MORPHOGENESIS OF THE GASTROINTESTINAL TRACT OF HELMETED GUINEA FOWL (*NUMIDA MELEAGRIS*) AT PRE AND POST-HATCH

BY

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DECLARATION

I hereby declare that the work in this thesis titled "Morphogenesis of the gastrointestinal tract of helmeted guinea fowl (*Numida meleagris*) at pre and post-hatch" was performed by me in the Department of Veterinary Anatomy. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

Innocent Jonah GOSOMJI	______________	__________
Name of Student	Signature	Date

CERTIFICATION

This project thesis titled " **MORPHOGENESIS OF THE GASTROINTESTINAL TRACT OF GUINEA FOWL (*Numida meleagris*) AT PRE AND POST-HATCH**" by Innocent Jonah GOSOMJI meets the regulation governing the award of the degree of Master of Science in Veterinary Anatomy of the Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to the loving memory of my teacher and model, Late Prof. Gregory Chibuzo.

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ABSTRACT

This study was conducted to investigate the development of the Helmeted Guinea fowl (*Numida meleagris*) gastrointestinal tract at pre- and post-hatch. One hundred and seventy five (175) eggs were purchased from the Poultry unit of National Veterinary Research Institute (NVRI), Vom out of which one hundred and thirty five (135) were used for pre-hatch and forty were allowed to hatch for post hatch studies. The samples were studied grossly, morphometrically, and microscopically. The result revealed that at day 8 of development a digestive tube appeared with a roundish structure in the middle of the tube. By day 10 of development, a dilatation had appeared cranial to the roundish structure identified as the proventriculus. At the same time a small outgrowth appeared at the caudal end of the tube also identified as one of the caeca. By day 11 of development, the crop had appeared separating the oesophagus into cervical and thoracic parts, respectively. By days 12 and 13 of development the second caecum and the duodenal loop became apparent with the appearance of the duodenal loop which marks the end of the gross anatomical development of the guinea fowl GIT. The study concludes that it took 6 days for the completion of gross anatomical development of the guinea fowl GIT. This study also revealed that the crop and the proventriculus did not follow craniocaudal pattern of development. The morphometric study revealed that there were positive correlation between the measured parameters at both pre and post-hatch. The r values between the body weight (BWT) versus GIT weight (GWT), between the BWT versus GIT length (LGT) as well as between the GWT versus LGT were 0.42, 0.96 and 0.95, respectively for pre-hatch while the r values of 0.70, 0.50 and 0.85 were for post-hatch respectively. The histological study revealed that the oesophagus was lined by simple columnar epithelium at the early embryonic life and then changed to stratified squamous epithelium with the oesophageal glands appearing in the submucosa by day 19 of development. The histology of the crop is similar to that of the oesophagus. The proventricular gland has the most prominent histological feature of the proventriculus with the primordial gland at the embryonic stage developing progressively through pre and post-hatch. The intestine has the typical mucosal lining which was pseudostratified initially but eventually changed to columnar epithelium with numerous villi at the small intestine. No goblet cells were found in the intestinal tract before hatching but appeared at post-hatch. This is a specific feature of this avian species. The residual yolk decreased with age until it became vestigial by day 8 post-hatch. The results of this study confirm previous reports on the GIT development that its growth and the digestive function are not fully formed in newly hatched birds. The study also revealed that days 8-13 of development are the most critical periods for the gross formation of the GIT in the guinea fowl when congenital malformation can occur.

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ABBREVIATIONS

GIT – Gastrointestinal tract

cm – Centimetre

g- Gram

BWT – Body weight

GWT – Weight of gastrointestinal tract

LGT – Length of gastrointestinal tract

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The formation of a functional vertebrate gut involves several processes. These include the induction and patterning of the endoderm, recruitment of the mesoderm and tube formation, proliferation and specification of sections along the gut tract, and renewal of the mature gut epithelium by the specification of the endoderm that will eventually form the complex epithelium of the mature digestive organ (Grapin-Botton and Melton, 2000; Stainier, 2002).

The gut tube is formed by two invaginations appearing on either end of the embryo, the anterior intestinal portal (AIP) and the posterior intestinal portal (PIP). These two areas move inward into the embryo, taking the specified endoderm along with them, and forming a primitive tube at both ends of the gut. Fusion occurs in the middle of the embryo, and the midgut must roll up, taking the endoderm and surrounding mesoderm along with it to form a final continuous tube. At the end of this process, the vertebrate gut is grossly separated into three main regions: foregut, midgut, and hindgut (Grapin-Botton and Melton, 2000). Ultimately, the foregut gives rise to the oesophagus (and crop in birds), stomach (including the gizzard and proventricularis in birds) and derivative organs – thyroid, lungs, pancreas, and liver while the midgut forms the small intestine, and the hindgut forms the ceca (in birds) or appendix (in mammals), the large intestine and the cloaca (in birds) or rectum (in mammals) (Drucilla *et al*., 1998). The accessory digestive organ buds develop as outgrowths of endodermal epithelium that intermingle with the surrounding mesenchyme, and together they proliferate and ultimately differentiate during fetal development into functional organs such as the pancrease and liver (Aaron and James, 2009).

Klasing (1999) studied the avian digestive system and found that the avian gastrointestinal tract is a double-ended open tube (as seen in mammals) that begins at the beak and finishes at the vent. In sequential order, it is composed of a mouth, oesophagus, crop, proventriculus, ventriculus, small intestine, caeca, rectum and cloaca. The gastrointestinal tract (GIT) becomes developmentally active in the early post-hatch period in poultry species (Uni *et al.,* 2000) undergoing changes in weight and length that are determined by genetic and environmental factors (Langenfeld, 1992).

Digestive tract has a major role in inducing growth during the early post hatch period. This was attributed to its more rapid increase in size compared to the whole body especially the small intestine (Nir *et al*., 1993).It possesses the functions of food content storage, secretion, digestion and absorption of nutrients (Sell *et al*., 1991).

1.2 Statement of Research Problem

The gastrointestinal tract is a critical organ system mediating nutrient uptake used by the animal (Frank, 2011). Understanding factors that influence GIT development, growth and function is critical in improving management and therapeutic approaches to maximize health and production efficiency. A lot of investigations on the development of avian digestive system have been made and also excellent reviews of the gastrointestinal of embryonic development have been given (Klasing, 1999; Uni *et al.*, 2000). However, all these information were on the domestic fowl, ducks, turkey and quails. Among the few works done on the anatomy of the guinea fowl are; effect of age and sex on digestive tract morphometry of guinea fowl (Daria *et al.*, 2012), studies on the onset of osteogenesis in grey-breasted helmeted guinea fowl (Salami, 2009), observations of the wattles of adult helmeted guinea fowl (Umosen *et al*., 2008), studies on the histochemistry of the proventriculus and gizzard of post-hatch guinea fowl (Senthanil *et al.*, 2008), studies on the major respiratory pathways of the West African guinea fowl (Ibe *et al.*, 2008), Studies on the digestive, respiratory, urogenital system and lymphoid organs of helmeted guinea fowl (Lakshminarasimhan *et al*., 1983) and Studies on the external morphology and skeletal system (Ojo *et al*., 1983).

A survey of literature revealed that there are global interests in guinea fowl production as an alternative to the chicken (Nahason *et al.*, 2006) but there is paucity of information on the gross and histology of this bird's GIT to enhance domestication and productivity of this species hence the present study.

1.3 Justification of the Study

The function of the digestive tract is to obtain the molecules necessary for the maintenance, growth and energy needs of the body from ingested food, in order to be able to perform its role of maximum production of meat and egg in this case. Therefore, the study of gastrointestinal development will shed light into the structural constituent of this organ and better understanding of its function(s). Information from this study will also equip the nutritionist with information to be able to formulate feeds that will be absorbable and helpful in enhancing production. This study will also give insight to the critical stages of GIT development and the changes that occurred at different stages of pre and post-hatch.

1.4 Aim of the Study

The aim of this research is to describe the morphological development of gastrointestinal tract of the helmeted guinea fowl during the pre- and post-hatch periods.

1.5 Objectives of the Study

i. To investigate the morphology and morphometry of gastrointestinal tract of the helmeted guinea fowl during the pre- and post-hatch periods.

ii. To investigate the histology of gastrointestinal tract of the helmeted guinea fowl during the pre- and post-hatch periods.

iii. To determine the period during which the GIT is fully developed at pre-hatch.

CHAPTER TWO

LITERATURE REVIEW

The food intake of an average Nigerian falls short of the Food and Agricultural Organization recommended level (David-West, 1978). According to Paarlberg (2000), the United Nation's Food and Agricultural Organization reported that one out of every five citizen from developing countries, totalling approximately 828 million people, is still suffering from chronic malnutrition. The situation may even be more disastrous for the whole of the African continent with an estimated population of about 776 million inhabitants (FAO, 2002).

Protein, an essential constituent of our daily diet, like fat and carbohydrates can also serve as a source of energy for the body. A regular daily intake is absolutely necessary to replace nitrogenous materials in the tissues (FAO, 2002), and also serve as the building blocks of human life, essential for normal growth (Marchuk, 1992). Lack of dietary protein can retard growth in children and in adult, it can be a contributing factor in chronic fatigue, depression, slow wound healing and the decreased resistance to infections (Gardner *et al*., 1996).

Man obtains his necessary proteins from either animal or plant source. The meat from cattle, goat, sheep, pig and poultry including their offal are the main sources of daily per capita consumption of animal protein (Alais and Lindel, 1990). According to Britton (2003), the main difference between animal protein and plant protein is that animal contains different ratios of amino acids and higher concentrations of protein than most plant products do. Most plants contain proteins at lower levels. Hence in a strictly vegetarian diet, it is important to mix and match different plants to get the variety of protein and amino acids necessary for good health. Animal proteins however are said to possess superiority over plan protein. Animal proteins are generally referred to as complete proteins and are the only available source (Oloyede, 2005; Fallon and Eign, 2001).

2.1 Origin and Distribution of Guinea fowl

The guinea fowl originates from African continent (Embury, 2001; Moreki, 2012). The strains are descendants of the helmeted guinea fowl, *Numida meleagris* (Moreki, 2012), which were first domesticated by ancient Egyptians (Oakland Zoo, 2001)from where they spread to other parts of the world such as France, Belgium, Canada and Australia where they are now produced commercially on a large scale (Robinson, 2000; Embury, 2001). However, in most African countries including Nigeria, Malawi and Zimbabwe, guinea fowl production is in its infancy (Dondofema, 2000; Ligomela, 2000; Smith, 2000).In Nigeria, there are two types of guinea fowl species - *Numida ptilorhycha* and *Numida meleagris*.The *N.ptilorhycha* is indigenous to the deciduous rain forest zone of southern Nigeria, while *N. meleagris* is currently domiciled in the Northern part of Nigeria but it is spreading to other smallholder farming areas (Ayorinde, 2004).

2.2 Breeding

Helmeted Guinea fowl are monogamous and occur in pairs during the breeding season, which is October-January in South Africa (Crowe, 2000). They are naturally seasonal breeders thereby restricting their reproductive activities to the rainy season as do the wild guinea fowl (Ayeni *et al.*, 1983 ; Ikani and Dafwang, 2004). The adult guinea fowl weighs over 1 kg with an average egg production of 55 to 100/year, each weighing 37 to 40 g (David, 2012). The egg shell is hard and thick, meaning less accidental cracks and longer preservation than most other poultry eggs, especially in the absence of refrigeration (Ayeni, 1980).

2.3 Social and Economic Importance of Guinea Fowl

In Africa, guinea fowl is truly the invisible animal, as it is hardly counted in wealth ranking as in the case of cattle, sheep and goats; yet, guinea fowl flock makes the best use of locally available resources in the quest to produce eggs and meat. Guinea fowl represents a reliable and active contributor to the survival of the rural folk since it has economic, cultural, livelihood and social values in their lives (Sonaiya *et al.*, 1999; Tye and Gyawu, 2001). In Nigeria, the guinea fowl ranks second to the chicken in poultry production with an estimated population of about 45.7 million in captivity (Anthony and Akinwumi, 1981) with very little known about the huge population of the bird in the wild. Hence, it contributes to family nutrition and income of rural populace (Sonaiya, 1999). Also a unique observation at study site was that, guinea fowl was being used as a tool in the promotion of ecotourism (Saina *et al.*, 2005). It contributes to family nutrition and income; however, the contribution is yet to be investigated. Cash income from guinea fowl has been used for food, school fees and unexpected expenses like medicines (Sonaiya, 1999). Guinea fowl provides the opportunity for better utilization of water and pasture resources in Africa that can generate additional food and income for rural communities. They are more heat tolerant and less susceptible to disease than chickens. Some farmers keep guinea fowls out of curiosity and as “watch animals” around homesteads because they have an excellent eye-sight, a harsh cry and they shriek at the slightest provocation (Smith, 2000). They are kept to control snakes, mice, ticks, other pests and weeds (Cactus, 2001; Frit’s Farm, 2001), thus encouraging its production.

Guinea fowl and guinea fowl products are generally accepted due to their quality and limited cultural barriers on consumption (Saina *et al.*, 2005). Nutritionally, guinea fowl meat is proving to be a potential competitor in the meat industry; it is rich in crude protein and is classified as white meat (Aisha *et al.,* 2012). In comparism with chicken meat; moisture, protein and ash content do not differ significantly, but fat as well as cholesterol content is lower in guinea fowl than in the chicken. Some minerals, vitamins and individual fatty acids differ somewhat between the species. The low sodium content of guinea fowl meat is an advantage to people who have to consume low sodium diet (Sales and VanNiekerk, 1996).

2.4 Functions of the Gastrointestinal Tract

The anatomy of an animal GIT clearly affects its food choices. In turn, the characteristics of the food are one of the primary forces that shape animal feeding behavior. For instance, carnivores must spend much time and energy searching for and capturing their rare preys that have mastered hiding, fleeing and fighting. In contrast, herbivores spend less time and energy finding and capturing bites of vegetation because plants are stationary and relatively abundant in the environment (Lisa, 1999).Thus, the digestive tract is an important system in living organisms, and plays a vital role in food processing and absorption (Hill *et al.*, 2008). In food animals, the digestive process ensures safety and healthiness of the animal product consumed by humans (Skriba, 2011). It provides an environment for the physical and chemical reduction in the size and molecular complexity of food and then absorbs the end products of digestion, which are in widely differing quantities (Klasing, 1999). The size of different portions of digestive tract of birds varies according to the type of alimentary habits (Jordano, 1987).

2.5 Oesophagus

Although the digestive system starts from the oral cavity, the gastrointestinal tract commences from the oesophagus. The oesophagus is a thin-walled, distensible tube which transports food from the pharynx to the stomach (Denbow, 2000) and performs an important storage function (Ziswiler and Farner, 1972). It contains a number of longitudinal folds which provide it with distensibility (Denbow, 2000; Gussekloo and London, 2006).

2.5.1 Developmental features

At first, it is a short tube that extends from the tracheal groove to the fusiform dilatation of the foregut. In association with the elongation of the cervical region of the embryo, the esophagus increases in length (McGeady *et al*., 2006).

2.5.2 Gross features

The avian oesophagus is on the right side of neck and it is placed between the pharynx and stomach glandular portion. It has thin and dilatable walls with a diameter relatively larger than that of mammals (Getty, 1975), due to limited ability of birds to break down food orally (Gussekloo and London, 2006).The avian oesophagus consists of two parts, cervical and thoracic; while in mammals it has three parts, cervical, thoracic and abdominal (Levin, 1984; Mule, 1991). The proximal oesophagus is more distensible and folded than the distal parts in the ostrich (Tivane, 2008), possibly due to their ability to accommodate cranio inertial feeding (Bonga, 2000). The oesophagus of birds lacks both upper and lower oesophageal sphincters which are present in mammals (Mule, 1991).

2.5.3 Histological features

The cervical portion of the oesophagus of aves has four layers; mucosa, submucousa, muscular and serosal layer. The mucosa is composed of the non keratinized stratified squamous epithelium with few mucous glands, nodular lymphatic tissue which are prominent within the connective tissue of lamina propria (Shehan, 2012). The submucosa is composed of loose connective tissue with glands, blood vessels and nervous plexus (Rodrigues *et al*, 2012). The muscular strauma consists of two layers, an inner which is circular and an outer longitudinal layer. The serosa is composed of loose connective tissue with collagen and elastic fibers (Shehan, 2012). The mucosal layer of thoracic part is thicker than mucosal layer of the crop. It is rich in lymphatic tissues called oesophageal tonsil. The submucosa is composed of loose connective tissue. The muscular layer consists of two layers, an inner circular and outer longitudinal. The serosa is composed of connective tissue, collagen and elastic fiber (Shehan, 2012).

2.6 Crop

Its structure and function vary according to the species and the diet. The crop functions to store food and may be spindle-shaped, bilobed or unilobed. The crop is absent in the gulls and penguin so food passes directly or it is stored in the tubular oesophagus (Nickel *et al,* 1977).

2.6.1 Developmental features

The embryonic crop appears as a sacullar diverticulum of the oesophagus (McGeady*et al*, 2006).

2.6.2 Gross feature

The crops is the extensive part of the cranial oesophagus to the entrance of thorax (Ensminger *et al*., 1990). The crop in most avian species is merely a fusiform enlargement of the oesophagus (Dyce *et al*., 2002).

2.6.3 Histological features

Crops of all studied birds were lined by a keratinised stratified squamous epithelium similar to that of domestic fowl (Dellmann and Eurell, 1998). The crop is structurally similar to oesophagus (Rodrigues *et al*., 2012).

2.7 Proventriculus

The proventriculus varies in size between different species. In graminivorous, it is of smaller size than carnivores where the proventriculus is quite larger and more distensible (Degen *et al*., 1994). In all birds except carnivore and piscivore species, the mucosal surface of the proventriculus lacks the longitudinal folds characteristic of the mucosal surface of the oesophagus and is lined by mucous secreting cells (McLelland, 1991).The proventricular folded mucosal surface is not smooth but it is covered by several projections or papillae. The proventricular glands which form the most thickness of the proventricular wall, open at the apex of these papilla by ducts elaborating pepsinogen, hydrochloric acid and mucus discharge into the stomach lumen (Liman *et al*., 2010). The surface lining epithelium of the proventriculus is of a simple columnar type (Rahman *et al*., 2003). However, the glandular epithelium is formed by only one principal exocrine cells of a simple cuboidal type; oxyntico-peptic cells and some gastric argyrophil endocrine cells (Langlois, 2003). The secretions of parietal and peptic cells are produced by a single cell type in birds. The distribution of the gastric glands is not consistent among avian species. Generally, they are distributed throughout the proventriculus, but sometimes are restricted to longitudinal tracts (owls), to a circular patch on the greater curvature (ratites), or to separate diverticular (King and McLelland, 1984).

2.7.1 Developmental features

The primordial epithelium is surrounded by different shapes of mesenchymal cells with myoblast cells arranged at the periphery. This represents the future tunica mucosa. Long tubular- shaped gland extends from the lumen as out pouching into the mesenchymal cells. Both the size and the number of the glands were increased and appeared as compound tubuleoalveolar glands (Hossam, 2008).

2.7.2 Gross features

Proventriculus or glandular stomach is a short, thick walled, spindled-shaped organ lying above the liver and between the oesophagus and the gizzard. The lumen is narrow and the thick spongy walls are composed mainly of tubular glands (Mina and Paria, 2011). The proventriculus arises from the oesophagus without a distinct demarcation; the wall is thicker than that of the oesophagus, and the caudal extent of the proventriculus is marked by a constriction, the isthmus gastris. The mucosal surface of the proventriculus posses raised papillae, papillae proventriculus, over its entire surface (Mostafa *et al*., 2012). According to Denbow (2000) and Taylor (2000), in birds that eat hard food items, the proventriculus is relatively thin-walled and glandular.

2.7.3 Histological features

The proventricular wall has all the four tunics of digestive organs; tunica mucosa, tunica submucosa, tunica muscularis and outermost tunica serosa. The surface mucosa of the proventriculus is invaginated at regular intervals forming mucosal plicae and sulci. The surface lining epithelium is simple columnar with vesicular nucleus located near the basement membrane. Under lamina epithelialis, the lamina propria is formed by loose connective tissue with lymphocytic infiltration and lymphatic nodules of different sizes; especially close to the mucosal papillae (Yasser *et al.,* 2011). The tunica submucosa is filled with proventricular glands. The mass of glands make up the greater part of the thickness of the proventricular wall. They are compound branched tubular glands. The glands are composed of numerous rounded or polymorphic lobules which are arranged in small groups. The secretory cells are pyramidal or cubical and they are deeply acidophilic. Surrounding the gland is a connective tissue septum consisting of collagenous and elastic fibers with a few muscle fibres, blood vessels and nerves (Mina and Paria, 2011). The secretory cells (oxyntico-peptic cells) of the glandular tubules or alveoli are basophilic and varied in size and shape. They are cuboidal, columnar, triangular, polyhedral or inverted flask in outline. Their nuclei are nearly rounded and located near the basement membrane. The secretory cells are oriented obliquely to the long axis of the glandular tubules of the proventriculus and are separated from each other by relatively narrow spaces giving the epithelial cells a serrated appearance (Yasser *et al.*, 2011)

The muscular tunic (tunica muscularis gastric), presents an inner layer of longitudinally disposed smooth muscle fibers (stratum longitudinal) and another intermediary of circularly disposed fibers (stratum circulare). Externally, the layer is constituted by bands of longitudinally arranged smooth muscular tissue (stratum longitudinal). Among the most external bundles of muscle fibers are nerves and nervous ganglions of the myenteric or of the Auerbach plexus. The serosa of the proventriculus (tunica serosa) is constituted by connective tissue lined by mesothelium, and is rich in blood vessels and nervous elements (nerves and ganglions) of the serous plexus (Catroxo and Lima, 1997).

2.8 Ventriculus (Gizzard)

Evolution of the gizzard or muscular stomach in the avian digestive system, allows for the mechanical digestion of foods without teeth (Yasunori and Tomotoshi, 2000). The gizzard is much larger and more muscular than proventriculus and it is important for grinding food. This grinding action of the gizzard prepares the food for further digestion in the intestine, where the first part of the small intestine, duodenum, is connected with the gizzard at gizzard- duodenal junction (Maya and Lucy, 2000).In granivores, herbivores, and insectivores, the main function of the ventriculus is to triturate the ingesta to decrease the size of food particles and increase their surface area to promote gastric proteolysis. This is accomplished through the strong contractions of the asymmetrical ventricular muscle masses and abrasive action of the cuticle. The ventricular contractions result in rotary and crushing movements, which reduce the particle size of hard diets and mix the ingesta with the digestive enzymes (Duke, 1986). In carnivorous and piscivorous species, the ventriculus is primarily a site of food storage rather than contributing to mechanical digestion, having evolved to digest relatively soft diets. In raptors, the ventriculus is also involved in the formation and regurgitation of pellets (Burthol, 1982).

Avian species with a less developed ventricular muscle, such as frugivores and nectarivores, has a softer and more uniformly distributed cuticle. Massive shedding of the cuticle occurs in some species such as the common magpie and the common starling. Some male hornbills regurgitate the cuticle as a seed-filled sac to feed the nesting female (King and McLelland, 1984). The pylorus arises from the right face of the ventriculus and connects the ventriculus to the duodenum (McLelland, 1991). It is poorly developed in some species, such as domestic fowl, and it forms a distinct chamber in aquatic species, such as the Great cormorant (King and McLelland, 1984). The pyloric fold regulates the rate of passage of food between the stomach and duodenum (Klasing, 1999), slowing down the movement of large particles into the duodenum (Vergara *et al*., 1989).

The development of the ventriculus varies among avian species and two basic types are recognized (King and McLelland, 1984). In granivores, insectivores, and herbivores, the ventriculus is well developed and distinct from the proventriculus. The ventriculus in these species consists of four semiautonomous smooth muscle regions: the thinner caudoventral and craniodorsal regions, and the thicker caudodorsal and cranioventral regions (McLelland, 1991). Each region originates and terminates on a circular tendinous area. In carnivores and piscivores, the ventricular muscle is poorly developed and uniform in thickness. The two pairs of semiautonomous muscles are lacking and no clear distinction exists between the proventriculus and the ventriculus in these species. In frugivores and testacivores, the development of the ventriculus is intermediate between these types and lean more towards the well-developed or poorly-developed type according to the species (King and McLelland, 1984). Ratites have a unique stomach anatomy. The gastric glands of ostriches and rheas are limited to a dorsally located patch (Fowler, 1991). The ostrich has a large, thin-walled proventriculus that passes dorsal to the ventriculus and empties into the ventriculus at its caudal aspect. The opening between the proventriculus and ventriculus is large, which makes removal of ventricular foreign bodies easier in this species. The ventriculus of the ostrich is similar to that of birds consuming a hard diet such as seeds and insects. In rheas, the proventriculus is much smaller than the ventriculus. Their ventriculus is elongated, with a koilin layer also present in the proventriculus. Emus and cassowaries have a large proventriculus slightly smaller than their ventriculus. Their ventriculus is less muscular than other ratites. Cassowaries lack a cuticle (Fowler, 1991).

2.8.1 Developmental features

The avian ventriculus arises from a simple gastric primodium, the caudal part (McGeady *et al*, 2006).

2.8.2 Gross features

The ventriculus is a small spheroidal organ. It lies in the left dorsal and ventral regions of the thoracoabdominal cavity, placed partly between the lobes and partly behind the left lobe of the liver. It is much larger and more muscular than the proventriculus. It consists of two pairs of opposing muscles. The caudoventral and craniodorsal thin muscles line the caudal and cranial sacs of the ventriculus, respectively. The ventriculus is lined by the koilin, a cuticle layer (Mostafa *et al*., 2012). The cuticle is water-resistant and usually brown, green or yellow due to the reflux of bile pigments from the duodenum. The cuticle is asymmetrically developed and is thickest opposite to the thicker semiautonomous muscle masses. It acts as an abrasive surface to improve the grinding function of the ventriculus and is continuously renewed as it wears down. It also protects the underlying mucosa from the digestive action of enzymes (Klasing, 1999).

2.8.3 Histological features

The gizzard wall also has the four layers; mucosa, submucosa, muscular layer and serosa. The gizzard mucosa shows low folds (plicae ventricularis) lined by simple columnar to cuboidal epithelium. Gizzard tubular glands are lined by cuboidal epithelium which is lower at the base and higher at the upper portion of the glands (Rossi *et al.*, 2005).The mucosa is covered with a thick keratinized laminated layer formed by the secretion of the glandular tubules and the degenerated surface epithelial cells. The glands are branched tubular glands located in the lamina propria and open in to the mucosal crypts by short neck. The glands within the mucosal ridges had fewer branches and had straight regular appearance while the glands in the depressions of the mucosal surfaces are more branched. The tubular glands are lined with low columnar or cuboidal cells with rounded vesicular basal nuclei and darkly stained basophilic cytoplasm. No muscularis mucosa was present thus making no partitions between lamina propria and submucosa. The muscular layer is well developed forming the main bulk of the gizzard wall and represented by smooth muscle fibers arranged mostly in a circular manner; however some fibers were arranged as outer longitudinal and inner oblique layers. Beneath the muscular layer is a tendinous layer of collagen bundles arranged in a parallel fashion (Yasser *et al*., 2011).

2.9 Small Intestine

Nutrient digestion, absorption, assimilation, and incorporation into developing and growing tissues depend directly on the functional capabilities of the intestine (Uni *et al*., 2011). The digestive system of the chick is anatomically complete at hatching but undergoes major morphological and functional changes in the immediate post-hatch period (Sklan, 2001). Morphological and functional development of the small intestine as well as growth is stimulated by early access to feed (Noy and Sklan, 1999).

2.9.1 Developmental features

The developing intestine undergoes morphogenesis and cytodifferentiation (Uni, 2006). As incubation progresses, the body weight of the embryo increases as does the weight of the small intestine. The morphology of small intestine also changes rapidly. Histology indicates that the intestine including the external muscular layers and the villi are growing rapidly. Villi at day 15 are rudimentary but obvious at day 17. At day 19 there is budding at the base of the existing villi which develop considerably and by day 20, an additional wave of small villi is seen, comprising 30% of the total (Uni, 2006).

2.9.2 Gross features

In birds, the small intestine is divided like in mammals, but the margins of the segments are not strictly separated; in herbivorous birds, the small intestine is longer than that in carnivorous ones (Whittow, 1999). The form and function of the small intestine is more variable than the anterior digestive organ, probably because the diverse physical nature of different food is reduced to relatively uniform and fluid chime (Klasing, 1999). The small intestine extends from the pyloric end of the stomach to the junction of the small intestine, caeca and colon. It is long and consists of a coiled mass forming a series of loops and lies within the abdominal cavity (Mostafa *et al.*, 2012). It is divided into 3 parts: duodenum, proximal small intestine (jejunum), and distal small intestine (ileum) (Andrew and Hickman, 1974). There is a distinct duodenal loop, the yolk stalk (diverticulum vitellinum; formally called Meckel's diverticulum) often used as a landmark to separate the jejunum and ileum (Denbow, 2000).

2.9.3 Histological features

The histology of the small intestine of the African pied crow showed that the wall of each of the three segments of the small intestine are composed of four tissue layers namely, tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa (Igwebuike and Eze, 2010).The tunica mucosa is modified into many finger-like projections, the villi. Each villus is lined by an epithelium while its center contained connective tissue. The jejunum has numerous villi, long and wavy, while the villi of the ileum are short. The lamina epithelialis mucosae of all components of the small intestine of the African pied crow are simple columnar epithelium with brush border. The epithelium contains many goblet cells especially in the ileum (Igwebuike and Eze, 2010).

Goblet cells are not very prominent in the duodenum and the jejunum. The lamina propria/sub mucosae consist of a loose connective tissue located just beneath the epithelial layer. The muscularis mucosa is absent, and the connective tissue of the lamina propria is continuous with the connective tissue of the tunica submucosa (Igwebuike and Eze, 2010). The submucosa is thin, narrow and hardly distinguished in some regions. The submucosa connective tissue holds few blood vessels. The muscular layer consists of two smooth muscle layers; outer longitudinal layer and a thick circular muscle layer. All muscle fibres are of the unstriated type, and allow for the mixing and propulsion of the digesta through the intestinal tract. The serosa is made up of flattened simple squamous epithelium (Mostafa*et al*., 2012).

2.10 Large Intestine

The alimentary tract extracts nutrients from the ingested diet, after which they are absorbed into the bloodstream. It therefore represents an interface between the metabolism of the animal and the external environment. Hydrolysis by enzymes is the most important mechanism for digestion in the small intestine. However, digestion also occurs by fermentation, an aspect of digestion most relevant to the large intestine of single-stomached animals (Ewing and Cole, 1994). The principal physiological functions of the large intestine have been considered to be the conservation of water and electrolytes (Dobbins and Binder, 1981; Ramakrishna *et al.* 1990), provision of a controllable route for the excretion of waste products of metabolism and toxic substances, and safe containment of the microorganisms present (Cummings, 1983). However, Bugaut (1987) included the salvage of energy and nutrients through its symbiotic relationship with the GIT microflora.

The carnivorous sparrow-hawk (*Accipiter nisus*) has small ceca, while its nocturnal counterpart, the barn owl (*Tyto alba*), has well-developed sacculate ceca. However, McLelland (1989) reported the absence of this organ in piciformes, apodiformes, corcaiiformes, columbiformes, coliiformes, cuculiformes and psittaciformes, although some of the species from these families (i.e. wood pigeon) consume fibrous diets .

2.10.1 Developmental features

It remains closed, by a cellular plug which originates on day 5 or 6 of incubation in chicks up to day 17 of incubation (Daniel, 1957).

2.10.2 Gross features

The large intestine consists of two ceca and the colorectum (McLeod *et al*., 1964). The cecum is an intestinal appendage in the midgut at the junction of the small and large intestine (Burns *et al*., 2004). Ceca are present in all domestic birds, but in some wild species they are missing (McLelland, 1989). The shape, size and capacity of this organ varies amongst different kinds of birds due to their evolutionary adaptation (Redig, 1989). The colorectum is the terminal segment of the alimentary canal. It is located in the dorsal part of the abdominal cavity and extends from the cranial part of the cavity to the cloaca. It is about 3 to 4 inches long and has a variable diameter, which appears to be influenced by the amount of content (McLeod *et al*., 1964).

2.10.3 Histological features

The wall of the colorectum is made up of tunica serosa, muscularis, submucosa, muscularis mucosa and mucosa. The serosa is a thin layer composed of simple squamous epithelium with flattened nuclei. The muscularis is made up of two muscle layers; an outer thin longitudinal and inner thick circular one. The submucosa consists of loose connective tissue holding blood vessels. The muscularis mucosa is composed of longitudinal muscle fibers. This layer extends inside the mucosal folds as vertical muscle fiber strands. The mucosa is thrown into numerous leaf-like villi, all covered by simple columnar epithelium containing goblet cells. The goblet cells are numerous in number and open into the lumen. At the base of the mucosal folds, rectal glands (simple tubular) are noticed. These glands are in crypts as in the small intestine, lined with simple columnar epithelium and goblet cells (Mostafa *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Egg Source and Preparation for Incubation.

One hundred and seventy-five (175) fertilized guinea fowl eggs were purchased from National Veterinary Research Institute (NVRI),Vom, Plateau State , Nigeria. The eggs were incubated at Dhenab hatchery, Angul-D, Jos, Plateau State. Before incubation, the eggs were first cleansed with cotton wool wetted with tap water and allowed to dry at normal room temperature. They were then set in automatic electrical incubator (ASE EURO, Belgium); a twenty thousand (20,000) capacity maintaining a temperature and relative humidity of 37.7^{0} C and 60-70 %, respectively.

3.2 Pre-hatch Study of the Embryos

Starting from day one **(**1) of incubation, five (5) eggs were picked at random from the incubator. The removal of the embryos from the eggs was done according to the method described by Salami (2009). The shells of the eggs were cracked at the broad end with a forcep to create an opening of approximately one (1) inch in diameter. The outer and inner shell membranes were removed using a small pointed-end scissors. The cracked eggs were preserved in 10% formalin for 1 week to arrest further development of the embryo and harden the egg content for easier handling and manipulation. The eggshells were removed and the embryos were exposed from under the shell membrane and placed on top of the yolk. With blunt forceps the embryos and the adherent extra-embryonic membranes were pulled away from the yolk and albumen. The extra-embryonic membranes were removed and the umbilical stalks were severed close to the body wall. The harvested embryos were rinsed thoroughly under running tap water and then preserved in 10 % formalin ready for use.

3.3 Post-hatch Study of Keets

Forty (40) hatched keets out of the one hundred and seventy-five (175) fertilized eggs were used for the post-hatch study for the period of eight (8) days. Five (5) keets were picked at random and sacrificed daily. The keets were euthanized with 0.1ml of phenobarbitone 200mg / ml via jugular vein (Igwebuike and Eze, 2010).

3.4 Gastrointestinal Tract Harvest

The gastrointestinal tracts of harvested embryos and keets were removed through the ventral incision into the thoraco-abdominal cavity. The gastrointestinal tract was carefully detached from the adhering structures and organs and brought out intact for further studies.

3.4.1 Morphological studies

Harvested gastrointestinal tract from the embryos and keets were carefully studied. Each segment of the gastrointestinal tract was identified with the aid of magnifying lens. Photographs of all the segments of the gastrointestinal tract were taken as soon as they were identified.

3.4.2 Morphometric studies

Harvested gastrointestinal tract from the embryos and keets were weighed at each point of collection with digital weighing balance. The lengths of each segment of the gastrointestinal tracts were also measured using a calibrated ruler. This was done by placing the calibrated side of the measuring ruler at the entire length of the segment (Shih *et al.*, 2005). All the measurements obtained were recorded in centimetre (cm).

3.4.3 Histological studies

Specimen samples were collected from each part or segments of the gastrointestinal tract for sectioning and histological studies. Samples taken were immediately fixed in 10% formalin where they were kept for two (2) days. The fixed tissues were dehydrated through a series of graded alcohol (70%, 80%, 90%, 95%, and 100%). They were cleared in xylene and infiltrated in molten paraffin wax. Transverse sections of 5µ thick were cut from the embedded tissue using disposable microtome knives. These sections were mounted on clean and grease free slides and were also stained at room temperature using Haematoxylin and Eosin (H and E) (Lee, 1968).

3.5 Microscopy and Photomicrography

The prepared slides were viewed under a binocular light microscope with a digital camera (AmScope, with resolution of 5 mega pixels) fitted to the eye piece of the binocular light microscope and adjusted at desired magnification and photomicrographs of the histological features taken.

3.6 Statistical Analysis

All data were analyzed using the Graphpad prism version 5.0 for windows (2008). Morphometry was expressed as Mean ± SEM. Correlation was used to determine the relationship between weight of embryo/keet, gastrointestinal tract and length of the gastrointestinal tract during pre-hatch and post hatch periods.

CHAPTER FOUR

RESULT

4.1 Morphology

4.1.1 Pre-hatch and post-hatch

The guinea fowl embryo showed no clear morphological development of gastrointestinal tract from days 1-7 of development. By day 8 of development there appeared a straight tube with a roundish structure at almost the centre, thus presenting what later developed into the oesophagus, ventriculus and intestine craniocaudally (Plate I). These structures increased gradually in length and size till day 10 of development when a spindle-like structure proximal to the roundish ventriculus appeared as proventriculus and also a tiny out pocket lateral to the caudal straight tube appeared as one of the two caeca. Caudal to the caecum is a short tube representing colorectum (Plate II). Thus, the proventriculus formed was as a result of dilation of the lower part of the proximal tube while the distal tube can at this stage of development be caecum and colorectum. At day 11 of development, an outpouch at the cranial part of the proximal tube appeared as the crop thereby dividing the oesophagus into the pre crop oesophagus (cervical oesophagus) and post crop oesophagus (thoracic oesophagus), respectively (Plate III). At day 12 of development, the second caeca appeared at the opposite side of the first (Plate IV). The duodenum which is the proximal part of the small intestine became apparent at day 13 of development. This part of the intestine became identifiable as a result of the tube having a U-shape (Plate V). It was also at this stage of development that the second caecum became conspicuous. Hence the two caeca now became clearly seen at both sides of the intestine. Thus, with the appearance of caeca at day 13 of development the entire segments of the GIT of guinea fowl were formed. This revealed that development of the guinea fowl GIT took six (6) days to be completed.

The gastrointestinal tract of guinea fowl at post-hatch showed no additional structural appearance except increase in size, length and weight of the formed structures. At post-hatch, the parts of the gastrointestinal tract took their normal position and location as found in the adult. The oesophagus of guinea fowl appeared at the right side of the neck. It appeared as cervical and thoracic oesophagus separated by an outpouch called crop (Plate VI) a diverticulum of cervical oesophagus.

The stomach of guinea fowl has two portions; the glandular part known as proventriculus and the muscular part known as the ventriculus or gizzard separated by isthmus gastris. The inner aspect of the ventriculus appeared to be lined by a hard structure called koilin or cuticular layer appearing partially green while that of proventriculus is by raised papillae (Plate VII). The guinea fowl has a small intestine in the abdominal cavity. It is a longitudinal tube that continues distal to the pyloric end of ventriculus and ends proximal to the junction of caeca and colorectum. The small intestine of guinea fowl like any other bird is divided into duodenum, jejunum and ileum. The duodenum appeared as a slightly elongated loop separated by pancrease into proximal descending and distal ascending parts (Plate VIII). The jejunum and ileum appeared long and coiled. They were separated by yolk sac that gradually reduced to become a rudimentary body called Meckel’s diverticulum (Plates VI and VIII).Caeca of guinea fowl are elongated, paired blind sac at both sides of the intestinal tube. The caeca are distal to the ileum and proximal to the colorectum (Plate VIII).

The guinea fowl colorectum appeared as a short and straight tube extending from the distal part of the ileum and it opened distally into the cloaca (Plate VIII).

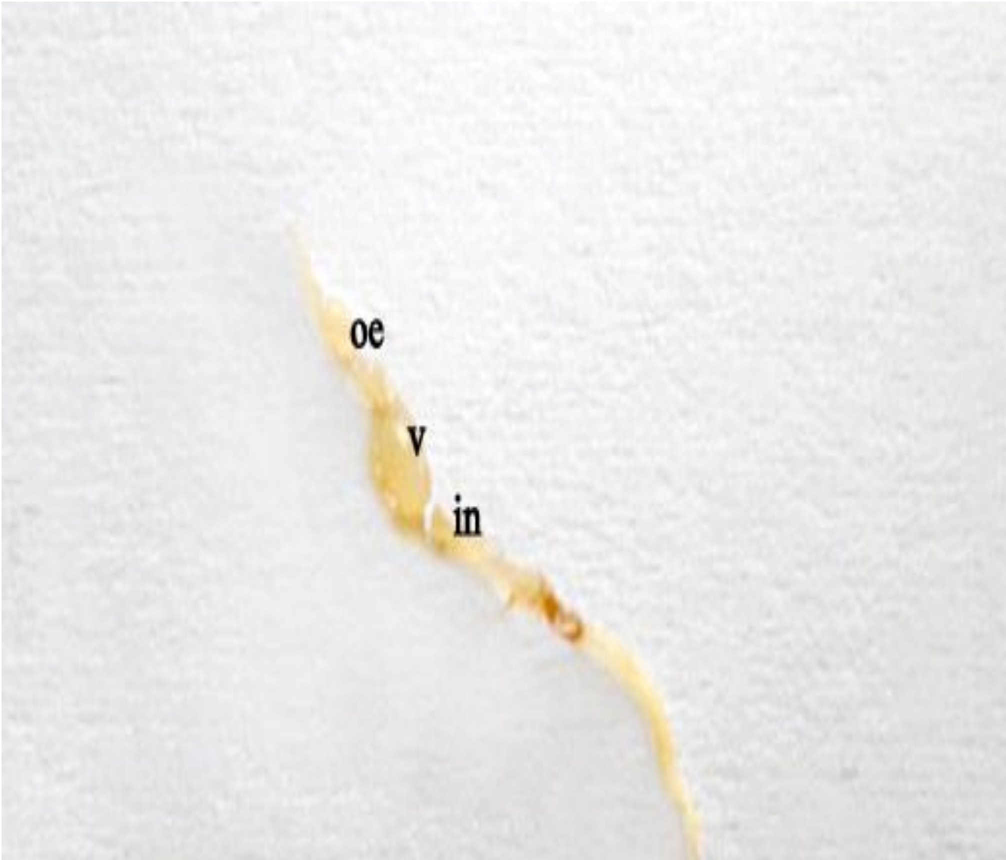

Plate I: Gastrointestinal tract of guinea fowl embryo at day 8 of development showing oesophagus (oe) ventriculus (v) and intestine (in).

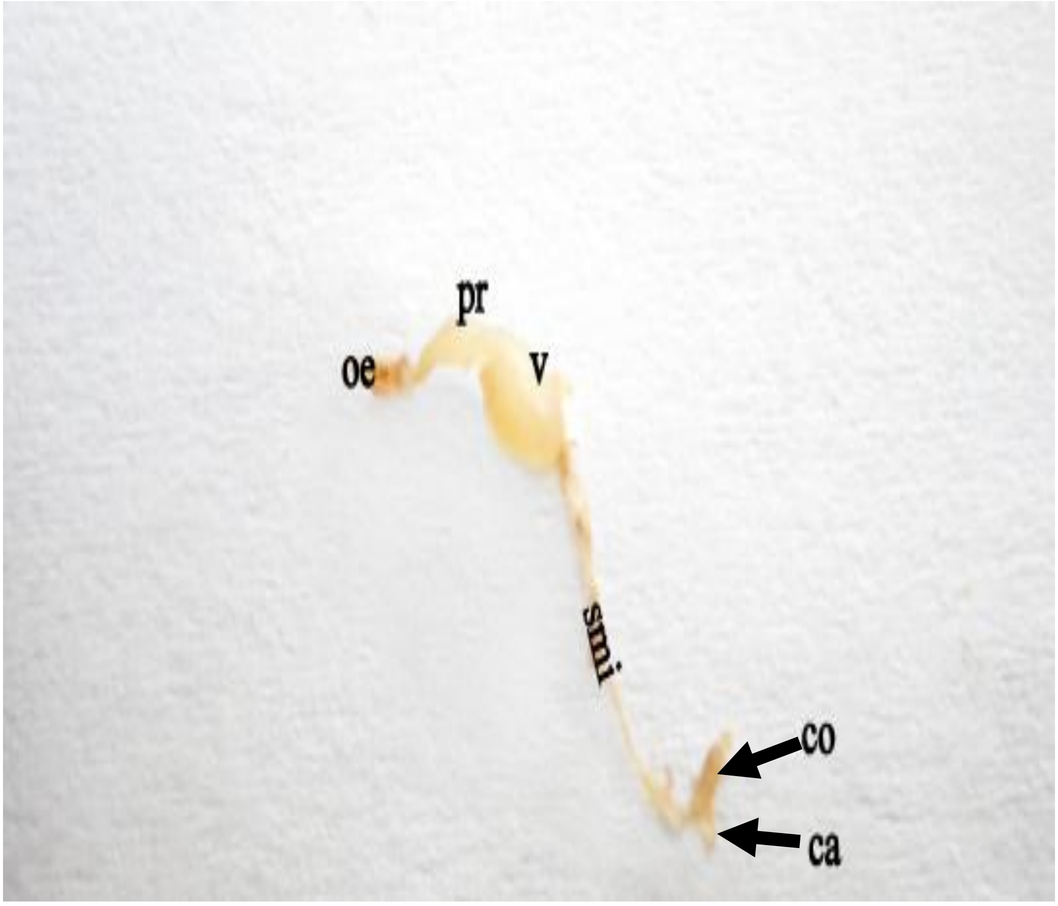

Plate II: Gastrointestinal tract of embryo at day 10 of development showing oesophagus (oe), ventriculus (v), proventriculus (pr), small intestine (smi), caecum (ca) and colorectum (co).

Plate III: Gastrointestinal tract at day 11 of development showing cervical oesophagus (coe), crop (cr) and thoracic oesophagus (toe).

Plate IV: Gastrointestinal tract at day 12 of development showing slight bulge, indicating the development of the second caecum (arrow).

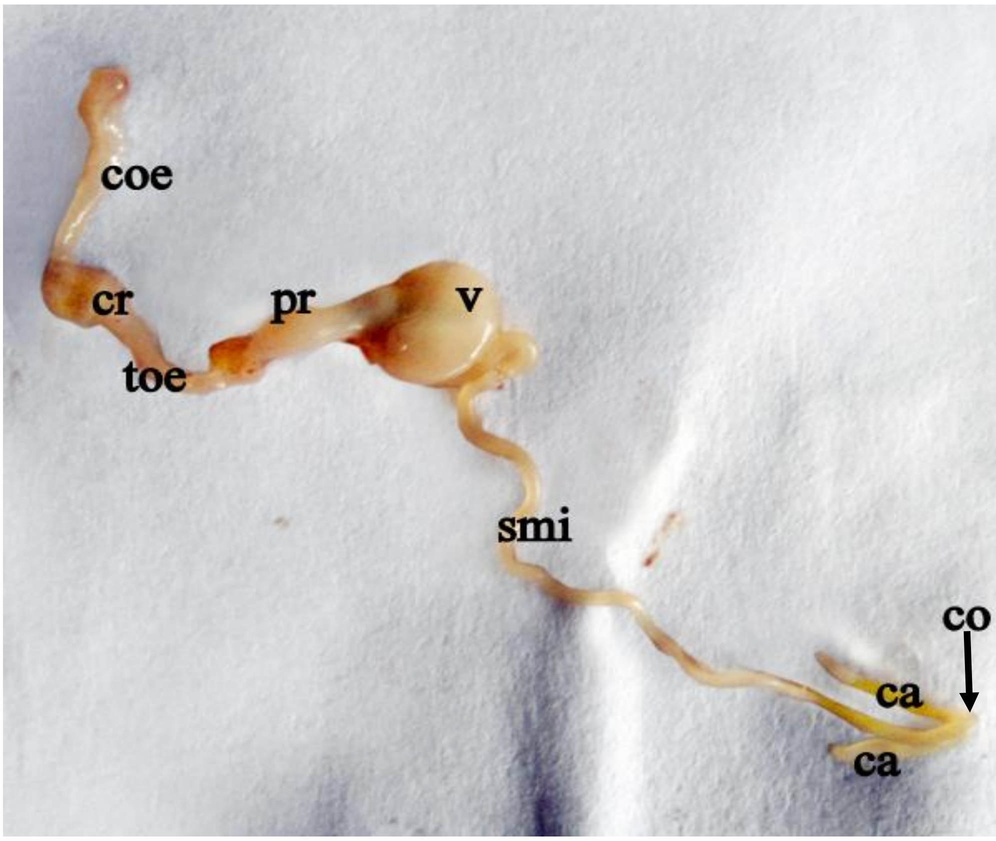

Plate V: Gastrointestinal tract at day 13 of development showing cervical oesophagus (coe), crop (cr), thoracic oesophagus (toe), proventriculus (pr), ventriculus (v), small intestine (smi), caeca (ca), colorectum (co) and the apparent duodenal loop (arrow).

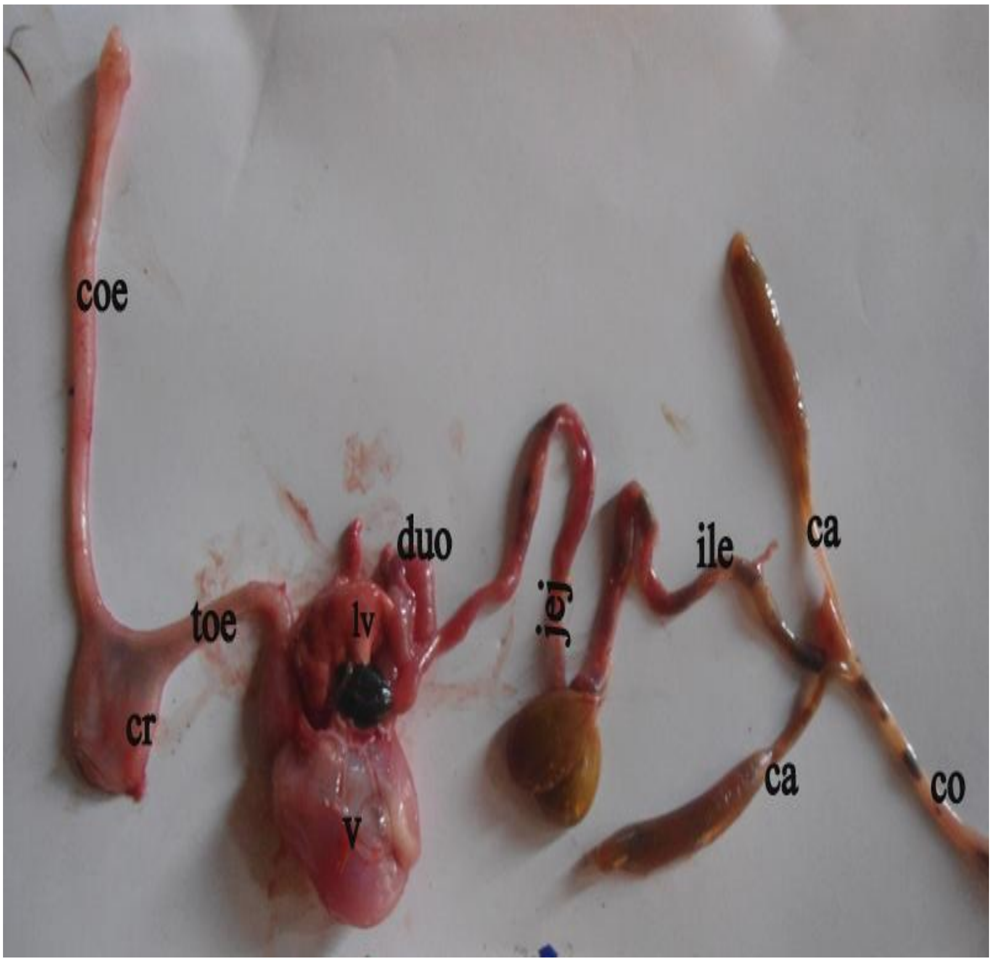

Plate VI: Gastrointestinal tract at day 1 post-hatch showing cervical oesophagus (coe), crop (cr), thoracic oesophagus (toe), proventriculus (pr), ventriculus (v), liver (lv), duodenum (duo), jejunum (jej), ileum (ile), caeca (ca), colorectum (co) and the apparent yolk sac (arrow).

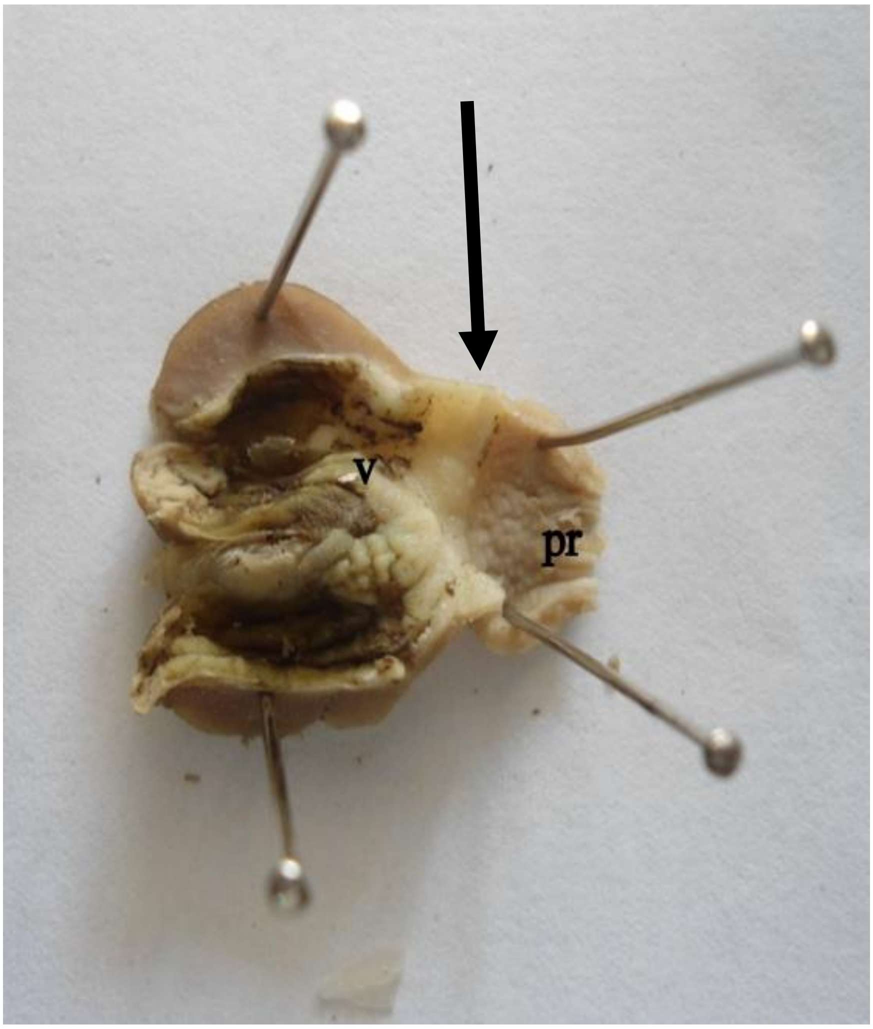

Plate VII: Cut open surface of proventriculus (pr) and ventriculus (v) separated by isthmus gastris (arrow).

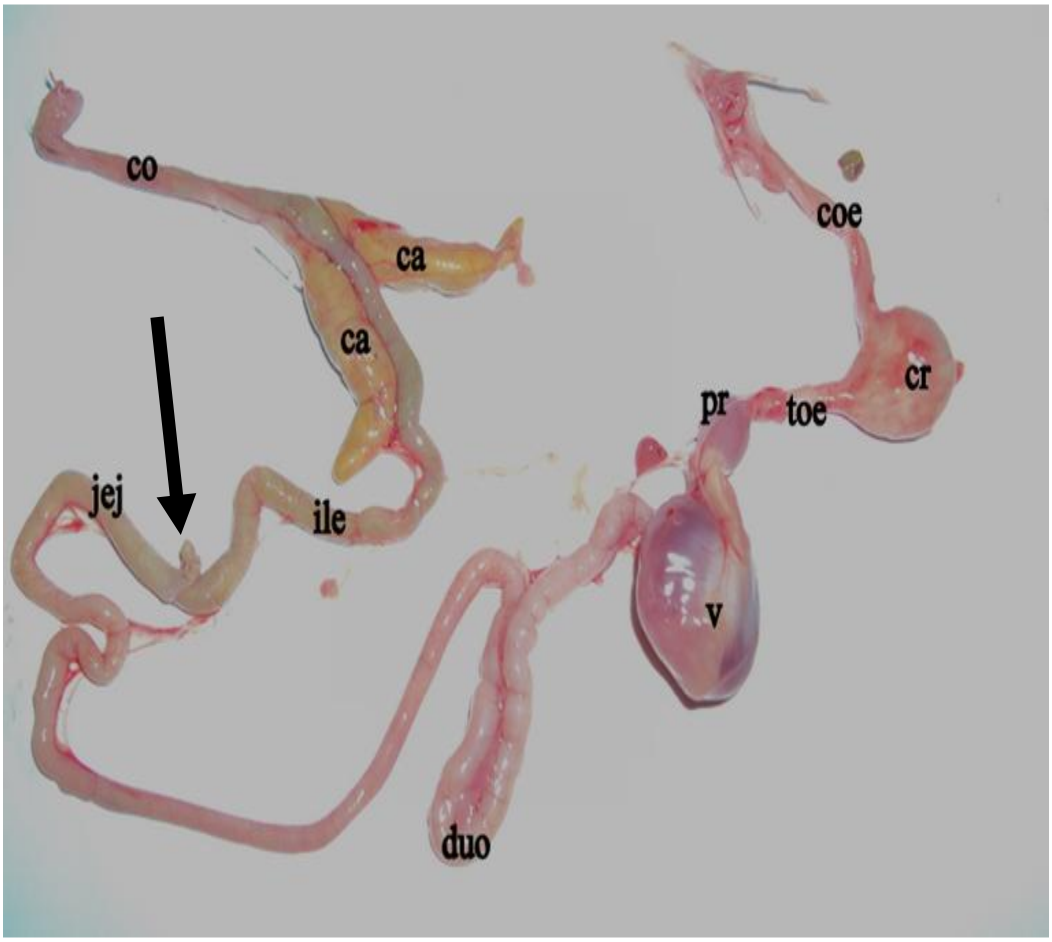

Plate VIII: Gastrointestinal tract at day 8 post-hatch showing complete morphological and functional GIT; cervical oesophagus (coe), crop (cr), thoracic oesophagus (toe), proventriculus (pr), ventriculus (v), duodenum (duo), jejenum (jej), ileum (ile), caeca (ca), colorectum (co) and vetillinum diverticulum (arrow).

4.2 Morphometry

4.2.1 Pre-hatch and post-hatch

The weights of embryos from days 1 to 6 were not measured because none of the gastrointestinal parts was recognizable. The relative weights of the gastrointestinal tract (GIT) of helmeted guinea fowl embryo were recorded in Table 4.1. It showed an unsteady increase of the embryonic GIT weight at different days of development. At days 9, 16, 21, and 25 there was a considerable decrease in the relative weight of the embryo accounting for 0.92%, 3.24%, 4.47% and 5.93%, respectively; this was further illustrated in Fig. 4.1.

Table 4.2 showed the relative weight of gastrointestinal tract of helmeted guinea fowl, post hatch. There was a progressive increase of the GIT weight from day 1 post hatch until at day 7 where it decreased considerably and then rose at the day 8. Fig. 4.2 illustrates the progressive increase and fall of gastrointestinal tract weight of helmeted guinea fowl.

The mean length of the different segments of gastrointestinal tract of helmeted guinea fowl at pre-hatch was recorded in Table 4.3. The increase and decrease in the length of all the segments were observed to be uniform; from day 8 to 10 of development, the whole segments increased progressively and then a decreased at day 11 of development. By day 12 of development, the length increased and maintained a steady increase daily till day 23 when it fell and then increased progressively again from day 24 till day 27. Fig. 4.3 illustrates the rise and fall in the mean length of the different segments of the gastrointestinal tract of helmeted guinea fowl at pre-hatch.

Table 4.4 showed the mean length of the different segments of GIT of helmeted guinea fowl at post-hatch. There was a progressive increase in length of the segments from day 1 to day 6 post-hatch and a sudden decrease at day 7 before increasing at day 8.

At pre-hatch, there was a positive correlation between the body weight (BWT) and gastrointestinal weight (GWT) ($r = 0.42$); between body weight (BWT) and length of gastrointestinal tract (GLT) ($r = 0.962$); and between weight of gastrointestinal tract and length of gastrointestinal tract ($r = 0.954$) (Table 4.3). At post-hatch, there was also a positive correlation between the body weight (BWT) and gastrointestinal weight (GWT) ($r = 0.697$); between body weight (BWT) and length of gastrointestinal tract (GLT) ($r = 0.597$); and between weight of gastrointestinal tract (GWT) and length of gastrointestinal tract (GLT) ($r = 0.849$) (Table 4.5).

There was a significant difference between the body weight (BWT) and gastrointestinal weight (GWT) ($P < 0.001$), between body weight (BWT) and length of gastrointestinal tract (GLT) ($P < 0.001$) and between weight of gastrointestinal tract (GWT) and length of gastrointestinal tract (GLT) ($P < 0.001$) (Table 4.5).

Table 4.1 Relative weight (%) of gastrointestinal tract of guinea fowl embryos during days 8-27 of pre-hatch period

Days	Relative Weight (%)
8	1.37
9	0.92
10	1.20
11	1.21
12	2.36
13	2.57
14	2.96
15	3.58
16	3.24
17	2.81
18	3.20
19	3.29
20	3.90
21	4.47
22	4.15
23	4.69
24	4.94
25	5.93
26	5.23
27	7.35

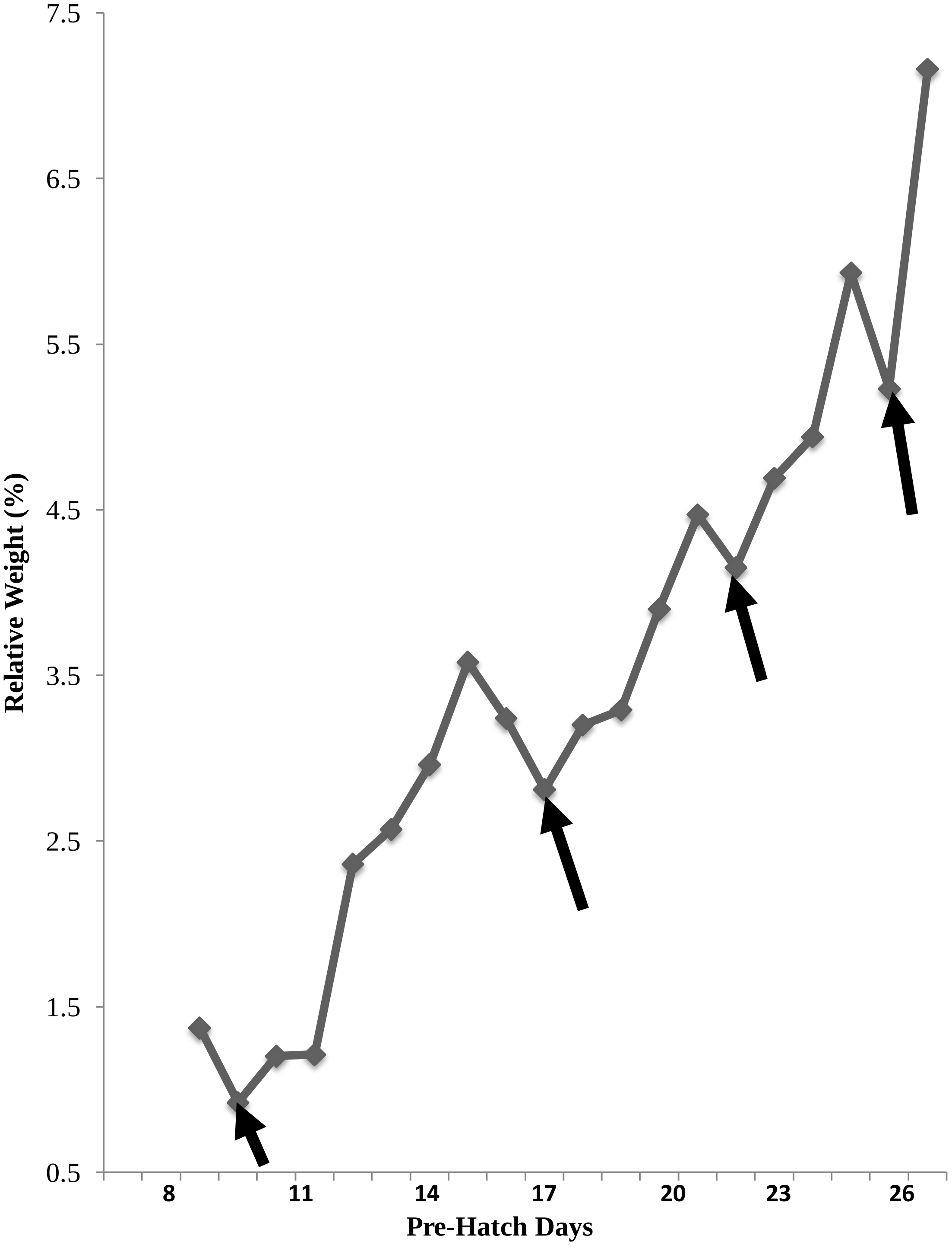

Figure 4.1 Relative weight (%) of the GIT of Guinea Fowl Embryos during pre-hatch period.

Table 4.2 Relative weight (%) of gastrointestinal tract of guinea fowl keets during days 1-8 of post-hatch period

Days	Relative weight (%)
1	10.83
2	13.11
3	17.20
4	18.00
5	20.10
6	23.16
7	21.80
8	31.03

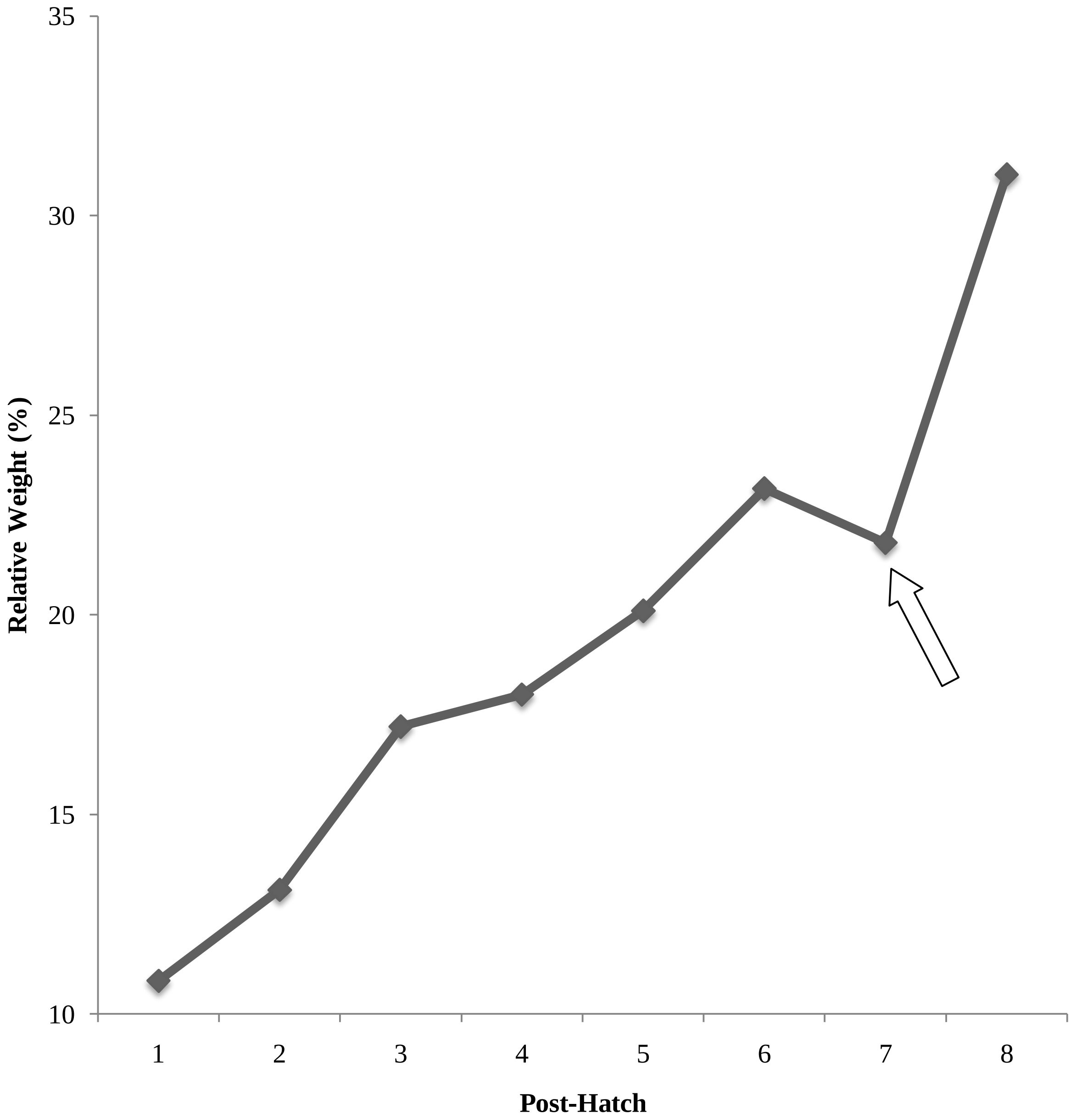

Figure 4.2 Relative weight (%) of the GIT of Guinea Fowl Keets during Post-hatch.

Table 4.3 Mean length (cm) of gastrointestinal tract of guinea fowl embryos during days 8-27 of pre-hatch period.

Days	Cer. Oes (cm)	Tho. Oes (cm)	Provent. (cm)	Ventricu. (cm)	Small Int. (cm)	Caeca (cm)	Colorect. (cm)	Total GIT Length (cm)
8	0.12	-	-	0.14	0.08	-	0.08	0.42
9	0.19	-	-	0.18	0.27	-	0.27	0.91
10	0.17	-	0.25	0.20	0.34	-	0.34	1.30
11	0.14	0.06	0.25	0.25	0.37	0.13	0.22	1.22
12	0.65	0.15	0.33	0.43	1.50	0.23	1.00	4.29
13	0.66	0.29	0.33	0.43	1.60	0.26	0.45	4.02
14	0.78	0.61	0.42	0.53	1.90	0.38	0.52	5.15
15	1.20	0.84	0.53	0.66	3.60	0.68	0.88	8.39
16	1.60	0.96	0.63	0.80	5.80	0.82	0.94	11.55
17	1.60	0.94	0.76	0.90	6.10	0.90	0.95	12.15
18	2.00	1.10	1.00	1.00	7.40	1.30	1.10	14.90
19	2.10	1.10	1.20	1.00	9.50	1.40	1.20	17.50
20	2.20	1.10	1.20	1.20	11.00	1.90	1.30	19.90
21	2.40	1.60	1.60	1.30	11.00	1.80	1.30	21.00
22	2.50	1.70	1.80	1.50	12.00	2.00	1.50	23.00
23	2.30	1.70	1.80	1.70	12.00	2.10	0.70	22.30
24	2.60	1.70	1.80	1.80	13.00	2.00	2.00	24.90
25	2.80	1.90	1.80	1.80	13.00	2.10	2.30	26.00
26	2.90	1.80	1.80	1.80	13.00	2.10	2.60	26.30
27	3.40	1.80	1.80	2.00	14.00	2.10	2.60	27.70

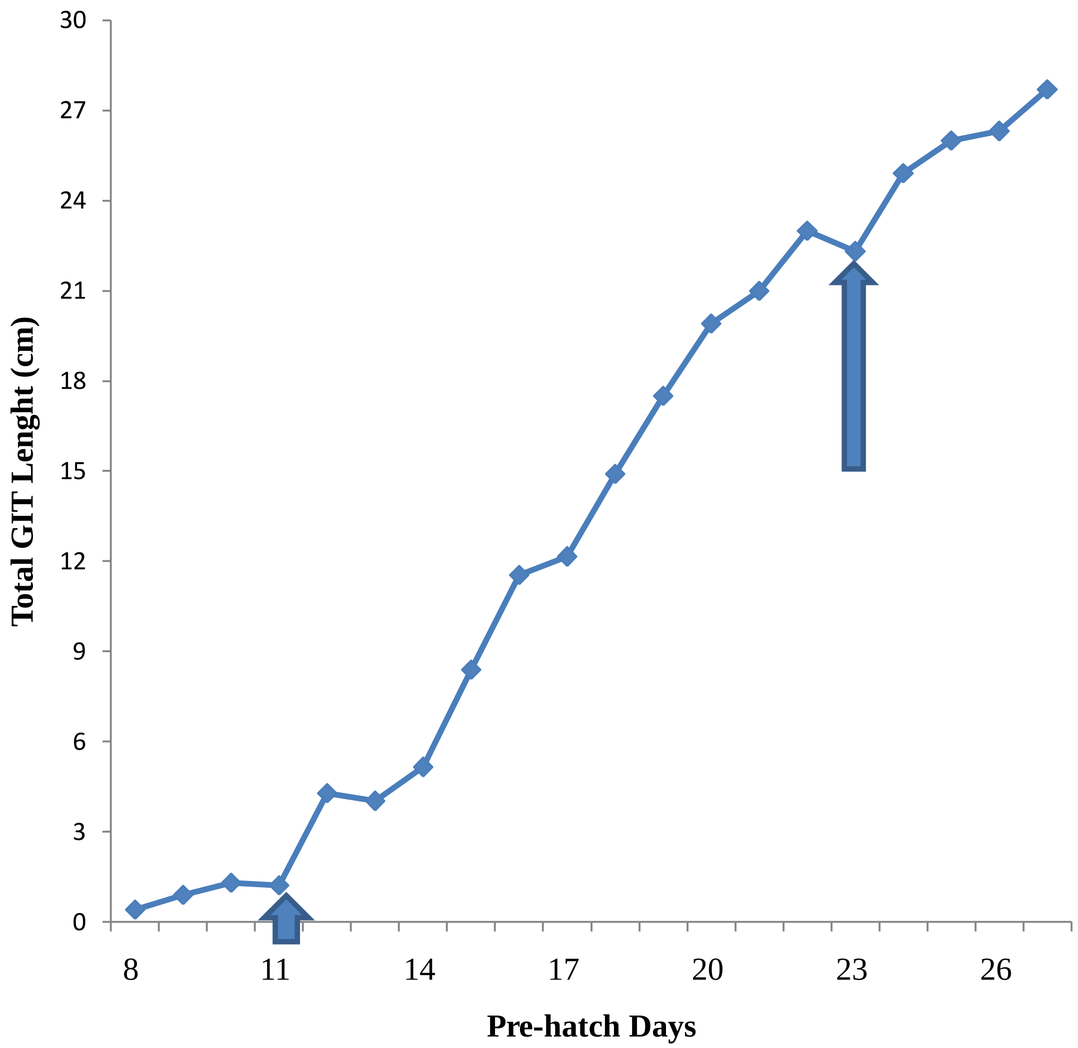

Figure 4.3 Mean Length (cm) of GIT of Guinea Fowl Embryos during Days 8-27 of Pre-hatch Period.

Table 4.4 Mean Length (cm) of gastrointestinal tract of guinea fowl keet during days 1-8 of post-hatch period

Days	Cer. Oes (cm)	Tho. Oes (cm)	Provent. (cm)	Ventricu. (cm)	Small Int. (cm)	Caeca (cm)	Colorect. (cm)	Total GIT Length (cm)
1	6.20	2.20	2.10	1.70	22.00	4.10	3.40	31.20
2	5.40	2.10	2.10	1.90	21.00	3.60	3.90	40.00
3	6.50	2.30	2.10	1.90	22.00	3.90	3.60	42.30
4	6.50	2.30	2.20	2.20	23.00	4.00	3.50	43.70
5	6.40	2.30	2.20	2.20	30.00	4.00	3.60	50.70
6	6.70	2.50	2.40	2.20	33.00	4.40	3.60	54.80
7	5.70	2.30	2.30	2.20	32.00	4.80	3.70	53.00
8	6.40	3.10	2.50	2.60	33.00	5.30	3.90	56.80

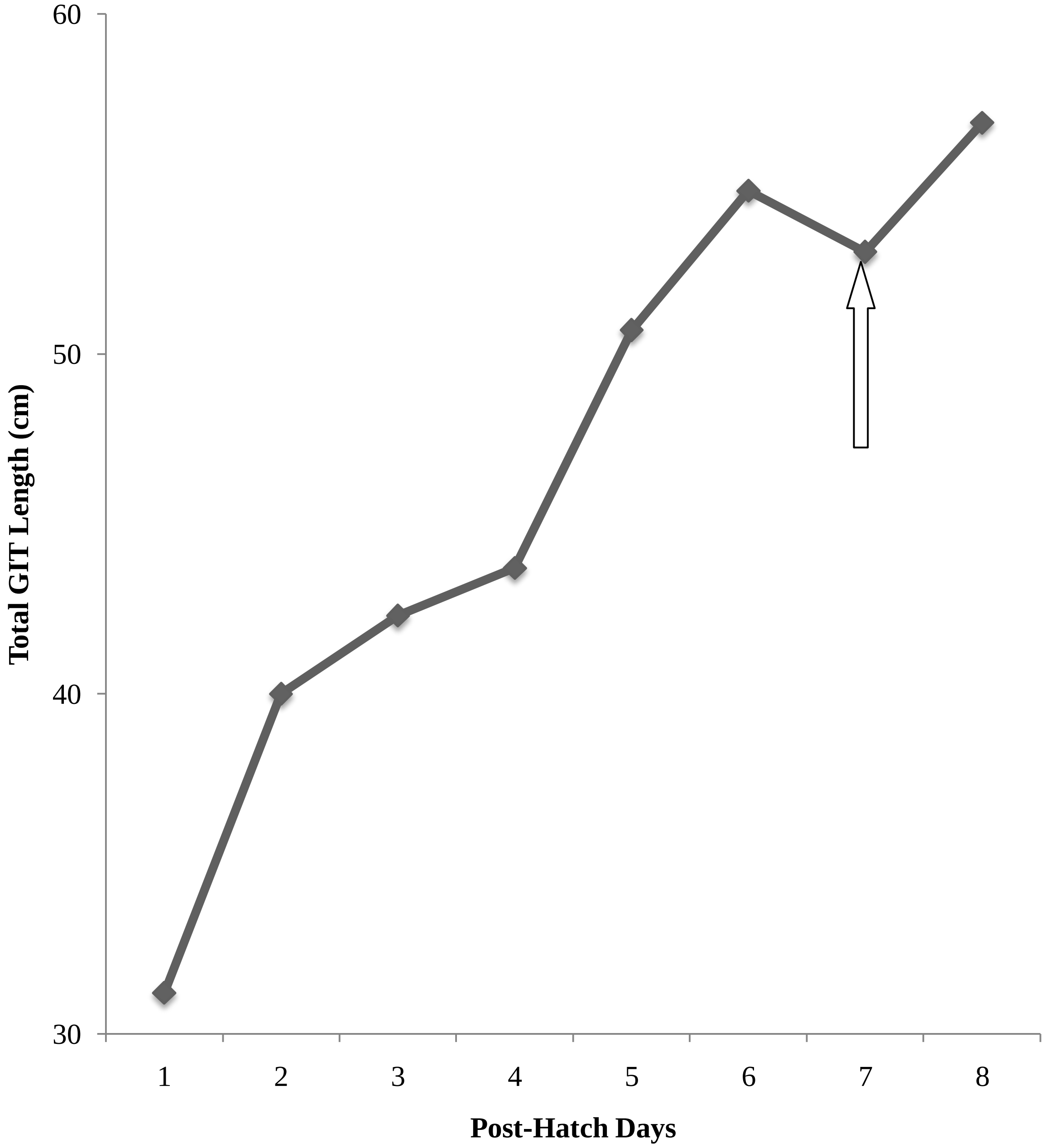

Figure 4.4 Mean length (cm) of Gastrointestinal Tract of Guinea Fowl Keet during days 1-8 of post-hatch period.

Table 4.5 Coefficient of correlation between parameters of guinea fowl during, pre-hatch and post hatch periods

Correlation parameter	Pre-hatch	Post-hatch	
	Correlation Values	Correlation Values	P value
BWT vs GWT	0.42	0.697	0.001
BWT vs GLT	0.962	0.597	0.001
GWT vs GLT	0.954	0.849	0.001

BWT = Body Weight

GWT = Gastrointestinal tract Weight

GLT = Gastrointestinal tract Length

Vs = Versus

4.3 Histology

4.3.1 Pre-hatch and post-hatch

The general histological features of the entire embryonic gastrointestinal tract of guinea fowl showed progressive development of the four typical layers of a tubular organ i.e tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa from inward.

The cervical oesophagus and the crop were observed to have similar histogenesis. These segments were observed at day 8 of development to be lined by simple columnar epithelium surrounded by mesenchymal cells (Plate IX). The longitudinal folds were observed to project into the lumen of cervical oesophaguson day 12 (Plate X). By day 19 of development, the epithelium had changed to stratifed squamous and also the cells hadbegun differentiating into submucosa and muscular layer (Plate XI). By day 27 of of development, the four typical histological layers of a tubular organ have matured (Plate XII) . At day 1 post-hatch, the histological features were similar except that the features had increased in size (Plates XIII).

The histogenesis observed in the thoracic oesophagus and crop of the helmeted guinea fowl was similar (Plates XIV to XXII) with the exception of the presence of mucus gland in the crop (XXII).

At day 10, the proventriculus was lined by stratified squamous epithelium surrounded by mesenchymal cells and betwen the mesenchymal cells were the primordial proventricular glands (Plate XXIII). Numerous primordial proventricular glands emerged by day 13 of development (Plate XXIV). By day 24, proventricular glands were compound tubuloalveolar and the muscular layer was apparent (Plate XXV). By day 27, the proventricular glands were separated by a tiny sheet of connective tissue septa, thus bringing the glands very close to each other (Plate XXVI). The histological features by day 27 of development and that of post-hatch period were similar, except that number of lobules of the glands was numerous; the glandular cells were prominent and the connective tissue septa was very thin (Plate XXVII).

At days 8-11 of development, the histological features of the ventriculus were similar with those of other segments of GIT; the lumen was lined with stratified squamous epithelium and surrounded by laminal propria and mesenchymal cells (Plate XXVIII). Tubular glands were observed on day 13 (Plate XXIX) and by day 23, a muscular layer had replaced the mesenchymal cells (Plate XXX). Keratinoid was observed at day 27 and also ventricular cells were apparent (Plates XXXI and XXXII). At day 1 post-hatch, the size and number of the tubular glands, ventricular cells were increased (Plate XXXIII).

The small intestine at pre-hatchhad an invaginated lumen lined by pseudostratified columnar epithelium surrounded by mesenchymal cells (Plate XXXIV). The villi were observed to erupt from the epithelium appearing thick and short while underlying connective tissue became differentiated into submucosa, muscular and serosal layers at day 13 of development (Plate XXXV). The villi were lined by simple columnar epithelium at day 19 of development (Plate XXXVI). The villi looked more organized, with their shapes appearing finger-like at day 24 (Plate XXXVII). At post-hatch, there was a rapid increase in number of villi; the height and width equally increased progressively; while the goblet cells and the crypts of Liberkuhm appeared at post-hatch (Plate XXXVIII).

The caeca at pre-hatch to had a starred shaped lumina surrounded by pseudostratified epithelium and mesenchymal cells (Plate XXXIX). By day 24, the villi had become branched with crypts (Plate XL). At day 8 post-hatch, the goblet cells were observed (Plate XLI).

The colorectum was lined by low columnar epithelium and mesenchymal cells by day 11 of development (Plate XLII). At day 19, numerous villi lined with simple columnar epithelium were observed. The submucosa, muscular layer and serosa were also observed by day 19 (Plate XLIII).At post-hatch,the villi were branched with underlying goblet cells and crypts of Liberkuhm (Plate XLIV).

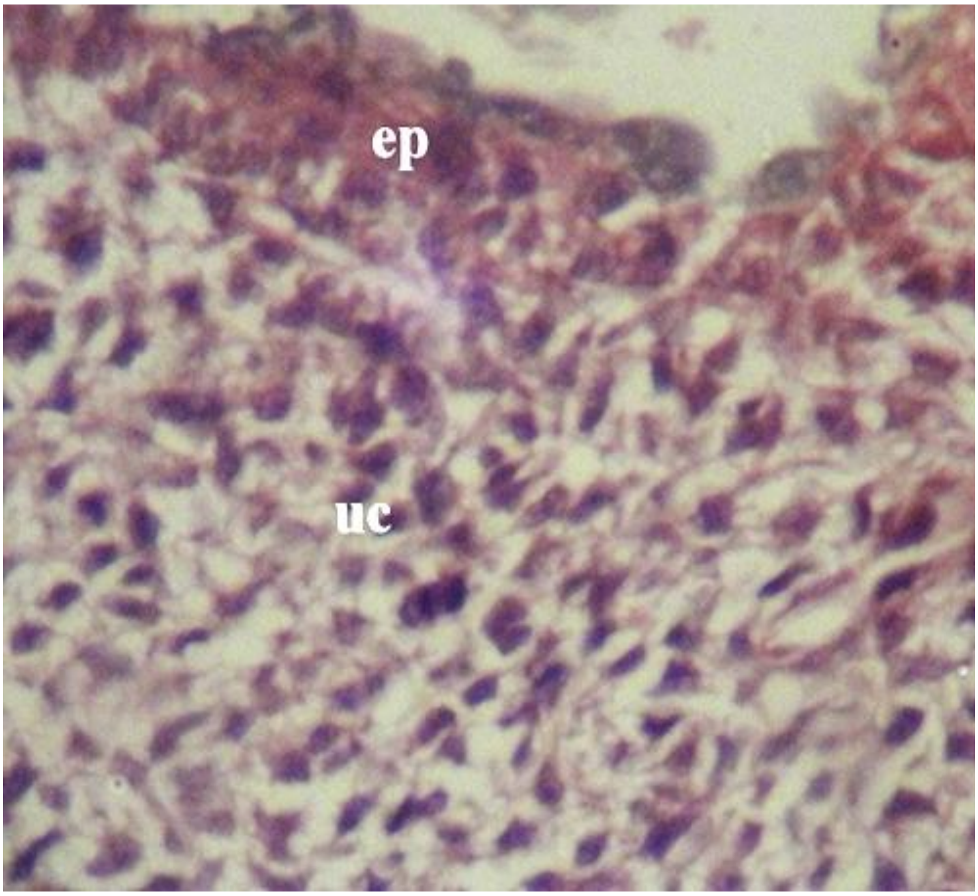

Plate IX. Transverse section of oesophagus at day 8 of development showing simple columnar epithelium (ep) surrounded by mesenchymal cells (uc). H and E x 400.

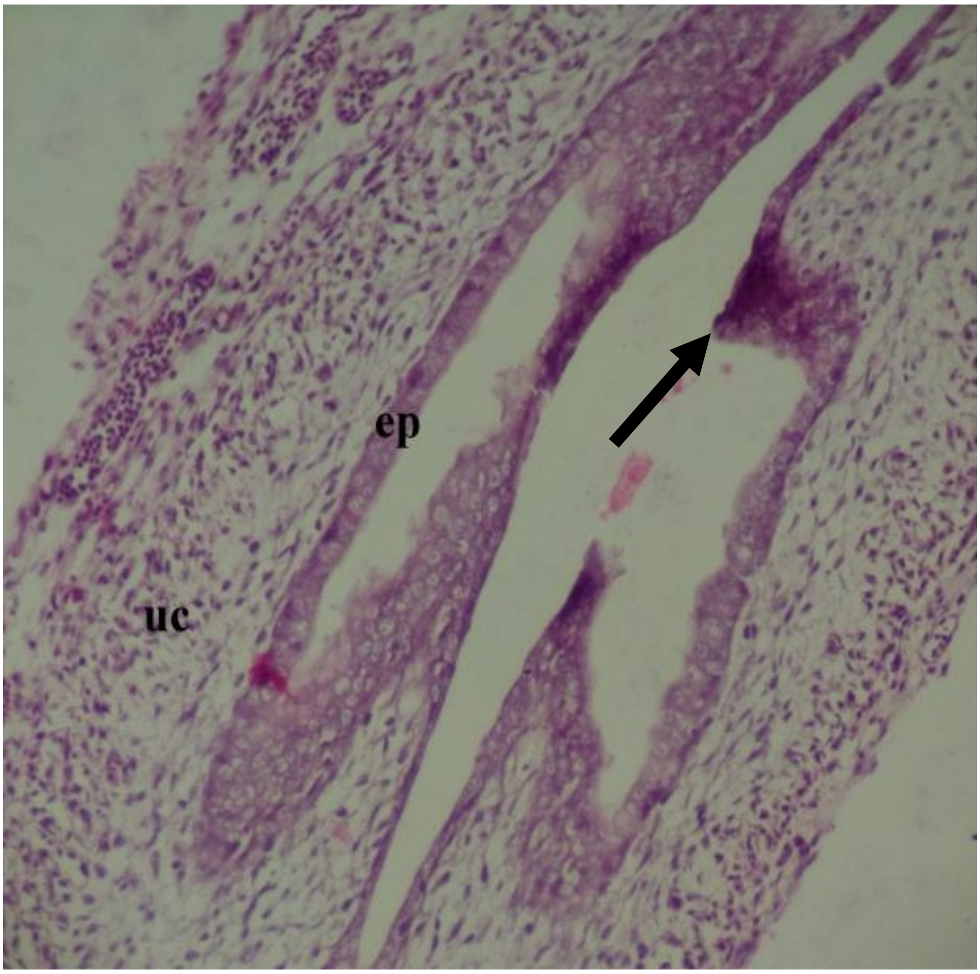

Plate X. Transverse section of cervical oesophagus at day 12 of development showing simple columnar epithelium (ep) surrounded by mesenchymal cells (uc) and appearance of longitudinal folds (arrow). H and E x 250.

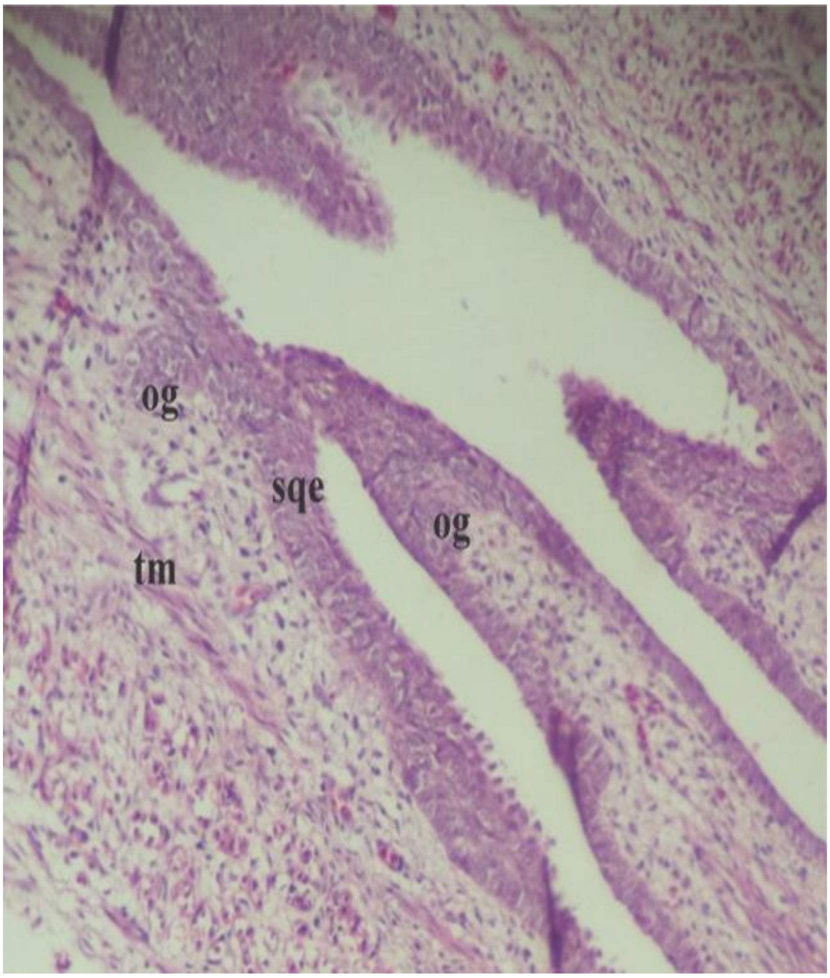

Plate XI. Tranverse section of cervical oesophagus at day 19 of development showing stratified squamous epithelium (sqe), oesophageal gland (og), tunica submucosa (ts) and tunica muscularis (tm). H and E x 100.

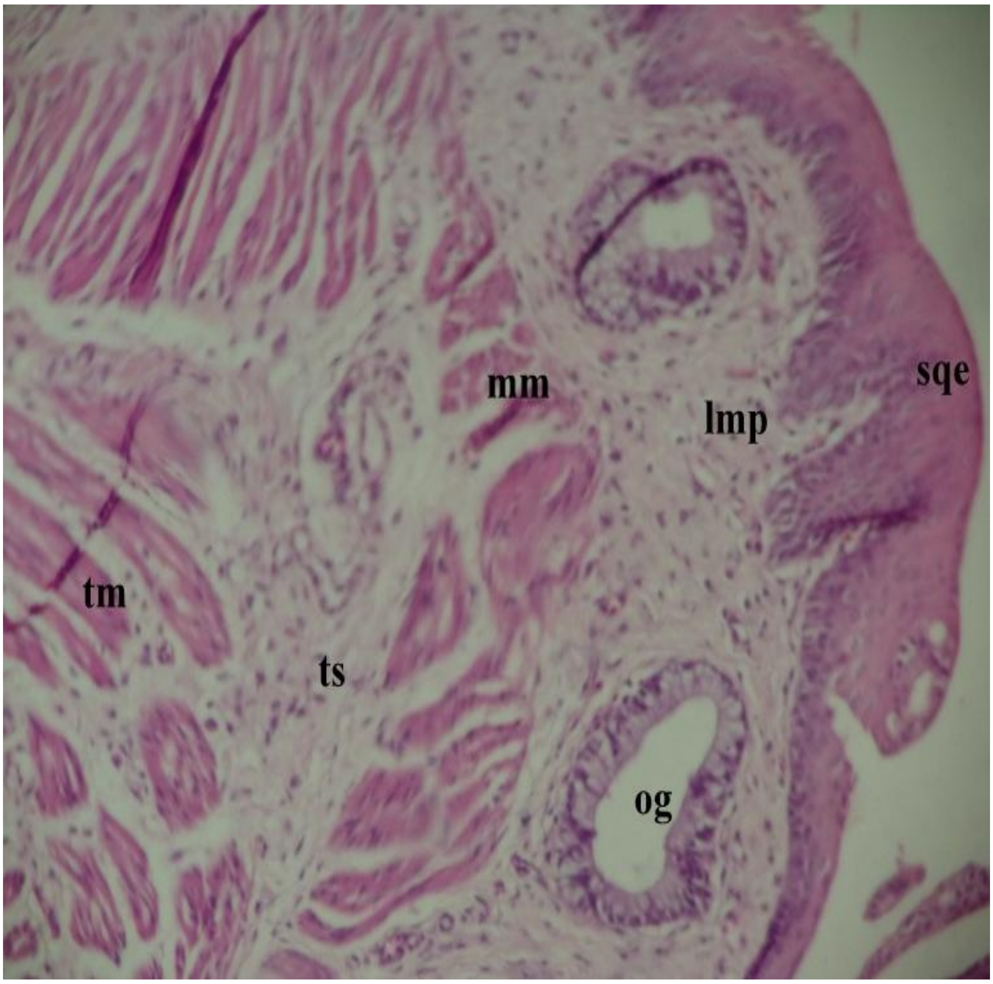

Plate XII. Transverse section of cervical oesophagus at day 27 of development showing stratified squamous epithelium (sqe), oeosophageal glands (og), lamina propria (lmp), muscularis mucosa (mm), tunica submucosa (ts), tunica muscularis (tm). H and E x100.

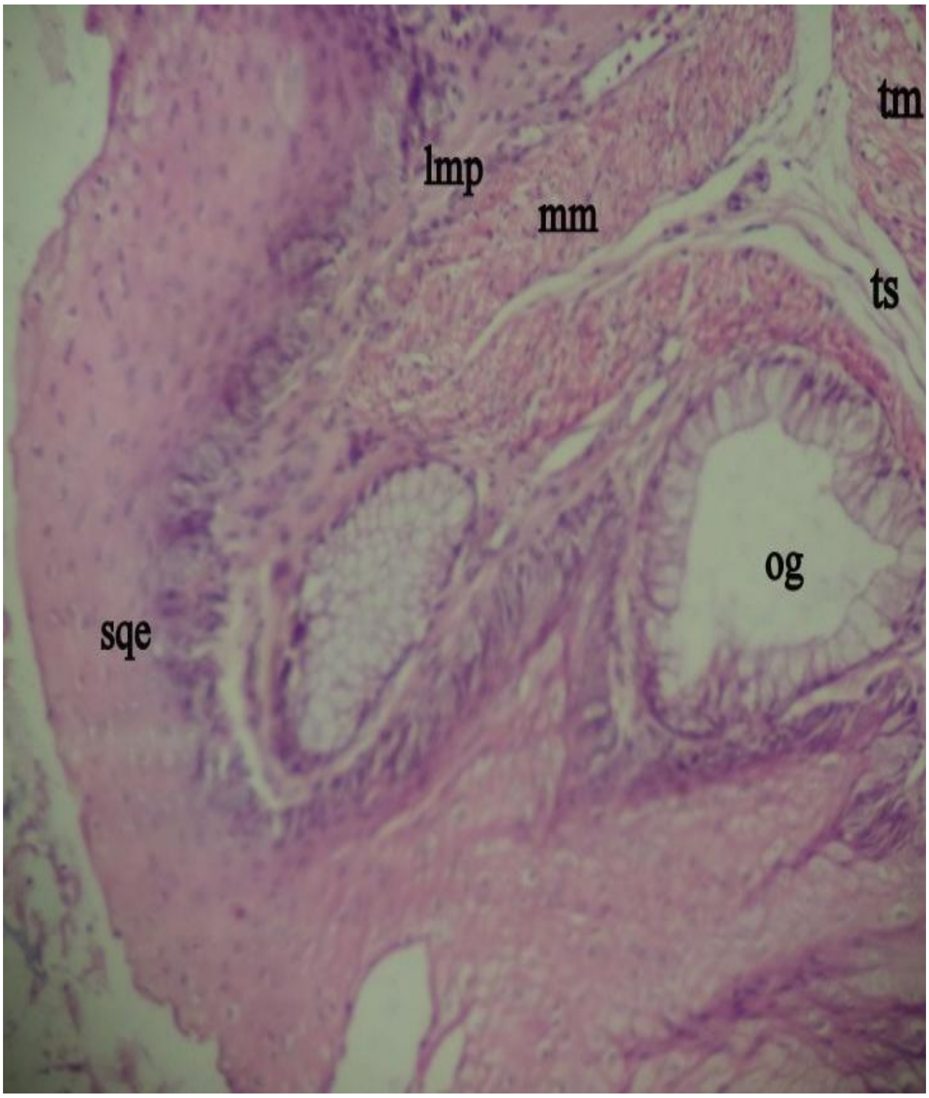

Plate XIII. Transverse section of cervical oesophagus at day 8 post hatch showing stratified squamous epithelium (sqe), oeosphageal gland (og), muscularis mucosa (mm),oesophageal gland (og) and tunica submucosa (ts). H and E x 100.

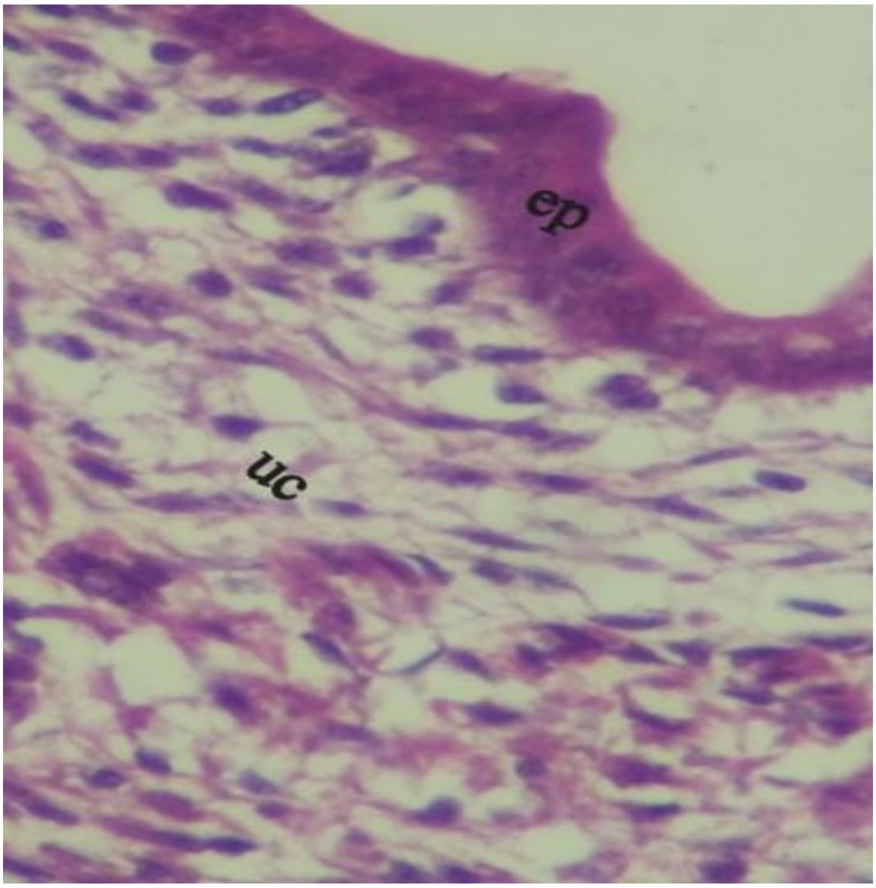

Plate XIV. Transverse section of thoracic oesophagus at day 11 of development showing simple columnar epithelium (ep) surrounded by mesenchymal cells (un). H and E x 400.

Plate XV. Transverse section of thoracic oesophagus at day 19 of development showing stratified epithelium (sqe), oesophageal gland (og), tunica submucosa (ts), tunica muscularis (tm) and tunica serosa (ts). H and E x 100.

Plate XVI. Transverse section of thoracic oesophagus at day 27 of development showing stratified squamous epithelium (sqe), oesophageal gland (og), muscularis mucosa (mm), tunica submucosa (ts), tunica muscularis(tm) and tunica serosa (ts). H and E x 250.

Plate XVII. Transverse section of thoracic oesophagus at day 8 post hatch showing stratified squamous epithelium (sqe), oesophageal gland (og), muscularis mucosa (mm), tunica submucosa (ts) andtunica muscularis (tm).H and E x 250.

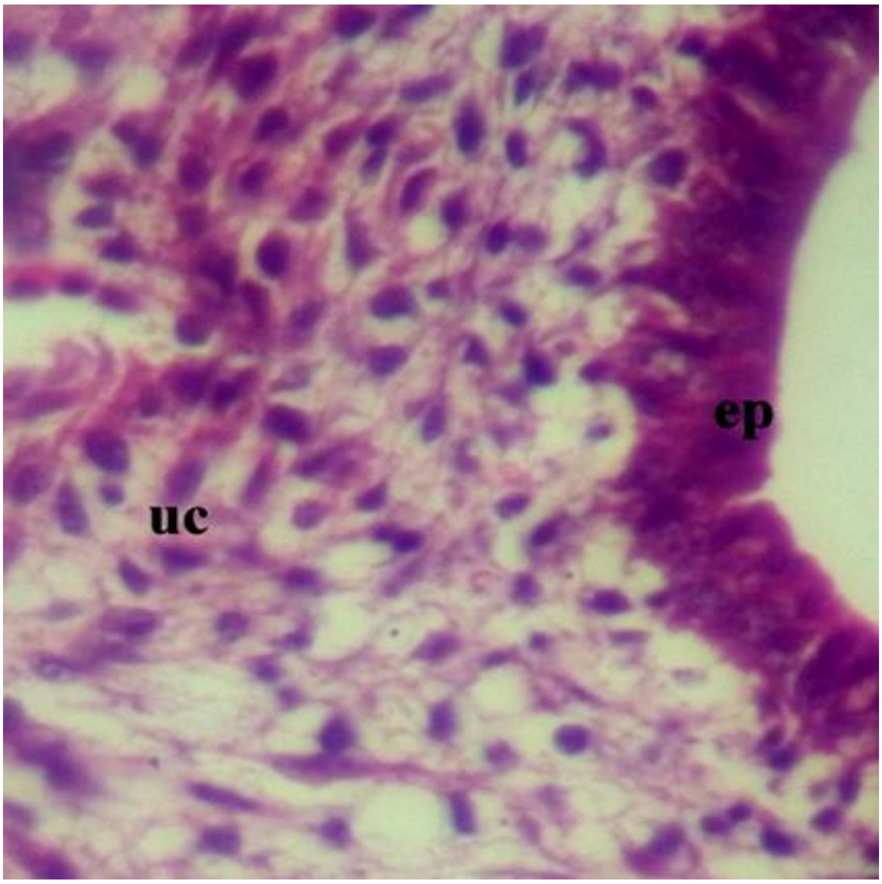

Plate XVIII. Transverse section of crop at day 11 of development showing simple columnar epithelium surrounded (ep) by mesenchymal cells (uc). H and E x 400.

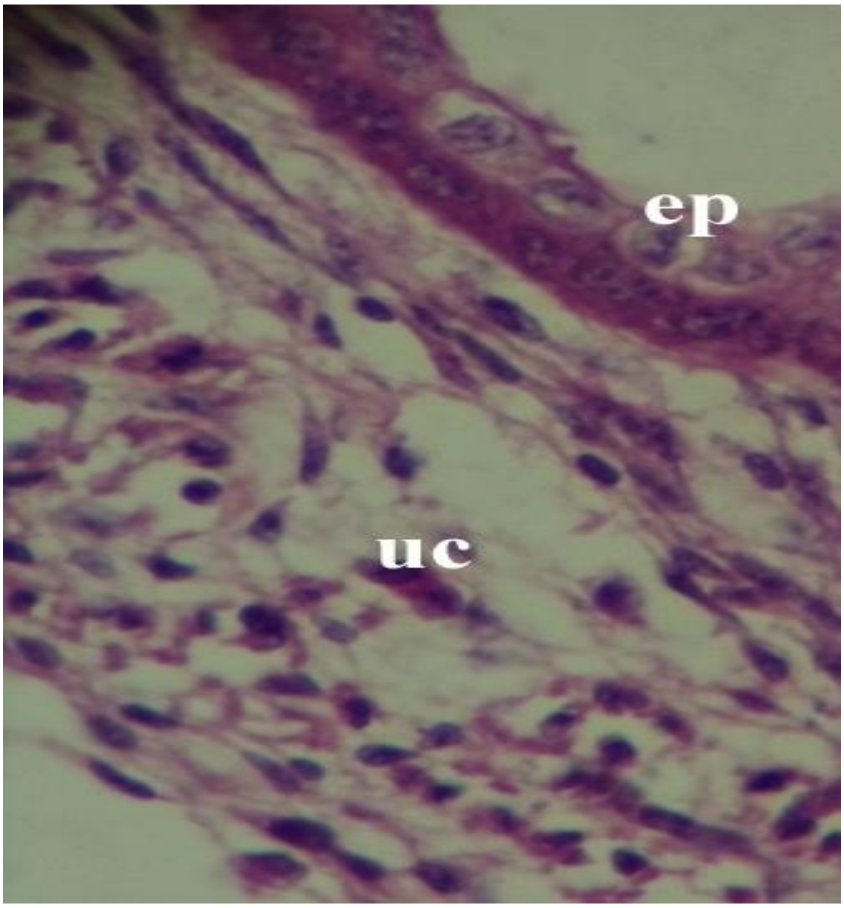

Plate XIX. Transverse section of crop at day 13 of development showing simple columnar epithelium (ep) surrounded by mesenchymal cells (uc) H and E x 250.

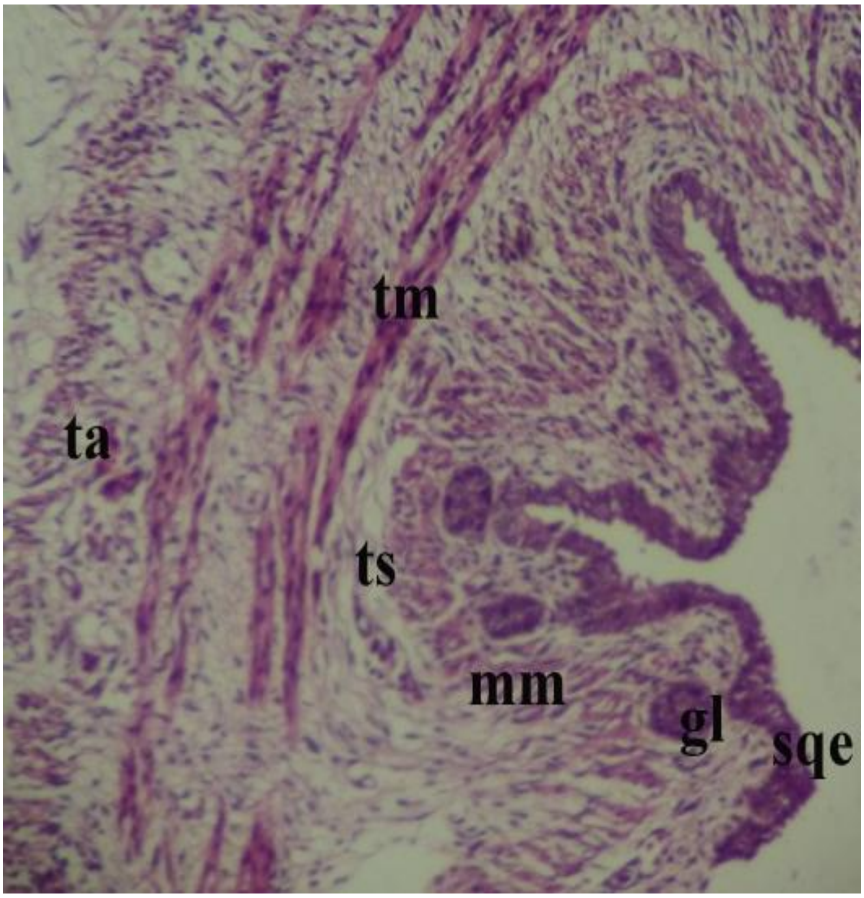

Plate XX. Transverse section of crop at day 19 of development showing stratified squamous epithelium (sqe), gland (gl), muscularis mucosa (mm), tunica submucosa (ts), tunica muscularis (tm), tunica adventitia (ta). H and E x 100.

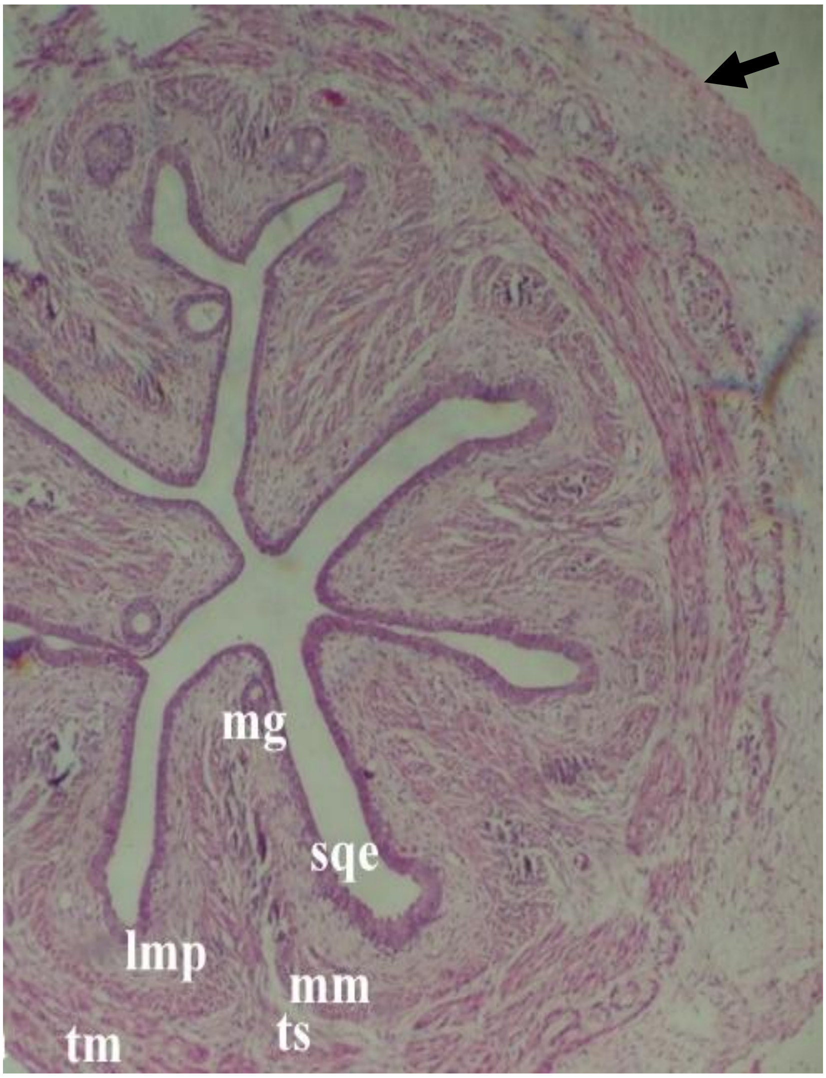

Plate XXI. Transverse section of crop at day 24 of development showing stratified squamous epithelium (sqe), mucosal gland (mg), lamina propria (lmp), muscularis mucosa (mm), tunica submucosa (ts), tunica muscularis (tm) and tunica serosa (arrow). H and E x 100.

Plate XXII. Transverse section of the crop at day 27 of development showing lumen (lm), stratified squamous epithelium (sqe), lamina propria (lmp), muscularis mucosa (mm) and mucus gland (mg). Hand E x 100.

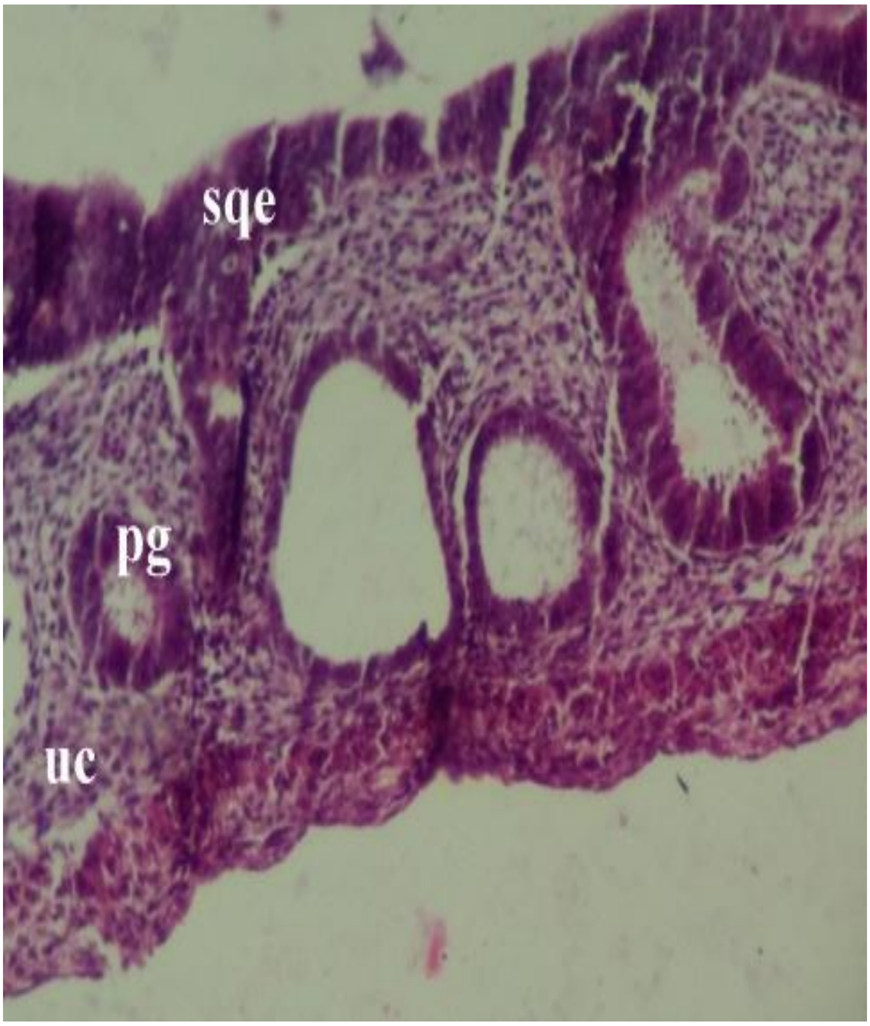

Plate XXIII. Transverse section of proventriculus of at day 10 of development showing stratified epithelium (se), primordial proventricular glands (pg) and mesenchymal cells (uc). H and E x 100.

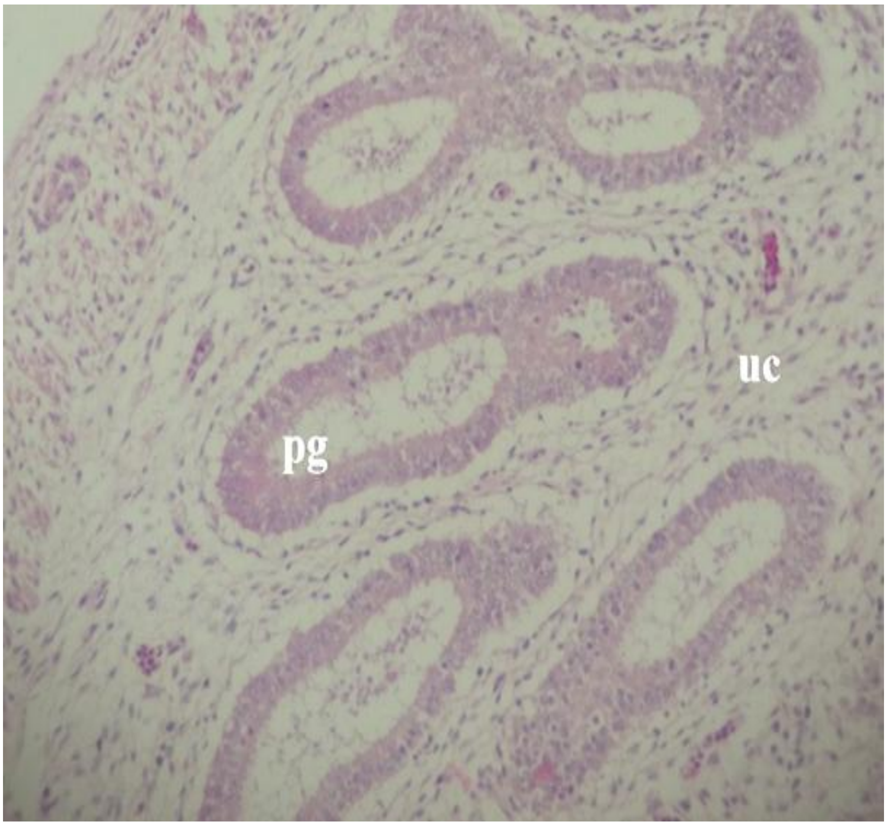

Plate XXIV. Transverse section of proventriculus at day 13 of development showing proventricular gland (pg) and mesenchymal cells (uc). Hand E x 250.

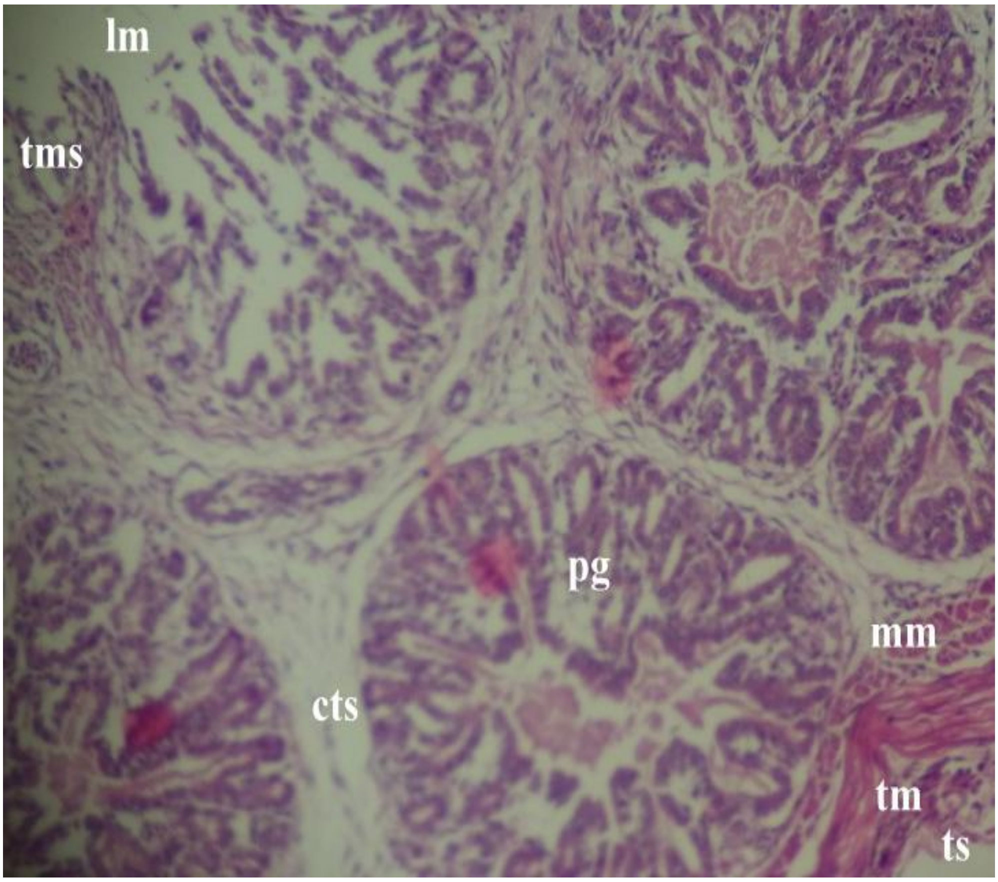

Plate XXV. Transverse section of proventriculus at day 24 of development showing lumen (lm), tunica mucosa (tms), proventricular gland (pg), connective tissue septa (cts), muscularis mucosa (mm), tunica muscularis (tm) and tunica serosa (tsa). H and E x 100.

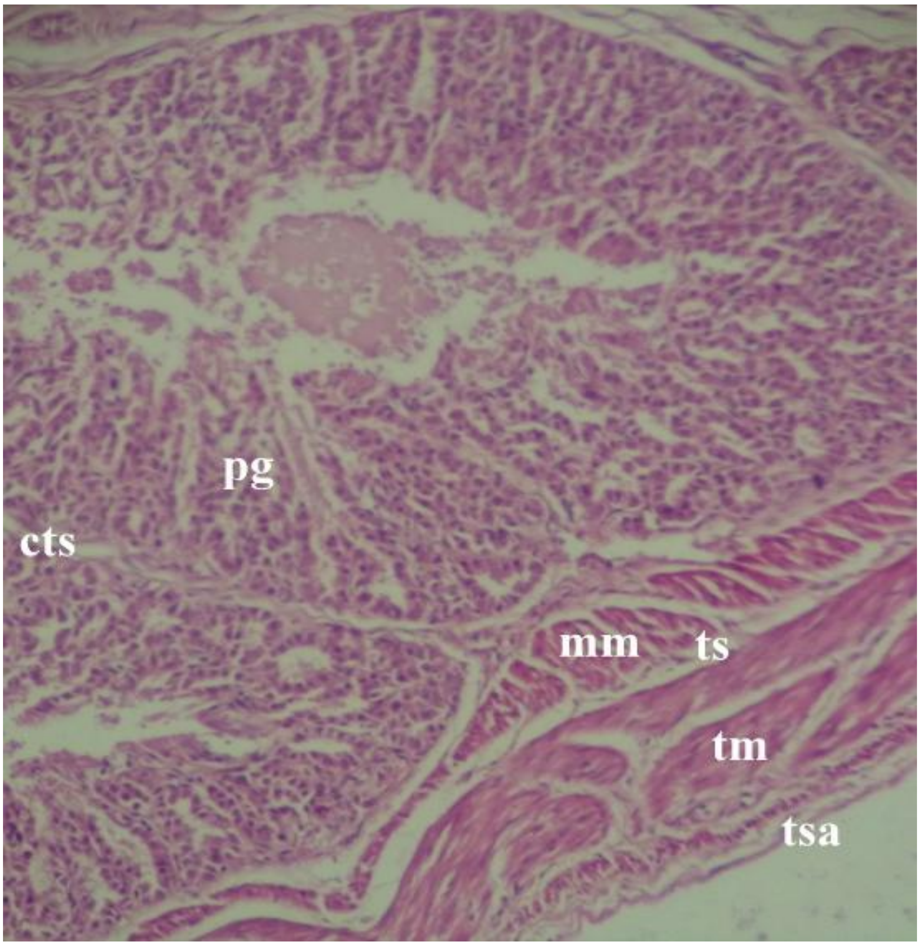

Plate XXVI. Transverse section of proventriculus at day 27 of development showing tunica mucosa (tms), connective tissue septa (cts), proventricula gland (pg), muscularis mucosa (mm), tunica submucosa (ts), tunica muscularis (tm) and tunica serosa (tsa). H and E x 100.

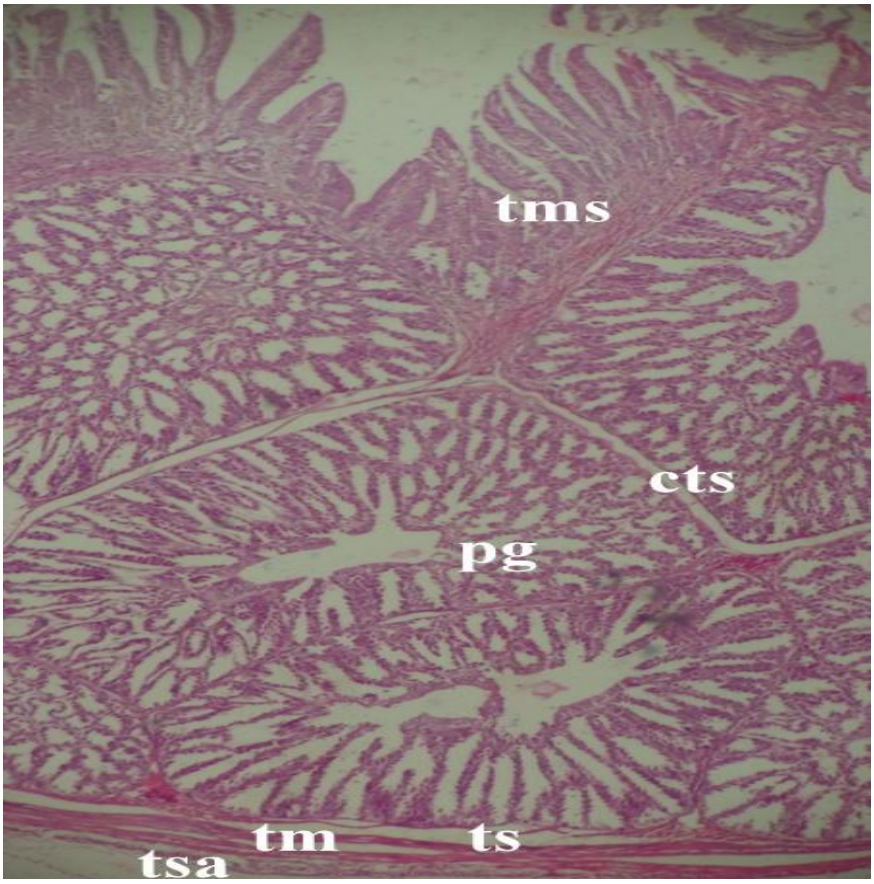

Plate XXVII. Transverse section of proventriculus at day 8 post-hatch showing tunica mucosa (tms), connective tissue septa (cts), proventricular gland(pg), tunica submcosa (ts), tunica muscularis (tm) and tunica serosa (tsa). H and E x 100.

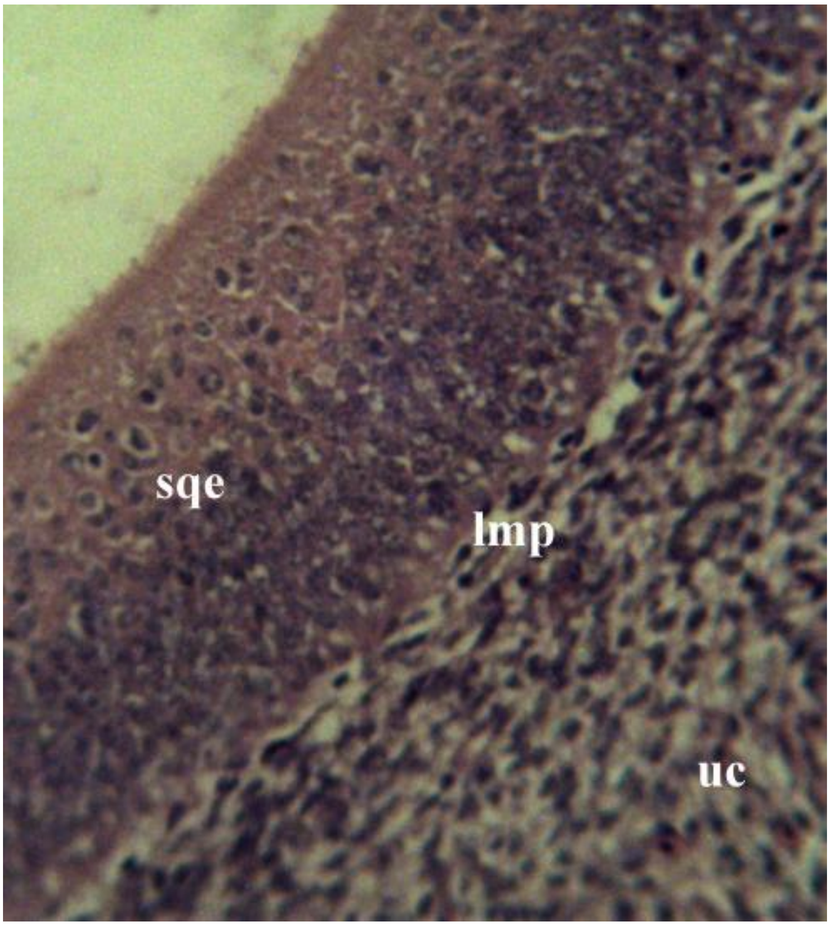

Plate XXVIII. Transverse section of ventriculus at day 11 of development showing stratified squamous epithelium (sqe), lamina propria (lm) and mesenchymal cells (uc). Hand E x 250.

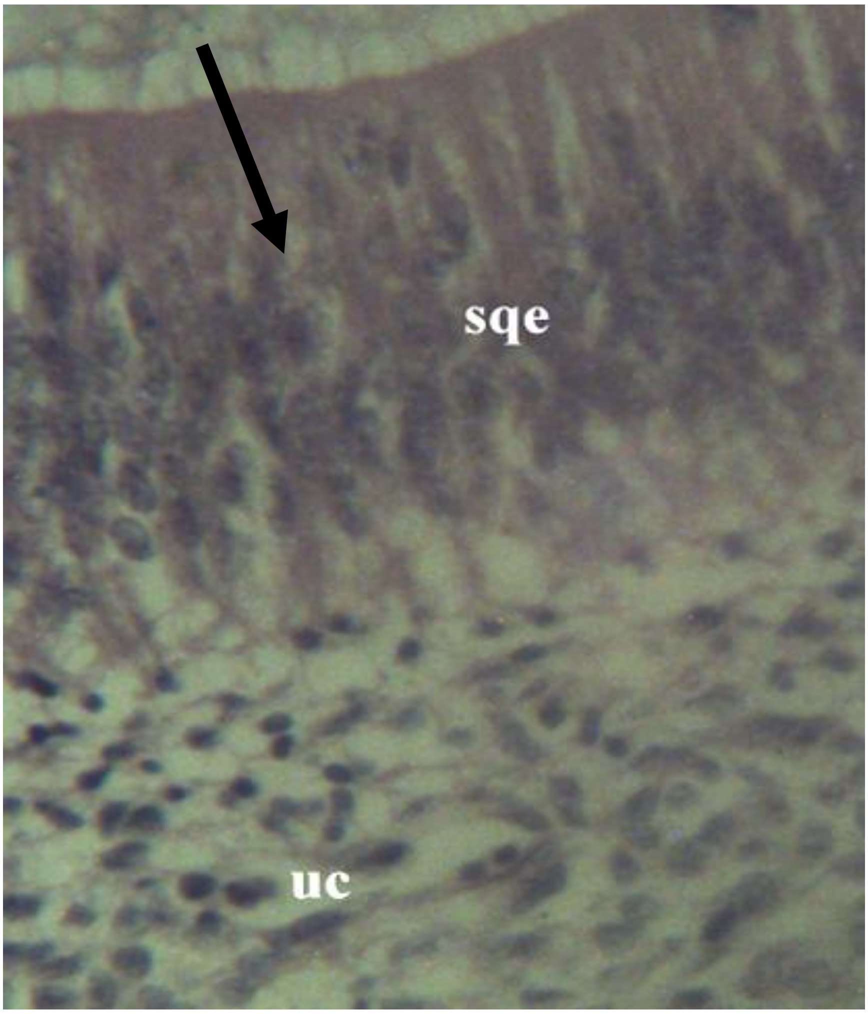

Plate XXIX. Transverse section of the ventriculus at day 13 of development showing stratified squamous epithelium (sqe), mesenchymal cells (uc) and the appearance of the tubular gland (blue arrow). H and E x 400.

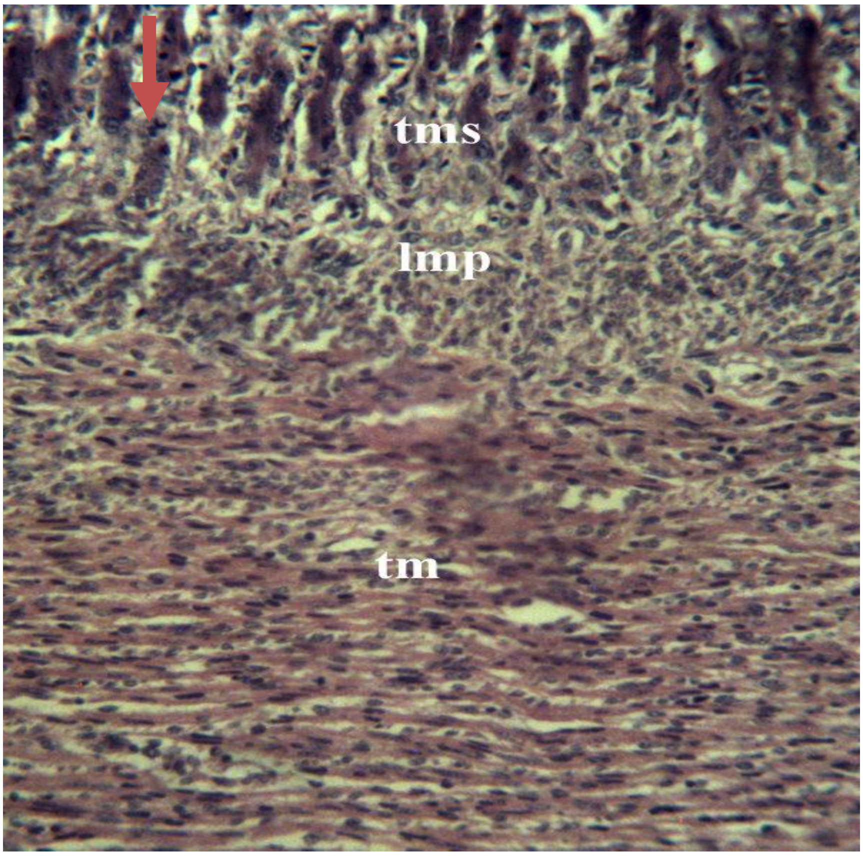

Plate XXX. Transverse section of ventriculus at day 23 of development showing tunica mucosa (tms) occupied by tubular glands (arrow), lamina propria (lmp) and tunica muscularis (tm). H and E x 250.

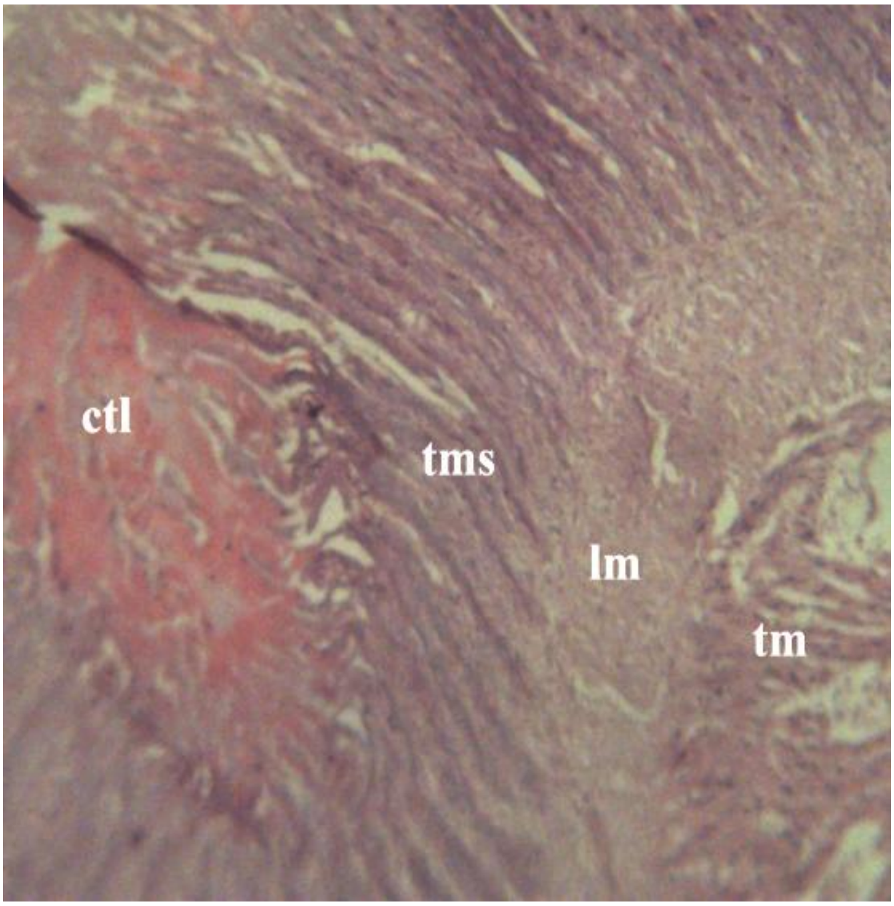

Plate XXXI. Transverse section of ventriculus at day 27 of development showing cuticular layer (ctl), tunica mucosa (tms), lamina propria (lm) and tunica muscularis (tm). Hand E x 100.

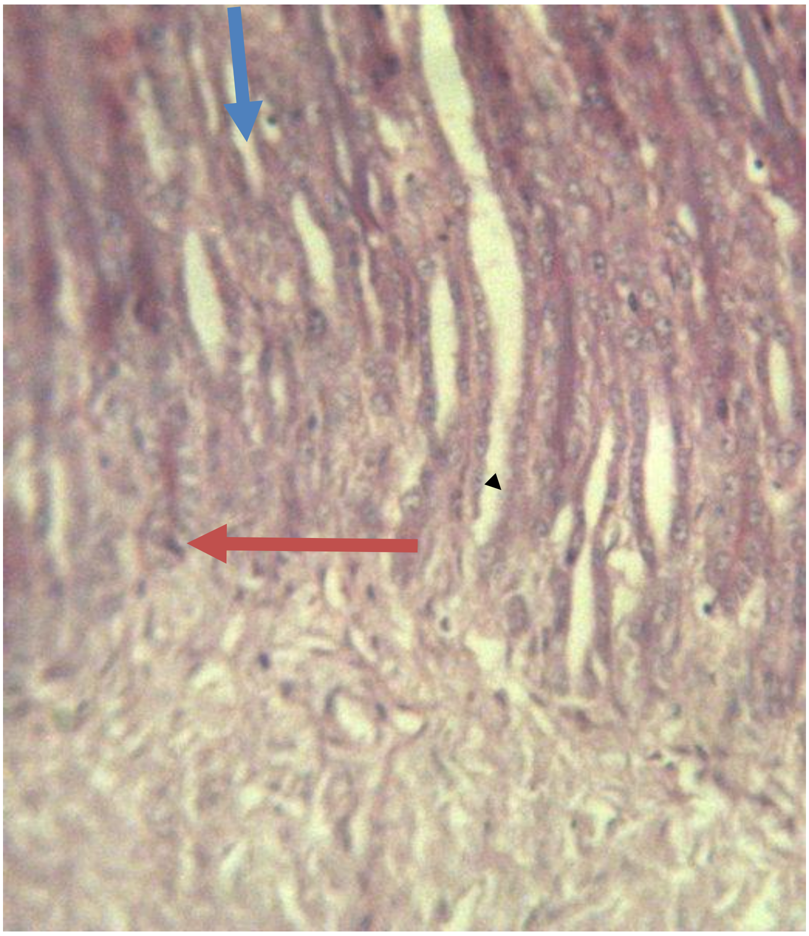

Plate XXXII. Transverse section of ventriculus at day 27 of development showing gastric pit (blue arrow) and ventricular cells (red arrow). Hand E x 250.

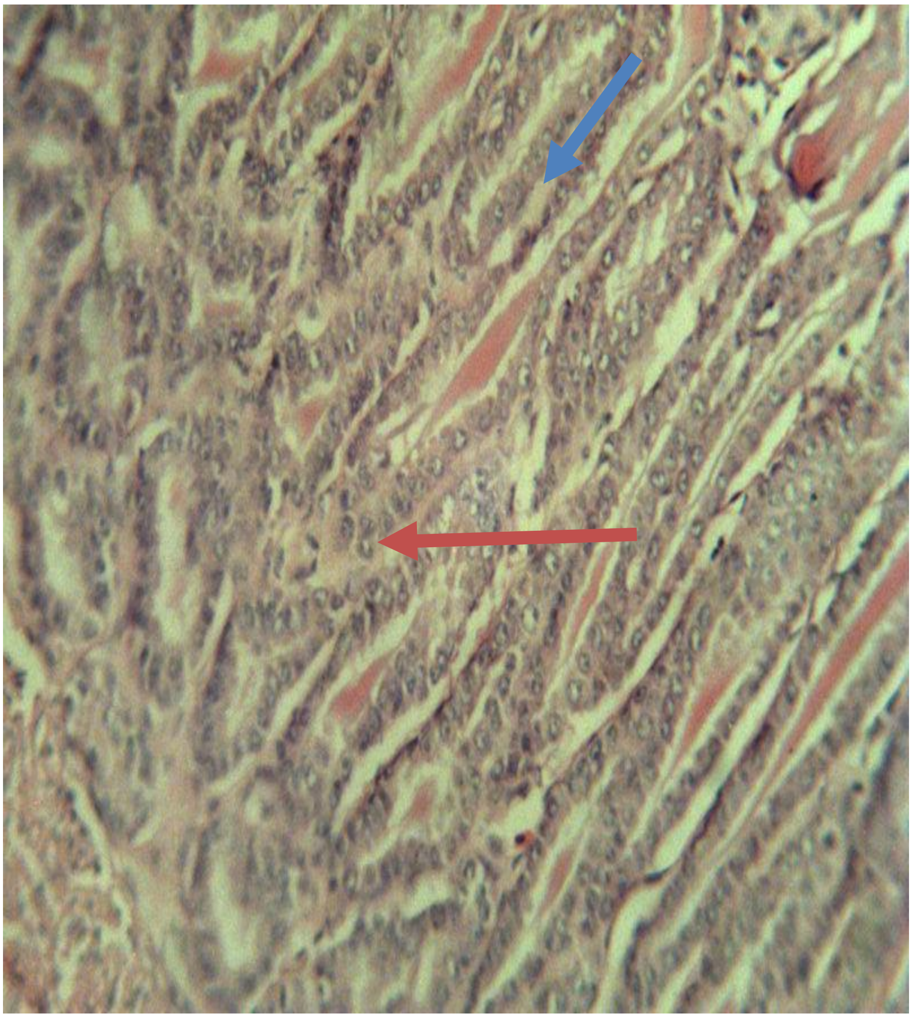

Plate XXXIII. Transverse section of ventriculus at day 8 post-hatch showing gastric pit (blue arrow) and ventricular cells (red arrow). H and E x 250.

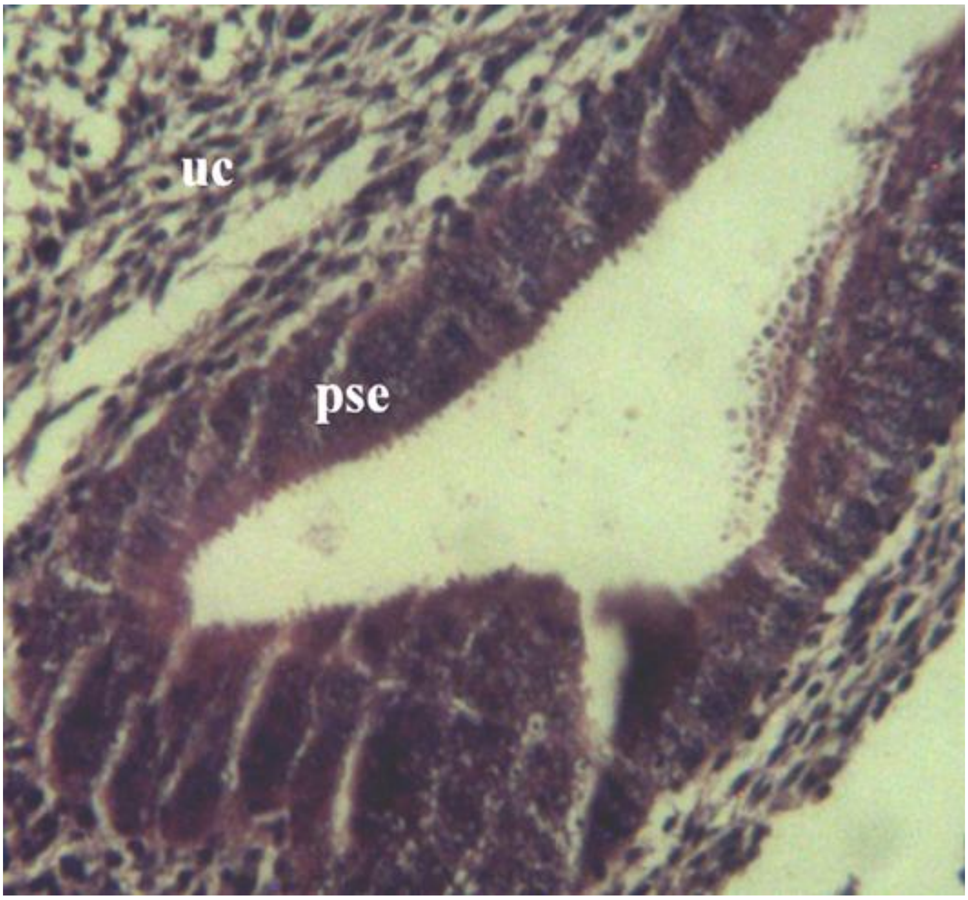

Plate XXXIV. Transverse section of small intestine at day 8 of development showing pseudostratified columnar epithelium (pse) and mesenchymal cells (uc). H and E x 250.

Plate XXXV. Transverse section of the small intestine at day 13 of development showing simple columnar epithelium (se), tunica submucosa (tms), tunica muscularis (tm), tunica serosa (ts) and villus (arrow). H and E x 100.

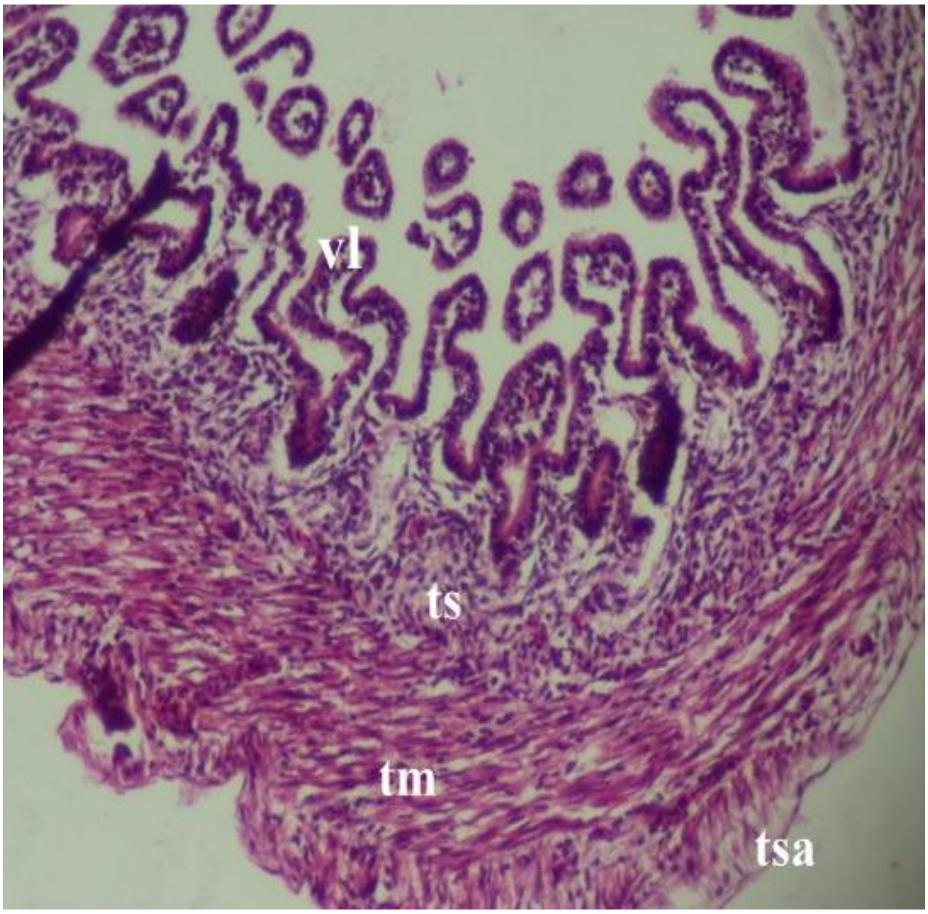

Plate XXXVI. Transverse section of small intestine at day 19 of development showing zigzag villi lined by simple columnar epithelium (vl), tunica submucosa (ts), tunica muscularis (tm) and tunica serosa (tsa). H and E x 100.

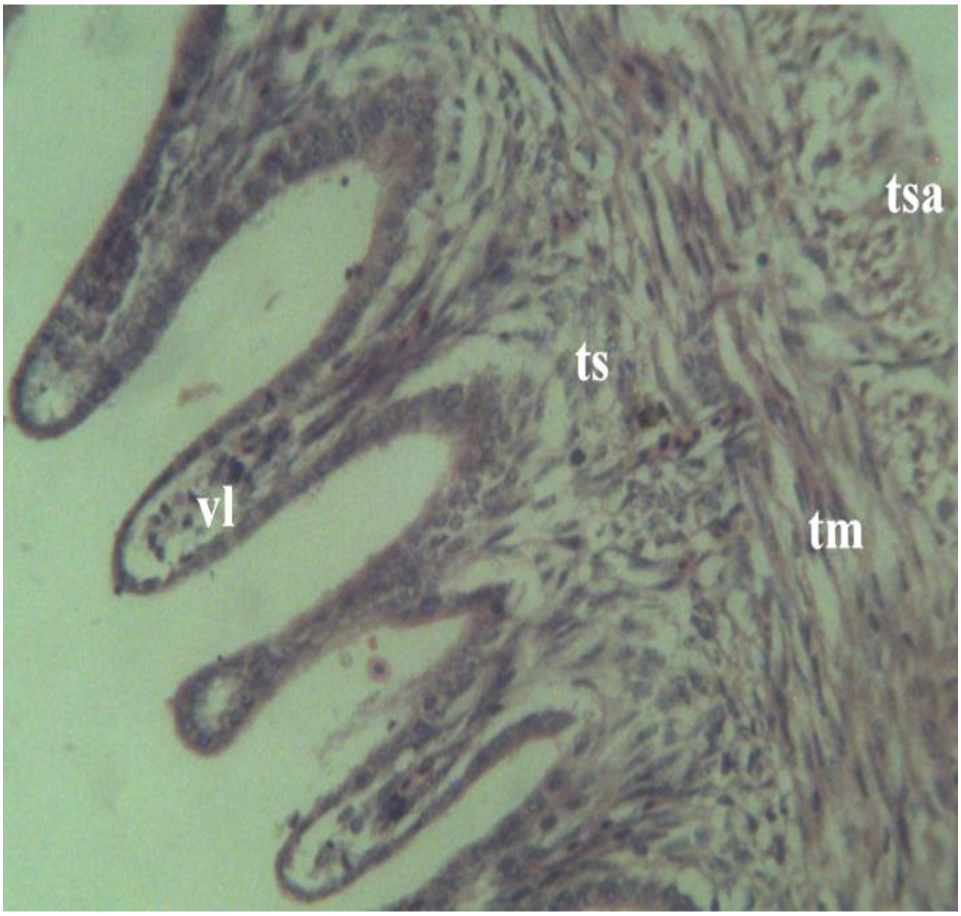

Plate XXXVII. Transverse section of small intestine at day 24 of development showing more mature villi lined by simple columnar epithelium (vl), tunica submucosa (ts), tunica muscularis (tm) and tunica serosa (tsa). H and E x 250.

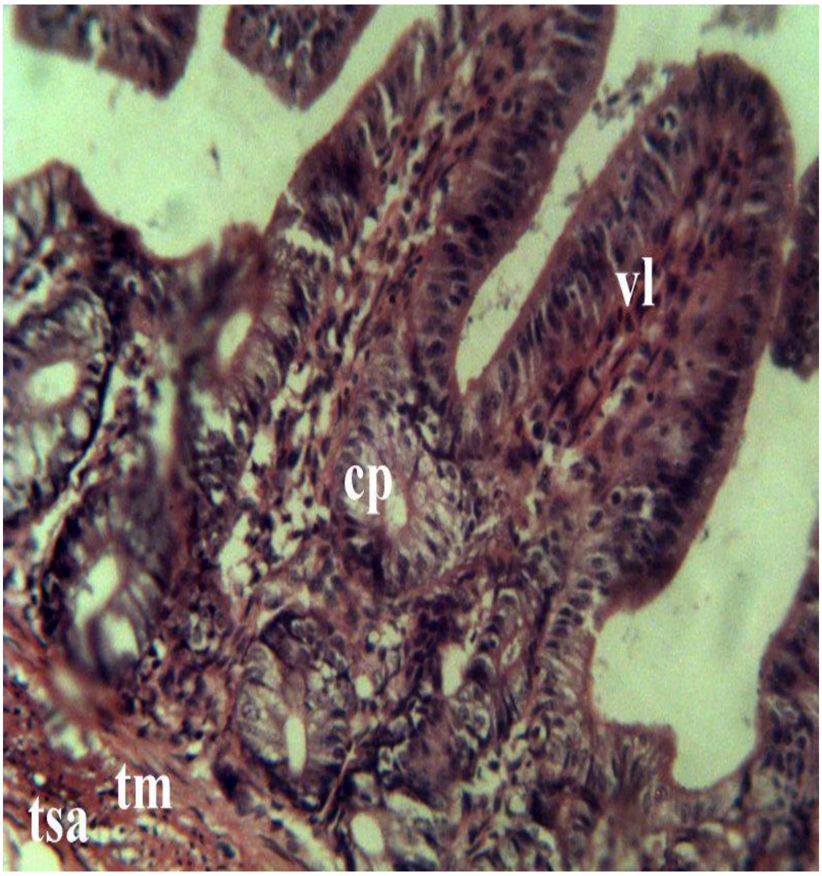

Plate XXXVIII. Transverse section of small intestine at day 8 post-hatch showing villi lined by simple columnar epithelium with goblet cell (vl), crypt of Liberkhum (cp), tunica muscularis (tm) and tunica serosa (tsa). H and E x 250.

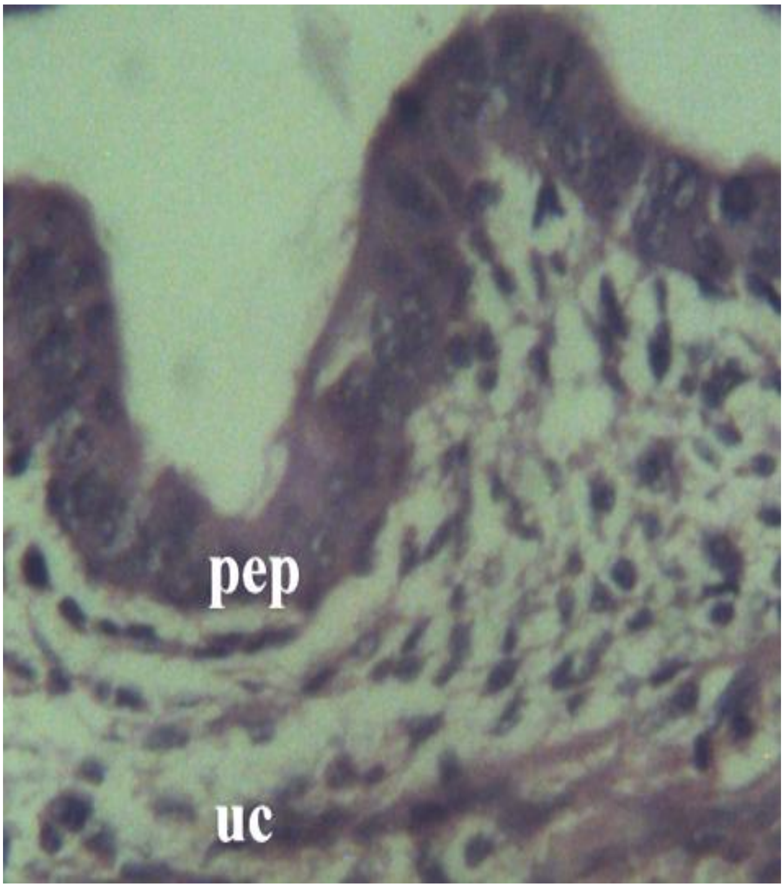

Plate XXXIX: Transverse section of caecum at day 11 of development showing pseudostratified columnar epithelium (pep) and mesenchymal cells (un). H and E x 400.

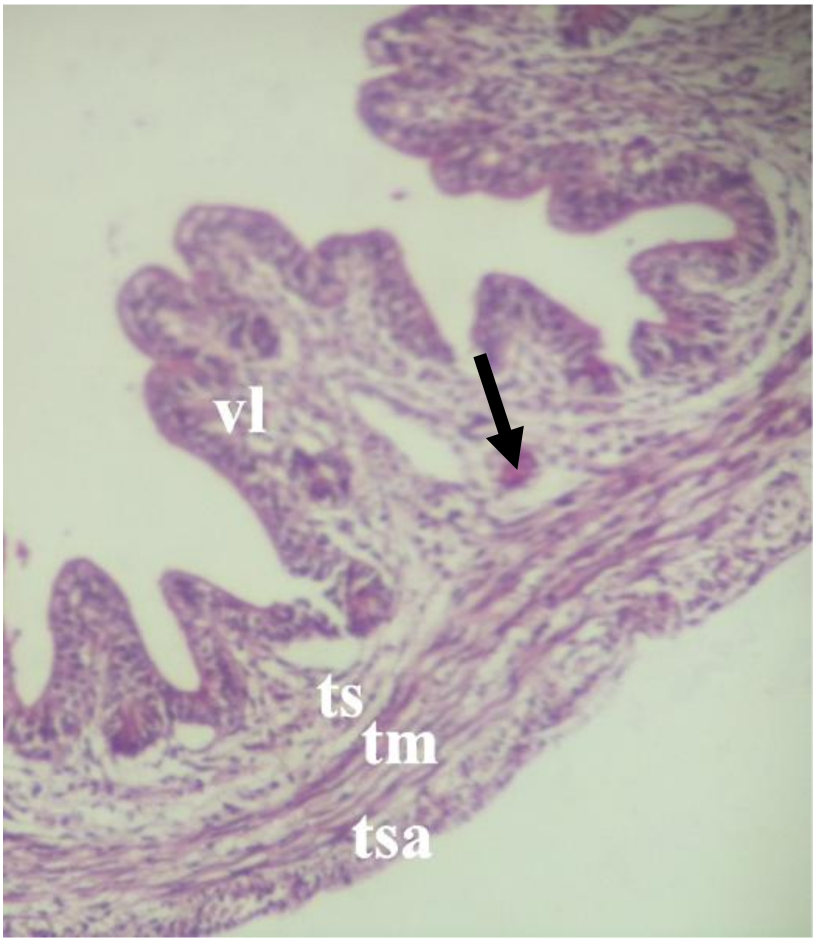

Plate XL. Transverse section of caecum of at day 24 of development showing thick branched villi lined by simple columnar epithelium (vl), crypts of Liberkhum (arrow), tunica submucosa (ts), tunica muscularis (tm) and tunica serosa (ts). H and Ex 100.

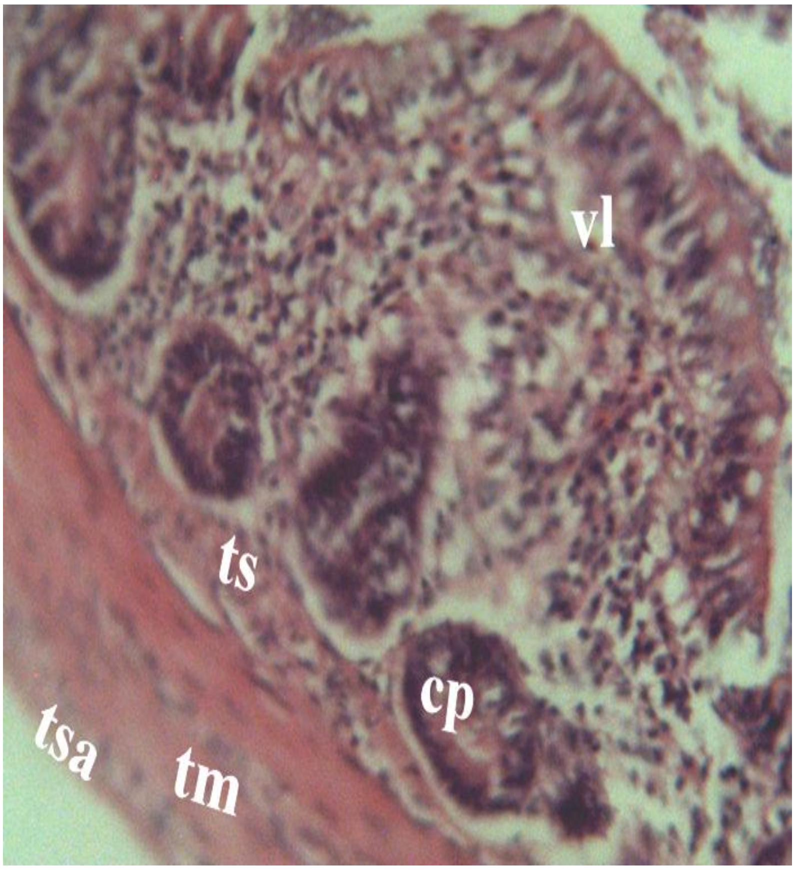

Plate XLI. Transverse section of the caecum at day 8 post–hatch showing villi lined by simple columnar epithelium with goblet cells (vl), crypt of Liberkuhm (cp), tunica submucosa (ts), tunica muscularis (tm) and tunica serosa (tsa). H and E x 250.

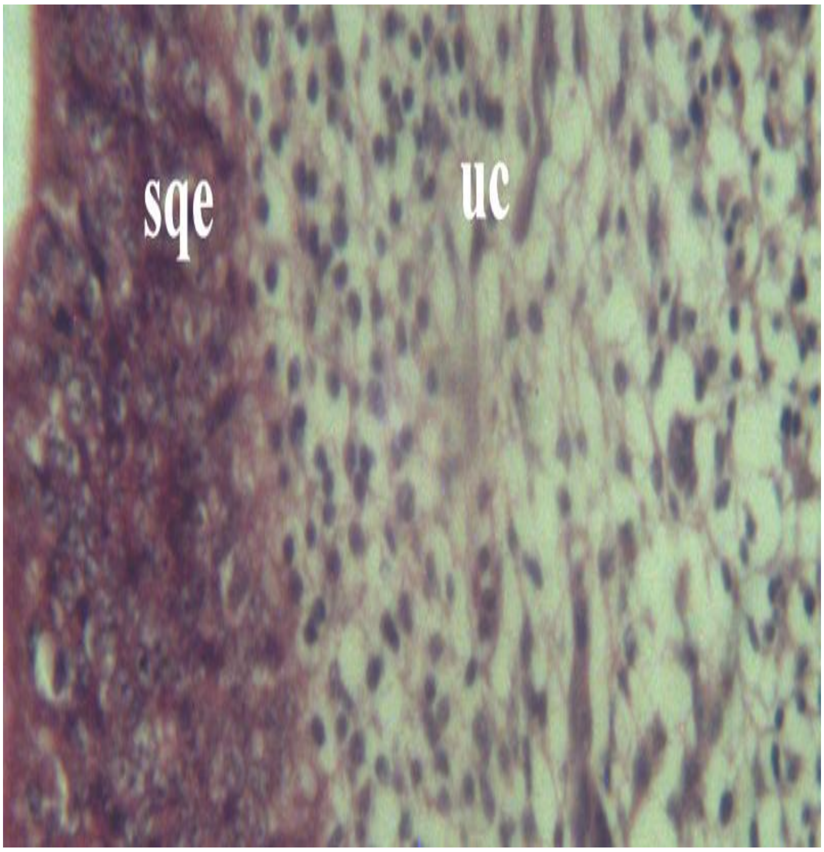

Plate XLVI. Transverse section of colorectum at day 11 of development showing stratified squamous epithelium (sqe) surrounded by mesenchymal cells (uc). H and E x 400.

Plate XLVII. Transverse section of the colorectum at day 19 of development showing villi lined by simple columnar epithelium (vl), tunica submucosa (ts), tunica muscularis (tm) and tunica serosa (ts). H and E x 100.

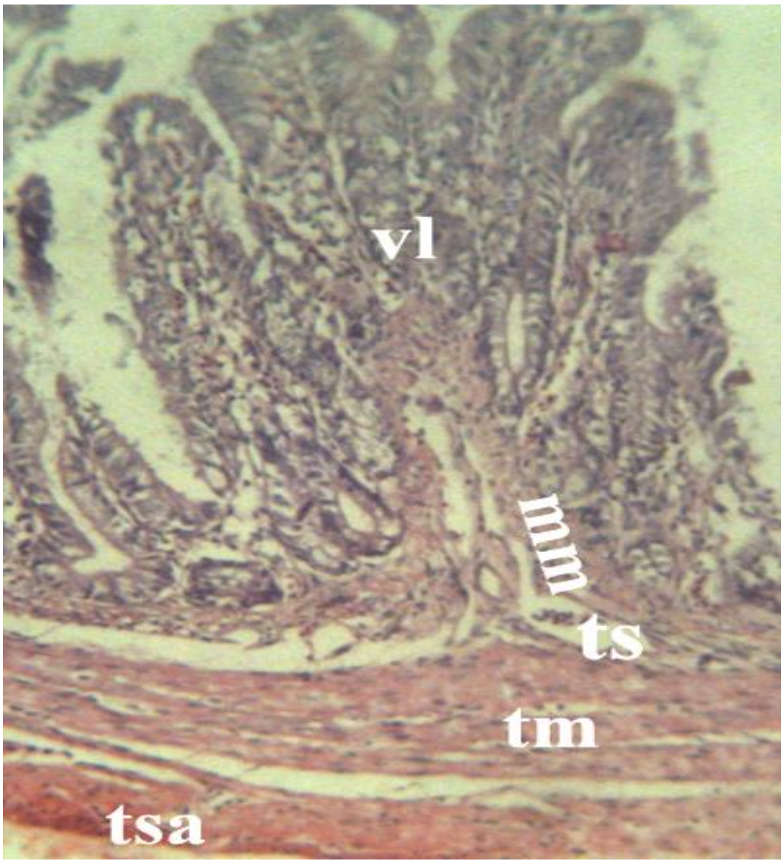

Plate XLVIII. Transverse section of colorectum at day 8 post-hatch showing branched villi lined by simple columnar epithelium (vl), muscularis mucosa (mm), tunica submucosa (ts), tunica muscularis (tm) and tunica serosa (tsa). H and E x 100.

CHAPTER FIVE

DISCUSSION

The development of early digestive tract is very significant for achieving maximal growth in animals (Tako, 2004). It helps in determining the patterns of growth in the animal species, especially at different ages (Mobini, 2011) as it is possible to affect the biology and behaviour of the species (Sherri *et al.*, 1988). In this study, the weight and length of gastrointestinal tract at pre-hatch were found to be lower than those of post-hatch since the gastrointestinal tract is still undergoing development. This agrees with the findings of Shih *et al.*, (2005) who observed the weight and length of gastrointestinal tract of geese at pre- and post-hatch. This observation may be explained from the fact that during early post-hatch life, the poultry undergoes a rapid transition in digestive function. The nutrients in developing bird are supplied from the yolk and development occurs due to feed uptake during the first few days after hatching (Shih *et al.*, 2005).

The appearance of oesophagus of the guinea fowl at day of development in this study agrees with the observation by McGeady*et al.*, (2006) in vertebrates. It extended from the fusiform dilation of the foregut and then separated into the cervical and thoracic oesophagus by the appearance of an out-pouch called the crop at day 11 of development. This observation agreed with what Daniel (1957) reported in chicken where the crop appeared at day 8 of development. The variation in time can be attributed to the difference in incubation period which is 21 days in domestic fowl and 28 days in the guinea fowl.

The increase in both absolute and relative lengths of the cervical oesophagus post-hatch compared to pre-hatch period agreed with what was reported in geese (Shehan, 2012), in *Rhynchotus rufescens* (Rossi *et al*., 2006) and in duck (Das and Biswal, 1967).

The changes observed in the oesophageal mucosal histogenesis from simple columnar cells pre-hatch to stratified squamous form post-hatch agreed with the observation of Van Alten and Fennell (1957) and Calhoun (1954) in chicken. The appearance of primordial oesophageal glands at day 19 of development in guinea fowl embryo was similar to that of chick embryo at day 16 of incubation (Daniel, 1957); the appearance of four typical layers of the GIT was between days 19-27 as was observed by Ventura *et al*., (2013) in chick embryo between 12-20 days of development.

At post-hatch, the mucosa of the oesophagus was distinguishable into the three laminae (i.e lamina epithialis, lamina propria and lamina muscularis mucosae) and lined by non-keratinized stratified squamous epithelium.This conforms to the observations in geese (Shehan, 2012), and in quail (Mostafa*et al*, 2012), but not with the observation in wild birds where the epithelium was stratified squamous but keratinized (Ebrahim and Abolghasem, 2009).

The appearance of the crop by day 11 of development separating the oesophagus into cervical and thoracic is in agreement with what McGeady *et al.*, (2006) described in other birds. The difference in time of appearance in different species is due to the difference in incubation period, like the chicken in which it appeared at day 8 of development (Daniel, 1957). The presence of mucus glands in the crop at pre and post-hatch is a striking feature observed in the guinea fowl. This agreed with the observation in quail (Mostafa *et al.*,2012), and in duck (Banks, 1992) but disagreed with what was observed in blue and yellow macaws (Rodrigues*et al.*, 2012), and in chicken and pigeon (Banks, 1992).

The avian stomach is peculiar in that it consists of two distinct parts morphologically and physiologically; the glandular portion or proventriculus and the muscular portion or gizzard (Thomson, 1969). The guinea fowl under study was no exception. The presence of isthmus, a constricted junction between the two portions of the stomach in the guinea fowl agreed with what was reported in the pigeon (Hassan and Moussa, 2012) but absent in *Elanus caeruleus* (Hamida *et al*., 2013)where the two portions form one large pear-shaped cavity.

One of the striking observations in the development of the guinea fowl stomach was the embryonic ventriculus which appeared two days earlier than the proventriculus. This observation revealed that the stomach development failed to follow the expected craniocaudal pattern.

The proventriculus was observed to be slightly longer than the ventriculus at pre and post-hatch periods of study. The observation agreed with what was reported earlier in the geese (Shih *et al*., 2005). Histologically, the primordial proventricular glands appeared at day 10 of development and became very conspicuous with each gland separated by the connective tissue septa by day 24 of development. This observation agreed with the findings of Sjogren (1941) and Hibbarb (1942) which reported that the primordial glands appeared by day 7 of development in domestic fowl.

The proventricular lumen of the embryonic guinea fowl appeared as irregular disagreeing with the star-shaped lumen in quail (Hossam, 2008) and inverted lumen in chicken (Thomson, 1969).The primordial proventricular glands were of varied shapes as early as day 10 of incubation and then became extended, branched and larger with age. This observation agreed with what was reported in geese and chicken embryo (Thomson, 1969;Sgambati *et al*., 1995), respectively. The finding by day 24 of development that the proventricular glands were similar to that of post-hatch (except for the connective tissue being loose in pre-hatch compared to the dense connective tissue in the post-hatch) agreed with what Ventura *et al*., (2013) reported, that the proventriculus of embryonic chicken showed similar histological features as adult by day 17 of development.

The mucosal layer of the ventriculus was observed to be folded, lined by simple columnar epithelium and covered by a thick layer called cuticle or keratinoid. This agreed with the observations in quails (Mostafa, 2012), in coot bird (Batah *et al*., 2012) and in pigeon and duck (Hassan and Moussa, 2012) but in contrast with the observation in cassowaries bird (Fowler, 1991) that its ventriculus lacks a cuticle. The fact that the chief and basal cells of this organ became conspicuous and better developed with advance in age from pre-hatch to post-hatch agreed with what was reported by William and Linda (2000).

The weights and lengths of small and large intestines of the developing guinea fowl significantly increased from incubation to 8 days post-hatch. This observation agreed with what was reported in other species of birds (Sell *et al*., 1991; Lu, 1999; Shih *et al*., 2005).

The segmentation of the intestine into small and large intestine became apparent with the appearance of one of the caeca at day 10 of development. The duodenal loop became apparent at day 13 of development making the distinction of the small intestine into the three segments of duodenum, jejunum and ileum possible. The transformation of the duodenal loop between day 1 and day 8 post-hatch was so marked. This observation goes to agree with previous study (Nitsan *et al*., 1991) emphasizing the importance of digestive capacity for mucosal growth and function of the duodenum in the chicks during the early growth period. This development was also reported in goslings by Shih *et al*., (2005).

The absolute lengths of duodenum increased many folds from day 1 to day 8 post-hatch while the development of the jejumal and ileum lengths were similar during this period. These data agreed with the observations reported in several other avian species (Lilja 1983; Lu 1999; Shih *et al*., 2005). This accelerated growth rate in avian gastrointestinal tract demonstrates the developmental changes in these organs (Jin *et al*., 1998). This claim was buttressed by the data in this study which revealed that the small intestine is the longest of all the segments of gastrointestinal tract in the guinea fowl. This is contrary to what was reported for the ostrich (Ilmars and Arnis, 2011).

The result of at pre-hatch showed that the luminal surface of the intestine was invaginated by day 8 of development. This observation was contrary to the report by Narin *et al*., (2002) that the lumen of the small intestine of geese was star-shaped. The luminal surface was lined by pseudostratified columnar epithelium in agreement with Narin *et al*., (2002) as reported in geese but contrary to Gheri *et al*., (1989) and Mamajiwalla *et al*., (1992) as seen in chicken embryo which was stratified squamous epithelium. The appearance of the previllus ridges at day 13 and the appearance of numerous zigzag folds lined by simple columnar epithelium at day 19 in the guinea fowl embryo were in agreement with observations in other avian species (Clark, 1967; Narin *et al*., 2002; Bohórquez *et al.*,2011). The absence of the goblet cells throughout the incubation period in any of the intestinal segment of the guinea fowl agreed with the report Narin *et al*., (2002) in geese embryo but disagreed with that of Uni *et al*.,(2003) in chick embryo where he reported the appearance of the goblet cells 3 days before hatching.

In this study, one of the two caeca appeared as a slight bulge at day 10 of development earlier than the second caecum at day 12. This observation disagreed with those of Daniel (1957) and Helen *et al*., (2003) in the domestic fowl where the two caeca appeared atdays 7 and 4 of development, respectively. In the guinea fowl, weight and length of the caeca increased with age and this is in agreement with the report of Shih *et al.*, (2005) in geese. It is however note worthy that caeca of avian species vary in sizes or may even be absent (Hamida *et al*., 2013). This variation is dependent on the type of diet that informed the presence of well developed caeca in the geese, a herbivores, where the caecal lengths accounted for 61.5% and 79.2% of the total length of the large intestine at pre-hatch and at 28 days post hatch, respectively. This is because the caecum is the main digestive segment degrading cellulose in the alimentary canal of geese (Clemens *et al*., 1975). The guinea fowl on the other hand has average sized caeca like any other granivorous species whose diet contained high level of plant fibre (Stevens and Hume, 1998).

The histogenesis of the caeca of guinea fowl was observed to have undergone series of changes similar to other segments of the gastrointestinal tract till day 24 of development when crypts in the luminal surface were observed. Numerous goblet cells were observed at post-hatch. The histology of the caeca from day 1 of hatching to day 8 post-hatch revealed no further observable histological differences. This agreed with what was reported in other avian species (Firdous and Lucy, 2012).

This study also showed that the colorectum became distinguishable by day of development in the guinea fowl when one of the two caeca appeared and served as the demarcation of the developing intestine into small and large intestine. In the guinea fowl, the colon was grossly distinguished from the rectum but both had similar histological features as the caecum. This observation is in line with what was reported for other galliform birds (Calhoun, 1954; Sell *et al*., 1991).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In this present study, the results indicate that gastrointestinal tract growth and digestive functions are not fully developed in the newly hatched birds and that the GIT development does not follow the expected sequential craniocaudal pattern in the guinea fowl.

The development of the guinea fowl embryonic GIT takes 6 days to be completed; starting from day 8 of incubation (with appearance of a straight tube having roundish structure at the centre) and ending on day 13 of development with the appearance of caeca.

Histologically, this study revealed the presence of mucus gland in the crop and absence of goblet cells in both small and large intestines during the pre-hatch period.

6.2 Recommendation

Further study should be conducted on the development of the accessory digestive organs of the guinea fowl so as to have a complete understanding of it morphogenesis. Study of the GIT enterocytes is also required.

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