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Morphological and morphometric characteristics of gastric mucosa in western grey kangaroo (*Macropus fuliginosus*)

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ABSTRACT

The present study was aimed to investigate the morphology and histomorphometry of stomach and gastric mucosa in western grey kangaroo (*Macropus fuliginosus*). The stomach was composed of three indistinctive separate parts namely sacciform forestomach, tubiform forestomach, and hindstomach. The tubiform forestomach was the main tubular section of the organ. The stomach had a compound lining. The non-glandular mucosa occupied the medial blind sac (MBS) of the sacciform forestomach; the layer covered about one-third of the tubiform forestomach (non-glandular region) and the entire length of the gastric sulcus. The glandular part lined the parietal blind sac (PBS) of sacciform forestomach and the cardiac gland region of tubiform forestomach as well as fundic and pyloric gland regions of the hindstomach. The cardiac mucosa had smooth and folded areas; these were filled with mixed glands. In the fundic glands, the parietal cells outnumbered the chief cells. The pyloric glands were of serous-like in characteristics. In conclusion, gross and histological structures of the stomach of western grey kangaroo are adaptive with its food habitat, which allows thorough mixing of highly fibrous grasses.

Keywords

Morphology, Morphometry, Stomach, Western grey kangaroo

ARTICLE HISTORY

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INTRODUCTION

The western grey kangaroo (*Macropus fuliginosus*) is one of the largest herbivorous marsupial animals belonging to the family Macropodidae. The term "Macropodid" was used to describe the largest species from this family that includes the red kangaroo, the eastern and western grey kangaroos. The family also includes smaller species such as wallabies, tree-kangaroos, and the pademelons (Groves, 2005). Western grey kangaroo is almost similar to the eastern grey kangaroo (*Macropus giganteus*), and this was treated as subspecies for many years. They vary in color, being anywhere from grayish-brown to chocolate brown. The muzzle is covered by fine hairs. The western group is slender and grayish-brown, and the southern group is brown in color. Herbivorous marsupials are classified according to the site of microbial fermentation into; foregut fermenters such as kangaroos, wallabies and rat-kangaroos, and hindgut fermenters such as wombats, possum and koala. The foregut fermenters have an expanded and differentiated forestomach, while the hindgut fermenters have an expanded cecum and proximal colon or both (Patricia et al., 2006; Tyndale-Biscoe, 2005). Formerly, many approaches have been described the morphology of macropod stomach in detail in eastern grey kangaroo (Shoaib, 2009), in Potoroinae (Langer, 1980), in tammar wallaby (*Macropus eugnii*) (Gemmell and Engelhardt, 1977, Richardson, 1980), and in marsupials (Hume, 1999). Further on, Pamment et al. (1994) described gastric morphology of the *M. fuliginosus* during his work. To the best of our knowledge, little information about the western grey kangaroo is available. Therefore, the objective of our study was to investigate the morphology and histomorphometry of stomach of the western grey kangaroo with special reference to its histological structures.

MATERIALS AND METHODS

Sample collection: Seven adult western grey kangaroos with average body weight of 57-kg were obtained from the post mortem room, Melbourne University, Australia, during conducting para cytological project.

Gross anatomy: The abdomens of four fresh carcasses were opened through a mid-ventral abdominal approach. The stomach was seperated from the attached omentum and the surroundings structures. The dissected stomach was photographed. The inner surface of stomach was revealed by opening through the lesser curvature as described by Trang et al. (2012). All remaining contents were removed using slow running tap water. Then, the regional distribution of the stomach was estimated.

Histology: Stomachs of three fresh carcasses were used for histological studies. Specimens from non-glandular region, parietal blind sac (PBS), cardiac, fundic, and pyloric gland regions (**Figure 1**) were dissected out and fixed in 10% neutral buffered formalin for 72h. The specimens were then dehydrated in ascending grades of ethanol, cleared in xylene, and impregnated in melted paraffin wax. Sections of 5 µm thickness were cut and stained with Hematoxylin and Eosin (Bancroft and Stevens, 1997). The slides were scanned prior to photograph utilizing the Australian Phenomics Network Histopathology and Organ Pathology Service, University of Melbourne, Australia. The nomenclature used in this study was adopted according to Hume (1999) and Nomina Anatomica Veterinaria (2005).

Histomorphometry: Histomorphometry was performed in 10 fields from stained slides of the different regions of stomach western grey kangaroo. The depth of the gastric mucosa in each region was measured in well-oriented sections using the MIRAX viewer program.

RESULTS AND DISCUSSION

Gross anatomy of the stomach: The stomach of the western grey kangaroo was a curved sacculated tubular organ with compound lining; both glandular and non-glandular mucosa. It occupied a major portion of the abdominal cavity with its cranial end on the left side and the caudal end at the right side. Based on the configuration of the stomach and the structure of its mucous membrane, it comprised three indistinctive separate parts, namely, the sacciform forestomach, tubiform forestomach, and hindstomach (**Figure 1, 2**). The gross anatomy of the stomach of western grey kangaroo did not differ significantly from its closely related species eastern grey kangaroo (Pamment et al., 1994; Shoaib, 2009).

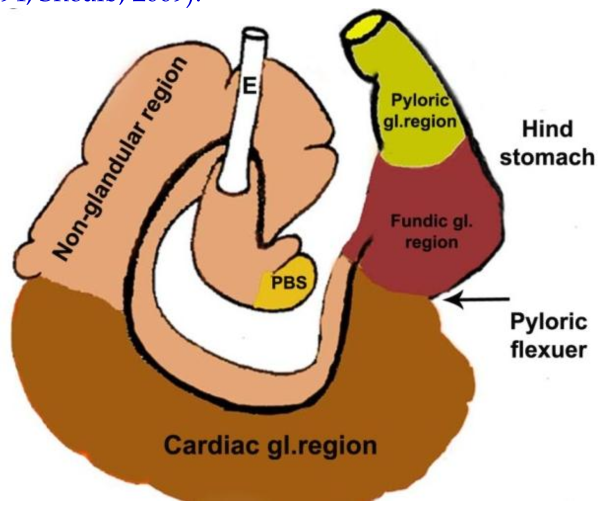

Figure 1. Schematic diagram showing the different regions of the gastric mucosa of the western grey kangaroo (E = esophagus, PBS = parietal blind sac). (this figure has been adapted from Hume (1999).

The terminology used in the current work in which the stomach is divided into sacciform forestomach, tubiform forestomach and hindstomach was in agreement with that mentioned by Stevens and Hume (1995), Tyndale-Biscoe (2005), and Shoaib (2009). The sacciform and the tubiform forestomach could not be distinguished internally due to the wide openings between the two regions in the eviscerated stomach.

According to the present study, there were two small blind sacs; PBS and medial blind sac (MBS); located close to the apex of the sacciform forestomach. Both the PBS and MBS open independently into the main cavity of the tubiform forestomach. These results reconfirmed the findings of Hume (1999) in the Macropodidae, and Shoaib (2009) in eastern grey kangaroo; both of them stated that in the eviscerated stomach, the sacciform and the tubiform forestomach were communicated internally with a wide opening. In contrary, Tyndale-Biscoe (2005) in Macropodidae recognized a permanent external fold separating between the two forestomach regions even in the eviscerated stomach. The internal surface of the PBS revealed a confined glandular mucosa which appeared brownish-red in color with moist texture, while that of the MBS was lined with non-glandular mucosa which appeared pale white in

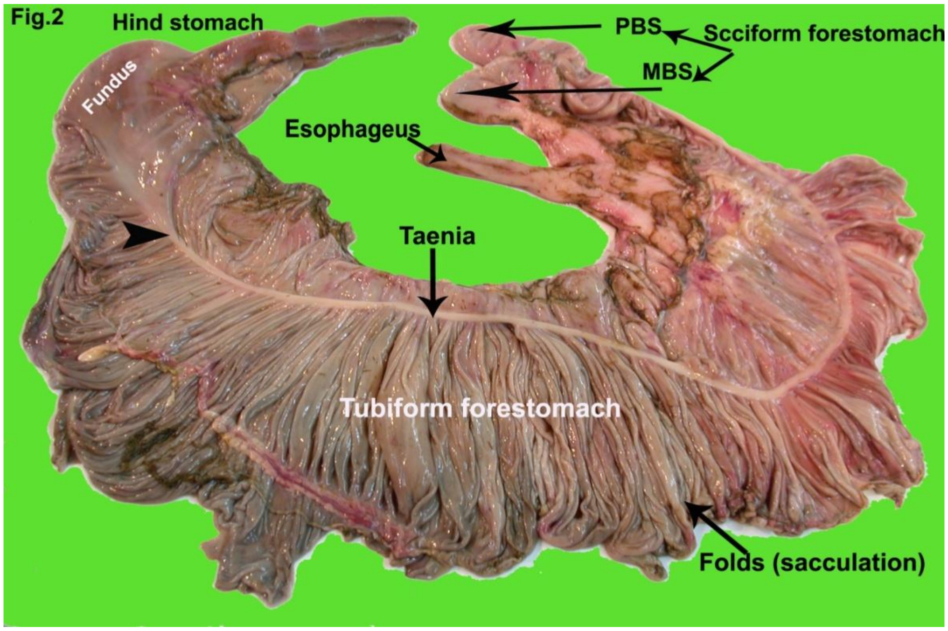

Figure 2. Photograph showing the external surface of the western grey kangaroo stomach (unopened), PBS = parietal blind sac, MBS = medial blind sac, the end of the taenia (arrow head).

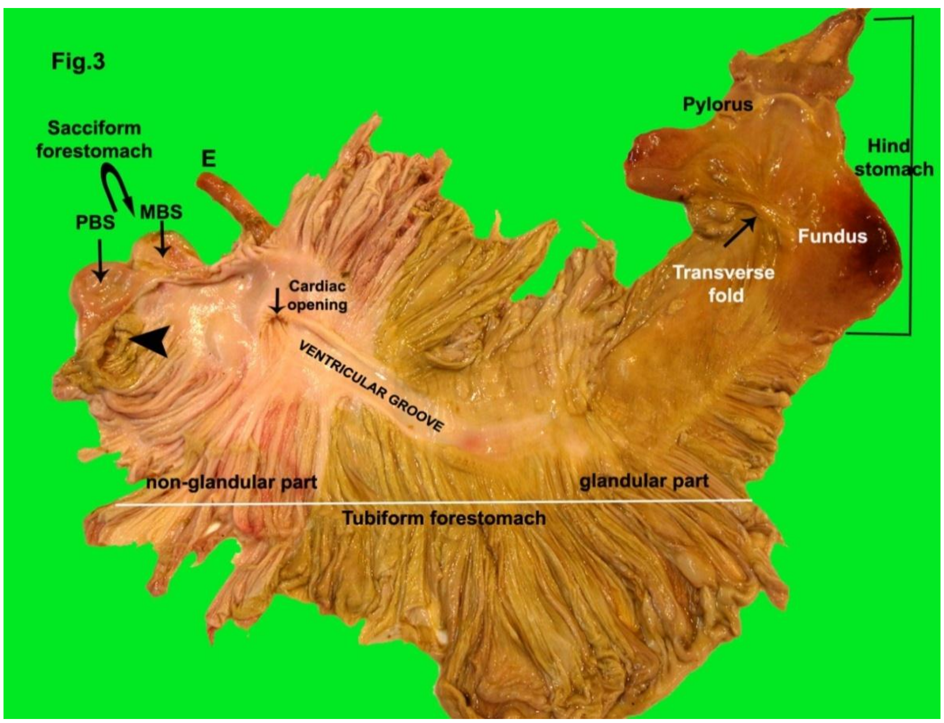

Figure 3. Photograph showing the internal surface of the western grey kangaroo stomach, PBS = parietal blind sac, MBS = medial blind sac, confined glandular mucosa in the PBS (arrow head), E = esophagus.

color with dry texture (**Figure 3**).

The tubiform forestomach, the main tubular section of the organ, extended from the level of cardiac opening to the pyloric flexure. It was in the form of sacculated tube, its outer surface had three taeniae, numerous semilunar folds and hustra which gradually disappeared toward the pyloric flexure (**Figure 2**). The presence of taeniae, haustra, and semilunar folds in the tubiform forestomach gave the stomach as sacculated appearance. This observation was in an accord with the results of Hume (1999) who described the shape of the kangaroo tubiform forestomach as a colon-like, similar to the proximal colon of the horse. Instead, the wall of the sacciform forestomach of the Potoroinae stomach was the part that shown sacculation, while the

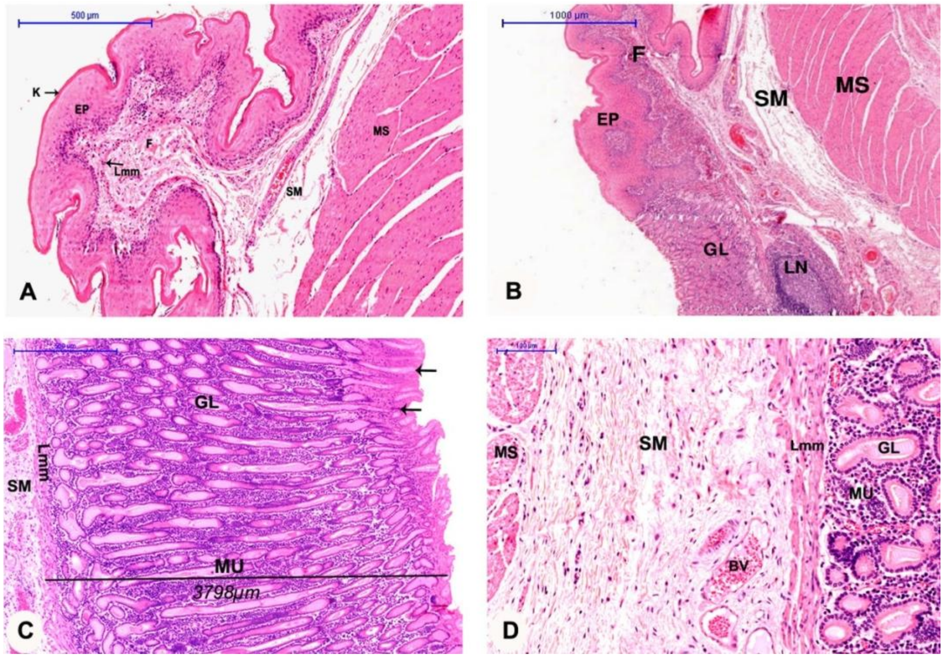

Figure 4. Photomicrographs of the gastric mucosa of the western grey kangaroo stained by H&E. **A**, non-glandular mucosa showing mucosal fold with stratified squamous keratinized (K) epithelium (Ep), lamina propria followed by lamina muscularis mucosae (Lmm), submucosa (SM) and thick muscularis (MS). **B**, junction between the glandular and non-glandular mucosa at the fold (F) that was covered by stratified sq. epithelium (EP) and glandular (GL) mucosa, with large lymphatic nodules (LN) in the submucosa (SM). **C**, PBS with thick mucosa (MU) densely packed with glands (G) and the surface columnar epithelium (arrow). **D**, higher magnification of C showing the basal portion of mucosa (MU) filled with the fluid-filled tubular glands (GL), lamina muscularis mucosae (Lmm), submucosa (SM) with wide blood vessels (BV) and tunica muscularis (MS).

tubiform forestomach was non-sacculated (Hume, 1999). In this concern, Langer and Takács (2004) observed that guts with taeniae and sacculation retained digesta transit, which was in agreement with functional necessities in herbivore digestive tracts, to make enzymatic digestion.

The internal surface tubiform forestomach was lined with both non-glandular and glandular (cardiac gland) regions. The former part was relatively small found around the cardiac opening of the stomach. It was pale white in color and uncovered with mucous. While the glandular part was quite extensive being brown in color and covered with mucous. A prominent ventricular groove was noticed along inside the tubiform forestomach (**Figure 3**). In this respect, the stomach of western grey kangaroo is almost similar to that of pig and differs from that of horse, as mentioned by Konig and liebich (2004) who stated that the non-glandular part is small in the pig but quiet extensive in horse.

The hindstomach constituted the caudal end of the stomach; it lies on the right side of the abdominal cavity and extended from the pyloric flexure till the pylorus. The hindstomach was separated externally from the tubiform forestomach by a shallow fissure while, internally it was separated by a transverse fold. It composed of fundus; a wider proximal portion and the pyloric canal which terminated at the pylorus (**Figure 3**). This findings was in parallel with the findings of Hume (1999) in Macropodidae and Shoaib (2009) in eastern grey kangaroo.

Histological features: The gastric mucosa of the western grey kangaroo showed both glandular and non-glandular regions. The non-glandular mucosa was found in the MBS, the esophageal region of the tubiform forestomach (non-glandular part) and along the gastric sulcus. Similar finding was reported by Pamment et al. (1994) in the same animal species and Hume (1999) in eastern grey kangaroo. In contrast, Hume (1999) in the tree kangaroos noted that the squamous epithelium was restricted to the gastric sulcus and to a small area close to the cardia. With regards to the glandular mucosa in the present work, it covered the PBS, cardiac region of the tubiform forestomach, and fundic and pyloric gland regions in the hindstomach (**Figure 1**). The histmorphometric results revealed distinct variation in the thickness of the gastric mucosa among the different parts that is summarised in **Table 1**. Similar finding was observed by Pamment et al. (1994) in western grey kangaroo

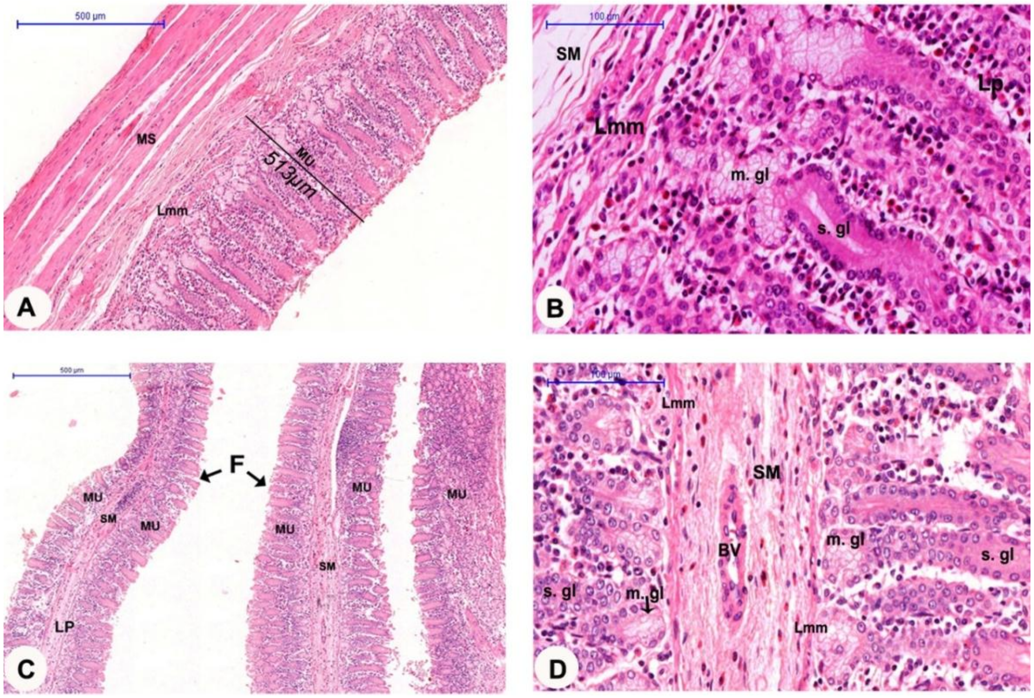

Figure 5. Photomicrographs of the cardiac gland region of the western grey kangaroo stained by H&E. **A**, showing unfolded, smooth cardiac mucosa (MU) separated from submucosa and muscularis (MS) by prominent lamina muscularis mucosae (Lmm). **B**, higher magnification of (A) showing the mixed cardiac glands (s.gl and m.gl) in the lamina propria (Lp) followed by lamina muscularis mucosae (Lmm) and tunica submucosa (SM). **C**, cardiac mucosal folds (F) covered with glandular mucosa from both sides (MU); LP, lamina propria; SM, submucosa with blood vessel (BV). **D**, higher magnification of (C) showing mixed glands similar to that in smooth portion.

Hume (1999) in Macropodidae, and Rosemary et al. (2005) in tammar wallaby.

The non-glandular mucosa: The non-glandular area of the stomach showed irregular mucosal folds with primary and secondary papillae. The average thickness of the non-glandular mucosa was 190 µm. It was formed from keratinized stratified squamous epithelium, lamina propria of dense connective tissue, and prominent lamina muscularis mucosa. The submucosa was a layer of loose connective tissue projected into the core of the folds, followed by thick tunica muscularis of smooth muscles (**Figure 4A**). The cellular structure of the squamous epithelium of the western grey kangaroo stomach is similar to that was described for other mammals and consistent with numerous approaches (**Table 1**) that investigated the thickness of the stratified squamous epithelium of several kangaroo species including *Macropus fuliginosus* (Pamment et al., 1994), *Macropus eugenii, Macropus giganteus*, and red-necked pademelon *(Thylogale thetis)* (Langer et al., 1980). At the esophagi-gastric junction, the epithelium changed abruptly from keratinized stratified squamous epithelium in the non-glandular region to simple columnar epithelium in the glandular part. Beside, we noticed at this region that, there were prominent lymphoid follicles in the submucosa (**Figure 4B**). Similar finding was reported by Wang (2003) in the stomach neck of Bactrian camel.

The glandular mucosa:

***Mucosa of the parietal blind sac*:** In the current study, the glandular mucosa in the PBS is formed from glandular tissue packed entirely with elongated tubular glands with wide lumen (approximately 70 µm), and contained an eosinophilic content (**Figure 4C**). The lamina propria showed many lymphocytic infiltrations, the lamina muscularis mucosa was thick and the submucosa was of loose connective tissue housed large blood vessels (**Figure 4D**). This observation is in parallel to that of Hume (1999) and Shoaib (2009) in eastern grey kangaroo, and Gemmell and Engelhardt (1977) in tammar wallaby. The thickness of the glandular mucosa of the PBS in the present work was 3,748 µm. This measure was much higher than that of Pamment et al. (1994) in the same animal species, and Langer et al. (1980) in eastern grey kangaroo and tammar wallaby who recorded 1,800 µm, 1,956 µm and 380 µm thickness, respectively. These results were relatively similar to the findings recorded in eastern

Table 1: Measurement of the average thickness of the mucosal regions (µm) in the stomach of western grey kangaroo. (n=3) and different kangaroo species.

Species	Sq. Epith.	PBS	Cardia mucosa	Fundic mucosa	Pyloric mucosa	References
Macropus fuliginosus	190	3798	513 & 276	2019	893	**This study**
Macropus fuliginosus	80-320	1, 800	600	1,550	1,200	Pamment et al. (1994)
Macropus giganteus	82	1956	306	-	-	Langer et al. (1980)
Macropus eugenii	69	380	341	-	-	Langer et al. (1980)
	300	-	250	1600	750	Gemmell and Engelhardt (1977)
Thylogale thetis	92	-	402	-	-	Langer et al. (1980)

M. eugenii = tammar wallaby, M. giganteus= eastern grey kangaroo, T. thetis = red-necked pademelon, M. fuliginosus = western grey kangaroo. Sq. Epith. = squamous epithelium, PBS = parietal blind sac.

grey kangaroo (3,500 µm) measured by Shoaib (2009). Based on the structure and thickness of the glandular mucosa of the PBS, we concluded that these mucosal glands might be called as atypical or pseudo-cardiac glands as these were collectively different in their structure from that of the typical cardiac glands. In spite of the presence of glandular mucosa in the PBS, the MBS is entirely lined by cornified squamous epithelium. This observation in the PBS may suggest a secretory function of this region. This speculation is substantiated by the idea that the secretory function of the glandular mucosa of the sacciform forestomach may increase the fermentation and digestibility of plant cell walls (Hume, 1978; Langer, 1980).

The cardiac gland region: The cardiac region showed smooth and folded areas of gastric mucosa. In the smooth (unfolded) area, the cardiac mucosa was thick (513 µm), filled with simple branched tubular glands that occupied almost the entire thickness of the mucosa with very little amount of connective tissue inbetween (**Figure 5A**). Similar findings were noticed in Macropode (Tyndale-Biscoe, 2005), in monkey (Fayed et al., 2010), and in domestic animals Konig and liebich (2004). The cardiac glands were of mixed type (seromucoid gland); the upper two-third portion is composed of serous cells with dense granular eosinophilic cytoplasm and rounded nuclei, while the basal one-third portion was of mucous cells with basal flattened nuclei and pale foamy cytoplasm (**Figure 5B**). The folded area was characterized by remarkable mucosal folds of variable heights resembling the omasal folds of ruminant stomach, covered in both sides by cardiac glands similar to that in smooth area although thinner in its thickness (276 µm) (**Figure 5C**). The mucosal folds showed prominent laminae muscularis and core of dense irregular fibrous connective tissue rich in blood vessels (**Figure 5D**). The function of these folds may be involed to increase the secretion of mucus and enzymes that help in digestion and fermentation of ingested food. In the previous approaches, the cardiac gland region showed unfolded (smooth) mucosal surface with shallow gastric pits and simple tubular mucus-secreting glands occupied the entire mucosal thickness (Hume, 1999) in Macropodae and in western grey kangaroo (Pamment et al., 1994).

Fundic (gastric) mucosa: The average thickness of the mucosa of the fundic gland region was 2,019 µm. It was composed of parallel, elongated glands with little amount of connective tissue in the lamina propria and thin distinct lamina muscularis mucosa (**Figure 6A**). The fundic glands appeared as long and straight, and consisted mainly of chief and parietal cells. The surface epithelium was simple columnar epithelium having oval nuclei and lightly eosinophilic cytoplasm. The neck of the fundic glands had mucous neck cells of low cuboidal type with basophilic cytoplasm. The parietal cells appeared as large with eosinophilic cytoplasm, rounded nuclei, and outnumbered the chief cells especially toward the neck of the gland. The chief cells had basophilic cytoplasm and rounded nuclei. Their number increased greatly toward the bases of the glands (**Figure 6B-D**). The fundic mucosa of the western grey hindstomach was analogous to the report of Eurell and Frappier (2006) in domestic animals. The present structural observation reconfirmed the presence of four different cell types as normally associated with mammalian fundic mucosa: surface epithelial cells, mucous neck cells, chief cells, and parietal cells. These findings are in agreement with the reports of Banks (1993) and Eurell and Frappier (2006) in domestic animals, and Hume (1999) and Pamment et al. 1994) in Macropodidae. However, the number of parietal cells, as reported here, is higher than chief cells especially in the upper part of the fundic glands. In this concern, Gemmell and Engelhardt (1977) mentioned that the high proportion of parietal cells in the tammar wallaby explains why the pH of the stomach contents changes abruptly from 7.0 in the cardiac region to as low as 2.0 in the fundic region. The relatively small proportion of chief cells in the fundic

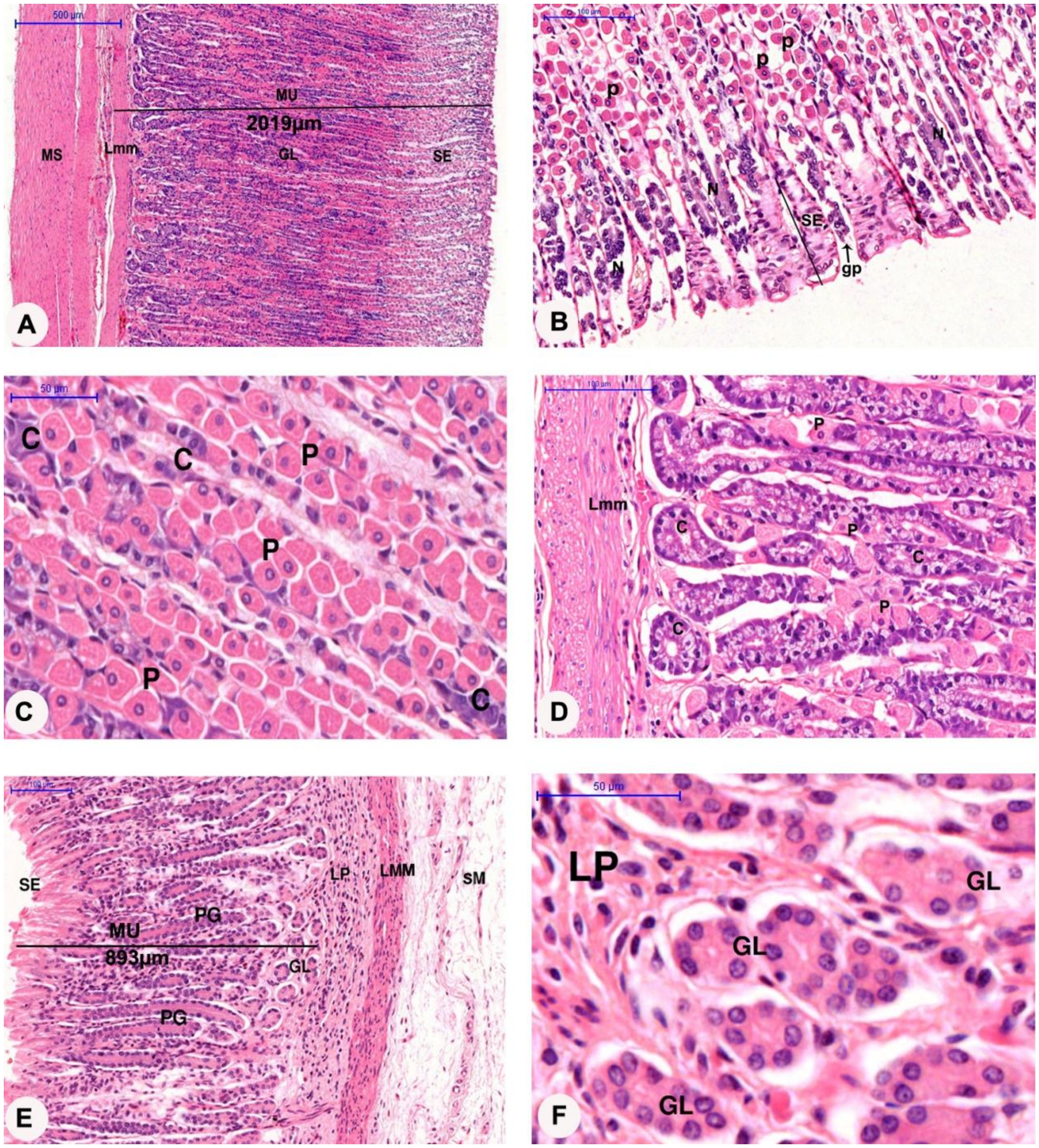

Figure 6. Photomicrographs of the fundic gland region (A-D) and pyloric gland (E-F) of the western grey kangaroo stomach stained by H&E. **A-D**, different levels and magnifications of fundic region showing gastric mucosa (MU) covered with surface epithelium (SE) and filled with fundic glands (GL), with gastric pits (gp), mucus neck cells (N), parietal (P) and chief cells and bounded by lamina muscularis mucosae (Lmm); MS (tunica muscularis). **E**, the mucosa (MU) of pyloric gland (PG) region showing surface epithelium (SE), long serous-like glands (GL) in the dense CT lamina propria (LP) and thick lamina muscularis mucosae (LM) separates between mucosa and submucosa (SM). **F**, higher magnification of E showing the serous-like pyloric glands (G) in the lamina propria (LP).

region may indicate that pepsin secretion is not as important in the western grey kangaroo as it is in other monogastric animals, and this may be attributed to the nature of food of this species.

***Pyloric gland region*:** In the current investigation, the pyloric mucosa had a relatively thick mucosal lining about half the thickness (893 µm) of the fundic mucosa. This measure is lower than that was recorded by Pamment et al. (1994) in the same animal species who stated that the pyloric epithelium was 1,200 µm in thickness. According to our work, the pyloric gland region was formed mainly of long branched tubular gland which was characterized by highly eosinophilic secretory granules. The surface epithelium was similar to that in the cardiac gland region with deep gastric pits. The lamina propria was occupied mainly by the pyloric glands with very little amount of dense irregular connective tissue inbetween. The lamina muscularis mucosa appeared as a thin layer of smooth muscles separated by the mucosa and loose submucosa (**Figure 6E**). Interestingly, in the present work the pyloric glands appeared serous-like in shape, with narrow lumen lined by cuboidal cells with eosinophilic cytoplasm and rounded basally located nuclei (**Figure. 6F**). In contrary, Hume (1999) in Macropodidae and Fayed et al. (2010) in monkey stomach stated that the pyloric glands are mucous-secreting in nature, similar to the cardiac glandular mucosa. These differences in the type of the pyloric gland among the different kangaroo species need further investigation.

CONCLUSION

The gross and histological structures of the western grey kangaroo stomach are adaptive with its food habit. It allows thorough mixing of highly fibrous grasses. Beside, the type of the PBS mucosa as well as the type of pyloric glands needs further investigation.

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