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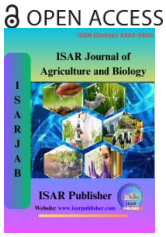

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NERVE SUPPLY OF THE HARDERIAN GLAND IN HENS

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Abstract: The Harderian gland in domestic birds is a major paraocular excretory gland that has an important role in tear production, as well as in the immune protection of the conjunctiva surface. The exocrine and lymphatic tissue of this gland has been described, but information about nerve supply is still very scarce in the present literature, so the main aim of this study was to provide more detailed and precise data about it. The research was conducted on 20 laying hens (Lohmann Brown) aged 4 months. Dissection and histomorphological examination show that the gland is innervated by the branch of the III (*n. oculomotorius*), V (*n. trigeminus*), and VII (*n. facialis*) cranial nerve. Before reaching the gland, the branches of these nerves enter into the *ganglion ethmoideum*, and later from this emerge several, thin postganglionic nerve fibers (*ramus glanduale membrane nictitanits*) that penetrate the capsule of the gland at its anterior extremity. The present study describes in detail the innervation of the Harderian gland, and this data will be useful for further research.

Keywords: Harderian gland, innervation, hens.

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Introduction

In domestic birds, the Harderian gland (*Glandula membranae nictitantis*) is the largest intraorbital excretory organ whose primary role is to lubricate, moisturize, and clean the surface of the eyeball (Wight et al. 2972, Maxwell and Burns 1979, Maxwell at el. 1986, Payne 1994, Bejdić et al. 2024). Besides this exocrine role, the lymphoid tissue of the gland participates in the immune defense of the conjunctiva and the gland represents an important part of the immune barrier **CALT**-*Conjunctiva-associated lymphoid tissue* (Scharm 1980, Olah et al. 1995, Davison et al. 2008, Bejdić et al. 2017, Bejdić et all. 2014). Although the exocrine and lymphatic tissue of the gland has been well investigated, literature data about the innervation of this organ are very scarce (Walcot et al 1989, Hiramatsu et al. 1998, Kohzy and Koji 2005). So far it has been reported that in chicken the Harderian gland is innervated only by the ventral, terminal branch of III cerebral nerve, *ramus ventralis n. oculomotori* (Wight et al. 1971), while, according to *Nomina Anatomica Avium* (Baumel et al. 1993), Koch (1973), and Nickel et al. 1977. the nerve supply comes from the terminal branch of V (*n. trigeminus*) and VII (*n. facialis*) cerebral nerve. Before entering the gland, those nerves enter into the *Ggl. ethmoideum* and later from it emerge under the name *ramus glandulae membrane nictitanitis*. The inerrvation by the branch of the III cerebral nerve, *ramus ventralis n. oculomotori* but this nerve branch is not mentioned in Nomina Anatomica Avium. Also, recent immunohistochemical research subcapsulary identified *Ggl. sphenopalatinum* and its postganglionic nerve fibers *radix autonomica* (Walcot et al. 1989). Considering these differences in the present literature, this study aimed to investigate precise and detailed innervation of the Harderian gland in laying hens and provide useful information for further research.

Material and Methods

The research was conducted on 20 laying hens (Lohmann Brown) aged 4 months. The innervation of the Harderian gland was investigated by dissection and light microscopy. The dissection procedure was done under the magnifier. To access the gland, the skin, eyelids, and entire eyeball were removed. For macroscopic nerve identification, we used *Nomina Anatomica Avium* (Baumel et al. 1993). Later, the gland was removed and fixed in 10% buffered formalin. The samples were treated in a rotary tissue processor (Microm Model STP-120) where they were spent for 48 hours in 70% alcohol, 24 hours in 96% alcohol, and 24 hours in 100% alcohol. Later, the samples were dehydrated in a solution of alcohol and toluene for 2 hours, and then another 4 hours in a toluene solution. Tissue samples were then embedded in paraffin blocks and cut onto the 4 µm slides (microtome LEICA RM 2145). Tissue sections were later stained with Haematoxylin and Eosin. All samples were observed under the light microscope Olympus UC 30 provided with a digital camera.

Results and Discussion

The Harderian gland in hens is situated behind the eyeball in the rostroventral part of the orbit, extending from the medial margin to the point where *n. opticus* penetrates the eyeball. The study shows that the gland is innervated by the branches of the III (*n. oculomotorius*), V (*n. trigeminus*), and VII (*n. facialis*) cerebral nerve (Figure 1). Nerve supply by the III (*n. oculomotorius*) cerebral nerve occurs through the small branch, *ramus ventralis nervi oculomotori*. This finding is consistent with the statements of Wight et al. 1971. The *ramus ventralis nervi oculomotori* passes over the lateral surface of the Harderian gland and innervates the ventral oblique eye muscle (Fig.1). Before reaching the muscle one small branch separates from this nerve and penetrates the capsule of the gland near the central narrowing of the gland.

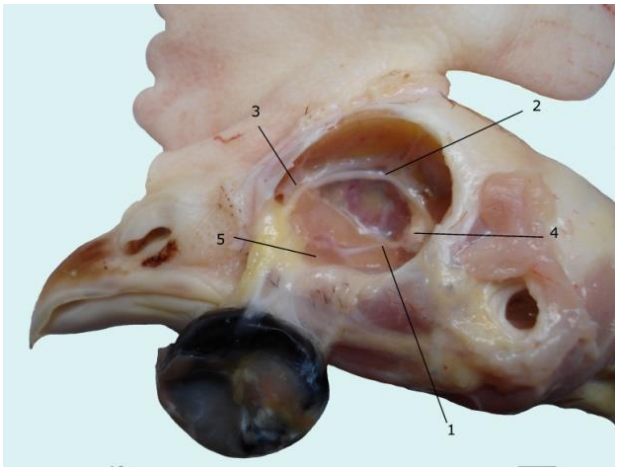

Figure 1. *Ramus ventralis n. oculomotori (*1), *n. ophthalmicus* (2), *Ggl. ethmoideum* (3), *n. opticus* (4), *m.*

The nerve supply by the V (*n. trigeminus*), cerebral nerve comes through the terminal branch of *n. ophthalmicus*. This nerve passes along the interorbital septum, following the ethmoidal artery and vein. In the nasal part of the orbit, the ophthalmic nerve gives one small branch that goes ventrally and enters into the *ganglion ethmoideum*. Also, into this ganglion enters the terminal, a dorsal, palatine branch of the VII cerebral nerve (*n. facialis*). The ethmoidal ganglion is situated close to the anterior extremity and upper margin of the gland (Figure 1 and 2). It is a very small ganglion, and its position is easiest to determine by following the nerves that enter and emerge from it. The postganglionic fibers that arise from this ganglion correspond to *ramus glandulae membranae nictitantis*, described in *Nomina Anatomica Avium* (Baumel et al. 1993). Those branches penetrate the gland capsule at its anterior extremity and pass along the upper margin of the gland. Subcapsullary these branches are ganglionated (Figure 3) and one of the ganglia probably corresponds to *radix autonomica* previously described by Walcot et al. 1989. Nerve fibers pass through the capsule of the gland and its septa, following the course of blood vessels, and some of them probably reach the stroma of the gland near the lymphoid cells. Further research is needed to confirm the termination of nerve fibers near lymphatic cells.

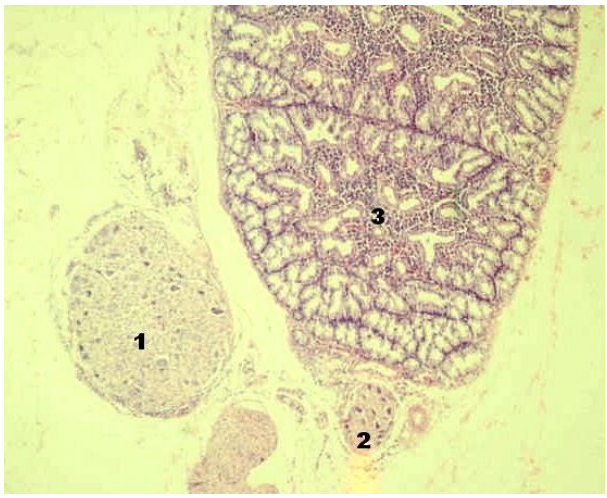

Figure 2. *Ethmoidal ganglion* (1) and one smaller ganglion (2) situated near the anterior extremity and

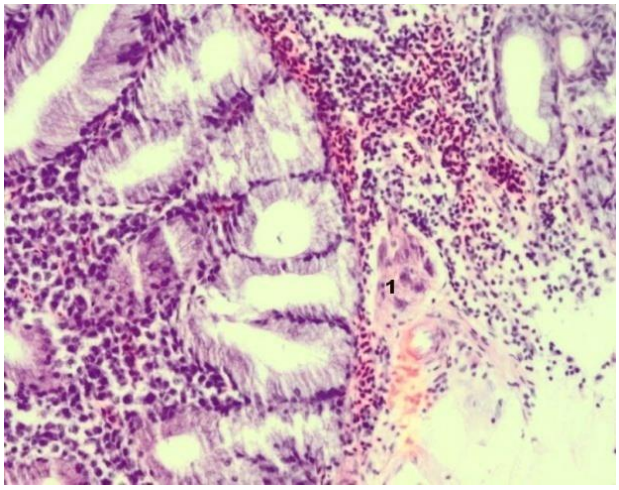

Figure 3. A small ganglion (1) situated subcapsular in the central narrowing of the Harderian gland. H&E 200X

Conclusion

The Harderian gland in hens is innervated by the branch of the III (*n. oculomotorius*), V (*n. trigeminus*), and VII (*n. facialis*) cranial nerve. The innervation by the III cranial nerve occurs through its smallest branch *ramus ventralis n. oculomotori*, while the branch of the V and VII cranial nerve, before reaching the gland, enters into the *ganglion ethmoideum*, and later from this emerge several, thin postganglionic nerve fibers (*ramus glanduale membrane nictitanits*) that penetrate the capsule of the gland at its anterior extremity. The present study describes in detail the innervation of the Harderian gland, and this data will be useful for further research.

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