Nigerian Veterinary Journal
Vol 36 (3) 1210-1221
ARTICLE

Ultramorphology of The Ventriculus of Nectarivorous, Granivorous and Omnivorous Species of Passerine Birds

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SUMMARY

The objective of this study was to investigate the effect of disparate diets on the ventriculi of three species of similar sized granivorous, nectarivorous and omnivorous passerine birds. A total of twelve birds were captured with mist net under license and ventriculi from these birds were processed for light and electron microscopy. Nectarivorousventriculus had thin, poorly developed muscle and sac-like in shape while the granivorous gizzard had the thick, well developed muscle with the classical shape of biconvex lens and the omnivorous gizzard was intermediate. The wall of the ventriculus consisted of mucosal, submucosal, muscularis and inconspicuous serosal layers. *Tunica muscularis*, the most prominent layer of the wall was thickest in granivore, thinnest in nectarivore, and intermediate in omnivore. The mucosal glands were individual, tubular units with slightly expanded basal ends, demarcated from each other by dense connective tissue and opened individually into the lumen of the organ. Each glandular unit consisted of basal, chief, endocrine, neck and surface epithelial cells.The chief cells of the glandular units produced the "tubular" portion of the cuticle while the neck and surface epithelial cells produced the "surface" portion of the cuticle and the merger of these two portions formed the gastric cuticle, a protein mucopolysaccharide complex. The overlaying gastric cuticle (luminal cuticle) of the ventriculus was hard, tough, and sieve-like in the granivore, soft and jelly-like in the nectarivore and intermediate in the omnivore. The structural adaptations of the ventriculi of these three species to their various diets are discussed. There is need for more studies to be done on composition and functional morphology of the cuticle in aves.

Keywords: Morphology Ventriculus Gizzard Passerine Birds

INTRODUCTION

There have been relatively few reports on the ultrastructure of the ventriculus of most avian species. While the structure of the gizzard as well as the histology of its epithelium and gastric cuticle have been described in the domestic fowl, *Gallus gallus domesticus* (Aitken, 1958; Chodnik, 1947; Toner, 1964, Hill, 1971; McLelland, 1975, 1979; Duke, 1986),the American robin (*Turdus migratorius*) and the house sparrow (*Passer*

domesticus) (Klem *et al.* 1983, 1984). Morphological reports especially at the electron microscopy level are incomplete and rather antiquated.

The composition of the membrane lining the ventriculus has been controversial. Earlier authors (Bradley & Grahame, 1950; Calhoun, 1954) are of the opinion that the gizzard cuticle is chitinous,,keratohyalin, keratinoid, and keratin-like. However, later researchers (Eglitis and Knouff, 1962; Akester, 1986) indicate that the gizzard membrane is a muco-protein complex and its nomenclature has varied (Grujic-Injac *et al.* 1977; Akester, 1986) but it is now referred to as the gastric cuticle (Baumel *et al.* 1993). The consistency of this cuticle is said to reflect the differences in diets seen in avian species, i.e. it is jelly-like in nectarivores; semi-solid in omnivores and hard, tough and abrasion resistant in granivores, insectivores and herbivores (Ziswiler&Farner, 1972; McLelland, 1975, 1979).

Whereas most morphological studies of the avian alimentary tract have focused on the intestine (Moss, 1972, Brugger, 1991) or both (Whyte & Bolen, 1985; Wooller and Richardson, 1988; Wooller *et al,* 1990) in relation to different diet of the birds, little information exist particularly on the ultrastructural peculiarities of the ventriculus of most avian species regarding their diet.

This paper therefore describes the light and electron microscopical appearance of the ventriculi of a nectarivore, the Scarlet breasted Sunbird (*Chalcomitra senegalensis*), an omnivore, the African yellow white eye (*Zosterops senegalensis*) and a granivore, the Red-billed fire finch (*Lagnostica senegala*) which are small, similar sized (9.9 -12 gm), African passerines.

MATERIALS and METHODS

Animals and ethical aspects

Four specimens each of nectarivorous Scarlet breasted sunbird, SBS, *Chalcomitra senegalensis,* omnivorous African yellow white eye, AYW, *Zosterops senegalensis* and the Red-billed fire finch, RBF, *Lagnostica senegala* were trapped under license issued by the Nigerian Federal Ministry of Agriculture and Rural Development and the Western Australian Department of Conservation and Land Management and with the approval of The Animal Experimentation Ethics Committee of Murdoch University. Samples were processed according to Ogunkoya and Cook (2009).

Biometry and histological processing

Briefly, the birds were weighed and euthanized with intraperitoneal barbiturate (sodium pentobarbitone, Sagatal© 60 mg/ml; May and Baker Pty Ltd., Sydney, Australia). The specimens were perfused with half strength Karnovsky fixative (Platt et al., 1997; Casotti, 2001; Ogunkoya and Cook, 2009) (1.5% glutaraldehyde, 0.8% paraformaldehyde in 0.1 m phosphate buffer, pH 7.3) via the left ventricle of the heart. Following *in situ* perfusion, the ventriculi were cut and separated from the other segments of the gastrointestinal tract (from proventriculo-ventricular junction to the ventriculo-duodenal junction) and was freed from its mesenteric attachments and fixed in half strength Karnovsky fixative overnight. The ventriculi were measured (vernier's caliper and a ruler graduated in millimeters) and immersed in the same fixative for electron microscopy and in neutral buffered formalin for light microscopy for 24 hours.

Samples of the ventriculi for light microscopy were dehydrated in alcohol, cleared in chloroform and embedded in paraffin wax. Longitudinal and transverse sections (6-µm thick) were cut and stained with haematoxylin and eosin (H & E).

Electron microscopy

Scanning electron microscopy

Tissues for scanning electron microscope (SEM) were cut longitudinally to show the luminal surface and specimens for TEM were cut transversely into 1 mm^3. The tissues were

washed several times in phosphate buffer and post-fixed in 1% Dalton's osmium tetroxide for 1.5 hours at 4°C. The tissues were dehydrated in a graded series of ethanol. Those for SEM were critically point dried (Balzers Union, Liechteinstein), mounted on individual stubs with double-sided tape, sputter coated (Balzers Union) with gold and examined with a Philips XL 20 scanning electron microscope (Eindhoven, The Netherlands).

Transmission electron microscopy

Specimens for transmission electron microscope (TEM) were also dehydrated in a graded series of ethanol, cleared in two changes of propylene oxide, infiltrated with a mixture of 60:40 propylene oxide and Epon for 1 hour at 4°C, transferred to pure Epon on a rotator overnight, embedded in pure Epon and allowed to polymerize for 24 hour at 60°C. Block surfaces were trimmed with hacksaw and single-edge razor blades. Using glass knives and a Reichert OMU3 ultramicrotome (Vienna, Austria), semi-thick, 1 µm sections were cut, stained with toluidine blue and viewed under the light microscope (Olympus BH2) for tissue orientation. Ultrathin sections of 70–90 nm thickness were cut using a diamond or glass knife on the ultramicrotome (Vienna, Austria), chloroformed in the boat, collected on 200 mesh copper grids and allowed to dry on filter paper. The sections were stained with aqueous uranyl acetate for 7 min, counterstained with freshly prepared lead citrate for 7 minutes, washed 40–60 times in double distilled water, dried on filter paper and carbon coated (Dynavac, Sydney, Australia). Sections were examined using a Philips 301 or CM100 BioTwin transmission electron microscopes (Eindhoven, The Netherlands) operated at an accelerating voltage of 80 KV.

RESULTS

Gross anatomy

The anatomy of the well-developed granivorous gizzard is the descriptive template.

Grossly, the gizzard was a musculo-tubular structure. This organ in the granivorous Red-billed fire finch (RBF) appeared darkish red (Fig. 1) and only the gizzard of

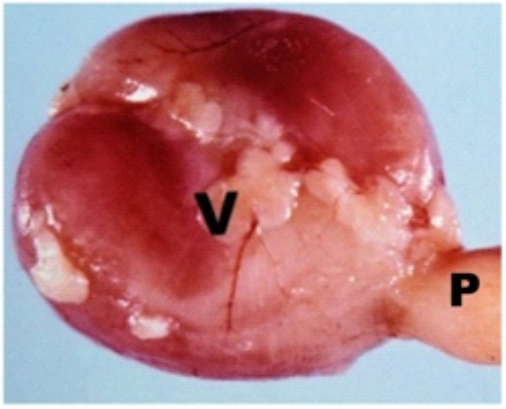

Fig.1: The stomach of the granivorous Red-billed fire finch, *Lagnosticasenegala*.P– remnant of the proventriculus; V– ventriculus. Bar 13 mm.

this species had the classical shape of a double-sided lens. It had two borders, two extremities and two surfaces. The organ had a convex dorsal border and a convex ventrolateral border. The proventriculus entered the gizzard from the cranial extremity, the cranial blind sac. The caudal extremity was in contact with the intestinal segments and other visceral organs. On its two surfaces were the flat tendinousaponeurotic sheets and vascular grooves. The tendinousaponeurotic sheets fanned out radially on the two surfaces of the organ. The two pairs of muscle originated and inserted into the aponeurotic sheets. The two groups of muscles were the paired thick and thin muscles. The thick muscles, the *musculi crassicranioventralis et caudodorsalis*, which formed the body of the organ. The paired thin muscles were the *musculi tenuis craniodorsalis et caudoventralis* which formed the extremities and the craniodorsal and the caudoventral blind sacs, the *saccicranialis et caudalis*. The caudodorsal thick muscle was continuous with the caudoventral thin muscle and at their junction was the transverse caudal groove, the *sulcus caudalis*. The cranioventral thick

muscle was continuous with the craniodorsal thin muscle and at their juncture was the transverse cranial groove, the *sulcus cranialis*. The ventriculo-pyloric opening was on the right tendon of the right surface of the organ. Internally, the cavity of the organ was lined by a firmly attached gastric cuticle, the lumen of which contained small pebbles or grits. The consistency of this membrane (described below) varied in the three species. The cuticle had a yellowish green to green colour in all the three species.

The cream colored, poorly developed, undifferentiated, sac-like gizzard of the nectarivore was lined by a soft, jelly-like gastric cuticle which readily detached from the underlying tissue with little or no manipulation. There were small dark pollen materials in the relatively larger lumen of the gastric cuticle.

The moderately developed gizzard of the omnivore was also pale colored, but more muscular than that of SBS. The outline of the tendinous sheets was more discernible and the gizzard lumen was lined by a semi-solid gastric cuticle that was more firmly attached to the underlying tissue than in the nectarivore.

Biometry

The average measurement of the small, sac-like musculo-tubular gizzard of the nectarivorous Scarlet breasted sunbird (SBS) measured 4.4 mm craniocaudally and 5.5 mm dorsoventrally. The omnivorous African yellow white eye (AYW) had a moderately sized, pale to cream colored gizzard. The craniocaudal diameter of which was 5.2 mm and 7.2 mm dorsoventrally and the granivorous Red-billed fire finch (RBF) had the biggest organ, the average measurement of which was 9.1 mm craniocaudally and 11.5 mm dorsoventrally.

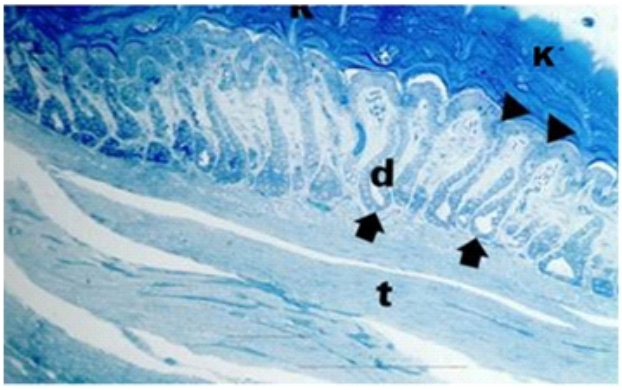

Fig. 5: Light micrograph of the ventricular wall of the nectarivorous *Chalcomitrasenegalensis* stained with toluidine blue. Note the slightly expanded (arrows) basal end of most of the glandular units and that each ventricular glandular unit opens individually (arrowheads) into the luminal surface of the ventriculus. Cuboidal chief cells (**d**) of the glandular unit, the luminal gastric cuticle (**k**) and the *tunica muscularis* (t). Bar 1μm.

Light microscopy

Toluidine blue stained specimen is described.Histologically (Fig. 5) the gizzard consisted of mucosal, thin submucosal, thick tunica muscularis and serosal layers. The gastric membrane overlaid the surface epithelium of simple columnar cells. The surface epithelium invaginated into the *lamina propria* to form the simple, tubular, mucosal glands. The invaginations were deeper in the longitudinal ridges than those seen in the grooves between the ridges. The columnar cells had large, spherical, basal nuclei. The *lamina propria* consisted of dense connective tissue and numerous capillaries and demarcated each glandular unit while the *lamina muscularis mucosae* was not seen, thin submucosal layer was only evident around large blood vessels. The *tunica muscularis* was the widest layer of the organ and it consisted of three layers: a thin, inner longitudinal, a thick middle and a thin outer longitudinal layer of smooth muscle fibers. The serosal layer was made of mesothelial cells, adipose cells, large blood vessels, bundles of axons and lymphatic tissue'

The glandular units were tubular. Each tubule

had a base, a neck and opened into the lumen of the organ through a small pore. Each glandular unit opened individually into the lumen of the ventriculus (Fig. 5). The structure of the glands was similar in all three species with the base of most being slightly expanded. However, not all glandular units were the same height.

The gastric membrane lining the gizzard (luminal cuticle) stained deep blue and bluish grey with toluidine blue.

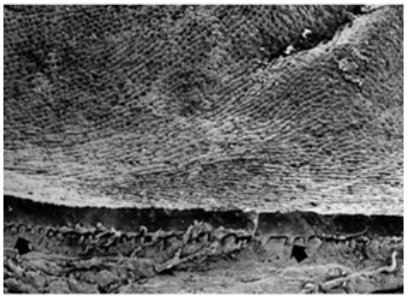

Fig. 2: Scanning electron micrograph showing the sieve-like, pitted surface of the gastric cuticle of the granivorous*Lagnostica senegala.* Note the rod-like projections (arrow) into the mucosa. Bar, 100 m.

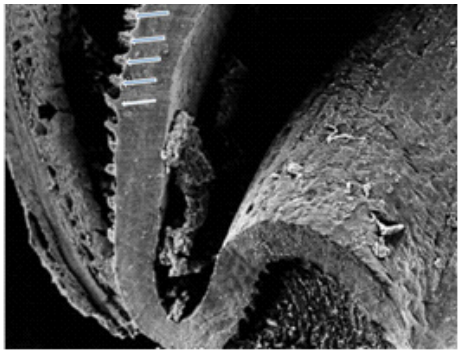

Fig 3: Scanning electron micrograph of the gastric cuticle of omnivorous *Zosterops senegalensis* showing 'the rod-like' projections of the tubular cuticle (white arrows) that have pulled away from the underlying mucosal membrane (black arrowhead). Note that the cuticle on the ridge appears thicker than that of the groove having luminal content. Bar, 100 m.

Fig 4: Scanning electron micrograph of the luminal surface of the gastric cuticle of the nectarivorous*Chalcomitrasenegalensis*. Note the longitudinal ridges and shallow grooves of the membrane. Bar 100 µm

Scanning electron microscopy

Scanning electron microscopy showed that generally, the luminal surface of the gastric cuticle had smooth longitudinal ridges with shallow grooves and the underside of the gastric cuticle conformed to the pattern of the underlying mucosal surface having projections that extended down into the underlying glands (Fig. 2 & 3). The ridges were finer in the omnivore (Fig. 2 & 3) than in the granivore and were not evident in the nectarivore (Fig 4). The openings (pores) of the ventricular glands were located in the grooves and appeared to form arch-like openings with the surface epithelial layer. The surface of the gastric cuticle of the granivore had a sieve-like appearance (Fig. 2)

Transmission electron microscopy

Ultrastructurally, there are four types of cell in the glandular units: light, chief, endocrine (described elsewhere) and neck and surface cells. The light cells (Fig. 6) were cuboidal, basal and had large, round, pale nuclei in a pale cytoplasm which contained round to oval mitochondria, polysomes and scattered profiles of rough endoplasmic reticulum (RER). The cell membrane had apical

microvilli, junctional complexes and lateral interdigitations.

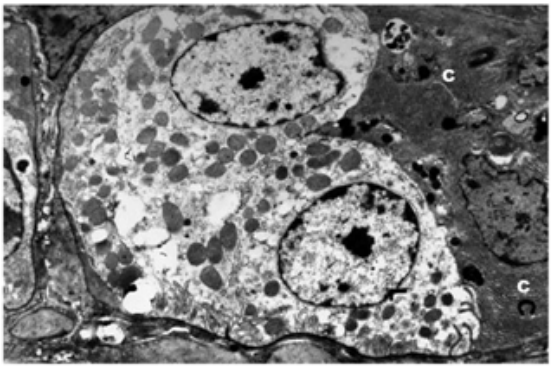

Fig 6: Transmission electron micrograph of two, light, basal cells of the glandular units of the ventriculus of nectarivorous *Chalcomitra senegalensis*. Note that these cells lack the extensive rough endoplasmic reticulum of the chief cells. Cuboidal chief cells (C), nucleus of light cell (L), mitochondria (M) and lysosomes (Y) Bar 1 μm.

encircled many organelles in the dense cytoplasm. These cells had apical junctional complexes and few lateral interdigitations. The electron-dense secretory granules were numerous at the apical portion of the cells. The dense secretory granules in the chief cells of the nectarivorous and omnivorous species were small in comparison with those in the

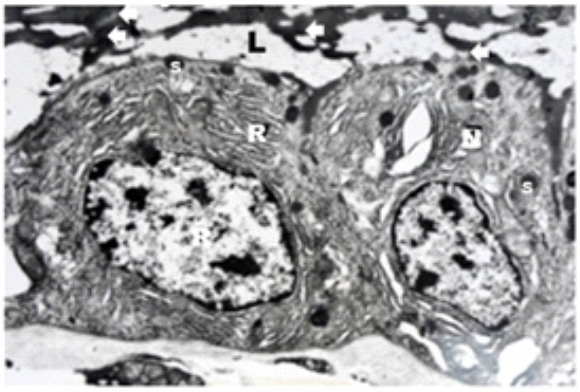

Fig 7: Transmission electron micrograph of cuboidal chief cells in a glandular unit of the ventriculus of granivorous *Lagnostica senegalensis*. Note the extensive rough endoplasmic reticulum (R), secretory granules (S) mitochondrion (N) and the dark tubular cuticle (arrows) in the lumen (L) of the gland. Bar 1μm

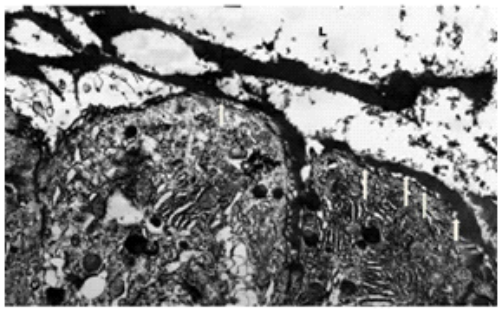

Fig 7a: Transmission electron micrograph of the coalescing tubular gastric cuticle in the lumen of the glandular unit of the granivorous RBF. The tubular gastric cuticle coalesces into filamentous stands. Lumen (L). Arrows show secretory products condensing on the apical microvilli and bending them toward the lumen. Bar 1μm.

granivore which were larger and less electron-dense. The granules appeared to be released into the lumen of the glandular unit by exocytosis between the bases of the short, stubby, microvilli and it encircled the microvilli without condensing on them and appeared to "flow" into the lumen of the glandular unit

where it coalesced or aggregated (Fig. 7a) into homogeneous structures (filamentous strands of tubular cuticle) towards the neck of the glands and became continuous with the surface membrane of the gizzard.

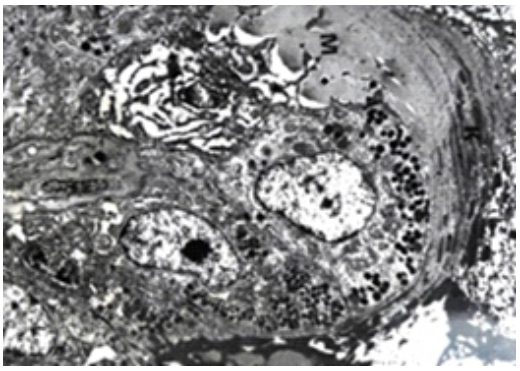

Fig 8: Transmission electron micrograph of surface columnar cells in the gizzard of *Lagnosticasenegalensis*. Note the cuticle filaments at the top left of the micrograph and the numerous apical secretory granules. Bar 1µm.

The mouth and surface epithelial cells (Fig. 8) had round to oval, pale, central nuclei. The cytoplasm contained polymorphic mitochondria, a supranuclear Golgi complex, numerous polysomes, and mostly basal RER. Small secretory granules of varying electron density occupied the supranuclear cytoplasm (Fig. 8a). The apical cell membrane had short, stubby microvilli and the lateral membranes had apical junctional complexes and many interdigitations. The secretion from the neck and surface cells

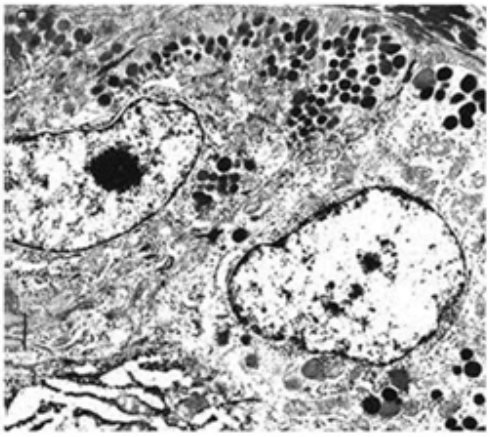

Fig 8a: Transmission electron micrograph of neck cells in the gizzard of *Lagnostica senegalensis*. Note the cuticle filaments at the top left of the micrograph and the numerous apical secretory granules. Secretory granules (S).

appeared to merge with the tubular cuticle to form the luminal cuticle.

TEM also revealed that the *lamina propria* consisted of collagen fibers, blood vessels and bundles of axons. Small bundles of unmyelinated axons, often closely associated with blood vessels, between the bundles of smooth muscle fibers of the three-layered *tunica muscularis* were also observed. Numerous intramural ganglia, especially between the outer longitudinal and middle circular muscle, were also seen in this muscle layer.

DISCUSSION

The ventriculi in these three bird species have the same basic morphology as that described for other avian species but some differences were identified. The observation of the current study agrees with the remark that the degree of development of the gizzard reflects the diet of that particular species (Ziswiler and Farner, 1972). Nectar- and fruit- eating birds have a reduced gizzard while insect- and grain- eating birds have a highly muscular gizzard (Smith, 1975; Guntert, 1981). Accordingly, the thinly muscled, sac-like gizzard of the nectarivore is a reflection of its liquid diet, nectar, which needs no processing or trituration before absorption. The well-developed, thickly muscled gizzard of the granivore is an indication of the amount of processing needed to digest grain. The peristaltic contractions of the gizzard musculature lead to grinding, pressing and rubbing effects (Ziswiler and Farner, 1972) of the grain against the grit in the cavity of the gastric cuticle (Moore, 1998) resulting in thorough crushing and digestion of the grain. Thus the observed grit in the cavity of the gastric cuticle enhances thorough and maximal performance of the gizzard in this species and is an indirect morphological, functional adaptation to the grain. The intermediate gizzard of the silvereye reflects the moderate processing of its fruit and nectar diet.

The shape of the gizzard is a functional, morphological, adaptation. Gross morphology in the current study revealed that the gizzard has two shapes: sac-like and bi-convex lens or double-sided lens. The nectarivorous gizzard in the Scarlet breasted sunbird is sac-like with poorly developed muscles while that of the granivorous Red-billed fire finch has the characteristic shape of a bi-convex lens with well-developed muscles. The sac-like, poorly-muscled gizzard of the nectarivore functions as a store to liquid diet, nectar which needs a relatively larger surface area to accommodate it. The well developed, thick muscled, bi-convex lens gizzard of the RBF is needed for thorough and maximal trituration of the grain/seed consumed by this species. The observation above supports the consensus of earlier workers (Cornselius, 1925; Pernkopf and Lehner, 1937; McLeland, 1975) that the shape of the gizzard determines the diet of an avian species. The shape of the gizzard in the above two species is a functional dietary adaptation to their respective diets. Thus, Scarlet breasted sunbird is a specialist nectarivore while Red-billed fire finch is a specialist granivore. The African yellow white eye with its moderately developed, intermediate gizzard combines the characteristics of the other two species.

The observation of the current study on the granivorous gizzard reinforces the earlier reports that the bi-convex gizzard has a main cavity and two smaller sacs while the sac-like gizzard has only one cavity (McLelland, 1979).
The consistency of the gastric cuticle in different species of birds varies from a soft to hard and tough structure. Aitken (1958), Eglitis and Knouff (1962) and Akester (1986) on the domestic fowl, Bezuidenhout and van Aswegen (1990) on the ostrich reported the membrane to be hard, tough and abrasion resistant, while Richardson and Wooller (1986, 1990) observed its softness in the nectarivorous birds. The observations in the present study support these earlier reports.
Gastric cuticular consistency is an indicator of the diet consumed by a particular species. Birds eating liquid diet tend to have a jelly-like gastric cuticle and those eating tough, fibrous diets have hard, tough and abrasion resistant gastric cuticle (McLelland, 1979). The observation in the present study tallies with these reports. The gastric cuticle of the nectarivorous gizzard was soft and jelly-like while that of the granivorous gizzard was tough and abrasion resistant with that of the omnivorous gizzard been intermediate in consistency.
The concept that gastric cuticle on top of the ridges is thicker than that in the grooves (Gabella, 1985) is consistent with the observation in the present study

The surface epithelium is thrown into ridges and grooves. This is suggestive of the ability of the organ to increase its surface area (Ziswiler, 1990), consequently, the gizzard of these three species can increase the luminal surface area.
The histology of the organ corresponds to that of the gizzard of other birds. However, certain differences are worth mentioning. Calhoun (1954) in the domestic fowl *Gallus gallus domesticus* and Klem *et al*. (1984) in the American robin, *Turdus migratorius* reported a well-developed submucosa in the gizzard. In contrast to their report, the current study observed a poorly developed submucosa in these three species of birds. The report that the gizzard lacks both the *lamina muscularis mucosae* and the submucosal layer agrees with the observation of the current study (Ziswiler and Farner, 1972).
The *tunica muscularis* in the gizzard of the current species consist of three layers: inner, thin, longitudinal; middle, thick, circular and outer thin, longitudinal layers (Hodges, 1974). Swenander (1902) reported only two layers and Bradley and Grahame (1960) identified only the middle circular layer. The observation that the muscle layer is divided into separate bundles by connective tissue fibers agrees with the reports of Hodges (1974); McLelland, (1979) and Klem *et al*. (1984).
In the current study, the simple columnar epithelium invaginated into the *lamina propria* to form the simple glandular units. This

observation agrees with the reports of Eglitis and Knouff (1962) and Bezuidenhout and van Aswegen (1990). However this observation does not support the concept that these glands are in groups, 10-30 glands per group, and that 5-8 glands open into a crypt (Eglitis and Knouff, 1962; Akester, 1986). The present study showed that each glandular unit opened individually into the lumen of the gizzard through the 'mouth' or pore.

Light microscopy revealed that the glands are not of the same height: those in the ridges were longer than those in the grooves. This observation may be analogous to the remark made by McLelland (1979) that the glands did not develop equally.

Ultrastructural observation in the current study located the paired light cells to the base of the glandular units. The light cells of the current study correspond to Toner's observation (1964) on basal cells that are limited to the basal part of the glands.

The chief cells of the glandular units of the gizzard have the ultrastructural features of protein-secreting cells, with their most striking features of dilated cisternae of RER, which formed whorls in the cytoplasm. In the extremely dense cytoplasm are the apical secretory granules (Toner, 1964). Overall, these chief cells had many features comparable with zymogen cells (Bloom and Fawcett, 1975). The characteristic, extensive, dilated RER of these cells are physiologically important in the production of protein needed for export (Hammersen, 1985; Leeson *et al*., 1988).

The surface epithelial cells have been suggested to be unusual mucus secreting cells (Toner, 1964). Buddington and Doroshov (1986) referred to this type of cell as goblet cells with microvilli and Boer and Kits (1990); Lobo-da-Cunha et al. (2010) referred to them as mucocytes.

The current ultrastructural study shows that the gastric cuticle consists of two components: a portion secreted by the chief cells which is probably proteinaceous and a second portion, probably a mucopolysaccharide, secreted by the neck and surface cells. The probable nature of the secretion is based on the ultrastructural features of the cells involved, the appearance of the secretion and the conclusion of previous reports (Aitken, 1958; Eglitis and Knouff, 1962; Akester, 1986). The current study confirms the report (Akester, 1986) that the gastric cuticle is produced and secreted by two portions of the glandular units. The chief cells at the base and neck region of the glands synthesize and secrete the tubular cuticle while the mouth and surface epithelial cells synthesize and secrete the surface cuticle. 'Tubular cuticle' is continuous with the 'surface cuticle' and the merger of the two fractions forms the luminal cuticle. However, there was no evidence in the species investigated in this study that microvilli of the secretory cells break off to be incorporated into either the tubular or the surface cuticle as previously suggested (Akester, 1986). The report that the chief cells had straight, stubby microvilli, covered with the dense membrane continuous with the surface membrane (Toner, 1964) tallies with the observation of the current study.

The concept that the tubular cuticle solidifies in the lumen of the glandular units (Eglitis and Knouff, 1962; Akester, 1986) was not supported by the current observations. The tubular cuticle was released by exocytosis into the lumen of the glandular unit. However, this appeared to be a liquid secretion since it encircled and covered the microvilli. Furthermore, a solid secretion would not readily 'flow' from the lumen of the glandular unit to the lumen of the gizzard.

The possible mechanism of the 'flow' of the tubular cuticle from the lumen of the glandular unit to the lumen of the organ is still not clear. It has been suggested that the movement of the tubular cuticle is associated with the upward movement of cells following mitotic division of basal cells and subsequent migration of these cells (Akester, 1986). However, it seems probable that the peristaltic movement of the gizzard muscles may also enhance the upward movement of the cuticle towards the lumen of the gizzard. In accordance with the report of

Ziswiler and Farner (1972) the contraction of the gizzard muscles results in rubbing, grinding and pressing effects and this may enhance the luminal flow of the tubular cuticle.

Mucous cells and some goblet cells have apical microvilli (Bayer *et al*., 1975; Buddignton and Doroshov, 1986).The microvilli present on the apices of cells are contractile (Hammersen, 1985) and they also assist in the distribution and maintenance of the mucus coat (Buddington and Doroshov, 1986). This may be true of these organelles in the gizzard since ultrastructure has shown that the cells of the glandular unit and surface epithelium have apical microvilli. It is suggested that the tubular cuticle flows to the lumen of the gizzard by the active assistance of the apical microvilli. At the surface, the luminal cuticle merges with the surface cuticle and is spread over the lumen by the action of microvilli on the surface cells.

The difference in the consistency of the gastric cuticle in the current species of passerines is related to their different diets. The different consistency of the gastric cuticle is probably due to the quantity and quality of the zymogen granules and transit time of the digesta. In the nectarivore few chief cells of the gizzard had "smaller sized" zymogen granules when compared to that of the omnivore and or the granivore. This suggests that the protein portion or the tubular cuticle in the luminal gastric cuticle is less in this species, signifying that the luminal gastric cuticle in the nectarivores is more of a mucopolysaccharide. Additionally, the diet of this species is liquid and has faster transit time in the gastrointestinal tract allowing little or no mixing of the acidic content from the proventriculus with that of the ventriculus resulting in little or no acidification of the gizzard cuticle in this species. The hard, tough, abrasive resistant, luminal gastric cuticle in the granivores presumably has more protein portion and the acidic content from the proventriculus stays longer in the ventriculus for trituration and thorough digestion. The longer exposure of the gizzard cuticle to the action of the HCl in the digesta from the proventriculus results in acidification of the cuticle and the differential contact with HCl results in its lamellar appearance. The omnivores have intermediate textured gastric cuticle which could be attributed to the intermediate production of the zymogen granules by the chief cells of the gizzard and intermediate transit time of its digesta.

CONCLUSIONS

The present work highlights the variations in the morphology of the ventriculus among three passerine species that live on different diets. This provided information will update the knowledge of the structure and the ultrastructure of the ventriculus in these species as well as enhance further work on the functional morphology of the organ among avian species.

Acknowledgments

The authors are grateful to Professors. A. Ezealor, J. Ogbajah and A. B Ogunkoya for assistance in obtaining the specimens, M. P. Fallon of the EM Unit and Murdoch University, and to USAID for funding the research.

Conflict of Interest

The authors declare no conflict of interest

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