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HISTOMORPHOMETRICAL STUDY OF SMALL INTESTINE IN IRANIAN *CHUKAR*

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ABSTRACT

Given the growing importance of *chukar* as a bird with high economic value as well as the lack of clear and comprehensive information about the bird's digestive system, the colon hystomorphology, in addition to providing basic information about the bird, also helps its comparison with other animal species as well as adjusting a suitable diet to breed the bird. Alectoris chukar chukar is in fact one of the sixteen strains of wild partridge. This sub-species has adapted to many industrial farming conditions. In this study, 20 mature healthy partridges of one-year-old, weighing approximately 500 to 600 grams and the mean age of 20 weeks were selected from of partridge training halls randomly and were transferred to the animal care center of university. After 12 hours of fasting they were transferred to a laboratory, then the Macroscopic and biometric characteristics (such as the animal's weight and height) were recorded. Intestinal samples were prepared as small sausage inside was full of fixative solution. In this stage, a piece of sample was separated, stained, and studied. Preparing the microscopic sections was conducted after fixation of the samples. The samples were prepared, for optical microscopic studies, by conventional staining methods: H & E method was used to assess overall tissue structure, periodic acid-Schiff (PAS) was used to study of goblet cells, Verhoeff staining and toluidine blue was used to study of connective tissue, mucosa, submucosa, muscular class and serous. Histologically, the intestinal structure of birds complies with four-layer layout of digestive tract of mammals and domestic birds, but has certain differences in the type of mucosal and glands cells. Unlike mammals, under the epithelium and lamina propria, the mucosal tissue membrane of the smooth is visible as a thick layer. There is a connective tissue under the sub

mucosal muscle. Submucosal layer of the bird is very thin and delicate. Unlike mammals, submucosa layer is lack of gland in the bird. Unlike mammals and domestic poultry, at the beginning of intestine the circular muscle layer is thicker than the longitudinal outer layer in chukar. There is a neural network between two muscular layers similar to mammalian and domestic poultries. Despite differences in some of the tissue properties of the gastrointestinal tract with domestic poultry and mammalian, its basic structure (including the mucosa, submucosa, muscle class and serous or adventitial) is common in all of them.

Keywords: Histology, morphology, small intestine, Iranian chukar.

INTRODUCTION

Although the world's population steadily increased over the centuries, however welfare and quality of human life is being upgraded due to sciences progress. Given the growing importance of *chukar* as a bird with high economic value and the lack of clear and comprehensive information about the bird's digestive system, the colon hystomorphology, in addition to provide basic information about the bird, also helps its comparison with other animal species as well as adjusting a suitable diet to breed the bird. Histologically, the intestinal structure of birds complies with four-layer layout of digestive tract of mammals and domestic birds, but has certain differences in the type of mucosal and glands cells. However, there is no exact definition for anatomical and morphological structure of chukar intestines. Alectoris chukar chukar is in fact one of the sixteen strains of wild partridge. This sub-species has adapted many industrial farming conditions. Chukar meat consumption is very high due to high protein and low fat. 72% of chukar weight can be used. The bird eats usually plants, including grass, plants leaf, seeds, legumes, fruits, as well as animal foods such as insects according to the abundance and seasonal availability (13). chukar resistance against heat and cold, its diet, its meat quality, particularly resistance to diseases that today have created many problems for the country's poultry industry will bring many people to the industrial farming of chukar (14).

METHODS AND MATERIALS

In this study, 20 mature healthy partridges of one-year-old, weighing approximately 500 to 600 grams and the mean age of 20 weeks were selected from of partridge training halls randomly and were transferred to the animal care center of university. The birds were killed using ether anesthesia and abdominal wall was cut by standard autopsy methods.

Intestinal macroscopic properties in terms of complexity and length of the entire digestive tract and its various parts were measured and recorded.

Morphologic examinations

Intestinal samples were prepared as small sausage inside was full of fixative solution. From each sample of intestine a piece, an area about 2-1.5 cm^2 was cut with scissors and placed into PAS staining solution for 3-5 minutes, then the sample was washed with saline and was placed on the solid paraffin in a Petri dish and finally the sample was placed under the loop and was prepared for cutting with magnification ×25.

Some cuts between the villi and in the longitudinal direction were created using Cataract Knife, so that the rows of villi were separated next to and connected to each other. After separating the rows of villi, all of them one by one was removed with tweezers or needle, and were placed on the slides (1and 16). Two or three drops of glycerin were poured on the prepared rows and then a conventional slide was put on it and finally the slide was placed under a microscope for study (17).

Histological study

After the macroscopic study, the middle and anterior parts of the small intestine were cut using a scalpel blade immediately were immersed in formalin-alcohol solution and 10% formalin to fix the samples. Microscopic Sectioning was begun after fixation of the samples with conventional method. The samples were prepared, for optical microscopic studies, by conventional staining methods: H & E method was used to assess overall tissue structure, periodic acid-Schiff (PAS) was used to study of goblet cells, Verhoeff staining and toluidine blue was used to study of connective tissue, mucosa, submucosa, muscular class and serous.

Macroscopic results

Average body weight, body length, total length of the gastrointestinal tract and its different parts as well as the relative average length of the gastrointestinal tract as a multiple of average body length is determined as follows in the table below.

Table 1: The average sizes of the body and the digestive tract

The average weight of males	**283.7 g**
The average weight of females	**270.5 g**
The average length	**34 cm**
The average length of the gastrointestinal tract	**79 cm**
The average length of the small intestine	**53 cm**
The average length of duodenum	**11 cm**
The average length of jejunum	**30 cm**
The average length of the ileum	**12 cm**
The average of the length of the gastrointestinal tract to body length	**2.3235**

Fig 1: Length of duodenum

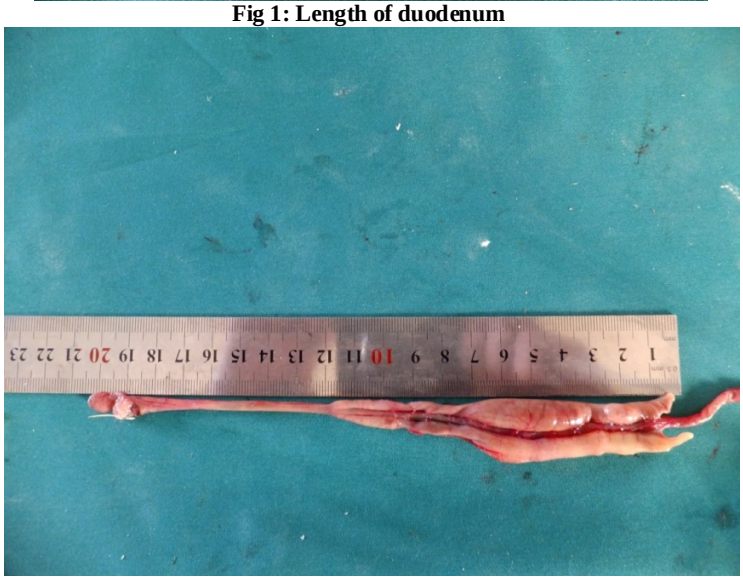

Fig 2: Length of the ileum

Small intestine villi

Small intestinal tissue is formed of different parts that the mucosal tissue is the innermost layer. This layer consists of the zigzag-shaped villi. The small intestine' villi are affected by several factors in terms of the shape and size. The villi are flattened and leaf-shaped in herbivores, while they are finger-shaped and long in carnivorous birds (4 and 5).

Intestine villi are significantly different in each section in terms of shape and size (7). According to Mouwen in 1971, the villi are divided into six categories in terms of shape: tongue, leaf, finger, bridge, filament, and complex shapes (16). With increasing the animal age and its food needs, the number and size of villi will be changed.

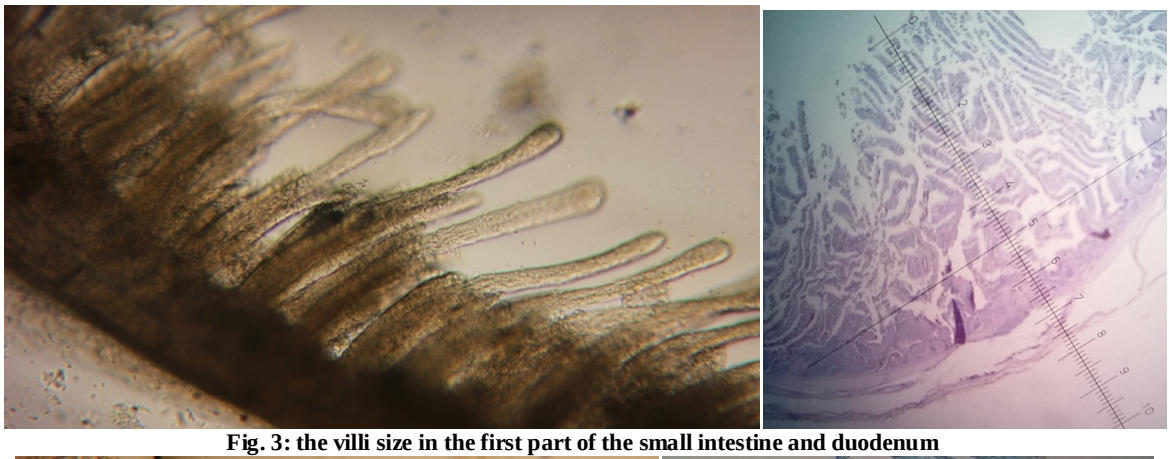

Fig. 3: the villi size in the first part of the small intestine and duodenum

Fig. 4: the villi size in the middle part of small intestine and jejunum

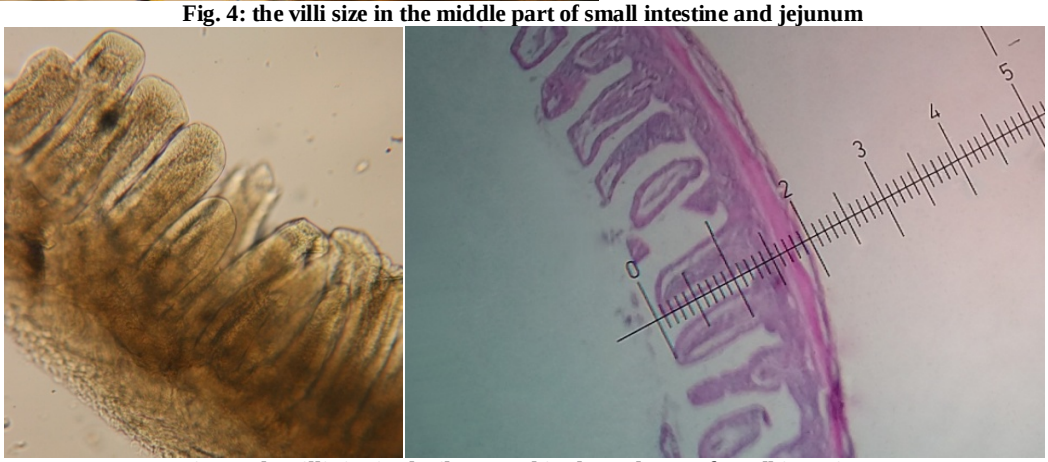

Fig. 5: the villi size in the ileum and in the end part of small intestine

Histology results

In this study, hematoxylin-eosin staining, which is the most common staining method in histological studies has been used to study the structure of small intestine tissue. In this method the nuclei are dark purple or blue and cytoplasm as well as intercellular material will be seen in pink. Periodic acid-Schiff (PAS) was used to study of goblet cells, Verhoeff staining and toluidine blue was used to study of connective tissue, mucosa, submucosa, muscular class and serous (18).

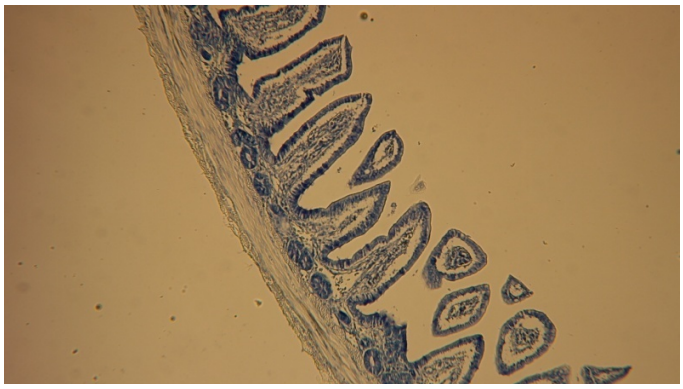

Fig. 6: Tissue layers of ileum. Observation of four-layer toluidine blue stained. Magnification ×10

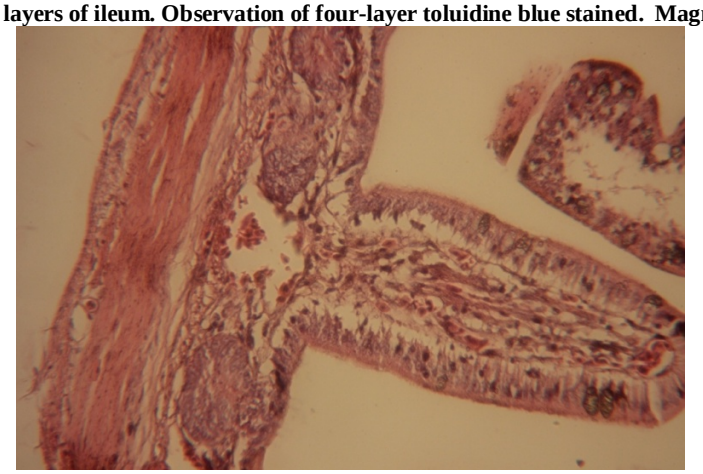

Fig. 7: Tissue layers of ileum. Observation of four-layer H&E stained. Magnification ×40

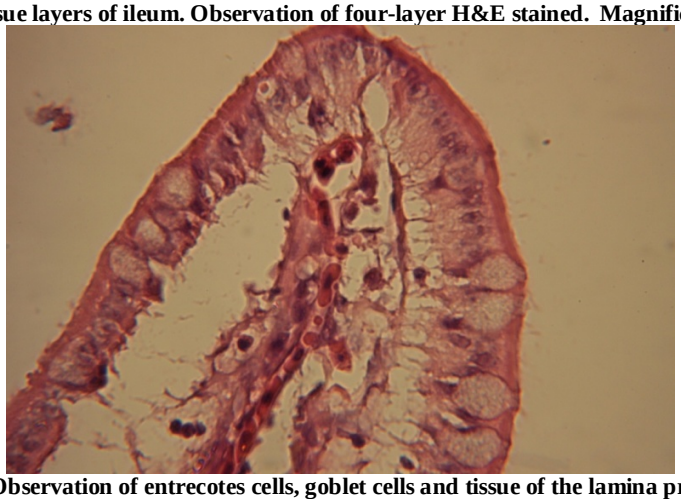

Fig. 8: Intestinal villi. Observation of entrecotes cells, goblet cells and tissue of the lamina propria along with blood vessels in the lamina propria. Hematoxylin and Eosin staining. Magnification ×100

The migratory lymphocytes with spherical and dense nuclei are located as black dots along with lamina propria connective cells tissue. To the end of the digestive tract, lymphatic cells accumulation becomes more, but in the free margin of intestine no lymphocyte is seen.

Most of intestinal goblet cells stained with periodic acid-shift are seen in purple in the case of having neutral mocopolysaccharides.

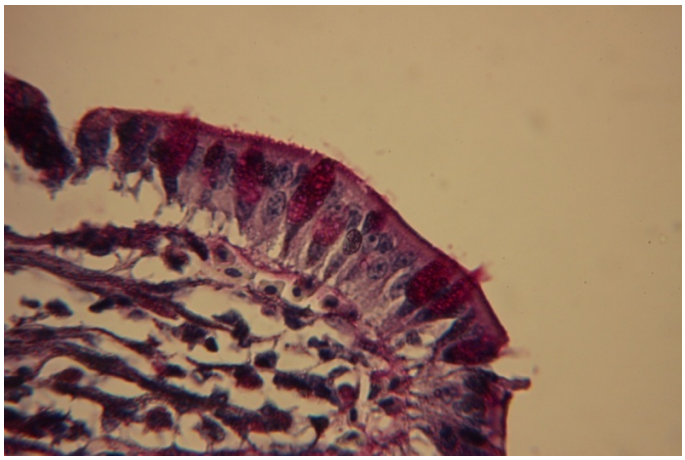

Fig. 9- goblet cells stained with periodic acid-Shift are seen in purple color. (Magnification ×100)

There is a delicate and loose connective tissue containing fibers and cells of connective type that is called the lamina propria layer. Lamina propria has a connective tissue rich in blood vessels and lymphatic organization. There are mucosal muscle filaments called Brook filaments into the villi.

Infiltration of epithelial into the lamina propria lining creates simple tubular glands known as the Lieberkuhn glands which have a tissue similar to the mucous. The glands types are simple and helical which elongated between the folds and villi.

Lieberkuhn glands are formed of three cylindrical cells called entrocytes, goblet cells and entroendocrine shape. Endocrine cells are as pyramidal forms which are stained with silver salts and located at the base of the Lieberkuhn glands. Most of the Lieberkuhn glands' cells are of mucous cells. There are specific differences in the type of mucous and gland cells. Tissue analyzing each of three sections of the small intestine in birds is not easy, because, at first, the duodenum is lack of Brunner's glands and secondly, the payers' patches are not seen in the ileum. Small intestinal epithelium in domestic poultry is similar to the mammalian small intestine and is formed of cylindrical cells called entrocytes, goblet cells and entroendocrine cells, but it is lack of Paneth cells. In the mammalian, the Paneth cells are usually located in the lower third of Lieberkuhn glands, in the form cylindrical cells with a thinner head. Eosinophilic granules on top of the cell are visible with a kind of lysosomal enzymes that are involved in the bacteria phagocyte and are effective in flour adjustment and removing the intestinal pathogens. The cells also are involved in the production of polysaccharides. However, the cells do not exist in poultry, dogs, cats and

birds. Paneth cells are of rare cells of the body that contain large amounts of zinc (18).

Brunner glands exist at the beginning of the small intestine in the duodenum of mammals. These glands secrete mucoid alkaline substance that keeps duodenal mucosa against gastric acidity. Theses glands are of combined type with the distance from the pyloric glands of the submucosa. Their number is high at the beginning of the duodenum but they decrease gradually. With a specific staining the mucus of the cells' cytoplasm can be shown. These glands don't exist in domestic poultry but mucosal secretion is done by goblet cells (18).

The Lieberkuhn glands in birds such as mammals are elongated as simple spiral tube in the connective tissue beneath the epithelial, among the intestinal villi and folds. As in mammals, the glands are seen across the small and large intestines of poultry, too.

Submucosal connective is located beneath the mucosal muscle. Submucosal layer is very thin and delicate in this bird. Submucosa lacks of gland. Smooth muscle is composed of two layers of smooth muscle cells which are annular inside and longitudinal outside the ring. Circular muscle layer has longitudinal nuclei at inner side, also the nuclei were observed spherical in longitudinal layer. The annular muscle layer is thicker at the beginning compared with the longitudinal outer layer. Auerbach neural network is seen between the two muscle layers.

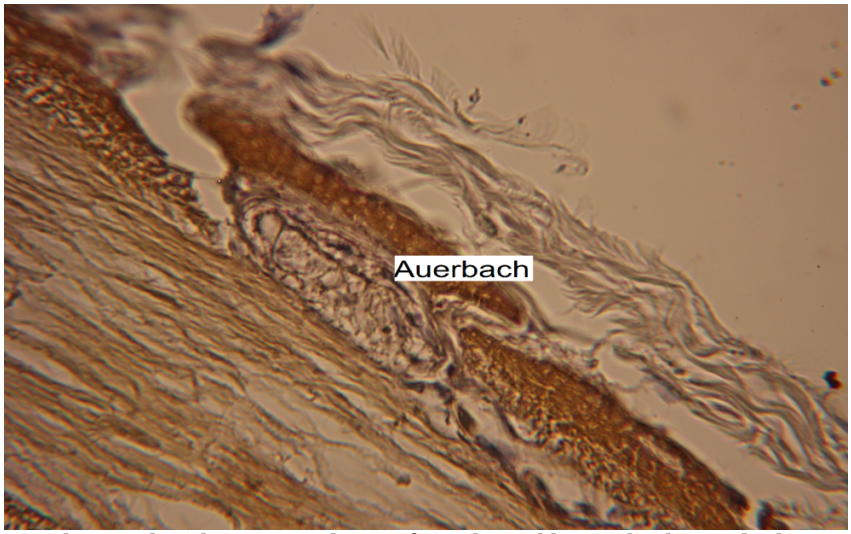

Fig. 10. Auerbach Neural Network (a) between two layers of circular and longitudinal muscular layers. Verhoeff staining-magnification ×100

The exterior part of the intestine has been covered with serous tissue which is composed of a loose connective tissue along with squamous cells on the outer surface.

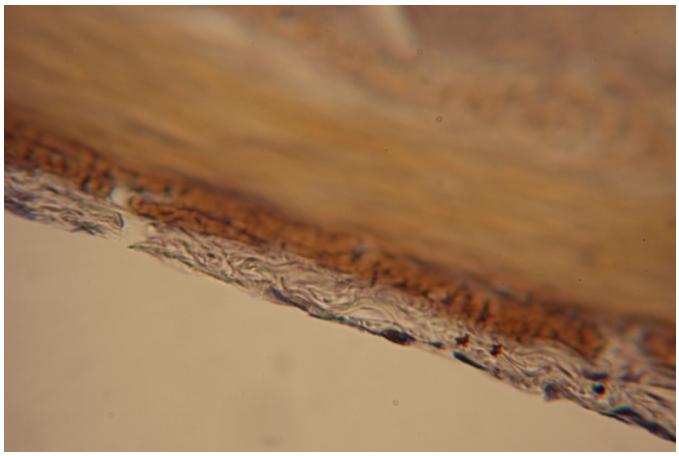

Fig. 11: Serous connective layer with a layer of squamous cells in the outermost part. Verhoeff staining - lens ×100

DISCUSSION

Digestive system of birds includes beak and mouth, esophagus, crop, proventriculus, gizzard, small intestine, cecum and cloaca. There is a reasonable relationship between different parts of the digestive system structure and digestion of certain types of feed. Birds have highly specialized digestive system, depending on the animal's eating habits is different from species to species. Mechanisms of digestion in the gastrointestinal tract cause the breaking of complex materials and changing to the suitable forms in order to absorb by the body. Some aspects of digestion are common among all species, but changes during the evolution of herbivores, carnivores and omnivores are important and it is necessary to pay special attention to them. There is a reasonable relationship among the structures of different parts of the digestive system and the digestion of certain types of feed. Even the size and length of the digestive tract in all kinds of birds which depends on their dietary habits vary considerably. The main components of animal diets are often polymeric materials that are made by the organisms forming diet. To absorb the nutrition three requirements must be met. At First, the food substance should be chemically suitable to cross the epithelial barrier (epithelium). Second, a large and adequate absorbent surface should be available and third, there should be enough time that directly associated with the length of the gastrointestinal tract (22).

The size and length of the digestive tract often depends on the birds dietary habits. The length of the birds' digestive tract is as a multiple of their body length determines their eating habits. The birds are divided into two general groups in terms of dietary: 1. the birds that have soft and bulk diet which composed of carnivorous and

ichthyophagous birds (18). The birds that have a relatively hard diet which composed of insectivorous, herbivorous, and gallinaceous birds (11).

The comparison of digestive tract as a multiple of body length in chukar shows that the bird is classified in the group with a relatively hard-substance diet, insectivorous, herbivorous, and gallinaceous. Generally, the digestive tract length of the birds is relatively short as a multiple of the body length; so, the intestine of chukar is different from mammalians' intestine due to the lack of colon.

There are notable variations among different species in terms of the folds and villi and often are seen in various and different forms. Annular folds which have villi are seen in interior part of the intestine. In general, all the villi and folds cause to increase the level of absorption (11).

Small intestinal tissue is formed of different parts that the mucosal tissue is the innermost layer. This layer consists of the zigzag-shaped villi. The small intestine' villi are affected by several factors in terms of the shape and size. The intestine villi are different significantly in terms of shape and size (7). According to Mouwen in 1971, the villi are divided into six categories in terms of shape: tongue, leaf, finger, bridge, filament, and complex shapes (16). The villi are flattened and leaf-shaped in herbivores, while they are finger-shaped and long in carnivorous birds (4 and 5).

Mucosa has long villi in all three sections of the intestine, but by approaching to the end of the small intestine the villi become shorter and thinner. Epithelial cells of the villi are in different types, including cells of the absorbent cells (entrocytes), goblet cells, and chromaffin cells. Entrocytes are the most important of them which are responsible for absorbing and are abundant at the top of the villi (4 and 21). The results of the research show that the means of Turnover is replacing old cells with young cells (24 and 25). Since, the nutrients, mineral and water in small intestine are absorbed and the Lieberkuhn crypts, water, and electrolytes are secreted by villi entrocytes, any change in the height of the villi causes and the depth of the Lieberkuhn crypts will have a significant effect on the digestion and absorption. Therefore, villi height changes (to a lesser extent) as well as the in-depth of Lieberkuhn glands lead to changes in the number of villi entrocytes and crypt cells (1, 6, and 27).

According to Creamer (1964) mucosa structure of small intestine is very flexible and any change in epithelial cells Turnover alters the shape and size of the villi (3). In

other words, the change in Turnover causes the change of villi shapes (27). According to Hampson (1986), the measurement of the villi length as well as their shape observation will be the evidences of the number of the villi entrocytes (8). In finger-shaped villi the length and the cell population are depend to each other to a large extent (1982, Wright) (27), although this phenomenon in complex forms leads to the reduced cell population in the intestine (3).

The greater number of simple villi like tongue-shaped and finger-shaped at first-degree, and the finger-shaped villi at the second degree, the more increase in improved intestinal absorption and the greater number of complex villi such as bridge-shaped, and filliform villi, the more decreased intestinal absorption (3 and 24).

So far, different studies on several species of poultry's tissue structure, tissue chemistry and ultra structure of the digestive tract have been reported and considerable differences in macroscopic, microscopic anatomy and its function have been observed. Histological studies have been conducted on small intestine of different animals such as cats (19), horses (17), camel (23), guinea pig (15), industrial poultry (12), red jungle fowl (12), broilers brand (12), Migrating Blackcaps Sylvia atricapilla (10), ostrich (2), African Ostrich Chicks (26), African peid crow (9) and Goslings (20).

Histologically, the intestinal structure of birds complies with four-layer layout of digestive tract of mammals and domestic birds (18) but has certain differences in the type of mucosal and glands cells.

Tissue analyzing each of three sections of the small intestine in birds is not easy, because, at first, the duodenum is lack of Brunner's glands and secondly, the payers' patches are not seen in the ileum. Small intestinal epithelium in domestic poultry is similar to the mammalian small intestine and is formed of cylindrical cells called entrocytes, goblet cells and entroendocrine cells, but it is lack of Paneth cells. In the mammalian, the Paneth cells are usually located in the lower third of Lieberkuhn glands, in the form cylindrical cells with a thinner head. Eosinophilic granules on top of the cell are visible with a kind of lysosomal enzymes that are involved in the bacteria phagocyte and are effective in flour adjustment and removing the intestinal pathogens. The cells also are involved in the production of polysaccharides. However, the cells do not exist in poultry, dogs, cats and birds. Paneth cells are of rare cells of the body that contain large amounts of zinc (18). Brunner glands exist at the beginning of the small intestine in

the duodenum of mammals. These glands secrete mucoid alkaline substance that keeps duodenal mucosa against gastric acidity. Theses glands are of combined type with the distance from the pyloric glands of the submucosa. Their number is high at the beginning of the duodenum but they decrease gradually. The birds lack the glands.

The Lieberkuhn glands, such mammals, are elongated as simple spiral tube in the connective tissue beneath the epithelial, among the intestinal villi and folds. As in mammals, the glands are seen across the small and large intestines of poultry, too. When become closer to the end of the digestive tract the Lieberkuhn glands become shorter and the number of goblet cells becomes greater. Beneath the epithelium and lamina propria tissue the mucosal tissue is of smooth muscle as a thick layer is observable in contrast of the mammalians. Submucosal connective is located beneath the mucosal muscle. Submucosal layer is very thin and delicate in this bird. Submucosa lacks of gland but riches of dispersed lymphatic muscle. The annular muscle layer is thicker at the beginning compared with the longitudinal outer layer. Auerbach neural network is seen between the two muscle layers. Despite differences in some tissue characteristics of poultry and mammalian digestive system, its basic structure (including the mucosa, submucosa, muscle, and serous or adventitial) is common in all of them.

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