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HISTOMETRIC INVESTIGATION OF THE THIRD EYELID GLAND IN MONGOLIAN PHEASANTS (*PHASIANUS COLCHICUS MONGOLICUS*)

D. S. DIMITROV

Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, 6000 Stara Zagora, Bulgaria

Summary

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The study was conducted on 40 Harderian glands obtained from 20 (10 male and 10 female) adult clinically healthy Mongolian pheasants. The glands were weighed and measured before placement in fixation fluids. Permanent histological preparations were prepared from fixed glands using conventional techniques. Histometric studies of all microstructural elements of the glands were done with a light microscope with a built-in eyepiece micrometer. The histometric analysis showed that the average size of pheasant Harderian gland lobules was 285.42 μm. The average outer diameters were as followed: acini – 46.24 μm, secretory ducts – 48.73 μm, lobular ducts – 167.96 μm and gland excretory ducts – 272.28 μm. The histometric investigation of 13 microstructural elements of the gland allowed concluding that by the time of the commercial realisation of Mongolian pheasants, the Harderian gland was a structurally and functionally complete organ.

Key words: Harderian gland, histometry, pheasant

INTRODUCTION

Intraorbital glands comprise the lacrimal gland and the third-eyelid gland (also called Harderian after its discoverer). All terrestrial vertebrates and some amphibians possess Harderian glands, while it is completely absent in aquatic organisms and rudimentary in humans. Birds, unlike most mammalian species, have a better developed and larger Harderian gland, dominating within the eye orbit (McLelland, 1975).

The research interest to intraorbital glands and the third-eyelid gland in particular has markedly increased after the discovery of immune bodies in the eye orbit. It was hypothesised that these glands were either a transitory depot on their pathway, or their source (Gallego *et al*., 1992; Payne, 1994; Burns, 1996; Shirama *et al*., 1996; Olah *et al*., 1998; Akaki *et al*., 2000; Khan *et al*., 2007).

Today, the prevailing opinion is that the Harderian gland in birds is a lymphoepithelial organ providing almost entirely the local immunity of the eye orbit.

The scientific literature provides data about the anatomical, structural, ultrastructural and immunomorphological features of this gland especially in chickens, ducks and turkeys (Burns, 1979). Few authors only have investigated the Harderian gland in different avian species, bred by hybridisation techniques for commercial purposes. A detailed study on phe-

asant's Harderian gland and its morphometry is not available so far.

The Mongolian pheasant (*Phasianus colchicus mongolicus*) in Bulgaria originates from an Italian imported hybrid, which is then spread and reared commercially in all regions of North Bulgaria due to favourable climatic conditions (Petkov & Kanakov, 2007). Also, this pheasant breed is the heaviest among all varieties reared in the country – the average weight of male well-nourished birds is 2,000 g, and that of females – from 900 to 1,300 g.

The scarce information on the subject and our long-standing interest in avian intraorbital glands (Dimitrov *et al.,* 1987; Dimitrov, 1997; 1999; 2001; 2009), the weight and morphometric studies on intraorbital glands of Mongolian pheasants (Dimitrov & Savov, 2009) were the incentive of the present study. It aimed to perform a light microscopy histometric analysis to determine the parameters of microstructural elements of the Harderian gland in this bird species by the time of its commercial realisation.

MATERIALS AND METHODS

The investigation was carried out on biological material obtained from 20 sexually mature Mongolian pheasants (10 male and 10 female) purchased from the pheasant farm near Rousse. The birds were housed and fed for 2 weeks as required for the species. They were housed in aviaries, each gender separately, at ambient temperature of 18–20 ^{o}C, mixed light regimen and ambient humidity of 55–60%. Feed and water were offered *ad libitum*.

All stages of the experiment were approved by the Trakia University Animal Ethics Committee. After inhalation anaesthesia followed by decapitation, a total of 40 Harderian glands (right and left) were obtained from birds (Aitken & Survache, 1976). After weighing and determination of metric parameters of glands, a part of the glands were put in 10% neutral formalin solution and another part – in Bouin's and Carnoy's fixatives.

Specimens were processed by routine histological techniques. Paraffin cross sections (5–7 μm), cut on a Reichert microtome (Austria) were stained with haematoxylin- (Ehrlich) eosin to obtain permanent histological preparations (Kiernan, 2008).

The micrometric parameters of Harderian gland's elements were determined using a light microscope Ergaval with built-in eyepiece micrometer (Carl Zeiss, Jena, Germany) by the methods of Avtandilov (1980).

Data were statistically processed by statistical software (Stat Most for Windows).

RESULTS

The entire surface of pheasant Harderian gland, protected by ocular muscles and the bony orbit, is covered by a connective tissue capsule. The histometric study showed that the average thickness of the capsule in all parts of the gland was 25 μm (Table 1). Along the surface of the gland, the capsule gave rise to connective tissue strands towards the inner part, thicker in their initial part, which divided the parenchyma to lobules of different shape and size. The average thickness of interlobular septa of this lobular organ was 15 μm. Using 10× magnification, 3 relatively closely situated glandular lobules were observed per observation field. The average size of glandular lobules was 285.42 μm (Table 1). Each lobule, regardless of its location, size or cut surface shape, possessed a very specific architec-

Table 1. Histometric parameters of the Harderian gland in Mongolian pheasants (*Phasianus Colchicus mongolicus Brandt*)

Histometric parameters	
Thickness of gland's capsule (μm)	25.2050 ± 1.6215
Thickness of interlobular septa (μm)	15.2650 ± 2.0151
Number of lobules per microscopic observation field	3.2777 ± 0.2777
Number of acini per microscopic observation field	18.7000 ± 2.3096
Number of glandular ducts per microscopic observation field	12.3333 ± 1.7105
tertiary tubules	18.0000 ± 2.1602
secondary tubules	11.0000 ± 0.8164
primary tubules	8.0000 ± 0.0000
Size of glandular lobules (μm)	285.4200 ± 4.1845
Outer diameter of acini (μm)	46.2400 ± 3.5519
Outer diameter of glandular ducts (μm)	48.7333 ± 2.8499
tertiary tubules	42.5000 ± 1.2241
secondary tubules	45.2200 ± 1.9299
primary tubules	58.4800 ± 1.1705
lobular duct	167.9600 ± 1.6862
excretory duct of the gland	272.2800 ± 1.6052

tonics, which suggested that the organ was composed by numerous lobules of the same structure. The peripheral zone of each lobule was always occupied by densely located glandular acini, despite the cutting technique used. Under 10× magnification, 19 acini could be averagely seen per observation field. The average outer diameter of the Harderian gland acinus in Mongolian pheasants was 46.24 μm (Table 2). Without being sharply delineated, a system of intricately branching tertiary, secondary and primary glandular ducts was found to originate from each acinus in the lobule's periphery. At magnification 20×, the average number of glandular ducts per one observation field was 18 (tertiary), 11 (secondary) and 8 (primary). The results showed that from the periphery to the centre of each lobule, the number of secretory tubules was reduced almost twice (Table 1).

Contrary to the decreased number of glandular tubules, the outer diameter of secretory ducts showed a moderate increase – 42.50 μm for tertiary, 45.22 μm for secondary and 58.48 μm for primary ducts (Table 1). All histological preparations exhibited glandular ducts of varying size, whose central part was occupied by the common collecting duct. The average size of the lobular duct was 167.96 μm. Although not all cuts were made across the duct passing through the gland and collecting the secretion of glandular lobules, the average diameter of the excretory duct was 272.88 μm.

In all preparations, the lining and glandular epithelia, covering the acini and all types of tubules, was actively functioning. The average epithelium height varied within a narrow range – 19.72 μm in acini, 18.02 μm in tertiary secretory ducts. It was almost identical for secondary and

tertiary ducts – 17.00 μm and 17.68 μm, respectively (Table 2).

Table 2. Height (μm) of the glandular lining epithelium of Harderian gland in Mongolian pheasants (*Phasianus colchicus mongolicus* Brandt). Data are presented as mean± SEM (n=20)

Epithelium height (μm)	
Acini	19.7200 ±1.4921
Tertiary tubules	18.0200 ±1.3141
Secondary tubules	17.0000 ±1.4135
Primary tubules	17.6800 ±1.4921
Average of all tubules	17.5666 ±0.1731
Lobular duct	21.7600 ±0.9557
Excretory duct	29.1100 ±2.6045

If epithelium height is interpreted as a morphological trait for functional activity, the present results showed the highest secretory activity in acini, which is almost preserved in the three types of secretory tubules. Histometrically, the average height of the epithelium lining the lobular duct was 21.76 μm, whereas in the excretory duct it attained 29.11 μm (Table 2), i.e. the epithelium height of the Mongolian pheasant Harderian gland increased by almost 10 μm from acini towards the excretory duct.

DISCUSSION

In an attempt to type the histological structure of Harderian glands, Burns (1996) have studied over 80 bird species. He determined the presence of 4 main histological types in birds, confirmed also by Shirama *et al.* (1996) and other researchers (Wight *et al.,* 1971). In a previous study of ours (Dimitrov & Savov, 2009), the Harderian gland of the Mongolian pheasant was identified as a compound tubuloacinar gland.

There are no data in available research literature related to histometric measurements of both intraorbital glands in pheasants. Thus, a comparison could be only made to data obtained in broiler chickens (Dimitrov, 1997; 2001; 2008). The Harderian gland of stock broiler chickens is also a compound tubuloacinar gland, with a similar microarchitectonics pattern.

By the time of the commercial realisation of birds, the glandular acini per microscopic observation field in pheasant Harderian gland were more numerous (3.27) as compared to 56-day-old broilers (1.15). The difference (2.12 lobules) was due to the larger size of lobules in chickens (599.95 μm) vs the respective dimension in Mongolian pheasants (285.42 μm).

A similar relationship was observed for the average number of acini per observation field – 18.70 in pheasants and 59.89 in broilers, but it could be stated that the outer diameter of chicken acini was by 20.51 μm (66.75 μm) larger than that of pheasant acini (46.24 μm).

The comparison of histometric data between pheasant and chicken Harderian glands showed differences in the dimensions of microstructural parameters, but similar patterns in glandular microarchitectonics. In both avian species, the number of secretory ducts (tertiary, secondary and primary) gradually decreased from the periphery to the centre of the lobule, in parallel to increasing tubular diameters.

The height of lining and glandular epithelium in both bird species was the highest in acini, insignificantly reduced in secretory ducts, but increased again in the lobular and excretory ducts of the gland, indicating a most intensive secretory activity of acinar epithelium, but also a

higher level of activity in secretory ducts of the gland.

The present histometric investigation of microstructural elements of the Harderian gland confirmed the maturity of its architectonics and allowed concluding that by the time of the commercial realisation of Mongolian pheasants, it was an active secretory gland.

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Correspondence:

Assoc. Prof. D. S. Dimitrov, PhD
Department of Veterinary Anatomy, Histology and Embryology,
Faculty of Veterinary Medicine,
6000 Stara Zagora, Bulgaria