Morphometric, Anatomical and histological features of Gastrointestinal Tract (GIT) of Freshwater Turtle, Pangshura tentoria

Monim Shah Rahman, Dhirendra Kumar Sharma

Abstract — The gastrointestinal tract (GIT) of reptiles draws our attention as numerous researches in the field of biological science have been going on using reptile as a model animal. The GIT morphology and physiology elaborates the variety in food and feeding habits. The aim of present investigation is to study the morphometrical and histological features of gastrointestinal tract of freshwater turtle, Pangshura tentoria. The oesophagus, stomach and intestine of Pangshura tentoria is buildup of four layers from outside inwards are serosa, muscularis, submucosa and mucosa. The oesophageal mucosa consists of simple columnar ciliated epithelial and goblet cells. The intestinal glands are absent. There are no much differences between the length of oesophagus and Stomach but the small intestine is larger in case of female. The length of whole gastrointestinal tract is sex dependent. In conclusion, the characteristic variation of the gastrointestinal tract is related to their ecology and physiology.

Index Terms — Morphometry, histology, Pangshura tentoria, gastrointestinal tract, freshwater turtle

1 INTRODUCTION

Gastrointestinal tract (GIT) is an interesting system in reptiles which have often drawn attention in many fields of biological sciences. The diversity of vertebrate food and feeding habits is matched by an array of adaptive intestinal morphologies and physiologies [1,2]. The intestinal tracts of herbivores are longer and more complex (many include fermentation chambers) than those of carnivores, and herbivore intestines hydrolyze and transport simple sugars at greater rates than they do amino acids, whereas the opposite tends to be true for carnivore intestinal tracts [3]. In general, tortoises and some turtles and few lizards eat vegetation. Small turtles feed on aquatic invertebrates. Large lizards, turtles, snakes and crocodiles eat various vertebrates from fishes to mammals [4,5,6]. The longer herbivore gut is necessary because plant material is more difficult to digest than animal material. Differences in substrate breakdown and transport between herbivores and carnivores express an adaptive emphasis to absorb the major biomolecules of their diet. Morphology, anatomy and histology have been studied extensively on lizards; Scincusofficinalis and Scincusscincus (Family: Scincidae), [7,8].Uromastyxaggyptia (family: Agamidae) [9] and Chamaeleon vulgaris (family: Chamaeleontidae). Yet, information on the gastrointestinal tract (GIT) on turtle species is extremely poor, especially on freshwater turtle species. The present study deals with the morphological adaptations in the freshwater turtle digestive system in relation to their food nature. Anatomical and physiological function of gastrointestinal tract of any organism is adjusted to their food preference and feeding habits. Many species of freshwater turtles in the family Emydidae undergo an dietary shift, from juvenile to mature, they change from a primarily carnivorous to a primarily herbivorous diet. It might be results from an unfavorable ratio of gut capacity to metabolic rate that prevents small reptiles from processing adequate volumes of plant material to meet their energetic demands. Ontogenetic dietary shifts in some reptiles have been attributed to a combination of these energetic differences and specific nutrient requirements for juveniles, including an increased need for calcium [10] and nitrogen [11,12]. Dietary intake may be regulated by numerous factors, including prey availability, energetic requirements, environmental conditions, social behavior, and diet quality [13,14,15,16,17,18]. This investigation was done to correlate between the anatomical, histological as well as morphometric configuration among differences between the sexes (male and female). This study could be useful with great importance in the field of turtle taxonomy and species conservation. The alimentary tracts of reptiles and higher vertebrates are mostly similar with some exceptions. The adaptive modifications of oesophagus vary from group to group. In turtles, heavily keratinized papillae are found in oesophagus that protect the mucosa and act as filtering devices. In lizards, it is formed of folds lined by ciliated columnar epithelium. The muscularis mucosa of theoesophagus is absent in many species of reptiles except some species of turtles [19].

The feeding habit of Pangshura genus is poorly understood.
However it has been shown that *Pangshurasylvethensis* and *Pangshura tentoria* were omnivorous species [20,21]. Many of the species has been shown as opportunistic feeders [22]. The carnivorous habit of marine turtle *Natator depressus* has been studied. However, the aim of this investigation is to study the morphometry and histology of gastrointestinal tract (GIT) organ length and to find any variation in adult male and female in relation to their feeding habit and sexual dimorphism.

2. MATERIAL AND METHODS

20 specimens (10 Male, 10 female) of freshwater turtle, *Pangshura tentoria* (trapped and killed by fishermen) were collected for this purpose during the year of 2008-2012. The morphometry of dead specimens were done by Vanier caliper. Dead animals were cut ventrally to remove the gut portions (oesophagus, stomach and intestine). These organs were measured and examined histologically.

2.1. ANATOMICAL Studies:

Specimens were measured from the tip of mouth to the cloacal opening, (snout-vent length). Lengths of the gastrointestinal tract (GIT) of males and females were expressed as indices and percentages of the total tract length. Statistical significance of female values versus male ones was determined by the application of student test [23]. T. test was also applied between the whole gut values versus male ones was determined by the application of student test [23].

2.2. HISTOLOGICAL Studies:

For histological study, different region of gut were taken. Cleaned sample were fixed in carnoy’s, then washed and dehydrated in ascending grades of ethyl alcohol cleared in xylene and embedded in paraffin, cut in section at 5 μm thickness and stained with haematoxylin and eosin [24] the stained section were examined and photographed using fluorescence microscope.

3 RESULTS AND DISCUSSION

3.1. ANATOMICAL Studies:

3.1.1 Gross anatomy:

The buccal cavity leads to the funnel-shaped pharynx which opens into the oesophagus. The liver is a large triangular gland and it is composed of two lobes. They are completely fused together anteriorly. Each lobe is more or less triangular, and is notched anteriorly. The stomach is left-curved and wider and shorter than the oesophagus. The stomach is opens in to the intestine without any constriction. The intestine is subdivided in to small intestine and large intestine. The large intestine is nearly straight and is again subdivided in to colon and rectum.

3.1.2 Morphometric analysis of GIT:

Collected data and t-test shows that there is a significant difference observed between the plastron length of male (8.04±0.92 cm) and females (10.64±2.32 cm). The alimentary tract length of female (53.44±11.37 cm) is always longer than that of males (40.72±3.55 cm) and there is significant different is detected in between them. The oesophagus and stomach length represents statistically non-significant in males and females. (Student t-test p>0.05 level) The length of small intestine of female (28.72±9.42 cm) is longer than that of males (21.34±2.33 cm) and this value is statistically highly significant (student t-test, p<0.001 level). There is no significant different is detected in the large intestine of males (8.2±0.95 cm) and females (12.14±1.59 cm) at p>0.05 level (Table: A). Among index values of alimentary tract organs of males and females, there is no significant difference is detected except oesophagus where male’s value is 7.90±0.38 cm and females value is 9.12±0.85 cm respectively as shown in Table: B. The percentage ratios of the GIT organs of male do not significantly differ from those of females in stomach and intestine. However there is significant difference is detected in oesophagus where males (12.68±0.61 cm) and females (11.03±1.08 cm) at p<0.05 level. These ratios increase progressively in both sexes through the oesophagus, stomach and intestine (Table: C).

### Table (A): Lengths (cm) of the Snout-vent and alimentary tract organs in both sexes of *Pangshura tentoria*

<table>
<thead>
<tr>
<th>GIT Organ</th>
<th>Males</th>
<th>Females</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastron Length (cm)</td>
<td>8.04±0.92</td>
<td>10.64±2.32</td>
<td>*</td>
</tr>
<tr>
<td>Total Alimentary tract length (cm)</td>
<td>40.72±3.55</td>
<td>53.44±11.37</td>
<td>*</td>
</tr>
<tr>
<td>Oesophagus (cm)</td>
<td>5.16±0.52</td>
<td>5.82±0.88</td>
<td>NS</td>
</tr>
<tr>
<td>Stomach (cm)</td>
<td>5.94±0.58</td>
<td>6.8±0.46</td>
<td>NS</td>
</tr>
<tr>
<td>Small intestine (cm)</td>
<td>21.34±2.33</td>
<td>28.72±9.42</td>
<td>**</td>
</tr>
<tr>
<td>Large intestine (cm)</td>
<td>8.2±0.95</td>
<td>12.14±1.59</td>
<td>NS</td>
</tr>
</tbody>
</table>

The values are means ± standard deviation (n = 10); ns: Non-Significant (student t-test, P > 0.05 level); *: Significant values (student t-test, p < 0.05 level); **: Significant values (student t-test, P < 0.001 level).

### Table (B): Index values of gastrointestinal tract (GIT) organs in both sexes of *P.tentoria*

<table>
<thead>
<tr>
<th>GIT Organ</th>
<th>Males</th>
<th>Females</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oesophagus (cm)</td>
<td>7.90±0.38</td>
<td>9.12±0.85</td>
<td>*</td>
</tr>
<tr>
<td>Stomach (cm)</td>
<td>6.86±0.37</td>
<td>8.03±1.21</td>
<td>NS</td>
</tr>
<tr>
<td>Small intestine (cm)</td>
<td>1.90±0.01</td>
<td>1.92±0.20</td>
<td>NS</td>
</tr>
<tr>
<td>Large intestine (cm)</td>
<td>4.93±0.41</td>
<td>4.42±0.94</td>
<td>NS</td>
</tr>
</tbody>
</table>

Index is the gastrointestinal tract length / organ length; Values are means ± standard deviation (n = 10); ns: Non-Significant (student t-test, P > 0.05 level); *: Significant values (student t-test, P < 0.05 level).

### Table (C): Percentage ratios of the gastrointestinal tract (GIT) organs in both sexes of *Pangshura tentoria*

<table>
<thead>
<tr>
<th>GIT Organ</th>
<th>Males</th>
<th>Females</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oesophagus (cm)</td>
<td>12.68±0.61</td>
<td>11.03±1.08</td>
<td>*</td>
</tr>
<tr>
<td>Stomach (cm)</td>
<td>14.58±0.80</td>
<td>12.68±2.03</td>
<td>NS</td>
</tr>
<tr>
<td>Case</td>
<td>Mean ± Standard Deviation</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---------------------------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Small intestine (cm)</td>
<td>53.38±2.76</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Large intestine (cm)</td>
<td>20.35±1.81</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

The values are means ± standard deviation (n = 10). Percentage ratio is the organ length × 100 / gastro-intestinal length; ns: Non-Significant (student t-test, p > 0.05 level); *: Significant values (student t-test, p < 0.05 level).

3.2 Histological observations:

3.2.1 Oesophagus: Under microscope, the oesophagus of *Pangshura tentoria* is divisible into serosa, muscularies, submucosa and mucosa (Figure 1. A, B). The serosa is a thin layer of squamous epithelium. The muscularies have two layers, an outer layer of longitudinal smooth muscle and an inner layer of circular smooth muscle. The submucosa is extended into many folds. The mucosa is arranged into many folds and are surrounded the lumen. The mucosa also comprises the lamina propria of connective tissue.

The epithelium of mucosa is composed of two types of cells, the large goblet cell and the thin elongated ciliated cells.

![Figure 1. A. Oesophagus of P.tentoria 10X, B. Oesophagus of P.tentoria 40X](image1)

The epithelium of stomach consists of a thin layer of spindle shaped squamous epithelial cells. The muscularies is composed of smooth circular muscle fibres. The submucosa is well developed (Figure 2. C, D, E).

Microscopic examination of the stomach revealed the presence of two types of gastric glands, the granular glands are mainly of simple tubular type and opens in to the gastric lumen by their pits. They are full of dark granules and have central rounded nuclei (Fig: 2 E). The light celled glands were found to be of the simple tubular or branched type.

![Figure 2. C. Stomach of P.tentoria 10X, D. Stomach of P.tentoria 40X, E. Stomach of P.tentoria 40X](image2)

3.2.3 Intestine: The lumen of this part of digestive tract is more or less narrow due to the presence of coiled and long villi. The serosa is formed of simple squamous epithelial cells. The muscularies which is formed of smooth muscles is arranged into an outer longitudinal layer and an inner circular layer (Figure 3. F, G, H).

The mucosal lining of the intestinal villi is composed of three types of cells, simple columnar epithelial cells, goblet cells and endocrine cells (Fig:H). The columnar cells have large elongated nuclei situated at the base of the cells. The goblet cells are fewer in number as compared with the columnar cell. The endocrine cells are small in size, limited in number and have a clear cytoplasm in which spherical and central nuclei were located (Fig:H).

![Figure 3. F. intestine of P.tentoria 10X, G. intestine of P.tentoria 10X, H. intestine of P.tentoria 40X](image3)

In both sexes examined, there is a narrow oesophagus and becomes gradually wider towards the stomach. The oesophagus of turtle is made up of spindle shaped longitudinal muscle fibre and...
inner surface is surrounded by circular muscle and having elasticity property as the neck is retractile. In female, the length of oesophagus is greater than that of male. On the contrary, the presence of a constriction between the oesophagus and the stomach was referred in turtles (Luppa, 1977). This is physiological adaptation to permit the entry of food item without any obstruction. The stomach of both sexes displays a large left convex curvature. It is attached to the livers left lobe by a gastrohepatic ligament and the left lung by a gastropulmonaryligament. The different part of the small intestine i.e. duodenum to jejunum and ilium are often difficult to identify. The morphometrical analysis of reptiles has got special attention for sexual dimorphism, for similar case present investigations were carried out.

Morphometrically there is no statistical significant difference in between the gastrointestinal organs of the males and the females of Pangshura tentoria. However the small intestine of female was found longer than that of males. The small intestine is the longest gut organ in Pangshura tentoria. This elongation is probably due to compensate the limited number of intestinal villi. The present morphometric data shows that in Pangshuratentoriathe plastron length represent 20% of the alimentary tract length. It means that when the plastron length is measured, the length of gastrointestinal tract as an internal system can be estimated without sacrifice the animal.

Histologically, the oesophageal mucosa of reptilian species showed a considerable variation in their structure. The present study revealed that the oesophageal mucosa of Pangshura tentoria is represented by a simple columnar ciliated and goblet cells. The oesophageal gland is mucous in nature and secret mucin to help the oesophagus to convey food from the buccal cavity to the stomach. In other reptilian species as for example in Lacertaagilis, the oesophageal mucosa is only represented by goblet cells (Przystalski A)

The presence of the goblet cells in the oesophageal mucosa of Pangshura tentoria as well as other reptiles, such as Chamaeleon vulgaris (Bishai, 1960) act as the same basic functions which conveys food from the buccal cavity to the stomach.

Histochemically, the presence of carbohydrates in the mucosal epithelium of Pangshura tentoria indicating the presence of mixed neutral and acidic mucins which helps in producing mucous necessary for lubricating food which is previously observed by Abo-Eleneen (2010) in Uromastyxacaegyptius and Varanusniloticus. The stomach mucosa of Pangshura tentoria is consists of gastric glands and gastric pits. The gastric glands are represented by pec tic cells which indicate that the Pangshura tentoria is carnivorous in habit in some extent. The presence of oxyntic cells in the gastric gland of Pangshura tentoria is the characteristic feature of herbivorous in nature. In this investigation, the mucosal epithelium of the small intestine shows endocrine cells. The absence of intestinal glands observed in the present work was similar to that found in Mabuyaquinquetaentiat25,26, in Uromastyxacaegyptius and Varanusniloticus[27].

Lack of intestinal gland or crypts of Lieberkhun in the small intestine was seen in this investigation. Microscopic investigation of the intestinal mucosa indicates the presence of extremely long and coiled villi, which increase the surface area for absorption of digested food.

Histo-chemically, the intestinal goblet cells of Pangshura tentoria are strongly stained with a mixture of neutral and acidic mucins. The secretion of these cells is probably responsible for mucous secretion.

4. CONCLUSION:

For this investigation it could be concluded that the morphometry, anatomy and histology of Pangshura tentoria show ecological and physiological adaptations.

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REFERENCES