

# Morphological Peculiarities of the Lumbosacral Region of Cattle Egret (*Bubulcus ibis*) with Special Reference to the Glycogen Body (Corpus gelatinosum)

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## Abstract

The current study aimed to study morphological peculiarities of the Lumbosacral Region of Cattle Egret with special reference to the glycogen body. The lumbosacral organ (LSO) is a unique modification in the spinal cord of all birds. Twenty adult cattle egret of both sexes are used to describe the morphological and histological peculiarities of this organ in cattle egret. The synsacrum of these birds was examined by gross, cross-sectional anatomy, Computed Tomography (CT), and transverse histological sections with different stains. The morphological peculiarities of the lumbosacral region of cattle egret includes enlarged vertebral canal in the region of synsacrum. This enlargement is due to the presence of a gelatinous glycogen body embedded in the rhomboid sinus of the spinal cord. Accessory lobes protrude at the ventrolateral end of the ventral horns in the vertebral canal. Transverse lumbosacral canals similar to semicircular canals above the spinal cord. The spinal cord is fixed to the vertebra by a network of dentate ligaments. Histologically both glycogen body and accessory lobes contain glycogen-containing glia cells. These cells were polygonal with narrow cytoplasmic rim and nucleus pushed to periphery by a central mass of glycogen. The blood capillaries were distributed throughout the glycogen body and accessory lobes. The connective tissue was very scanty except in the vicinity of the blood capillaries and central canal. The accessory lobes contain multipolar neurons scattered between the glia cells. The transverse lumbosacral canals were fluid-filled meningeal tubes that arch dorsally over the spinal cord and open laterally above the accessory lobes. The network of dentate ligaments formed from regular dense fibrous connective tissues mainly collagenous fibers. Therefore this work concluded that the proposition of the anatomical and histological modifications of the lumbosacral region might act as a sense organ of equilibrium control the balanced walking on the ground.

## KEYWORDS

Cattle egret, Glycogen body, Accessory lobes, Dentate ligament, Lumbosacral region, Morphology.

## INTRODUCTION

All birds show unique and unlike locomotor behaviors, including the highly-strung ability to balance on two feet and control their movements. These functions can be partly explained by a set of exciting collectively modifications in their vertebral column which are known as the lumbosacral organ (LSO). They are particularly found in the fused lumbosacral vertebrae called the synsacrum (Stanchak *et al.*, 2020). At this region the spinal cord splits dorsally, along the sagittal plane, and within this split rests an ovoid organ of glycogen-rich cells called the glycogen body (De Gennaro, 1982). The latter is a one of these main a unique lumbosacral organ (LSO) which occupying the dorsal rhomboid sinus in the lumbosacral region of their spinal cords (Uehara *et al.*, 1982).

Glycogen body is a controversial and very complicated issue and there is no clear-cut function till now, but many literature had suggested that the function of the glycogen body is mainly related to transmission of hydrostatic pressure changes during movements of the bird on the ground (Necker, 1999; Necker *et al.*, 2000), distributing substances originating in the glycogen

body to the CNS (Wilhelm and Wolfgang, 2003), playing a role in the metabolism of the neurons of the spinal cord (Azcoitia *et al.*, 1985), and sharing in the processes of myelin formation as well as lipid synthesis in the central nervous system (Benzo and De Gennaro, 1983).

Anatomically, The glycogen body is located partly intrapial and subpial with its dorsal surface is covered by the spinal pia mater, from which a pial septum enters the interior of the organ. The organ is separated it into a larger dorsal part and a small ventral part by apial septum. Through the ventral part of the glycogen body, the central canal of the spinal cord passes (Vukovic and Lucic, 2005). Uehara and Ueshima (1982) reported that the glycogen body extended along the whole length of the spinal cord. Azcoitia *et al.* (1985); Mammen (1997); Necker (2005; 2006); Ebraheim (2016) and Raja *et al.* (2019) observed that, the extension variety of the glycogen body along rhomboid sinus of spinal cord in the lumbosacral region (Synsacrum). Stanchak *et al.* (2020) found that, it difficult to determine the number and the extentation of lumbosacral region. The orientation of the LSTCs relative to the longitudinal axis of the synsacral vertebral canal varies both across species and within an individual. The shape

of the canals also varies as some canals are expanded dorsally and then constrict near the vertebral canal, forming a balloon- or mushroom-like shape. The constrictions may allow the arachnoid membranes to form a more defined canal that optimizes the flow of cerebrospinal fluid toward the accessory lobes

Histologically, the body classified into central and peripheral parts. The cells in the peripheral portion showing a more regular arrangement than the cells in the central portion (Uehara and Ueshima, 1982)). The nature of these cells is distinctive as it composed from highly specialized and morphologically modified astroglial cells (Vukovic and Luci-Uehara, 2005, Lyser, 1973; De Gennaro, 1993). In general, these cells are polygonal with narrow cytoplasmic rim and peripheral nuclei (Hodges, 1974). The cytoplasm is fully laden with glycogen which has strong positive reaction to both Best's carmine stain and PAS reaction ( Lyser, 1973). The blood supplies of the glycogen body ramify from a sinusoidal network which is better developed in the central portion than in the peripheral one (Hodges, 1974). Therefore this work aimed to study study morphological peculiarities of of the Lumbosacral region of Cattle Egret with special reference to the glycogen body.

## MATERIALS AND METHODS

### Birds used

The present work was carried out on twenty apparent healthy adult cattle egret of both sexes which were hunted from the fields of Sharkia governorate, Egypt. The birds were housed separately, one in each cage and fed synthetic ration and water ad libitum. They were kept in the laboratory for two days before euthanization. They were euthanized by injection of sodium Phenobarbital (100 mg/kg body weight) in the wing vein.

### Institutional Review Board Statement

The present study was duly approved by the Institutional Animal Care and Use Committee, Zagazig University, Egypt (ZU-IA-CUC/2/F/283/2023).

### Macroscopical and morphometric examination

Six birds were freshly dissected just after euthanization. The abdomen was opened and eviscerated to expose the synsacrum containing the glycogen body against the sciatic nerve roots. Then the synsacrum was excised and fixed without further dissection in 5% formaline solution. The lumbosacral part of spinal cord with intact glycogen body was exposed very carefully with the help of forceps, scissors, and scalpel. Gross morphology of the organ in situ and after separation was observed by the naked eye and photographed by Nikon digital camera with resolution (16.1 megapixels, Sony DSC-W690, 36v, and 10x optical zoom).

The body weight of the bird vs. the weight of glycogen body was evaluated. The length and width of rhomboid sinus and glycogen body was measured by using digital balance and Vernier caliper. All morphometric data for all pervious specimens were recorded and expressed as means  $\pm$  SE.

### Computed tomography (CT) and cross section

Computed tomographic (CT) studies were done on four birds; the birds were undergone consecutive CT scan using at AL-Bayan center in Belbes, Sharkia Governorate, Egypt. Then the four birds were sectioned transversally at the lumbosacral segments to inspect different parts of lumbosacral organ under stereomicro-

scope.

### Histological examination

Synsacrum and lumbosacral segments of spinal cord were removed en bloc from ten birds. Then fixed with 10% natural buffered formalin for 24 hours. All specimens were decalcified in buffered 19% Ethylene di-amine tetra-acetic Acid (EDTA) (pH 7.2-7.4) for two days at room temperature. The decalcified specimens were dehydrated and embedded in paraffin. Five- $\mu$ m-thick sections were obtained and stained with Hematoxylin and Eosin for routine histological examinations, Periodic acid- Schiff (PAS) stain for the demonstration of glycoprotein, Crossman-Trichrome and silver impregnation stains for collagen fibers.

## RESULTS

The cattle egret vertebral column contained a complex distinctive structure was synsacrum which formed from junction of lumbar and sacral vertebrae with each other and with the pelvic girdle Fig. 1a, b, c and d. In, the synsacrum the joined lumbosacral vertebrae formed circumfluent and large space, ultimately larger than the spinal cord. Within this peculiar bony structure of the cattle egret had unconventional soft tissue morphological features was Glycogen body (GB).

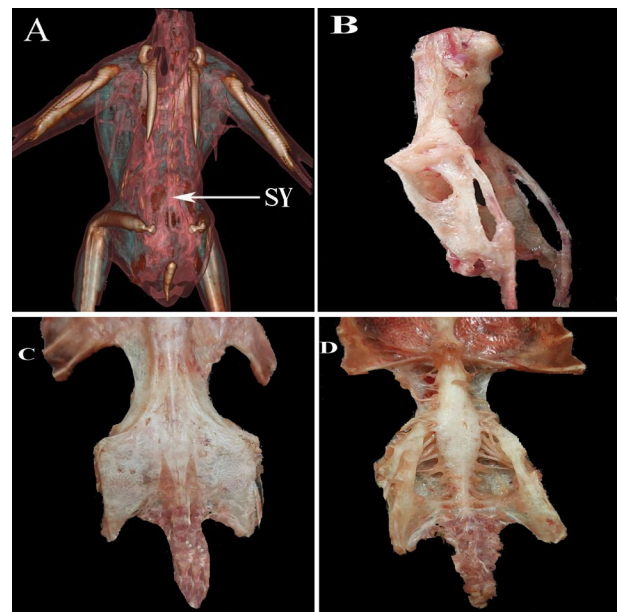


Fig. 1. A photograph shows (a) scout view of CT of cattle egret (SY) synsacrum (b) lateral view of synsacrum, (c) dorsal view of synsacrum, (d) ventral view of synsacrum.

The glycogen body was a diaphanous, subtle, gelatinous, uncommon cellular mass was located at the dorsal rhomboid sinus in Ibis bifurcated parts of the lumbosacral segment of spinal cord Fig. 2a and b. The glycogen body was specialized structure present only in birds and it had specific position above the lumbosacral segment of spinal cord. The glycogen body was easily detached from vertebral column and secluded from the spinal cord tissues without any injury to their tissues Fig. 2 (c and d). In the current study, the shape of the glycogen body was elongated oval (ovoid) (Fig. 2c and d).

It extended from the level of fourth lumbar vertebra to the second sacral vertebra (L4-S2) on the the rhomboidal sinus Fig. 3 a, b, c and d. The weight of glycogen body was  $17.5 \pm 3.03$  mg, its length was  $7.25 \pm 1.51$  mm and its width was  $1.95 \pm 0.30$  mm, however the length of rhomboid sinus was  $12.6 \pm 2.12$  mm, its width

4.26±0.29 mm. So the rhomboidal sinus was longer and wider than the glycogen body, also there were positive relationship between the weight of glycogen body and the weight of the bird.

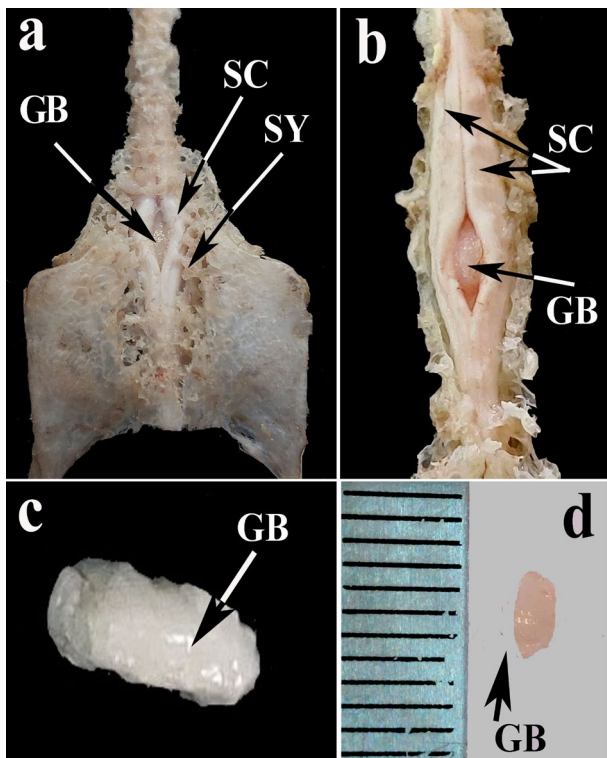


Fig. 2. A photograph shows (a) synsacrum skeleton with spinal cord after removal of bone cover (SY) synsacrum (GB) glycogen body (SC) spinal cord (b) After extraction of spinal cord from synsacrum (c) and (d) Separated glycogen body.

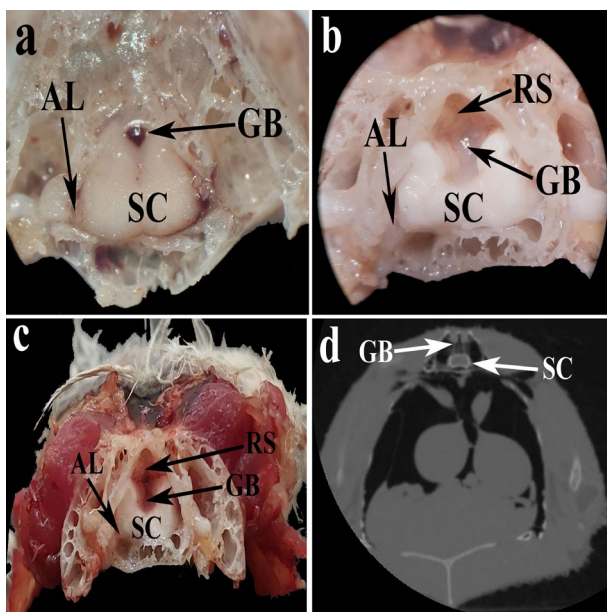


Fig. 3. A photograph shows (a) cross section on lumbosacral vertebral column at the level of L (x 5 Stereomicroscope) (b) at the level of S1 x15 Stereomicroscope (c) fresh ssp lumbosacral vertebral column specimen at the level of S1 (d) CT cross section scan (GB) glycogen body (SC) spinal cord (AL) accessory lobe (SC) rhomboidal sinus.

After removal of the spinal cord and the glycogen body the cattle egret synsacral vertebral canal showed a group of the lumbosacral canals its dorsal surface Fig. 4 (a and b).

The accessory lobes was segmental bilateral protrusions of neural tissue along the margins of the spinal cord, these lobes occur near the intersections of denticulate ligaments, a network of ligaments that support the spinal cord. These accessory lobes were located at the level of the processes of the dentate ligament

which contacted the wall of the vertebral canal and hence at the border of successive spinal segments (Fig. 3c and Fig. 4c).

The dentate ligaments where it attached and supported the spinal canal through ventral process. The dentate ligaments formed from lateral, middle longitudinal ligaments and transverse ligament (Fig. 4d).

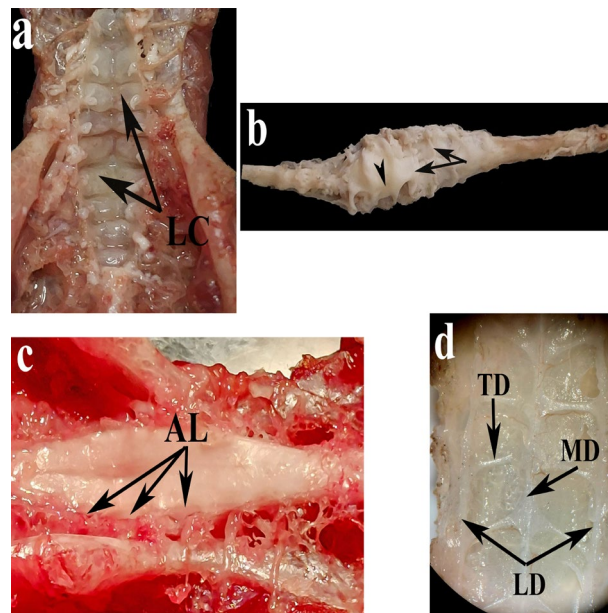


Fig. 4. A photograph shows (a) ventral view of synsacrum after removal of the spinal cord (LC) lumbosacral canals (b) a cast of the lumbosacral vertebral canal (arrows) lumbosacral canals (arrow head) site of glycogen body (c) ventral view of synsacrum (AL) accessory lobes (d) the dentate ligaments (x 5 Stereomicroscope) (LD) lateral dentate ligament (TD) transverse dentate ligament (MD) middle dentate ligament.

#### Histological specializations of the lumbosacral region

The specializations of the lumbosacral region were; an enlarged vertebral canal contained the lumbosacral intumescens with a rhomboid sinus in its dorsal part. The latter occupied by the glycogen body, accessory lobes, lumbosacral canals and specialized network of dentate ligaments (Fig. 5a, b).

The bony wall of the vertebral canal of adult ibis was made up of thin lamellae with many hollow spaces owing to the typical pneumatization (Fig. 5c). There was a dramatic increase in the depth of the rhomboid sinus in dependency to the size and level of the glycogen body. Serially, this body expanded and contracted rapidly, over just three segments (from L4 to S2). When comparing segment L2 with that of S1, it reached a maximum at S1 with a subsequent decrease toward more caudal segments (Fig. 5d, e, f, g).

Transverse sections of the lumbosacral region elucidate that the glycogen body appeared between the two halves of the spinal cord in sinus rhomboideus as an inverted triangle or mashrome-like. It was slightly protruded from the dorsal surface of the spinal cord and covered dorsally by pia mater (Fig. 5g). The glycogen body divided into large dorsal and small ventral parts by a pial septum. The ventral part appeared enclosed the central canal (Fig. 5h).

Histologically, The glycogen body formed of a mass of single kind of large polygonal cells, classified into central and peripheral portions. the cells in the peripheral portion were more regulary arranged than that of the central portion. The nuclei and perinuclear cytoplasm were displaced to the cell periphery due to the presence of an empty-appearing structure - when seen by H & E stain. Nuclei were round or oval in shape with irregular borders(-

Fig. 6a, b). The center of the cells and cytoplasm appeared filled with PAS +ve material (Fig. 6c).

The glycogen body was highly vascularized by numerous longish diagonal and transverse capillary sections. Capillaries contained blood cells and lined with endothelium were observed throughout the glycogen body (Fig. 6a, b, d, h). Connective tissue was observed in the vicinity of the blood capillaries. Numerous collagen fibers were seen in the perivascular connective tissue space. (Fig. 6e, f). The glycogen body was a separate structure and upon its removal there was no injury occurred to the spinal cord (Fig. 6g).

The central canal (*Canalis centralis*) passed through the ventral portion of glycogen body. It was lined by ependymal cells

without intrusion of a basement membrane between the ependymal and glycogen body cells (Figs. 5f, g, h, 6h). Connective tissue was noticed around the central canal in the subependymal region (Figs. 6f, 7a). No evidence of innervation inside the glycogen body was seen.

In the lumbosacral enlargement of cattle egret, there are marginal nuclei appeared as obvious accessory lobes protruding from the spinal cord ventrolaterally into the vertebral canal. They mostly looked rounded with great space separated it from the wall of the vertebral canal. These lobes were located at the fusion site of the transverse and lateral dentate ligament (Figs. 5a, b, 7b, c, g). It contained multipolar neurons scattered between glycogen-containing glia cells similar to those found in the glycogen

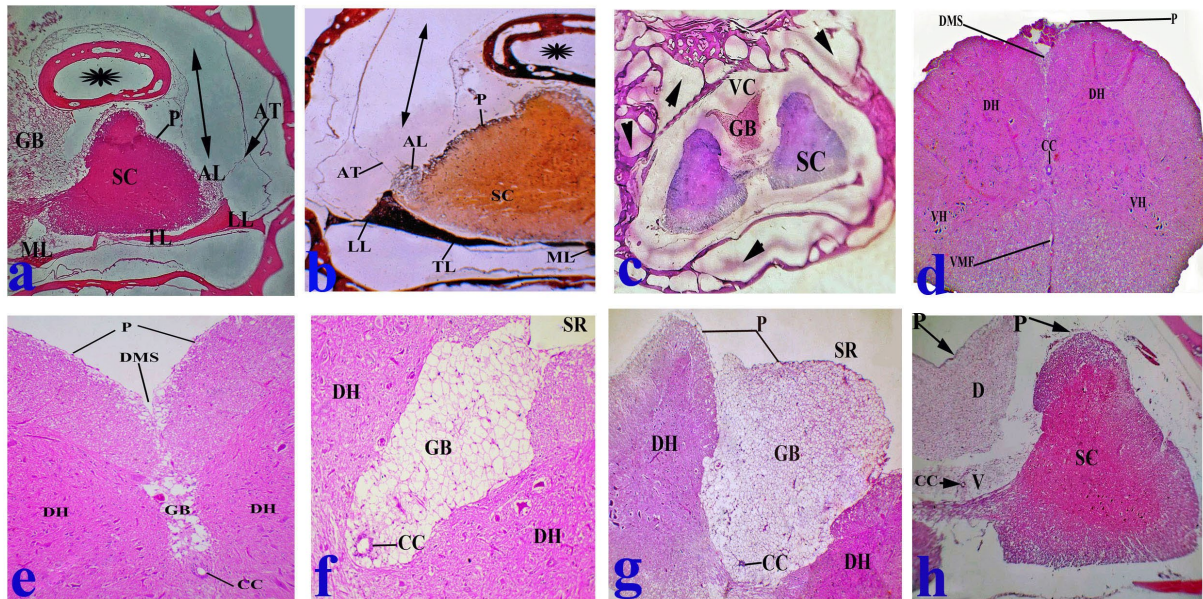


Fig. 5. Transverse paraffin sections showing the specializations in lumbosacral spinal cord of adult cattle egret (a, H&E X 40, b, silver impregnation X40); the enlargement of the vertebral canal (c, H&E X 1.6 Stereomicroscope); dramatic increase in the depth of the rhomboid sinus in dependency to the size and level of the glycogen body (d, H&E X 40) at L2, (e, H&E X 40) at L3, (f, H&E X 40) at L4 and (g, H&E X 40) at S1: SC spinal cord; GB glycogen body; AL Accessory lobe; LL lateral (dentate) ligament; ML middle ligament; TL transverse ligament; AT arachnoidal trabecle; P pia mater; ↔ lumbosacral canal; asterisk, bony lumbosacral canal; VC vertebral canal; arrow heads, hollow spaces in synsacrum; DH dorsal horn; VH ventral horn; DMS dorsal median sulcus; VME ventral median fissure; SR Sinus rhomoides; CC central canal, D dorsal part and V ventral part of glycogen body.

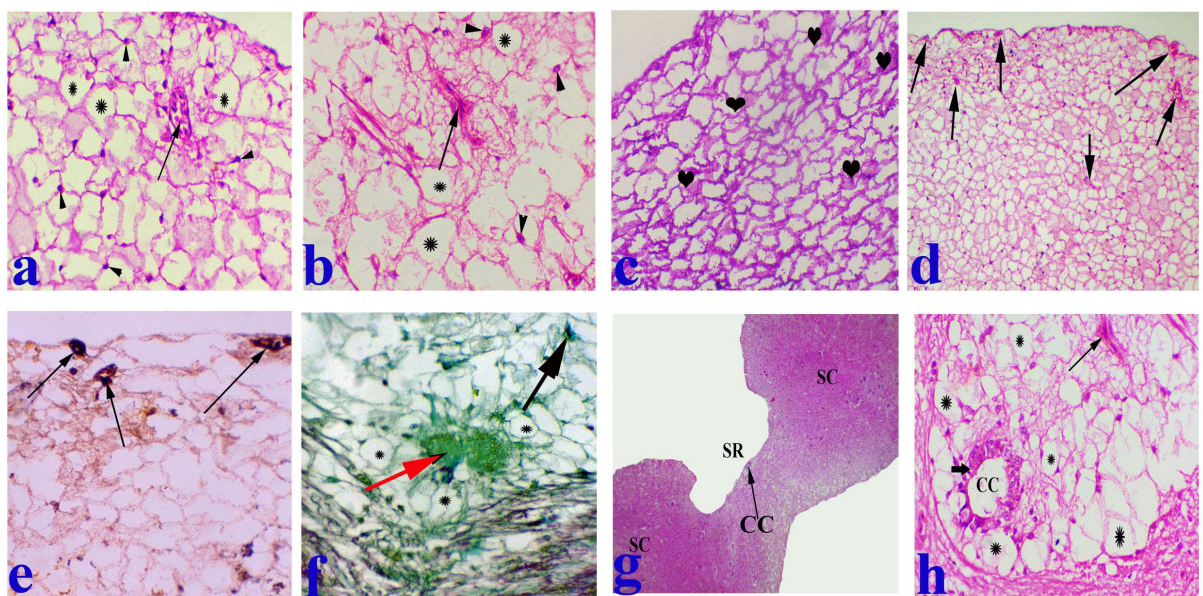


Fig. 6. Photomicrographs of the glycogen body showing its peripheral (a) and central portions (b H&E X 400) contained large polygonal cells (asterisks) with peripheral thin layer of cytoplasm and round or oval irregular borders nuclei (arrow heads). The glycogen body cells appeared filled with PAS +ve material (♥)(c, PAS stain X 400). The whole glycogen body was well vascularised by numerous capillaries (black arrows) (d, H&E X 100). Collagen fibers were detected in the vicinity of the blood capillaries (black arrows) (e, Silver impregnation stain X 400) and in the subependymal region of the central canal (red arrows) (f, Masson Trichrome stain X 400). By removal of the glycogen body there was no injury occurred to the spinal cord with clear sinus rhomoides (SR) and central canal (CC) (g, H&E X 40). The central canal was lined by ependymal cells and passed through the ventral portion of glycogen body (h, H&E X 400).

body and blood capillaries (Fig. 7d).

A multilayered arachnoidal trabecle arose from the ligament, in close contact with the ventrolateral aspect of the lobe. Then left the lobe and coursed dorsolaterally to join the arachnoid. The trabecle separated a wide dorsal fluid compartment that included the lobes and shared in the formation of lumbosacral canals (Figs. 5a, b, 7b, c, d, e).

The dorsal part of the lobe was loosely covered by pia mater. The loose mesh of pial cells means that the accessory lobe was separated from the large subarachnoidal space only by the basal lamina (Figs. 5b, 7b, d, e, f). The latter was formed by peripheral glycogen cells which make up the superficial layer, then continued dorsally onto the spinal cord (Fig. 7d).

The cytoplasm of the glia cells appeared filled with PAS +ve material (Fig. 7e). Small capillaries were noticed throughout the accessory lobes similar to those in the glycogen body (Fig. 7d). Connective tissue mainly collagenous fibers was observed in the vicinity of the blood capillaries (Fig. 7f).

The lumbosacral canals were regularly structured, transverse, fluid-filled meningeal tubes formed by arachnoidal trabecle. They arched dorsally over the spinal cord and opened laterally above the accessory lobes. These canals occupy expansions of the neural canal at former intervertebral joints in the synsacrum (bony lumbosacral canals) (Fig. 5a,b) .

The network of dentate ligaments (middle, transverse and lateral dentate ligaments) formed from regular dense fibrous connective tissues mainly collagenous fibers as detected by Masson Trichrome and Silver Impregnation stains (Figs.5a, b and 7b, c, e,g,h).

## DISCUSSION

The glycogen body extended from L4 to S2 part of lumbosacral zone of the spinal cord. This result was in agreement with Mammen (1997) ; Ebraheim (2016); Raja *et al.* (2019) and Kamska *et al.* (2020). In the contrast, Ebraheim (2016) revealed that the gelatinous glycogen body embedded in the lumbosacral region of spinal cord from L2- S2 segments. Meanwhile Singh *et al.*

(2021) reported that the glycogen body stretched from the level of 26<sup>th</sup> to 29<sup>th</sup> spinal nerves i.e. in lumbo –sacral (L-S) plexus.

The glycogen body was ovoid, diaphanous and like structure in the rhomboid sinus of spinal cord (De Gennaro, 1982). In the contrary Uehara and Ueshima (1982) stated that the glycogen body protracted throughout the whole length of the spinal cord. In accordance with Azcoitia *et al.* (1985); Mammen (1997); Necker (2005; 2006) and Ebraheim (2016) explained that, the glycogen body lodged at the dorsal aspect of rhomboid sinus of spinal cord.

The glycogen body was a very delicate structure and it is easily detached from vertebral column and secluded from the spinal cord tissues without any injury to their tissues Necker (2005); Ebraheim (2016) and Singh *et al.* (2021).

Kamska *et al.* (2020) revealed that, the denticulate ligament network showed a pennate angular arrangement with an angle close to perpendicular at the S1–L4 fusion. The denticulate ligaments extend from the ventral process to attach to the spinal canal walls at the fusion zones between vertebrae. The spinal cord in this region was supported by thickened dentate ligaments (Schroeder and Murray, 1987). In contrast with Necker (1999), the synsacral canal appeared in a series of transverse recesses over its dorsal surface. However, the present study passed in hand with Necker (2005) that cattle egret synsacral vertebral canal showed a group of the lumbosacral canals its dorsal surface.

In line with Stanchak *et al.* (2020), the lumbosacral organ (LSO) was present in all birds and may vary in morphology relying on a species' locomotor habits.

The CT scan. C, A sagittal section through the vertebral canal of the synsacrum CT scan shows the dorsal expansion of the vertebral canal that held the glycogen body (Stanchak *et al.*, 2022). Furthermore, the vertebral canal was enlarged considerably. This enlargement wasn't due to an increase in the size of the nervous tissue of the spinal cord, but to a conspicuous glycogen body embedded in a dorsal groove of the spinal cord.

The current study explained that the dentate ligaments formed from lateral, middle longitudinal ligaments and transverse ligament, however, Necker (2006) showed that the dentate ligaments formed from lateral, middle longitudinal ligaments and transverse ligament

In comparison to other Vertebrata the spinal cord of adult cattle egret is atypical because it contains an unusual cellular

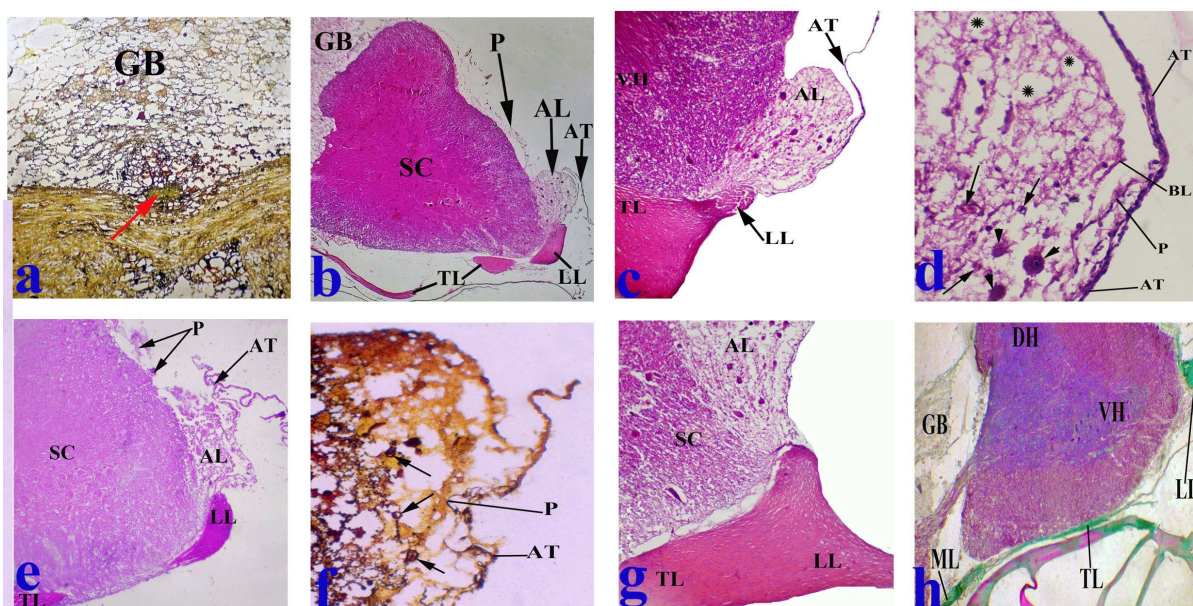


Fig. 7. Transverse sections of the spinal cord at the level of glycogen body and accessory lobes showing (a) Collagen fiber in the subependymal region and around the central canal (red arrow) by silver impregnation (x 400). (b, c) Rounded accessory lobes (AL) were located at the fusion site of the transverse (TL) and lateral (LL) dentate ligaments. The lobes were covered by pia mater (p) and bounded ventrolaterally by arachnoidal trabecle (AT) (b, x 40 & c, X 10). (d) The lobe contained multipolar neurons (arrow heads) scattered between glia cells (asterisks) and blood capillaries (black arrows) (X 400). (e) The cells of the accessory lobes filled with PAS +ve material (X 40). (f) collagen fibers were detected in the vicinity of the blood capillaries (black arrows), the pia mater (p) and arachnoidal trabecle (AT) by silver impregnation (x 400). (g)The net work of dentate ligaments formed from dense regular fibrous connective tissues (X, 100) which mainly collagen fiber as detected by Masson Trichrome stain (h, X, 40).

mass known as the glycogen body similar to other birds. The microscopic structure of the glycogen body had been described in chicken (Hodges, 1974; Sansone, 1980), Japanese quail (De Gennaro, 1982), pigeon (Schroeder and Murray, 1987) turkey (Vukovic and Luci-Uehara, 2005) and different species of wild birds (Peternel, 1994).

Glycogen body cells were of glial origin (De Gennaro, 1993), possibly astrocytes, having undergone extreme differentiation (Lyser, 1973; Sansone, 1980; Lee *et al.*, 2001).

The glycogen body of adult cattle egret was divided into dorsal and ventral parts. The ventral part enclosed the central canal of spinal cord. The glycogen body cells were polygonal with a peripheral cytoplasmic frame encircling a central PAS +ve mass and peripherally placed nuclei. This structure, is assumed to be the glycogen derivatives stores, as it was stained bright red with the PAS-stain. The nuclei Nuclei were round or oval with irregular edges. Similar results were observed by De Gennaro (1993); Hodges (1974); Ebraheim (2016) and Raja *et al.* (2019) in chicken, while in the pigeon, the nuclei were lobular (Schroeder and Murray, 1987). Benzo and De Gennaro (1983) supposed that this glycogen had a role in the processes of lipid synthesis and myelin formation in the nervous system.

Collagen fibers were detected in the pia mater and also in the vicinity of the blood capillaries and central canal. These observations were concurred with the findings of Ebraheim (2016) and Raja *et al.* (2019) in chicken. The present study revealed blood capillaries throughout the glycogen body, on the other hand Ebraheim (2016) and Raja *et al.* (2019) in chicken stated that the central portion was more highly vascularized.

The Accessory lobes of cattle egret contain multipolar neurons scattered between glycogen-containing glia cells similar to those found in the glycogen body (Breazile and Hartwig, 1989). The glycogen gave these lobes a gelatinous appearance as revealed by stereomicroscope. These neurons were provided with mechanoreceptors (Necker, 2002) and were located at the ventrolateral end of the ventral horns (Schroeder and Murray, 1987).

Functionally, The relatively dense glycogen body has the potential to apply loads sufficient to pre-stress dentate ligaments, enabling external accelerations to excite tuned fluctuations in the lumbosacral organ soft tissue, leading to strain-based mechanosensing in the accessory lobe neurons (Kamska *et al.*, 2020). The bipedal walking of birds considered a special challenge as their legs are present caudal to the center of gravity, and so birds should have an extralabyrinthine sense of equilibrium in their abdomen (Delius and Vollrath, 1973). The peculiar glycogen body in the lumbosacral spinal cord might represent such a sense organ (Grimm *et al.*, 1997). Also, The presence of lumbosacral canals which analogous to the semicircular canals in the inner ear propose that the specializations in the lumbosacral region may act as a sense organ of equilibrium that control the bipedal walking (Necker, 1999).

## CONCLUSION

The peculiar lumbosacral organ of cattle egret is morphologically similar to other birds. The results of this study support the assumption that the anatomical and histological modifications of the lumbosacral region might act as a sense organ of equilibrium control the balanced walking on the ground.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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