

Histological Changes Of Uropygial Gland In Indigenous Chicken During Summer And Winter Seasons

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ABSTRACT

The histological changes of uropygial gland (UG) were studied on 20 mature indigenous chickens during summer and winter seasons to highlight the significant function of the gland. The glands were collected by autopsy with a maximum of 0.5 cm thickness and fixed in 10% buffered formalin saline, followed by dehydration, then embedded into hard paraffin and sectioned at 3 μ m, stained with special stains and examined under light microscope. The histological investigations showed that seasonal changes had a significant effect on weight and histological architectures of chicken UG. Grossly, the mass of the glands was larger in summer than winter samples. The surrounding capsule and glandular epithelium of summer were thinner with lower amount of adipose tissue, collagen and elastic fibers than winter. At uropygial papillae, the epithelium of secretory tubules of summer appeared thinner with wider lumen full of secretory contents than winter. However, some of winter samples showed accumulation of calcified granules in clumps at uropygial duct system and sinus which may indicated microbial infection. Histochemical changes of glands showed that PAS activity of summer was less intense in capsule, interlobular and secretory tubules than winter. Seasonal changes revealed that indigenous UG is more active which played a vital role during summer than winter season.

التغيرات النسيجية للغدة الزمكية في الدجاج المحلي خلال فصلي الصيف والشتاء

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الكلمات المفتاحية:

التغيرات المناخية
الغدة الزمكية
الدجاج المحلي

المخلص

تمت دراسة التغيرات النسيجية للغدة الزمكية على 20 من الدجاج المحلي خلال فصلي الصيف والشتاء لتسليط الضوء على الدور الحيوي للذي تلعبه الغدة الزمكية. تم تجميع العينات بسماك حوالي 0.5 سم و حفظها في مادة الفورمالين، بعد ذلك تم تمريرها بتراكيز مختلفة من الكحول و الزايلين و تقطيعها بسماك 3 ميكرومتر و صبغها بصباغات خاصة و فحصها تحت المجهر الضوئي. أظهرت الدراسة المظهرية تأثير المناخ على الغدة الزمكية، تمثلت في زيادة وزن الغدة خلال فصل الصيف مقارنة بفصل الشتاء. في حين أظهرت الفحوصات النسيجية للعينات التي تم جمعها خلال فصل الصيف، انخفاض سمك المحفظة المحاطة بالغدة و الظهارة المبطنة للنبيبات الإفرازية. أيضا ملاحظة القليل من ألياف الكولاجين و المرنة التي تخللت المحفظة و متن الغدة مع زيادة تجويف النبيبات الإفرازية و تجمع كميات كبيرة من إفرازات الغدة داخل التجويف، بينما أظهرت الفحوصات النسيجية للعينات التي تم جمعها خلال فصل الشتاء زيادة في كلا من سمك المحفظة، الظهارة المبطنة للنبيبات الإفرازية و ألياف الكولاجين في المحفظة و المتن مع تقلص حجم تجويف النبيبات الإفرازية و تجمع كميات قليلة من الإفرازات الدهنية. بعض العينات التي تم جمعها خلال فصل الشتاء أظهرت تجمعات من حبيبات التكلس مما يدل على وجود التهابات في القنوات الإفرازية للغدة الزمكية. أيضا أظهرت الفحوصات

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النسجية الكيميائية للعينات التي تم جمعها خلال فصل الشتاء زيادة حبيبات الجلايكوجن في كلا من المحفظة والتجاويف ما بين النيبات الإفرازية مقارنة بعينات فصل الصيف. مما سبق نستنتج أن التغيرات المناخية لها تأثير على التركيب النسيجي للغدة الزمكية في الدجاج المحلي حيث تكون أكثر نشاطا و تلعب دورا حيويا خلال فصل الصيف مقارنة بفصل الشتاء.

Introduction

The uropygial gland (UG) is also known as *oil or preen gland*, it is an epidermal derivative, arises from ectoderm germinal layer during embryonic development[1]. Paired invaginations of ectoderm on dorsal surface of the birds tail begin the UG development, this process occurs on the 8th day in both Zebra Finch and pigeon, 9th day in chickens and 10th day in ducks. A mesenchymal layer develops at the base from epithelial cells and as this layer proliferates, a simple tubular layer is formed around a central cavity then differentiated into the follicles of the lobes and produces connective tissue which develops into capsule, interfollicular and interlobular septa, the lumina develop within the follicles and secretion droplets are formed in the secretory layer of cells[2].

The UG is situated dorsally and medially in the synsacrocaudal region of the bird's body[3]. In the most avian species, it appears bilobed organ varies in both size and shape, each lobe contains the secretory tissue which produce oil, and an intricate duct system returns secretion to the papillae[4]. The papillae is situated just above the tail, excretes its secretion through the uropygial duct which project from the primary cavity and extends for the apex of the glands papillae which opens at the ductus uropygialis, contact of the beak with the papilla induces a flow of secretion [5], [6].

The composition of avian UG secretions is a complex mixture of ester waxes, fatty acids, lipids, and wax alcohols vary with species, gender, season, diet, and hormonal regulation, spread within the plumage by preening[7]. The exact function of the UG is not yet known but to date four main explanatory hypotheses have been identified for the function of the gland: (1) The feather maintenance hypothesis suggested that the UG secretion maintains the keratin in feathers, keeps them in a good condition [8]; (2) The waterproofing hypothesis suggested that the UG could has waterproofing mechanism depending on chemical compounds of gland secretion[4]; (3) The intraspecific communication hypothesis proposed that the gland plays a significant role in attraction to the opposite sex[9]; (4) The defense hypothesis showed the uropygial secretions could play a significant action against toxic and microbial infections [10].

Although several studies showed seasonal changes associated with chemical components of the gland, they have not been studied in chickens histologically. Therefore, this study was designed to investigate the histological changes of the UG of indigenous chickens during summer and winter seasons to highlight the vital role of the gland.

Materials and Methods

The study was conducted in Tripoli, Libya which is located about 2 km along the Mediterranean Sea. Its annual temperature ranges from 8 to 46 °C. The mean annual rainfall of the area is 266 mm. The studied animals included 20 mature indigenous chickens which were bred under the same environmental condition in summer and winter seasons. The study was conducted at Aboud's Farm, Tripoli – Libya, Department of Histology and Embryology, Faculty of Veterinary Medicine, Department of Histology and Genetics, Faculty of Medicine, University of Tripoli; Tripoli – Libya. All the

chickens were supplied with water and poultry food, handled accordingly as proposed by the guidelines for the care and use of animals for experiment set by W.H.O.

The Sampling time was August 10th in summer and January 15th in winter. The UG samples were taken from 10 chickens in summer and 10 in winter in the same farm. The chickens were taken to slaughter house in order to take specimens. The uropygial glands were removed using a scalpel and collected by autopsy (the mass of glands was observed anatomically), with maximum 0.5 cm thickness and were fixed in 10 % neutral buffered formalin.

All the samples were washed with distilled water, Followed by

dehydration by immersing the tissue in a series of gradually increasing concentrations of alcohol (50%, 70%, 80%, 95% and absolute alcohol) and xylene, then immersing in soft paraffin overnight in oven at 60 °C then embedded into hard paraffin for making blocks and sectioned at 3 µm. In order to show the seasonal changes in general structure of UG, smooth muscle fibers, adipose connective tissue, collagen and elastic fibers and glycogen; Hematoxylin & Eosin (H&E), Masson Trichrome, Van Geison and Periodic-acid Schiff (PAS) were used and examined under light microscope[11].

Results and Discussion

In addition to the UG, avian species have a wide variety of glands that produce sebaceous substances including salivary gland, ear (wax) glands, salt gland, anal gland and epidermal cells called sebokeratocytes[12].

The histological changes of indigenous UG in Tripoli, which has a hot summer and relatively cold winter were studied. Several studies have reported differences related UG weight connecting them to factors like seasonal changes[13]. In this study, the gross observations found that the mass of the UG collected during summer season was larger than collected during winter season. Earlier studies; found that the UG size greatly influenced by weather conditions which being larger during summer compared to winter seasons[14], [15], [16].

The histological investigations of this study showed significant differences between seasonal samples, the UG samples collected during summer season showed thin capsule consisting of thin layers of smooth muscle with low amount of adipose connective tissue (Fig.1a), collagen and elastic fibers (Fig.4). These findings confirmed by other special stain (Fig.6). In contrast, the UG samples collected during winter season showed thick capsule demonstrated high amount of fatty tissue and thick layers of smooth muscle fibers (Fig.2 a) (Fig.3a), abundant amount of collagen and elastic fibers (Fig.5). These findings confirmed by other special stain (Fig.7).

Another important histological changes observed in the studied groups, were reduced thickness and number of glandular epithelium secretory tubules of summer samples appeared with wider lumen filled with higher amount of secretory contents (Fig.1b) than winter (Fig.2b). This indicated the higher producing of gland secretion during summer compared during winter seasons. The neutral lipids, phospholipids, glycolipids and acidic mucins were found normal components of UG with significant variations in fatty acids of the gland secretion due to seasonal conditions[17]. Other findings suggested that volatility of gland components is attributing to the environmental temperature which observed in tropical birds with higher molecular weights[18].

The oily secretions are a good medium for the removal of toxic substances from body surface [19], [20]. The different compositions of the preen oil revealed decreased the growth of several skin fungi in *Gallus gallus domesticus* [21]. In this study, the UG sinuses of winter samples showed large number of calcified granules, widely scattered throughout the uropygial duct system as well as gland sinus arranged in clumps as well as singles (Fig.3b); which may indicate microbial infection. Some studies found that gland waxes acting as defensive barrier of skin and plumage against microbial and fungal organisms[22].

Previous studies proposed that seasonal changes also have significant affects on the chemical compositions of the gland secretions with changes in viscosity and texture [23]. The histochemical investigations of glands revealed that PAS activity of summer were weak in capsule, interubular septa, and all surface epithelium of secretory tubules (Fig8a), whereas it appeared moderate to intense in secretory tubules of winter samples (Fig.8b). These findings suggested that weak intense of glycogen activity indicates the

continuous production of secretory materials in the gland. These glycoproteins may play an important role in protection of pathogenic agents that observed in some cutaneous glands of wild birds and marine mammals [24],[25]. Similar results were demonstrated glycoproteins in the lobes of rock pigeon (*Columba livia*) [26].

Conclusion

The UG of indigenous chickens is more active in summer than winter, indicated the vital role of the glands during summer season.

Conflicts of Interest

The authors declare that there is no conflict of interest.

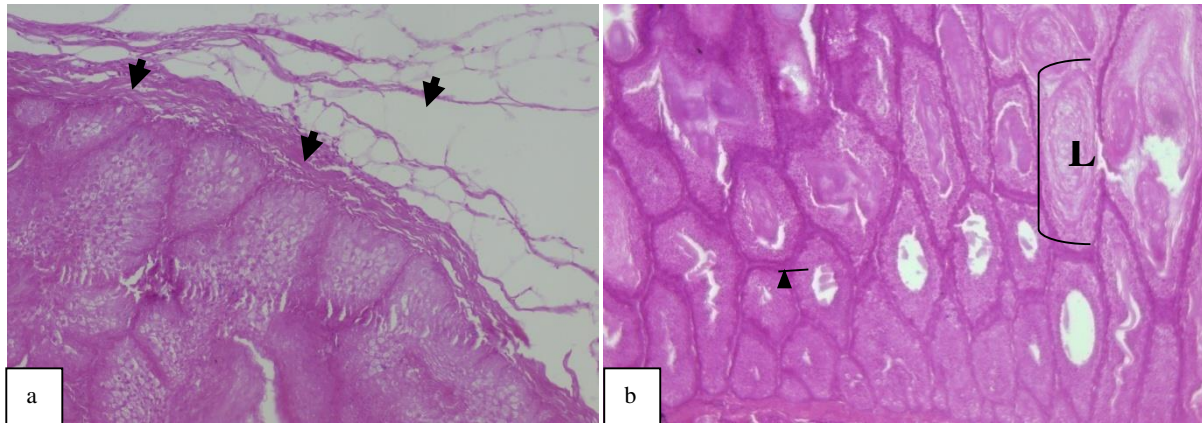


Figure1 : Photomicrographs of summer UG; showing : (a) Thin capsule consisting of thin layers of smooth muscle with low amount of adipose connective tissue, collagen and elastic fibers (**arrows**), H&E ($\times 10$). (b) Decreasing thickness of glandular epithelium at uropygial papillae appeared with wide lumen (**L**) filled with high amount of secretory contents (**arrow head**), H&E ($\times 10$).

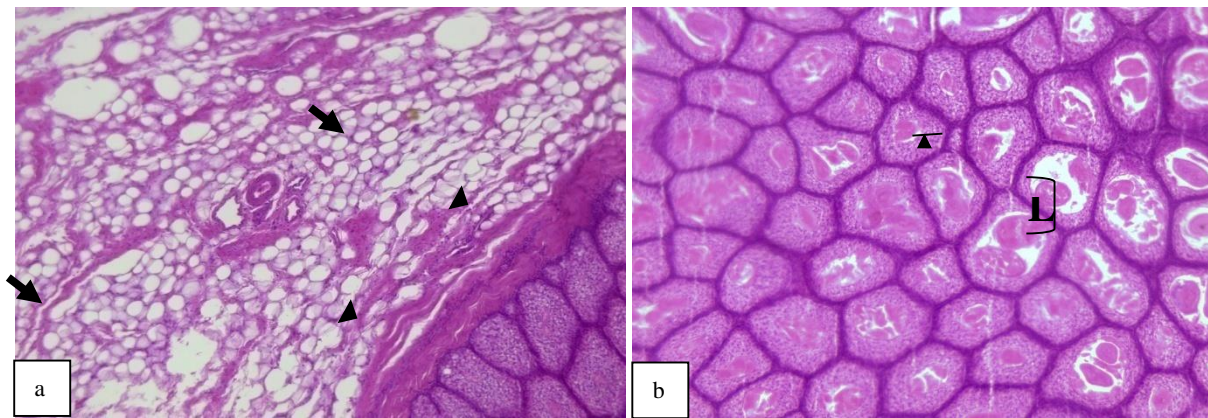


Figure2 : Photomicrographs of winter UG; showing : (a) Thick capsule consisting of thick layers of smooth muscle with high amount of collagen and elastic fibers (**arrow heads**) adipose connective tissue (**arrows**), H&E ($\times 20$). (b) Increasing thickness of glandular epithelium at uropygial papillae appeared with narrow lumen (**L**) filled with low amount of secretory contents (**arrow heads**), H&E ($\times 10$).

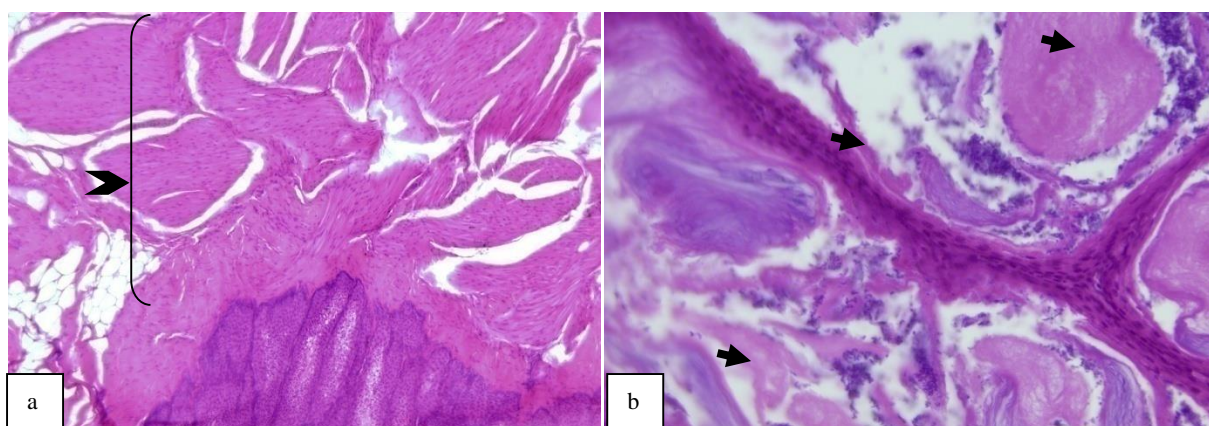


Figure3 : Photomicrographs of winter UG; showing : (a) Thick capsule consisting of thick layers of smooth muscle (**arrow head**), H&E ($\times 20$). (b) Large number of calcified granules widely scattered throughout the uropygial in clumps (**arrows**), H&E ($\times 40$).

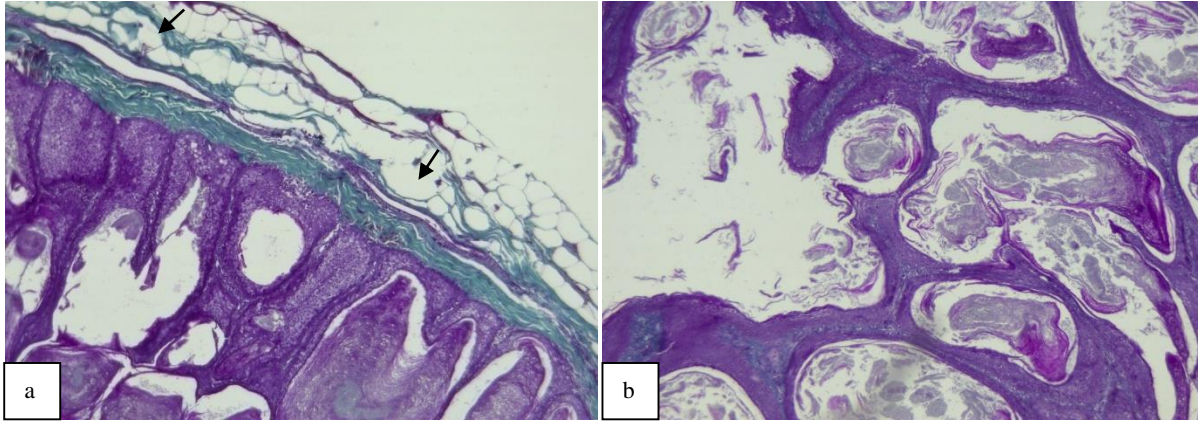


Figure4 : Photomicrographs of summer UG; showing : (a) Thin capsule consisting of thin layers of smooth muscle with low amount of adipose (**arrows**), collagen (**green colour**) and elastic fibers (**red colour**), Masson Trichrome ($\times 10$). (b) Low amount of collagen fibers (**green colour**) distributed between glandular epithelium at uropygial papillae. Masson Trichrome ($\times 10$).

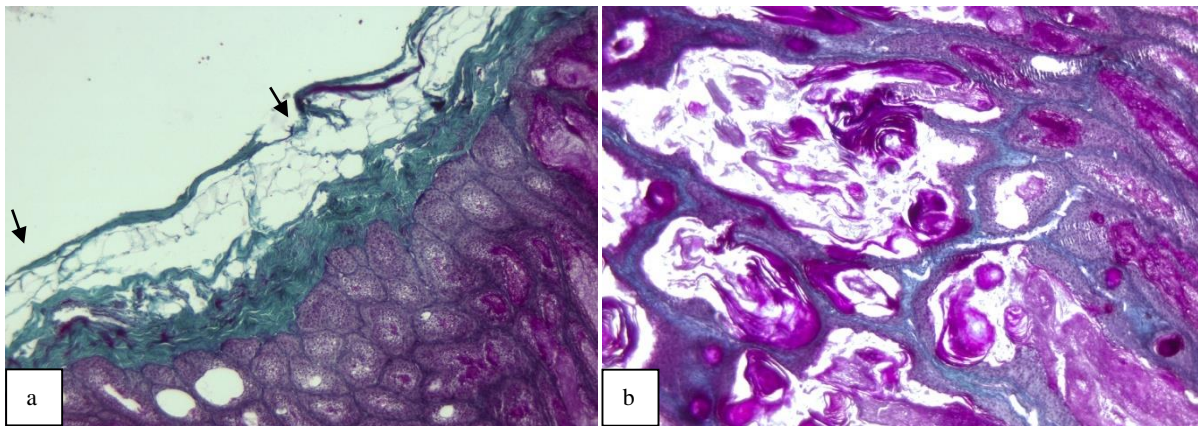


Figure5 : Photomicrographs of winter UG; showing : (a) Thick capsule consisting of thin layers of smooth muscle with high amount of adipose (**arrows**), collagen (**green colour**) and elastic fibers (**red colour**), Masson Trichrome ($\times 10$). (b) High amount of collagen fibers (**green colour**) distributed between glandular epithelium at uropygial papillae. Masson Trichrome ($\times 10$).

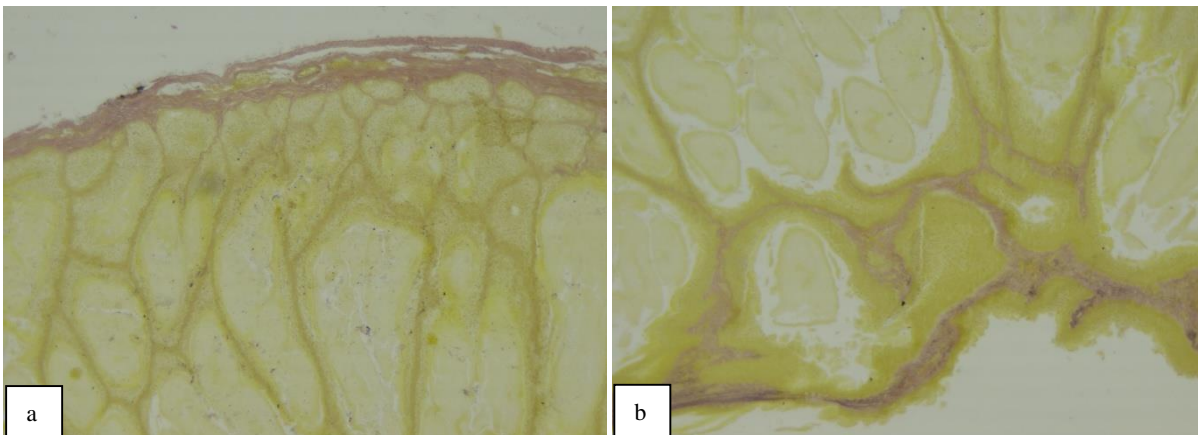


Figure 6 : Photomicrographs of summer UG; showing : (a) Thin capsule consisting of thin layers of smooth muscle with low amount of collagen (**red colour**) and elastic fibers (**black colour**), Van Gieson ($\times 10$). (b) Low amount of collagen fibers (**red colour**) distributed between glandular epithelium at uropygial papillae, Van Gieson ($\times 10$).

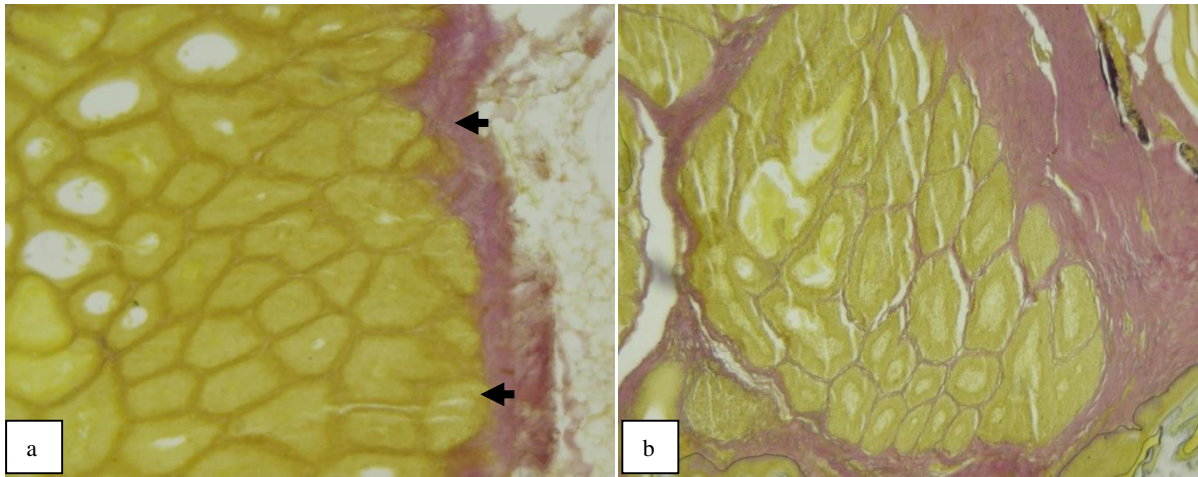


Figure7 : Photomicrographs of winter UG; showing : (a) Thick capsule consisting of thick layers of smooth muscle with high amount adipose tissue (arrows) of collagen (red colour) and elastic fibers (black colour), Van Gieson ($\times 10$). (b) High amount of collagen fibers (red colour) distributed between glandular epithelium at uropygial papillae. Van Gieson ($\times 10$).

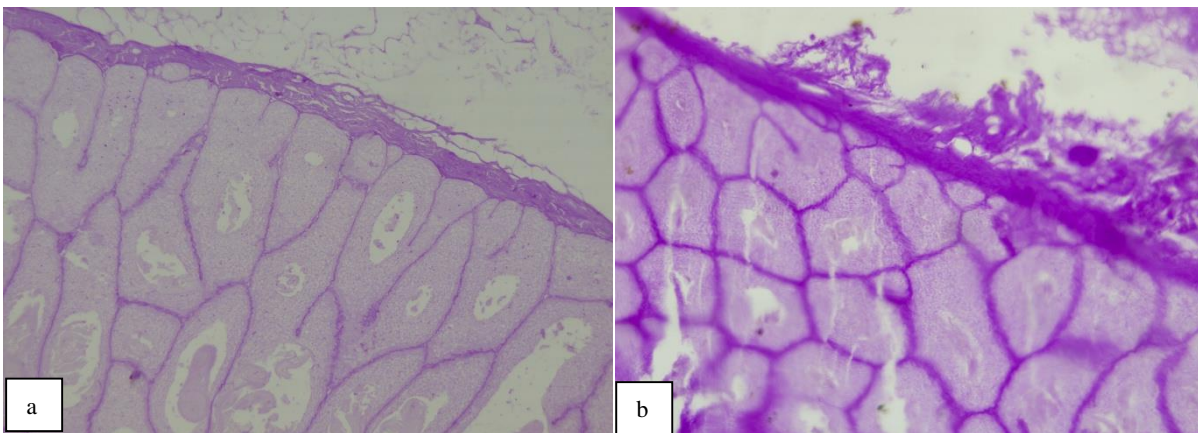


Figure8 : (a) Photomicrographs of summer UG; showing : weak PAS reaction in capsule, intertubular septa, and all surface epithelium of secretory tubules of summer samples (purple colour), PAS ($\times 10$). (b) Photomicrographs of winter UG; showing : strong PAS reaction in capsule, intertubular septa, and all surface epithelium of secretory tubules (purple colour), PAS ($\times 10$).

References

- [1]- Bride J, Gomot L. (1978). Changes in the ecto-mesodermal interface during development of the duck preen gland. *Cell Tissue Res* 197: 141–149.
- [2]- Jacob, J. and Ziswiler, V., (1982). The uropygial gland. In FARNER, DS., KING, JR. and PARKES, KC. (Eds.). *Avian Biology*. vol. 6. New York: Academic Press, p. 199-324.
- [3]- Harem IS, Kocak-Harem M, Turan-Kozlu T, Akaydin-Bozkur Y, Kardag-Sari E, Altunay H (2010). Histologic structure of the uropygial gland of the osprey (*Pandion haliaetus*). *J Zoo Wildl Med*, 41: 148-151.
- [4]- Salibian, A. & Montalti, D. (2009). Physiological and biochemical aspects of the avian uropygial gland. *Brazilian Journal of Biology* 69, 437–446.
- [5]- Stettenheim PR (2000). The integumentary morphology of modern birds – an overview. *American Zoologist* 40, 461–477.
- [6]- Shawkey, M.D., S.R. Pillai and G.E. Hill, (2003). Chemical warfare effects of uropygial oil on feather degrading Bacteria. *J. Avian Biol.*, pp: 34: 345-349.
- [7]- Mayer, J., and B. V. S. D. Thomas M. Donnelly, (2012). *Clinical Veterinary Advisor: Birds and Exotic Pets*. Elsevier Health Sciences.
- [8]- Martín-Vivaldi, M., Ruiz-Rodríguez, M., Soler, J. J., PeraltaSa´nchez, J. M., Me´ndez, M., Valdivia, E., Marti´n-Platero, A. M. & Marti´nez-Bueno, M. (2009). Seasonal, sexual and developmental differences in hoopoe preen gland morphology and secretions. Evidence for a role of bacteria. *J. Avian Biol.* 40, 191 –205.
- [9]- Amat et al. (2011). Amat JA, Rendón MA, Garrido-Fernández J, Garrido A, Rendón-Martos M, Pérez-Gálvez A. Greater flamingos *Phoenicopterus roseus* use uropygial secretions as make-up. *Behavioral Ecology and Sociobiology*.
- [10]- Møller, A.P., Czirja´k, G.A. & Heeb, P. (2009). Feather microorganisms and uropygial antimicrobial defences in a colonial passerine bird. *Funct. Ecol.* 23: 1097–1102.
- [11]- Bancroft, J. D., & Gamble, M. (Eds.), (2008). *Theory and practice of histological techniques*. Elsevier health sciences.
- [12]- Menon, GK. and Menon, J., (2000). Avian epidermal lipids: functional considerations and relationships to feathering. *American Zoologist*, vol. 40, no. 4, p. 540-552.
- [13]- Kennedy, R. J. (1971). Preen gland weights. *Ibis*, 113(3), 369-372.
- [14]- González, C. A. (2014). Changes in mass of the preen gland in rock ptarmigans (*Lagopus muta*) in relation to sex, age and parasite burden 2007-2012 (Doctoral dissertation).
- [15]- Møller, A. P., & Laursen, K. (2019). Function of the uropygial gland in eiders (*Somateria mollissima*). *Avian Research*, 10(1), 1-6.
- [16]- Giraudeau, M., Stikeleather, R., McKenna, J., Hutton, P., & McGraw, K. J. (2017). Plumage micro-organisms and preen gland size in an urbanizing context. *Science of the Total Environment*, 580, 425-429.
- [17]- Martín-Platero, A. M., Valdivia, E., Ruíz-Rodríguez, M., Soler, J. J., Martín-Vivaldi, M., Maqueda, M., & Martínez-Bueno, M. (2006). Characterization of antimicrobial substances produced by *Enterococcus faecalis* MRR 10-3, isolated from the uropygial

- gland of the hoopoe (*Upupa epops*). *Applied and Environmental Microbiology*, 72(6), 4245-4249.
- [18]- Pan, P. R., Dilworth, B. C., DAY, E. J., & Chen, T. C. (1979). Effect of season of the year, sex, and dietary fats on broiler performance, abdominal fat, and preen gland secretion. *Poultry science*, 58(6), 1564-1574.
- [19]- Rajchard, J. (2010). Biologically active substances of bird skin: a review. *Vet Med*, 55(9), 413-421.
- [20]- Vincze, O., Vágási, C. I., Kovács, I., Galván, I., & Pap, P. L. (2013). Sources of variation in uropygial gland size in European birds. *Biological Journal of the Linnean Society*, 110(3), 543-563
- [21]- Moreno-Rueda, G. (2017). Preen oil and bird fitness: a critical review of the evidence. *Biological Reviews*, 92(4), 2131-2143.
- [22]- Bandyopadhyay, A., & Bhattacharyya, S. P. (1999). Influence of fowl uropygial gland and its secretory lipid components on the growth of skin surface fungi of fowl.
- [23]- CzirjákG.Á.PapP.L.VágásiC. I. GiraudeauM. MureşanC. MirleauP. HeebP.(2013). Preen gland removal increases plumage bacterial load but not that of feather-degrading bacteria. *Naturwissenschaften* 100: 145– 151.
- [24]- Soler, J. J., Peralta-Sánchez, J. M., Martín-Platero, A. M., Martín-Vivaldi, M., Martínez-Bueno, M., & Møller, A. P. (2012). The evolution of size of the uropygial gland: mutualistic feather mites and uropygial secretion reduce bacterial loads of eggshells and hatching failures of European birds. *Journal of Evolutionary Biology*, 25(9), 1779-1791.
- [25]- Meyer, W., Seegers, U., Herrmann, J., and Schnapper, A. (2003). Further aspects of the general antimicrobial properties of pinniped skin secretions. *Diseases of Aquatic Organisms* 53, 177–179. doi:10.3354/dao05317.
- [26]- Montaltl, D., Quiroga, A., Massone, A., Idiart, JR. and Salibian, A., (2001). Histochemical and lectin histochemical studies on the uropygial gland of rock dove *Columba livia*. *Brazilian Journal of Morphological Sciences*, vol. 18, no. 1, p. 33-39.