

## Seasonal changes in the Thymus gland of Tilapia Nilotica Fis

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### Abstract

This study was carried out on 100 thymus gland of apparent healthy Tilapia fish. The thymus was situated on the superior edge of the gill cover close opercular cavity; the thymus was surrounded by connective tissue capsule consisted of collagen fibers and reticular fibers. The thymocytes (T- lymphocyte) the main type of cells present in thymus embedded within network of epithelial cells. The thymus was characterized by presence of Hassall's body-like stromyoid cells, mucin secreting cells and melanomacrophage cells. The thymus showed great involution in winter in form of decrease number of lymphocytes, proliferative connective tissue and adipose tissue. Thymus showed regeneration of tissue in spring. Sharp increase in lymphocytes number was observed during summer. Lymphocytic foci became denser and increased in size. In autumn the parenchyma was divided into cortico-medullary like zones with increase in connective tissue proliferation especially in the medulla. The lymphocytic foci become less dense and decreased in size.

### Introduction

*Oreochromis niloticus* is probably the most important of the Tilapia species. (Adepo-Gourence, Abban and Fermon, 1997). Because of its rapid growth, large size, good taste and its economical price (Bakr, 2002). In teleost fishes, the immune system consist of the spleen, pronephros and thymus. (Zwollo Cole, Bromage and K 2005). Some researchers believe that photoperiod and temperature are both principle seasonal cues in aquatic animals (Bromage, Porter and Randall, 2000). It is clear that the environment affects the immune system of fishes. If seasonal associated changes can be anticipated, it may be possible to bolster the immune system at times when they are known to be immunosuppressed, as under photoperiod regimens, with the application of immunomodulators such as immunostimulants prior to exposure to stressful events. Ultimately, understanding seasonal effects on the immune function of fish may provide a better understanding of epidemiology of specific fish pathogens, Bowden Thompson, Morgan, Gratac Nikoskelainen (2007). The aim of this study to show the influence of seasonal changes on the thymus gland as one of the immune organs.

### Materials and Methods

Hundred apparent healthy Tilapia nilotica fish (*Oreochromis niloticus*) of both sexes were collected from different fish farms in El-Kanater el-khaieria. The thymus glands were collected and put in Susa and Bouin's fixatives then dehydrated, cleared, and cut at 4-5 microns. The tissues stained with general and special stains according to the methods given by Bancroft and Cook (1994). For TEM, 1mm<sup>3</sup> sample was used. Samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate and postfixated for 2 h in 1% osmium tetroxide buffered to pH 7.4 at 4°C. Fragments were dehydrated in a graded series of ethyl alcohol and embedded in resin embedding medium to produce medium hardness blocks prior to sectioning.

Semithin sections 0.5  $\mu\text{m}$  in thickness were used for histological observation and toluidine blue staining. The ultrathin sections were prepared with Ultracut microtome, stained with uranyl acetate and lead citrate (Chiu, Schmidt, and Prasad, 1993), examined by a Jeol JEM-100S transmission electron microscope (25KV) in national cancer institute.

## Results

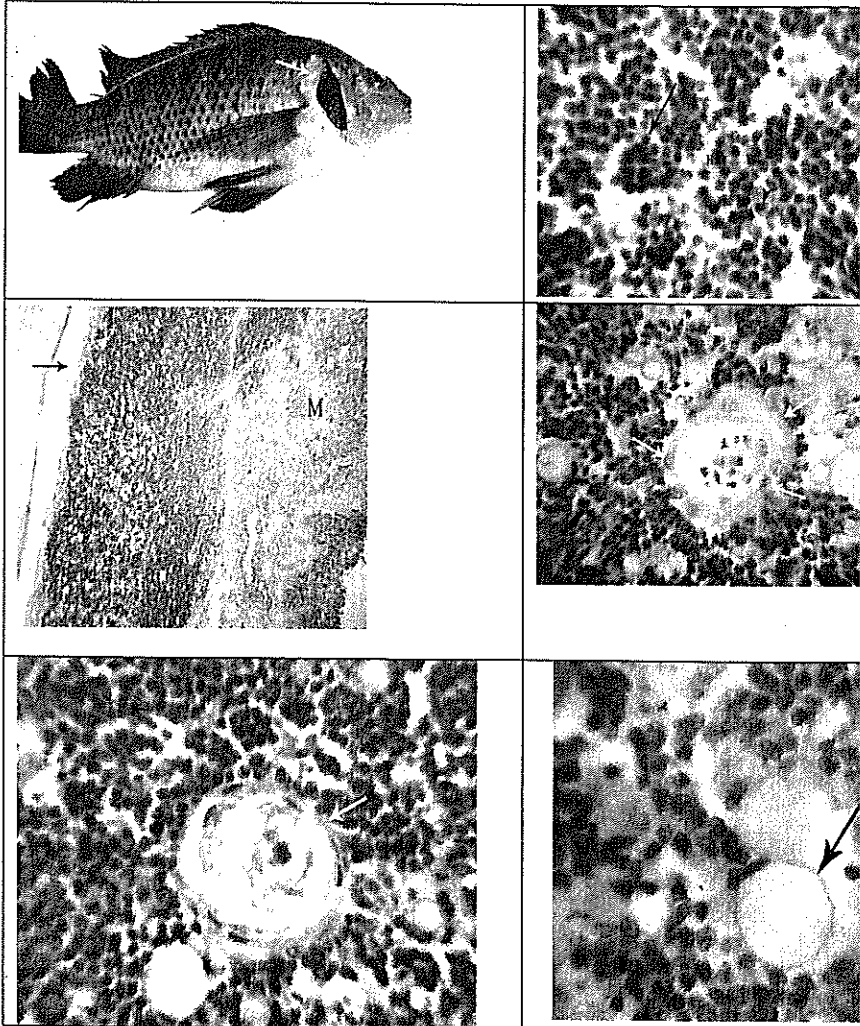
The thymus was bilobed organ and situated on the superior edge of the gill cover to the opercular cavity in contact with the water current (Fig.1). The thymus gland consisted of darkly stained cortex and lightly stained medulla, and covered by connective tissue capsule (Fig. 2), which formed from collagen fibers and reticular fibers, from the capsule short trabeculae emerge into the parenchyma.

The thymus gland parenchyma was formed from thymocytes which were numerous and arranged in two forms, small sized lymphocytes which mainly condensed in cortex with centrally located, darkly stained nucleus and lightly stained rim of cytoplasm. Large sized lymphocytes were located in the medulla with darkly stained nucleus and faint eosinophilic cytoplasm. The lymphoblasts were located between thymocytes especially in the cortex (Fig. 3). Reticuloepithelial cells were located between the thymocytes especially in the medulla. They were large cells with lightly stained basophilic cytoplasm and oval to ovoid nuclei (Fig. 4). The reticuloepithelial cells form Hassall's body-like structures which composed of concentrically arranged reticuloepithelial cells with degenerated center (Fig. 5). Another type of reticuloepithelial cells was found in thymic parenchyma, which were large cells with elongated periphery situated nucleus (Fig.6) and mucin filled cytoplasm which consisted of acid (simple, or non-sulfated) mucins typical of epithelial cells containing sialic acid which stained positively with PAS stain. Some epithelial cells showed no reaction to PAS stain others not. The reaction varied according to amount of mucin in the cytoplasm of these cells (Fig.7), also the cytoplasm of these cells stained with a blue PH 0.1 (Fig. 8). The thymic medulla also consisted of another type of cells, which were large cells with acidophilic (fibrillar) cytoplasm and large spherical centrally situated nucleus with prominent nucleolus which could be named myoid cells (Fig. 9). Melanomacrophage cell also found in thymus which was macrophage containing melanin granules. These macrophages appeared usually in solitary form (Fig. 10). Sometimes form clusters of group of melanin containing macrophages. The melanin granules may be few dispersed in cytoplasm or may fill it and even obscure the nucleus.

The thymus showed great involution in winter in form of decreased number of lymphocytes and proliferation of connective tissue and adipose tissue so the size of thymus decreased as a whole. It was formed from three zones, inner and outer zones consisted of thymocytes and reticuloepithelial cells while middle zone consisted of adipocytes and collagen fibers (Fig. 11). The thymus showed regeneration of thymus during spring season, the parenchyma divided into cortico-medullary like zones. Lymphocytes number increased in comparison with winter with decrease in connective tissue, the lymphocytes were more condensed with numerous blood cells dispersed between the medullary cells while few cells escaped between the cortical cells (Fig. 12). The thymus increased in size in summer season and covered by connective tissue capsule enclosing the parenchyma which divided into cortico-medullary like zones (Fig.13). Sharp increase in lymphocyte number observed during this season as the cortex become denser and highly populated with thymocytes.

Decrease in lymphocytes number was observed during autumn season lymphocytic foci became less dense and decreased in size, with noticeable in myoid cells (Fig. 14).

**Legend of figures**



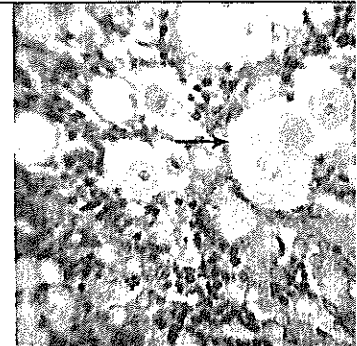
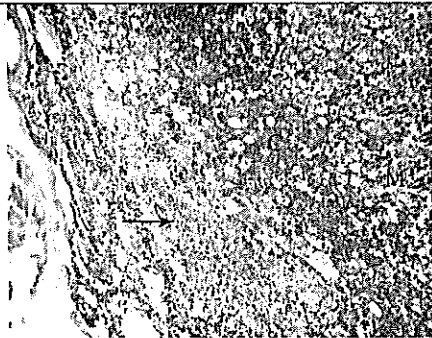
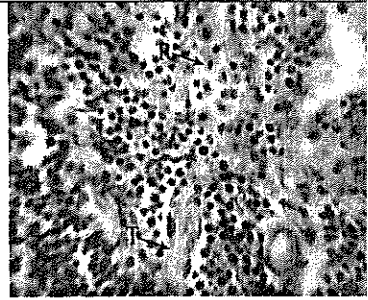
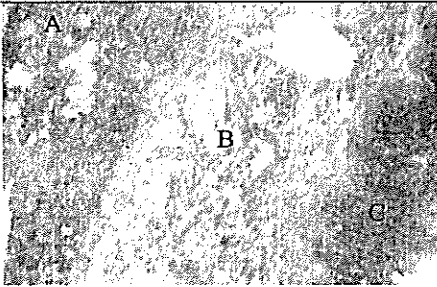
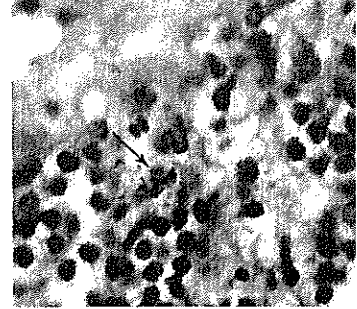
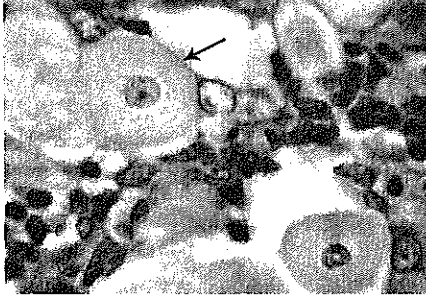
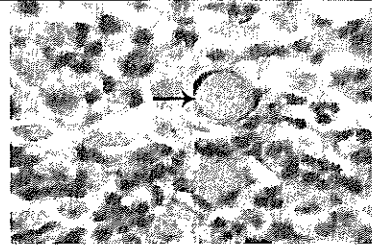
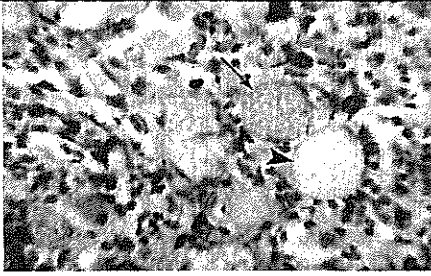


Fig.1: *Tilapia nilotica* fish showing the thymus gland (arrow) the thymus located superior edge of the gill cover close to the opercular cavity.

Fig. 2: Section in the thymus showing connective tissue capsule (arrow) and differentiating lymphoid cells (thymocytes) within a network of epithelial cells generally organized in dense cortical (C) and light medullary (M) like zone (X100).

Fig.3: Section in the thymus showing parenchyma of thymus which is composed of small sized lymphocytes (arrow), large sized lymphocytes (head arrow) and lymphoblast (b). H&E (X1000).

Fig.4: Section in the thymus showing reticulo-epithelial cells (arrows). H&E (X100)

Fig.5: Section in the thymus showing Hassal's like corpuscle which composed of concentrically arranged epithelial reticular cells with degenerated center (arrow) (X1000).

Fig.6: Section in the thymus showing one of the epithelial reticular cell which was oval and spherical in shape with elongated and periphery situated nucleus and mucopolysaccharide cytoplasm (arrow). H&E (X1000).

Fig.7: Section in the thymus showing epithelial reticular cells containing mucins. Mucicarmum cells show high reaction to stain (arrow) others show negative reaction (head arrow). PAS stain (X 1000).

Fig.8: Section in the thymus showing thymic medulla which consisted of alveolar epithelial reticular cells containing mucins (arrow). Alcian blue at pH 0.1 stain (X1000).

Fig. 9: Section in the thymus showing myoid cell which is characterized by large nucleus, prominent nucleolus and fibrillar cytoplasm (arrow). H&E (X1000).

Fig.10: Section in the thymus showing macrophage containing melanin granules (arrow). H&E (X1000).

Fig.11: Section in the thymus during winter season showing three zones outer zone (O) and inner zone (C) consisted of lymphocytes and epithelial reticular cells and medullary zone (B). H&E (X100).

Fig.12: Section in the thymus during spring showing presence of red blood cells under capsule (C) which extend trabeculae (T) into the parenchyma. H&E (X400)

Fig. 13: Section in the thymus during summer showing connective tissue capsule (C) envelope the parenchyma which composed of outer dense cortex (C) and inner medulla (M). H&E (X100).

Fig.14: Section in the thymus showing melanomacrophage center (arrow). Modified Fontana method with hematoxylin stain. (X1000).

## Discussion

This study revealed that the thymus was surrounded by connective tissue capsule which consisted of collagen fibers and reticular fibers sending short trabeculae into the parenchyma dividing it into incomplete compartments. This was described by Huez et al. (1995) in Brown trout.

The thymus was formed from an outer layer or cortex and an inner layer or medulla which can be distinguished, although the delineation between the cortex and medulla is indistinct. This result was similar to that detected by Press and Ewing (1999) in teleost fish.

The cortex usually contained a higher density of thymocytes than the medulla. The medulla contained relatively few epithelial reticular cells. The medulla was less densely populated with cells but the epithelial cells were more prominent and form supporting meshwork.

These cell types were referred to as reticulocytes and the epitheliocytes. This result reported by Becker et al. (2001) in Carp and Petrie-Hanson and Peterman (2005) in American paddlefish. The thymus in winter was formed from three zones, inner and outer zones contained thymocytes and epithelial reticular cells while the middle zone contained adipocytes and collagen fibers. This result indicated that during winter seasonal involution had occurred in thymus which means that a drop in immune response was found during this season, this result agreed with Alvarez et al. (1998) in wild brown trout, and Hueza et al. (1995) in *Salmo trutta*. The thymus gland was composed generally from thymocytes (T-lymphocytes) which form the main type of cells present in thymus embedded within network of epithelial reticular cells. Thymocytes were numerous and arranged in two forms, small sized lymphocytes which were condensed in the cortex with centrally located, darkly stained nucleus and lightly stained rim of cytoplasm. Large sized lymphocytes were located in the medulla with darkly stained nucleus and faint eosinophilic cytoplasm. The lymphoblasts were located between the thymocytes especially in the cortex, this result is augmented with Mani (1994) in teleost fishes. Corresponding with Romano et al. (1999) in Carp, this study revealed that the epithelial-reticular cells were large cells with lightly stained basophilic cytoplasm and oval to ovoid nuclei, they formed Hassall's body-like structures which were composed of concentrically arranged epithelial reticular cells with degenerated centers. Also the medulla of thymus contains mucin-secreting cells (type of reticuloepithelial cells) which were large cells with elongated periphery situated nucleus and mucin-filled cytoplasm, these cells stained positively with PAS, Alcian blue PH 0.1 and 2.5. This result was found by Hussein (2007) in *Oreochromis niloticus*.

The thymus was characterized by the presence of large cells with acidophilic (fibrous) cytoplasm and large spherical centrally situated nucleus with prominent nucleolus which termed myoid cells. This result was augmented by Fänge and Pulsford (1983) in teleost fish who found that myoid cells are noted in the teleostean thymus and are thymocytes and epithelial cells and characterized by their large size, a clear cytoplasm and a large nucleus with a central prominent nucleolus. In agreement with Gorge (1983) in Cling fish, Heemstra and Randall (1993) in grouper, rockcod, hind, and grouper and lyretail species, the present study revealed that melanomacrophages were also found in thymus which were macrophages containing melanin granules. These macrophages were present usually in solitary form sometimes form centers from groups of melanin-containing macrophages. The melanin granules may be few dispersed in cytoplasm or may fill it and even obscure the nucleus. Kendall (1991) in teleost and Romano et al. (1999) in Carp. In agreement with Alvarez et al. (1994) in Rainbow trout the present work found that erythrocytes were also observed in thymus and their number changes with season. It was few in winter and autumn but in spring thymic erythropoietic activities were increased which appeared in the form of increased number of erythrocytes inside thymic parenchyma especially under the capsule. A decrease in erythrocytes number was observed in summer.

The thymus gland showed great involution in winter in form of proliferation of connective tissue and appearance of high number of adipocytes, this result indicates that low temperature adversely affects immune organs in fish (poikilothermic animals) and this result is in agreement with Tamura et al. (1981) in viviparous surfperch and Takemura (2003) in *Tilapia niloticus*. The thymus showed regeneration of tissue during spring, the lymphocyte number reached its maximum level during summer and then decreased again during autumn, this result was previously mentioned by Sharaf El-Din (1993) in *Oreochromis niloticus*.

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خص العربي

بيرات الموسمية للغدة الزعترية في سمك البلطي النيلي

أم فؤاد عطية، إيهاب محمود الزغبي، منى نصر عبد النعيم، حاتم حسين بكري  
الانسجه والخلايا وقسم الطب الشرعي- كلية الطب البيطري-جامعه بنها

بت هذه الدراسة علي الغدة الزعترية أثناء المواسم المختلفة لمعرفة تأثير الحرارة وفترة الإضاءة علي عة. وقد أجريت هذه الدراسة علي مائة من سمك البلطي النيلي جمعت من مزارع مختلفة بالقناطر الخيرية طة القلوبية. وجدت الغدة الزعترية تحت الجزء العلوي من غشاء الخياشيم بجانب التجويف الفمي وكانت : مغطاة بطبقة طلائية ترسل تفرعات صغيرة إلي داخل النسيج البرنشيمي الذي يتكون من قشرة ونخاع :ة من مجموعة من الخلايا الليمفاوية داخل شبكة من الخلايا الطلائية. تتميز الغدة الزعترية بوجود تركيب كرات هاسل وخلايا شبه عضلية وبعض الخلايا المفرزة لمادة لزجة (نوع من الخلايا الطلائية) والخلايا :ة الصبغية وكرات الدم الحمراء. الغدة الزعترية تضمحل في فصل الشتاء في صورة زيادة نسبة النسيج :مي والنسيج الدهني في الجزء الأوسط أما الجزء الداخلي والخارجي فيحتويان علي خلايا ليمفاوية داخل : من الخلايا الطلائية والشبكية. في فصل الربيع تستعيد الغدة الزعترية تكوينها من قشرة ونخاع مع زيادة :طة في عدد كرات الدم الحمراء وخاصة تحت النسيج الطلائي الشبكي. في فصل الصيف وجدت زيادة :طة في عدد الخلايا الليمفاوية والخلايا البالعة للميلانين وتكون في هيئة تجمعات بدلا من وجودها منفردة :ني المواسم الأخرى. أما في فصل الحريف فقد وجد هناك نقص في عدد الخلايا الليمفاوية.