Original Article

Immunoreactivity of Somatolactin (SL)-Producing Cells During Larval Development of Nile Tilapia

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ABSTRACT

Introduction: A growing body of evidence confirmed the involvement of somatolactin (SL) in various physiological processes including the acid-base as well as calcium regulation, fat metabolism and osmoregulation. However, less is known about the immunoreactivity of SL- producing cells during developmental stages of Nile tilapia, Oreochromis niloticus.

Aim of the Work: The aim of the present work was to examine the immunoreactivity of SL-producing cells during different developmental larval stages of Nile tilapia, O. niloticus.

Material and Methods: We performed immunohistochemical technique using specific antibody against chum salmon SL to identify the anatomical distribution and change in the activity of SL-producing cells accompanied the different larval stages of Oreochromis niloticus, and study the possible involvement of this hormone in the early endocrine integration.

Results: The present immunocytochemical assessments revealed a few and dispersed numbers of SL-positive cells at the first day after hatching. Moreover, we observed a gradual increase in the activity of SL-positive cells in the pituitary gland as reflected with the increased cell number and size as well as SL immunoreactivity concomitant with the continued larval development from the first day post-hatching onwards and reached the highest levels on day 35 post- hatching. In parallel to the advanced development of larvae, the number of SL-positive cells and the intensity of immunoreaction were clearly and gradually increased especially after the onset of exogenous feeding at 21-28 days post-hatching. Consequently, there is an obvious increase in both the larval body length and weight during the experimental period of rearing.

Conclusion: The early appearance of SL-producing cells and the gradual changes in their immunoreactivity during development suggest crucial effects of this hormone in different physiological processes during larval rearing of O. niloticus especially in the period after yolk sac resorption.

Received: 23 May 2021, **Accepted:** 31 May 2021

Key Words: Development; fish larvae; immunocytochemistry; oreochromis niloticus; SL.

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ISSN: 1110-0559, Vol. 45, No. 3

INTRODUCTION

It is well established that Nile tilapia (Oreochromis niloticus) play a crucial role in polyculture of freshwater fishes in the world with specific regards in Egypt. The importance of this species is related to the fast-growing rate and worldwide distribution. Recently, there is a growing demand for sufficient supply of fry and fingerlings of this species among fishpond operators. However, the rearing process of O. niloticus in the hatcheries failed to produce a reliable amount of fry and this failure is related to the heavy larval mortalities; commonly happening during the second and third week after hatching. The main reason of high larval mortality may relate to their starvation due to the physical nutritional shortage after utilization of yolk sac and/or environmental changes. Mostly, high larval mortality rate was prominent during the early yolk sac stage of teleost in hatcheries[1,2]. However, the true causes are complex process and still unknown.

The period after hatching is the most interesting variable and decisive time during the teleost species life. Fish larvae are very small functionally independent vertebrates, subject to a daily increase in adaptive capacity, and many organs are characterized by becoming active. Accumulating data confirmed the involvement of SL in different physiological processes, such as the acid-base as well as calcium regulation[3-5], fat metabolism[6-8] and osmoregulation[9]. SL is a new hormone in the pituitary gland and is linked to mobilize energy in response to stress[10,11], exhaustive exercise[12], and fasting[8,13]. SL is a fish-restricted peptide whose synthesis and release are changed in organisms during background adaptation, and consequently regulates fish skin pigmentation[14-19]. In addition, a several recent studies revealed a seasonal change in the number, size, and activity of the SL-producing cells concomitant with the gonad maturation and spawning in O. niloticus and Mugil cephalus[20-23]. SL-producing cells in early stages of development were identified in some teleosts; Plecoglossus altivelis[24], Sparus aurata[25,26], Oreochromis mossambicus[27], Cichlasoma dimerus[28] and Phreatichthys andruzzii[29]. However, the changes of SL- producing cells during different larval stages of Nile tilapia, O. niloticus are still lacking. Therefore, additional information on the immunoreactivity of SL- secreting cells during larval stages of Nile tilapia may increase our knowledge of the physiological functions of this hormone during the larval rearing of this species.

 DOI: 10.21608/ejh.2021.75219.1477

MATERIAL AND METHODS

Study Site

The experiments of this study were performed at the Research Station of El-Matareyya and Zoology Department, Faculty of Science - Menoufia University during the period extending from 1 January until 30 August 2020.

Obtaining the larvae

Both sexes of mature Nile tilapia breeders (weight ranged from 150 g to 250 g) were separated into two groups in two different ponds. To ensure the quality and quantity of gonads, breeders were fed a daily 40% protein diet. Spawning of O. niloticus breeders was performed in the spawning tanks as previously obtained[30]. We monitored daily the breeding activity. After the spawning was completed, only the brooding female left to brood the progeny.

Directly after hatching, the larvae were transferred into glass aquaria (50 L) at a density of 10 larvae/l. Larvae were incubated, nursed, and fed as previously described[30].

Sampling of larvae and measurements

Both standard length (L) and weight (W) of larvae were measured at day 1 and weekly during rearing period of 35 post-hatching. At least twenty larvae were randomly sampled as previously mentioned[30].

For histological and immunohistochemical experiments, the larvae were anaesthetized using clove oil (Sigma), fixed in Bouin's fluid and histologically prepared as previously illustrated[20,21,30].

Immunohistochemical Procedure

Antibody

The source of specific antiserum against chum salmon somatolactin (chum SL) (Lot No. 8906) was Dr. H. Kawauchi (School of Fisheries Science, Kitasato University, Iwate, Japan).

Immunohistochemical reactions

Immunohistochemical staining was accomplished using vectastain ABC (Avidin-biotin peroxidase complex) Kit (Vector Laboratories) as previously described[20,21]. In brief, dewaxed mounted tissue sections were rehydrated and washed in phosphate-buffered saline (PBS; pH 7.4) then incubated with the primary antibody against chum salmon SL (1:1000) overnight at 4°C. Thereafter, the sections were incubated with the secondary antibody (Vector Laboratories) and with avidin-biotin-conjugated peroxidase. Finally, the specific immunoreactivity was visualized with 3, 3- diaminobenzidine tetrahydrochloride (DAB) (Sigma). Finally, the sections were mounted in DPX.

The immunoreactive procedure specificity was confirmed as follow: a) according to the above-described protocol adjacent sections were stained without SL antiserum. Moreover, primary antibody was replaced with normal bovine serum. Indeed, the specific immunoreactivity disappeared in control sections.

Semi-quantification of immunostaining

Semi-quantification of SL-positive cells was determined per larva as previously described[30]. Briefly, both the number and size of SL-positive cells were counted and measured using the Zeiss microscope.

Statistical analysis

Comparison between the numbers and sizes (mean±SD) of SL-positive cells during the developmental stages were calculated by one-way ANOVA. To obtain significant differences between all developmental stages, Student-Newman-Keuls test or Dunn's test were used. Differences were considered significant if $P< 0.05$.

RESULTS

I. Immunoreactivity of SL cells

At the first day post-hatching

At first day post-hatching, the mean weight of the larvae was 11.15±0.08 mg. The pituitary of larvae at first day post-hatching has small mass of cells attached to the brain floor (Figures 1, 2). In parallel, our immunohistochemical staining detected a weak SL-immunoreactivity in the posterior part of adenohypophysis of the all pituitary examined at first day post-hatching.

After seven days post-hatching

After 7 days post-hatching the mean total weight of larvae was 15.15±0.45 mg. At this developmental stage, the pituitary gland consists of an oval group of cells within demarcated rostral and caudal regions of adenohypophysis (Figures 3, 4). However, SL-positive cells were located in both rostral and caudal adenohypophyseal regions and exhibit a moderate immunoreactivity (Figure 4).

After 14 days post-hatching

After 14 days post-hatching, the mean total weight of the larvae was 29.10±1.35 mg. At this stage of development, the pituitary gland became apparently developed with elongated shape and more recognizable neurohypophysis, but there is no innervation within the adenohypophysis. Importantly, the distinctive parts of the pituitary can be easily distinguished into the pars intermedia and pars distalis (Figures 5, 6). Moreover, SL-positive cells were located mainly in the pars intermedia (Figure 6). However, some SL-immunoreactive cells were identified in the region between the two main parts of pars distalis; rostral and proximal (Figure 6).

After 21 days post-hatching

The mean total weight of larvae after 21 days post-hatching was 115.25±7.25 mg. At this stage, the SL-positive cells increased in both the size and number and

became more obvious (Figures 7, 8). In parallel, the size of both glandular adenohypophysis and neural lobe increased with notable development of the all examined 28 days old larvae. In addition, the pituitary gland appearance of the larvae at this stage is similar to those of adult fish (Figures 9, 10). Importantly, the immunoreactivity of SL-positive cells was apparently multiplied with larval growth.

After 28- and 35-days post-hatching

After 28 day post-hatching, the mean total weight of the larvae was 270.75±9.15 mg. In parallel, the number, size and the immunoreactivity of SL-positive cells were increased after 28 days post-hatching (Figure 9). Moreover, these cells located dorsally, in both the pars distalis (PD) and pars intermedia (PI), in close contact with neural innervation (Figure 10). However, the mean total weight of the larvae was 545.85±25.75 mg during 35 days post-hatching. In parallel, the size of pituitary gland increased. In addition, the number of SL-positive cells showed a noticeable increase and exhibited moderate immunoreactivity (Figures 11, 12).

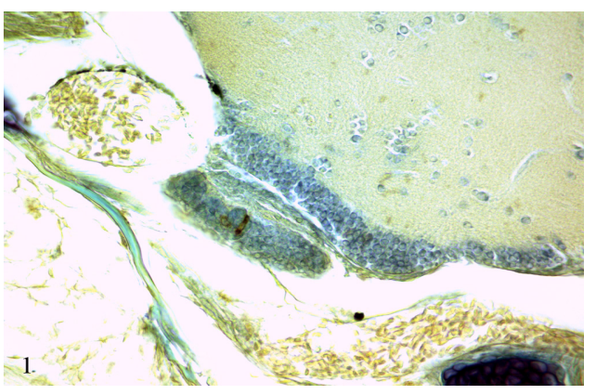

Fig. 1: Sagittal section of O. niloticus larvae at the first day post-hatching immunostained with chum salmon somatolactin (SL) antibody. During this stage, the pituitary gland consists of small group of dispersed cells beneath the brain. X200.

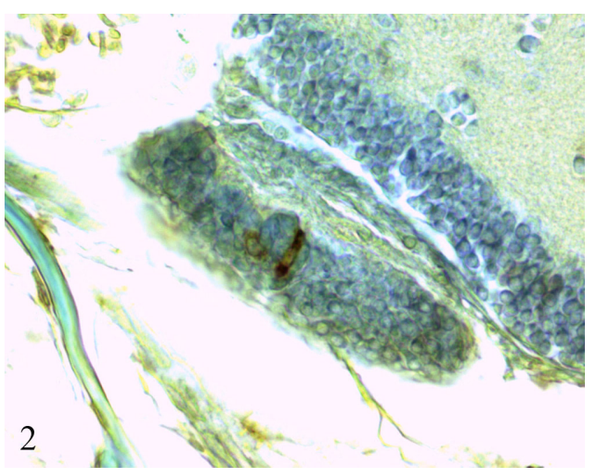

Fig. 2: A higher magnification of figure (1); showing a weak SL-immunoreactivity as indicated with brown color in the posterior part of adenohypophysis. X400.

II. Larval growth

i) Growth in length

The normal development of O. niloticus from the first day of hatching and through several larval stages was presented in the (Table 1). During the first day of hatching, the mean total length of the larvae was 8.44±0.025 mm. Importantly, our data reveal that the length of larvae was significantly (P<0.05) increased during the developmental period. It achieved 35.65±2.05 mm after five weeks as represented in (Table 1).

ii) Growth in weight

The normal development of O. niloticus, as reflected with the increased body weight from the first day of hatching and through several larval stages, was presented in the (Table 1). At the first day post-hatching, the mean weight of the larvae was 11.15±0.08 mg. Interestingly, the obtained results indicated that the weight of O. niloticus larvae was significantly increased (P<0.05) during the developmental period. In parallel, the body weight attained 545.85±25.75 mg after five weeks as shown in (Table 1).

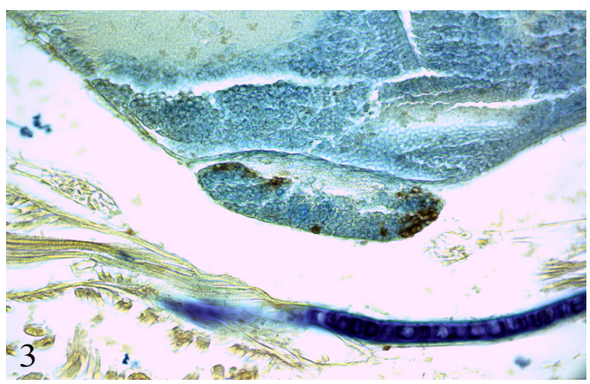

Fig. 3: Sagittal section O. niloticus larvae at 7 days post-hatching immunostained with anti-chum salmon somatolactin (SL). Note that; the pituitary gland is a group of cells that are oval in shape and the rostral and caudal regions of the pituitary gland can be distinguished by clear borders. X200.

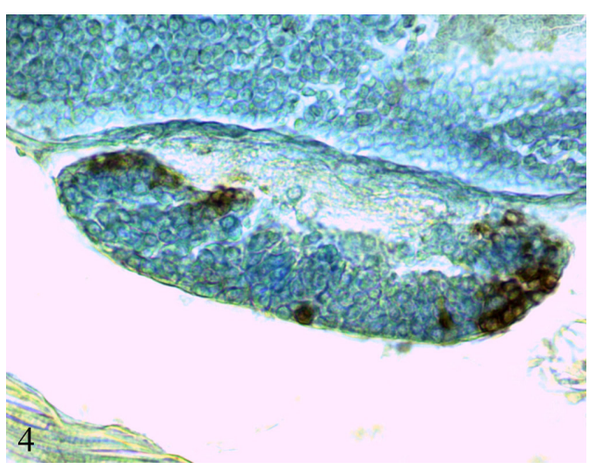

Fig. 4: A higher magnification of figure (3), showing SL-positive cells as reflected with brown color recognized in both rostral and caudal regions in adenohypophysis with moderate immunoreactivity. X400.

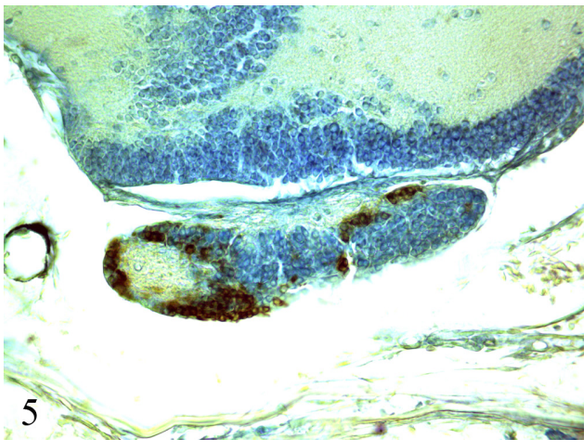

Fig. 5: Sagittal section of O. niloticus larvae 14 days post-hatching stained with chum salmon-SL antiserum. Note that, the pituitary gland exhibited a distinct elongated shape and the neurohypophysis was well distinguished although, there is no neural innervation in the adenohypophyseal region. X200.

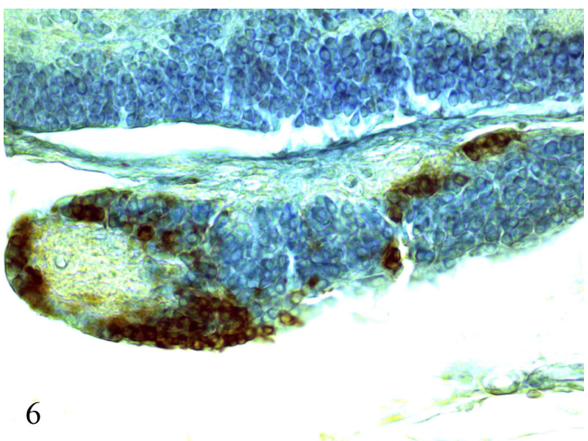

Fig. 6: A higher magnification of figure (5), showing SL-positive cells located mainly in the pars intermedia. In addition, some of SL-positive cells were located in the region between the two parts of pars distalis; rostral and proximal. X400.

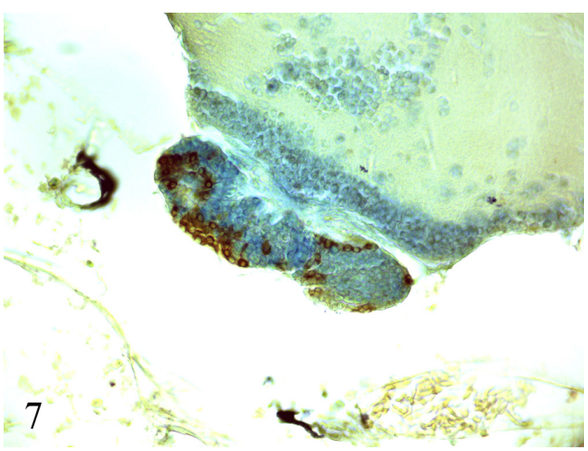

Fig. 7: Sagittal section of O. niloticus larvae 21 days post-hatching, immunostained with anti-chum salmon somatolactin (SL), showing more SL-positive cells were found in the pituitary gland compared to the previous stage. X200.

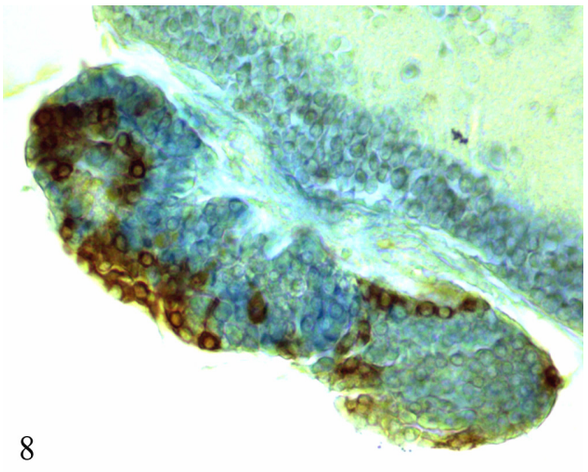

Fig. 8: A higher magnification of figure (7), showing the SL-positive cells increased in both size and number and became more obvious. X400.

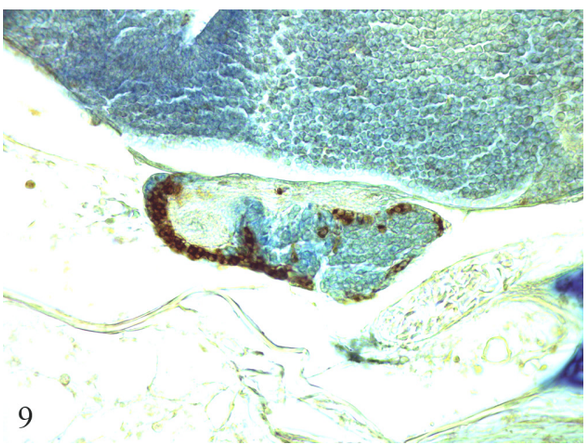

Fig. 9: Sagittal section of O. niloticus larvae 28 days post-hatching immunostained with anti-chum salmon somatolactin (SL). The size of pituitary gland was increased with advanced development, and it was similar to that of adults. X200.

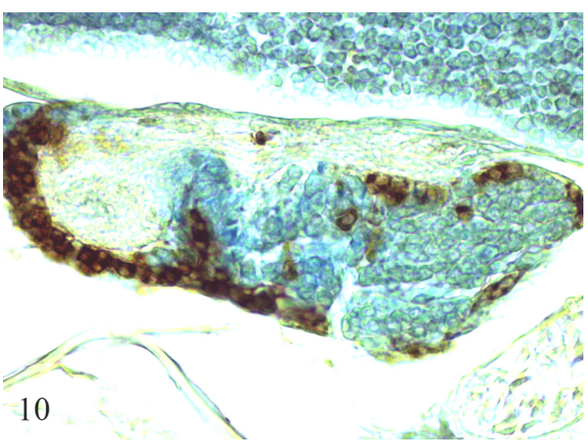

Fig. 10: A higher magnification of figure (9), showing that the increase of number and immunoreaction of SL cells with advanced development. SL-positive cells were located more dorsally in contact with branches of neurohypophyseal tissue that penetrated the pars distalis (PD) and pars intermedia (PI). X400.

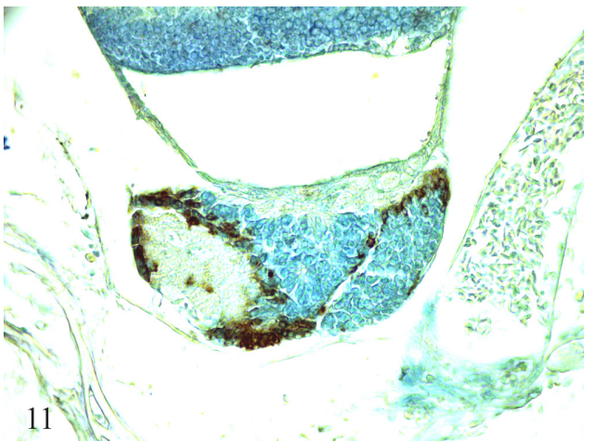

Fig. 11: Sagittal section of O. niloticus larvae 35 days post-hatching immunostained with anti-chum salmon somatolactin (SL). Note that; the size of the pituitary gland was increased. X200.

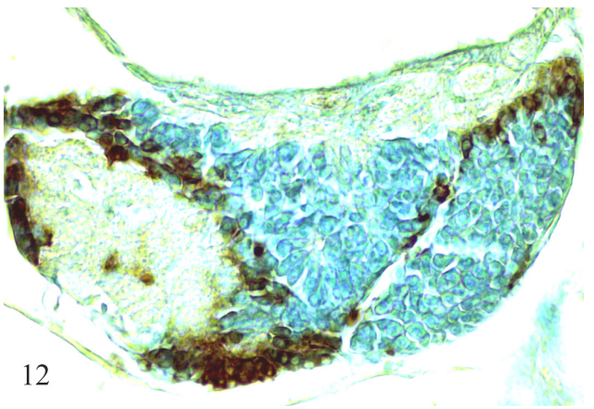

Fig. 12: A higher magnification of figure (11), showing SL-positive cells were markedly increased in the number and exhibited a moderate immunoreactivity. X400.

Table 1: Total length (mm), total weight (mg) and SL-immunoreactive cells; cell number and cell size (µm2) at different ages of O niloticus larvae

Days after hatching	Larval growth		SL-immunoreactive cells	
	TL (mean±SD mm)	TW (mean±SD mg)	Cell number	Cell size
1 day	08.44±0.025	011.15±0.08	025±2.5	40±3.55
7 day	09.86±0.028[a]	015.15±0.45[a]	025±2.5	45±2.95[a]
14 day	12.85±0.45[a]	029.10±1.35[a]	035±3.5[a]	33±1.85[a]
21 day	16.95±0.25[a]	115.25±7.25[a]	040±2.5[a]	35±2.50[a]
28 day	24.95±1.44[a]	270.75±9.15[a]	055±4.0[a]	45±2.65[a]
35 day	35.65±2.05[a]	545.85±25.75[a]	065±3.0[a]	40±2.55[a]

a: Significant differences when compared to the previous age (P<0.005)

DISCUSSION

It is well established that fish pituitary is endocrine gland which produce and secrets a various type of hormones. Importantly, these hormones play essential roles not only in reproduction but also in embryonic development and larval growth[9,29-33]. Previous investigations of hypophyseal cells of fish species from different habitats suggested that the start of their activity exhibit prominent different patterns among fish species. Consistently, our present findings confirmed the early appearance of SL-producing cells from the first day post-hatching and the gradually increase in their immunoreactivity during development of larvae as reflected with the increased number, size and immunoreaction suggest a decisive role of this hormone in different physiological processes during the growth of O. niloticus.

Pituitary cells of the O. niloticus larvae, juveniles and adults were subjected to immunohistochemical technique using specific antibody against SL that specifically reacts with SL cells in fish, as well confirmed in the previous studies[34-41]. In the present study, we revealed SL-producing cells within the posterior part of adenohypophysis from the first day post-hatching. In older larvae of O. niloticus, the activity of SL-positive cells was apparently increased from day 28 onwards and reached the highest levels on day 35 post-hatching, as reflected with the increased number, size and immunoreactivity. Our findings are in agreement with previous study in Sparus aurata[25] in which the authors used Northern blot analysis to detect only SL mRNA. The authors confirmed the appearance of SL specific mRNA from the first day post-hatching. However, SL immunoreactivity appeared at 1.5 post-hatching in Phreatichthys andruzzii[29]. In C. dimerus, SL-positive cells appeared on the second day after hatching[28]. In Sparus aurata[26] they appeared on newly hatched specimens as in Plecoglossus altivelis[24]. Taken together, these findings demonstrated SL from the first day post-hatching and a strong relation between the increased activity of these cells with the developmental rate and in the duration of the embryonic period in several species.

It is well accepted that SL is a hypophyseal hormone belonging to the superfamily of growth hormone/prolactin and has a high affinity to the SL-receptor. SL is the last pituitary hormone to be discovered among the GH/PRL family of fish[10]. The previous studies showed that SL synthesis and secretion is strongly connected with broad physiological processes in teleost such as calcium regulation, acid–base balance, phosphate and fat metabolism[8], reproductive physiology, stress response[21,22,23,42], yolk reabsorption and reserve tissue mobilization[13,26]. Also, the SL immunoreactivity is changed during fishes background chronically adaptation, and controls fish color appearance[14,15,16,17,18,19,43]. Moreover, emerging evidence suggested that SL might also play a role in embryo and larval development[29,31,44,45]. Therefore, the early appearance of SL-positive cells from the first day post-hatching in the present experiments support this notion and hints to a similar role in O. niloticus.

Until now the information supporting the physiological significance of the SL in larvae remains scarce, although, as is likely for other pituitary hormones, it may participate in development of fish larvae especially in the early days

after hatching through an autocrine or paracrine way[46]. In newly hatched S. aurata larvae, the involvement of SL in the mobilization of tissue reserves could be supported by the rapid reabsorption of the yolk sac. Several previous studies revealed that the plasma SL concentration increased during acute stress[10,12]. Therefore, the presence of SL-positive cells in newly hatched larvae of O. niloticus demonstrated in the current study may suggests that they are involved in larvae adaptation to physiological stress associated with hatching and their new environment. Here, the adenohypophysial SL-producing cells developed before the appearance of neurohypophysis in O. niloticus and this is in contrast to the previous observation in O nerka in which the adenohypophysis exhibits a distinctive zonation[39]. It is likely that the appearance of the first cell types in the pituitary during embryo development arise independently of exogenous cues. In contrast, the cell type differentiations at larval later stages are more dependent on paracrine or endocrine factors[31].

CONCLUSION

The present work indicated the early appearance of SL-producing cells in the pituitary gland of O. niloticus from the first day post-hatching. Moreover, there is a gradual increase in the activity of SL- positive cells as reflected with the increase in their number, size and immunoreactivity during larval development. The obtained results indicate the possible physiological role of SL in development during larval growth of O. niloticus. Also, the appearance of SL from the first day post-hatching suggests its role in the survival of larvae but further studies is required in order to focus on the effect of environmental factors that affect the hormone synthesis and secretion.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التفاعل المناعى للخلايا المفرزة لهرمون السوماتولاكتين أثناء التطور اليرقى لسمكة البلطى النيلى

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المقدمة: يشارك هرمون السوماتولاكتين في العمليات الفسيولوجية المختلفة في الأسماك العظمية.
الهدف من الدراسة: تم تصميم هذا العمل لدراسة تطور النشاط المناعي لخلايا إفراز السوماتولاكتين أثناء تطور البلطي النيلي.
المادة والطرق: فى هذا البحث تمت دراسة تتبع ظهور وتوزيع التفاعل المناعى لهرمون السوماتولاكتين أثناء التطور اليرقى لسمكة البلطى النيلى باستخدام طريقة الهستوكيميائية المناعية، وذلك بهدف التعرف على المساهمة المحتملة لهذا الهرمون فى تنشيط التكامل المبكر للغدد الصماء.
النتائج: أوضحت النتائج السيتوكيميائية المناعية وجود عدد قليل من الخلايا المفرزة لهرمون السوماتولاكتين مبكرا عند عمر يوم بعد الفقس. إزداد عدد الخلايا المفرزة لهرمون السوماتولاكتين وكذلك كثافة التفاعل المناعى أثناء التطور اليرقى عند المراحل التى تمت دراستها (من بعد الفقس مباشرة وحتى عمر ٣٥ يوم). مع تطور اليرقات، إزداد عدد الخلايا المفرزة لهرمون السوماتولاكتين وكذلك كثافة التفاعل المناعى بوضوح وبشكل تدريجي خاصة بعد بدء التغذية الخارجية في المدة من ٢١- ٢٨ يومًا بعد الفقس. ونتيجة لذلك، حدثت زيادة ملحوظة في كل من طول ووزن اليرقات خلال الفترة التجريبية لتحضين اليرقات.
الخلاصة: يشير الظهور المبكر والتوزيع التشريحي لنشاط هرمون السوماتولاكتين إلى الأهمية الوظيفية لهذا الهرمون في التنظيم الأزموزى والتكيف مع البيئة وتناول الطعام والنمو أثناء تطور يرقات البلطى النيلى خاصة في الفترة التي تلي إمتصاص كيس المح.