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Effect of different light intensities on prolactin and cortisol plasma concentration in farming African catfish (*Clarias gariepinus*) in RAS with low-water exchange

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Abstract

In the Bulgarian aquaculture sector, focusing research efforts on the technical specifications of the cultivation of African catfish (*Clarias gariepinus*) is important due to the need for more information. We aimed to assess the impact of light intensities on prolactin and cortisol levels as stress markers in African catfish. Fish were acclimated over an appropriate period to the short photoperiod (16D and 8L) and then distributed randomly to three groups, each separated by sex. Fish exposed to different light intensities (63, 51, and 40 lux, respectively) were followed for 45 days by lux meter. Blood samples were collected from the caudal vein using a 2 ml K2EDTA-coated syringe. A laser fluorescence reader assayed plasma prolactin and cortisol. The pituitary was removed from anesthetized fish and fixed for histological examinations. The results of our study suggest that increasing light intensity increases prolactin and cortisol levels in African catfish.

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Introduction

In the Bulgarian economy aquaculture sector is a share of the GDP of less than 1%, but it is extremely important to livelihoods in some regions of the country (Massa et al., 2021). The aquaculture objects in Bulgaria are mainly four species of fish – carp, trout, sturgeon, and catfish. In the period 2007-2012 were introduced two new species for marine and freshwater aquaculture – the barramundi (*Lates calcarifer*) and African catfish (*Clarias gariepinus*) (IRA-STRATEGMA, 2020). As a relatively new species, the latter has shown growth in production last ten years, although they are not well known in the local market. Therefore, focusing research efforts on the technical specifications of African catfish (*Clarias gariepinus*) is extremely important due to the lack of information.

In connection with the Bulgarian climate and environmental condition and contrast to the European catfish (*Silurus glanis*), cultivated over 100 farms, African catfish is produced only in two farms equipment with a recirculation aquaculture system (RAS). The success of the catfish industry has been determined by a few main factors, including water temperature, stocking density, feed, and light intensities. Studies have been carried out to evaluate the impact of rising temperatures (Kasihmuddin et al., 2021), different salinity levels (Zidan et al., 2022), feed (Mustafa, 2021), and light intensity (Prokešová et al., 2017) on behavioral responses, biochemical stress parameters and growth performance of catfishes. For example, catfish have different light preferences that may negatively affect growth (Santos et al., 2019) or can directly affect survival rates and cause adverse physiological response changes (Costas et al., 2016). Naturally, these conclusions gave us reason to assess the impact of light intensities on prolactin and cortisol levels as stress markers and determine the optimum light intensity in cultivating African catfish in RAS with a low-water exchange.

Materials and Methods

Fish and experimental designs

The experiment was performed according to the Guidelines of the European Union (2010/63/UE) and the Animal Welfare Act and approved by the Committee on Animal Experimentation at the Bulgarian Food Safety Agency (4803/2022).

Thirty fish (778.86±39.53g, 47.16±0.94 cm) were acclimated over an appropriate time frame to the short photoperiod (8 light (L): 16 dark (D) and then distributed in RAS with low-water exchange randomly in three light intensities groups. For each light condition, 10 fish separated by sex (five female and five male) were placed into a tank with 900 L of water. The duration of the study was 45 days, and the light/dark cycle was adjusted to 8L: 16D. To maintain a uniform and fixed intensity, the light lamps were cleaned weekly, and light intensity was measured at the tanks using a portable digital lux meter (Lutron EM9300SD, Taiwan). The fish were exposed to three different levels of incidence of light in a tank: 63, 51, and 40 lux, respectively. The treatment with the lowest light intensity (40 lux) was constructed with no light input, and a black Styrofoam lid covered the tank. In the group with an intensity of light of 51 lux, the tank was covered with a 50% black Styrofoam lid and 50% mesh, respectively. The tank, in the intensity of light of 63 lux, was covered with 100% mesh.

The aeration was constant, and fish were fed three times daily at 8^{00}, $12^{00,}$ and 16^{00} h using a commercial feed (Aqua Wels Swim, Aqua-Garant GmbH, Austria), with a ration of 3% of their body weight per day. The hydro chemical parameters (SO_4-, NO_3-N, NO_2-N, NH_3-N, PO_4- and permanganate oxidizability) were measured daily before feeding and one hour after the last feeding of fish, using a portable colorimeter (Hach DR/850, USA). The water quality parameter was: 50-53 mg L^{-1} for sulfates, 5.1-7 mg L^{-1} for nitrate nitrogen, 0.169-0.185 mg L^{-1} for nitrite nitrogen, 0.46-0.65 mg L^{-1} for nitrogen, 1.12-1.17 mg L^{-1} for phosphates and 1.20-1.36 mg L^{-1} for permanganate oxidizability.

Blood collection analysis

Blood samples (six fish from each group) were collected from the caudal vein using a 2 ml K2EDTA-coated syringe. Plasma was separated and stored at -20° C for later analysis. A laser fluorescence reader (i-chroma TM Reader Boditech, Korea) assayed prolactin and cortisol. The test uses a competitive immunodetection method in which the target material in the sample binds to the fluorescence (FL)-labeled detection antibody. This complex is loaded onto the nitrocellulose matrix, where a covalent couple of cortisol and bovine serum albumin (BSA) is immobilized on a test strip and interferes with the binding of target material FL-labeled antibody.

Histological examination

Three fish from each sex (six from each group) were stunned with eugenol solution at 55 mg.L^{-1} in water. After removing the pituitary gland of the fish undergoing a craniectomy, the pituitary gland was fixed in 10% buffered formalin, dehydrated, and embedded in paraffin. Histological sections (4µm) were stained with hematoxylin and eosin (H&E), and examined to a morphometric analysis by light microscopy (Bullock, 1978).

Statistical analysis

The statistical analysis was performed using a one-way analysis of variance (ANOVA). The results are presented as mean and standard deviation of the mean (Mean ± SD).

Results

This study was designed to determine the plasma levels of prolactin and cortisol from African catfish exposed to different light intensities. The acquired results are shown in table (**Table 1**).

Table 1 Levels of prolactin and cortisol levels from African catfish exposed to different light intensity.

		Table feature	
Group	*Sex*	*Prolactin ng/ml*	*Cortisol ng/ml*
Light intensity 40 lux	♂	$0.95±0.18^{A}$	$19.41±1.84^{A}$
	♀	$0.98±0.28^{A}$	$20.76±2.56^{A}$
Light intensity 51 lux	♂	$1.54±0.20^{a}$	$34.95±2.12^{a}$
	♀	$2.04±0.38^{b}$	$35.11±1.48^{b}$
Light intensity 63 lux	♂	$6.83±0.66^{A,ab}$	$46.22±3.84^{A,ab}$
	♀	$7.52±0.97^{A,ab}$	$51.78±2.09^{A,ab}$

a,b,c: Values within a column followed by different superscript letters indicate significant differences (p<0.05)

A, B: Values within the row followed by different superscript letters indicate significant differences (p<0.05)

During the experiment it was observed that on light intensity 63 lux, the plasma prolactin level in both sexes significantly increased (male 6.83±0.66 and female 7.52±0.97) compared to Light intensity 40 lux (male 0.95±0.18 and female 0.98±0.28) and Light intensity 51 lux (male 1.54±0.20 and female 2.04±0.38). Regarding the second examined hormone, the results showed that light intensity had significant effect on the cortisol level. The catfish reared under light intensity of 40 lux has significantly lower cortisol level than the catfish reared on light intensity 63 lux.

Histologically, it was determined that the size of rostral pars distal (rpd) of the pituitary gland enlarged in size in catfish exposed to light intensities, because of the increase in prolactin level of plasma and environmental stress (**Figure 1,2**). In contrast, it was noted that the rostral pars distalis shrinkage in fish kept in the darkness (**Figure 3**).

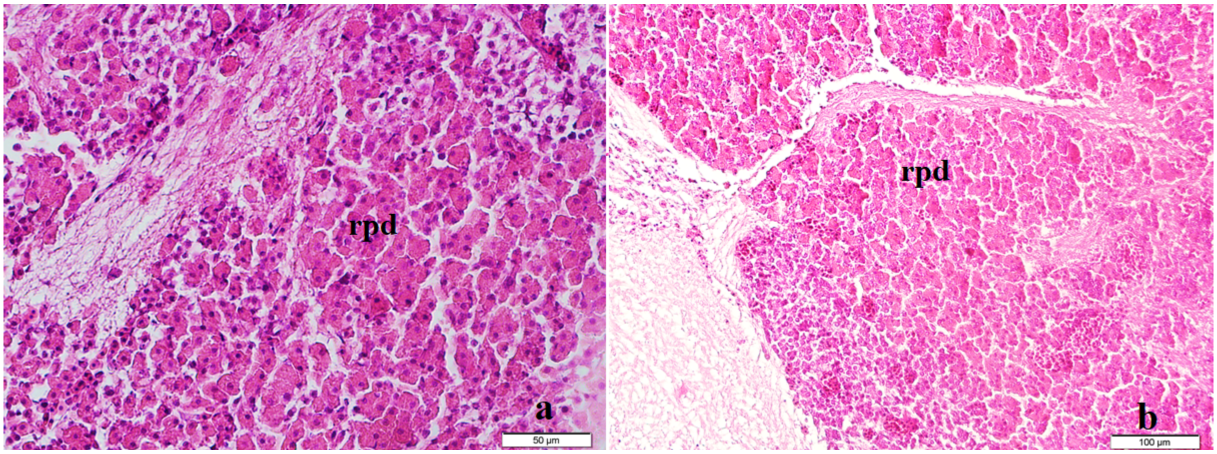

Figure 1 Histological section of the pituitary gland of African catfish exposed to 63 lx. (**a**) male (**b**) female (H&E) (**rpd**: rostral pars distalis, **ppd**: proximal pars distalis).

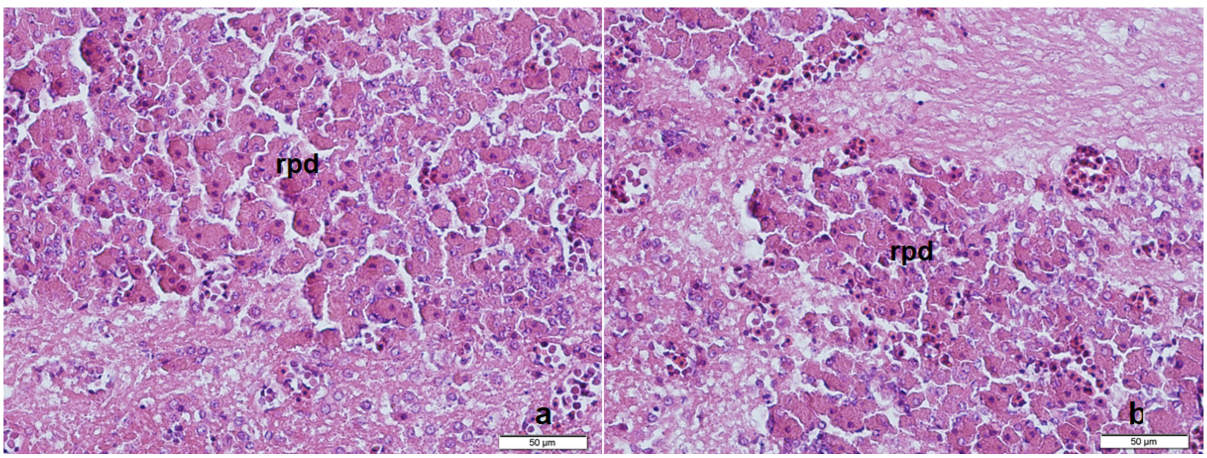

Figure 2 Histological section of the pituitary gland of African catfish exposed to 51 lx. (**a**) male (**b**) female (H&E) (**rpd**: rostral pars distalis, **ppd**: proximal pars distalis).

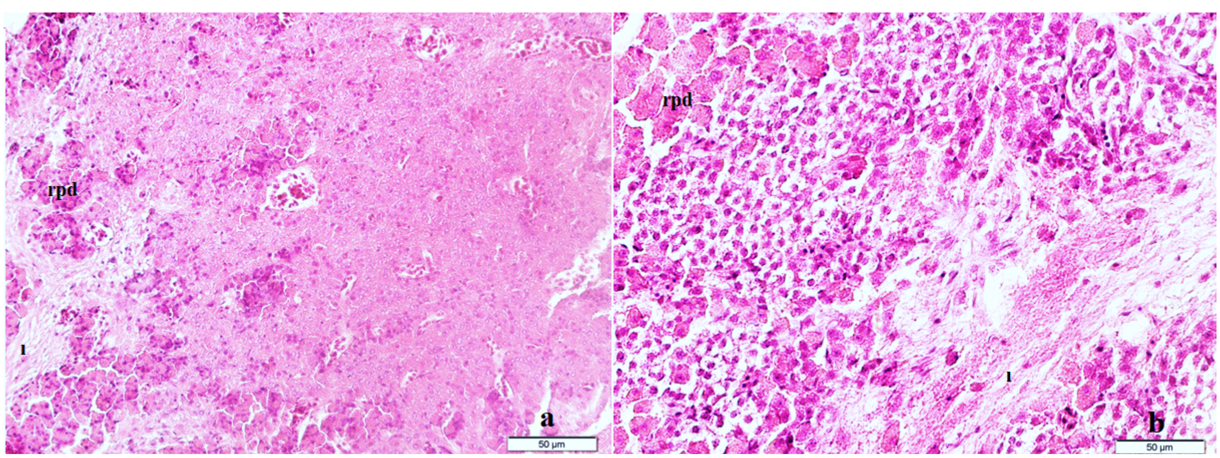

Figure 3 Histological section of the pituitary gland of African catfish exposed to 40 lx. (**a**) female (**b**) male (H&E) (**rpd**: rostral pars distalis **ppd**: proximal pars distalis **i**: infundibulum).

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Discussion

Biotic factors have a severe impact on hormone activity. By hormones, fish can adapt to constantly changing environments. Therefore, measures of the stress hormones can be used as an indicator of environmental quality and how well a fish is coping in this environment. African catfish is easy to cultivate because of its resilience to suboptimal environmental conditions (Baßmann et al., 2017; Secer et al., 2019).

In teleost fish, the thyroid gland is scattered elements (follicles) around the ventral aorta (Atanasoff et al., 2021). In them, thyrotropin-releasing factor (thyrotropin-releasing hormone TRH) stimulates the adenohypophysis (anterior pituitary) to secrete prolactin. Endocrine neurons regulate pituitary prolactin secretion in the hypothalamus. Prolactin is a multifunctional polypeptide hormone released from specific cells (lactotrophs) located in the anterior lobe of the pituitary (adenohypophysis) into the bloodstream that exerts a particularly diverse impact on growth and development, endocrinology, and metabolism in fish (Whittington and Wilson, 2013). In fish, in contrast with mammals where lactotrophs spontaneously release, secretion seems to be affected by different factors like reduced osmolarity, stress, etc.

Environmental stress has profoundly harmful effects on many endocrine functions in teleost fish. Prolactin is also considered a stress hormone associated with changes in environmental conditions. In case of chronic stress, their respond to it by increased plasma prolactin levels (Avella et al., 1991). Similarly, Figueroa et al. (1997) reported increases in plasma prolactin levels in long-term photoperiod applications in carp. In other fish, such as the *Carassius auratus*, longer photoperiods caused pituitary PRL (McKeown and Peter,1976) release than trout *Oncorhynchus mykiss*. Sage and Vlaming (1971) reported that the prolactin hormone was increased during seasonal adaptations to the annual cycle of day length (long-photoperiod). These may be the reasons for increasing plasma prolactin levels during the current study.

The fish from both sexes were included in the current study to address the hormonal changes associated with the reproductive cycle during seasonal (photoperiod) changes. In females, plasma PRL is associated with ovarian maturation, whose level increases along with 17β-estradiol and thus directly affects reproduction. The maximum levels are reached during the pre-spawning period. Opposite in males, no such evidence of prolactin was obtained, and levels were lower than ever in females (Verma and Alim, 2014).

Under certain stress conditions, the teleost fish use the hypothalamic-pituitary-interrenal (HPI) axis to regulate their metabolism. The central nervous system, provoked by the stressor, in the hypothalamus starts to produce a corticotropin-releasing hormone (CRH) that stimulates the anterior pituitary gland to release adrenocorticotropic hormone (ACTH). When ACTH is transported to the interrenal cells of the fish, cortisol is emitted (Baßmann et al., 2017). Cortisol, the main glucocorticoid, plays an important role in the primary response to stress (MartínezPorchas et al., 2009).

The juveniles of African catfish (< 90g), showed a cortisol baseline level of about 20 ng mL^{-1}. Larger African catfish (< 300g) had cortisol baseline levels under 135 ng mL^{-1} (Baßmann et al., 2017). In the present study, the plasma cortisol levels were like our earlier studies (Zapryanova et al., 2018). We observed significantly lower plasma levels in light intensity 40 lux, possibly indicating better well-being of these groups. On the other hand, other researchers verified the increase in *C. gariepinus* cortisol after exposure to high light intensity (350 lux) (Manuel et al., 2016).

The rostral pars distalis (RPD) is anterodorsal in position and is the smallest portion of the pituitary in fish. It has been reported that prolactin cells are in this part of the gland. The catfish cells of this region are cyanophils, acidophils, and a few chromophobes (Gill et al., 1977). The responses of fish to stress factors vary according to the fish species; more precisely, the pituitary cells react differently in species. Bonga et al. (1984) observed the increases in size and number of prolactin cells in tilapia (*Oreochromis mossambicus*) due to the enlarged dimension of the rostral pars distalis of the pituitary. Similarly, the current study determined that the application of light caused stress in catfish, and the size of the rostral pars distalis of the pituitary gland was enlarged.

Since African catfish prefer continuous darkness, it may be concluded that they react to a high light intensity with increasing plasma prolactin and cortisol levels and the described histological changes in the pituitary gland.

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