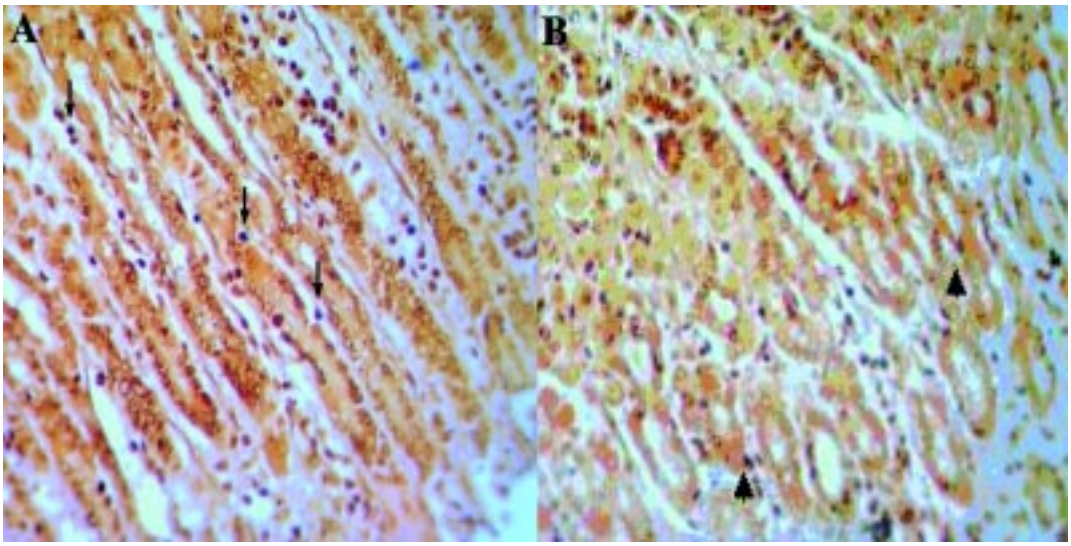

Fig. 9. Endocrine cells stained with Grimelius. (A) Endocrine cells (arrow) in connective tissue. Stained with Grimelius X 100. (B) Endocrine cells (arrow) in glandular epithelium. Stained with Grimelius X 100.

Pyloric glandular area (Table I). The reactions to PAS were weakly positive in the surface mucosal epithelial, and more strongly positive in gastric pit epithelium. There was a narrow negative zone between the gastric pits and isthmus in glands. From the neck of glands, the strength of positive reaction was gradually increasing, until the bottom of the gland was most strongly positive (Fig. 7B).

In the AB staining, the mucosal epithelial showed weak positive reaction. All gastric pit epithelium cells showed a moderate positive reaction, there were nuclei stained weakly red and positive granules in the cytoplasm. A negative zone to AB can be observed between the gastric pits and isthmus in glands. The result of positive reaction was gradually strengthened from the neck to the bottom of the glands, and the bottom showed a strongly positive reaction with lots of positive granules.

In the AB-PAS staining, the mucosal epithelial showed lightly red. The upper and lower gastric pit epithelium was red and purple, respectively. The isthmus, neck and body of mucosal epithelial were all stained red, and the upper and lower at bottom of mucosal epithelial was red and purple, respectively (Fig. 7C). Moreover, the surface of mucosal epithelial and gastric pit epithelium in pyloric gland mainly secreted neutral glycoconjugates, including the glands.

The results of silver staining in endocrine cells. Grimelius silver staining was widely used in the identification of the amine precursor uptake and the argyrophilic of decarboxylation cells. We can investigate endocrine cells varying in shape in the fanclus abomasi and corpus abomasi and pars pylorica, such as tapered, round, fusiform, irregular, etc. (Fig. 9A). Secretory granules of endocrine cells were different in argyrophilic. Portion of large cells were stained dark brown, with strong argyrophilic granules located in the basal region. In addition to non-colored nuclei and brown yellow background, it is easily recognizable. Another portion of small cells was stained weak pale brown so that its contrast was less obvious. The statistical results demonstrated that a large amount of endocrine cells were observed in the pyloric gland area (Table II). Following the fundic gland area, the density of the endocrine cells was the least in the cardiac glands area Endocrine cells individually dispersed among gland epithelial cells, occasionally in groups of 3-5. There were filled with argyrophilic granules in the cytoplasm and cell protrusions, where some granules can also be found in the extracellular. Most of endocrine cells were tapered, the smaller tip-shape toward the luminal or mucosal epithelium, the larger base containing argyrophilic granules. And some branches in the base of the cells, which pass through the basement membrane, were close to capillaries in surrounding tissue. More argyrophilic granules sometimes can be observed in glandular cavity or near the mucosal epithelium, which was the extracellular secretion phenomenon. Moreover, the argyrophil cells were scattered in connective tissue in the lamina propria, especially near the epithelium or glands (Fig. 9B).

Table I. Results of histochemical staining of epithelium cells in the yak abomasum glands.

Section	Cell type	PAS	AB pH2.5	AB-PAS pH2.5
Cardiac glandular area	Surface mucosal epithelium	2R	0	2BP
	Gastric pit epithelium	3R	2B	3BP
	The sthmus of glandular epithelium	0	0	1BP
	The neck of glandular epithelium	2R	1B	2BP
	The body of glandular epithelium	0	2B	1BP
	The bottom of glandular epithelium	2R	1B	2BP
Fundic glandular area	Surface mucosal epithelium	1R	0	1BP
	Gastric pit epithelium	2R	2B	2BP
	The isthmus of glandular epithelium	1R	1B	1BP
	The neck of glandular epithelium	2R	3B	2BP
	The body of glandular epithelium	3R	3B	1BP
	The bottom of glandular epithelium	0	0	0
Pyloric glandular area	Surface mucosal epithelium	1R	1B	1BP
	Gastric pit epithelium	3R	2B	2BP
	The isthmus of glandular epithelium	0	0	1BP
	The neck of glandular epithelium	2R	1B	2BP
	The body of glandular epithelium	2R	2B	2BP
	The bottom of glandular epithelium	3R	3B	3BP

Intensity of staining reaction: 0, no staining; 1-3, weak to very strong staining. Colour of reaction: R, red; B, blue; BP, bluish purple.

Tabla II. The different amounts of argyrophilic cells in the yak abomasum (n= 10).

	Fanclus abomasi (I)	Corpus abomasi (II)	Pars pylorica (III)
X±SD	3.64±2.45	5.58±3.22	10.59±5.63

I vs. II, P>0.05; I vs. III, P<0.05; II vs. III, P<0.05, respectively.

DISCUSSION

According to the distribution of the glands in the abomasum (Eerdunchaolu *et al*., 1999), the yak's abomasum also contained cardiac, gastric and pyloric glands. The mucosa epithelium was simple columnar epithelium. The cardiac glands, which were curved branch tubular glands, as same as other ruminants, belonged to mucous glands that mainly secrete mucus. The surface of the mucosa was covered with mucus, except for lubricating effect, which can also protect mucosa from mechanical damage, like rough food, etc. It had been known that the cardiac glands contained mucous and endocrine cells, except for mucous and endocrine cells, the parietal and chief cells were also observed in the deep of lamina propria of the yaks. Parietal cell, also known as oxyntic cell, helps to digest rough, hard and indigestible ingredients. Compared to humans, the yak's endocrine cells were provided with more complex function. It was, therefore, believed that the digestive capacity of the yak had been strengthened from the cardia, which was the main features of the cardiac glands area.

The yak fundic glandular area had well-developed mucosal folds and thick mucous layer. Lamina propria was filled with long and straight simple tubular glands or branched tubular glands, which was similar to the fundic glandular area of other ruminants (Kitamura *et al*., 1986). However, the number of parietal and chief cells was abundant. Therefore, it was suggested that the yak had strong ability of secretion for hydrochloric acid and pepsinogen. The bracket, constituted by reticular fibers closely, was distributed along the basement membrane. Developed muscularis mucosa, especially the outer mucosal muscle formed the coarse longitudinal bundle in the pleats, which was beneficial to the movement of the developed mucosal pleats and the secretion of glands.

Mucous neck cells generally distributed in the neck of the glands, occasionally single neck mucous cell at the bottom and body of the glands. But the mucous neck cells distributed in all of the glands in the pig, especially at the bottom of glands (Agungpriyono *et al*., 2000). In the yak, the distribution area of the mucous neck cell was the neck of glands, of course, a small amount of mucous neck cells, which were white vacuoles in shape and stained lightly, can also be found at the body of the glands. Yet the mucous neck cell was not observed at the bottom of the gland. The result of glycoconjugate staining showed that the mucous neck cells can secrete not only neutral glycoconjugate but also acid glycoconjugate, the latter being the main ingredients. It was consistent with that of the Bactrian camel and dromedary (Eerdunchaolu *et al*.).

In the yak, parietal cell was as same as other ruminants. The larger parietal cells with small and round nuclei, which distributed in the fundic glands, were filled with strong eosinophilic granules in the cytoplasm. Occasionally, two-nuclei also can be observed clearly. Unlike the pig with clustering distribution, the yak's parietal cells were neatly arranged in the outside of chief and mucous neck cells. Previous reports showed that the distribution of parietal cells in plateau animals was the same, such as yak, Bactrian camel and Mongolian gazelle. However, it was different from some animals, such as mice, rats, hamsters, gerbils, guinea and rabbits, the parietal cells mainly distributed in the 1/2 -1/3 of the fundic gland and extended toward the neck and spread in mucous neck cells, occasionally in chief cells (Imai *et al*., 1990; Ghoshal & Bal, 1989). According to published data, PAS-positive parietal cells can be seen in dromedary, cat, rhesus monkeys and baboons (Sheahan & Jervis, 1976).

According to the result of glycoconjugate staining, the pylorus gland belonging to mixed glands in the yak secreted not only neutral glycoconjugate but also acid glycoconjugate. The acid glycoconjugate was a main constituent, which was the same as pigs and cats. But pyloric gland mainly secreted acid glycoconjugate in the Bactrian camel, dromedarys and rabbits, while it only secreted neutral glycoconjugates in dogs. Both the surface mucosal and gastric pit epithelium were mixed gland, which was as same as Bactrian camels, cats and dogs. Yet the surface mucosal and gastric pit epithelium of pigs, rabbits and humans only secreted neutral glycoconjugate (Dougbad & Berg, 1981; Sheahan *et al*.).

In the yak, the mucosa-associated lymphoid tissues were concentrated in the gastric glands in the abomasum. Generally speaking, where there was gastric gland, there

was lymphoid tissue, which indicated that the larger gland stores more lymphoid tissue. However, the yak abomasum, in which the surface area of mucosa was broadened due to a large number of pleats, was greater and more developed than that of the cattle and other animals. So there was a large amount of lymphoid tissues in the abomasum in the yak. Aggregated lymphoid nodule, which lies in intestinal tract in other animals and humans, was the key transferred position that the lymphoid tissue executed immune response (Voutilainen *et al*., 2002). It is noteworthy that there were aggregated lymphoid nodules in both gastric glands and pylorus in the yak. It was, therefore, believed that the yaks grazed throughout the year on diverse natural grasslands had evolved morphological characteristics in intestinal tract enabling them to adapt harsh plateau environment.

In vivo, neuroendocrine forms a completely integrated regulating network, which plays an important role in the organism's health and defense capability for animals (Calingasam *et al*., 1984). In contrast, the yak gastric mucosa is an ideal organ to study neuroendocrine-immune network due to containing a large number of lymphocytes and a variety of endocrine cells. So it may be a good prospect for drug development, especially new vaccines or oral medicines.

It was suggested that the gastrointestinal was the largest and most complex endocrine organ *in vivo* (Sjölund *et al*., 1983). In the yak, the number of endocrine cells in the pylorus were the most, followed by gastric glands. In addition, the number of endocrine cells in the mucosal glandular epithelium was significantly higher than the surface epithelium. Although there has been much research on the distribution of gastrointestinal endocrine cells in other animals (Kitamura *et al*., 1984), few reported in ruminants, especially plateau ruminants. In this study, it was found that the distribution of endocrine cells was the most in the pylorus gland, the more in the fundic gland, and the less in the cardiac glands. So the pyloric gland may be a key position existing a variety of endocrine cells, including EC cells.

In conclusion, it was suggested that the histology features of the yak abomasum enhanced the function of digestion and absorption that enabled them to adapt to the highland of the Qinghai-Tibetan Plateau environment, China. Further research regarding stomach of this species is needed. These findings may also improve our understanding of metabolism-related diseases, thereby benefiting human health.

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WANG, J.; ZHANG, R.; ZHANG, L.; WANG, CH.; SHAO, B. & WANG, J. Adaptación histomorfométrica del abomaso de yak (*Bos grunniens*) al medio ambiente de la meseta tibetana Qinghai. ***Int. J. Morphol., 33(2)***:764-776, 2015.

RESUMEN: Seis abomaso yak (*Bos grunniens*) fueron estudiados con disección y métodos histológicos. Se encontró que la mucosa del abomaso yak estaba cubierta de epitelio columnar simple. Se observaron pliegues en espiral (≥10) en la zona glandular fúndica. La membrana desarrollada de la lámina propia contenía glándulas de alta densidad. De acuerdo con las características morfológicas de las glándulas, el abomaso se dividió en las glándulas cardíacas, gástricas y pilórica. Las glándulas cardíacas se curvan en glándulas tubulares con la parte inferior intumescente y una pequeña cavidad glandular. Las glándulas fúndicas eran glándulas tubulares simples o glándulas tubulares ramificadas, donde se pueden observar con claridad las células principales, parietales y mucosas del cuello. Las glándulas pilóricas fueron glándulas tubulares curvadas, cuanto más cercanas a la lámina propia, más evidente fue su forma ondulada. La tinción glucoconjugada reveló que el epitelio de la mucosa de las glándulas gástricas cardiacas, pilóricas y el epitelio de las fosas gástricas secretaron principalmente un glucoconjugado neutro, pero otras porciones cárdicas y de las glándulas gástricas secretaron un glucoconjugado mixto y ácido, respectivamente. A la tinción de fibras reticulares, se encontró que las células mucosas del cuello poseían características argirófilas. Se observó un gran número de gránulos en el citoplasma supranuclear en contraste con las células principales. Además, no fueron aislados los nódulos linfoides y presentaba tejido linfoide difuso en las glándulas de abomaso, especialmente en el cuerpo del abomaso. La tinción Gordon Sweet indicó que las células argirofílicas se localizaron en el epitelio y lámina propia glandular, lo que también se observó en el tejido conectivo. Estas células endocrinas se dispersan individualmente en las células epiteliales, de vez en cuando en grupos celulares de 3-5. De esta forma, los yak pastorean durante todo el año, en diversos pastizales naturales, y han evolucionado sus características morfológicas que les permiten consumir una amplia variedad de especies de plantas, con lo que se adaptan mejor a las condiciones inhóspitas.

PALABRAS CLAVE: Yak; Abomaso; Adaptación histomorfométrica; Meseta Tibetana Qinghai.

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