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Research Article

HISTOCHEMICAL STUDY OF BRUNNER GLANDS IN IRANIAN BUFFALO

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ABSTRACT
Brunner glands or duodenal glands are located in the sub mucosa of the small intestine of mammals. The principle aims of this study have been to elucidate the morphological and histochemical properties of duodenal sub mucosal glands in the small intestine of Iranian buffalo (murrah breed). The duodenum of 15 healthy animals of both sexes constituted the material of this study. After dissecting them, three parts of duodenum were determined. For histological studies, part of tissue samples taken from different part of duodenum were first fixed in 10% buffered formalin and then subjected to routine tissue processing for light microscopy, then PAS and alcian blue staining performed. Result showed that the glands were branched tubulo-alveolar which composed of acini densely packed within the sub mucosa. The Brunner glands in this breed contained mucous glands. Histochemical examination revealed that the mucous glands and excretory duct react with the periodic acid Schiff stain, furthermore; mucous glands reacted positively with alcian blue ph. 2.5. When applied the combined aldehyde fuchsine-alcian blue ph. 2.5 staining procedure, mucous glands were determined to be aldehyde fuchsine (-) and alcian blue (+). These results showed that the secretion of mucous cells of the duodenal glands in this breed of buffalo was composed of neutral carbohydrates and a limited amount of acidic carbohydrates which this acidity being due to the presence of carboxyl groups.

Keywords: *Histochemy, Iranian Buffalo, Duodenum, Small Intestine,*

INTRODUCTION
Brunner glands or duodenal glands are located in the sub mucosa of the small intestine of mammals (Grossman 1958). These glands which in general, produces a mucous secretion are located in sub mucosa of the proximal duodenum (Ainsworth *et al.,* 1995; Krause, 1981, 2000; Takehana *et al.,* 2000; Verdiglione *et al.,* 2002), Brunner glands were determined to be started from gastrointestinal junction in the majority of the species studied (Alogninouwa *et al.,* 1996; Krause, 1981, 2000; Takehana *et al.,* 2000; Verdiglione *et al.,* 2002) however, how far Brunner gland extended distally along the intestinal tract is variable and species dependent (Krause, 1981, 2000; Takehana *et al.,* 2000; Verdiglione *et al.,* 2002). The existence of duodenal sub mucosal glands in the duodenum is uncontestable (Boutros *et al.,* 1990; Bloom and Fawcett, 1994; Burkitt *et al.,* 2000). However, there remain doubts as to their exact location along the full extent of the duodenal wall, given that the existing opinions in the specialized literature are often incomplete (Coutinho *et al.,* 1996; Gartner and Hiatt, 2003). Various studies dealing with the mucosubstance histochemistry of duodenal submucosl glands, pyloric glands and goblet cell in a large number of mammals show marked inter–species and even within-species variation (Poddar and Jacob, 1979). Brunner glands were reported to contain neutral or acidic mucin glycoproteins or the combination of both types of mucin (Crescenzi *et al.,* 1988; Krause, 2000; Takehana *et al.,* 1989, 1991a, 2000; Verdiglione *et al.,* 2002). Generally, the duodenal glands are believed to protect the duodenal mucosa from the gastric hydrochloric acid. Brunner glands of the buffalo (murrah breed), which is a species endemic to Iran have not been studied previously. The aim of this study is to demonstrate the distribution, morphological and histochemical properties of duodenal glands in this species.

MATERIAL AND METHODS
A total of 15 healthy adult male and female buffalo duodenum were used for this study. All samples were gathered from slaughter house in Khuzestan province. After dissecting duodenum, part of tissue samples

were fixed in 10%neutral buffered formalin and then subjected to routine tissue processing for light microscopy, dehydrated in graded series of ethanol, cleared in xylene and embedded in paraffin wax, The blocks of tissues of duodenum from proximal, middle and distal parts were sectioned into 5 micron thickness and these sections were stained with periodic acid Schiff (PAS) for neutral mucosubstance, alcian blue pH 2.5 for acidic mucosubstance, PAS/AbPH 2.5 from the combined assessment of neutral and acidic mucosubstances (Culling *et al.,* 1985). The aldehyde fuchsine-alcian blue method was employed for the demonstration of acidic mucosubstances contain sulphate and carboxyl groups. Selected sections were photographed with photomicroscope. The morphological features were noted.

RESULTS AND DISCUSSION

Cells which compose the Brunner gland vary with species. These glands were reported to be composed of two types of cells, serouse and mucous cells in the rabbit (Takehana *et al.,* 1989, 1991b) and horse (Oduor-Okelo, `1976; Pfeiffer and Dabareiner, 1992; Takehana *et al.,* 1989, 1991b), while they were demonstrated to be composed of only mucous cells in other species (Krause, 1981, 2000). In Iranian buffalo (murrah breed) there were only mucous cells. The glands were branched tubulo-alveolar which composed of acini densely packed within the submucosa. In most species Brunner gland are distributed In an area starting from the gastrointestinal junction and extending to varying distances in the proximal small intestine (Alogninouwa *et al.,* 1996; Krause, 2000; Takehana *et al.,* 2000; Verdiglione *et al.,* 2002). While in humans they extend almost to the level of the papillae of vater (Treasure, 1978). In rats the area extends one half ways down to the entry of the bile duct (Treasure, 1978). In several mammals Brunner gland are located within the first few mm of the proximal duodenum, just distal to the pyloric sphincters (Krause, 2000). In horses Brunner glands occupy a very large area and extend approximately 6 m caudal to the pylorus. They are known to exist also in the jejunum in pigs and large herbivores (Verdiglione *et al.,* 2002). In rabbits the distributions of duodenal sub mucosal glands were determined to start from the pyloroduodenal junction and to extend near the jejunum (Emel *et al.,* 2010). In the pony mucous glands were reported to be present along the duodenum while serous glands were determined to be located in the upper part of duodenum within the region of extending (Takehana *et al.,* 1991b). In Guiana pig duodenal sub mucosal glands are compound tubuloalveolar composed only of mucous acini densely packed within the submucosa and the glands were well developed in the cranial part of the duodenum (Mohamadpour, 2011). In bovine Brunner glands extended through the duodenum and discontinuously in the jejunum (Verdiglione *et al.,* 2002). In Iranian buffalo (murrah breed) these glands were located in all parts of duodenum, proximal, middle and distal part, but distribution of these glands in proximal portion is more tangible. They were branched tubulo-alveolar glands organized in lobules by well-defined sub mucosal connective tissues. In the lobules tubular and alveolar end pieces, terminal tracts-were located in the peripheral portion, while the branched secretory tubules –preterminal tracts-opening in to the excretory duct and the same excretory duct were located in the central portion of the lobules. The excretory duct penetrated the muscularis mucosae to empty mainly in to the base or side of intestinal crypts. Occasionally, glands were found within the deeper part of the lamina properia, these glands had a simplified structure and opened directly in to the intestinal crypts. The cells of the excretory ducts being lower than the secretory cells. Brunner glands were stained by PAS (preterminal tracts), although the terminal tracts and excretory duct seemed to show mild reaction. The secretion of the terminal portion of Brunner glands is viscous mucus similar to the secretion of pyloric glands and to a certain degree to duodenal goblet cells. The secretion on the other end of the preterminal tracts shows a unique composition that differs from the secretion of the absorptive cells as well highlighting a specific though not completely understood role in the digestive function. Although Brunner gland were observed to be present in all three regions of the duodenum the number of these glands was greater in the proximal region in the murrah breed. While the majority of the glands in the proximal duodenum were composed of mucous cells the number of these glands decreased evidently in the other region of the duodenum. In human (Crescenzi *et al.,* 1988), cat, dog and rat the glandular secretion is composed of neutral mucin (Schumacher *et al.,* 2004). Acidic mucins are the primary secretory product of burners glands in only a

few species (Krause, 2000; Takehana *et al.,* 2000). Oduor-Okelo (1976) has demonstrated the presence of acidic groups in the mucosubstances secreted by the horse's duodenal glands. The general morphology of the duodenum of buffalo examined in this study was in accordance with that described for mammals in general (Figure 1).

Despite their similar morphological appearance in the H&E sections, the PAS and alcian blue (ph. 1 and 2.5) staining properties of duodenal sub mucosal glands showed marked differences in our study. in bison, deer, voles and domestic rabbit they contain acidic sulphated and carboxylate mucins, whereas in humans, cats, raccoons and rats they contain neutral mucins. this variation could not be attributed to either order or the diet of the mammals (Schumacher, 2004). The Brunner's glands of some ruminants including the American bison (Krause, 1981) and Holstein cow (Takehana *et al.,* 1991a) are characterized by an unusual feature.

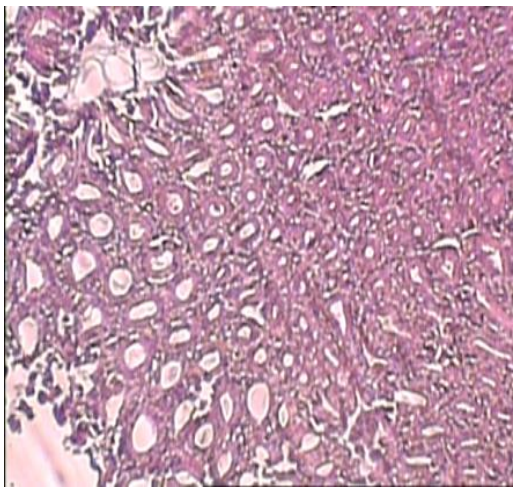

Figure 1: Photomicrograph of the cranial part of the duodenal glands. The glands are only mucous and compound tubuloalveolar gland. H&E120X

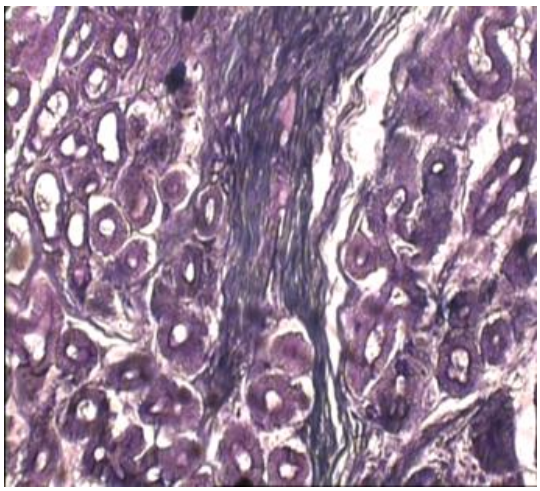

Figure 2: Photomicrograph of the cranial part of the duodenal glands. Mucous gland reacted positively with periodic acid shiff.periodic acid shiff staining, 120x

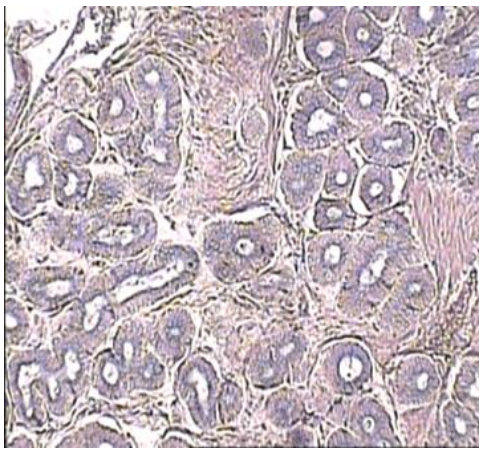

Figure 3: Photomicrograph of the cranial part of the duodenal glands. Most of mucous gland reacted positively with periodic acid shiff and some of the secretory cell positively reacted with alcian blue too. Periodic acid shiff and alcian blue staining, 120 x

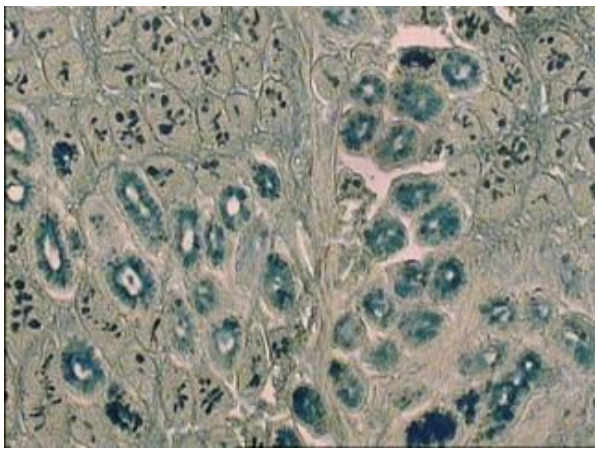

Figure 4: Photomicrograph of the cranial part of the duodenal glands. Acidic secretory unit cells of duodenal sub mucosal glands reacted positively with alcian blue.alcian blue aldehyde fusion staining performed, 120x

While, the cells in the central region of the lobules produce neutral mucin, acidic mucins were determined to be present in only the peripheral cells of the glands. It has been reported that in the domestic rabbit and American (Cottontail) rabbit (Sylvilagus floridanus) the mucous cells in the acini of Brunner's glands contained neutral, carboxylic and sulpho acidic mucin, while serous cells contained neutral mucin (Krause, 2000).

In the murrah breed, the secretion granules in mucous cells of Brunner's glands react with PAS (Figure 2), and reacted positively with alcian blue pH 2.5 (Figure 3). Furthermore, PAS (+) cells, resembling goblet cells in their morphology were determined to exist among mucous cells. When employed combined aldehyde fuchsine-alcian blue pH 2.5 staining, mucous cells were determined to be alcian blue (+) and aldehyde fuchsine (-) (Figure 4). Thus, it was determined the secretion of the ducts and mucous cells of duodenal glands in the murrah breed contained neutral carbohydrates and a limited amount of acidic carbohydrates, which this acidity being due to the presence of carboxyl groups. Males and females did not differ in the histochemical staining properties of the duodenal secretion.

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