Immunohistochemical evaluation of the pituitary gland of carp as a source of hormones needed to stimulate spawning in marine fish

Original Article

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ABSTRACT

Introduction: Several hormonal types, including pituitary hormones of common carp, were used for induced spawning in marine fish.

Aim of the work: The aim of this study is to evaluate the hormonal content of the pituitary gland for different types of carp fish, used to stimulate spawning in marine fish, by using immunohistochemical technique.

Material and methods: In this study, the pituitary glands of mature males and females in three types of carp; common carp (Cyprinus carpio), grass carp (Ctenopharyngodon idella) and silver carp (Hypophthalmichthys molitrix), were selected for evaluation as a source of hormones. In this regard, these pituitaries were immunohistochemically stained using corticotropin-releasing factor (CRF), adrenocorticotropin hormone (ACTH), gonadotropin hormones IIβ (GTH IIβ) and somatolactin hormone (SL).

Results: The results showed that the pituitary gland of silver carp had higher immunoreactivity; the number, size, and immunostaining of GTH IIβ and SL hormones than those of common carp and grass carp. The integrated optical density (IOD) of immunoreactivity of the two hormones in silver carp was significantly higher than those of common carp and grass carp. However, the immunoreactivity of stress-response hormones; CRF and ACTH in silver carp was significantly lower than those of common carp and grass carp; since lower number and size of ACTH-immunoreactive cells were obtained. Furthermore, significantly lower IOD of both CRF and ACTH were obtained in silver carp.

Conclusion: It could be concluded that the use of the pituitary gland of silver carp was effective to stimulate the spawning of mullets and less expensive.

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Key Words: Economic efficiency, hormone, liza ramada, spawning.

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INTRODUCTION

Reproduction in fish, as in all vertebrates, is ultimately controlled by the brain, via hormones from the pituitary gland (the gonadotropins (GTH)) which control gonadal development in both sexes. In fish, two gonadotropins have been identified[1] which were originally referred to as GTH I and GTH II, but more recently have been found to be similar to tetrapod FSH and LH, respectively[2]. It is now considered that GTH I and II play roles in fish similar to those played by FSH and LH, respectively, in mammals; this is reflected in the seasonal profiles of plasma FSH and LH concentrations in the female rainbow trout[3] which mimic those in the oestrus cycle in mammals. GTHI and II are therefore referred to as FSH and LH, respectively.

The physiological role of ACTH is the stimulation of synthesis and release of cortisol from the inter-renal tissue[4]. Also, the ACTH secreted cells is activated by several factors such as stress, temperature, pollution, etc.[5-8]. CRF, a 41-amino-acid peptide produced by neurons in the brain, is pivotal in the coordination of the stress response, mainly by regulation of the pituitary–interrenal axis activity. CRF is considered to be the dominant stimulatory factor and plays a key role in ACTH release during the stress response[9-11]. In addition, CRF has been reported to regulate the reproductive system[12], body temperature, food intake, growth, and thyroid functions[13,14]. The role of CRF in the teleost endocrine stress response has been reported to be species specific[10]. Furthermore, hormonally induced ovulation in L. ramada was accompanied with elevation of plasma cortisol and depletion of CRF and ACTH immunoreactivity within the brain and the pituitary gland, supporting the possible role of these hormones during stress and reproduction in L. ramada[11].

The identification and the distribution of the different cell types in the pituitary gland of teleosts have been studied using immunocytochemical techniques using antisera against mammalian and piscine hormones[11,15-20]. Seven different classes of hormones, grouped into three main families have been described: (i) growth hormone

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(GH)/prolactin (PRL) family, containing PRL, GH and somatolactin (SL); (ii) glycoprotein hormones including gonadotrophins (GTHs) and thyrotropin (TSH); and (iii) proopiomelanocortin-derived hormones such as adrenocorticotropic (ACTH) and melanotropic hormone (MSH)[16, 18, 20-22].

Several studies have been carried out on spawning of marine fish using several hormones, including human chorionic gonadotropin, pituitary hormones of common carp and gonadotropin releasing hormones[23-26]. The pituitary gland is commonly used from common carp without other types of carp. Recently, the use of the pituitary gland of silver carp has given higher ovulation rate than those of common carp and human chorionic gonadotropin[26]. In light of the prices increasing of synthetically hormones, it is necessary to evaluate the hormonal content of the pituitary gland for different types of carp fish used to stimulate spawning of marine fish. To determine the usefulness of using pituitary hormones for other types carp, it is necessary to determine the types and quantities of hormones in the pituitary gland of the different carp types; common carp (Cyprinus carpio), grass carp (Ctenopharyngodon idella) and silver carp (Hypophthalmichthys molitrix).

Therefore, the objective of the present study was to evaluate the hormonal content of the pituitary gland for different types of carp fish used to stimulate spawning of marine fish. Corticotropin-releasing factor (CRF), adrenocorticotropin hormone (ACTH), gonadotropin hormones IIβ (GTH IIβ) and somatolactin hormone (SL) were investigated in the pituitary gland of the different carp types; common carp (C. carpio), grass carp (C. idella) and silver carp (H. molitrix). The immunohistochemical technique was used in these investigations.

MATERIAL AND METHODS

Study Site:

The present study was carried out at both El-Serw Fish Research Farm and El-Matareyya Research Station.

Tissue Preparation:

Pituitary glands were collected from sexually mature 2-year-old male and female common carp, grass carp and silver carp. Fishes were anesthetized in a solution (40 mg/l) of clove oil (Sigma) before handling[27] and then perfused via the ascending aorta with 20 ml of normal saline, followed by 50 ml of Bouin's fluid at 4°C. The pituitary gland attached to the brain was immediately removed and postfixed in Bouin's fluid for 24 h at 4°C. The fixed brain and pituitaries were thereafter dehydrated through graded ethanol solution, cleared and embedded in paraplast (M.P.: 56–58 °C). Consecutive median sagittal sections (5 µm thick) of the brain and the pituitary gland prepared on microtome were mounted onto gelatin coated slides.

Immunohistochemical procedure:

Antibodies: Rabbit polyclonal antibody against ovine CRF was obtained from Dr. Nigel Brooks, MRC Reproductive Biology Unit, Centre for Reproductive Biology, Edinburgh, Scotland. Rabbit antibody directed against human ACTH was obtained from National Institute of Health. Antisera to chum salmon (Oncorhynchus keta) GTH IIβ subunit (Lot No.8506) and chum salmon somatolactin (Lot No. 8906) were obtained from Dr. H. Kawauchi (School of Fisheries Science, Kitasato University, Iwate, Japan).

Immunocytochemical reactions: Immunohistochemical staining of the pituitary gland and brain serial sections was performed with a vectastain avidin-biotin peroxidase complex (vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA), as described previously[28]. Unless otherwise stated, all incubations were done at room temperature and PBS was used for washing (three times for 20 min) after each step. The sections were incubated with PBS, 0.3 % H_2O_2 and 10 % methanol for 45 min to block endogenous peroxidase. To prevent nonspecific binding, the sections were incubated for 60 min in PBS containing 0.3 % Triton X-100, 1 % BSA, 4 % goat serum (GS) and 4 % horse serum (block solution). The sections were then incubated overnight at 4°C with the following antibodies: a rabbit polyclonal antibody against human ACTH (1:500), rabbit polyclonal antibody against ovine CRF (1:1000), and antisera to chum salmon (Oncorhynchus keta) GTH IIβ subunit and somatolactin (1:5000). Thereafter, the sections were incubated for 1 hr with a goat anti-rabbit biotinylated secondary antibody (Vector Laboratories). Sections were then incubated with avidin-biotin-conjugated peroxidase for 45 min. Finally, the sections were washed and stained with 3`, 3`- diaminobenzidine tetrahydrochloride (DAB) (Sigma) containing 0.01 % H_2O_2 in 0.05 M Tris-buffered saline (pH 7.6) for 3-5 min. After the enzyme reaction, the sections were washed in tap water, counterstained with thionin, then dehydrated in alcohol, cleared in xylene and mounted in DPX (Merck, Darmstadt, Germany).

In order to confirm the specificity of the immunoreactive procedures, adjacent sections were stained according to the above described protocol but incubation in the primary antisera was omitted. In addition, normal bovine serum was used instead of primary antiserum. No positive structures or cells were found in these sections.

Semi-Quantification of Immunostaining

Semi-quantification of each hormone-expressing cells in the pituitary gland was calculated from five sections of each individual animal (10 fish for each treatment) cut at 5 mm. Briefly, we obtained five sections starting from the middle of the pituitary gland of each fish for hormone-ir cell counting using the microscope (40× objective). Five squares (0.03 mm2 each) per section were analyzed using a Zeiss microscope. The hormone-ir cell number for each animal was expressed as the mean±SD. The hormone-ir cell size was measured using computerized analysis (the Image-Pro Analysis package, Media Cybernetics) of digital images viewed via microscope (Axioskop; Zeiss,

Oberkochen, Germany). Cell immunoreactivity was semi-quantified by Java Image processing and analysis software (Image J; open-source image software downloaded from http://rsb.info.nih.gov/ij/). The area and density of pixels within the threshold values representing immunoreactivity were measured, and the integrated optical density (IOD) (the product of the area and mean of gray value) was calculated. The IOD of the three carp species were compared, and statistically analyzed.

Statistical analysis:

Differences between treatments were tested by one-way ANOVA using the treatment as factor of variance. Statistical significance was accepted at $P<0.05$.

RESULTS

The pituitary gland of the three carp types; common carp (Cyprinus carpio), grass carp (Ctenopharyngodon idella) and silver carp (Hypophthalmichthys molitrix) consists of the neurohypophysis, and the adenohypophysis, which showed the three major subdivisions typical of teleost; an anterior rostral pars distalis (RPD), a medium proximal pars distalis (PPD) and posterior pars intermedia (PI) (Figs. 1-3).

SL-Immunoreactive (-ir) Cells

PAS cells of pars intermedia (PI) showed strong immunoreactivity to anti-chum salmon somatolactin (SL) (Figs. 1-6). These cells appeared with high activity in both grass carp and silver carp as reflected by their hypertrophy and hyperplasia (Figs. 2, 3, 5 and 6). The immunoreactivity of SL cells in common carp was low as reflected by weak immunostaining and size decrease (Figs. 1 and 4). The IOD of SL immunoreactivity was higher by 22.2% for grass carp and by 40.8% for silver carp compared to those of common carp *(P<0.05)* (Table 1).

GTH- Immunoreactive Cells

In carp gonadotrops (GTH cells) occupied the major part of the PPD, and also recognized in the periphery of the PI. GTH IIβ (LH) secreting cells exhibited variable sizes and shapes with secretory vacuoles (Figs. 7-9). Antisera to chum salmon GTH IIβ and ovine Luteinizing hormone (o-LH) bound strongly and specifically to the GTH cells. In silver carp GTH cells showed strong immunoreactivity and increase in both size and number (Fig. 9). The IOD of GTH IIβ immunoreactivity was significantly increased by 7.5% for silver carp and by 1.9% for grass carp compared to that of common carp *(P<0.05)* (Table 1). In grass carp; GTH cells showed increase in the number and size in comparison with common carp (Figs. 8 and 9). Furthermore, in common carp GTH cells showed decrease in the immunoreactivity (Fig. 7).

ACTH- Immunoreactive Cells

The ACTH cells appear as cords bordering the PRL cells or as islets between PRL cells and the neurohypophysis (NH) (Figs. 10-12). Antiserum to human ACTH bound strongly to the ACTH cells (Figs. 10-12). The immunoreactivity of the ACTH cells in silver carp was lower than that of grass carp and common carp as shown by the decrease in their number, size, and immunostaining (Figs. 10-12). The IOD of the ACTH immunoreactivity was lower by 22.7% for silver carp and by 14.2% for grass carp compared to that of common carp *(P<0.05)* (Table 1). In common carp and grass carp, ACTH cells showed hyperplasia and strong immunoreactivity (Figs. 11 and 12).

MSH- Immunoreactive Cells

The second type of cells in the pars intermedia exhibited small variable sizes and shapes. These cells were cross reacted and immunostained with anti-human ACTH (Figs. 13-15). These cells had small size and lower number in silver carp (Fig. 15). However, in grass carp and common carp MSH cells showed strong immunoreactivity, increase in number and size (Figs. 13 and 14). The IOD of ACTH in MSH cells immunoreactivity was significantly decreased by 19.2% for silver carp and by 7.1% for grass carp compared to that of common carp *(P<0.05)* (Table 1).

CRF- immunoreactivity

Immunohistochemical staining of pituitary section with ovine CRF antiserum showed that CRF-immunoreactive fibers were found in close with the ACTH-producing cells in the rostral pars distalis (Figs. 16-18) and in close with the MSH cells in the pars intermedia (Figs. 19-21). The immunoreactivity of CRF in silver carp was lower than that of grass carp and common carp (Figs. 16-21). The IOD of CRF immunoreactivity was significantly decreased by 17.6% for silver carp and by 6.7% for grass carp compared to that of common carp *(P<0.05)* (Table 1).

As for the economic evaluation, the results showed that the pituitary gland of silver carp as a source of hormones is less expensive compared to the use of other carp hormones.

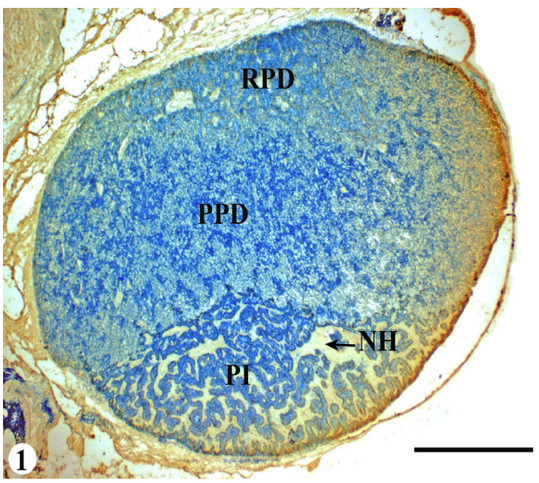

Fig. 1: Sagittal section of the pituitary gland of common carp immunostained with anti-chum salmon SL and counterstained with thionin showing the rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI) which comprise the adenohypophysis, and neurohypophysis (NH). Scale bar = 250 µm.

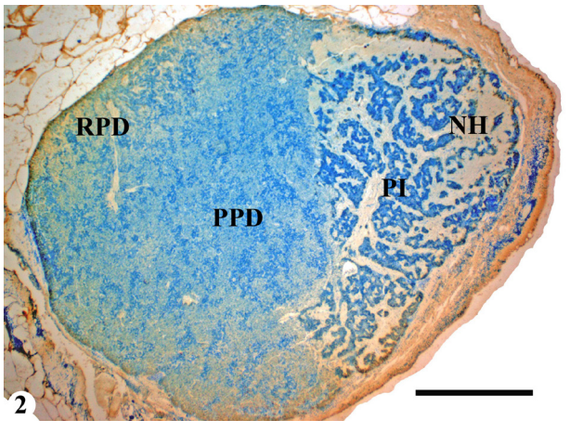

Fig. 2: Sagittal section of the pituitary gland of grass carp immunostained with anti-chum salmon somatolactin and counterstained with thionin showing the rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI) which comprise the adenohypophysis, and neurohypophysis (NH). Scale bar = 250 µm.

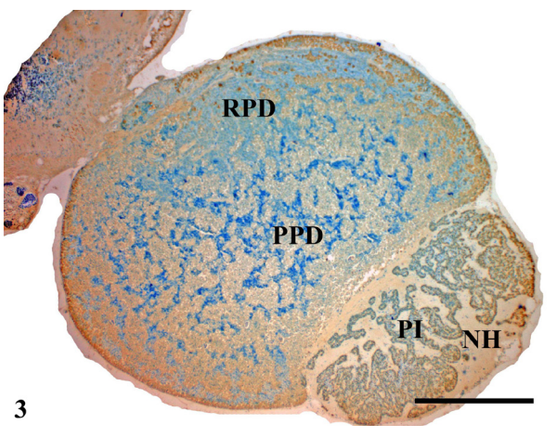

Fig. 3: Sagittal section of the pituitary gland of silver carp immunostained with anti-chum salmon somatolactin and counterstained with thionin showing the rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI) which comprise the adenohypophysis, and neurohypophysis (NH). Scale bar = 250 µm.

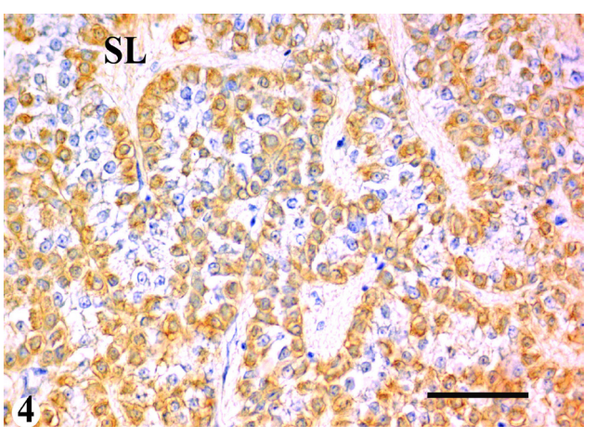

Fig. 4: Sagittal section of the pituitary gland of common carp immunostained with anti-chum salmon SL and counterstained with thionin displaying SL-immunoreactive (ir) cells with irregular shapes and have spherical nuclei. SL cells are few in number, small in size and with weak immunoreactivity. Scale bar = 50 µm.

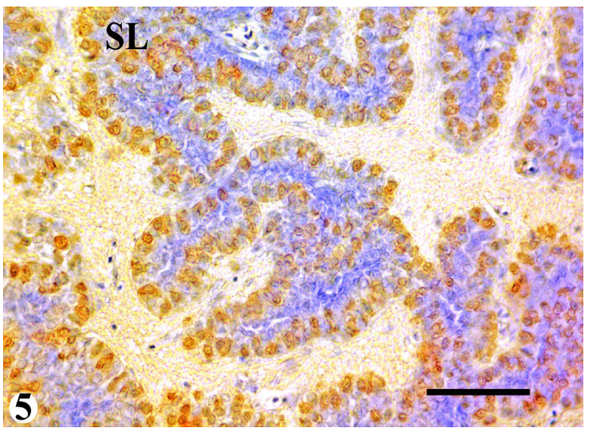

Fig. 5: Sagittal section of the pituitary gland of grass carp immunostained with anti-chum salmon SL and counterstained with thionin displaying SL-ir cells moderate in number and size and with moderate immunoreactivity. Scale bar = 50 µm.

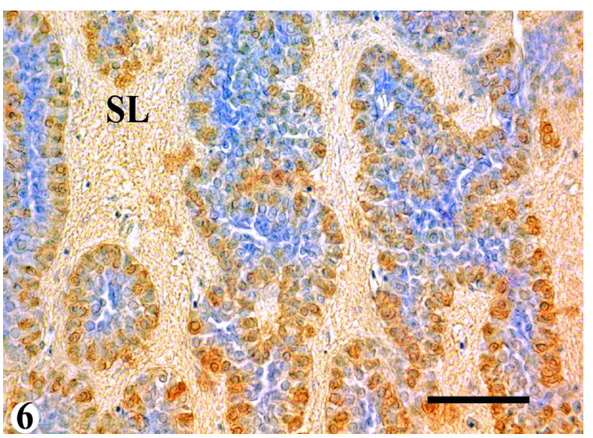

Fig. 6: Sagittal section of the pituitary gland of silver carp immunostained with anti-chum salmon SL and counterstained with thionin displaying SL-ir cells with increased number, large in size and with strong immunoreactivity. Scale bar = 50 µm.

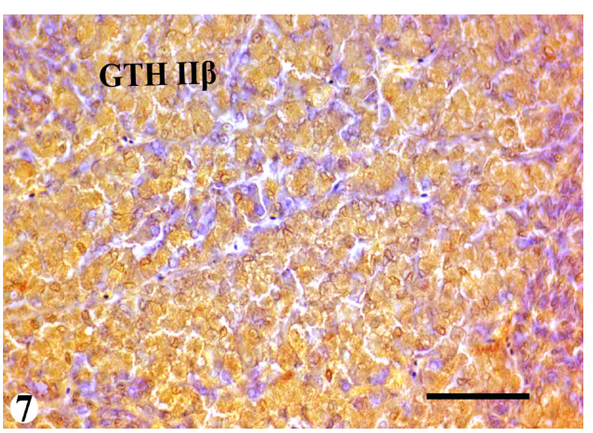

Fig. 7: Sagittal section of the pituitary gland of common carp immunostained with anti-chum salmon GTH IIβ and counterstained with thionin displaying GTH IIβ-ir cells moderate in number and size and with moderate immunoreactivity. Scale bar = 50 µm.

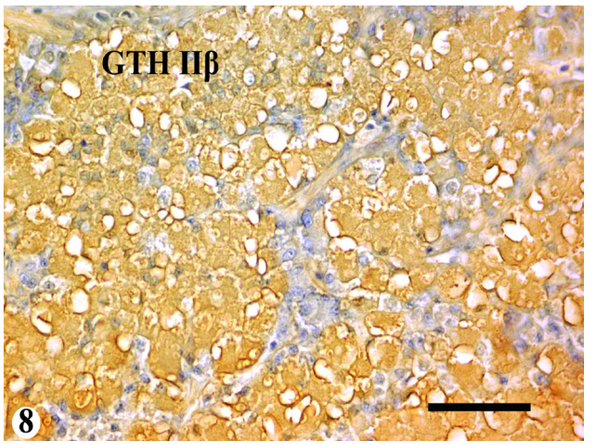

Fig. 8: Sagittal section of the pituitary gland of grass carp immunostained with anti-chum salmon GTH IIβ and counterstained with thionin displaying GTH IIβ-ir cells moderate in number and size and with moderate immunoreactivity. Scale bar = 50 µm.

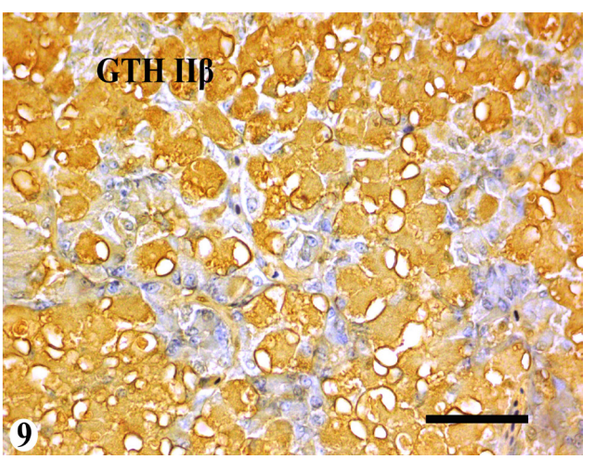

Fig. 9: Sagittal section of the pituitary gland of silver carp immunostained with anti-chum salmon GTH IIβ and counterstained with thionin displaying GTH IIβ-ir cells with increased number, large in size and with strong immunoreactivity. Scale bar = 50 µm.

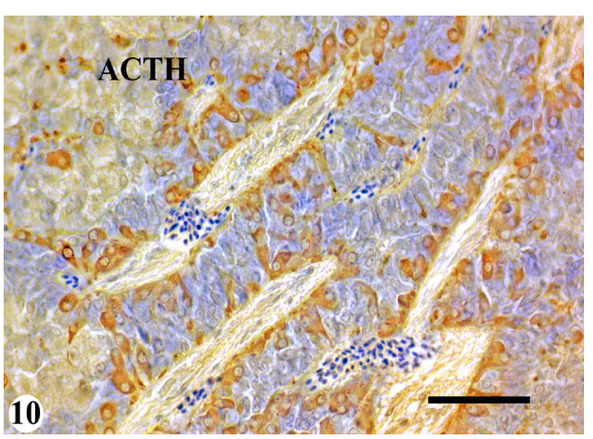

Fig. 10: Sagittal section of the pituitary gland of common carp immunostained with anti-human ACTH and counterstained with thionin displaying ACTH-ir cells increased in number and large in size and with strong immunoreactivity. Scale bar = 50 µm.

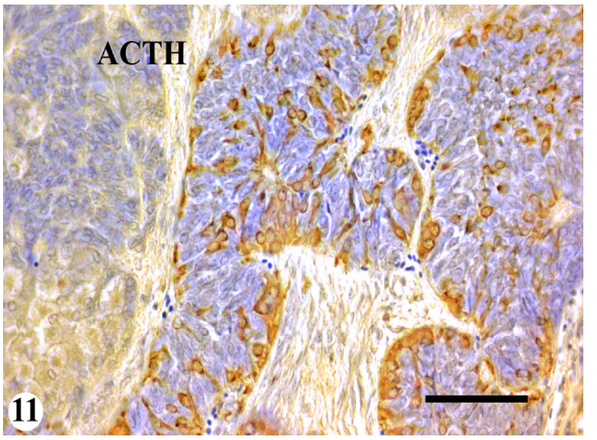

Fig. 11: Sagittal section of the pituitary gland of grass carp immunostained with anti-human ACTH and counterstained with thionin displaying ACTH-ir cells moderate in number and size and with moderate immunoreactivity. Scale bar = 50 µm.

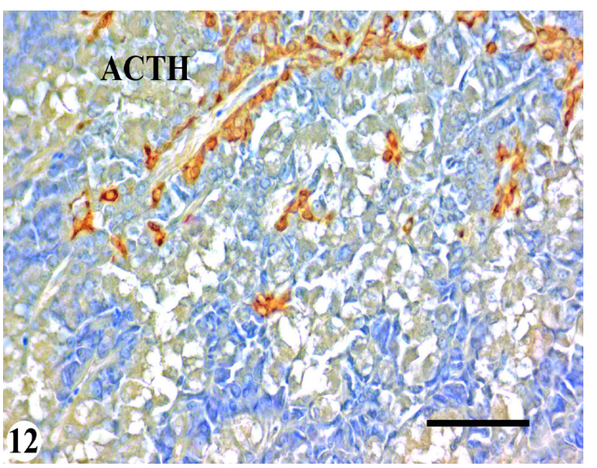

Fig. 12: Sagittal section of the pituitary gland of silver carp immunostained with anti-human ACTH and counterstained with thionin displaying ACTH-ir cells with few number, small in size and with moderate immunoreactivity. Scale bar = 50 µm.

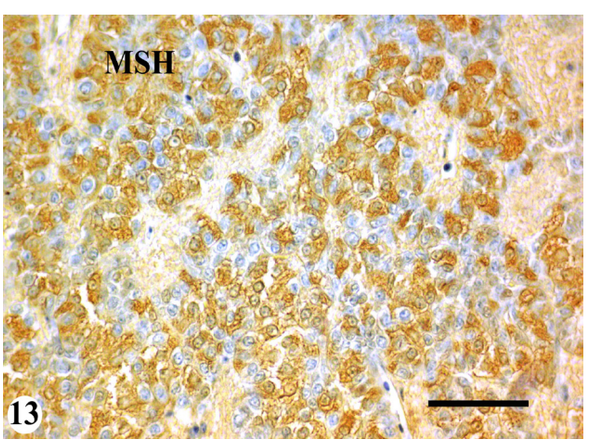

Fig. 13: Sagittal section of the pituitary gland of common carp immunostained with anti-human ACTH and counterstained with thionin displaying MSH-ir cells increased in number and large in size and with strong immunoreactivity. Scale bar = 50 µm.

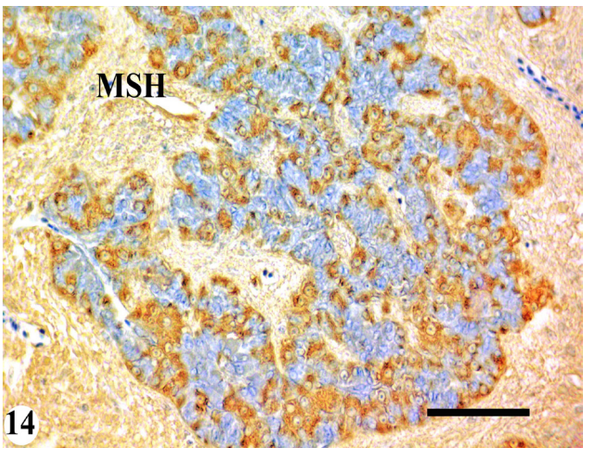

Fig. 14: Sagittal section of the pituitary gland of grass carp immunostained with anti-human ACTH and counterstained with thionin displaying MSH-ir cells moderate in number and size and with moderate immunoreactivity. Scale bar = 50 µm.

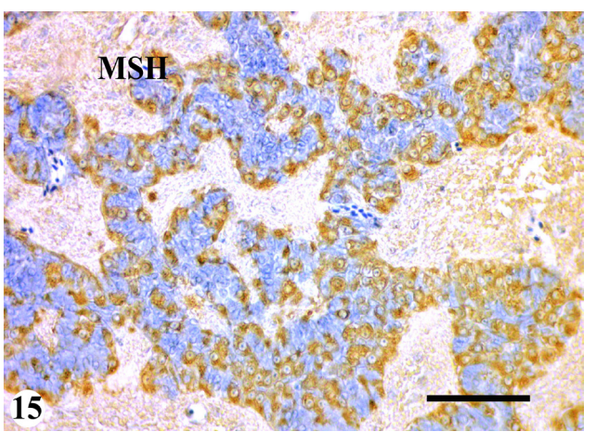

Fig. 15: Sagittal section of the pituitary gland of silver carp immunostained with anti-human ACTH and counterstained with thionin displaying MSH-ir cells with few number, small in size and with moderate immunoreactivity. Scale bar = 50 µm.

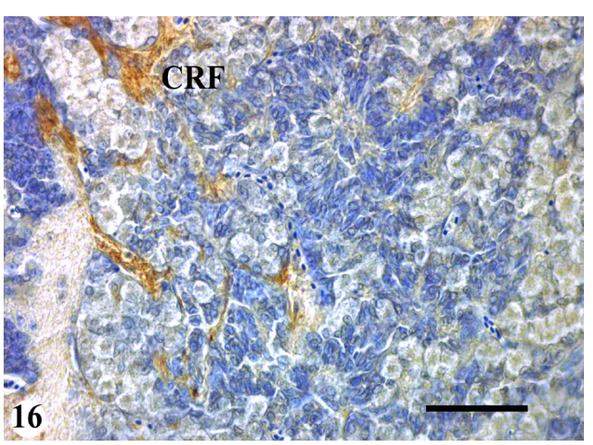

Fig. 16: Sagittal section of the pituitary gland of common carp immunostained with anti-ovine CRF and counterstained with thionin displaying many of CRF-ir fibers, in close with the ACTH-producing cells in the rostral pars distalis, with moderate immunoreactivity. Scale bar = 50 µm.

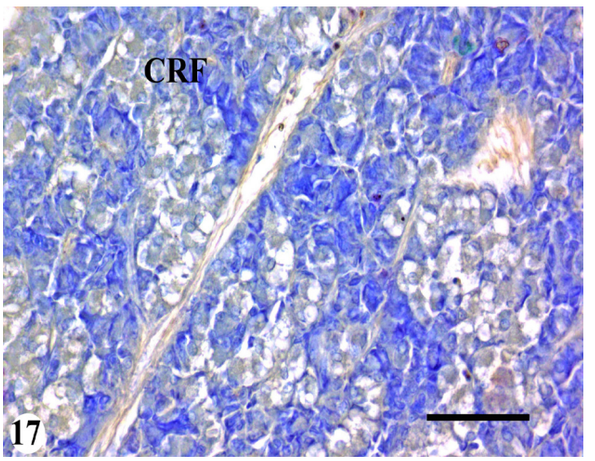

Fig. 17: Sagittal section of the pituitary gland of grass carp immunostained with anti-ovine CRF and counterstained with thionin displaying few of CRF-ir fibers, in close with the ACTH-producing cells in the rostral pars distalis, with moderate immunoreactivity. Scale bar = 50 µm.

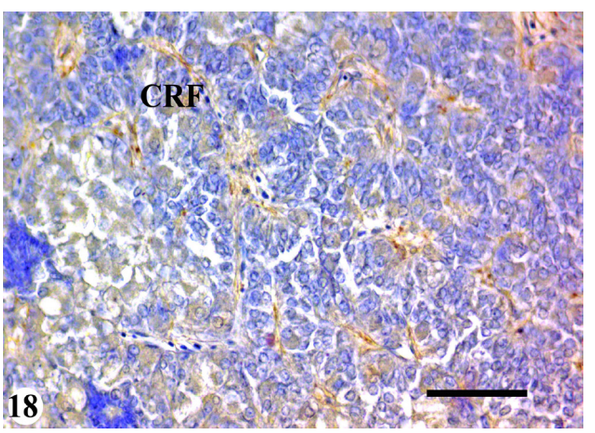

Fig. 18: Sagittal section of the pituitary gland of silver carp immunostained with anti-ovine CRF and counterstained with thionin displaying few of CRF-ir fibers, in close with the ACTH-producing cells in the rostral pars distalis, with moderate immunoreactivity. Scale bar = 50 µm.

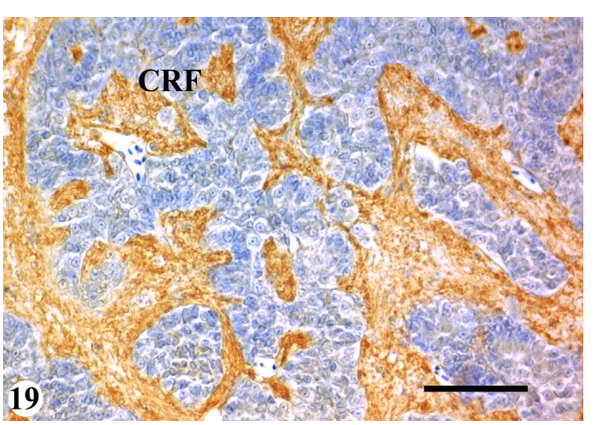

Fig. 19: Sagittal section of the pituitary gland of common carp immunostained with anti-ovine CRF and counterstained with thionin displaying many of CRF-ir fibers, in close with the MSH cells in the pars intermedia, with strong immunoreactivity. Scale bar = 50 µm.

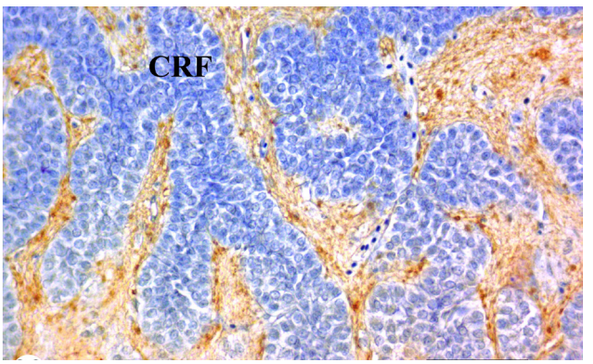

Fig. 20: Sagittal section of the pituitary gland of grass carp immunostained with anti-ovine CRF and counterstained with thionin displaying many of CRF-ir fibers, in close with the MSH cells in the pars intermedia, with moderate immunoreactivity. Scale bar = 50 µm.

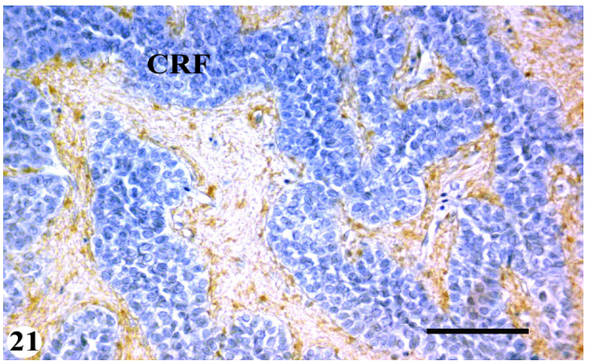

Fig. 21: Sagittal section of the pituitary gland of silver carp immunostained with anti-ovine CRF and counterstained with thionin displaying few of CRF-ir fibers, in close with the MSH cells in the pars intermedia, with weak immunoreactivity. Scale bar = 50 µm.

Table (1): Hormonal immunoreactivity of different carp types; cell number, cell size (µm2), integrated optical density (IOD) and IOD% (% from common carp).

Hormonal Immunoreactivity		Carp Type		
		Common carp	Grass carp	Silver carp
SL-ir cells	Cell number	65±0.95	75±1.28[a]	85±2.25[a,b]
	Cell size	60±1.36	65±1.45[a]	75±1.15[a,b]
	IOD	49±2.85	59.9±3.93[a]	69±3.19[a,b]
	IOD%	100	122.2±1.66[a]	140.8±1.25[a,b]
GTH IIβ-ir cells	Cell number	130±10.25	147±11.82[a]	150±13.5[a]
	Cell size	70±1.76	75±1.65[a]	85±1.93[a,b]
	IOD	73.5±4.9	74.9±3.82	79±3.15[a,b]
	IOD%	100	101.9±1.45	107.5±1.54[a,b]
ACTH-ir cells	Cell number	155±15.5	145±12.4[a]	100±10.75[a,b]
	Cell size	45±2.35	40±2.93[a]	35±1.83[a,b]
	IOD	76.6±4.52	65.7±3.82[a]	59.2±2.55[a,b]
	IOD%	100	85.8±2.35[a]	77.3±1.85[a,b]
MSH-ir cells	Cell number	140±11.5	125±13.6[a]	110±8.85[a,b]
	Cell size	70±1.77	65±2.55[a]	55±1.33[a,b]
	IOD	66.5±3.51	61.8±2.95[a]	53.7±2.36[a,b]
	IOD%	100	92.9±1.84[a]	80.8±2.35[a,b]
CRF-ir	IOD	73.3±3.13	68.4±2.62[a]	60.4±1.91[a,b]
	IOD%	100	93.3±2.92[a]	82.4±1.53[a,b]

a: Significant differences when compared to common carp *(P<0.05)*.
b: Significant differences when compared to common carp and grass carp *(P<0.05)*.

DISCUSSION

The pituitary gland of the three carp types; common carp (Cyprinus carpio), grass carp (Ctenopharyngodon idella) and silver carp (Hypophthalmichthys molitrix) consists of the neurohypophysis, and the adenohypophysis, which showed the three major subdivisions typical of teleost; an anterior rostral pars distalis (RPD), a medium proximal pars distalis (PPD) and posterior pars intermedia The immunohistochemical results showed that the pituitary gland of silver carp had higher immunoreactivity; the number, size, and immunostaining of GTH IIβ and SL hormones than those of common carp and grass carp. The integrated optical density (IOD) of immunoreactivity of the two hormones in silver carp was significantly higher than those of common carp and grass carp. However, the immunoreactivity of stress-response hormones; CRF and ACTH in silver carp was significantly lower than those of common carp and grass carp; since lower number and size of ACTH-immunoreactive cells were obtained. Furthermore, significantly lower IOD of both CRF and ACTH were obtained in silver carp.

Induced spawning of marine fish was done by using several hormones, including human chorionic gonadotropin, pituitary hormones of common carp and gonadotropin releasing hormones[23-26]. The pituitary gland is commonly used from common carp without other types of carp. In this respect, the use of the pituitary gland of silver carp has given higher ovulation rate than those of common carp and human chorionic gonadotropin[26]. In carp gonadotrops (GTH cells) occupied the major part of the PPD, and also recognized in the periphery of the PI. The immunohistochemical results showed that the pituitary gland of silver carp had higher immunoreactivity of GTH IIβ hormone than those of common carp and grass carp. The gonadotropic hormones are released from the pituitary in fish and control the annual cycle of gonadal growth, ovulation in females, sperm release in males, and production of sex steroids in both sexes[29,30]. Therefore, high content of gonadotropin in donor pituitary can have a significant positive effect on the induction of spawning in fish. This may explain why the pituitary gland of the silver carp is superior to those of both common carp and grass carp during induced spawning of L. ramada[26].

A definite physiological function has not yet been attributed to SL in fish. SL is involved in different physiological processes, such as the regulation of some aspects of reproduction[31-35] and in response to stress[36,37]. Other studies reported on the relation between SL and acid-based balance[38], calcium regulation[39], phosphate and fat metabolism[40], background color adaptation[41-43], and development and regulation of chromatophores during morphological body color adaptation to different backgrounds[44]. Also, the SL cells in the PI were activated during the reproductive phase in two species of the genus Oncorhynchus[45]. In addition, seasonal variations in the number, size, and intensity of SL-immunoreactive cells concomitant with the development of the gonads and spawning in O. niloticus and Mugil cephalus (Mugiliformes; Mugilidae)[28,33]. Furthermore, the response of SL- expressing cells in the PI in parallel with changes in hydro mineral balance induced by stress was supporting the possible role of SL in the adaptive response of L. ramada to stress[46]. The immunohistochemical results showed that the pituitary gland of silver carp had higher immunoreactivity of SL hormone than those of common carp and grass carp. This may be one of the reasons for the high success of the pituitary gland of silver carp in induced spawning of L. ramada[26].

The present immunohistochemical results showed that the ACTH cells appear in the RPD as cords bordering the PRL cells or as islets between PRL cells and the neurohypophysis. Antiserum to human ACTH bound strongly to the ACTH cells In addition, the antiserum to human ACTH also showed a cross-reaction with presumptive MSH cells in the pars intermedia of carp. These findings are in good agreement with previous studies on the specificity of this antiserum[47-49]. This positive reaction of ACTH antiserum to the MSH cells is possibly due to the presence of ACTH in the cells as a precursor of MSH[50]. The presence of CRF immunoreactivity in close with ACTH- and MSH- producing cells in the pituitary gland of carp is consistent with its function as a releasing factor in the pituitary and in European eels[51,52]. Interestingly, our results showed that CRF immunoreactivity beside ACTH- and MSH-producing cells in the pituitary gland suggesting that this peptide could act as a classic pituitary hormone like ACTH and/or may play a role in the regulation of ACTH secretion in fish. Similarly, CRF immunoreactivity was demonstrated in MSH-producing cells in the pituitary gland of the Lungfish[53]. In support of this findings, CRF stimulated ACTH and MSH release from the goldfish pituitary[54]. CRF produced by neurons in the brain plays an important role in the coordination of the stress response in teleost, mainly by regulation of the pituitary–interrenal axis activity[9,10]. In addition, CRF regulates cardiac output and ACTH secretion from circulating leukocytes of catfish during stress[55-57]. CRF has also been reported to regulate the reproductive system[12], body temperature, food intake, growth, and thyroid functions[13,14]. The ACTH secreted by the RPD controls interrenal synthesis and release of cortisol in fish[4,58] and is activated by several factors such as stress, temperature, pollution, etc.[5-8, 28,59]. Furthermore, the activation of ACTH-ir cells in the pituitary and CRF-ir in the brain and pituitary during seawater acclimation as well as gonad maturation and spawning induction by hormonal injection, in addition to the elevation of cortisol plasma during ovulation strongly confirm earlier findings[9,12, 28 ,60-62] which support the possible involvement of cortisol, ACTH, and CRF on stress, reproductive cycle, and spawning in L. ramada[11]. However, the MSH cells are responsible for the colour background adaptation[63]. Taking all of the above into consideration, caution should be taken while stimulating the spawning of marine fish using the pituitary gland to minimize stress as possible. Minimizing stress was obtained during induced spawning of L. ramada

using the pituitary gland of silver carp with low stress-response hormones (ACTH and CRF) immunoreactivity as observed in the present study[26].

In conclusion, the use of the pituitary gland of silver carp, with high content of GTH IIβ and SL hormones and low content of stress-response hormones (ACTH and CRF), was effective to stimulate the spawning of mullets and less expensive.

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CONFLICT OF INTEREST

The author declares there are no conflicts of interest.

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الملخص العربى

تقييم هستوكيميائى مناعى للغدة النخامية لأسماك المبروك كمصدر للهرمونات اللازمة لتحفيز التفريخ فى الأسماك البحرية

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المقدمة: يستخدم العديد من الهرمونات، من بينها هرمونات الغدة النخامية لأسماك المبروك العادى، لتحفيز التفريخ فى الأسماك البحرية.

الهدف من البحث: يهدف هذا البحث إلى تقييم المحتوى الهرمونى للغدة النخامية لأنواع مختلفة من أسماك المبروك، المستخدمة لتحفيز التفريخ فى الأسماك البحرية، باستخدام الطريقة الهستوكيميائية المناعية.

المادة والطرق: فى هذه الدراسة تم إختيار إناث وذكور ثلاثة أنواع من أسماك المبروك الناضجة جنسيا وهى: المبروك العادى، مبروك الحشائش، والمبروك الفضى وذلك للتقييم كمصدر للهرمونات. ولتحقيق هذا التقييم تم صبغ قطاعات الغدد النخامية بالطريقة الهستوكيميائية المناعية باستخدام الأجسام المضادة لهرمون السوماتولاكتين، هرمون الجونادوتروبين 2 بيتا، هرمون الأدرينوكورتيكوتروبين، والعامل المحرر لهرمون الكورتيكوتروبين.

النتائج: أوضحت النتائج أن الغدة النخامية لأسماك المبروك الفضى ذات نشاط مناعى مرتفع من حيث العدد والحجم والتفاعل المناعى لكل من هرمون الجونادوتروبين 2 بيتا وهرمون السوماتولاكتين مقارنة بتلك الموجودة فى أسماك المبروك العادى أو مبروك الحشائش. كانت الكثافة البصرية المتكاملة للتفاعل المناعى لكل من هرمون الجونادوتروبين 2 بيتا وهرمون السوماتولاكتين فى المبروك الفضى مرتفع عن تلك الموجودة فى المبروك العادى ومبروك الحشائش. بينما كان النشاط المناعى للهرمونات المستجيبة للإجهاد؛ العامل المحرر لهرمون الكورتيكوتروبين وهرمون الأدرينوكورتيكوتروبين فى المبروك الفضى أقل من تلك الموجودة فى المبروك العادى ومبروك الحشائش؛ حيث وجدت أعداد قليلة ذات أحجام صغيرة من الخلايا المفرزة لهرمون الأدرينوكورتيكوتروبين. علاوة على ذلك تم الحصول على أقل كثافة بصرية متكاملة لكل من العامل المحرر لهرمون الكورتيكوتروبين وهرمون الأدرينوكورتيكوتروبين لأسماك المبروك الفضى.

الخلاصة: مما سبق يمكن التوصية بأن إستخدام الغدة النخامية لأسماك المبروك الفضى أكثر فعالية وأقل تكلفة لتحفيز التفريخ فى أسماك العائلة البورية.