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Morphological and histological investigation of proventriculus structure in common kestrel (*Falco tinnunculus*), steppe eagle (*Aquila nipalensis*), golden eagle (*Aquila chrysaetos*), and imperial eagle (*Aquila heliacal*)

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Abstract

The proventriculus is one of the most important parts of digestive tract in birds for mechanical and chemical digestion and can vary depending on the bird's eating habits. In the present study, the morphological and histological structure of proventriculus in common kestrel, steppe eagle, golden eagle and imperial eagle have been investigated and compared. In this study, Proventriculus of common kestrel (n=4), steppe eagle (n=5), golden eagle (n=4) and imperial eagle (n=6) were collected and fixed in 10% formalin buffer solution and the histological process were carried. Finally, H&E, Masson's trichrome, Orcein and PAS stainings were utilized. Histologically, slight differences were observed between the proventriculus of common kestrel and steppe eagle. Notable differences included a continuous and relatively thicker layer of mucosal muscle in the steppe eagle's proventriculus, and the distinct simple columnar epithelium of the submucosal glands type II ducts. The only notable structural difference in golden eagle was the presence of relatively dense connective tissue of lamina propria and one to three layers of continuous smooth muscle in its structure. The histological structure of imperial eagle's proventriculus had two differences with its structure in common kestrel. Firstly, the lamina propria was composed of relatively dense connective tissue and secondly, the muscle layer was relatively thinner than other species examined in this study, while the serosa layer had a considerable thickness. It can be concluded that the structure of proventriculus was similar in three species of eagles and common kestrel, and the main significant difference was related to the layers of mucosal muscle.

Keywords: Histology, Gastrointestinal tract, Birds of prey, Lamina propria, Mucosal layer, Muscular layer

Introduction

One of the most important parts of digestive system in birds, which is responsible for the mechanical and chemical digestion of food, is the stomach. In birds, stomach is anatomically and functionally composed of two distinct parts; the glandular stomach or proventriculus and the muscular stomach or gizzard. The proventriculus is attached to esophagus and the chemical digestion of food (by the secretion of the pepsin enzyme and hydrochloric acid) takes place in proventriculus. The gizzard on one side is connected to proventriculus by isthmus and on the other side is connected to duodenum. It is also responsible for the mechanical digestion of food. Depending on the type of diet, there are two types of stomachs, one of

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them belongs to carnivorous and fish-eating birds, which due to the use of relatively soft and bulky foods, is compatible to store this type of food. The stomach in this type of bird is bag-shaped and has a thin wall. Another type of stomach belongs to birds that eat foods such as insects, plants and seeds. Since the food of these birds is slow to digest, they need a stomach that can also do physical digestion. In this type of bird, gizzard clearly has thick and expanded muscle layers and the proventriculus-gizzard junction is easily recognizable from the outside (Bacha Jr and Bacha, 2012; McLeland, 1990).

The common kestrel belongs to Falco tinnunculus family and lives in open areas, groves, lagoons and near towns and villages. This bird is smaller than other birds of prey but larger than most sparrows. The common kestrel is found in abundance in Iran (Groombridge et al., 2002).

The Golden Eagle (*Aquila chrysaetos*) is one of the best birds of prey in the Northern Hemisphere. Like all eagles, golden eagle belongs to the eagle family. The color of these birds is dark brown, along with light brown or golden feathers on the head and neck. The golden eagle is sometimes known as the best bird among other eagles and other birds of prey (Brown, 1976). In Iran, this animal is found in many different parts of the country. Golden eagle can be found in deserts of Iran and cities such as Ardabil, Yazd, and other cities from northwest to east of the country.

The steppe eagle (*Aquila nipalensis*) is a bird of prey from the eagle family. This bird is found as an immigrant in Iran and is protected. This bird is 75 cm long. The adult bird has dark brown color or lighter, with a variety of under-wing covering, similar to other parts of the body. Beneath the wing they have uniform color with darker or lighter flight feathers, and broad dark bands are seen at the end margin of their wings, which is darker at the end of the wings. The bird's habitat is in open areas, semi-desert steppes and foothills, and near wetlands and garbage dumps in desert cities (Vazhov et al., 2013).

The Imperial Eagle (*Aquila heliaca*) is a species of bird of prey from the eagle family, which has dark brown ornamental feathers during adulthood. This bird is considered a large eagle. Females are about 25% larger than males. The imperial eagle is very similar to the golden eagle, and can be distinguished from the golden eagle by its taller beak, smoother wings during flight, white spots on the shoulders and wings, lighter and more colorful shoulders, and darker color on the rest of the body. Also, immature imperial eagles are much lighter in color than immature golden eagles. The imperial eagle's favorite habitat is open plains with few trees, and unlike many other eagles, it does not live in mountains and forests. Rabbits, hamsters and pheasants are the main prey of this eagle (Forsman, 1999).

The histological structure of proventriculus and gizzard has been reported in native sparrows (Raji and Asadi, 2013), common starlings (Sayrafi and Aghagolzadeh, 2020), domestic ducks and pigeons (Hassan and Moussa, 2012), seagulls (Selvan et al., 2008), red junglefowl (Kadhim et al., 2011), and yellow-billed grosbeak (Zhu et al., 2013). However, so far, no study has been done on the histological structure of proventriculus in common kestrel, steppe eagle, golden eagle and imperial eagle. Therefore, in the present study, the proventriculus structure in these birds was investigated and compared.

Materials and Methods

In this study, common kestrel (n=4), steppe eagle (n=5), golden eagle (n=4) and imperial eagle (n=6) which were died because of leg and wing broken, sent to the faculty of veterinary science at Bu-Ali Sina University of Hamadan. Proventriculus samples were taken and placed in 10% buffer formalin solution for fixation. After 72 hours, the samples entered the tissue passage stages (using the Tissue Processor DS9602). After completing the passage

steps and preparing the paraffin blocks, 5µm slides (Rotary Microtome DS4055) were prepared from the samples. The prepared slides entered the H&E staining process and after drying were examined by optical microscope (Medic M-107 BN). Histomorphometrical examination was performed by a Dino-Lite lens digital camera and Dino-capture 2 software (Akbari et al., 2018; Kalantari-Hesari et al., 2015; Shahrooz et al., 2018). For histochemical evaluation of proventriculus structure in four species of birds of prey in current study, three types of staining consist of Masson's trichrome (for investigation and tracking collagen fibers), Orcein (for evaluation and tracing elastic fibers), and Periodic acid Schiff (PAS) (for considering of carbohydrates density) stainings were utilized (Sayrafi and Aghagolzadeh, 2020).

Results

Histological structure of the proventriculus in common kestrel

The proventriculus of common kestrel is located along the esophagus. At the esophagus-proventriculus junction, the lamina propria glands of esophagus disappear and the squamous epithelium tissue turns to proventriculus epithelium (Fig. 1A). The structure of proventriculus in common kestrel has the general shape of tubular organs and consists of four layers of mucosa (epithelial tissue, parenchyma, and mucosal muscle), submucosa, muscular layer (muscularis) and the outermost layer (serosa), (Fig. 1B). The mucosa contains of several layers. The epithelial tissue of proventriculus in this bird is made of simple columnar tissue to long cuboidal tissue, so that in the middle part of lamina propria the cells are taller but in both ends they become shorter (Table 1). The mucosal glands originate from the epithelial tissue and penetrate into the parenchyma. The mucosal glands in this bird are mainly made of simple columnar tissue and rarely of simple branched columnar tissue, which are covered by simple cuboidal epithelial tissue. The secretions of lamina propria glands enter the end of the mucosal folds. The lamina propria of proventriculus in common kestrel is made of loose connective tissue. The mucosal muscle in proventriculus of this bird consists of one to three layers of continuous smooth muscle that separate the lamina propria from the submucosa (Fig. 1C).

The submucosa has become very large due to the presence of large glands (submucosal glands). These glands are made of composite branched columnar tissues. The secretory units in these glands as concentric rays are located around the central duct. The epithelial tissue of these glands is made of simple cuboidal type and part of the apex of these cells is separated from each other due to the lack of intercellular connections, which is so-called grape cluster shape. These glands are made of only one cell type. The cuboidal cells of these glands grow taller at the apex and turn into simple columnar type, and their cytoplasm is slightly dimmer (Figs. 1D, 1E, and 1F). The outlet ducts of these glands can be divided into three parts; the space between simple columnar cells (type one duct), the central space of each gland (type two duct) and the continuation of this space that opens between the mucosal folds (type three duct). The epithelium of all ducts was made of simple columnar tissue (Fig. 1G).

The muscular layer in common kestrel proventriculus contained three layers of smooth muscle, including the inner longitudinal muscle layer that penetrates between the submucosal glands, the middle muscle layer that is the thickest muscle layer, and finally the outer longitudinal muscle layer, which is less thick than the other layers (Fig. 1H). The outermost layer in the common kestrel proventriculus was of serosa type and contained blood vessels and nerve bundles (Fig. 1I).

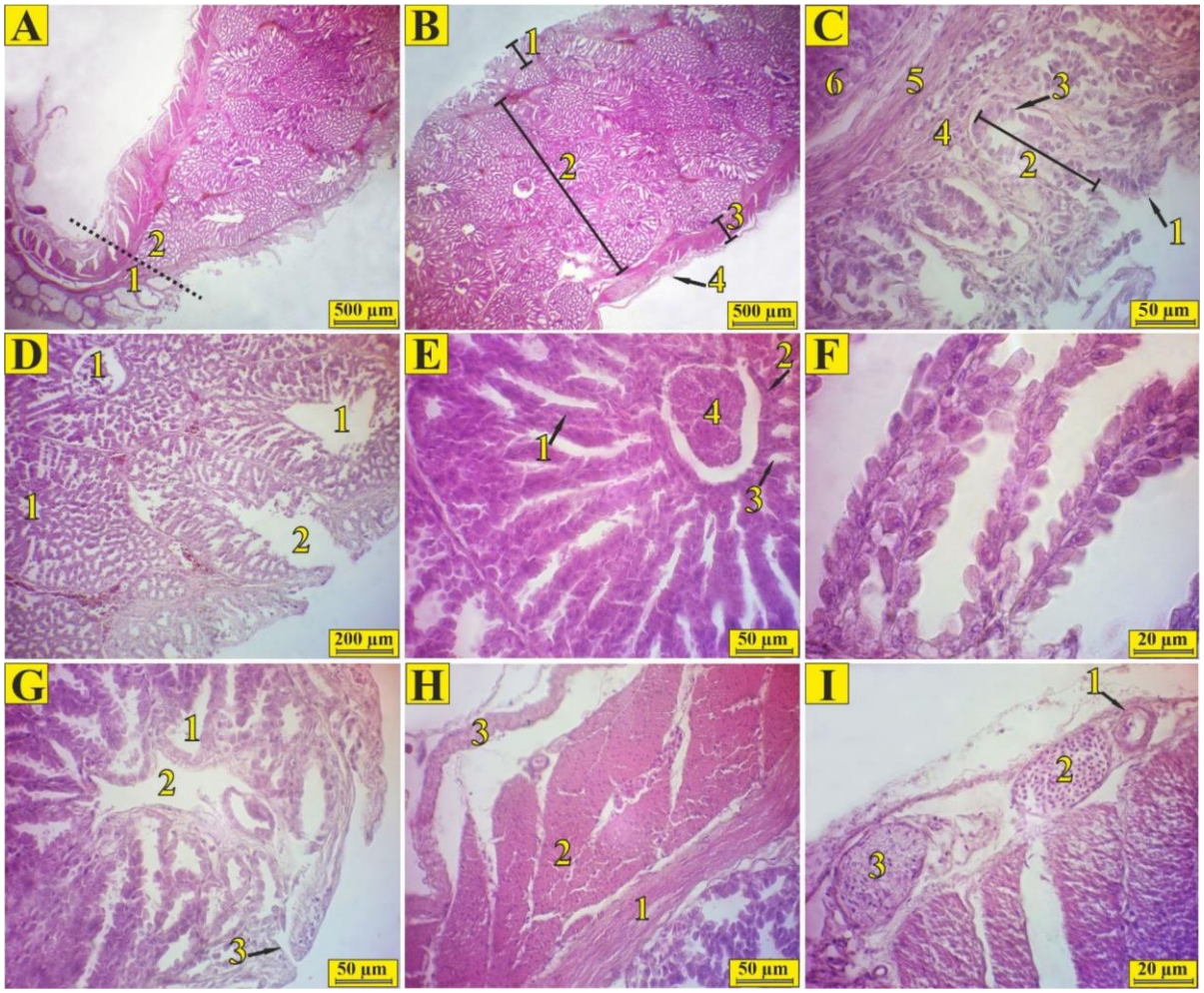

Figure 1: Histological structure of proventriculus in common kestrel (H&E staining). A: Cross section of the esophagus-proventriculus junction. 1: Esophageal tissue with parenchymal glands. 2: Proventriculus tissue with submucosal glands. B: Cross section of the initial part of proventriculus. 1: Mucosa 2: Submucosa 3: Muscularis 4: Serosa. C: Cross section of the mucosa and submucosa of proventriculus in common kestrel. 1: Simple columnar epithelial tissue 2: Simple mucosal tubular glands 3: Simple cuboidal epithelial tissue of mucosal glands 4: lamina propria 5: Mucosal muscle layer 6: Submucosa. D: Cross section of the submucosal glands' ducts of proventriculus in common kestrel. 1: Type II ducts. 2: Type I duct. E: Cross section of the submucosal gland structure in proventriculus of common kestrel. 1: Simple cuboidal epithelium covering the glands 2: Simple apical columnar cells 3: Type III ducts. 4: Type II ducts. F: Cross section of submucosal glands' secretory units in the proventriculus of common kestrel. 1: These units are covered by simple cuboidal epithelium 2: The absence of cellular apex connections 3: These cells are seen as grape clusters. G: Cross section of submucosal glands' ducts in the atrium of proventriculus of common kestrel. 1: Type III ducts. 2: Type II ducts. 3: Type I duct. H: Cross section of muscle layer in the proventriculus of common kestrel. 1: Longitudinal inner layer 2: Circular middle layer 3: Longitudinal outer layer. I: Cross section of the serosa layer in the proventriculus of common kestrel. 1: Artery 2: Vein 3: Nerve.

Histological structure of proventriculus in steppe eagle

Histologically, slight differences were observed between the proventriculus of common kestrel and steppe eagle. However, the proventriculus in steppe eagle also had tubular shape and consisted of four layers of mucosa (epithelial tissue, parenchyma, and mucosal muscle), submucosa, muscle layer, and serosa (Fig. 2A). The differences between the eagle's and common kestrel's proventriculus included; the proventriculus mucosa in the steppe eagle contained multiple and relatively short folds, and the epithelial tissue in all three initial, middle and end parts of this organ was made of

simple columnar and long cuboidal tissues (Fig. 2C). The mucosal glands in the proventriculus of this bird were made of simple tubular tissue and to a lesser extent the simple branched tissue, and the secretions of these glands were entering the end part of the folds. lamina propria was made of relatively loose connective tissue (Fig. 2B).

The mucosal muscle in the proventriculus of this bird can be seen as a continuous and relatively thick layer of smooth muscle (Fig. 2D).

The submucosa in this bird had a structure similar to that of common kestrel, only the epithelial tissue of type II ducts of the submucosal glands was made of taller simple columnar cells and had a more specific shape. These cells are easily recognizable by their lighter color (Fig. 2E). The muscle layer in the proventriculus of steppe eagle was similar to its histological structure in common kestrel. The outermost layer of this organ was made of serosa (Fig. 2F).

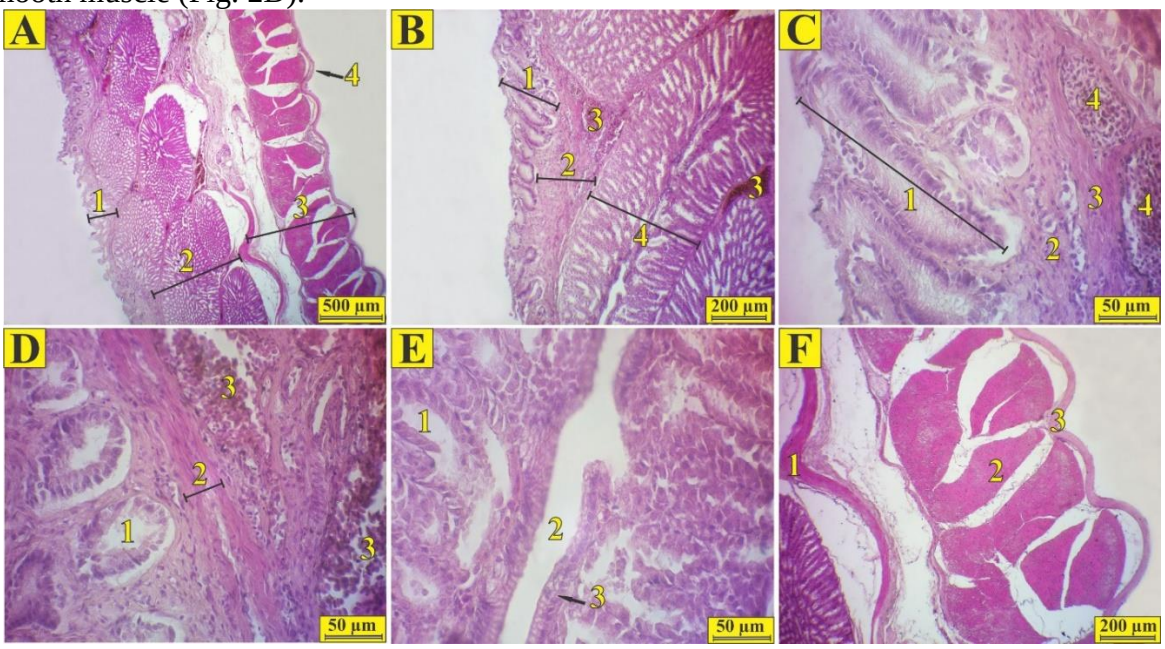

Figure 2: Histological structure of proventriculus in the steppe eagle (H&E staining). A: Cross section of the middle part of proventriculus in steppe eagle. 1: Mucosa 2: Submucosa 3: Muscle layer 4: Serosa. B: Cross section of the mucosa and submucosa of proventriculus in steppe eagle. 1: Mucosal glands 2: lamina propria 3: Blood vessels 4: Submucosal glands. C: Cross section of the proventriculus mucosa in steppe eagle. 1: Mucosal glands 2: lamina propria 3: Mucosal muscle 4: Blood vessels. D: Cross section of the mucosa and submucosa of proventriculus in steppe eagle. 1: Mucosal glands 2: Mucosal muscle 3: Blood vessels. E: Cross section of the central part of submucosal glands in the proventriculus of steppe eagle. 1: Space between glandular cells 2: Type II duct. 3: The columnar apical epithelium of submucosal glands. F: Cross section of the muscle layer of proventriculus in steppe eagle. 1: Longitudinal inner layer 2: Circular middle layer 3: Longitudinal outer layer.

Histological structure of proventriculus in golden eagle

Most of the histological structure of the proventriculus in the golden eagle was similar to the structure of this organ in the common kestrel. Significant structural differences included relatively dense connective tissue of lamina propria and the presence of mucosal muscle with one to three layers of continuous smooth muscle in its structure (Fig. 3).

Histological structure of proventriculus in imperial eagle

The structure of proventriculus in imperial eagle had only two histological differences with its structure in common kestrel. The lamina propria was composed of relatively dense connective tissue. Also,

the muscle layer that was relatively thinner compared to other species was not investigated in this study, while the serosa layer was significantly thicker (Fig. 4).

The results of histomorphometrical examination of different parts of the proventriculus in common kestrel, steppe eagle, golden eagle and imperial eagle are presented in Table 1 and 2.

Figure 3: Histological structure of the proventriculus in golden eagle (H&E staining). A: Cross section of the proventriculus mucosa and submucosa in golden eagle. 1: Mucosal folds 2: Mucosal muscle (1 to 3 layers) 3: Submucosal glands. B: Cross section of golden eagle's proventriculus mucosa. 1: Mucosal glands 2: lamina propria 3: Mucosal muscle. C: Cross section of the central part of submucosal glands in the proventriculus of golden eagle. 1: Space between secretory cells 2: Type II ducts 3: The apical columnar cells of the glands.

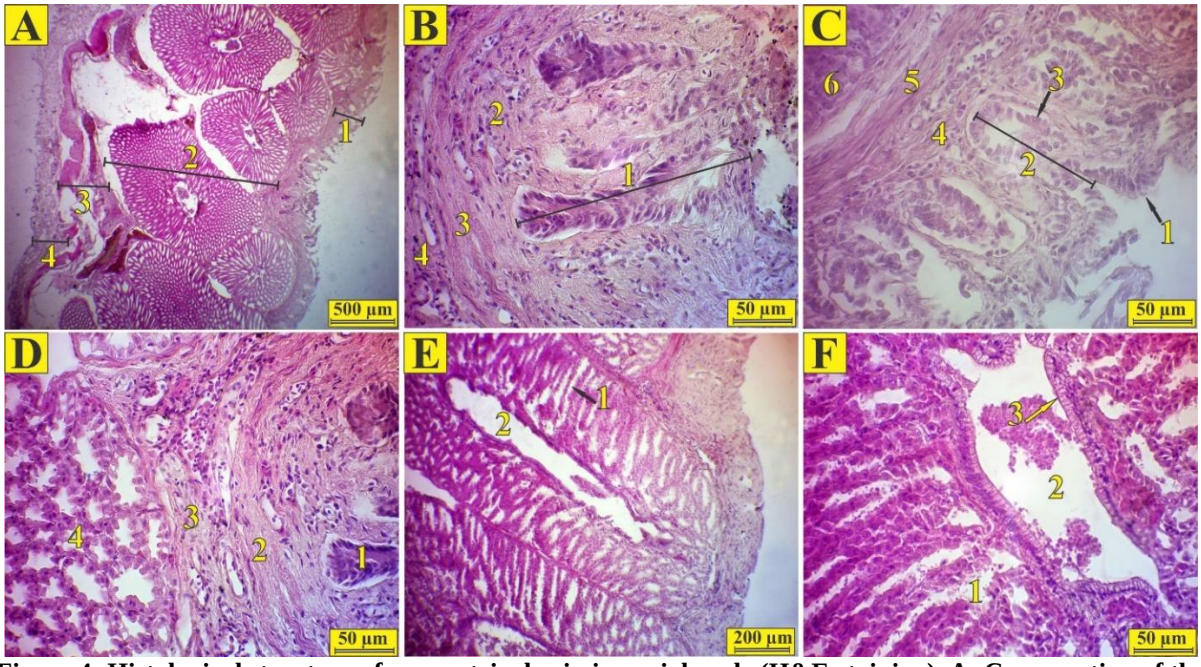

Figure 4: Histological structure of proventriculus in imperial eagle (H&E staining). A: Cross section of the end part of proventriculus in imperial eagle. 1: Mucosa 2: Submucosa 3: Muscle layer 4: Thick serosa layer. B: Cross section of the proventriculus mucosa in imperial eagle. 1: Mucosal glands 2: lamina propria 3: Mucosal muscle 4: Submucosa. C: Cross section of the proventriculus mucosa and submucosa in imperial eagle. 1: Simple superficial columnar epithelium 2: Mucosal glands 3: Simple cuboidal epithelium of glands 4: lamina propria 5: mucosal muscle 6: submucosa. D: Cross section of the proventriculus mucosa and submucosa in imperial eagle. 1: Mucosal glands 2: Mucosal muscles 3: Submucosa 4: Submucosal glands. E: Cross section of the proventriculus submucosal glands in imperial eagle. 1: Space between glandular cells 2: Type II duct. F: Cross section of submucosal gland in the proventriculus of imperial eagle. 1: Space between glandular cells 2: Type II duct. 3: Simple apical columnar cells of the glands.

Table 1: Results of proventriculus morphometry in common kestrel and steppe eagle

	Parameter	The initial one third	The middle one third	The end one third
Common kestrel	Epithelium height (µm)	9.325 ± 1.247	12.787 ± 2.235	10.437 ± 1.170
	Thickness of mucosal layer (µm)	157.970 ± 30.204	155.380 ± 41.365	60.300 ± 11.236
	Thickness of mucosal muscle (µm)	12.867 ± 2.344	9.895 ± 2.806	9.045 ± 0.904
	Thickness of submucosa (µm)	1150.365±225.947	1530.513 ± 79.149	1519.918 ± 15.341
	Thickness of muscle layer (µm)	334.235 ± 42.058	208.625 ± 32.598	208.187 ± 37.336
	Diameter of sub-mucosal glands (µm)	378.125 ± 40.294	364.497 ± 27.212	443.260 ± 46.654
Steppe eagle	Epithelium height (micrometers)	10.210 ± 0.898	9.157 ± 2.877	11.272 ± 1.543
	Thickness of mucosal layer (µm)	184.130 ± 25.453	179.180 ± 23.411	211.537 ± 12.050
	Thickness of mucosal muscle (µm)	17.250 ± 3.758	10.437 ± 1.777	37.065 ± 4.045
	Thickness of submucosa (µm)	1462.395 ± 29.718	1035.735 ± 86.339	1013.570 ± 78.546
	Thickness of muscle layer (µm)	856.680 ± 79.644	798.810 ± 78.002	887.932 ± 83.190
	Diameter of sub-mucosal glands (µm)	421.060 ± 32.189	402.487 ± 111.242	429.955 ± 29.115

All data were represented as the mean ± standard deviation.

Table 2. Results of proventriculus morphometry in golden eagle and imperial eagle

	Parameter	The initial one third	The middle one third	The end one third
Golden eagle eagle	Epithelium height (micrometers)	11.315 ± 1.718	10.211 ± 1.957	11.742 ± 1.236
	Thickness of mucosal layer (µm)	195.309 ± 29.748	166.350 ± 20.713	225.827 ± 11.117
	Thickness of mucosal muscle (µm)	19.346 ± 2.478	11.879 ± 1.465	40.088 ± 5.593
	Thickness of submucosa (µm)	1399.574 ± 35.998	1163.587 ± 75.402	999.325 ± 80.568
	Thickness of muscle layer (µm)	938.985 ± 81.471	810.856 ± 88.118	935.114 ± 86.274
	Diameter of sub-mucosal glands (µm)	318.117 ± 35.765	438.555 ± 123.298	440.875 ± 36.479
Imperial eagle	Epithelium height (µm)	10.405 ± 2.664	10.558 ± 1.872	12.456 ± 1.232
	Thickness of mucosal layer (µm)	205.569 ± 31.478	178.406 ± 21.117	230.741 ± 14.207
	Thickness of mucosal muscle (µm)	20.555 ± 1.568	12.689 ± 0.751	45.247 ± 6.704
	Thickness of submucosa (µm)	1409.785 ± 37.741	1203.607 ± 82.367	1009.744 ± 83.599
	Thickness of muscle layer (µm)	910.417 ± 80.351	863.888 ± 84.201	941.187 ± 84.354
	Diameter of sub-mucosal glands (µm)	327.124 ± 30.117	478.583 ± 118.217	432.841 ± 28.471

All data were represented as the mean ± standard deviation.

Histochemical results

Masson's trichrome staining

Histochemical examination of the proventriculus structure in all four species of birds of prey showed the same results. Thus, in Masson's trichrome staining (to examine collagen fibers) showed that these fibers were mainly present in the lamina propria of the proventriculus of all four species of birds. However, very thin streaks of collagen fibers were visible in the basement membrane and thin connective tissue around the glands, around the muscular layer, and also in the proventriculus serosa. No noteworthy differences were observed between species (Figs. 5A, 5D, 5G, and 5J).

Orcein staining

The density of the elastic fibers in the proventriculus was not very noticeable. But thin streaks of these fibers were visible in the lamina propria, basement membrane, and connective tissue around the glands, as

well as the tissue around the muscle layer. Noticeable differences were not observed between species (Figs. 5B, 5E, 5H, and 5K).

PAS staining

The density of tissues that responded positively to PAS staining was mainly in the lamina propria and basement membrane and connective tissue around the glands. However, the apical part of the epithelial tissue of some type two ducts also responded positively to PAS staining. Considerable differences were not seen between four species of birds of prey (Figs. 5C, 5F, 5I, and 5L).

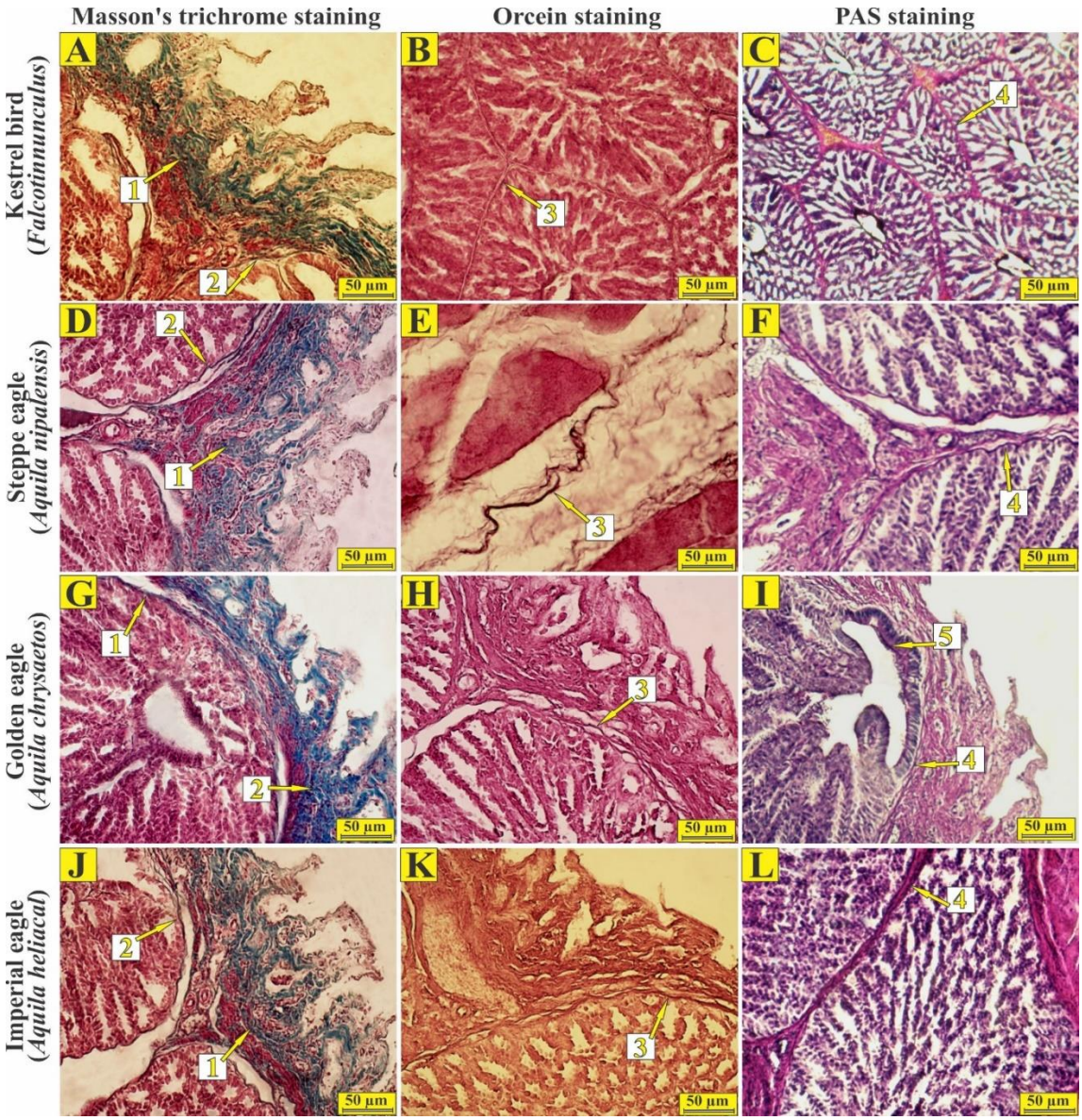

Figure 5: Histological sections of proventriculus in Common kestrel (*Falco tinnunculus*), Steppe eagle (*Aquila nipalensis*), Golden eagle (*Aquila chrysaetos*), and Imperial eagle (*Aquila heliaca*). Histochemical stainings. A, D, G, and J respectively related to Masson's trichrome staining of proventriculus in Common kestrel, Steppe eagle, Golden eagle, and Imperial eagle, 1) Density of collagen fibers in the lamina propria, 2) Density of collagen fibers in the basement membrane around the glands. B, E, H, and K respectively related to Orcein staining of proventriculus in Common kestrel, Steppe eagle, Golden eagle, and Imperial eagle, 3) Elastic fibers in lamina propria, basement membrane, and connective tissue around the glands, as well as the tissue around the muscle layer. C, F, I, and L respectively related to Masson's trichrome staining of proventriculus in Common kestrel, Steppe eagle, Golden eagle, and Imperial eagle, 4) basement membrane around glands, 5) Apical part of the epithelial tissue of type two ducts.

Discussion

The proventriculus in the native sparrow, like other birds, consists of four layers of mucosa, submucosa, muscular, and serosa (Raji and Asadi, 2013). The structure of starling proventriculus has also been reported to have four layers (Sayrafi and Aghagolzadeh, 2020). Also, in domestic ducks and pigeons (Hassan and Moussa, 2012), seagulls (Selvan et al., 2008) and red junglefowl (Kadhim et al., 2011) reports show proventriculus to have four layers. However, reports show that the structure of proventriculus in the yellow-billed grosbeak only has three layers of mucosa, muscularis, and serosa (Zhu et al., 2013). In the present study, the histological structure of proventriculus in common kestrel, steppe eagle, golden eagle and imperial eagle was observed with four tissue layers.

Proventriculus mucosa

In Jain (1976) study that was conducted on the proventriculus mucosal folds in three species of omnivorous, frugivorous, and carnivorous birds, it was shown that the mucosal folds in the proventriculus of omnivorous birds were larger than those of the frugivorous and carnivorous birds. The mucosal folds in different species of birds have branched, reticulate and leaf shapes (Catroxo et al., 1997; Jain, 1976). In other reports, the epithelial tissue of proventriculus in chicken is made of simple long columnar cells (Glevean and Katchourian, 1964), and in the American red starling is made of simple columnar cells (Catroxo et al., 1997). In starling, simple columnar epithelium has also been reported (Sayrafi and Aghagolzadeh, 2020). In most studies conducted on the proventriculus mucosa of chicken, epithelium has been reported to be made of simple columnar cells (Hassan and Moussa, 2012; Kadhim et al., 2011; Ogunkova and Cook, 2009; Selvan et al., 2008). It has also been reported that, the mucosa in the sparrow's proventriculus has longitudinal and epithelial folds that are made of simple columnar tissues. Also, the presence of two types of mucosal and submucosal glands has been reported in the sparrow's proventriculus (Raji and Asadi, 2013). The presence of longitudinal folds and simple columnar epithelium was also observed in the histological structure of proventriculus in common kestrel, steppe eagle, golden eagle and imperial eagle, which is consistent with the previous reports.

Lamina propria of the proventriculus

The lamina propria in the sparrow’s proventriculus is made of loose connective tissue, containing many blood vessels and lymphocytes. Mucosal glands have been reported to be made of simple branched tubular tissue with a simple cuboidal epithelium (Raji and Asadi, 2013). The glands in the pigeon's proventriculus have been reported to be made of branched tubular tissue (De Lima and Da Silva Sasso, 1985), with the glands making up the most thickness of proventriculus wall (Kadhim et al., 2011; Ogunkova and Cook, 2009; Zhu et al., 2013). The mucosal glands in the starling are opened in the anterior part at the base of the folds, and are made of simple tubular tissue, while in other parts the mucosal glands have been reported to be made of branched tubular tissue (Sayrafi and Aghagolzadeh, 2020). lamina propria has been reported in the American red finch, chicken, pigeons, and owls to be made of loose connective tissue, containing simple tubular glands with simple cuboidal epithelium surrounded by a capsule (De Oliveira Rocha and Inforzato De Lima, 1998). In terms of the type of connective tissue in parenchyma, the present study showed that in common kestrel and steppe eagle, this tissue was made of loose tissue and in golden eagle and imperial eagle was made of relatively dense tissue. In terms of mucosal glands and simple cuboidal epithelium, the present study is consistent with the previous reports.

Mucosal muscle of proventriculus

The mucosal muscle layer in the sparrow is made of a longitudinal smooth muscle layer that separates the lamina propria and submucosa (Raji and Asadi, 2013). In the study of proventriculus structure of starlings and ducks, the mucosal muscle layer has been reported as a thin layer in the mucosal layer. This layer of mucosal muscle surrounds the alveolar tubular glands (Hassan and Moussa, 2012; Sayrafi and Aghagolzadeh, 2020). In the present study, the mucosal muscle in common kestrel, golden eagle and imperial eagle consisted of 1 to 3 continuous smooth muscle layers, but in the steppe eagle, it consisted of a continuous and relatively thick smooth muscle layer.

Submucosa of proventriculus

The submucosal glands in the sparrow's proventriculus are surrounded by a capsule of connective tissue and are made of the branched composite tubular tissue. The secretory cells of these glands are cuboidal and cylindrical (Raji and Asadi, 2013). Submucosal glands have been reported to be made of composite alveolar tubular in starlings and three species of sparrow (Ogunkova and Cook, 2009; Sayrafi and Aghagolzadeh, 2020). In red junglefowl, the submucosal glands of proventriculus have been reported to be made of alveolar tubular tissue (Kadhim et al., 2011), while these glands have been reported as composite tubular in yellow-billed grosbeak and African pied crow (Udoumoh and Ikejiobi, 2017; Zhu et al., 2013). In most reports, the cells that make up the alveolar tubular glands have a jagged appearance (Kadhim et al., 2011; Zhu et al., 2013). It has been reported that in the starling, proventriculus glands are located in the parenchyma. These glands are surrounded by muscular cells branching from muscle layer (Sayrafi and Aghagolzadeh, 2020). In a study, Ogunkoya and Cook (2009) found that, the proventriculus glands are located between the mucosal muscle layers in three species of sparrow (Ogunkova and Cook, 2009). This is while Selvan et al. (2008) have reported these glands as submucosa in the proventriculus of Guinea fowl (Selvan et al., 2008). Each of the lobules of the proventriculus glands is surrounded by connective tissue that contains collagen and elastic fibers (Das et al., 2017). The proventriculus glands in pigeons and ducks are surrounded by muscle fibers branching from the mucosal muscle layer. Although these glands are located between the inner and outer layers of mucosal muscle layer (Sayrafi and Aghagolzadeh, 2020). the report of proventriculus structure in the starling show that the submucosal layer, as a layer of thin connective tissue, is located between the main mucosal muscle layer and inner muscle layer (Sayrafi and Aghagolzadeh, 2020). This is while another report found that, there are no submucosal glands in three Australian species of sparrow (Ogunkoya and Cook, 2009). In the present study, the submucosa was extended in all four species of birds of prey (common kestrel, steppe eagle, golden eagle and imperial eagle) and contained composite branched tubular glands composed of simple cuboidal epithelium containing three types of ducts.

Proventriculus muscle layer

Reports indicate that the sparrow's proventriculus muscle layer, like other birds, consists of three layers of smooth muscle (inner longitudinal, meddle circular, and outer longitudinal). The Auerbach neural network was visible between the muscle layers (Raji and Asadi, 2013). In starlings, Eurasian hobby and Partridge (*Rhynchotus rufescens*), the muscle layer has been reported to include the inner longitudinal layer and the outer layer (Abumandour, 2014; Rossi et al., 2005; Sayrafi and Aghagolzadeh, 2020). This is while in red junglefowl and chicken, muscle layers have been reported as inner latitudinal layers and outer longitudinal layers (Hodges, 1974; Kadhim et al., 2011).

It has been reported that in most birds, the proventriculus muscle has two layers (Hodges, 1974; Kadhim et al., 2011; Rossi et al., 2005), while Banks (1992) in his book reports three smooth muscle layers (inner longitudinal, meddle circular, and outer longitudinal) (Banks, 1992). In a study conducted on Eurasian Hobby, Abumandour (2014) reported that the muscle layer forms the major structure of proventriculus wall (Abumandour, 2014). But in the starling and Australian sparrows, it has been reported that glands make up the major structure of proventriculus wall (Ogunkoya and Cook, 2009; Sayrafi and Aghagolzadeh, 2020). The muscle layer in chicken, American red finches, and owls has been reported to have three smooth muscle layers (inner longitudinal, meddle circular, and outer longitudinal) (De Oliveira Rocha and Inforzato De Lima, 1998). In the present study, the investigation of muscle layer structure of proventriculus in all four species of birds of prey consisted of three muscle layers (inner longitudinal, middle circular and outer longitudinal). However, in the imperial eagle, the thickness of muscle layer was relatively more than other species.

Serosa

The serosa layer in the sparrow's proventriculus structure consists of connective tissue along with mesothelial cells, blood vessels, and nerves (Raji and Asadi, 2013). The serosa layer in other birds has been reported to have the same structure (Eurell and Frappier, 2013), which is the same in most birds (Deka, 2017; Hassan and Moussa, 2012; Kadhim et al., 2011; Ogunkoya and Cook, 2009). This is while the serosa layer in partridge has been reported to include connective tissue and a layer of columnar cells. In this report, the presence of smooth muscle in the serosa layer has also been mentioned (Rossi et al., 2005). Nerve ganglia in the serosa layer of proventriculus have been reported in starlings and three species of Australian sparrow (Ogunkoya and Cook, 2009; Sayrafi and Aghagolzadeh, 2020). In the present study, the outermost layer in the proventriculus of all four species of birds of prey was made of serosa and only the serosa layer was slightly thicker in the imperial eagle.

Conclusion

According to available sources, diet, eating habits, and amount of food consumed have been shown to affect the histology of gastrointestinal tract. Structural differences in the histology of proventriculus and stomach can be due to the variety and type of food consumed by the bird. In the present study, it was shown that the structure of proventriculus in four birds of prey, such as kestrel, steppe eagle, golden eagle and imperial eagle was similar to the structure of these organs in other birds, and slight structure differences were observed in our study compared to some reports.

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Conflict of interest

The authors declare that they have no conflict of interests.

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بررسی مورفولوژیکی و بافت‌شناسی ساختار پیش معده در دلیجه معمولی (*Falco tinnunculus*)، عقاب صحرایی (*Aquila nipalensis*)، عقاب طلایی (*Aquila chrysaetos*) و عقاب شاهی (*Aquila heliacal*)

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چکیده

پیش‌معده یکی از مهم‌ترین قسمت‌های لوله‌ی گوارش در پرندگان برای هضم مکانیکی و شیمیایی است و بسته به عادات غذایی پرنده می‌تواند متفاوت باشد. در پژوهش حاضر، ساختار مورفولوژیکی و بافت‌شناسی پیش‌معده در دلیجه‌ی معمولی، عقاب صحرایی، عقاب طلایی و عقاب شاهی بررسی و مقایسه شده است. در این مطالعه، پیش‌معده دلیجه‌ی معمولی (n=4)، عقاب دشتی (n=5)، عقاب طلایی (n=4) و عقاب شاهی (n=6) جمع‌آوری و در محلول بافر فرمالین ۱۰ درصد تثبیت شده و فرآیند بافت‌شناسی انجام شد. در نهایت از رنگ‌آمیزی‌های هماتوکسیلین و ائوزین، تری کروم ماسون، اورسئین و PAS استفاده شد. از نظر بافت‌شناسی، تفاوت‌های اندکی بین پیش‌معده دلیجه‌ی معمولی و عقاب صحرایی مشاهده شد. تفاوت‌های قابل ذکر شامل یک لایه‌ی ممتد و نسبتاً ضخیم از ماهیچه‌ی مخاطی در پیش‌معده عقاب صحرایی و بافت پوششی استوانه‌ای ساده مشخص در مجاری نوع II غدد زیرمخاطی بود. تنها تفاوت ساختاری قابل توجه در عقاب طلایی وجود بافت همبند پارین نسبتاً متراکم و یک تا سه لایه ماهیچه‌ی صاف پیوسته در ساختار آن بود. ساختار بافت‌شناسی پیش‌معده‌ی عقاب شاهی با ساختار آن در دلیجه‌ی معمولی دو تفاوت داشت. اولاً پارین از بافت همبند نسبتاً متراکم تشکیل شده بود و ثانیاً لایه‌ی عضلانی نسبتاً نازک‌تر در مقایسه با سایر گونه‌های مورد بررسی در این مطالعه بود، در حالی که لایه‌ی سروز ضخامت قابل توجهی داشت. می‌توان چنین نتیجه گرفت که ساختار پیش‌معده در سه گونه عقاب و دلیجه‌ی معمولی مشابه بوده و عمده تفاوت معنی‌دار مربوط به ردیف لایه‌های ماهیچه‌ی مخاطی است.

کلمات کلیدی: بافت‌شناسی، دستگاه گوارش، پرندگان شکاری، پارین، لایه مخاطی، لایه عضلانی

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