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Histochemical Structure of Stomach (Proventriculus and Gizzard) in Some Bird Species

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Abstract: Stomach samples (proventriculus and gizzard) of sparrow hawk (*Accipiter nisus*), crow (*Corvus corone*) and sparrow (*Passer domesticus*) were investigated in this study. In histochemical investigations, strong sulfated mucins were not determined in compound glands of proventriculus, but a little reaction to AB (pH 0.5) in compound glands was observed in proventriculus of sparrow hawk and crow. While weak AB (pH 1.0) (+) character was found in sparrow gizzard, it was found as dense in superficial glands of sparrow hawk. However, it was not observed in crow gizzard. While AF (+) character (sulfated mucins) was detected as dense in epithelium and as weak in glands of gizzard of sparrow hawk, this character was not observed in glands of gizzards of crow and sparrow. Any reactivity to Periodic Acid-Shiff (PAS) staining was not observed in both epithelium and glands of sparrow proventriculus.

Bazı Kuş Türlerinde Mide (Proventrikulus ve Gizzard)'nin Histokimyasal Yapısı

Anahtar Kelimeler:
Mide
Atmaca
Karga
Serçe
Mukosubstans
Histokimya

Özet: Bu çalışmada atmaca (*Accipiter nisus*), karga (*Corvus corone*) ve serçeden (*Passer domesticus*) alınan mide örnekleri (proventrikulus ve gizzard) incelendi. Histokimyasal incelemelerde, proventrikulusun bileşik bezlerinde güçlü sülfatlı musinlere rastlanmadı; fakat atmaca ve karga proventrikulusunda bileşik bezlerde AB (pH 0.5)'e karşı zayıf bir reaksiyon gözlendi. Serçe gizzardında zayıf AB (pH 1.0) (+) karakter bulunurken, atmacanın superficial bezlerinde yoğundu. Bununla birlikte karga gizzardında bu karaktere rastlanmadı. AF(+) karakterin (sülfatlı musinler) atmaca gizzardının epitelinde yoğun, bezlerinde zayıf olduğu tespit edilirken; karga ve serçe gizzardlarının bezlerinde bu karakter gözlenmedi. Serçe proventrikulusunun hem epiteli hem de bezlerinde Periyodik Asit-Shiff (PAS) boyamasına karşı hiç bir reaksiyon gözlenmedi.

1. Introduction

The mucosal tissues of the systems associated with the external environment such as gastrointestinal, respiratory, reproductive, and urinary tracts are covered with resident microbial flora, which vary considerably in composition and complexity (Linden et al., 2008). Mucus is a sticky and viscous complex secretion secreted by goblet cells in columnar epithelium which is surrounding and connected with all organs. (Allen, 1981; Neutra and Forstner, 1987). In addition this, mucus glycoproteins are secreted by sweat glands, exocrine glands, mammal and tumor cells (Straus and Dekker, 1992). Mucus in this locations have a lot of functions that substance transportation, protection of hydrate level above epithelial barrier for pathogenic and toxical materials, and a transparent gel level for epithelium change under gas and foods. In addition to protection function, the mucus includes a lot of disease period (Allen, 1981). Mucus include that %95 water (maximum level) %1 salts, lipid acids, phospholipids and cholesterol, lisosomes, immunoglobulins, defensins and protecting proteins same as growing factors. Also, this material has glycoprotein consist important portion of the material. The mucus composes of glycoprotein (%2), synthesing viscous elastic features and an important portion of the material (Boat and Cheng, 1980). Mucins are high molecular weight glycoproteins which constitute the major component of the mucus layer and are produce by

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many epithelial tissues in vertebrates (Liman et al., 2010). The mucins such as sulfated or sialic acids that are classified as neutral, acidic and high-sulfated connection tissue mucins by contents of acidic groups (Jones, 1977).

Histochemical studies on the distribution of various mucosubstances in chick (Altamirano et al., 1984; Pastor et al., 1988; Jamroz et al., 2006), pigeon (Inforzato de Lima and Sasso, 1985), fowl (Imai et al., 1991), guinea fowl (Selvan et al., 2008); mucins and lipids in duck (Shyla et al., 1992) were available yet, the present study was performed to further unveil the distribution and localization of various histochemical components and to compare them in bird species (sparrow hawk, crow and sparrow) having different feeding.

2. Materials and Methods

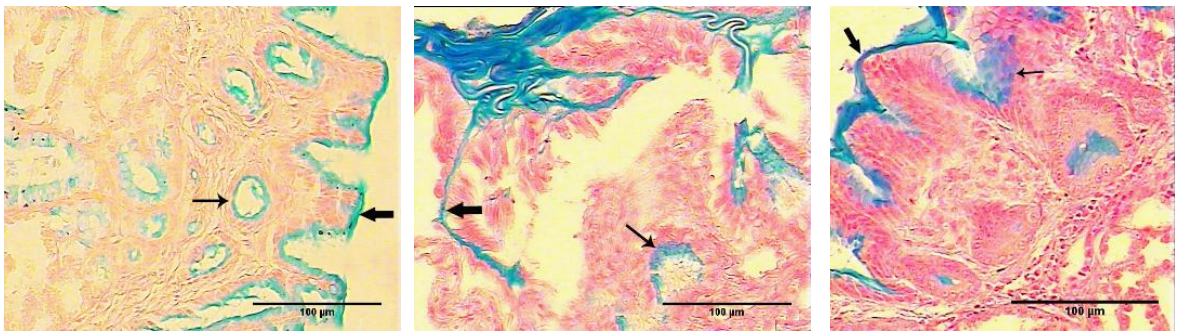
Stomach samples (proventriculus and gizzard) for the present study were removed from five sparrow hawk (*Accipiter nisus*), crow (*Corvus corone*) and sparrow (*Passer domesticus*). Apparently healthy and adult sparrow hawk (*Accipiter nisus*), crow (*Corvus corone*) and sparrow (*Passer domesticus*) were sacrificed for the present study. Tissue samples were fixed in 10% formaldehyde solution during 24 hours and then fixed materials were thoroughly washed in running water. After, these materials dehydrated in a series of alcohol series and cleared in xylene, they were embedded in paraffin wax. Sections of 6-7µm thickness were cut. The sections were then subjected histochemical staining techniques listed in Table1. The sections passed through the series of alcohol and xylene series after that were investigated under light microscopy after having been covered with entellan. The preparations were examined by light microscope that Olympus CX 41 and photographs of related regions were taken.

3. Results

It was determined that strong sulfated mucins (AB pH 0.5) were dense in *Lamina epithelialis* (*L. epithelialis)* of proventriculus of sparrow hawk (*A. nisus*), crow (*C. corone)* and sparrow (*P. domesticus)* while the mucins were few in simple glands (Fig. 1). However it was not determined strong sulfated mucins in compound glands of proventriculus of sparrow, there were weak reaction to AB (pH 0.5) in compound glands of proventriculus of sparrow hawk and crow.

Table 1: The staining techniques used in this study to determine some mucosubtances

STAINING TECHNIQUES	**MUCINS**
Alcian Blue (AB) pH 0.5 (Lev and Spicer, 1964)	Strong sulfated mucins
AB pH 1.0 (Lev and Spicer, 1964)	O-sulfate estered mucins
AB pH 2.5 (Lev and Spicer, 1964)	Carboxylated (sialic or uranic acide) mucins
Aldehyde Fuchsin (AF) (Gomari, 1952)	Sulfated mucins
Periodic Acid-Shiff (PAS) (McManus, 1948)	Neutral mucins
AB/PAS (Mowry, 1956)	Distinction between acidic and neutral mucins
AF/AB (Spicer and Mayer, 1960)	Distinction between sulfomucins and sialomucins



A B C

Figure 1: Strong sulfated mucins (AB pH 0.5) in *L.epithelialis* (thick arrows) and simple glands (thin arrows) of proventriculus. (A) Sparrow hawk, (B) Crow, (C) Sparrow. Scale Bar: 100 µm.

Strong sulfated mucins were determined as dense in goblet cells (*L. epithelialis*) of gizzard of sparrow hawk (Fig. 2A). And also, that reaction was observed as little on 2/3 of epithelial cells of gizzard in crow (Fig. 2B) and poor in sparrow. All glands of gizzard of sparrow hawk and in superficial glands of crow were observed weak and moderate reactions to AB (pH 0.5). But, it was not determined strong sulfated mucins in glands of gizzard of sparrow (Fig 2C).

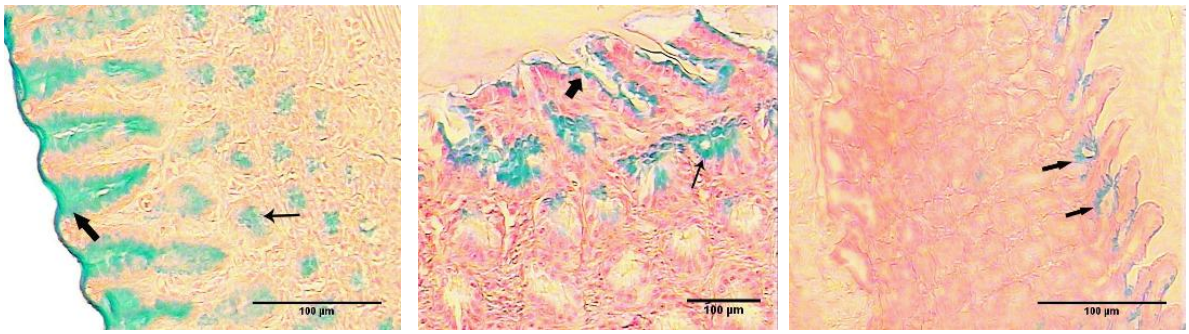


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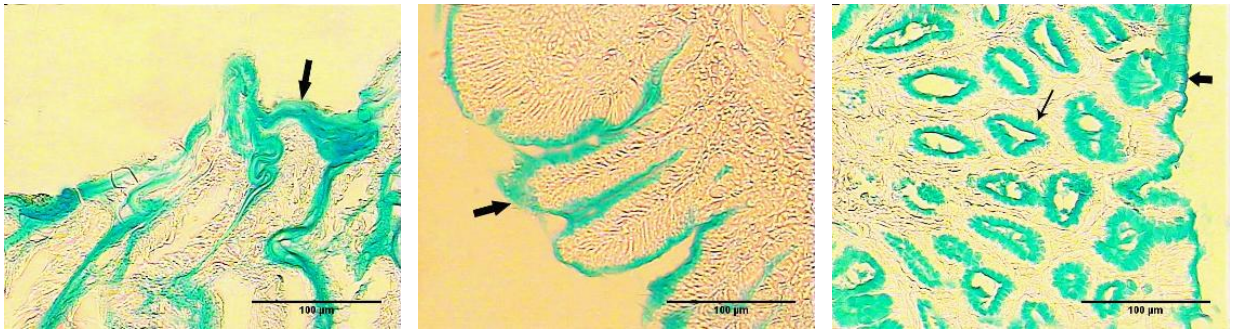
Figure 2: Strong sulfated mucins (AB pH 0.5) in *L.epithelialis* (thick arrows) and glands (thin arrows) of gizzard. (A) Sparrow hawk, (B) Crow, (C) Sparrow. Scale Bar: 100 µm.

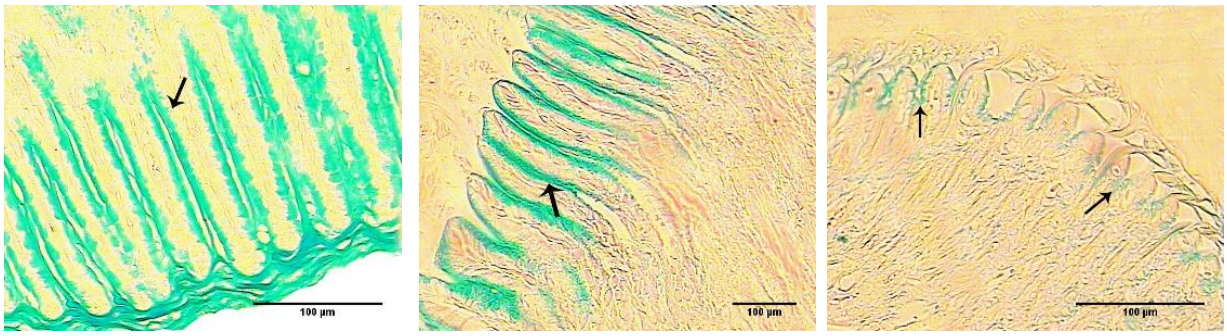
In epithelial cells, massive reactivity of AB (pH 1.0) (O-sulfate estered mucins) was determined in proventriculus of studied three species (Fig. 3A, 3B, 3C). Reactions were massive in simple and compound glands in proventriculus of sparrow hawk and in simple glands in proventriculus of sparrow (Fig. 3A, 3C). While reactivity was not observed simple and compound glands in proventriculus of crow and compound glands of sparrow.



A B C

Figure 3: Sulfate estered mucins (AB pH 1.0) in *L.epithelialis* (thick arrows) and glands (thin arrows) of proventriculus. (A) Sparrow hawk, (B) Crow, (C) Sparrow. Scale Bar: 100 µm.

While sulfate estered mucins were found little in *L.epithelialis* of gizzard of sparrow, they were determined as dense in *L.epithelialis* of gizzard of sparrow hawk and crow (Fig. 4). Positive reaction was as weak (AB pH 1.0) in sparrow gizzard and as dense in superficial glands of sparrow hawk gizzard, but this character was not observed in crow gizzard.



A B C

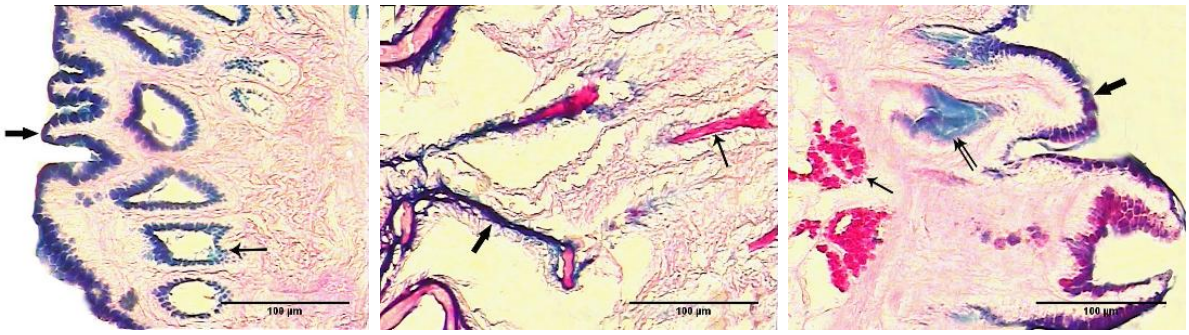
Figure 4: Sulfate estered mucins (AB pH 1.0) in *L.epithelialis* of gizzard (arrows). (A) Sparrow hawk, (B) Crow, (C) Sparrow. Scale Bar: 100 µm.

It was observed reaction was weak to carboxylated (acidic) mucins (AB pH 2.5) in simple glands of proventriculus in the studied three species. Reactivity of AB (pH 2.5) was found in *L.epithelialis* and compound glands of proventriculus of sparrow hawk densely. While carboxylated (acidic) mucin reactivity was found little in *L.epithelialis* of proventriculus of crow and sparrow, this was more little in goblet cells of crypts. In addition to, AB (pH 2.5) (+) character was observed as dense in *L.epithelialis* and as weak in glands of gizzard of sparrow hawk. Reaction was absent near to epithelial surface of gizzard of crow and sparrow but it was defined crypts and near to crypts. While reactivity for AB (pH 2.5) was defined in glands of sparrow gizzard, it was not observed in glands of gizzard of crow.

In proventriculus of sparrow hawk, sulfated mucins (AF) were defined as massive in *L.epithelialis* and simple and compound glands. In *L.epithelialis* of proventriculus of crow and sparrow, it was observed a dense reaction to AF while as weak reaction to AF was defined in simple glands. It was identified as weak AF reactivity in compound glands of proventriculus of crow but the reactivity was not observed in sparrow in this locality. While AF (+) character was detected as dense in *L.epithelialis* and as weak in glands of gizzard of sparrow hawk, this reaction was not observed in glands of gizzards of crow and sparrow. However, AF reactivity was detected as weak near to epithelial surface of gizzard of crow and sparrow.

In histochemical investigations, any reactivity to PAS staining was not observed in both *L.epithelialis* and glands of proventriculus of sparrow. PAS reaction was detected as dense (1/3 ratio) in mucous cells of gizzard *L. epithelialis* of sparrow, but this reaction was defined as weak in glands.
While glycogenic mucins were detected as dense in *L.epithelialis* of proventriculus of sparrow hawk, these were observed as weak in simple and compound glands. And also, PAS (+) character was defined as dense in mucous cells of sparrow hawk but a weak reaction was observed in superficial glands of gizzard. While the reaction was not observed in simple glands of crow proventriculus, a weak reaction was detected in *L.epithelialis* and compound glands. As the same findings at gizzard of sparrow hawk, in gizzard of crow, PAS (+) character was observed as dense in *L.epithelialis* and as weak superficial glands.

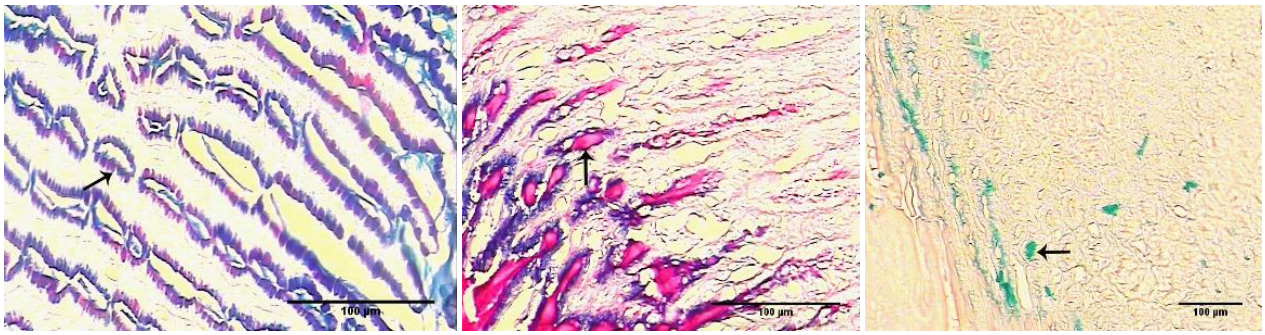
In AB/PAS (AB pH 2.5) combination staining, both AB (+) and PAS (+) characters were observed in epithelial surface and simple glands but AB (pH 2.5) (+) character (carboxylated mucins) were defined as dominant in regions near to crypts and in compound glands of sparrow hawk proventriculus (Fig. 5A). However, it was observed that mucous cells in *L. epithelialis* of proventriculus of crow and sparrow included both strong acidic and neutral mucins. PAS reactivity was defined as dominant in simple and compound glands of crow proventriculus, AB reactivity was detected as dense in these regions of sparrow (Fig. 5B, 5C).



A B C

Figure 5: Neutral and carboxylated mucins (PAS/AB) in *L.epithelialis* (thick arrows) of proventriculus of sparrow hawk (A), crow (B) and sparrow (C). Carboxylated mucins (AB pH 2.5) in simple glands of proventriculus of sparrow hawk (thin arrow) (A) and sparrow (double arrow) (C). Neutral mucins (PAS) in glands (thin arrows) of proventriculus of crow (B) and sparrow (C) (PAS/AB combination staining) Scale Bar: 100 µm.

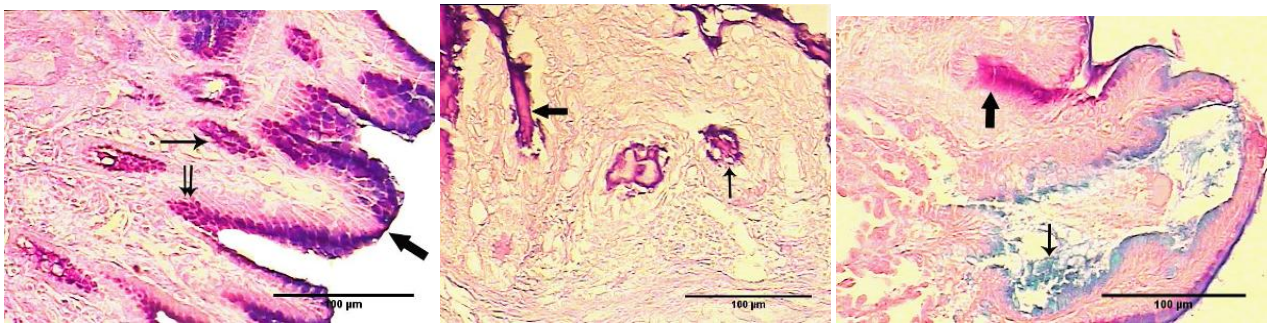
It was defined that in gizzard of sparrow hawk in both *L. epithelialis* and glands included both two mucins (Fig. 6A). While PAS (+) character was detected in these regions of crow (Fig. 6B), strong acidic mucins (AB pH 2.5) were defined at the same area in sparrow (Fig. 6C).



A B C

Figure 6: Neutral and carboxylated mucins (PAS/AB) in glands of gizzard of sparrow hawk (A); neutral mucins (PAS) in glands of gizzard of crow (B); carboxylated mucins (AB pH 2.5) in glands of gizzard of sparrow (C) (arrows) (PAS/AB combination staining). Scale Bar: 100 µm.

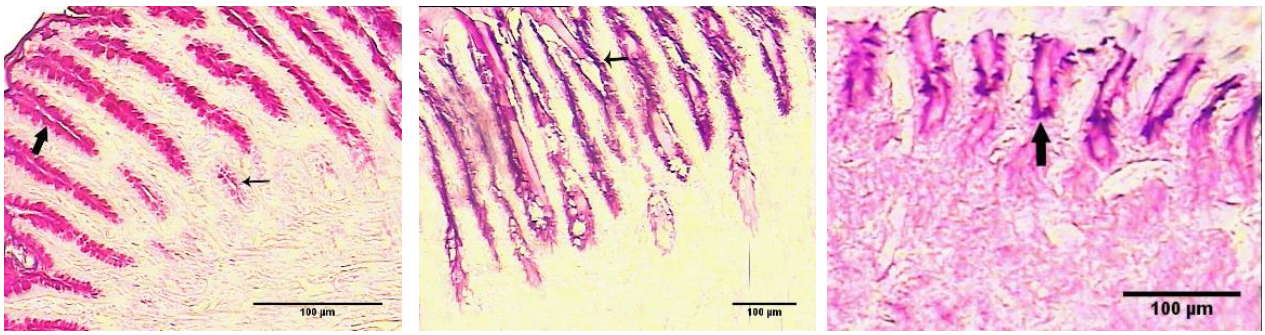
It was defined that sulfated mucins (AF) were as dominant subject to in simple glands of proventriculuses of the three species with used AF/AB (AB pH 2.5) combination staining in order to compare sulfated and carboxylated mucins. While both AF and AB (pH 2.5) reactivity were observed near to surfaces of *L. epithelialis* of sparrow hawk proventriculus, AF (+) character was dominant in crypts of epithelium and compound glands (Fig. 7A). AF reactivity was as dense in *L. epithelialis* and simple glands of crow and sparrow proventriculus (Fig. 7B, 7C). There were both sulfated and carboxylated mucins in simple glands of crow. While AB (pH 2.5)(+) character was defined dominant in bottom of compound glands, AF (+) character was detected as dense in crypts of compound glands in sparrow.



A B C

Figure 7: Sulfated and carboxylated mucins (AF/AB) in *L.epithelialis* (thick arrow), sulfated mucins (AF) in glands (thin arrow) and crypts (double arrow) of proventriculus of sparrow hawk (A); sulfated mucins (AF) in epithelial cells (thick arrow) and gland (thin arrow) of proventriculus of crow (B); sulfated mucins (AF) (thick arrow) and carboxylated mucins (AB pH 2.5) (thin arrow) in crypts of proventriculus of sparrow (C) (AF/AB combination staining). Scale Bar: 100 µm.

Although sulfated and carboxylated mucins were observed as equal density at the same local area of crow and sparrow (Fig. 8B, 8C), sulfated mucins (AF) were determined as dense in *L. epithelialis* and glands of sparrow hawk gizzard (Fig. 8A).



A B C

Figure 8: Sulfated mucins (AF) in epithelial cells (thick arrow) and gland (thin arrow) of gizzard of sparrow hawk (A); sulfated and carboxylated mucins (AF/AB) in epithelial cells of gizzard of crow (B) and sparrow (C) (arrows) (AF/AB combination staining). Scale Bar: 100 µm.

The distribution of mucosubstances and their reaction densities in proventriculus and gizzard of sparrow hawk (*A. nisus*), crow (*C. corone)* and sparrow (*P. domesticus)* was summarized in Table 2.

Table 2. Mucosubstances and reaction densities in proventriculus and gizzard of sparrow hawk, crow and sparrow

Regions / Methods	Sparrow hawk					Crow					Sparrow				
	Proventriculus			Gizzard		Proventriculus			Gizzard		Proventriculus			Gizzard	
	Le	SG	CG	Le	SG	Le	SG	CG	Le	SG	Le	SG	CG	Le	SG
AB pH 0.5	3	3	1	3	1-2	3	3	1	3	1-2	3	3	0	1	0
AB pH 1.0	3	3	3	3	3	3	0	0	2-3	0	3	3	0	0-1	0
AB pH 2.5	3	1	3	3	1	1-2	1	0	0-1	0	1-2	0-2	0-1	0-1	1
PAS	3	1	1	3	1	1	1	1	3	1	0-1	0-1	0	0-3	0
AF	3	3	3	3	1	3	1	1	1	0	3	1	0-2	1	0
PAS/AB	AB^ CD	AB^ CD	AB^	CD	CD	CD	PAS^	PAS*	PAS^	PAS*	CD	AB^	AB^	AB^	AB*
AF/AB	CD AF^	AF^	AF^	AF^	AF^	AF^	AF^	CD AB^	CD	CD	AF^	AF^	AF^ AB^	CD	CD

Le: Lamina epithelialis, SG: Simple Gland, CG: Compound Gland
Reactions: 0: negative, 1: weak, 2: moderate, 3: strong
AB^: AB dominant, PAS^: PAS dominant, AF^: AF dominant, AB*: only AB, PAS*: only PAS, AF*: only AF, CD: Co-dominant

4. Discussion and Conclusion

Chikilian and De Speroni (1996) reported that mucin heterogenity was determined not only near to surfaces of digestion tract but also in the gland cells of three species (Tinamidae).

Pastor et al. (1988) defined that sialo and sulfomucins were shown a large distribution through the digestion tract of chicken. They also reported that neutral glycoproteins (PAS) were observed in some mucous cells of proximal segment of proventriculus, in superficial cells of medial segment included only sialomucins. In addition, the authors found that PAS (+) material placed in lumen of gizzard glands but superficial cells included both sialomucins and sulfomucins. As the same findings of researchers in this study, we found that epithelial cells in proventriculus and gizzard included sialo and sulfomucins and neutral mucins located in glands of gizzard in studied three species of birds. In addition, neutral glycoproteins were observed in mucous cells of proventriculus of sparrow hawk and crow but neutral mucins were not determined at the same regions of sparrow.

In their histochemical study on poultry, Imai et al. (1991) reported columnar glandular cells were placed as a line and these cells were showed a strong reaction subject to PAS, AB (pH 2.5) and AB (pH 1.0) staining. We defined that columnar glandular cells in proventriculus of sparrow hawk and crow were respond as a positive reaction to PAS, AB (pH 1.0) and AB (pH 2.5) same as the findings of Imai et al. (1991). In sparrow, we observed that there was a respond to AB (pH 1.0) and AB (pH 2.5) but there was not PAS reaction in these cells. The authors (Imai et al., 1991) observed superficial gastric glands included neutral, weak and stronged acids, sulfuric and acid mucopolysaccharides and sialomucins. In addition, they (Imai et al., 1991) determined glandular cells of compound glands in telasubmucosa were responded as reactive to PAS in turn to AB (pH 2.5 and pH 0.5) and PAS/AB (pH 2.5). In this study, we observed that simple glands of proventriculus in the three species with strong acidic and sulfuric mucins and sialomucins but simple glands of proventriculus in sparrow hawk were contained neutral and weak acids. And then, we defined that there were a reaction the compound glands of proventriculus in three species respond to PAS/AB (pH 2.5), the compound glands of proventriculus to AB (pH 2.5) in sparrow, respond to AB (pH 0.5) compound glands of proventriculus in crow and hawk in turn to findings of Imai et al. (1991) and were not reactivity subject to PAS in compound glands of sparrow. Same as the findings of researchers (Imai et al., 1991) it was defined that compound glands of

proventriculus in sparrow hawk and crow showed PAS (+) character.

In relation to their digestion study with glycoproteins of pigeon stomach, in Inforzato de Lima and Sasso (1985) reported that there were neutral and sulfated glycoproteins in proventriculus surfaces and simple tubular glands; glycoproteins were not in gizzard and there were only glycoprotein in cuticular level on surfaces of epithelium. In this study, we determined that sulfomucins were observed epithelial surface and simple glands of proventriculus in per species although neutral mucins were only determined *L. epithelialis* of crow proventriculus and epithelium and glands of sparrow hawk proventriculus.

It was defined that epithelium and glands of gizzards of every three species included different mucosubstance which was the opposite of findings of Inforzato de Lima and Sasso (1985). The authors (Inforzato de Lima and Sasso, 1985) reported that there was no glycoprotein in tubular glands of proventriculus. The same authors (Inforzato de Lima and Sasso, 1985) defined that glycoprotein was absent in oxynticopeptic cells of branched tubular glands of proventriculus. They determined that there were neutral glycoproteins and little number of sulfoglycoproteins on epithelial surfaces of folds and lumens of crypts and also sialoglycoprotein in cuticular layer of gizzard, in the other epithelial surfaces and epithelium of glands. In this study, we defined that sialomucins were on epithelial surfaces of gizzard in three species and in epithelium of glands of sparrow gizzard but were not observed in glands of crow gizzard.

In their histochemical study on stomach of chick feed with plant materials, Jamroz et al. (2006) found that mobilization of mucocyts in epithelial surface of gizzard and secretion of neutral mucopolysaccharides were high and there were little sialomucins. Our findings on neutral and sialomucins in *L. epithelialis* of gizzards of the three species were parallel with Jamroz et al. (2006).
As result, it was defined that histochemical character and mucosubstance composition in stomach mucosa were changed with feeding manner.

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