

Seasonal changes in the Thymus gland of Tilapia Nilotica Fish

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Abstract

This study was carried out on 100 thymus gland of apparent healthy Tilapia Nilotica fish. The thymus was situated on the superior edge of the gill cover close to the opercular cavity; the thymus was surrounded by connective tissue capsule which consisted of collagen fibers and reticular fibers. The thymocytes (T- lymphocytes) form the main type of cells which were embedded within a network of epithelial reticular cells. The thymus was characterized by presence of Hassall's body-like structures, myoid cells, mucin secreting cells and melanomacrophage cells. The thymus showed great involution in winter in the form of decreased number of lymphocytes, proliferation of connective tissue and adipose tissue. Regeneration of thymic tissue was noticed during spring. Sharp increase in lymphocytes was observed during summer as the lymphocytic foci became denser and increased in size. In autumn the parenchyma was divided into cortico-medullary like zones showing increased 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. (Agnese, 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 organs consist of the spleen, pronephros and thymus. (Zwollo Cole, Bromage and Kaattari, 2005). Some researchers believe that photoperiod and temperature are both reliable principle seasonal cues in aquatic animals (Bromage, Porter and Randall, 2001). It is clear that the environment affects the immune system of fishes. If seasonally associated changes can be anticipated, it may be possible to bolster the immune system at times when they are known to be immunosuppressed. This can be checked by artificial photoperiod regimns, with the application of immunomodulators such as immunostimulants prior to exposure to stressful events. Ultimately, understanding the seasonal effects on the immune function of fish may provide a better understanding of epidemiology of specific fish pathogens, Bowden Thompson, Morgan, Gratacap and Nikoskelainen (2007). The aim of this study is to show the influence of seasonal changes on the thymus gland as one of the immune organs.

Materials and Methods

Hundred apparently healthy Tilapia nilotica fishes (*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, embedded and cut at 4-5 microns. The tissues stained with general and special stained according to the methods given by Bancroft and Cook (1994).

Results

The thymus was bilobed organ and situated on the superior edge of the gill cover close to the opercular cavity in contact with the water current (Fig.1).The gland was 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 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 (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 reticular epithelial cells form Hassall's body-like structures which were 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 high reaction to PAS stain others not. The reaction varied according to amount of mucins in the cytoplasm of this cells (Fig.7), also the cytoplasm of this cells stained with alcian 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 cells were also found in the thymus which appeared either in solitary form or in clusters (Fig.10). The melanin granules may be few dispersed in cytoplasm or may fill it and even obscured the nucleus.

The thymus showed great involution in winter in the form of decreased density of lymphocytes and proliferation of connective tissue and increase in adipose tissue which result in decreased size of the gland. 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 tissue during spring season, the parenchyma divided into cortico-medullary like zones. Increased condensation of lymphocytes with a decrease in connective tissue was obvious if compared with winter. Numerous blood cells were dispersed in the in 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 as the lymphocytic foci became less dense and decreased in size, with noticeable increase in myoid cells (Fig. 14).

Legend of Figures:

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

Fig. 2: Section in the thymus showing connective tissue capsule (arrow) under it differentiating lymphoid cells (thymocytes) within a network of epithelial cells and is generally organized in dense cortical (C) and light medullary (M) like zones. H&E (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).

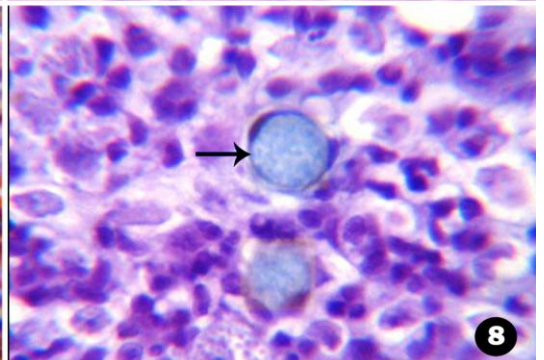
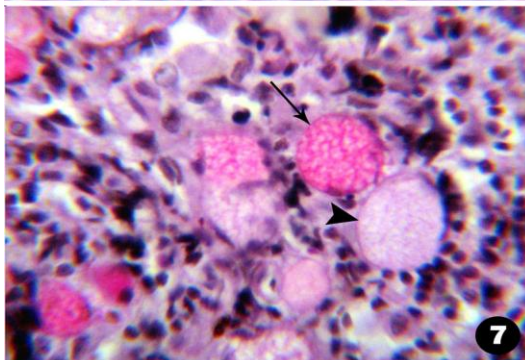
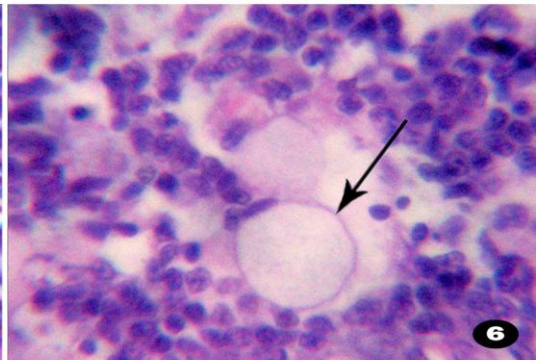
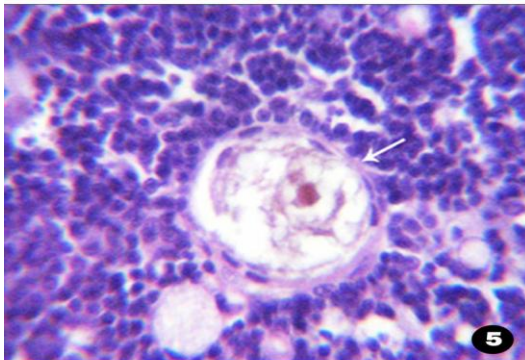
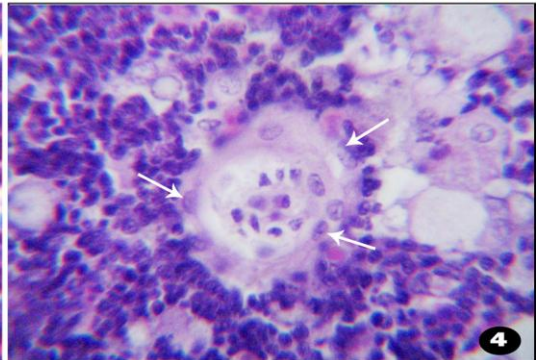
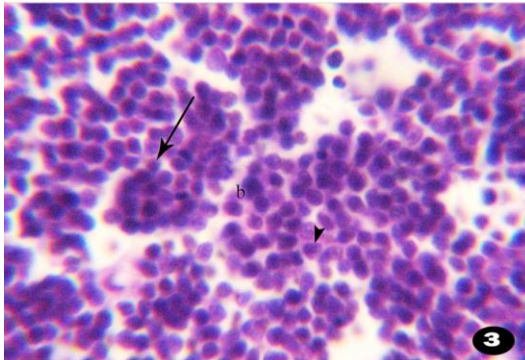
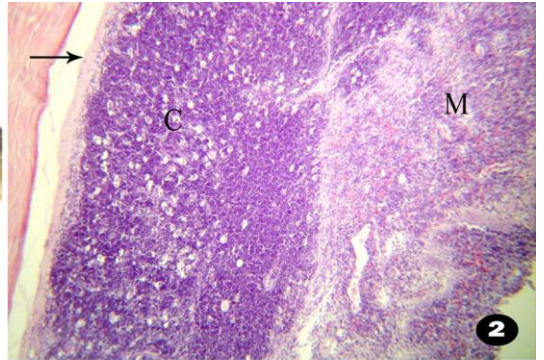
Fig.4: Section in the thymus showing reticulo-epithelial cells (arrows). H&E (X1000).

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

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

Fig.7: Section in the thymus showing epithelial reticular cells containing mucins, some 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 alcianophilic epithelial reticular cells containing mucins (arrow). Alcian blue at pH 0.1 stain (X 1000).



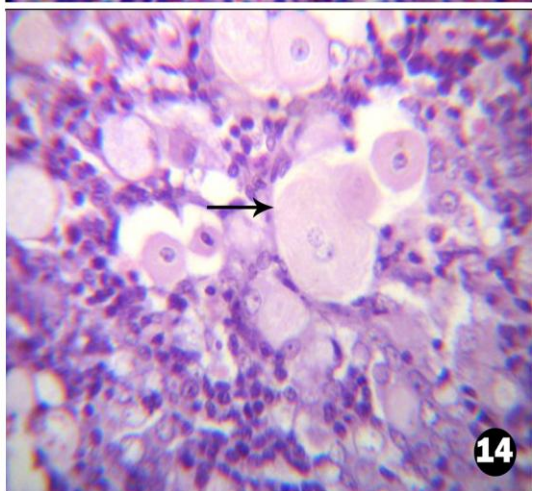
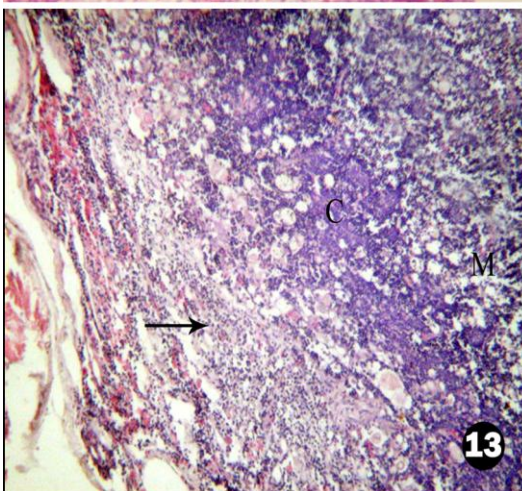
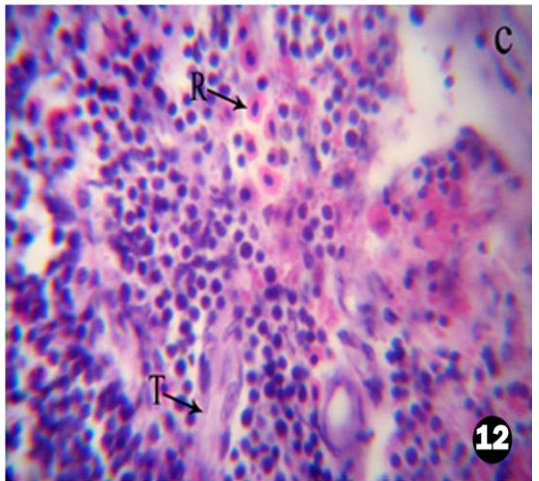
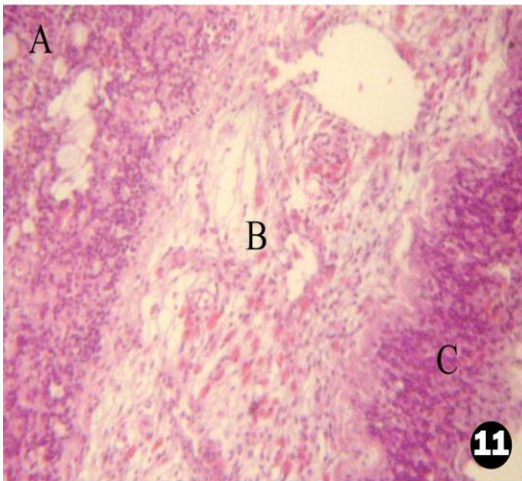
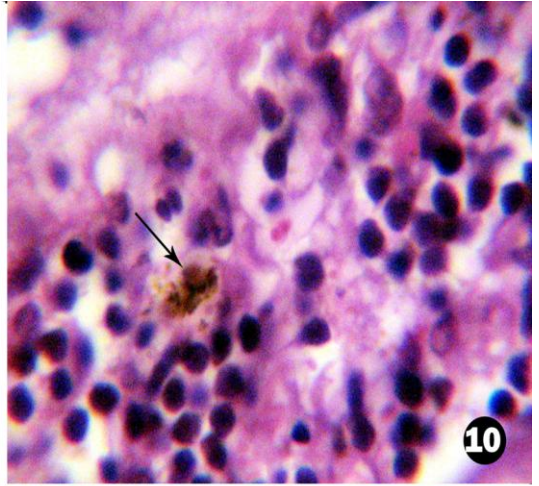
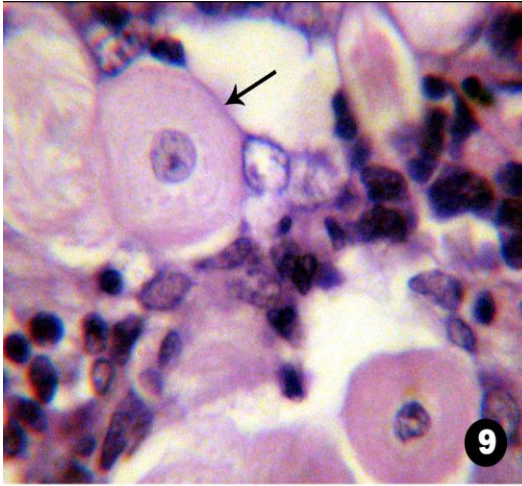


Fig. 9: Section in the thymus showing myoid cell which is characterized by large nucleus, prominent nucleolus and fibrillar cytoplasm (arrow). H&E (X1000).

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 (A) and inner zone (C) consisted of lymphocytes and epithelial reticular cells and middle zone (B). H&E (X100).

Fig.12: Section in the thymus during spring showing presence of red blood cells (R) 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 envelope the parenchyma which composed of outer dense cortex (C) and inner light medulla (M). H&E (X100).

Fig.14: Section in the thymus during autumn season showing, noticeable increase in myoid cells. H&E (X1000).

Discussion

This study revealed that the thymus was surrounded by connective tissue capsule consisted of collagen fibers and reticular fibers sending short trabeculae into the parenchyma dividing it into incomplete compartments. This was described also by **Hueza 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 the medulla is indistinct. This result was similar to that detected by **Press and Evensen (1999)** in teleost fish.

The cortex usually contained a higher density of thymocytes than the medulla and 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 some 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 which were numerous and arranged in two forms, small sized lymphocytes which mainly 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 lymphoblast were located between the thymocytes especially in the cortex, this result is augmented with **Manning (1994)** in teleost fishes

Corresponding with **Romano et al. (1999)** in Carp, this work 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 composed of concentrically arranged epithelial reticular cells with degenerated center. Also the medulla of thymus contain mucin secreting cells (type of reticuloepithelial cells) which were large cells with elongated periphery situated nucleus and mucin filled cytoplasm, this 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 (fibrillar) cytoplasm and large spherical centrally situated nucleus with prominent nucleolus which termed myoid cells this result was augmented by **Fange and pulsford (1985)** in teleost fish who found that myoid cells are noted in the teleostean thymus among thymocytes and epithelial cells and

characterized by their large size, a clear cytoplasm and a large nucleus with a central prominent nucleolus. These myoid cells may protect thymocytes from apoptosis and modulate their differentiation as mentioned by **(Le Panse and Aknin, 2005)**

In agreement with **Gorgollon (1983)** in Cling fish, **Heemstra and Randall (1993)** in grouper, rockcod, hind, coral grouper and lyretail species, the present study revealed that melanomacrophage cell also was found in thymus which was macrophage containing melanin granules. These macrophages were present usually in solitary form sometimes form centers from group 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 fish, and **Romano et al. (1999)** in Carp

In agreement with **Alvarez et al. (1998)** in the Rainbow trout the present work found that erythrocytes also observed in thymus and its number change with season. It was few in winter and autumn but in spring the thymic erythropoietic activities were increased which appeared in the form of increased number of erythrocytes inside thymic parenchyma especially under the capsule. Slight decrease in erythrocytes number was occurred in summer.

The thymus gland showed great involution in winter in form of proliferation of connective tissue and appearance of high number of adipocyte, this result indicated that low temperature adversely affect immune organs in fish (poikilothermic animals) this result is in agreement with **Tamura et al. (1981)** in viviparous surffperch and **Kim and Takemura (2003)** in *Tilapia niloticus*.

The thymus showed regeneration of tissue during spring, the lymphocyte number reached its maximum level during summer then re decreased again during autumn, this result previously mentioned by **Sharaf El-Dien (1993)** in *Oreochromis niloticus*.

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التغيرات الفصلية للغده الزعترية في سمك البلطي النيلي

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*قسم الأنسجة والخلايا و**قسم الطب الشرعي - كلية الطب البيطري - جامعته بنها

- أجريت هذه الدراسة علي الغدة الزعترية أثناء المواسم المختلفة لمعرفة تأثير الحرارة وفترة الإضاءة علي المناعة. وقد أجريت هذه الدراسة علي مائة من سمك البلطي النيلي جمعت من مزارع مختلفة بالقناطر الخيرية محافظة القليوبية.
- وجدت الغدة الزعترية تحت الجزء العلوي من غشاء الخياشيم بجانب التجويف الفمي وكانت الغدة مغطاة بمحفظة من النسيج الضام والتي ترسل حويجزات الي داخل متن الغده يتكون المتن من قشرة ونخاع مؤلفة من مجموعة من الخلايا الليمفاوية داخل شبكة من الخلايا الظهارية.
- تتميز الغدة الزعترية بوجود تركيب يشبه كرات هاسل وخلايا شبه عضلية وبعض الخلايا المفرزة لمادة لزجة (نوع من الخلايا الطلائية) والخلايا البالعة الصبغية وكرات الدم الحمراء.
- الغدة الزعترية تضمحل في فصل الشتاء في صورة زيادة نسبة النسيج الدعامي والنسيج الدهني في الجزء الأوسط أما الجزء الداخلي والخارجي فيحتويان علي خلايا ليمفاوية داخل شبكة من الخلايا الطلائية والشبكية.
- في فصل الربيع تستعيد الغدة الزعترية تكوينها من قشرة ونخاع مع زيادة ملحوظة في عدد كرات الدم الحمراء وخاصة تحت النسيج الطلاني الشبكي .
- في فصل الصيف وجدت زيادة ملحوظة في عدد الخلايا الليمفاوية والخلايا البالعة للميلانين وتكون في هيئة تجمعات بدلا من وجودها منفردة كما في المواسم الأخرى.
- أما في فصل الخريف فقد وجد هناك نقص في عدد الخلايا الليمفاوية.