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HISTOLOGY OF THE SPLEEN OF FARMED JUVENILE AFRICAN CATFISH (*Clarias gariepinus* B.)

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ABSTRACT

The histology of the spleen of the farmed juvenile African catfish was investigated as there is dearth of such information in available literature. Juveniles were used for the study because most investigative research of the species is usually carried out with the age group. The organ was harvested from apparently healthy juveniles from a commercial aquaculture. It was subjected to routine histological procedure of dehydration, clearing and embedding, and subsequently stained appropriately. Histologically, the capsule covering the splenic parenchyma was of dense regular connective tissue fibres. The red pulp contained sinusoids, capillaries and splenic cords of erythrocytes, macrophages and lymphocytes. The white pulp comprised of melanomacrophage centres, lymphocytes and surrounding arterial vessels. The parenchyma was divided by connective tissue trabeculae into lobules containing mostly white pulp melanomacrophage centres. The melanomacrophage centres consisted of brown pigments and leukocytes. The spherical melanomacrophage centres were characterized mostly by an outer basophilic region and an inner region containing dark reddish spherical bodies. The melanomacrophage centres were periodic acid Schiff positive and alcian blue negative.

***Keywords*:** Spleen, Histology, Farmed African Catfish

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INTRODUCTION

The teleost spleen is a vital organ associated with haemopoiesis since no medullary cavity has been described in their bones [1]. The spleen, cranial kidney and liver play important role in defense against pathogens as lymph nodes are absent in teleost [2,3]. The teleost spleen is also involved in the circulation of blood through sequestration, storage and release of blood cells [4]. The teleost splenic parenchyma consists of a red pulp and white pulp. The red pulp contains the interconnecting system of splenic cords

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and sinusoids while the white pulp comprises mainly lymphoid cells, arterial vessels and melanomacrophage centres (MMCs). The variation in the ratio of red pulp to white pulp quantity in spleen parenchyma has been reported in some teleosts [5]. Melanomacrophage centres are part of the normal histology of fish spleen and kidneys, serving as the phagocytic centres [1,6]. The MMC is teleost equivalent of mammalian splenic white pulp and the lymph node germinal centres [7,8]. The MMC usually contain pigments like melanin, lipofuscin, ceroid and hemosiderin [9]. They have been suggested to serve as a biomarker for fish health status [10,11]. Clinically, MMCs have been associated with resistance to pathogens [12].

The structure of MMCs varies in different fish species. In non-salmonid species, clusters of densely packed MMCs form melanomacrophage centers, which are usually bound by a thin argyrophilic capsule, surrounded by white pulp and associated with thin-walled, narrow blood vessels [1,13]. However, in salmonids, the concentration of MMCs are not properly defined and a capsule is absent, but they are also the associated with blood vessels and lymphocytes [14]. Despite the increasing importance of commercial aquaculture in Nigeria, there is dearth of information in on the normal histology of the vital organs of the African catfish including the spleen –the major lymphoid and haemopoietic organ of this species [12,15]. Juvenile African catfish were used in this study as they are frequently the specimen of choice in most investigative research on the species [16,17].

This study was therefore designed to provide information on the descriptive histology of apparently healthy farmed juvenile African.

MATERIALS AND METHODS

Ten apparently healthy juvenile African catfish of different sexes sourced from a commercial aquaculture in Umuahia, Eastern Nigeria were used for the study. Their mean weight and length were 30.76 ± 3.30 g and12.03 ± 0.27 cm respectively. The fish were euthanized with chloroform. The body cavity was cut open through the ventral surface and the spleen dissected out. It was immediately fixed in 10% neutral buffered formalin.

The tissues were passed through graded levels of ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Sections 5µm thick were obtained with Leitz microtome model 1512. They were stained with haematoxylin and eosin for light microscopy examination [18]. Mucins were demonstrated using alcian blue (AB) at pH 2.5 [19,20] and periodic acid schiff (PAS) procedure with and without prior digestion with diastase [21,22]. In addition, the PAS technique was employed in combination with AB for neutral and acid mucin [18]. The slides were routinely examined and photomicrographs taken with a Motican 2001 camera (Motican UK) attached to an Olympus microscope.

RESULTS

The spleen was covered by a capsule containing dense regular connective tissue (Fig. 1). The sub-scapular cavity contained collagen fibres, lymphocytes, macrophages and other haematopoeitic cells (Fig. 2). The sub-scapular cavity contained sinusoids (Fig. 3). The red pulp contained sinusoids, capillaries and splenic cord of haematopoeitic cells, lymphocytes and macrophages (Fig. 2 and 3). The white pulp which constituted the majority of the splenic parenchyma was comprised of the MMCs, lymphocytes and surrounding arterial vessels and capillaries (Fig. 2).The splenic trabeculae divided the highly vascularized spleen into small lobules. The lobules contained clusters of white pulp spherical MMCs.

Four major MMCs were recognized as distributed in the white pulp: Type I or MMCs with basophilic outer region containing clear ovoid to pear-shaped vacuoles and a slightly eosinophillic central palour containing dark reddish inclusion bodies (Fig.4); Type II or MMCs that were completely basophilic without inclusion bodies (Fig. 5); Type III or MMCs with few dark reddish inclusion bodies (Fig.5) and

Type IV or MMCs characterized by three regions- very basophilic outer region, middle slightly clear region and weak eosinophillic centre with dark reddish inclusion bodies (Fig. 5).
Mucin histochemistry revealed that the outer region of the MMC was PAS positive and AB negative while the central region was PAS and AB negative.

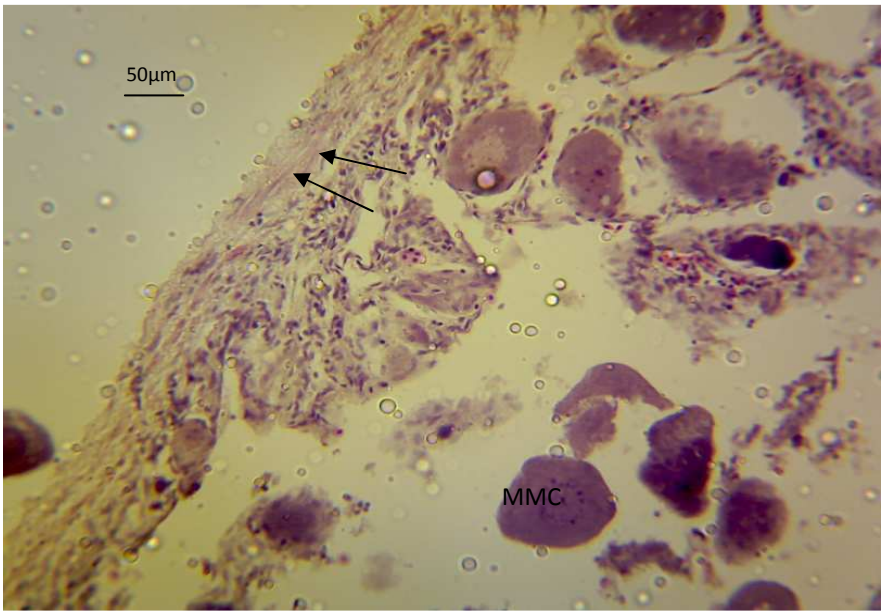

Fig.1. Section of juvenile spleen showing the splenic capsule (arrows) of dense regular connective tissue. Note the splenic MMC. H and E.

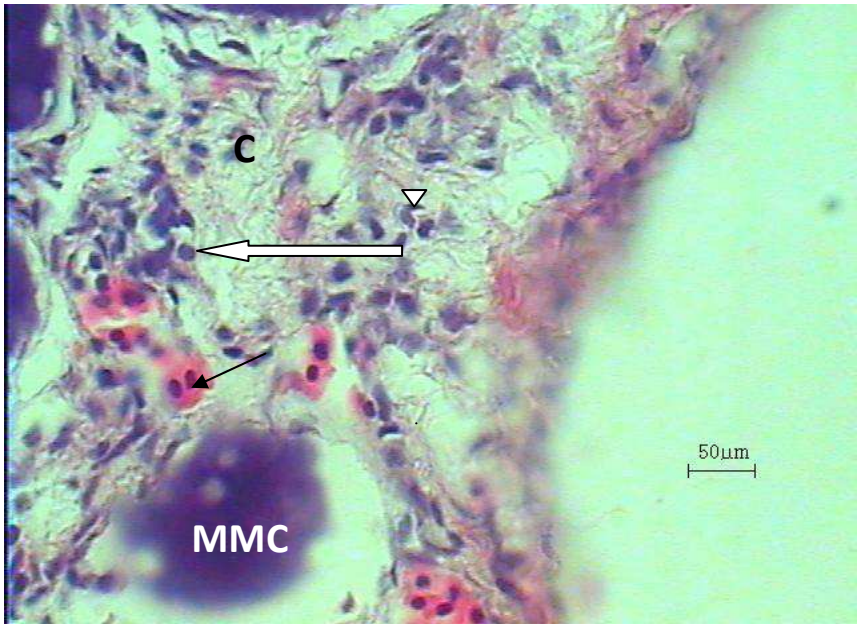

Fig.2. Section of juvenile spleen showing the red pulp splenic cords (C), containing erythrocytes (black arrow), lymphocytes (white arrow), and macrophages (white arrow head). Note the white pulp melanomacrophage centre. H and E.

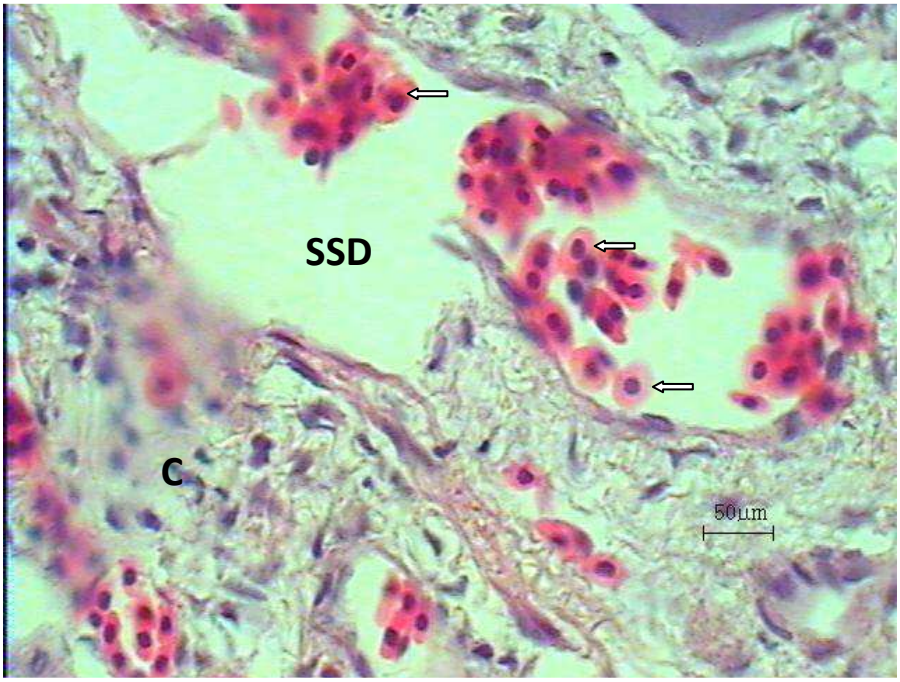

Fig.3. Section of the splenic red pulp containing sinusoid (SSD) and splenic cords (C). Note the nucleated erythrocytes (arrows). H and E.

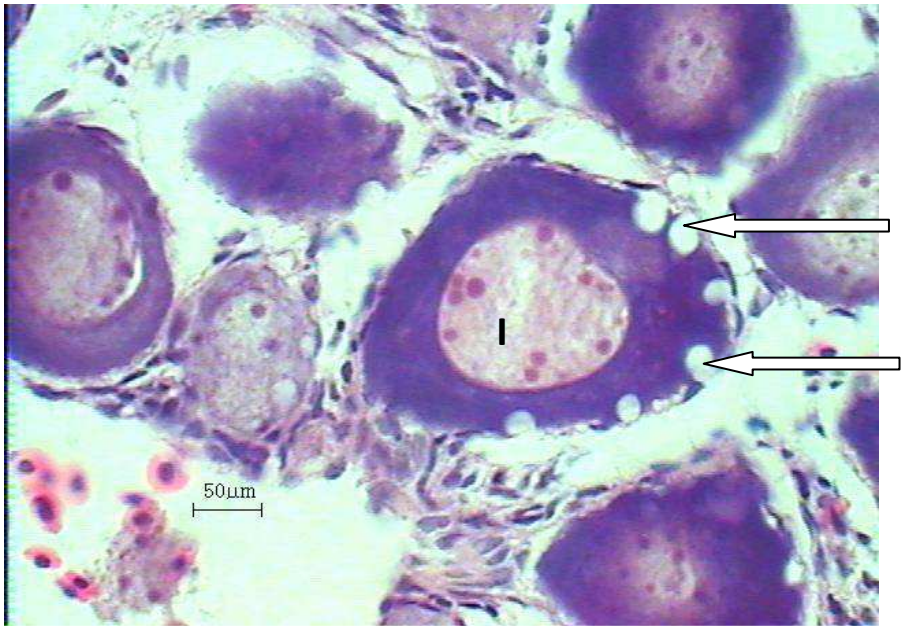

Fig.4. Section of the splenic parenchyma containing white pulp melanomacrophage centres. Note type I MMC containing phagocytic vesicles (arrows). H and E.

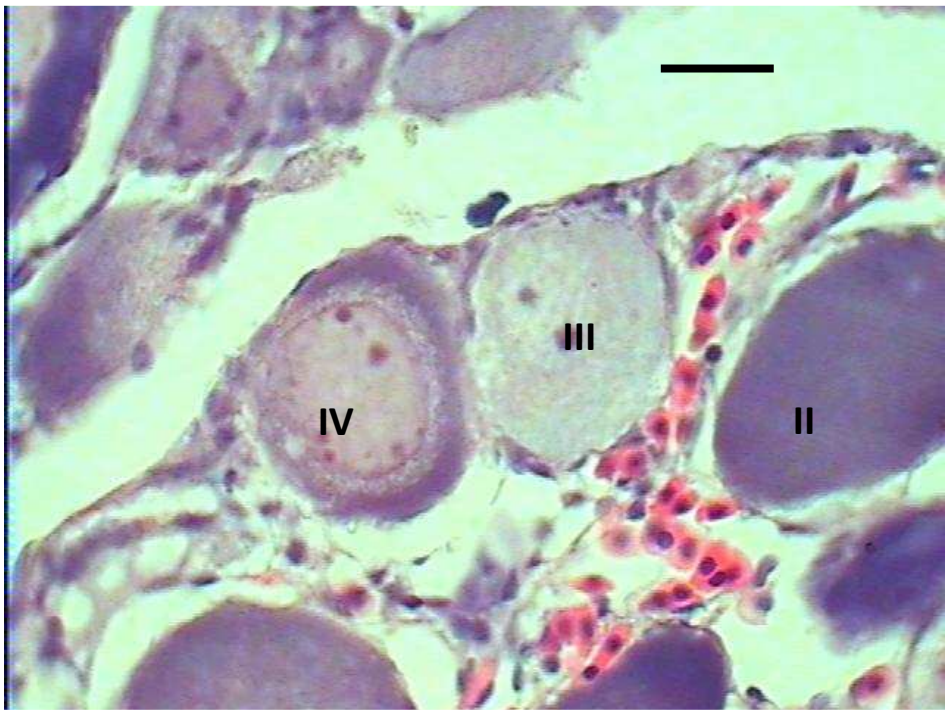

Fig.5. Section of the splenic parenchyma containing white pulp melanomacrophage centres. Note types II, III and IV MMCs. H and E. (Scale bar = 50 µm).

DISCUSSION

The splenic capsule of fibrous coat is for protection of the inner parenchyma containing the red and white pulp. The high level of vascularization and presence of haematopoietic cells in the spleen in this study has previously been reported [1]. The presence of sinusoids implies good perfusion of the organ for removal of pathogens and senescent cells in blood by the MMC and free leukocytes. The variation in colour of the MMC represents a correlation of the quantities of pigments like melanin, lipofuscin, ceroid and hemosiderin [11]. This colour variation has been reported in the *Dicentrarchus labral* [6].

The type I, MMCs may represent active stage involved in phagocytosis, thus accumulating pigments and inclusion bodies. The ovoid vacuoles in them suggest evidence of phagocytosis through pinocytosis. The type II may represent MMCs containing mostly leukocytes, hence the predominating basophilic colour. The Type III MMCs suggest accumulation of pigments yet to be infiltrated by leukocytes. The type IV MMC may represent a transitional stage to type I, where the 3 regions present will ultimately be reduced to 2 regions as the pigments accumulate in the central region making the middle region to disappear.

The finding in this study of the white pulp constituting majority of the splenic parenchyma is at variance with the report of the red pulp occupying majority of the organ in *Barbus pectoralis* [5], and *Huso huso* [23].

The PAS positive mucin histochemistry result indicates presence of carbohydrate moieties in the MMC, while AB negative result represents the absence of acid mucin. These suggest that the mechanism of defense elaborated by the teleost spleen is by phagocytosis and not by destroying pathogens through changing the pH of the body fluids as reported in teleost digestive tract acid mucin [24,25,26].

In conclusion, the results of this study suggest that melanomacrophage centers are part of the normal histology of the spleen of the African catfish. The report being the first on the histology of the juvenile African catfish spleen from Nigerian commercial aquaculture may serve as baseline for further investigative research or analysis of catfish health status in the country.

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