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Structural Differences of the Harderian Gland between Common Pheasants (Phasianus Colchicus Talischensis) and Hybrids of Italian Amber and Common Pheasants

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■Keywords

Harderian gland, histology, histochemistry, common pheasant, hybrid of Italian amber and common pheasant.

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ABSTRACT

The aim of the present study was to demonstrate the histological and histochemical structure of the Harderian gland in wild and hybrid of wild and domestic birds. The samples were stained with haematoxylin-eosin, methyl green-pyronin Y, periodic acid-Schiff, alcian blue pH 2.5, aldehyde fuchsin and Hale's dialyzed iron staining's. In both species, the glands had multilobar tubuloacinar structure type I. The Harderian gland was located in the orbit near the inter-orbital septum, between the medial rectus muscle, the pyramidal muscle of the third eyelid, and the ventral oblique muscle. In the common pheasant, the gland was wider in the proximal and distal part. The common pheasant had more elongated lobes of the Harderian gland than in the hybrid. In the common pheasant, the glandular cells presented darkly-stained serous secretion and lightly-stained mucous secretion. In the hybrid, the glandular cells presented seromucous secretion. The central lobar space, interacinar space, and apical parts of the acini of the Harderian glands were filled with many lymphocytes and plasma cells, particularly in the common pheasant, where centers of all large lobes were abundantly filled with plasma cells. The plasma cells dominated in common pheasant's Harderian gland, while in the hybrid, lymphocytes and plasma cells were present at similar quantities. The cells positive for periodic acid of Schiff staining were dominant in hybrid. Periodic acid-Schiff, Hale's dialyzed iron and alcian blue pH 2.5 stainings demonstrated acid-carboxylated mucopolysaccharides in the glandular cells cytoplasm of the examined birds.

INTRODUCTION

The Harderian gland (HG) of birds is a dominant orbital gland which plays an important role in the immune response of the ocular region and of the upper respiratory system (Mobini, 2012). According to Burns (1992), birds may present three types of HG. The HG with compound tubuloacinar structures, lobule composed of a one type of epithelial cells and a large age-dependent population of plasma cells in the interstitium of the gland are characteristic for the first type of gland observed in domestic fowl. The second type has compound tubular structures and a lobe with two types of epithelial cells in the tubule and a much smaller population of plasma cells, like in the duck. The third type is regarded as "mixed" and is typical of rooks (Burns, 1992). The HG produces lacrimal fluid; hence, the main function of this gland is to lubricate the surface of the eyeball and third eyelid (Baba et al., 1990). This gland is also as a source of pheromones and growth factors (Frahmand & Mohammadpour, 2015; Khan *et al.*, 2007; Kozlu & Altunay, 2011; Klećkowska-Nawrot *et al.*, 2015). Furthermore in birds, the HG is a lymphoepithelial organ which, together with the spleen, the bursa of Fabricius, and the caecal tonsils, belongs to a system of avian organs that determines both general and local immunity (Khan

et al., 2007; Nasrin *et al.*, 2013). The gland is a site of activation and terminal differentiation of B-cells, as well as plasma cell proliferation (Khan *et al.*, 2011; Koskela *et al.*, 2003; Savage *et al.*, 1992; Scott *et al.*, 1993; Tsuji *et al.*, 1993). The fowl HG has a large age-dependent populations of plasma cells and is capable to produce antibodies both to systemically and locally applied antigens (Burns, 1992). The plasma cells of HG produce or participate in the transmission of four classes of immunoglobulins: IgA, IgG, IgM and IgY (Ohshima & Hiramatsu, 2002; Bejdic *et al*., 2014).

Information on the anatomy and histochemical analysis of the HG in birds are available in literature (Mobini, 2012; Dimitrov & Nikiforov, 2005). However, HG structural differences, including immune cell component, between wild and hybrids of wild and domestic birds have not been reported yet. Thus, the present study aimed investigating the histological structure, histochemistry of the HG of common pheasants (*Phasianidae*, *Phasianus colchicus talischensis*) and of a hybrid strain of Italian amber (*Phasianidae, Gallus gallus f. domestica*) with common pheasant.

MATERIAL AND METHODS

2.1 Animals

The HGs used in the study were obtained from six female common pheasants and six female hybrids of Italian amber and common pheasant. All birds were clinically healthy, adults (from three to six years of age), and were kept under the same environmental conditions (free-range). These birds came from the collection of the Institute of Animal Breeding, Division of Poultry Breeding, University of Environmental and Life Sciences in Wroclaw. This material was obtained as a result of natural death of birds. The samples for this study (HG), were collected directly after the birds' death. Under the Polish law, the *post mortem* examination of tissues derived from animals that died naturally does not require the Ethics Committee approval (Parliament of the Republic of Poland, 2012).

2.2 Macroscopic study

The glands were grossly examined before fixation. The studies were conducted with stereoscopic Zeiss Stemi 2000-C microscope (Carl Zeiss, Jena, Germany). The shape of HGs were described in all of examined birds. Morphometric measurements (length, width, thickness) of the glands were done using electronic slide caliper (accuracy 0.1 mm). Measurements were submitted to analysis of variance and means were compared by the Student's *t*-test ($p<0.05$).

2.3 Microscopical examination

2.3.1 Histological study

For histological examination, the entire glands were fixed in 4% buffered formaldehyde. After fixation, samples were rinsed under running water for 24 h, processed in a vacuum tissue processor (ETP; model RVG3, INTELSINT, Italy), embedded in paraffin, and cut on sliding microtome Slide 2003 (Pfm A.g., Germany) into 3-4 µm sections. All samples were stained with hematoxylin-eosin (H&E) to examine the general structure, and the methyl green-pyronin Y (MGP Y) method was used to examine the plasma cells.

2.3.2 Histomorphometric study

The histological measurements of main glandular structures, including capsule and interlobar septa thickness, and the diameters of the lobes, acini, and ducts (tertiary, secondary and primary) were conducted with use of Axio Vision 4.8 (Carl Zeiss MicroImaging GmbH, Jena, Germany) program. Data were statistically processed with Student's *t*-test ($p<0.05$).

2.3.3 Histochemical analysis

The histochemical analysis of HGs was conducted in order to identify the presence of neutral glycoproteins, glycogen, glycolipids, and phospholipids using periodic acid-Schiff staining (PAS); acidic sialylated glycoproteins and acidic sulfated mucosubstances using Alcian blue at pH 2.5 (AB pH 2.5); sulfated acid mucopolysaccharides (SAM) and carboxylated acid mucopolysaccharides (CAM) using Hale's dialyzed iron staining (HDI); and sulfated acid mucopolysaccharides (SAM) with aldehyde fuchsine staining (AF). All obtained slides were examined under a Zeiss Axio Scope A1 light microscope (Carl Zeiss, Jena, Germany), applying Axio Vision Release 4.8.2 SP2 program for histological and histochemical description. The histochemical staining scoring system was based on the standard protocol, where (-) indicated a negative reaction; (+) a weak reaction; (++) a mild reaction and (+++) a strong reaction (Spicer & Henson, 1967).

RESULTS

3.1. Gross anatomy

The HGs of the common pheasant and hybrid of Italian amber and common pheasant were located in the orbit near the inter-orbital septum, between the medial rectus muscle, the pyramidal muscle of the third eyelid and the ventral oblique muscle. In the examined birds, an efferent duct was located on the periorbital

surface of the gland, and its exit was situated in the lower conjunctival sac, between the third eyelid and the cornea. The HGs, both in the common pheasant and the hybrid strain, were elongated in shape and light pink in color (Figure 1a, 1b), and presented similar shape. However, in common pheasant, the gland was wider in the proximal and distal part (Figure 1a). The central part of HG was markedly narrower in the common pheasant, while the gland was insignificantly longer in hybrids. The mean size of the HG in common pheasant was 13.18 (±0.58) x 5.25 (±0.15) x 1.61 (±0.05) mm and 14.34 (±2.98) x 2.75 (±0.16) x 1.58 (±0.07) mm in the hybrids. The results of macroscopic measurements (HG length x width x thickness) are shown in Figure 2 and Table 1 (mean ± standard deviation).

3.2 Histological study

The histological examination revealed that the structure of HG of common pheasants and hybrids is multilobar tubuloacinar, which is consistent with the first type of Burns' (1992) classification. The results of histological measurements (capsule and interlobar septa thickness, and the diameters of the lobes, acini, and tertiary, secondary and primary diameters of the ducts are presented in Figure 3 and Table 1.

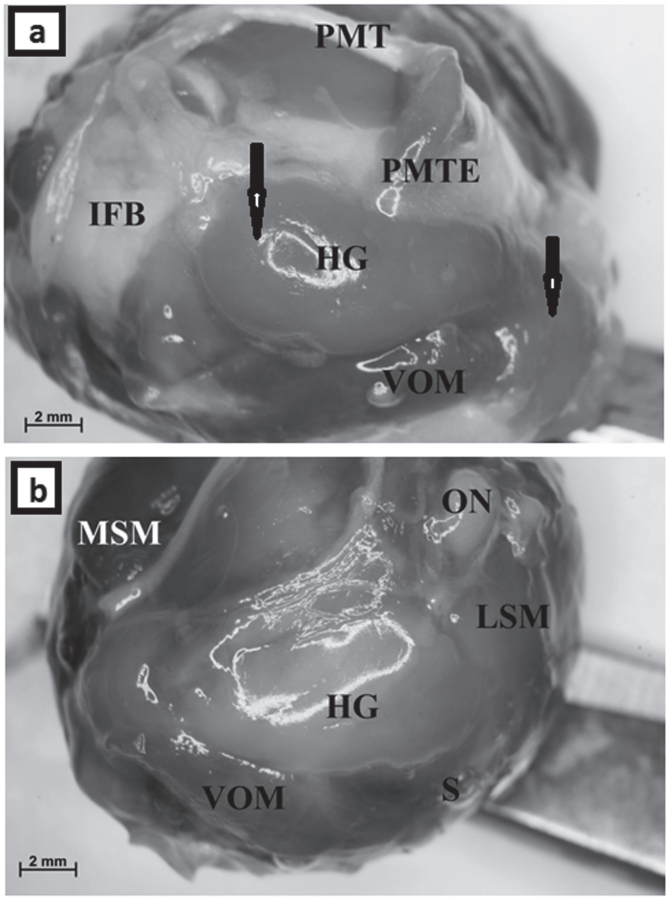

Figure 1 – Macroscopic view of posterior part of common pheasant (a) and hybrid of Italian amber and common pheasant (b) eyeball before fixation. Bar = 2 mm.

HG – Harderian gland, ON – optic nerve, S – sclera, VOM – ventral oblique muscle, LSM – lateral straight muscle, MSM – medial straight muscle, PMTE – pyramidal muscle of the third eyelid, PMT – pyramidal muscle tendo, IFB – intraperiorbital fat body.

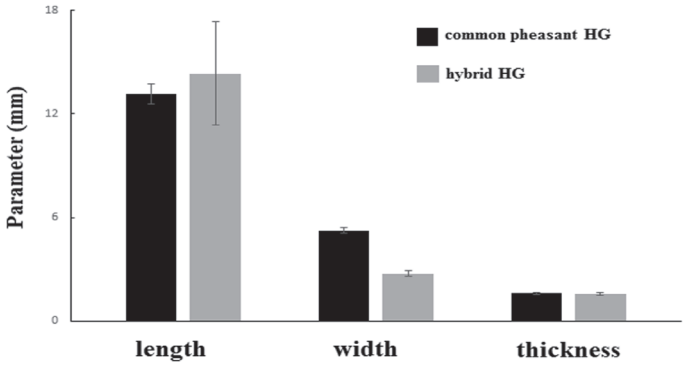

Figure 2 – Morphometric parameters (mm) of the Harderian gland in common pheasant and hybrid of Italian amber and common pheasant. Explanations: HG – Harderian gland. Values are expressed as mean ± standard deviations.

Table 1 – Morphometric parameters of Harderian gland in common pheasant and hybrid of Italian amber and common pheasant. Values are expressed as mean ±standard deviations.

Parameters	Common pheasant	Hybrid
length (mm)	13.18 (±0.58)	14.34 (±2.98)
width (mm)	5.25 (±0.15)	2.75 (±0.16)
thickness (mm)	1.61 (±0.05)	1.58 (±0.07)
thickness of capsule (μm)	39.21 (±3.51)	43.17 (±3.96)
thickness of interlobares septa (μm)	27.23 (±2.14)	34.15 (±2.14)
outer diameter of lobes (μm)	265.96 (±14.1)	187.83 (±10.03)
outer diameter of acini (μm)	68.39 (±7.94)	45.75 (±7.53)
outer diameter of ducts (μm)		
tertiary	71.39 (±3.16)	69.16 (±2.95)
secondary	119.12 (±8.57)	121.32 (±9.61)
primary	169.39 (±9.93)	173.18 (±10.12)

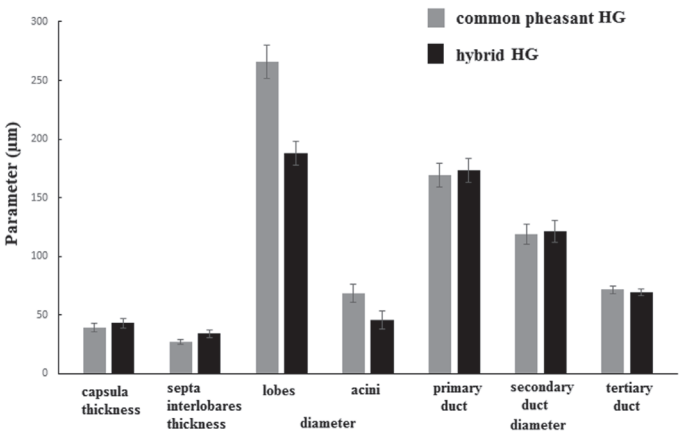

Figure 3 – Morphometric parameters (μm) in acini, primary, secondary and tertiary ducts, septa interlobares, lobes and capsula of the Harderian gland in common pheasant and hybrid of Italian amber and common pheasant. Values are expressed as mean ± standard deviations.

The glands in the examined birds were surrounded by a thin connective tissue capsule. The average thickness of the capsule was 39.21 µm (±3.51) in the common pheasant and 43.17 µm (±3.96) in the hybrids, respectively. The connective tissue capsule was composed of tightly-packed layers of fibroblasts and elastic and collagen fibers (Figures 4a, 4b). The capsule was continuous with the interlobar trabeculae, which contained blood vessels, in addition of fibroblasts, elastic and collagen fibers (Figure 4c). The average thickness of the interlobar septa was 27.23 µm (±2.14) in the common pheasant and 34.15 µm (±3.01) in the hybrids. There were no significant differences in capsule and interlobar septa thickness between both species. The septa of this capsule penetrated into the glands and divided them into lobes of varying sizes (Figures 4a, 4b). The lobes emptied into a wide lumen of a primary duct (Figures 4b, 6c and 6d) lined with columnar epithelial cells of varying heights. In the common pheasant, the apical cells of the corpus glandulae were darkly stained and contained serous secretion (Figure 4a), while deeper portions of the corpus glandulae were lightly stained and contained mucous secretion (Figure 4c). In the hybrids, the apical cells of the corpus and also deeper portions of the corpus glandulae had visible seromucous secretion (Figure 4b).

Beyond the similarities, some evident differences were also observed in the HG histological structure between the common pheasants and the hybrids. The HG lobes of the common pheasant were much more elongated (Figure 4a). The average outer diameter of the lobes as determined by histometry was 265.96 µm (±14.1) in the common pheasant, and 187.83 µm (±10.03) in the hybrids (Figure 3 and Table 1). Both in the common pheasant and in the hybrids, some HG lobes were associated with solitary lymph nodules (Figures 4b, 4d and 5a). The acini were lined with columnar epithelium. The acini lumen was spherical and elongated in the hybrids, and markedly elongated in the common pheasant (Figures 6c, 6d). The mean outer diameter of the glandular acini was 68.39 µm (±7.94) in common pheasant and 45.75 µm (±7.53) in hybrids (Figure 3 and Table 1). There were no significant

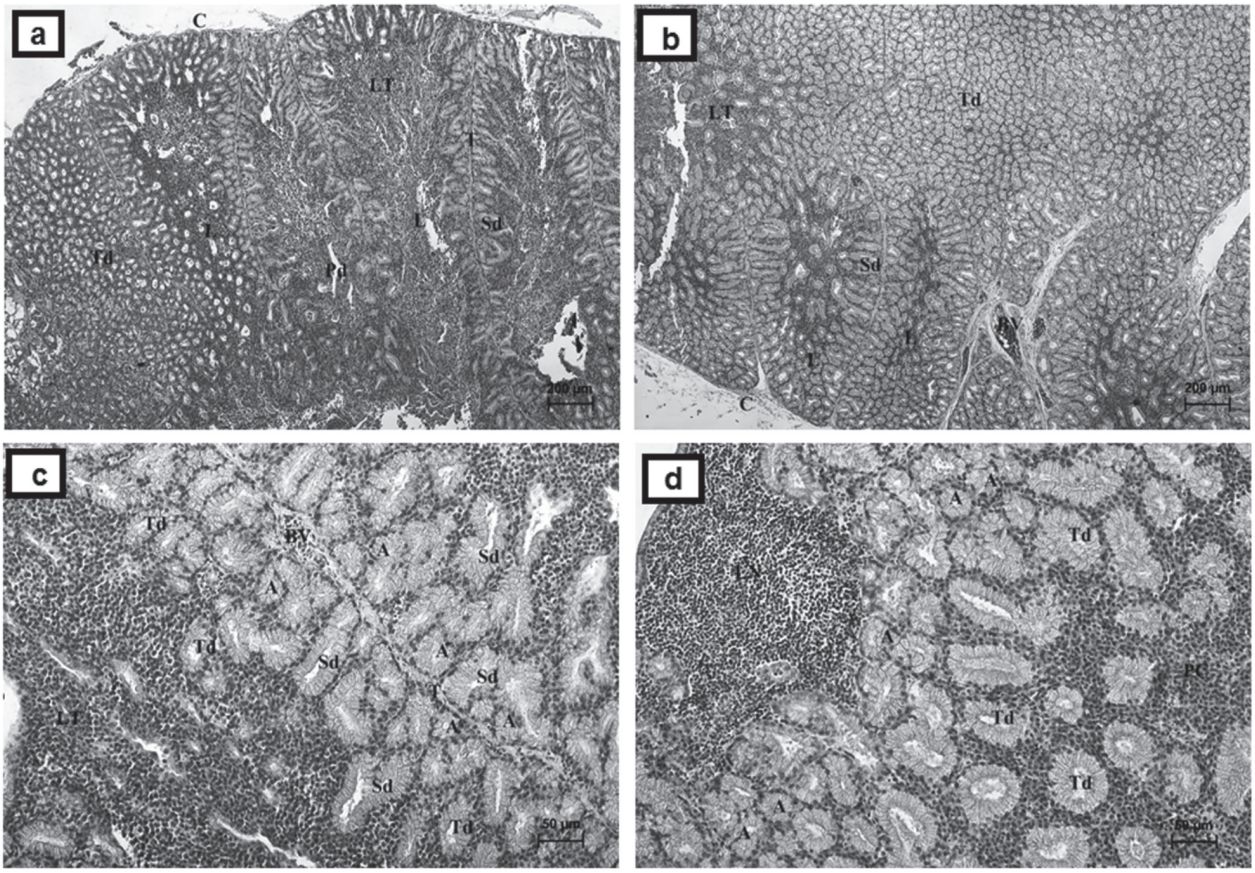

Figure 4 – Light micrograph of Harderian gland. H&E stain **a.** common pheasant. Bar = 200 µm; **b.** hybrid of Italian amber and common pheasant. Bar = 200 µm; **c**. common pheasant. Bar = 50 µm; **d**. hybrid of Italian amber and common pheasant. Bar = 50 µm.

C – capsule, L – lobes, T – trabeculae, A – acini, Pd – primary duct, Sd – secondary duct, Td – tertiary duct, BV – blood vessels, LT – lymphatic tissue, PC – plasma cells, LN – lymph nodule.

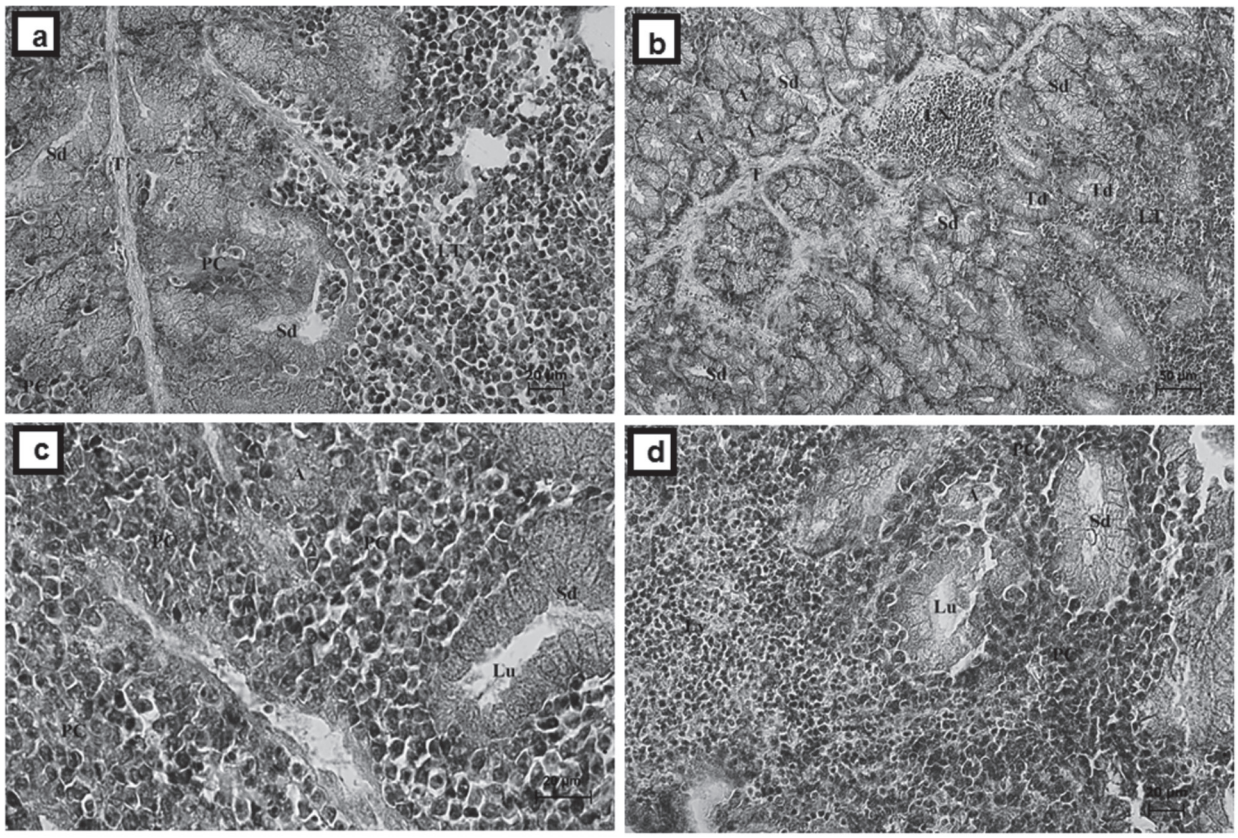

Figure 5 – Light micrograph of Harderian gland. MGP Y stain. **a**. common pheasant. Bar = 20 μm; **b**. hybrid of Italian amber and common pheasant. Bar = 50 μm; **c**. common pheasant. Bar = 20 μm; **d**. hybrid of Italian amber and common pheasant. Bar = 20 μm.

T – trabeculae, Lu – lumen, A – acini, Sd – secondary duct, Td – tertiary duct, LT – lymphatic tissue, PC – plasma cells, LN – lymph nodule, Ly – lymphocytes.

differences in the diameter of the lobes and the acini between birds. Acini were observed at the periphery of the lobes and their short tertiary ducts led to secondary ducts, which in turn emptied into a primary duct (Figures 4b, 6c). The secondary and tertiary ducts were lined with basal layer of cuboidal cells forming its large and irregular lumen (Figures 4c, 4d). The mean outer diameters of the three types of ducts of the common pheasant were different: 71.39 μm (±3.16) for the tertiary, 119.12 μm (±8.57) for the secondary, and 169.39 μm (±9.93) for the primary ducts (Figure 3 and Table 1). Also, the mean outer diameters for the three types of ducts of the hybrids were different: 69.16 μm (±2.95) for the tertiary, 121.32μm (±9.61) for the secondary, and 173.18 μm (±10.12) for the primary ducts (Figure 3 and Table 1).

3.3 Histochemical study

Small differences in the histochemistry of the HG structure were detected between the common pheasant and hybrids. The MGP Y staining method, applied to demonstrate the presence of plasma cells, showed that these cells were more abundant in the HG of common pheasants than in hybrids (Figures 5a, 5c). However in hybrids, the higher number of lymphocytes were located among plasma cells (as a single cells or aggregation of cells) (Figures 5b, 5d). The plasma cells had characteristic blue nucleus and pink cytoplasm (Figures 5c, 5d). In PAS staining, rare PAS-positive cells with blue nuclei and slightly-pink or colorless cytoplasm were observed in epithelial cells of glandular ducts (Figures 6a, 6b). In both species, the vestigial amount of slightly-pink mucus was demonstrated (Figures 6a, 6b). However, significantly more PAS-positive cells, classified as weakly (+) and averagely (++) positive, were observed in the HG of hybrids (Figure 5b). The HDI staining indicated the presence of carboxylated acid mucopolysaccharides (CAM), stained blue, in the HG epithelial cells of both species (Figures 6c, 6d) as well as of lymphatic cells. The AF staining showed a negative reaction (-) in the acini and ducts of all birds (Figures 7c, 6d). Additionally, the AB pH 2.5 staining indicated the presence of plasma cells (pink nuclei), acidic sialylated glycoproteins (blue) and acidic sulfated mucosubstances in the cytoplasm of glandular cells (Figures 7a, 7b). All epithelial cells of the corpus glandulae and of the duct systems reacted positively to AB pH 2.5 staining both in common pheasants and

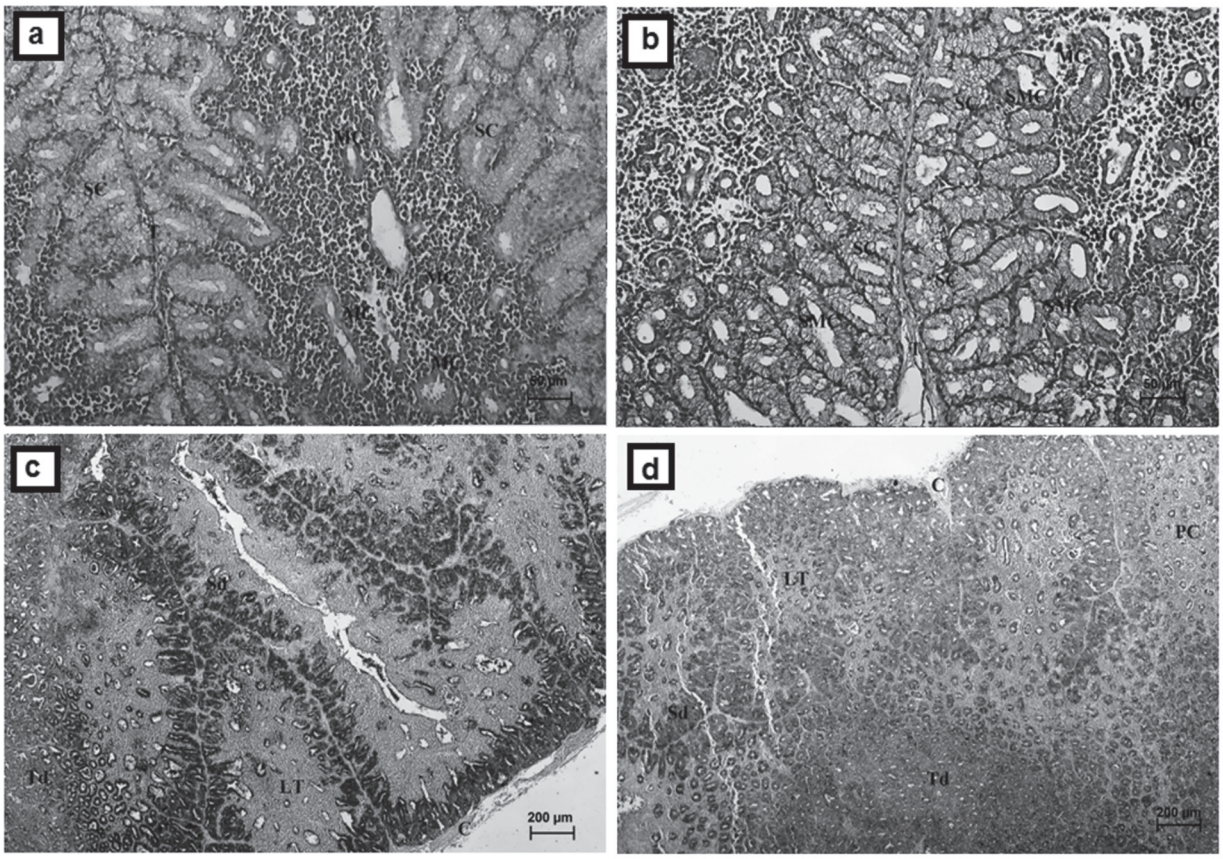

Figure 6 – Light micrograph of Harderian gland. PAS and HDI stain. **a**. common pheasant. Visible different PAS staining of mucous cells (MC) – PAS (+) and serous cells (SC) – PAS (–).Bar = 50 µm; **b**. hybrid of Italian amber and common pheasant. Visible different PAS staining of mucous cells (MC) – PAS (++), seromucous cells (SMC) – PAS (+) and serous cells (SC) – PAS (–). Bar = 50 µm; **c**. common pheasant. Visible HDI staining the presence of strong positive reaction (++/+++) in acini and tertiary and secondary ducts. Bar = 200 µm; **d**. hybrid of Italian amber and common pheasant. Visible HDI staining the presence of strong positive reaction (++/+++) in acini and tertiary and secondary ducts. Bar = 200 µm.

C – capsule, T – trabeculae, Sd – secondary duct, Td – tertiary duct, LT – lymphatic tissue, PC – plasma cells, MC – mucous cells, SC – serous cells, SMC – seromucous cells.

hybrids (Figures 7a, 7b). The frequencies of plasma cells in the HG between common pheasant and hybrid were compared. The central lobular space, interacinar space, and apical parts of the acini of the HG were filled with many lymphocytes and plasma cells in both species (Figures 5a, 5c, 5d, 6a and 6b) but in the common pheasant, differently from the hybrids, all centers of the large lobes were abundantly filled with plasma cells (Figure 6c).

DISCUSSION

The studies of HG include different bird species, including of birds also primitive avian species (Oliveira *et al.*, 2006). In the present study, the HG of two popular birds from *Phasianidae* family of the *Galli* order were examined. The Italian amber (*Phasianidae, Gallus gallus f. domestica*) is one of the most popular species of hen. The birds of this very old breed, derived from Italy, are respected for their high utility as laying hens (Brzóska *et al*., 2012). The common pheasant (*Phasianidae*, *Phasianus colchicus talischensis*) is smaller than Italian amber hen, and it is one of the largest gallinaceous bird in Europe. Its adaptability makes the common pheasant the most common bird in Central Europe, together with the Italian amber hen, and it is characterized by its high reproductive value (Mróz, 2003).

This study demonstrated small HG dimension differences between the common pheasant and the hybrid of the common pheasant with the Italian amber. This gland was slightly longer in the hybrids, but wider in common pheasant. According to Burns (Burns, 1992), the gross morphology of avian HGs does not differ substantially. Like in the other studies, also here it was shown in common pheasants and hybrids that the HG is covered by a thin capsule, and the connective tissue septa divide the gland into numerous unequal-sized lobes (Frahmand & Mohammadpour, 2015; Burns, 1992; Boydak & Aydin, 2009). In general, the

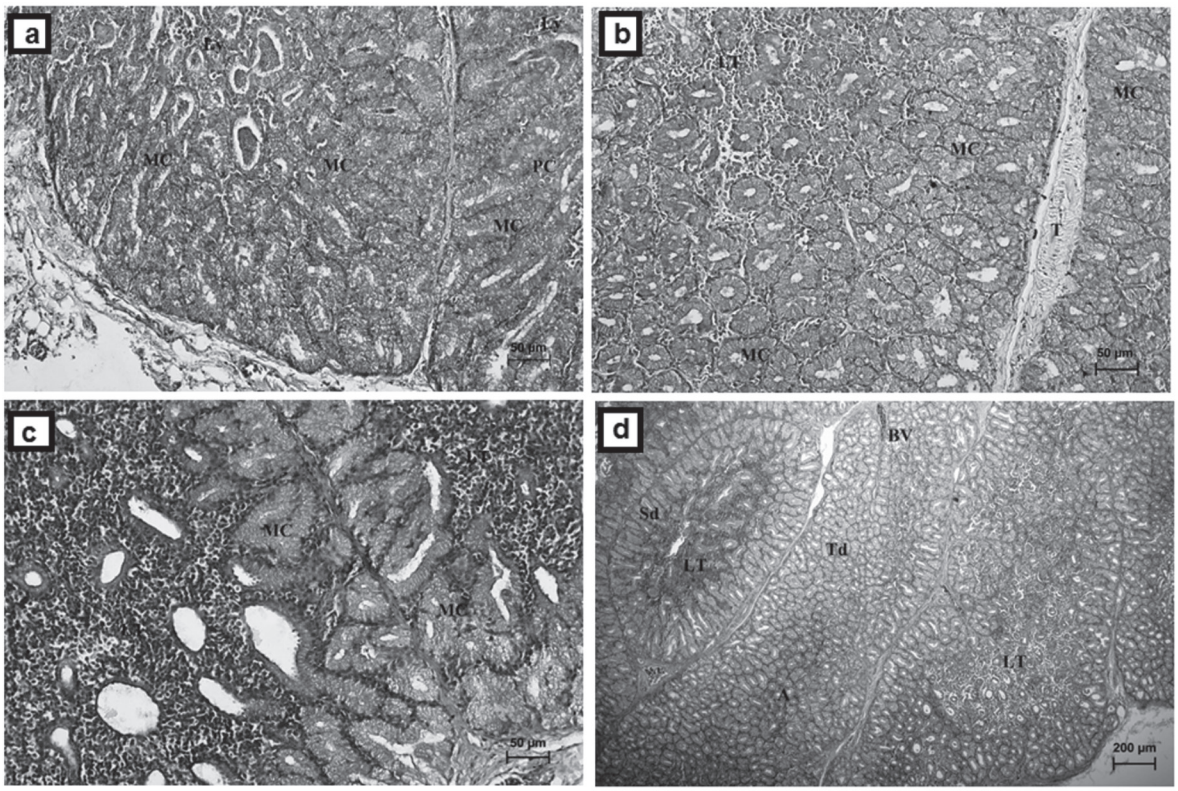

Figure 7 – Light micrograph of Harderian gland. AB pH 2.5 and AF stain. **a**. common pheasant. Note AB pH 2.5 staining of mucous cells (MC) – (++). Bar = 50 µm; **b**. hybrid of Italian amber and common pheasant. Note AB pH 2.5 staining of mucous cells (MC) – (++). Bar = 50 µm; **c**. common pheasant. Visible AF staining the presence of strong positive reaction (++/+++) in acini and tertiary and secondary ducts. Bar = 50 µm; **d**. hybrid of Italian amber and common pheasant. Visible AF staining the presence of negative reaction (–) in acini and tertiary and secondary ducts. Bar = 200 µm.

T – trabeculae, A – acini, Sd – secondary duct, Td – tertiary duct, BV – blood vessels, LT – lymphatic tissue, PC – plasma cells, Ly – lymphocytes, MC – mucous cells.

interlobar trabeculae, which contain blood vessels and fibroblasts, were also described in many other avian species (Dimitrov & Nikiforov, 2005; Boydak & Aydin, 2009; Dimitrov, 2011). Likewise, the HG of the native chicken (*Phasianidae, Gallus gallus f. domestica*) is covered with a thin capsule of adipose tissue (Mobini, 2012). The present study indicated that both examined birds presented the first type of gland, similarly to Canadian ostriches (*Struthionidae, Struthio domesticus*) (Frahmand & Mohammadpour, 2015), domestic fowl (*Phasianidae, Gallus gallus f. domestica*) (Burns, 1992), and African black ostriches (*Struthionidae, Struthio camelus domesticus*) (Klećkowska-Nawrot *et al.*, 2015). Dimitrov also measured the HG of adult (*Numididae, Numida meleagris meleagris*) and common pheasant (*Phasianidae, Phasianus colchicus colchicus*) (Dimitrov, 2012; Dimitrov, 2014). The histometrical study conducted by Dimitrov (2011; 2012) indicated that the HG was structurally mature and functionally active, as also observed in our studies.

The HG of the common pheasant and the hybrid is composed of many lobes. Similar results were obtained by Boydak and Aydin (2009) in domestic geese (*Anatidae*, *Anser anser f. domestica*), Canadian ostriches (Frahmand & Mohammadpour, 2015), and African black ostriches (Klećkowska-Nawrot *et al.*, 2015), and also by Kozlu *et al*. (2010) in osprey (*Pandionidae*, *Pandion haliaetus*). The average size of lobes of turkeys and chickens, measured by Dimitrov (2011), is larger compared with the common pheasant and the hybrid. A similar study performed by Dimitrov (2014) with the common pheasant (*Phasianidae, Phasianus colchicus colchicus*) showed that outer diameter of the lobes was larger than that found in the present study, whereas the mean outer diameter of the glandular acini of turkeys and common pheasants (*Phasianidae, Phasianus colchicus colchicus*) was smaller (Dimitrov, 2011; Dimitrov, 2014). On the other hand, Dimitrov (Dimitrov, 2011; Dimitrov, 2014) also found that the mean outer diameter of ducts was different and the primary ducts presented larger outer diameter compared with the secondary and tertiary ducts.

Consistent with Mobini's (2012) study, the apical cells of the corpus glandulae were darkly stained and contained serous secretion, while deeper portion of the corpus glandulae were lightly stained

and contained mucous secretion, particularly in the common pheasant. These two types of cells were also described by Kozlu *et al.* (2010) in the HG of osprey and by Frahmand & Mohammadpour (2015) in the HG of Canadian ostrich.

According to Burns (1992), the first type of gland secretes weakly acidic sulfated and neutral mucosubstances together with a small proportion of sialic acid and hyaluronic acid, while the second type secrete a mixture of acid and neutral mucosubstances. The histochemical analysis in the present study showed that the HG of both common pheasants and hybrids secreted neutral glycoproteins, as well as acidic sialylated glycoproteins and carboxylated acid mucopolysaccharides. Similar results were obtained with adult domestic geese (*Anatidae*, *Anser anser f. domestica*) (Boydak & Aydin, 2009; Liman & Gülmez, 1996), mallard ducks (A*natidae*, *Anas sterillis*) (Dimitrov & Nikiforov, 2005), Canadian ostriches (Frahmand & Mohammadpour, 2015) and African black ostriches (Klećkowska-Nawrot *et al.*, 2015). In native chickens (*Phasianidae, Gallus gallus f. domestica*), all epithelial cells of the corpus glandulae and duct system reacted positively to neutral mucopolysaccharides and were positive in AB pH 2.5 staining (Mobini, 2012). On the other hand, in osprey, histochemical staining showed that most HG secretory cells contained acidic mucins, while the remaining secretory cells contained both neutral and acidic mucins, and the duct epithelium contained secretory vesicles with only neutral mucins (Kozlu *et al.*, 2010).

The proper function and distribution of the lymphatic cells is very important in the protection against infectious diseases (Guo *et al.*, 2008). In the examined birds, the lymphatic cells were abundant in the HG walls and around the draining ducts. The glands of all birds were infiltrated with varying degrees of plasma cells, but foci of lymphocytes were scant. The MGP Y staining demonstrated numerous plasma cells in the glandular tissue. The plasma cells were more numerous in the common pheasant compare with the hybrid. Consistent with the study of Khan *et al.* (2007), the plasma cells in the examined birds were located in the interstitial space, interacinar space, apical part of the lobule, and lumina of the lobules of the HG.

The HG has a decisive role in the immune system of birds, as it is related with immunoglobulin production and has a protective effect both on the eye and the nasopharynx. The main classes of immunoglobulins (Ig) produced in the HG of chickens are probably related with the secretory feature of this gland (Baba *et al.,* 1990). In our study, significant differences in lymphatic cells components were observed. The common pheasant presented a higher number of immunoglobulin-producing plasma cells. The hybrid had a lower number of plasma cells, but a higher number of lymphocytes in the gland tissue in comparison with the common pheasant. Ohishima *et al.* (2002) made various observations concerning the proportions of plasma cells. Differences in HG plasma cell numbers between broilers and native chickens were reported by Khan *et al.* (2007). The epithelial cells of the HG ducts play the crucial role in secretion of Ig by plasma cells. We demonstrated that, in addition of a higher number of plasma cells in the tissue gland, the common pheasant had lower number of ducts in comparison with the hybrid. The plasma cells were in direct contact with the epithelial cells and closely surrounded the ducts. This strongly suggests that the B cells of the bursa migrate through the HG interstitial cells via blood circulation. These B cells further differentiate into plasma cells and move to the apical parts of the lobules, producing Ig that are released into the duct system (Khan *et al.*, 2007). In the HG of the common pheasant, the plasma cells were densely located in the central part of lobes, where relatively lower number of ducts were also observed. The acini were located only on periphery of the HG lobes. In the studies conducted by Mobini (2012), plasma cells were observed beneath the capsule, in the interlobar septa, and near the crypts of the main duct. A lower number of plasma cells were present in the hybrid's HG; however, in central part of lobes, more abundant ducts were observed. The HG of osprey presents low numbers of plasma cells in the interlobular trabeculae (Kozlu *et al.*, 2010), while in our study, the plasma cells also filled the center of the lobes.

In the HG, the plasma cells synthesize mainly IgA, especially during the early post-infection stage. No other avian organ appears to have such an extensive population of plasma cells (Burns, 1992). In the present study, these extensive populations of plasma cells in the HG were demonstrated especially in the common pheasant. The histochemical study confirmed significant differences in quantity of plasma cells between the common pheasant and the hybrid, as previously observed in the histological analysis. In addition, the AB pH 2.5 staining showed that, among the immune cells, the plasma cells were predominant in the HG of the common pheasant, while in the hybrid, the amounts of lymphocytes and plasma cells were similar. Precise knowledge of the structure of this

gland, and of the components of the immune system in particular, may have a large impact on the clinical practice and on the selection of birds for crossbreeding (Gallego *et al.*, 1992).

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REFERENCES

Baba T, Kawata T, Matsumoto K, Kajiwara T. Role of the Harderian gland in immunoglobulin A production in chicken lacrimal fluid. Research Veterinary Science 1990;49:20-24.

Bejdic P, Avdic R, Amidzic L, Cutahija V, Tandir F, Hadziomerovic N. Developmental changes of lymphoid tissue in the Harderian gland of laying hens. Macedonian Veterinary Review 2014;37:83-88.

Boydak M, Aydin MF. Histology of the Harderian Gland of domestic geese (*Anser anser domesticus*). Acta Veterinaria Brno 2009;78:199-204.

Brzóska F, Bobrowolska D, Kłopotek E, Pietras M. Drób ozdobny – hodowany przez człowieka dla przyjemności. Wiadomości Zootechniczne 2012;4:67-76.

Burns RB. The Harderian glands in birds: Histology and immunology. Berlin: Springer-Verlag; 1992. p.155-163.

Dimitrov D. Histometrical investigation on the turkey broiler's third eyelid (Harderian) gland. Journal of Agricultural Science and Technology 2011; 3: 246-248.

Dimitrov D. Histometry of the third eyelid (Harderian) gland in Helmeted guinea fowl (*Numida meleagris*). Journal of Agricultural Science and Technology 2012;4:368.

Dimitrov D. Histometrical parameters in third eyelid (Harderian) gland of the common pheasant (*Phasianus colchicus colchicus*). Journal of Agricultural Science and Technology 2014;6:24-27.

Dimitrov DS, Nikiforov IP. Histological and histochemical studies of Harderian gland, lacrimal gland and bursa of fabricius in mallard ducks (*Anas sterilis*) with chlamydial infection. Bulgarian Journal Veterinay Medicine 2005;8:119-127.

Frahmand S, Mohammadpour AA. Harderian gland in Canadian ostrich (*Struthio camelus*): A morphological and histochemical study. Anatomia Histologia Embryologia 2015;44(3):178-85.

Gallego M, del Cacho E, Felices C, Bascuas JA. Immunoglobulin classes synthesized by the chicken Harderian gland after local immunization. Research Veterinary Science 1992;52:44-47.

Guo X, Rosa AJ, Chen DG, Wang X. Molecular mechanisms of primary and secondary mucosal immunity using avian infectious bronchitis virus as a model system. Veterinary Immunology and Immunopathology 2008;121:332-343.

Khan MZI, Jahan MR, Islam MN, Haque Z, Islam MR, Kon Y. Immunoglobulin (Ig)- containing plasma cells in the Harderian gland in broiler and native chickens of Bangladesh. Tissue Cell 2007;39:141-149.

Klećkowska-Nawrot J, Goździewska-Harłajczuk K, Barszcz K, Kowalczyk A. Morphological studies on the Harderian gland in the Ostrich (*Struthio camelus domesticus*) on the embryonic and post-natal period. Anatomia Histologia, Embryologia 2015;44:146-156.

Koskela K, Kohonen P, Nieminen P, Buerstedde JM, Lassila O. Insight into lymphoid development by gene expression profiling af Avian B cells. Immunogenetics 2003;55:412-422.

Kozlu T, Altunay H. Light and electron microscopic studies of the quail (*Coturnix coturnix*) Harderian gland. Journal of Animal Veterinary Advances 2011;10:932-938.

Kozlu T, Bozkurt YA, Altunay H, Sari EK. Histological and histochemical studies on the Harderian gland of the osprey (*Pandion haliaetus*). Journal of Animal and Veterinay Advances 2010;9:1875-1879.

Liman N, Gülmez N. The light microscopic examination on the development of the Harderian gland of the geese (*Anser anser*). Veterinary Journal of Ankara University 1996;43:25-30.

Mobini B. Histological and histochemical studies on the Harderian gland in native chickens. Veterinary Medicine 2012;57:404-409.

Mróz E. Bażanty. Warszawa; Oficyna Wydawnicza Hoża; 2003.

Nasrin MK, Khna MZI, Siddiqi MNH, Masum MA. Mobilization of immunoglobulin (Ig) – containing plasma cells in Harderian gland, cecal tonsil and trachea of broilers vaccinated with Newcastle Disease Vaccine. Tissue Cell 2013;45:191-197.

Ohshima K, Hiramatsu K. Immunohistochemical localization of three different immunoglobulin classes in the Harderian gland of young chickens. Tissue Cell 2002;34:129-133.

Oliveira CA, Telles LF, Oliveira AG, Kalapothakis E, Gonçalves-Dornelas H, Mahecha GA. Expression of different classes of immunoglobulin in intraepithelial plasma cells of the Harderian gland of domestic ducks *Anas platyrhynchos.* Veterinary Immunology and Immunopathology 2006;113:257-266.

Savage ML, Oláh I, Scott TR. Plasma cell proliferation in the chicken Hardeian gland. Cell Proliferation 1992;25:337-344.

Scott TR, Savage ML, Oláh I. Plasma cells of the chicken Harderian gland. Poultry Science 1993;72:1273-1279.

Spicer SC, Henson JG. Methods for localizing mucosubstances in epithelial and connective tissue. In: Bajusz E, Jamin F, editors. Series on methods and achievements in experimental pathology. Basal: S Karger Press; 1967. v.2.

Tsuji S, Baba T, Kawata T, Kajikawa T. Role of Harderian gland on differentiation and proliferation of immunoglobulin A-bearing lymphocytes in chickens. Veterinary Immunology Immunopathology 1993;37:271-283.