HISTOGENESIS OF THE FORESTOMACH OF RED SOKOTO GOATS

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ABSTRACT

Data are presented on the prenatal development of the forestomach of red Sokoto goats. Three stages of fetal development were studied by gross and light microscopy techniques. The findings demonstrate that the primordia of all the components of the forestomach were present at 39 days of gestation. The mucosal surfaces of the rumen were smooth and later became granular at 30 and 96 days of gestation, respectively. Granules were observed on the mucosal surface of the omasum at 95 days of gestation. The rumen, reticulum and omasum differentiated into epithelium, blastemic tissue and serosa at 40 days of gestation. The epithelium of the entire forestomach consisted of superficial stellate cells and basal globus shaped cell layers at 53 days. The underlying bastemic tissue differentiated into disorganized arrays of myoblastic tissues at about 53 days. The primordia of the ruminal papillae appeared as protuberances of the stratum basale at 53 days. The ruminal papillae progressively increased in dimension with advance in age of the fetus. Reticular ribs were first observed on the mucosa of the reticulum at 65 days of gestation. In the reticulum, the lamina propria and submucosa were poorly demarcated throughout the study. The myoblastic tissues differentiated into isolated muscle bundles at the tips of the primary reticular folds at 124 days of gestation. Reticular cells were observed first at 145 days of gestation. Omasal leaves were first identified at 120 days. The forestomach of this goat differentiated earlier and developed faster than most of the other breeds.

Keywords: Red Sokoto goat, Histogenesis, Omasum, Reticulum, Rumen

INTRODUCTION

Red Sokoto goats and West African Dwarf goats are the two most important goat breeds found in Nigeria (Shaib *et al*., 1997). The breed is economically important in northern Nigeria, where they constitute an important source of animal protein (Akpa *et al*., 2002).

The stomach of ruminants has four compartments. Each of these organs has a very distinctive mucosa structure. The rumen appears to be the most important component of the forestomach as it is involved in breakdown of feed and serves as the primary site for microbial fermentation. The ability to browse and optimise the use of grazing land has been linked to the peculiar nature of the stomach of ruminants (Garcia *et al*., 2012). From embryological findings, the compartments of the forestomach are derived exclusively from the stomach spindle (Dyce *et al*., 2002). The prenatal developments of the forestomach have been studied in different breeds of ruminants such as deer (Franco *et al*., 2004), West African dwarf goat (Nwaogu and Ezeasor, 2008; Garcia *et al*., 2012). The findings of these studies may not be deduced to that of red Sokoto goat because of variations in genetic composition, climate and feeding habits. Information on the prenatal development of forestomach of red Sokoto goat appears to be unavailable. The paucity of this information is worrisome as it has been

demonstrated that animal health in post-natal life could be influenced by events during the pre-natal life (Greenwood *et al*., 2010). The value of organ embryology has led to increased interest in management of pre-natal development to improve livestock health and productivity (Evans and Sack, 1973). The purpose of the present study is therefore to trace the prenatal histogenesis of the forestomach in red Sokoto goat.

MATERIALS AND METHODS

Ninety goat (*Capra hircus)* embryos and fetuses of red Sokoto goats were used for the study. All embryos and fetuses were obtained at Nsukka abattoir in Enugu State, Nigeria. Gestational age was estimated using the method of Nwaogu and Ezeasor (2008). The fetal ages were determined using the formula X = (Y + 17) 2.1, where X is the age in days and Y the crown rump length (CRL). The embryos and fetuses were divided into three groups depending on their ages and corresponding to the three trimesters of gestation. The rumen, reticulum and omasum were isolated by dissection; the outer and inner surfaces of each of these stomach components were grossly examined.

Samples were taken from each component of the forestomach and processed for light microscopy, by fixing in 10 % buffered formalin, dehydrated in increasing concentrations of alcohol, cleared in xylene and embedded in paraffin wax. 6µm thick sections were stained in Haematoxylin and Eosin (H & E). Sections were photographed using a Moticam 1000 digital camera attached to a computer. Morphometric parameters were determined using a calibrated Moticam 1000 digital camera.

RESULTS

Gross Anatomy: The primordia of the three compartments of the ruminant stomach were differentiated both externally and internally at the first trimester of gestation. However, at about 41 days of gestation, the stomach was observed as a spindle-shaped tube with two dilations at the position of the future rumen and reticulum respectively.

The dorsal and ventral sacs of the rumen were apparent at this age. The dorsal sac appeared to be growing faster than the ventral sac as the fetus advanced in age. The transverse grooves appeared at day 42, while the longitudinal grooves appeared at day 43. The coronary grooves were outstanding at day 45. The mucosal surface of the rumen was smooth in appearance from 30 days fetal life, at 96 days fetal life, it was granular in appearance. The size and numbers of the granules increased with advance in gestational age. Reticular ribs were first seen as tiny grain-like dots on the central portion of the organ at 65 days fetal life. These dots gradually widened in diameter and coalesced to form honeycomb like structures at 100 days fetal life. The horney comb like appearance of the mucosa was consistently seen throughout the older fetal life. Omasal leaves first appeared on the mucosa of the omasum at 52 days fetal life. Granules were observed on the apical surfaces of the omasal leaves at 74 days fetal life. These granules spread to the bases of omasal leaves at 96 days fetal life. This pattern of granule distribution was observed in all the other more advanced fetal age groups 100 – 148 days fetal life.

Histology

Group 1 (30 – 50 days fetal life): Differentiation of the three components of the forestomach occurred at this fetal age range (Figure 1a). The primordium of the rumino-reticular groove was populated by cords of dark staining mesenchymal cells. A narrow lumen (3 ± 0.20 µm wide) was observed between the surface epithelium of the rumen at 42 days of fetal life. Also at 42 days fetal-life, the wall of the rumen was relatively thin, irregularly shaped, and consisted of inner light epithelial layer, middle dense staining mesenchyme blastermic tissue and outermost layer of serosa (Figure 1b). The epithelial cell layer (25 ± 0.28 µm thick) was composed of stratified elliptically shaped cells that posses round shaped centrally located nuclei surrounded by thin band of light stained cytoplasm.

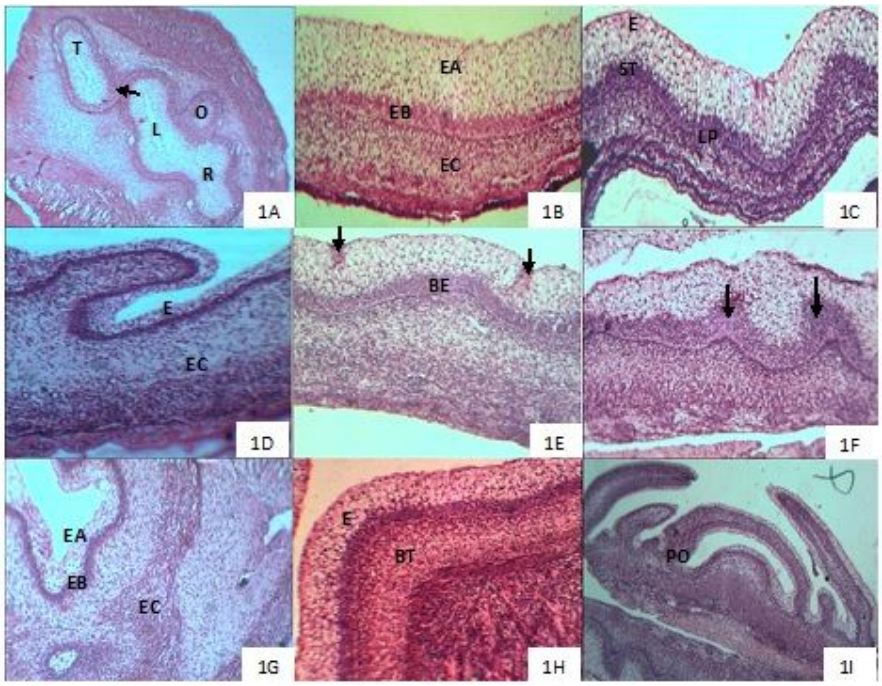

Figure 1: A) a cross section of the forestomach at 40 days, showing narrow lumen (L), rumen (R), reticulum (T) and omasum (O). Note the connection between the rumen and reticulum (arrow) none canalized and is occupied by mesenchyme cells. H&E X 200; B) Photomicrograph of a transverse section of the ruminal wall at 40 days. Note light staining epithelia zone (EA) and dark staining zone (EB), eosinophilic blastemic zone (EC) and serosa (S) H & E, X 200; C) Photomicrograph of a transverse section of the ruminal wall at 50 days, showing epithelium stratum basale (ST) evaginating towards the epithelia surface (E). Note the primodium of the lamina propria (LP). H & E, X 200; D) Photomicrograph of a transverse section of the reticular wall at 40days, showing epithelia layer (E) with light apical and dark basal regions. Note thick blastemic tissue (EC). H & E, X 200; E) Photomicrograph of a transverse section of the reticular wall at, 46 days. Note the undulating basal layer of the epithelium (BE) and epithelia furrows (arrow). H & E, X 200; F) Photomicrograph of a cross section of reticular wall at 50 days Note the increased evagination of the basal epithelia cells (arrow) towards the surface. H & E, X 200; G) Photomicrograph of a transverse section of the omasal wall at 40 days. Note the stratified epithelium with a light apical (EA) and dark basal zone (EB). The blastemic tissues (EC) were densely distributed towards the base. H & E, X 200; H) Photomicrograph of a transverse section of the omasal wall at 43 days. Note the stratified epithelium (ES) and the dark staining basally located blastemic tissue (BT) and I) Photomicrograph of a transverse section of omasal wall at 50 days, showing primary omasal leaves (PO) containing evaginations of stratum basale.

The blastemic layer (10 ± 0.52 µm thick) consisted of randomly distributed elliptically shaped stratified cells, characterized by round nuclei and eosinophilic cytoplasm. At 50 days fetal life (Figure 1c), the population of epithelial cells in the superficial light zone increased remarkably while the basal dark staining zone, appeared to have reduced in number and thickness (3.14 ± 0.73 µm thick). The primodium of the lamina propria (2.15 ± 0.73 µm thick) was an eosinophilic staining epithelia band at the junction between the superficial light staining epithelia zone and the blastemic tissue. The reticulum differentiated as a separate compartment at 30 days fetal life. At 42 days, the wall of the reticulum comprised a light staining thin epithelial layer, a very thin dark staining sub epithelial layer and abundant blastemic tissue (Figure 1d). At 46 days (Figure 1e), the light staining epithelial layer of the

mucosa has increased in size, epithelial furrows and undulations of the stratum basale were observed. The underlining blastemic tissue has differentiated into myoblasts forming the muscular tunic lined by serosa. At 50 days, increased condensation of cells of the stratum basale and pronounced evagination of the cells towards the surface epithelium differentiated into primary reticular crests (Figure 1f). The dark staining blastemic tissue differentiated into myoblasts that formed muscular tunic.

At 40 days of gestation (Figure 1g), the wall of the omasum comprised the mucosa (7.34 ± 0.23 µm), submucosa (5.89 ± 0.78 µm), muscularis (30.45 ± 0.71 µm) and serosa (1.38 ± 0.25 µm). The light staining epithelial layer was 3 – 4 cell layer thick, a sub adjacent layer was composed of a thin ribbon of dark staining cells (2 – 3 cell layer thick). The blastermic tissue and the myoblasts formed a very broad band. At 43 days (Figure 1h) the epithelial layer was thin. The light staining surface epithelium and the underlining dark staining blastermic tissue were condensed. At 50 days (Figure 1i) proliferated and evaginated light stained surface epithelial cells and the sub adjacent dark stained blastemic tissue cells formed finger like projection, the primary omasal leaves. On the lateral surfaces of the omasal leaves the blastemic tissue cells differentiated into myoblasts forming the lamina muscularis mucosae. The sub mucosa and the tunica muscularis has differentiated at this age.

Group 2 (52 – 98 days fetal Life): The epithelial layer comprised a basal layer (15.23 ± 0.58 µm) of globuse shaped cells and a superficial layer (9.25 ± 1.23 µm) stellate shaped cells. At 55 days of fetal-life, small evaginations (nipple-like) of the epithelia cells of the stratum germinativum were observed (Figure 2a). These protuberances were the primordial of the ruminal papillae. These projections increased in size with advance in age of the fetus. The morphogenesis of the ruminal papillae was characterised by condensation of cells of the basal layers and oscillation of the surface epithelium of the light zone. The basal blastemic tissue condensed and transformed into myoblastic tissues lacking definite orientation.

At 84 days, proliferating germinativum cells and basal blastermic tissue differentiated into lamina propria and submucosa (Figure 2b). The height and width of the papillae at 8.7 – 30 cm CRL were 7.25 ± 0.32 µm and 2. 65 ± 0.72 µm, respectively, suggesting an increase in the dimensions of the ruminal papillae with advance in fetal age. The underlining blastemic tissue differentiated into myoblasts which rearranged to form tunica muscularis.

Growth of the cells of the stratum basale towards the surface epithelium resulted in the formation of finger like primary reticular ribs at 55 days (Figure 2c). The growth of the reticular ribs later involved incorporation of tissues from the lamina propria. The epithelium at this fetal age comprised two zones, a thick apical zone (10 – 20 layers of cells and 25.34 ± 0.62 µm thick) containing light staining cells and a thin basal zone (2 – 3 layers of cells and 3.45 ±0. 76 µm thick) comprising of basophilic staining cells and abundant ground substance. At 85 days of fetal life, buds of secondary reticular ribs emerged from proliferating cells of the stratum basale (Figure 2d). These secondary reticular ribs were shorter and located by the sides of the primary ribs. The growth of the secondary ribs was observed to increase with advance in age.

At 55 days, the core of the omasal laminae consisted of two layers of lamina muscularis, a submucosa (thin collagenous fibres) and a central muscle band that originated from the inner muscle coat of the tunica muscularis (Figure 2e). The outer surfaces of the laminae were covered by light staining vacuolated stratified epithelium. The myoblastic fibres of the inner circular and outer longitudinal smooth muscle bundles were well differentiated and highly vascularised. At 84 days, the primary and secondary laminae increased in length, but decreased in width. The tertiary laminae (third order laminae) budded out in between the secondary and primary laminae.

The submucosa, tunica muscularis and serosa were morphologically similar to that of the preceding fetuses.

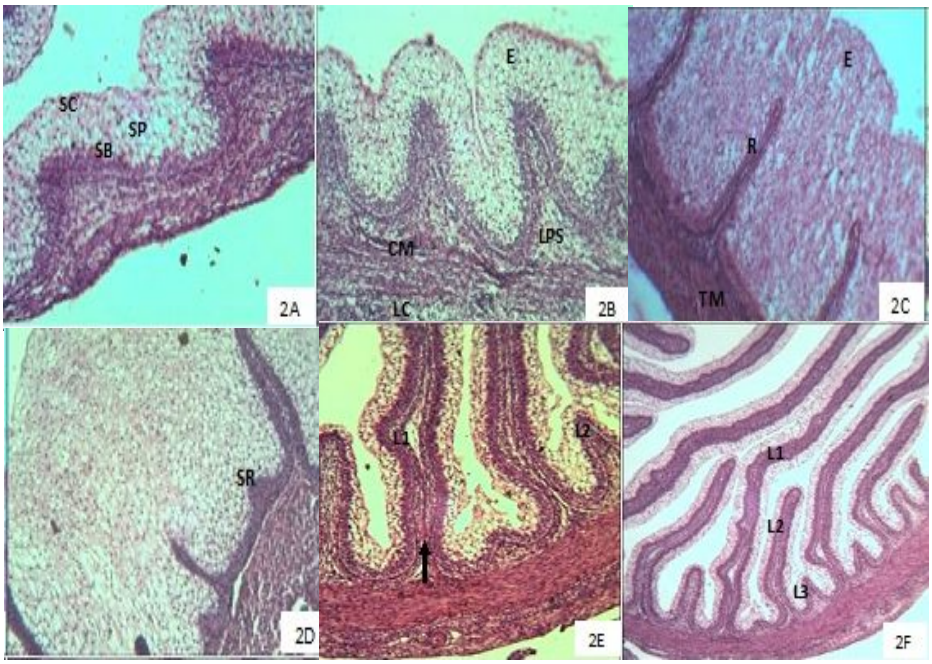

Figure 2: A) Photomicrograph of a transversal section of the ruminal wall at, 55 days, showing stratum basale (SB), stratum spinosum (SP) and stratum corneum (SC). H & E, X 200; B) Photomicrograph of transverse section of the rumen at 84 days, showing folding of the epithelial layer (E) forming the ruminal papillae. Note, dispersed blastemic tissues that formed lamina propria submucosa (LPS) and prominent circularly arranged muscle fibres (CM), longitudinal muscle coat (LM); C) Photomicrograph of a transversal section of the reticular wall at, 55 days, showing stratified epithelium (E), nail like primary reticular ribs (R) complements of tunica muscularis (TM).H & E, X 200; D) Photomicrograph of a transversal section of the reticular wall at 84 days, showing buds of secondary reticular ribs (SR) in-between the primary ribs. H & E, X 200; E) Photomicrograph of a transverse section of the omasal wall at 55 days. Note the primary laminae (L1) secondary laminae (L2). The inner circular muscle coats extended into the core of the primary laminae (arrow). H & E,X 200; F) Photomicrograph of a transverse section of the omasal wall at 84days. Note the increased length of the primary (L1) and secondary (L2) laminae and buds of tertiary laminae (L3).

Group 3 (102 – 148 days fetal life): The wall of the rumen consisted of mucosa (30 ± 0.22 µm thick), submucosa (15.20 ± 0.74 µm), muscularis (40.25 ± 0.34 µm thick) and serosa (8.45 ± 0.78 µm thick) (Figures 3a-c). The mucosa was subdivided into epithelium (20.24 ± 0.13 µm thick) and lamina propria (9.76 ± 0.23 µm thick). The epithelium has differentiated into four distinct layers, the corneum, spinosum, granulosum and basale. The basale contained basophilic staining cells, granulosum cells stained very lightly. Thin collagen fibres were observed in the lamina propria and tunica submucosa. Increased height, thickness and width of the papillae were observed. The number of papillae per fixed area of the rumen (papillary density) also increased at this fetal age range.

At 109 days the primary reticular ribs grew upwards and deep into the surface epithelium. The epithelium (20 – 40 layers thick) was keratinized stratified squamous resembling

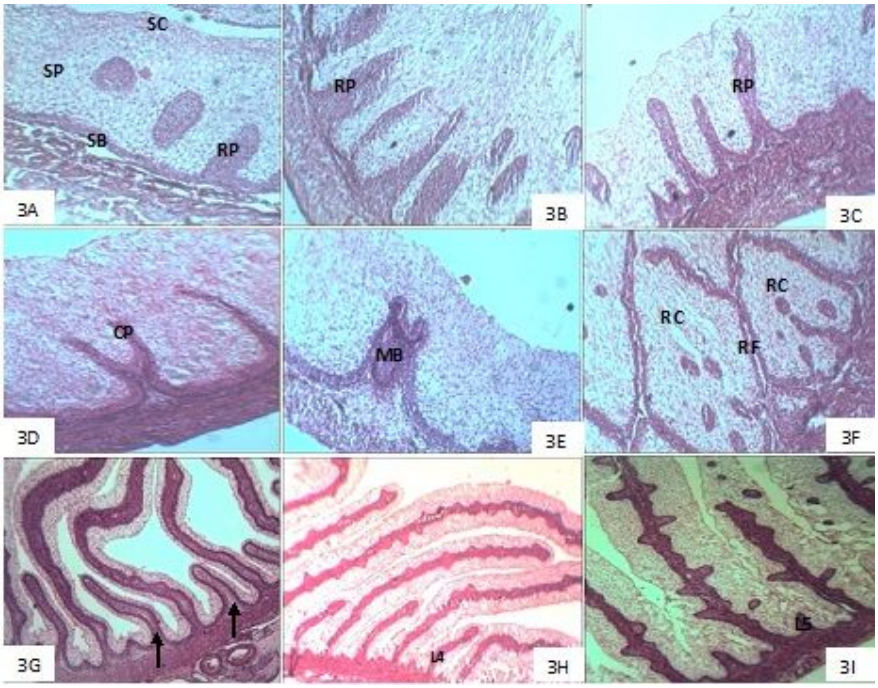

Figure 3: A) Photomicrograph of a transversal section of the ruminal wall at 109 days. Note stratum basale (SB), stratum spinosum (SP) and stratum corneum (SC) short but broad ruminal papillae (RP), H&E X 200; B) Photomicrograph of a transversal section of the ruminal wall at 124 days. Note elongated ruminal papillae (RP), H & E, X 200; C) Photomicrograph of a transverse section of the ruminal wall at 145 days. The ruminal papillae (RP) are very close to the last layer of the lining epithelium. H&E X 200; D) Photomicrograph of a transverse section of the reticular wall at 109 days. The primary papillae formed fork shaped structures as a result of differentiation of corneum papillae (CP); E) Photomicrograph of a transverse section of the reticular wall at 124 days. The blastemic tissues differentiated into muscle bundles (MB) at the tip of the primary reticular folds; F) Photomicrograph of a transverse section of the reticular wall at 145days. Note the union of the primary folds (RF) forming reticular cells (RC). H & E, X 200; G) Photomicrograph of a transverse section of the omasal wall at wall at 109 days. The lamina muscularis (arrow heads) can be observed in the cores of the primary and secondary laminae. H & E,X 200. H) Photomicrograph of a transverse section of the omasal wall at 124 days. Fourth order laminae (L4) are shown. H & E, X 200; I) Photomicrograph of a transverse section of the omasal wall at 145 days, showing fifth order laminae (L5), H & E, X 200.

that of the skin. Progressive proliferation and condensation of cells of the stratum germinativum resulted in lateral outgrowth of corium papillae from the primary reticular ribs giving it a fork shaped appearance (Figure 3d). At 124 days, the blastemic tissues differentiated into myoblastic fibres that formed muscle bundles at the tips of the primary reticular ribs. These muscle bundles represent the lamina muscularis mucosae (Figure 3e). As the fetal age advances, extensive branching and anatomoses of the primary and secondary papillae were observed (Figure 3f). These networks resulted in the transformation of the mucosal surface into several rectangular shaped structures (horney comb), the reticular cells. The tunica muscularis consisted of inner circular and outer longitudinal muscle coats. The thickness of the tunica muscularis of the rumen increased as the fetal age progresses. The serosa comprised areola connective tissue bound by a layer of squamous shaped cells.

In the omasum, central muscle core of the second, third and fourth order laminae were observed in this age group (Figures 3g-i). A fifth order laminea were observed at 145 days of gestation. The mean thickness of the different layers of the wall were 4.32 ±0.19 µm

(mucosa), 2.56 ± 0.71 µm (submucosa), 9.72 ± 0.14 µm (muscularis) and 1.21 ±0.05 µm (serosa).

DISCUSSION

From the findings in the present study, the forestomach of red Sokoto goat developed as enlargements from the spindle shaped stomach primordium. Similar observations were earlier reported (Dyce *et al*., 2002). The primordia of the rumen and omasum were prominent at 30 days of gestation however; the reticulum became distinguishable as a distinct organ at 38 days of fetal life. McGeady *et al.* (2006) reported the appearance of the rumen primordium at 36 days of gestation in goats. The discrepancy of our finding from that of the later could be attributed to variations in breed, fetal age determination technique and feeding habitat. The absence of a distinguishable reticulum at 30 days fetal life indicates a later differentiation of this organ compared to the other compartments of the forestomach. Our observation is similar to the findings of Vivo *et al.* (1990) and Mutoh and Wakuri (1989) in bovine fetuses. These Authors observed that the primordial reticulum originated from an area between the already formed primordial rumen and omasum in a goat embryo.

The dorsal and ventral sacs of the rumen were separated by transverse and longitudinally oriented grooves. These grooves projected as pillars on the mucosal surfaces of the rumen at day 40 of gestation. However, Hejazi and Frikaghaji (2013) reported the appearance of the longitudinal grooves at 50 days of gestation in sheep. The variation in the timing of the appearance of these grooves most probably resulted from differences in species and duration of gestation.

In the present study, the mucosal surfaces of the forestomach were smooth during the first three months of gestation, thereafter, it became granular. Smooth surfaced mucosa was reported by other authors (Garcia *et al*., 2012; Gupta *et al*., 2015) up to 75 days of gestation. The type of epithelium seen in all the compartments is similar to those observed by Ramkrishna and Tiwari (1979) in goats, and Redondo *et al.* (2011) in sheep and deer. The hexagonal shape of the cells above the basal layer could be attributed to the presence of cytoplasmic accumulations in them, as has been reported (Ramkrishna and Tiwari 1979; Franco *et al*., 2004). This could also be responsible for the denser appearance of the cells of light zone towards the lumen. The progressively flattened and darkened cells of the superficial epithelial layer were described as being parakeratotic later in gestation (Ramkrishna and Tiwari, 1979; Kitamura *et al*., 2003). Although they reported an increase in the thickness of the epithelium, this may not be due to increase in the number of cells (hyperplasia), as Arias *et al.* (1980) observed a significant decrease in the number of both basal and superficial cells of the epithelium. Hence, the apparent increase in the thickness of the epithelial layer could be attributed to increase in the size of individual cells, particularly at the middle and basal layers, as was observed in this study. The invaginations of the epithelial cells to form furrows could be responsible for the gross granular appearance of the rumen mucosa. The time of formation of corial papillae and epithelial furrows, as well as the observation of muscularis mucosae at the tips of the reticular ribs were consistent with the findings of Garcia *et al*. (2012).

The corial papillae of the reticulum were observed early in the development of the fetus and formed the future reticular ribs. The epithelial undulations seen around the papillae were also observed grossly, dividing the surface of the organ into typical honeycomb-like structures or 'cells'. The development of these characteristic features of the reticulum occurred much earlier and faster than those of the rumen, although the reticulum evolved later than the rumen.

The different laminae of the omasum developed later in red sokoto goats than what has been reported in other breeds of ruminants (Garcia *et al*., 2012). The variation could be due to difference in genetic composition.

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