

## THE LACHRYMAL GLAND IN MANCHURIAN AND PHARAOH JAPANESE QUAILS – A HISTOMETRICAL INVESTIGATION

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### Abstract

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The study was carried out on 40 sexually mature and clinically healthy Japanese quails (20 females and 20 males) from the stock hybrids Manchurian Golden and Pharaoh. After weighing and determination of metric parameters, 80 lachrymal glands were processed by routine histological techniques. On the permanent histological preparations the principal microstructural elements of the Japanese quail lachrymal gland were determined using a light microscope with built-in eye-piece micrometer. Data were statistically processed. The aim of this study was to determine the parameters of main microstructural elements of the lachrymal gland in the two Japanese quail breeds – Manchurian Golden and Pharaoh.

The present study showed that the determined parameters in the two breeds of Japanese quails were different and contradictory. The histometric investigation demonstrated that the average size of glandular lobules of the female Manchurian Golden was 483.93  $\mu\text{m}$  and 521.56  $\mu\text{m}$  for male and 280.84  $\mu\text{m}$  and 322.32  $\mu\text{m}$  from the Pharaoh female and male, respectively. The mean outer diameter of glandular acini in the female Manchurian Golden was 35.73  $\mu\text{m}$  and 42.22  $\mu\text{m}$  for the male, but those of the female Pharaoh quails was 22.21  $\mu\text{m}$  and 31.00  $\mu\text{m}$  for the males.

The results from this histometric study of 13 parameters allowed us to assume that by the time of realization on the market, the Japanese quail lachrymal gland from the Manchurian Golden and Pharaoh breeds is a structurally mature and functionally active organ.

*Key words:* Lachrymal gland, Japanese quails – Manchurian Golden and Pharaoh breeds, histometry

### Introduction

Today it is acknowledged that all terrestrial vertebrates possess intraorbital glands (Popivanov et al., 1995). In birds, intraorbital glands are the classic lachrymal gland and third eyelid gland, also called Harderian after its discoverer. The Harderian gland is better developed and of a greater size in avian eye orbit (McLelland, 1975; Vraikin and Sidorova, 1984). After the first assumption (Mueller et al., 1971) that lachrymal gland and Harderian gland in chickens are a source of antibody – producing cells, made it raised a significant interest among researchers.

Japanese quails (*Coturnix Coturnix Japonica*) are the smallest representative of galliform birds reared for meat and egg production. According to Bondarenko (2002), Manchu-

rian Golden quails are created as a light egg-producing breed. As a result of domestication, a number of phenotype alterations have occurred (Genchev et al., 2008), related to high egg and meat production traits. Manchurian Golden (egg-type) together with the Pharaoh quail (meat-type), they are among the six Japanese quail breeds and strains registered in the International Registry of Poultry Genetic Stocks and they are more popularity in Bulgarian industrial scale. According to March (1977), Bondarenko (2002) and Genchev et al. (2008), female Japanese quails are usually heavier than males with sex-related differences in body weight up to 20%. In a previous study of ours (Dimitrov and Genchev, 2011), we determined there was no correlation between body weight of Manchurian Golden and Pharaoh Japanese quails and the weight of the their studied intraorbital glands. The lachry-

mal gland of heaviest birds- Manchurian Golden females- was by 1.5 mg lighter than that of males from the same breed whereas the lachrymal gland of female Pharaoh was heavier by 3.2 mg as compared to the male quails. In same investigation, intraorbital gland morphometry carried out in both quail breeds did not show differences of the same extent as for live body weight.

In scientific literature, data about the anatomical, structural, ultrastructural, histochemical and immunomorphological characteristics of the lachrymal gland are available with special reference to some avian species. According to Vrakin and Sidorova (1984), all gallinaceous birds have identical type lachrymal gland. The first full light microscopy description of lachrymal gland structure is that of Burns (1976), which compares the lachrymal glands of domestic fowl and ducks. According to Burns (1976), the lachrymal gland in domestic fowl was specified as a compound tubuloacinar gland. By light microscopy, Burns and Maxwell (1979) compared the structure of ducts in Harderian and lachrymal glands in chickens, ducks and turkeys but do not provide information to breed or age of birds. A few researchers only have focused on lachrymal gland of the Japanese quails. There are no morphometric investigations on intraorbital glands in quails. There is not a complex investigation of Japanese quails lachrymal gland. Data about the structure and morphometric traits of lachrymal gland in industrial hybrids are almost lacking.

The scarce information on the subject and our long-standing interest on the intraorbital glands in industrial avian hybrids (Dimitrov, 1997; 1999; 2001; 2005; 2009; Dimitrov and Savov, 2009) as well as weight and metric investigations on lachrymal and Harderian glands in Japanese quails (Dimitrov and Genchev, 2011), has motivated the present study. It aimed to determine the parameters of main microstructural elements of the lachrymal gland in the two Japanese quail breeds – Manchurian golden and Pharaoh, by the time of their realization on retail markets by means of light microscopy histometric analysis.

## Materials and Methods

The investigation was carried out on biological material obtained from 40 sexually mature and clinically healthy Japanese quails (20 males and 20 females) from the stock hybrids Manchurian Golden (20 birds, equally from both sexes) and Pharaoh (20 birds, equally from both sexes) reared in the Poultry Breeding Unit of the Faculty of Agriculture, Trakia University – Stara Zagora. The Japanese quails were housed in aviaries, each gender separately, at ambient temperature of 20-22°C, mixed light regimen and ambient humidity of 55-60%. The birds received feed and water *ad libitum*. Immedi-

ately prior to obtaining the material, every bird was weighed to determine its live body weight.

All stages of the experiment were approved by the Trakia University Animal Ethics Committee. After inhalation anaesthesia followed by decapitation, a pair of lachrymal glands (right and left) were obtained from each quail by the method of Aitken and Survashe (1976). After weighing and determination of metric parameters of glands, they were put in 10% buffered neutral formalin solution and in Bouin's and Carnoy's fixatives. Fixed specimens were processed by routine histological techniques. Single and serial paraffin cross and longitudinal sections (5-6 µm), cut on a microtome (Reichert, Austria) were stained with haematoxylin (Ehrlich) – eosin to obtain permanent histological preparations (Kiernan, 2008). The histometrical parameters of the principal microstructural elements of the Japanese quail lachrymal gland were determined as per Avtandilov (1990), using a light microscope Ergaval with built-in eye-piece micrometer (Carl Zeiss Jena, Germany). Data were statistically processed by statistical software (StatMost for Windows, Data Most Co., USA, 2004). Statistical relationship between studied parameters were determined by Pearson's regression analysis.

## Results

According to Burns (1976), Vrakin and Sidorova (1984) and in a previous study of ours (Dimitrov and Genchev, 2011), the lachrymal gland of Manchurian Golden and Pharaoh Japanese quails was specified as a compound tubuloacinar gland.

The results of present study showed that the determined histometric parameters in the two breed Japanese quails were different and contradictory (Table 1). Out of a total of 13 investigated micro structural elements in Japanese quail lachrymal gland, the males Manchurian Golden dominated over the females in 9 parameters while in the male Pharaoh quails these parameters were only 6. Light microscopy of all histological preparations in this study has shown that the entire surface of the lachrymal gland was covered by a connective tissue capsule. The results showed (Table 1) that in the male Manchurian Golden quails thickness of the capsule were with 2.20 µm higher from the female, while in the male Pharaoh there are 2.72 µm higher from the female. The average thickness of connective tissue interlobular septa in the male Manchurian Golden was 8.26 µm higher from the female but in the female Pharaoh there are 0.42 µm higher from the male quails. Using the same magnification (objective 10X) it was found out that the male Manchurian Golden had a higher number of lobules per one microscopic observation field (0.56) than the females, but the female Pharaoh had

a 0.69 number higher from the male quails. Size of glandular lobules in the males Manchurian Golden and in the Pharaoh was higher with 37.62  $\mu\text{m}$  and with 41.48  $\mu\text{m}$  respectively than the female Japanese quails. The lachrymal gland of the male Manchurian Golden exhibited a lower (2.85) number of glandular acini per observation field than the females and the males Pharaoh than the females with 2.1 numbers. The outer diameter of acini in the males Manchurian Golden and in the Pharaoh was higher with 6.49  $\mu\text{m}$  and with 8.79  $\mu\text{m}$  respectively than the female quails.

The light microscopy has shown that a system of complex branching ducts – tertiary, secondary and primary, stems up from each acinus in the periphery towards the central part of lobules without strict demarcation between them. The results in this study shown (Table 1) that average number of glandular ducts that could be identified per microscopic field, had a higher number in the female Manchurian Golden – tertiary tubules with 7.68; secondary with 8.19; primary with 5.57

and in the female Pharaoh – tertiary tubules with 1.8; secondary with 0.5; primary with 0.60, than the males Manchurian Golden and the Pharaoh Japanese quails.

The mean outer diameter for the glandular tubules in the two Japanese quail breeds was different (Table 1). The results from histometrical investigation showed that had a higher diameter in the males Manchurian Golden – tertiary tubules with 6.13  $\mu\text{m}$ ; secondary with 4.40  $\mu\text{m}$ ; primary with 3.74  $\mu\text{m}$ , and in the male Pharaoh – tertiary tubules with 6.82  $\mu\text{m}$ ; secondary with 3.24  $\mu\text{m}$ ; primary with 3.94  $\mu\text{m}$ , than the females Manchurian Golden and the Pharaoh Japanese quails.

The secretion produced by acini and the three categories secretory tubules is emptied in a duct occupying the central part of each lobule via the primary ducts. Each lobule in the glandular parenchyma empties its secretions in a common draining duct passing through the entire gland and ending on the inner surface of the third eyelid. The results showed (Table 1) that outer diameter of central lobular ducts in the males Manchu-

**Table 1**  
**The lachrymal gland in Manchurian and Pharaoh Japanese quails - A histometrical investigation (mean  $\pm$  SEM)**

Parameters	Manchurian	Pharaoh
Thickness of capsule, $\mu\text{m}$	27.0640 $\pm$ 0.5531	16.6770 $\pm$ 0.7106
Thickness of interlobular septa, $\mu\text{m}$	39.4360 $\pm$ 0.5911	17.6430 $\pm$ 0.9640
Number of lobules	2.3500 $\pm$ 0.2944	2.3750 $\pm$ 0.2642
Number of acini	44.5000 $\pm$ 0.7498	22.3000 $\pm$ 0.7127
Number of glandular ducts		
tertiary tubules	35.5000 $\pm$ 0.9154	20.4000 $\pm$ 0.4418
secondary tubules	23.2000 $\pm$ 0.6836	14.3000 $\pm$ 0.2575
primary tubules	14.1000 $\pm$ 0.6883	11.6000 $\pm$ 0.9583
All ducts	24.2666 $\pm$ 0.5799	15.4333 $\pm$ 0.5027
Size of glandular lobules, $\mu\text{m}$	521.5600 $\pm$ 0.4807	280.8400 $\pm$ 0.6862
Outer diameter of acini, $\mu\text{m}$	42.2280 $\pm$ 0.1760	22.2100 $\pm$ 0.9045
Outer diameter of glandular ducts, $\mu\text{m}$		
tertiary tubules	41.0720 $\pm$ 0.6428	24.1200 $\pm$ 0.5626
secondary tubules	35.5930 $\pm$ 0.1175	29.4530 $\pm$ 0.6082
primary tubules	39.6470 $\pm$ 0.4279	30.1470 $\pm$ 0.8935
lobular duct	202.6400 $\pm$ 0.7363	243.0080 $\pm$ 0.0863
excretory duct of the gland	448.9200 $\pm$ 0.0049	447.4410 $\pm$ 0.4409
Height of the epithelium, $\mu\text{m}$		
Acini	16.3200 $\pm$ 0.7851	14.1520 $\pm$ 0.4607
tertiary tubules	13.6000 $\pm$ 0.5011	13.8020 $\pm$ 0.1835
secondary tubules	14.0760 $\pm$ 0.8551	13.0700 $\pm$ 0.4795
primary tubules	14.4160 $\pm$ 0.5832	16.8710 $\pm$ 0.6825
Lobular duct	17.5440 $\pm$ 0.7764	15.4000 $\pm$ 0.7592
Excretory duct of the gland	21.7600 $\pm$ 0.8276	19.3360 $\pm$ 0.4899
All ducts	16.2860 $\pm$ 0.0235	15.6484 $\pm$ 0.8391

rian Golden and in the males Pharaoh, higher with 9.48  $\mu\text{m}$  and 19.19  $\mu\text{m}$  respectively than the female Japanese quails. Mean outer diameter of the common drainage excretory glandular duct in the two breed male Japanese quails was higher with 29.60  $\mu\text{m}$  and with 10.06  $\mu\text{m}$  respectively than the female's quails. The light microscopy revealed an actively functioning lining and glandular epithelium that covered the acini and all categories of ducts. The histometric study showed a variable height of the epithelium in each microstructural element of the Japanese quail lachrymal gland (Table 1).

## Discussion

No histometric data referring to lachrymal gland in Japanese quail are available and therefore we could not compare the results obtained in the present investigation with those of other researchers. Determined parameters in present study on the Manchurian Golden and Pharaoh Japanese quail lachrymal gland were different and contradictory, yet there were similar trends in the glandular micro architectonics. The glandular secretory ducts (tertiary, secondary and primary) in both breeds decreased in number from the periphery towards the central part of glandular lobules, while the outer diameter of these tubules increased in the same direction (Table 1). If the height of the lining and glandular epithelium is accepted as a morphological functional activity marker, it was different for the various structural lachrymal gland elements in the both breeds of Japanese quails. The height of the epithelium was biggest in their acini, slightly lower in some glandular secretory ducts and again high in lobular and drainage excretory ducts. These results makes us believe that the secretory activity in the lachrymal gland acini for the two breeds Japanese quails was the most pronounced, and the one in the secretory ducts is almost equal as well.

## Conclusion

The results from this histometric study of 13 parameters allowed us to assume that by the time of Manchurian Golden and Pharaoh Japanese quail realization on the market, the lachrymal gland is a structurally mature and functionally active organ.

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