Research article:

Title: Changes in the Uropygial (preen) gland in Fulani ecotype chicken (*gallus gallus domestica*) a post-hatch study.

Alhaji Zubair Jaji[a], Latifah Ozohu Abdulkarim[a], Furo Nathan Ahmadu[b], Esther Solomon Kigir[a], Kenechukwu Tobechukwu Onwuama[a], AbdulGaniu Olawale Ibrahim[a] Shaibu Mohammed Atabo[c], Sulaiman Olawoye Salami[a] and Wanmi Nathaniel[a]

[a]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ilorin, Nigeria.
[b]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ilorin, Nigeria.
[c]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Bayero University, Kano, Nigeria.

Corresponding author: Dr. Kenechukwu Tobechukwu Onwuama

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Abstract: The Uropygial (Preen) gland, located dorso-cranial to the pygostyle and rectrices in birds was observed to have two pear-shaped lobes. It is a bi-lobed structure secreting oil for plumage maintenance. Its morphologic and histologic features during post-natal development were studied in the Fulani Ecotype chicken (*Gallus gallus domestica*) to document anatomical information that will be useful for further biomedical and embryological studies in this breed of chicken. A total of fifty six (56) Fulani Ecotype chicken were sampled, and studied in four phases of development. Each phase had 7 males and 7 females with live weight, weight of uropygial gland and weight of preen oil documented in each case. The morphometric traits of the excised gland were documented before squeezing and measuring the volume of the preen oil. Four Uropygial glands per developmental phase were also excised, and fixed in 10% buffered formalin solution for gross and histological analysis. The Uropygial gland of the Fulani ecotype chicken was observed to have two pear shaped lobes and a short papilla as early as week 2-3 of development. Three layers of the secretory cells were recognised at 7-8 months

post hatch. Findings support that uropygial gland of the Fulani ecotype chicken develop as early as at week 2-3 post hatch and the weight increases as the body weight increases. Histological findings indicate that the gland was fully developed from 7-8 months post hatch.

Introduction

The Nigerian indigenous chicken is one of the major sources of protein to the Nigerian people. The industry encompasses about 180 million birds, the second largest chicken population in Africa after South Africa [1].

Based on location, the Nigerian indigenous chickens are classified mainly into two breeds; The Fulani Ecotype and the Forest savannah (Yoruba) Ecotypes [3]. The Fulani Ecotypes (heavy ecotypes) are found in the Sahel and guinea savannah parts of Nigeria as well as the cattle Kraals and Montane regions of the north [4]. To survive some weather conditions such as rain, the chicken adapts by waterproofing its feathers through oily secretions from its uropygial (Preen) gland [5].

The Uropygial (Preen) gland is a prominent feature of most birds [6]. It is a bi-lobed structure enclosed in an irregular connective tissue capsule of collagen, elastic and reticular fibre [7]. The secretory tube which consists of four cell types; a germinative layer (basal), an intermediate layer, a secretory layer and a degenerative layer [8].

The Oleaginous secretion of the preen gland is a combination of extruded cells, ester waxes, fatty acids, fat and secretory granules [9]. Its chemical composition is highly variable at interspecific and intraspecific levels [10]. The gland is also of current interest because it provides source of chemicals used for communication in birds. Its anatomy has been investigated in the Kiwi [6], ducks [11] and gull [12] to mention a few. Literature search however, revealed little on post-natal developmental changes. This study examines the gross morphology and histological development of the uropygial gland in Fulani ecotype chickens to fill gaps in the literature on avian gland development.

Fulani ecotype cock

Fulani ecotype hen

Materials and methods.

Animals and ethics

A total of fifty-six (56) Fulani ecotype birds sourced from local backyard poultry farms in Ilorin metropolis (8.4882° N, 4.5341° E), Ilorin, Kwara state, Nigeria, were used for the study. The micro morphological study was carried out at the Veterinary Gross Anatomy Laboratory, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Kwara state, Nigeria. The birds were housed in a ventilated apartment, food and water were given ad libitum for a week. The protocols for this study were approved by the institutional Animal care and Use Committee (IACUC) of University of Ilorin, Ilorin, Nigeria with reference number FVM/UERC/0012021.

Study design and data collection

The birds were grouped into 4 phases of development with each group having 7 males and 7 females. Thus: the chick phase (2-3-week-old), the young phase (2-3-month-old), the young adult phase (6–9-month-old) and the adult phase (18 – 24 Months). Live weight of each bird was determined in gram (g) using the Harvard trip weighing balance (Citizen® with 0.1 g – 100 kg range) and the in-situ location of the preen gland documented in each case. The gland

was excised after humane slaughter, weighed using the Golden-Metler electronic balance (U.S.A., GF-300 Analytical Balance 310 * 0.001g, (A&D Weighing, India) and photographed with a digital camera (Nikkon Coolpix A100). The preen gland oil was then squeezed and weighed using the Golden–Mettler electronic balance.

Preen glands from two birds (male and female) per developmental phase were excised, and preserved in 10 % buffered formalin solution for processing onto histological slides. The prepared slides were viewed under the microscope (Olympus) with micrographs captured with the AMscope 500 microscope software.

Statistical analysis

Descriptive statistics (Mean ± SD) of the dimensions were carried out using the Microsoft excel worksheet (Microsoft office 2013 software, Microsoft©). Inferential statistics (analysis of Variance), to compare mean SD across the age and gender groups were carried out using the Graph pad software (Graph pad prism 5). Pearson's correlation coefficient of Graph pad prism version 5.0 was also used to determine the relationship between the live weight of each bird and gland weight of each bird and also the oil weight respectively. P-values less than 0.05 was considered significant.

Results

Gross morphology

The Preen gland of the Fulani ecotype chicken was located at the base of the tail, between the fourth caudal vertebra and the pygostyle (Fig. 1). It was made up of two pear shaped lobes and a short nipple like papilla. Each papilla was surrounded by 5-7 tufts of fine down, circlet, feathers (Fig. 2), depicting a type 2 circlet arrangement, as adopted from a classification by[13].

The gland was found in the birds as early as week 2 - 3 of age and continued to develop and function as the birds grew.

Morphometry

The respective means and standard deviations of weights in gram, (g) of preen glands and preen oils of genders and growth phases were compared to the live weights in gram, (g) of the birds and the significant differences across groups were obtained. The weights showed levels of significant increases across the age groups ($p < 0.001$) see Table 1. It was observed that there were age gender related levels of significant difference between the live weights of the male and female birds and that of their preen glands and oils right from the chick stage (Table 2).

Marked significant difference also exists between the weight of the preen gland and preen oil of male birds. This was also observed in the female (Table 3). There was no significant difference between the weights of preen glands of the male and female birds studied. Weight of the preen oil in the male bird was significantly higher than that of the female at 18-24 months of age (Table 3).

From age 2-3 months, the preen gland and its oil continued to increase in weight in both sexes possibly due to increase levels of metabolic and hormonal demand and need to maintain feather hygiene. (Table 3).

Histology

Though the secretory tubules of the Fulani ecotype chicken had sparing preen secretions in their lumens, the parenchyma was yet to be fully developed at the first phase of development at week 2-3. The cellular stratifications were poorly outlined resulting from highly spread parenchyma tissues. Distinct cellular aggregations or granules ranging from spherical, oval to ecliptic shapes were observed throughout the developing preen gland (Fig. 3).

The secretory tubules were separated by thick connective tissue septae, non-classical luminization, void of the capsule, blood vessels and had haphazardly arranged cells at 2-3

months (Fig. 4). Subsequent developmental stages were characterized by developed states of the preen gland. There were improved organization and size of the secretory tubules, and the overall parenchyma, translating into improved levels of preen oil secretion. The black spotted bodies were replaced by secretory cells thereby taking the orientation of a tripartite cellular strata (Fig. 4).

The parenchyma continued development at the second phase of development studied (4-6 months). At this stage, the secretory tubules were separated by thinner connective tissue septae and were clearly lumened. The cells of the secretory tubules were better arranged (Fig. 5).

At 6-9 months of development, the cells of the secretory tubules were arranged into three distinct layers: basal layer (cuboidal cells next to the basement membrane), intermediate layer of polyhedral cells and the secretory layer (had more secretory vacuoles, next to the tubular lumen). The cytoplasm of the cells became more vacuolated and increases in size due to their increasing secretory content as they approach the tubular lumen (fig. 6).

At 18 – 24 months post hatch development, numerous simple branched tubular secretory units which blindly end near the capsule. The entire gland was enclosed in a thick capsule of dense irregular, elastic, adipose and smooth muscle tissues. The capsule divides the gland into two separate lobes, was vascularised and innervated. It sent radiating septae into the substance of the parenchyma of each lobe, demarcating secretory tubules, establishing and linking its drainage channels to a central canal which in turn drained to the exterior by the papilla (Fig. 7).

Discussion

In this study, the uropygial gland of the Fulani ecotype chicken is situated between the fourth caudal vertebra and the pygostyle, dorsal to the levator caudalis muscle, at the base of the tail, agreeing with report on uropygial Glands of most birds [14] which are known to be situated

dorsally and medially to the synsacocaudal region visible to the naked. The Uropygial gland of Fulani ecotype chickens have a bi-lobed, conical flask-like structure with a single opening on each lobe and a short nipple-like papilla (Uropygial papilla) that lays dorsocaudal to the gland. Preen glands have been observed in a variety of configurations, including a heart-shaped preen gland with a broad bean-sized base in Ankra putra chickens [15]. However, according to [16] the duck's uropygial gland is not developed in this manner, therefore size is not a significant determinant in this species.

According to [16], the uropygial gland of birds is crucial for preserving feather hygiene and integrity, regardless of the shape of the feathers [18], preening, and dust bathing [19]. These could be one of the main reasons for the uropygial gland's growth at week 2-3, which coincides with the formation of feathers.

According to [20], the uropygial papilla is long and thin in chicken, wide and short in turkey, has two openings in geese and absent in musk ducks. [21] reported the papilla to be slightly above the tail and resembles a nipple in appearance [14]. An isthmus made of highly sturdy connective tissue clearly divides the papilla from the gland's lobes [22].

Each papilla is surrounded by 5-7 tufts of fine down and circlet feathers at week 2-3, which is a type 2 circlet arrangement according to a classification by Johnston 1988. This is in agreement with [21] and [14]. According to [21], the circlets help smear the oily secretion onto the bill.

With the exception of the female's preen oil, the live bird weight and preen gland weight continuously grow. This is consistent with other observations, like those of [22] on wild rock pigeons (*Columbia livia*) and [23] on the helmeted guinea fowls, which show that male birds weights are higher than those of female birds of the same species.

Due to its holocrine form and a close relationship between the histology of fowl and guinea fowl, the uropygial gland of the Fulani ecotype chicken corresponds to the mammalian sebaceous gland [24]. According to [25], the preen gland is surrounded by an irregular

connective tissue capsule made up of adipocytes and smooth muscles in domestic ducks, however [26] found that smooth muscles are absent from the capsule in kiwis. Smooth muscle is necessary for contraction, which leads to the opening of primary ducts and the ejection of secretion from the gland. There are other supportive systems in existence to compress secretions, therefore the lack of this smooth does not imply a lack of secretion [27].

In order to develop and link its drainage channels to a central canal, which is then drained to the exterior by the papilla, the capsules were vascularized and sent septae into the substance of the parenchyma of each lobe, demarcating secretory tubules. According to [28], the gland is made up of a lot of simple branched tubular secretory units that end blindly close to the capsule. Its holocrine nature is demonstrated by fragmentation of cells from the transitional cell layers of secretary tubules as seen in guinea fowl [29].

At week 2-3 post hatch, the interfollicular septae were not clearly visible. This demonstrates that interfollicular septae develops lobules of follicular cells as the chicken grows. The thickness of the interfollicular septae varies between species. The interfollicular septae are narrow in Indian peafowl and thick in water rails, respectively [30].

The glandular zones present in the secretory tubules were separated into a peripherally greater outer zone near to the tubular wall bordered with stratified epithelium and proportionally a lesser interior zone of large cells that was close to the lumen.

Between the formal layer and the bottom layer, which is made up of the tiniest cells that border the basement membrane, is an intermediate zone of relatively smaller cells. This finding is consistent with a prior work by [31]. In this investigation, the basal layer cells had a low cuboidal shape. They make up the top two layers of the secretory tubules' glandular cells. The cytoplasm of the cells in this layer is highly basophilic and darker than the cytoplasm of other guinea fowl cells, and the nuclei of these cells are spherical and darkly pigmented [7]. The Gallus domestica lack this exhibition.

Conclusion

It was concluded from this study that relationship exist between age of the Fulani ecotype chicken to the weight of the gland and sex. The preen gland from micromorphological perspectives, at week 2- 3 was not developed as it was devoid of capsules, septae and cellular layers that were fully formed from 6-9 months post Hatch. Having concluded with the above statement, it should be noted that limitations to this study may include sample size and environmental factors such as temperature and humidity

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Figures

Figure 1: Photographs showing in situ position of the Preen gland (arrow) of the Fulani ecotype chicken as seen at the base of the tail (Week 2-3).

Figure 2: Photograph of the excised preen gland of the Fulani ecotype chicken with circlet feathers (arrow heads) (Week 2-3).

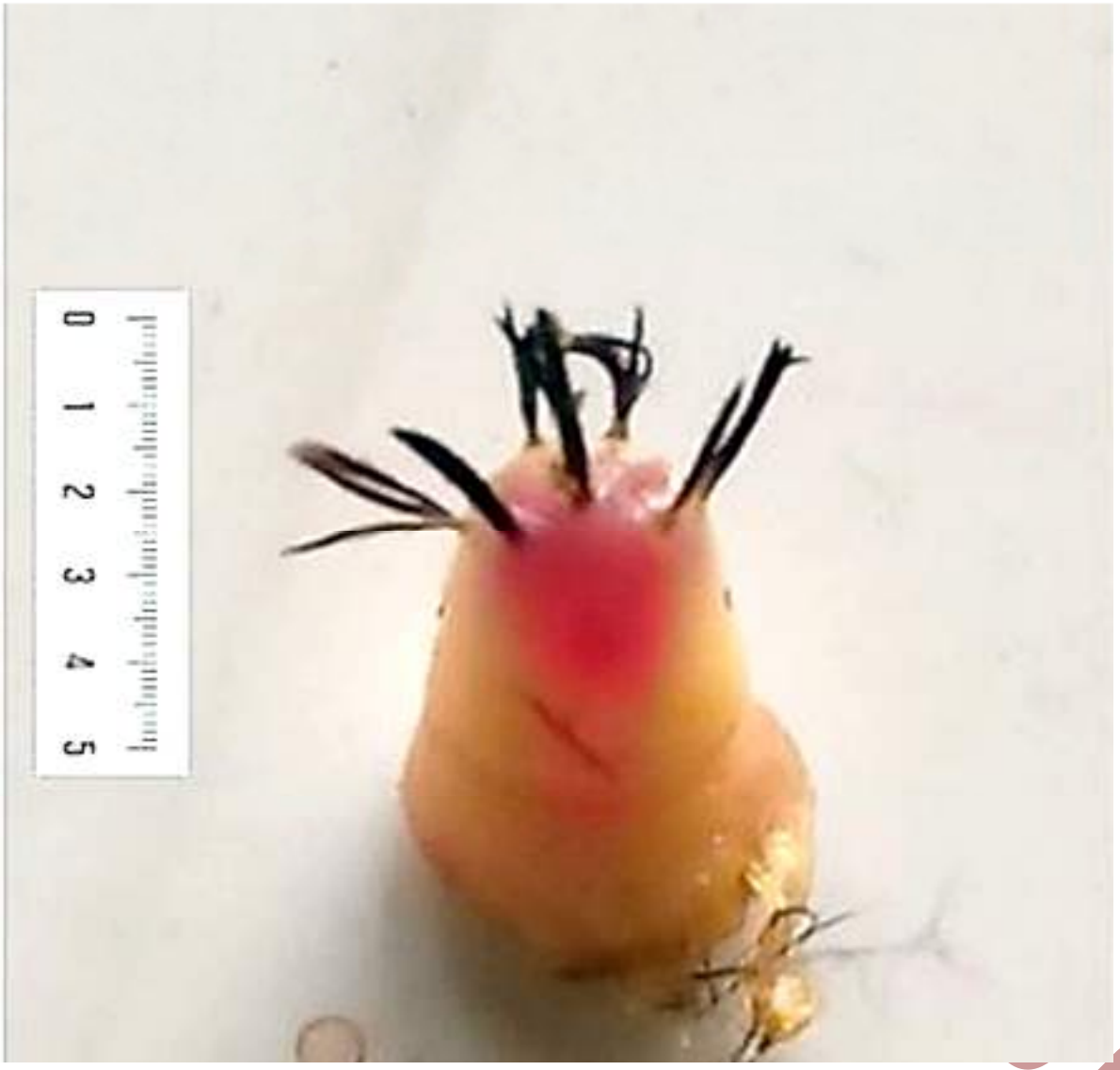

Figure 3: Micrograph of the Preen gland in a 2–3-week-old chicken. A: Depicts thick connective tissue septae (S) demarcating poorly lumenized developing secretory tubules (T). H&E X 40. B: Depicts poorly arranged glandular cells. H&E X 400.

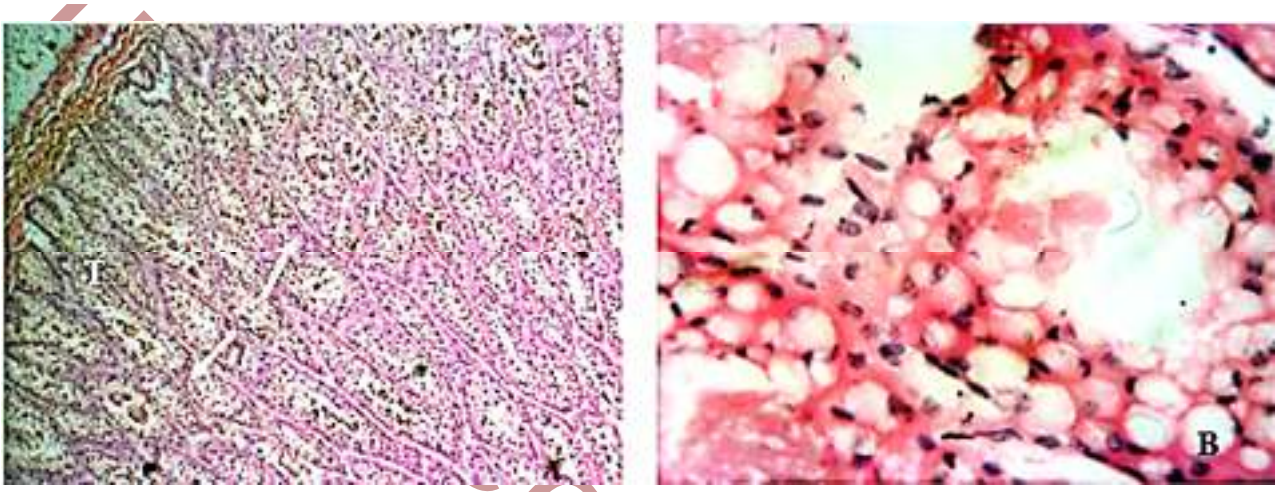

Figure 4: Micrograph of the Preen gland in a 2-3 month old chicken depicting thin connective tissue septae (S) demarcation of secretory tubules and clearer tubular lumen (L). The cells (C) of the tubules were better arranged. H&E X 100.

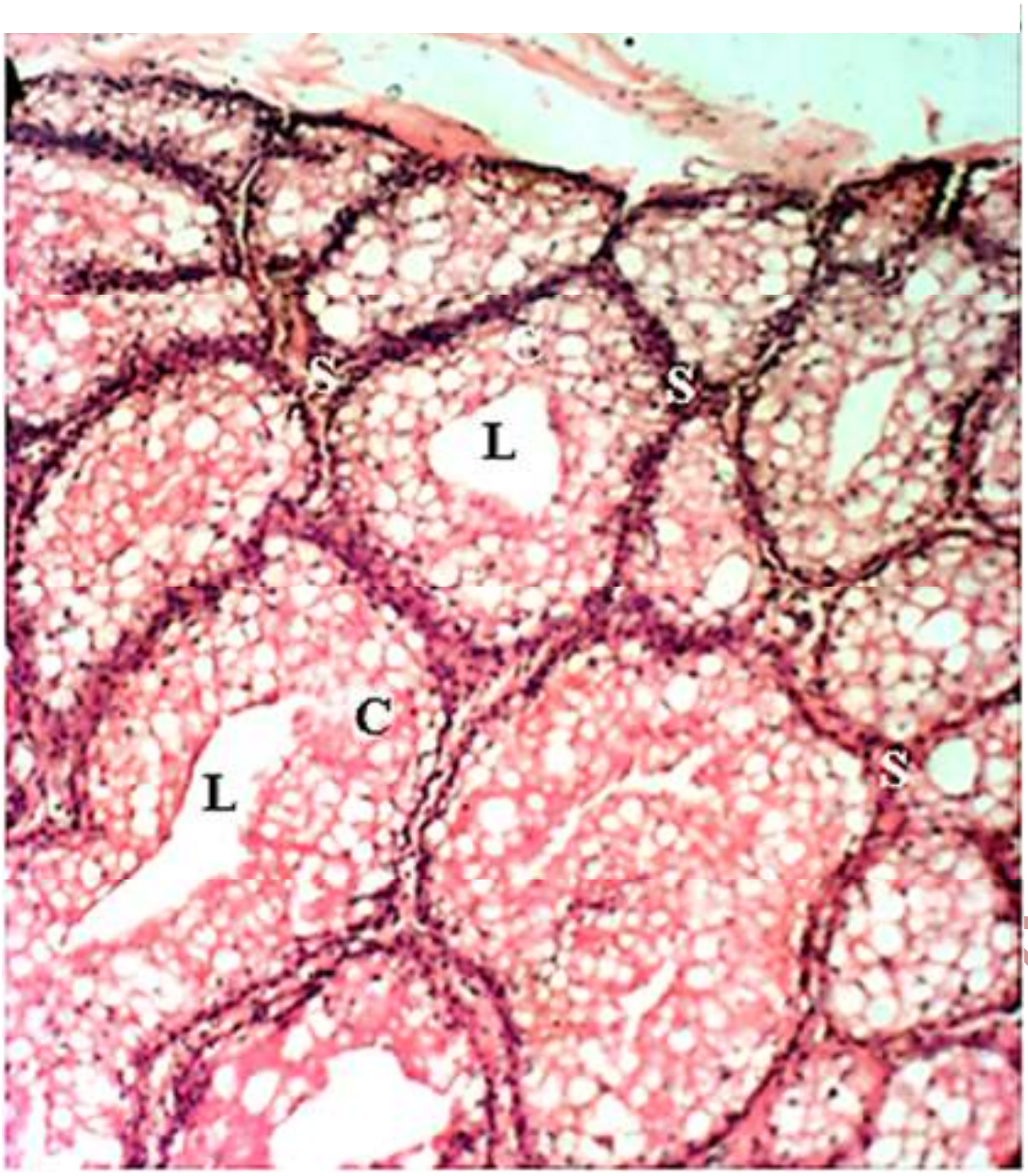

Figure 5: Micrograph of the Preen gland in a 4-6 month old chicken depicting the dense irregular and elastic connective tissue (CT) and smooth muscle (SM) fibres that make its thick capsule. The capsule is vascularized (V) and septae (S) into the glandular parenchyma to separate the secretory tubules (T). H&E, X 40.

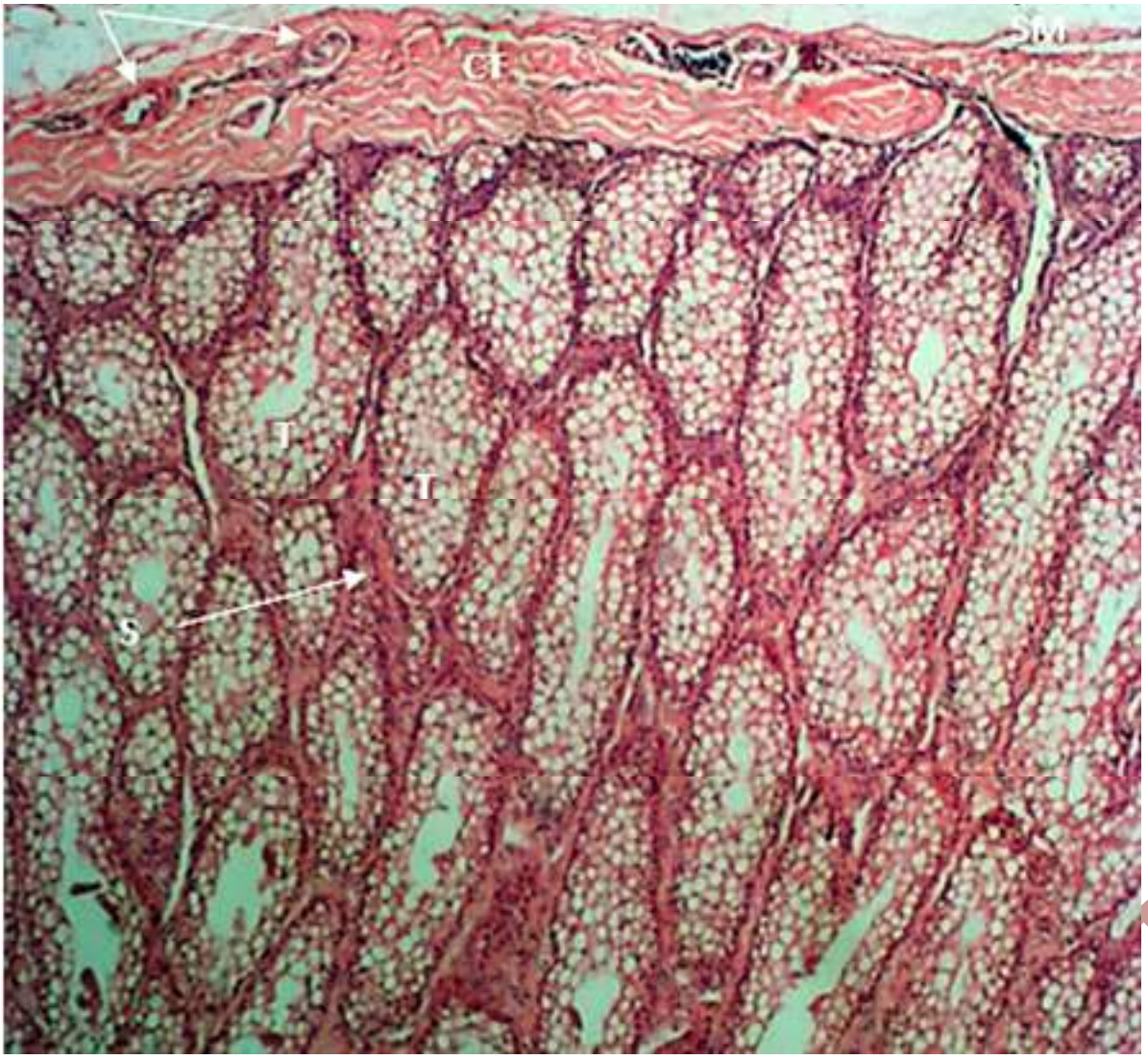

Figure 6: Micrograph of a fully developed Preen gland in a 6- 9 month old chicken depicting layers of i) basal cells (B), ii) intermediate cells (I) and iii) secretory cell (S) of a secretory tubule. The basal cells (cuboid shaped) are next to the basement membrane (BM), H&E X 400.

Figure 7: Micrograph of a fully developed Preen gland in a 6-9 month old chicken depicting layers of i) basal cells (B), ii) intermediate cells (I) and iii) secretory cell (S) of a secretory tubule. The basal cells (cuboid shaped) are next to the basement membrane (BM), H&E X 400.

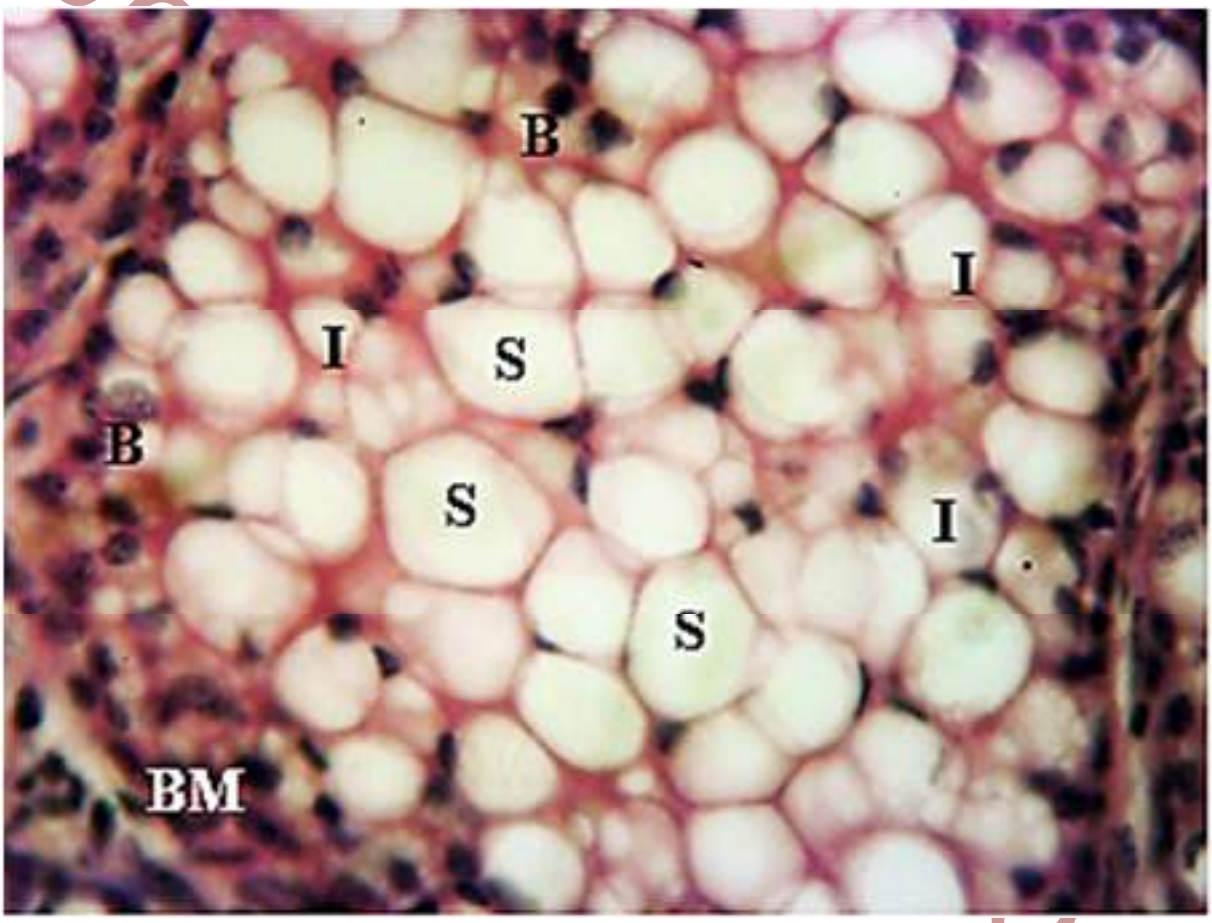

Figure 8: Micrographs of the parenchyma of the Preen gland in an 18-24-month old chicken. Depicting connective tissue septae (S) demarcating its secretory tubules (T) and linking drainage tributaries (Arrow) to the central canal (CC) H&E X 40.

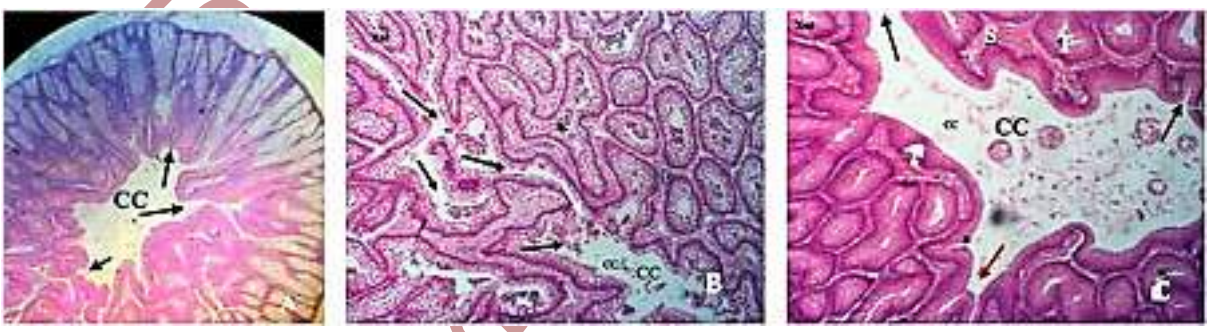

Table 1
Mean ± Standard Deviation of the Weights of Preen Oil, Preen Gland and Live Birds.

Parameters (Mean ± S.D)	**Sex**	**Postnatal Growth Phases (n = 6 per growth phase)**			
		2-3 weeks	**2-3 months**	**7-8 months**	**18-24 months**
Bird Live Weight (g)	Male	33.94±3.09	427.00±63.00 [b]	969.67±111.70 [c]	1090.67±417.21 [c]
	Female	32.35±0.87	229.67±37.82 [a]	931.33±79.86 [c]	1279.67±174.08 [c]
Preen gland Weight (g)	Male	0.10±0.01	0.40±0.08 [b]	0.74±0.18 [c]	0.86±0.25 [c]
	Female	0.04±0.01	0.45±0.02 [c]	0.77±0.02 [c]	0.90±0.07 [c]
Oil weight (g)	Male	0.01±0.01	0.05±0.02	0.15±0.03 [c]	0.43±0.07 [c]
	Female	0.02±0.01	0.07±0.03 [b]	0.09±0.07 [c]	0.12±0.07 [c]

Key: [a] = significant ($p < 0.05$, [b] = very significant ($p < 0.005$), [c] = extremely significant ($p < 0.001$).

Table 2
Comparisons of the Mean ± Standard Deviation of the Weights of Preen Oil, Preen Gland and Live Birds

Features	**Sex**	**2-3 weeks**	**2-3 months**	**7-8 months**	**18–24 Months**
Male vs female live weights	Male	ns	ns	c	a
Male vs female preen gland weights	**Female**	**ns**	b	c	b
Male vs female preen oils weights	**Male**	**ns**	**ns**	c	**ns**
Weight of gland vs Weight of oil	Female	ns	b	c	b
		ns	ns	b	ns
Relative weight of pineal gland	Male	c	c	c	c
		c	c	c	c
Relative weight of pineal oil	Female	c	c	c	c
		c	c	c	c

Key: ns = not significant at p= > 0.05; [a] = significant ($p < 0.05$, [b] = very significant ($p < 0.005$), [c] = extremely significant ($p < 0.001$), Vs= Versus

Table 3:
Relationship between the mean live weight, mean gland weight and mean oil weight of Fulani ecotype chicken during four post-natal growth phases.

Pearson's correlation coefficients (r)			
Correlated parameters	Male (n=3)	Female (n=3)	Both sexes (n=6)
Mean live weights vs mean gland weights	0.9994***	0.9460^{n}	0.9833*
Mean live weights vs mean oil weights	0.8395^{n}	0.2468^{n}	0.8918^{n}

*= Significant correlation ($P < 0.05$) *** = Highly significant correlation ($P < 0.001$) n = Non significant correlation, vs= Versus.