Conference Paper

Evaluation of Hormone-Induced Stress Responses Using Endogenous Cortisol in Carp *(Cyprinus carpio)*

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Abstract

With stress studies in fish it is often difficult to determine the degree of response to various stressors, and the interpretation of this influence is usually based on an increase in endogenous cortisol levels. Simulation of stress with synthetic corticosteroids is widely used in ichthyological practice, which raises the question of whether endogenous cortisol is the most appropriate parameter for measuring stress levels in such studies. This work presents the dynamics of the plasma cortisol level in simulating acute and chronic stress in 24 carps by a single injection of dexamethasone in the first experimental group and betamethasone in the second experimental group, in comparison with the control group (without injection) for 21 days. The analysis was performed before injection, as well as after 7, 14 and 21 days of treatment. The hormonal response was compared with that of fish stressed by natural factors (hypoxia). It was found that betamethasone inactivates the production of endogenous cortisol during all subsequent days of the experiment after injection from 353.68±66.39 ng/ml to 7.28±1.27 ng/ml by day 21, while the effect of dexamethasone caused multidirectional fluctuations in its level: from 346.25±43.16 ng/ml to 242.25±58.49 ng/ml on the 7th day, 388.25±37.51 ng/ml on the 14th day and 264.25±21.21 ng/ml on day 21 compared with smooth dynamics in control fish: 376.25±44.04 ng/ml, 366.75±42.82 ng/ml, 335.33±8.57 ng/ml and 366.00±89.22 ng/ml, respectively. It was concluded that measuring the level of endogenous cortisol is not recommended when assessing the degree of stress imitation by these hormones, and in studies of this type it is necessary to search for other indicators.

Keywords: carp, cortisol, stress, dexamethasone, betamethasone, hormone

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1. Introduction

Growing fish under intensive aquaculture conditions is associated with the constant influence of a number of damaging environmental factors (stress factors) on it. Stress

in cultured fish, in particular carp, is an increasingly studied topic due to its effects on growth [1], reproduction [2], functioning of defense systems: immunity and hemostasis [3–5], and ultimately on the fitness of the animal.

The primary response to stress in fish is endocrine changes, including, first of all, the production of catecholamines and glucocorticoids [6, 7]. They cause secondary metabolic, osmotic, and other changes [8, 9]. The cortical response in teleosts is regulated by the hypothalamic-pituitary-interrenal (HPI) axis [10]. The quantitative characterization of this response, in particular cortisol, in different fish species has been well studied: cortisol levels before stress vary significantly and are 2-42 ng/ml, post-stress cortisol levels range from 20 to 500 ng/ml [11].

In stress research, it is often difficult to measure the degree of response to various stressors, and the interpretation of this effect is usually based on an increase in endogenous cortisol levels. The influence of stress stimuli on the fluctuations of this hormone in carp has been described, including fishing [12, 13], transportation [14, 15], and high stocking density [16], hypoxia [5, 17], temperature fluctuations [18], toxic pollution [19–21] and acidity of the [22, 23]. In other studies, the following are used as the main or additional stress markers: the level of catecholamines (adrenaline and norepinephrine), the concentration of glucose and chlorides [24–26]. It should be borne in mind that the innate response to stress can be different even in different carp subspecies [27].

In ichthyological practice, the imitation of stress of various durations is widely used by the introduction of exogenous cortisol [28–30], cortisone [31], or synthetic corticosteroids —dexamethasone [32–34] and triamcinolone [35, 36]. A legitimate question may arise as to whether endogenous cortisol is the most appropriate parameter for measuring stress levels in such studies.

An artificial increase in the concentration of blood cortisol and thus the induction of stress reactions can be carried out by introducing exogenous cortisol orally, by intraperitoneal and intramuscular injections, as well as the introduction of oil implants [37]. The introduction of cortisol with food provokes an increase in the level of endogenous cortisol [1], and also has a number of advantages over other methods, since it does not cause additional stress associated with manipulation. The disadvantage of this method, however, is the uneven consumption of food by fish and the impossibility of accurate hormonal modulation. Cortisol levels achieved by this method are best used to simulate chronic stress responses.

Administration of cortisol by intraperitoneal [28], intravascular injection [31], and implants [1, 30] also increases the level of endogenous cortisol and is effective for

the induction of acute stress, and in in the latter case, these changes are directly proportional to the dose of the implant.

The issue of controlling the level of endogenous cortisol in modeling stress responses in different ways is discussed in detail in a review of studies by AK Gamperl et al. [37], which reported that dexamethasone and betamethasone suppress the production of endogenous cortisol by inhibiting adrenocorticotropic hormone (ACTH) in effects on the hypothalamus and pituitary gland from 80 to 100%. In this case, it is necessary to take into account the handling stress during injection, which can raise the level of endogenous cortisol. The authors conclude that dexamethasone and betamethasone have cortisol-like effects, but their value in assessing hormonal response during stress may be limited.

Information on the effect of another synthetic glucocorticoid, triamcinolone, on the plasma level of cortisol in modulating stress reactions [35, 36] is absent in the literature.

The influence of natural stress factors (hypoxia, temperature, toxicants, etc.) and imitation of stress of different nature and duration in ecology, biology and veterinary medicine are often used to study its effect on the functioning of the physiological systems of common carp. Synthetic corticosteroids with cortisol-like effects, including dexamethasone, betamethasone, and triamcinolone, are often used for this purpose. The accuracy of such modulation in the practice of using exogenous cortisol is determined by the level of endogenous (plasma) cortisol in the blood of fish. However, using synthetic analogs, the practice of using this method may be limited.

Research Questions

3.1. The first question to be solved within the framework of this study is how the use of dexamethasone and betamethasone affects the dynamics of the level of endogenous cortisol in carp when simulating acute and chronic stress.

3.2. Another question is whether the assessment of plasma cortisol is objective for a quantitative and qualitative assessment of the induced stress reactions, or is it necessary to search for other indicators.

The aim of this work is: (1) to assess the effects of synthetic glucocorticosteroids inducing acute stress (dexamethasone) and chronic stress (betamethasone) on plasma cortisol levels in the long term (21 days) compared with the effects of a natural stress factor (hypoxia); (2) analysis of the effectiveness of monitoring cortisol for characterizing stress tension in studies with modulation of stress responses in carp.

2. Methods and Equipment

An experiment with hormonal induction of stress reactions was carried out on 24 carps of Cyprinus carpio carpio L., which were preliminarily divided into three groups: fish with simulation of acute stress (first experimental group), fish with simulation of chronic stress (second experimental group), and control animals. To simulate acute stress, dexamethasone phosphate (4 mg/ml) [38] was used, which is metabolized within 4 hours. The animals were treated once with Dexamethasone (Ellara, Russia) by parenteral injections at a dose of 0.2 ml or 0.8 mg of the active substance dexamethasone-phosphate per animal. A suspension of betamethasone (2.63 mg of betamethasone sodium phosphate + 6.43 mg of betamethasone dipropionate/ml) was used once as a glucocorticoid that simulates chronic stress, with a withdrawal period of more than 10 days. Diprospan (Schering-Plow Labo N.V., Belgium) was injected at 0.5 ml per individual, which corresponds to 3.5 mg of the active substance. The control group remained intact.

The fish were kept in an experimental setup providing continuous circulation of water between aquariums with aeration and a water temperature of 18–20 °C. Feeding was once a day. Before blood sampling, the fish were anesthetized by adding clove oil to the water at a dose of 0.033 ml/l [39] for 15 minutes. Blood sampling was carried out from the tail vein after fish acclimatization, and then after 7, 14, and 21 days [38] after drug treatment.

The quantitative analysis of serum cortisol was determined by the method of solid-phase chemiluminescent immunoassay with the participation of LLC “Center for laboratory diagnostics “Tseldi”.

The results obtained in the work are presented as the mean and standard error of the mean (M±m). The significance of differences in indicators for multiple independent samples was determined using the Croskell–Wallace test; for paired dependent samples, the Wilcoxon test was used. The results of the study with the value of the probability of making an alpha error equal to or less than 5% ($p < 0.05$) were regarded as statistically significant. The difference between the two indicators was considered significant if it was equal to or exceeded its mean error of the difference by two or more times.

3. Results

As a result of our study, it was noted that the level of plasma cortisol in fish that did not receive hormone injection underwent a gradual decrease by the 14th day and a further

TABLE 1: Dynamics of endogenous cortisol in fish before and after treatment with hormones

Indicator	Before treatment			7th day			14th day			21st day		
	Control (n = 8)	First group (n = 8)	Second group (n = 8)	Control (n = 8)	First group (n = 8)	Second group (n = 8)	Control (n = 8)	First group (n = 8)	Second group (n = 8)	Control (n = 8)	First group (n = 8)	Second group (n = 8)
Cortisol [ng/ml]	376.25 ± 44.04	346.25 ± 43.16^{ac}	353.68 ± 66.39^{abc}	366.75 ± 42.82*	242.25 ± 58.49^{*bc}	5.25 ± 1.49^{c}	335.33 ± 8.57*	388.25 ± 37.51^{*c}	6.00 ± 1.08	366.00 ± 89.22*	264.25 ± 21.21*	7.28 ± 1.27

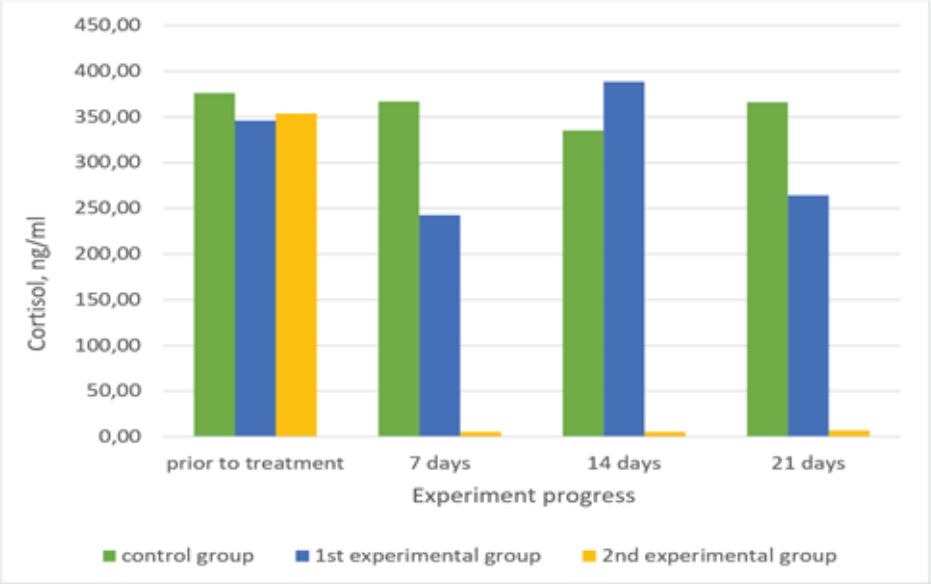

Figure 1: Dynamics of endogenous cortisol in fish before and after hormone treatment

restoration of concentration by the 21st day of the experiment, and its spread was 335-376 ng/ml per all time. In fish stressed with dexamethasone, the content of endogenous cortisol changed abruptly and in different directions, having sharply decreased twice on the 7th and 21st days of the experiment and amounted to 246-346 ng/ml during the study. Probably, 80-100% of blocking of cortisol synthesis by dexamethasone in fish, mentioned by A. K. Gamperl [37], manifests itself in the period of 24-96 hours [40], causing subsequent fluctuations. In the group of fish that were injected with a prolonged-release hormone (betamethasone), the production of endogenous cortisol was completely inhibited by the 7th day of the experiment from 353 to 5 ng/ml, which was not restored by the end of the experiment, which is associated with the gradual release of the active substance from the repository. In both cases, fluctuations in the hormonal composition of the blood of the studied fish occur, which differ from those in the control. In this case, the additional stress effect of the injection in experimental animals can be excluded, since the injection of the preparations took place immediately after the initial blood sampling in all groups of fish, and therefore the effect of the handling stress was the same.

As part of the assessment of the cortisol response, it is also necessary to compare the described results with the data on the effect on carp Cyprinus carpio carpio L. of a natural stress factor—hypoxia for 3 days, which we conducted earlier [4]. According to this study, the lack of dissolved oxygen in comparison with the control group led to the stimulation of the production of endogenous cortisol during all days of the experiment: 118.0±32.3 ng/ml on the 1st day; 224.8±69.0 ng/ml on the 2nd day; 235.5±74.5 ng/ml on the 3rd day, while the indicators of intact fish by the end of the experiment decreased compared to the initial level: 251.7±92.0, 319.3±10.2 and 200.6±64.4 ng/ml on the 1st, 2nd and 3rd days, respectively.

4. Discussion

In the case of studying the effect of abiotic environmental factors (in this case, hypoxia) on carp, the level of plasma cortisol naturally reflects the degree of influence of stress stimuli, which is confirmed by the studies described in the introduction. In comparison, simulating stressful conditions by injecting synthetic corticosteroids leads to a decrease in production and further fluctuations (dexamethasone) until a significant and long-term blockage (betamethasone) of endogenous hormone production. Such data undoubtedly confirm the thesis of colleagues [37] that the use of dexamethasone and betamethasone is not recommended as indicators of the stress response in carp in such experiments. Presumably, this indicator can be the concentration of blood glucose in the greatest number of cases in other fish, as well as having a tendency to increase with the use of corticosteroids in carps [20, 21, 26], although sensitive to the effects of other factors. Also, glucocorticoids have a clear immunosuppressive [3] and hypercoagulant [4, 5] effect in carps, and therefore the effectiveness of an artificially induced stress reaction can be determined by indicators of immunity and hemostasis when studying other physiological systems. In any case, the question of the suitability of these indicators for these purposes requires further research

5. Conclusion

Thus, the stated factual material allows us to make a number of generalizations concerning the characteristics of the cortisol response of the studied carp to hormonal treatments, usually performed in the framework of homeostasis studies.

In comparison with biotic and abiotic stress stimuli, modulation of the stress response by injection of synthetic corticosteroids, in comparison with control animals, leads to a

general decrease in the level of endogenous cortisol during the first hours and one day, and in the following days it fluctuates under the influence of dexamethasone, up to complete inactivation of the hormonal response as a result of treatment with betamethasone.

In this regard, the problem of assessing the tension and duration of the stress reaction in fish using synthetic analogs of cortisol becomes urgent.

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Conflict of Interest

The authors have no conflict of interest to declare.

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