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Muscular Stomach in Birds of Prey, Common Kestrel (Falco tinnunculus), Steppe Eagle (Aquila nipalensis), Golden Eagle (Aquila chrysaetos) and Imperial Eagle (Aquila heliacal); A Morphological Evaluation

Ali Kalantari-Hesari[1*], Mohammad Babaei[2], Kaveh Esfandiyari[3] and Hassan Morovvati[4]

[1] Assistant Professor, Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
[2] Assistant Professor, Department of Clinical Sciences, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
[3] PhD Graduated of Histology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[4] Professor, Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

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Abstract

Muscular stomach or gizzard is one of the most important parts of gastrointestinal tract in birds for mechanical and chemical digestion and can vary depending on the bird's eating habits. In the present study, the morphology of muscular stomach in common kestrel, steppe eagle, golden eagle and imperial eagle has been investigated and compared. *Materials & Methods:* In this study, common kestrel (n=4), steppe eagle (n=5), golden eagle (n=4) and imperial eagle (n=6) which died because of broken leg and wing, sent to the faculty of veterinary science at Bu-Ali Sina University of Hamadan. The gizzard samples were fixed in 10% formalin buffer solution and the histological process was carried. Finally, H&E, Masson's trichrome, Orcein and PAS staining were utilized. *Results:* The most important difference between the stomach of steppe, golden, and imperial eagles and common kestrel was the lack of keratinous layer in the surface of stomach in eagles. The histological structure of stomach in imperial eagle was more similar to that of steppe eagle. The differences of the stomach of imperial eagle were due to the presence of a thick muscle layer in several different rows with different directions in comparison with steppe and golden eagles. *Conclusions:* It can be concluded that, the structure of muscular stomach in common kestrel was similar to gizzard, while the stomach in three species of eagles did not have a keratinous layer.

Keywords: Birds of prey, Common kestrel, Eagles, Histology, Muscular stomach

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 enzyme pepsin and hydrochloric acid) takes place in proventriculus. The gizzard, on one side, is connected to proventriculus by strait and on

[*] **Corresponding Author**: Ali Kalantari-Hesari, Assistant Professor, Department of Pathobiology, Faculty of Veterinary Science, Bu-Ali Sina University, Hamedan, Iran
E-mail: a.kalantarihesari@basu.ac.ir

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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 which belongs to carnivorous and fish-eating birds, which is, due to the use of relatively soft and bulky foods, compatible to store this type of food. The stomach in this type of birds 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 (Hristov, 2020; Hanafy et al, 2020; El-Mansi et al, 2021; Madkour et al, 2022).

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 (Collopy et al, 2017). 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 (HorvátH et al, 2018).

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 & 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 muscular stomach (gizzard) in common kestrel, steppe eagle, golden eagle and imperial eagle. Therefore, in the present study, the structure of this organ was investigated and compared in these birds.

Materials and method

In this study, common kestrel (n=4), steppe eagle (n=5), golden eagle (n=4) and imperial eagle (n=6) which died because of broken leg and wing, were sent to the

faculty of veterinary science at Bu-Ali Sina University of Hamadan. Muscular stomach 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). Histomorphometric examination was performed by a Dino-Lite lens digital camera and Dino-capture 2 software (Shahrooz et al, 2018; Kalantari et al, 2015; Akbari et al, 2018). For histochemical evaluation of muscular stomach 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, 2019).

Results

Histological structure of the stomach in common kestrel

The structure of stomach in common kestrel has the general shape of tubular organs and consists of four layers of mucosa (epithelial tissue, lamina propria, and mucosal muscle), submucosa, muscular layer (muscularis) and the outermost layer (serosa), (Figure 1B). The stomach mucosa in common kestrel has large folds. The surface of mucosa in this bird contains a relatively thick keratinous layer (Figures 1A and 1B).

The epithelium in all three initial, middle, and end parts of common kestrel stomach is composed of long or short columnar epithelium with basal nucleus. Superficial epithelial cells turn into simple cuboidal mucosal gland cells (Figures 1C and 1D). The secretions of rugged layer generate from the mucosal glands and after passing through the glands' ducts and superficial epithelium turn into a relatively thick layer (Figures 1E and 1F). The mucosal glands are made of simple tubular tissues that are located within lamina propria. The lamina propria is made of loose connective tissue and is filled with diffuse lymphatic tissue that fills the space between the glands (Figure 1G).

The mucosal muscle, as a continuous layer of smooth muscle, separates the lamina propria and submucosa. The submucosa is made of dense connective tissue (Figure 1H).

The not-so-thick muscle layer in the common kestrel stomach contains several relatively thin layers of smooth muscle, all in the same direction and separated from each other by a thick connective tissue. Thick serosa covers the outside of the organ and contains nerves and blood vessels (Figure 1I).

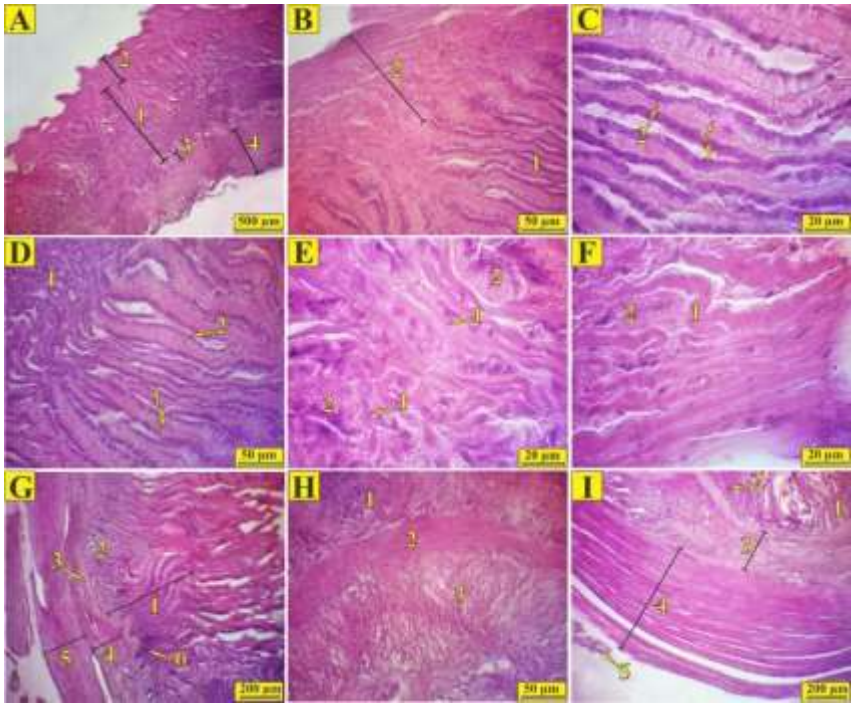

Figure 1. Histological structure of stomach in common kestrel (H&E staining). A: Cross section of the initial part of stomach in common kestrel, 1) Mucosa, 2) Keratinous layer, 3) Submucosa, 4) Muscle layer. B: Cross section of the stomach mucosa in common kestrel, 1) Mucosal glands, 2) Keratinous layer. C: Cross section of the stomach mucosa in common kestrel, 1) Simple apical columnar epithelium, 2) Simple cuboidal epithelium of mucosal glands. D: Cross section of the stomach mucosa in common kestrel, 1) Mucosal glands, 2) Simple apical columnar epithelium, 3) Simple cuboidal epithelium of mucosal glands. E, F: Cross section of the stomach secretory glands in common kestrel, 1) Secreting keratinous layer, 2) Simple apical columnar cells. G: Cross section of the middle part of stomach in common kestrel, 1) Mucosal glands, 2) lamina propria, 3) Mucosal muscle, 4) Submucosa, 5) Muscle layer, 6) Diffuse lymphatic tissue. H: Cross section of stomach mucosal muscle in common kestrel, 1) lamina propria, 2) Mucosal muscle, 3) Submucosa. I: Cross section of stomach muscle layer in common kestrel, 1) Mucus, 2) Mucosal muscle, 3) Submucosa, 4) Muscle layer, 5) Serosa.

Histological structure of stomach in steppe eagle

Histologically, slight differences were observed between the stomach of common kestrel and steppe eagle. Stomach in steppe eagle had a tubular shape and consisted of four layers of mucosa (epithelial tissue, lamina propria, and mucosal muscle), submucosa, muscle layer, and serosa (Figures 2A and 2B). The differences between the stomach of the eagle and common kestrel are due to the fact that; the stomach in the steppe eagle contained short folds and multiple villis. The most important difference was the lack of keratinous layer in the superficial part of the steppe eagle's stomach.

The epithelial tissue in all three initial, middle and end parts of this organ was made of short to tall columnar tissues (Figure 2C). The mucosal glands with simple tubular tissue and epithelium with simple columnar to simple cuboidal tissues were visible (Figure 2D). The mucosal muscle was also visible in two layers, with the upper layer being continuous and the lower one being discontinuous in some areas (Figure 2E).

The submucosa was made of relatively thick and dense connective tissue that contained blood vessels and nerves (Figure 2B). The muscle layer was relatively thick and was visible as separate bundles (separated by connective tissue). The innermost muscle layer seemed to have a different direction than the other layers. The outermost layer of this organ was made of thick serosa that contained nerves and blood vessels (Figures 2B and 2F).

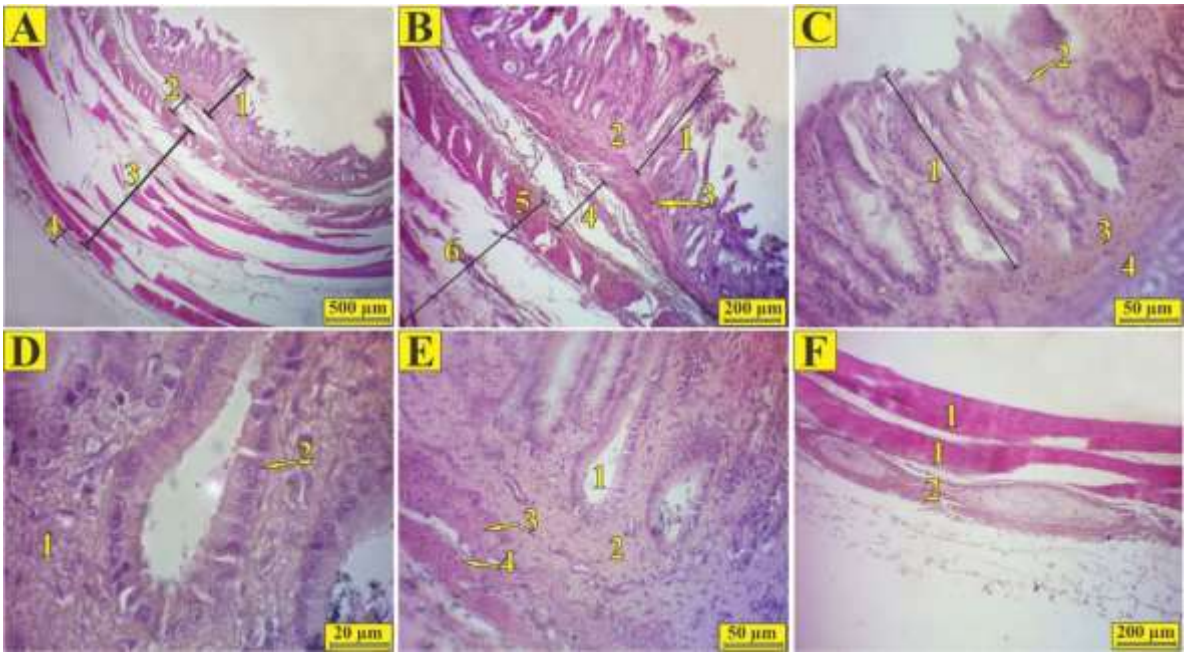

Figure 2. Histological structure of stomach in the steppe eagle (H&E staining). A: Cross section of the middle part of stomach in steppe eagle, 1) Mucosa, 2) Submucosa, 3) Muscle layer, 4) Serosa. B: Cross section of the middle part of stomach in steppe eagle, 1) Mucosal glands, 2) lamina propria, 3) Mucosal muscle, 4) Submucosa, 5) First muscle layer, 6) others muscle layers. C: Cross section of mucosa in the stomach of steppe eagle, 1) Mucosal glands, 2) Simple apical columnar epithelium, 3) lamina propria, 4) Mucosal muscle. D: Cross section of stomach mucosa in steppe eagle, 1) lamina propria, 2) Glandular epithelium. E: Cross section of the mucosal muscle layer in the stomach of steppe eagle, 1) Mucosal gland, 2) lamina propria, 3) Upper mucosal muscle, 4) Lower mucosal muscle. F: Cross section of the muscle layer in the stomach of steppe eagle, 1) Muscle layers, 2) Serosa.

Histological structure of stomach in golden eagle

The histological structure of stomach in the golden eagle was similar to that of the steppe eagle's (Figure 3). Significant structural difference between the stomach of golden eagle and common kestrel was the lack of keratinous layer in the stomach of golden eagle. Also, the mucosal muscle in the stomach of golden eagle contained two layers of smooth muscles, with the upper layer being continuous and the lower one being discontinuous in some areas (Figure 3C).

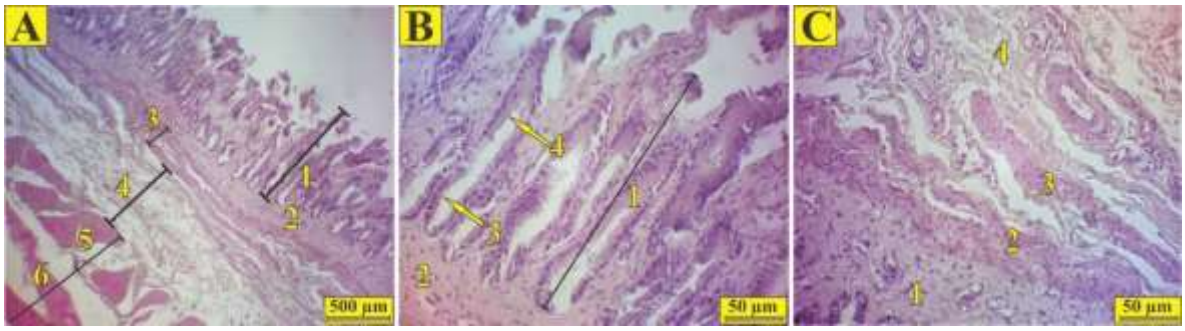

Figure 3: Histological structure of stomach in golden eagle (H&E staining). A: Cross section of the end part of stomach in golden eagle, 1) Mucosal glands, 2) lamina propria, 3) Mucosal muscle, 4) Submucosa, 5) First muscle layer, 6) Other muscle layers. B: Cross section of stomach mucosa in golden eagle, 1) Mucosal glands, 2) lamina propria, 3) Simple cuboidal epithelium of the glands, 4) simple apical columnar epithelium. C: Cross section of mucosa and submucosa in golden eagle stomach, 1) lamina propria, 2) Upper layer of mucosal muscle, 3) Lower layer of mucosal muscle, 4) Submucosa.

Histological structure of stomach in imperial eagle

The structure of stomach in imperial eagle was similar to that of the steppe eagle's (Figure 4). The mucosal layer was visible as two layers of smooth muscles, with the upper layer being continuous and the lower one being discontinuous (Figure 4A). The most significant structural difference between the stomach of golden eagle and common kestrel was the lack of clear superficial keratinous layer in the stomach of imperial eagle, although the secretion of keratinous layer between mucosal glands was visible (Figure 4B). The difference between the stomach of imperial eagle and stomach of steppe and golden eagles was due to the presence of thick muscle layer in several rows with different directions (Figure 4C).

The results of histomorphometric examination of different parts of the stomach in common kestrel, steppe eagle, golden eagle and imperial eagle are presented in Tables 1 and 2.

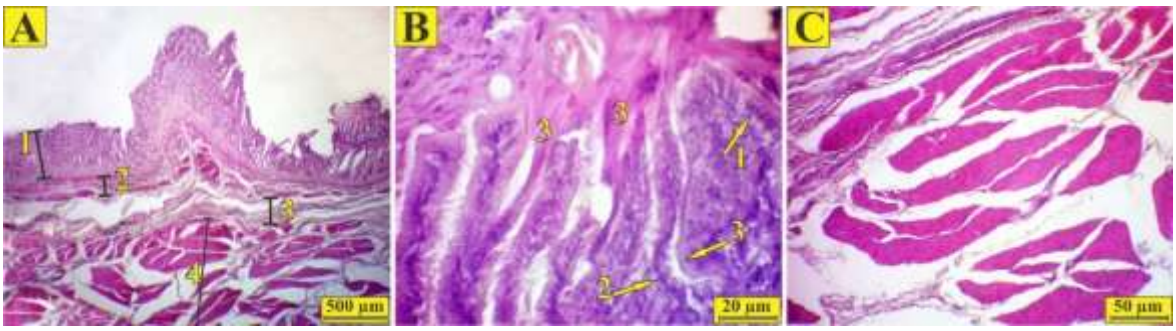

Figure 4. Histological structure of stomach in imperial eagle (H&E staining). A: Cross section of the middle part of stomach in imperial eagle, 1) Mucosa and lamina propria, 2) Mucosal muscle layers, 3) Submucosa, 4) Muscle layer. B: Cross section of the secreting stage of keratinous layer in the mucosal glands, 1) Simple apical columnar epithelium, 2) Cuboidal epithelium of the glands, 3) The secreting stratum keratinous layer. C: Cross section of the muscle layer in the stomach of imperil eagle.

Table 1. Results of stomach 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)	14.435 ± 1.174	12.640 ± 3.817	15.007 ± 0.686
	Thickness of mucosal layer (µm)	374.970 ± 74.458	322.815 ± 8.088	558.120 ± 39.037
	Thickness of mucosal muscle (µm)	39.247 ± 1.174	50.605 ± 13.611	24.697 ± 10.117
	Thickness of submucosa (µm)	261.460 ± 81.339	74.217 ± 17.078	33.242 ± 9.923
	Thickness of muscle layer (µm)	499.047 ± 3.661	969.650 ± 42.273	171.280 ± 35.604
	Diameter of sub-mucosal glands (µm)	11.515 ± 2.716	12.120 ± 0.913	13.837 ± 2.090
Steppe eagle	Epithelium height (µm)	345.407 ± 23.999	442.780 ± 51.387	489.092 ± 54.247
	Thickness of mucosal layer (µm)	31.740 ± 1.890	31.572 ± 7.722	63.637 ± 17.115
	Thickness of mucosal muscle (µm)	152.027 ± 53.876	101.402 ± 19.186	226.877 ± 25.993
	Thickness of submucosa (µm)	595.407 ± 82.824	1149.105 ± 41.552	852.255 ± 31.637
	Thickness of muscle layer (µm)	12.441 ± 2.547	11.208 ± 1.112	12.857 ± 1.226
	Diameter of sub-mucosal glands (µm)	327.446 ± 21.784	469.719 ± 47.456	441.777 ± 50.374

All data were represented as the mean ± standard deviation.

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

	Parameter	The initial one third	The middle one third	The end one third
Golden eagle	Epithelium height (µm)	29.583 ± 1.786	33.547± 5.458	67.614± 14.415
	Thickness of mucosal layer (µm)	171.372 ± 42.574	119.486± 22.104	241.417± 21.578
	Thickness of mucosal muscle (µm)	624.415 ± 71.803	1264.475± 61.741	846.475± 33.345
	Thickness of submucosa (µm)	10.499 ± 1.574756	12.145± 2.432	11.843± 1.476
	Thickness of muscle layer (µm)	362.477 ± 22.464	439.419± 50.447	455.771± 38.437
	Diameter of sub-mucosal glands (µm)	36.443 ± 2.189	29.554± 2.472	72.603± 16.347
Imperial eagle	Epithelium height (µm)	185.547 ± 40.568	127.574± 19.144	255.478± 18.774
	Thickness of mucosal layer (µm)	683.159 ± 73.458	1309.447± 475	831.499± 32.255
	Thickness of mucosal muscle (µm)	14.435± 1.174	12.640± 3.817	15.007± 0.686
	Thickness of submucosa (µm)	374.370± 74.458	322.815± 7.088	558.120± 39.037
	Thickness of muscle layer (µm)	39.247± 5.661	50.605± 13.611	24.697± 10.117
	Diameter of sub-mucosal glands (µm)	261.460± 81.339	74.217± 17.078	33.242± 9.923

All data were represented as the mean ± standard deviation.

Histochemical results

Masson's trichrome staining: Histochemical examination of the Stomach 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 stomach of all four species of birds. However, very thin streaks of collagen fibers were visible in around the muscular layer, and also in the stomach serosa. No noteworthy differences were observed between species (Figures 5A, 5D, 5G, and 5J).

Orcein staining: The density of the elastic fibers in the stomach was not very noticeable. These fibers were seen only in the submucosa layer. Noticeable differences were not observed between species (Figures 5B, 5E, 5H, and 5K).

PAS staining: In the investigation of sections stained with PAS, it was observed that the most part of lamina propria had positive reaction to PAS staining. Also, the thick basement membrane of the mucosal layer epithelium in the stomach was well visible. Considerable differences were not seen between four species of birds of prey (Figures 5C, 5F, 5I, and 5L).

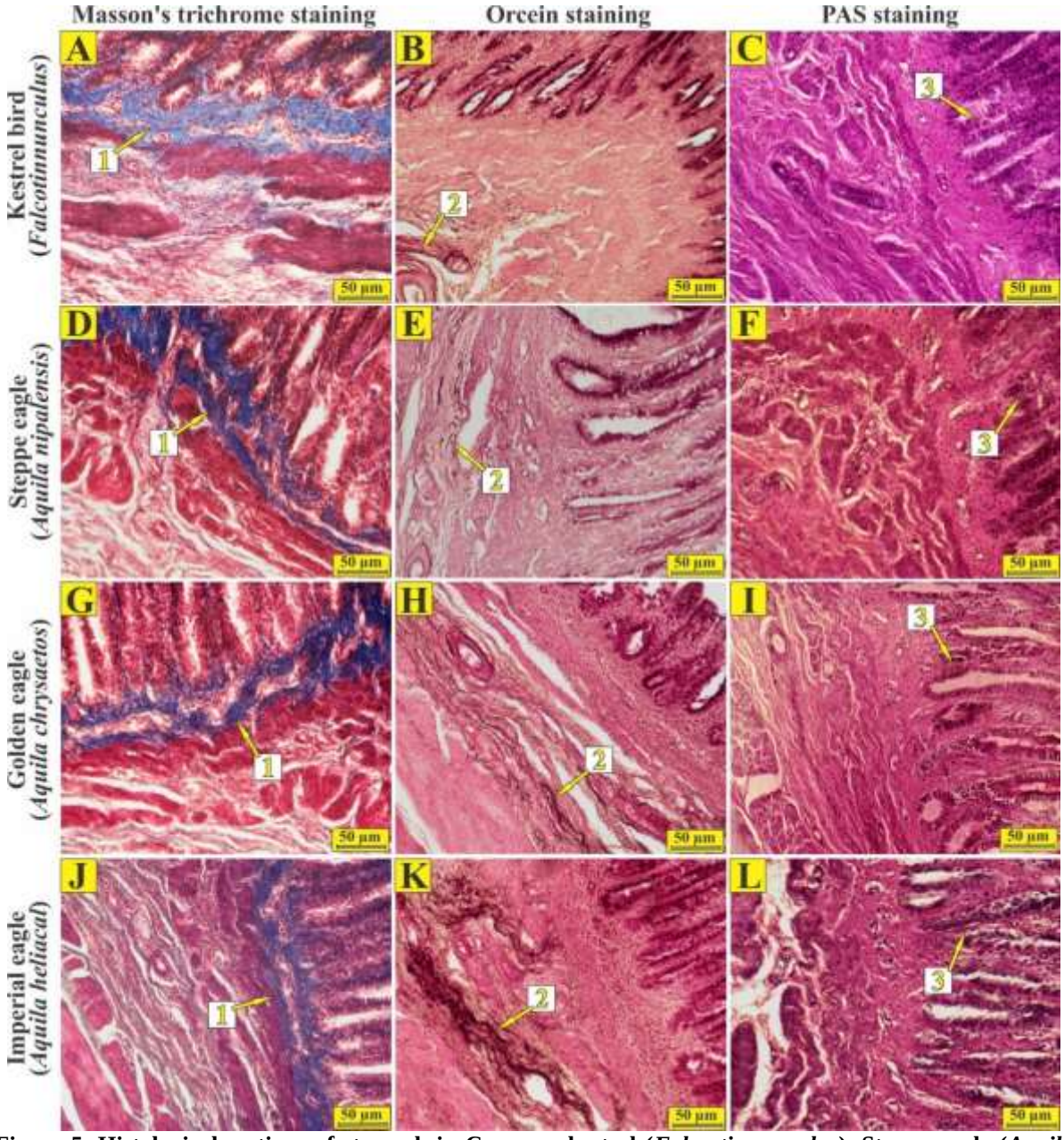

Figure 5. Histological sections of stomach in Common kestrel (*Falco tinnunculus*), Steppe eagle (*Aquila nipalensis*), Golden eagle (*Aquila chrysaetos*), and Imperial eagle (*Aquila heliaca*). Histochemical stainings. ×100. A, D, G, and J respectively related to Masson's trichrome staining of stomach in Common kestrel, Steppe eagle, Golden eagle, and Imperial eagle, 1) Density of collagen fibers in the lamina propria. B, E, H, and K respectively related to Orcein staining of stomach in Common kestrel, Steppe eagle, Golden eagle, and Imperial eagle, 2) Elastic fibers. C, F, I, and L respectively related to Masson's trichrome staining of stomach in Common kestrel, Steppe eagle, Golden eagle, and Imperial eagle, 4) basement membrane of the epithelium layer.

Discussion

The stomach in sparrow, like other birds, consists of four layers of mucosa, submucosa, muscularis, and serosa (Raji and Asadi, 2013). The stomach in starling also consists of 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 that stomach has four layers, while in yellow-billed grosbeak it only has three layers of mucosa, muscularis, and serosa

(Zhu et al, 2013). In the present study, the histological structure of muscular stomach in common kestrel, steppe eagle, golden eagle and imperial eagle was observed in four layers.

In sparrows and chickens, the mucosal folds are big and leaf-shaped. In the American red starling, mucosal folds are leaf shaped (Klem et al, 1976). The mucosal folds in owls are short and oval-shaped (De Oliveira Rocha & Inforzato de Lima, 1998). The number of mucosal folds in the red-crested finch is low (Catroxo et al, 1997). The type of food is involved in the formation and thickness of keratinous layer, and probably the higher the animal's diet in terms of wood content is, the thicker the keratinous layer will be. Studies show that the thickness of keratinous layer in omnivorous birds is thicker than in carnivorous and frugivorous birds (Jain, 1976). The mucosal layer of muscular stomach in sparrows has short longitudinal folds and its epithelium is made of simple columnar tissues. This mucosal layer is covered by a thick layer of glycoprotein (Raji and Asadi, 2013). The mucosal epithelium in chickens, owls, and red-crested finches is simple cylindrical (Catroxo et al, 1997; De Oliveira Rocha and Inforzato de Lima, 1998). In the present study, the mucosa in the muscular stomach of common kestrel was observed to have large folds containing the keratinous layer; whereas nd in the steppe eagle, the golden eagle and the imperial eagle it had large folds and multiple villis without the keratinous layer. Also, the epithelium in all four types of birds of prey studied in the present study was made of long or short columnar tissues.

The lamina propria in the sparrows, chickens, American red-crested finches and owls has been reported to be made of loose connective tissue. It is also being reported that the epithelium of the secretory units is made of simple columnar tissue. But the epithelium in some species, such as pigeons and hawks, is made of simple cuboidal tissue (Catroxo et al, 1997; Klem et al, 1976). In another report, lamina propria in the sparrow's gizzard was made of loose connective tissue and a large number of simple tubular glands were observed parallel to each other. The secretory units in the mucosa of sparrow's gizzard are made of simple tubular tissue with a simple short columnar epithelium at the base of the glands and a long columnar tissue near the epithelium (Raji and Asadi, 2013). In the present study, lamina propria was made of loose connective tissue in common kestrel and contained diffuse lymphatic tissue, and in the steppe, golden and imperial eagles, it was made of relatively dense connective tissue. lamina propria in common kestrel was also made of simple tubular glands with simple cuboidal epithelium, but in the steppe, golden and imperial eagles, it was made of simple tubular glands with simple columnar to simple cuboidal epithelium.

It has been said that mucosal muscle does not exist in owls and American red-crested finches and submucosa- lamina propria is composed of loose connective tissue. It has also been reported that mucosal muscle does not exist in sparrow's gizzard and lamina propria and therefore submucosa cannot be distinguished (Raji and Asadi, 2013). In the present study, the mucosal muscle in common kestrel contained continuous smooth muscle layer and in all three species of eagles, contained two smooth muscle layers, with the upper layer being continuous and the lower one being discontinuous.

The submucosal in all four species studied in the present study was made of dense connective tissue, but it was thicker in the steppe, golden and imperial eagles. In this regard the results of the present study are consistent with all previous reports (Catroxo et al, 1997; Klem et al, 1976).

The muscular layer in sparrow's gizzard is very thick and visible in both inner longitudinal layer and outer circular layer. Between the loose connective tissue muscles, the blood vessels and Auerbach

network were visible (Raji and Asadi, 2013). In the present study, the muscle layer in common kestrel was not so thick and different muscle layers had different directions, while in the steppe eagle and the golden eagle, the muscle layer was relatively thick and the innermost muscle layer had a different direction than other layers. In regard to the muscle layer of imperial eagle, it was observed that the muscle layer was relatively thick and the muscle layers had different directions in relation to each other.

The structure of serosa layer in chickens, sparrows, red-crested finches and owls consists of loose connective tissue along with blood vessels, nerves and fat cells (Catroxo et al, 1997; De Oliveira Rocha and Inforzato de Lima, 1998). The serosa in sparrow's gizzard is made of loose connective tissue, containing mesothelial cells, blood vessels, nerves and fat cells (Raji and Asadi, 2013). In the present study, the outermost layer of serosa was thickened in all four species.

According to the 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 stomach 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 ساختار بافت‌شناسی معده در پرندگان شکاری دلیجه (*Falco tinnunculus*)، عقاب صحرایی (*Aquila nipalensis*)، عقاب طلایی (*Aquila chrysaetos*) و عقاب شاهی (*Aquila heliacal*)

علی کلانتری‌حصاری[1*]، محمد بابائی[2]، کاوه اسفندیاری[3] و حسن مروتی[3]

[1] استادیار گروه پاتوبیولوژی، دانشکده پیرادامپزشکی، دانشگاه بوعلی سینا، همدان، ایران
[2] استادیار گروه علوم درمانگاهی، دانشکده پیرادامپزشکی، دانشگاه بوعلی سینا، همدان، ایران
[3] دانش آموخته دکترای تخصصی بافت‌شناسی، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران
[3] استاد گروه علوم پایه، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران

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چکیده

معده عضلانی یا سنگدان یکی از مهمترن قسمت‌های دستگاه گوارش پرندگان است که نقش مکانیکی و شیمیایی در گوارش داشته و ساختار آن به عادات غذایی پرنده بستگی دارد. در مطالعه حاضر، ریخت‌شناسی معده عضلانی در دلیجه، عقاب صحرایی، عقاب طلایی و عقاب امپراتور مورد مطالعه و بررسی قرار گرفت. در این مطالعه تعداد ۴ عدد دلیجه، ۵ عدد عقاب صحرایی، ۴ عدد عقاب طلایی و ۶ عدد عقاب امپراتور که به دلیل شکستگی پا یا بال تلف شده و به دانشکده پیرادامپزشکی بوعلی سینا همدان ارسال شده بودند مورد استفاده قرار گرفتند. نمونه‌های سنگدان در داخل محلول فرمالین ۱۰ درصد بافری تثبیت و وارد مراحل تهیه مقطع شدند. نهایتاً از روش‌های رنگ‌آمیزی هماتوکسیلین- ائوزین، تری‌کروماسون و اورسئین برای رنگ‌آمیزی لام‌ها استفاده شد. تفاوت عمده در ساختار سنگدان عقاب‌های صحرایی، طلایی و امپراتور و دلیجه، نبود لایه شاخی در قسمت سطحی سنگدان عقاب‌ها بود. ساختار بافت‌شناسی سنگدان در عقاب امپراتور بسیار شبیه به عقاب صحرایی بود. تفاوت قابل بیان سنگدان عقاب امپراتور وجود لایه عضلانی در چندین لایه با جهت‌گیری مختلف در مقایسه با عقاب‌های صحرایی و طلایی بود. می‌توان نتیجه گرفت که ساختار معده عضلانی در دلیجه مشابه ساختار سنگدان پرندگان دیگر است، در حالی که معده عضلانی سه گونه دیگر مورد مطالعه فاقد لایه شاخی سطحی بودند.

کلمات کلیدی: بافت‌شناسی، پرندگان شکاری، دلیجه، عقاب‌ها، معده عضلانی

* نویسنده مسئول: علی کلانتری‌حصاری، استادیار گروه پاتوبیولوژی، دانشکده پیرادامپزشکی، دانشگاه بوعلی سینا، همدان، ایران
E-mail: a.kalantarihesari@basu.ac.ir

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