Research Article

The effect of different doses of GnRH on stress responses in female koi carp (*Cyprinus carpio*)

M. M. Eslami[1], S. R. Javadian [1*], S. Bahram [1]

[1] Department of Fisheries, Qaemshahr Branch, Islamic Azad University, Qaemshahr, Iran

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Abstract

This study was conducted to evaluate the effect of different doses of GnRH on stress response in female koi carp (*Cyprinus carpio*). For this purpose, forty sexually mature female koi carp with an average weight of 102.05 ± 9.03 g were divided to four groups and fish received with a single intraperitoneal 0.9% NaCl with 20 mg kg^{-1} metoclopramide (C); 10 µg kg body $weight^{-1}$ (BW) GnRH with 20 mg kg^{-1} metoclopramide (Gn10); 20 µg kg BW^{-1} GnRH with 20 mg kg^{-1} metoclopramide (Gn20); 50 µg kg BW^{-1} GnRH with 20 mg kg^{-1} metoclopramide (Gn50). Blood samples were collected before and after ovulation (approximately 24 h post-injection). The concentrations of plasma cortisol, lactate, and glucose were measured. Broodstocks who received GnRH spawned, while broodstocks of the group C did not spawn. The Gn10, Gn20, and Gn50 treatments led to significantly higher cortisol, lactate, and glucose concentration after ovulation compared to before injection ($p< 0.05$).

[*]**Corresponding author E-mail:** Ro.javadian@gmail.com

The present results showed that females are highly sensitive to manipulation during reproduction and higher levels of the hormone cause more stress broodstocks, so they must be held with minimal disorders, especially during spawning period.

Keywords: *Cyprinus carpio*, GnRH, Artificial spawning, Hormonal treatment, Cortisol

Introduction

Artificial reproduction of brood stock is one of the most important part in aquculture activity to develop aquatic production (Mylonas and Zohar, 2001). The control of reproductive process is essential for obtaining high-quality eggs (Mohammadzadeh *et al*., 2021). Reproductive process should be managed by controlling environmental factors such as light period, water temperature, and spawning net (Mylonas and Zohar, 2001). Since for some new species, the natural requorments are not well recognized so the use of maturation inducing factores are the only alternative to induce final maturation in these species

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(Mohammadzadeh *et al*., 2021). As a result, hormonal therapy may be used to induce gamete maturation and provide gametes for generating intraspecific hybrids, chromosomal manipulation, or artificial insemination for genetic selection (Mylonas and Zohar, 2001). A variety of hormonal approaches have been used successfully such as ground pituitaries and different synthetic hormones (Linhart *et al*., 2000, 2003; Piros *et al*., 2002; Podhorec *et al*., 2016). Gonadotropin-releasing hormone (GnRH) has been used extensively to release LH needed to stimulate final oocyte maturation, ovulation, and spermiation (Mylonas and Zohar, 2001), but there are various problems associated with these methodologies. Multiple hormonal applications are very often necessary for a successful response. Consequently, the fish must be monitored and handled extensively, which is not only labor-intensive but also stressful to the fish and often leads to mortalities of valuable and painstakingly reared broodstock (Harmin and Crim, 1992). Various studies have evaluated the effects of stress-induced by hormone injection and manipulation on the reproductive process and have reported adverse impacts on the reproductive function (Schreck *et al*. 2001; Milla *et al*., 2009; Pourhosein Sarameh *et al*., 2012). Generally, Cortisol levels in animals, including fish, indicate a state of stress (Wendelaar Bonga, 1997; Barton, 2002). Koi carp (*Cyprinus carpio*) is a ornamental fish from Cyprinidae family (Mohammadzadeh *et al*., 2021). High fecundity, short embryonic period and simple breeding technique are reported for this species (Ghosh *et al*., 2012). It is not clear whether hormonal levels in higher doses will alter stress indicators. To date, there are no studies about the effects of different GnRH doses on stress factors in koi carp. Therefore, this study was designed to evaluate different doses of GnRH on stress response in koi carp brood stock (*Cyprinus carpio*).

Materials and methods

Broodstock rearing condition and treatment

A total of 120 mature female koi carp (102.05 ± 9.03 g) were obtained from a local fish center (Sari, Iran) and carried into praivet orimental fish center. The selection of female brood stock was made according to anatomical appearance (soft and protruding abdomen), (Podhorec *et al*., 2016). The selected fish were randomly distributed into 4 experimental groups with 3 replication (n = 10) and each experimental group was placed in a 250-liter aquarium. The selected fish was adopted to experimental condistion for two days and then they were injected by: 0.9% NaCl with 20 mg kg^{-1} metoclopramide (C); 10 µg kg body $weight^{-1}$ (BW) GnRH with 20 mg kg^{-1} metoclopramide (Gn10); 20 µg kg BW^{-1} GnRH with 20 mg kg^{-1} metoclopramide (Gn20); 50 µg kg BW^{-1} GnRH with 20 mg kg^{-1} metoclopramide (Gn50) (Mohammadzadeh *et al*., 2021). Before injection, fish were anesthetized with clove powder at a dose of 15 ppm. Breeders were tested for ovulation 8 hours after the injection, and this manipulation was repeated at two hours intervals up to 24 hours post-injection (Mohammadzadeh *et al*., 2021). In response to gentle pressure, the spawning-ready fish were stripped to collect the eggs in a dry container.

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The latency period was recorded as the lapse of time between the hormone injection and ovulation.

Sampling and Hormone analysis

First, fish were anesthetized with 15 ppm clove powder extract (Ahmadifar *et al*., 2016). Blood samples were collected from the behind of the anal fin with a 2 mL heparinized syringe at the time of injection and after ovulation (approximately 24 h post-injection). Then the samples were transferred to the tubes, centrifuged (1,600 × g for 10 min) to separate plasma, and stored at -20° C for later analysis.

The concentrations of lactate and glucose were measured using standard kits from Pars Azmoon (Karaj, Iran). Plasma cortisol was determined by using competitive enzyme immunoassay kits (ELISA Micro wells, Diaplus, USA).

Statistical analysis

Data normality and homogeneity of variances was tested by Shapiro-Wilk and Levene's tests, respectively. The effect of the treatments on reproductive performance and stress factors was examined by one-way analysis of variance (ANOVA). Differences between several treatments were determined by Tukey's post-hoc tests.

Results

Reproduction performance

eight females in Gn10, ten females in Gn20, and five females in Gn 50 treaments spawn but in control group there was no spawned females (Table 1). The females injected with 20 µg/kg of GnRH showed an increased ovulation rate than the two other groups injected with GnRH.

Table 1. Reproduction performance in koi carp (*Cyprinus carpio*) females after injection 0.9% NaCl with 20 mg kg^{-1} metoclopramide (C); 10 µg kg body $weight^{-1}$ (BW) GnRH with 20 mg kg^{-1} metoclopramide (Gn10); 20 µg kg BW^{-1} GnRH with 20 mg kg^{-1} metoclopramide (Gn20); 50 µg kg BW^{-1} GnRH with 20 mg kg^{-1} metoclopramide (Gn50)

Treatment	No. of injected fish	No. of spawned fish	Spawning success (%)[1]
0.9% NaCl	10	0	-
GnRH (10 µg kg-^{1})	10	8	80
GnRH (20 µg kg^{-1})	10	10	100
GnRH (50 µg kg^{-1})	10	5	50

[1]Spawning success (%): the number of females that ovulated after injection divided by the total number of injected females.

Stress factors

While the cortisol level was approximately similar among the groups before injection (Fig 1, A; $p > 0.05$). After injection, a significant increase of cortisol was obtained in Gn10, Gn20, and Gn50 treatments (Fig 1, A; $p < 0.05$).

No approximately difference in glucose levels was observed among treatments before injection (Fig. 3; $p > 0.05$). After injection, a significant increase of glucose was observed in Gn10, Gn20, and Gn50 treatments (Fig, 1, B; $p < 0.05$).

In Gn10, Gn20, and Gn50 treatments 24 h post-injection, lactate levels were significantly higher than before injection (Fig. 1, C; $p < 0.05$). While the lactate level was approximately similar among the groups before injection (Fig. 1, C; $p > 0.05$).

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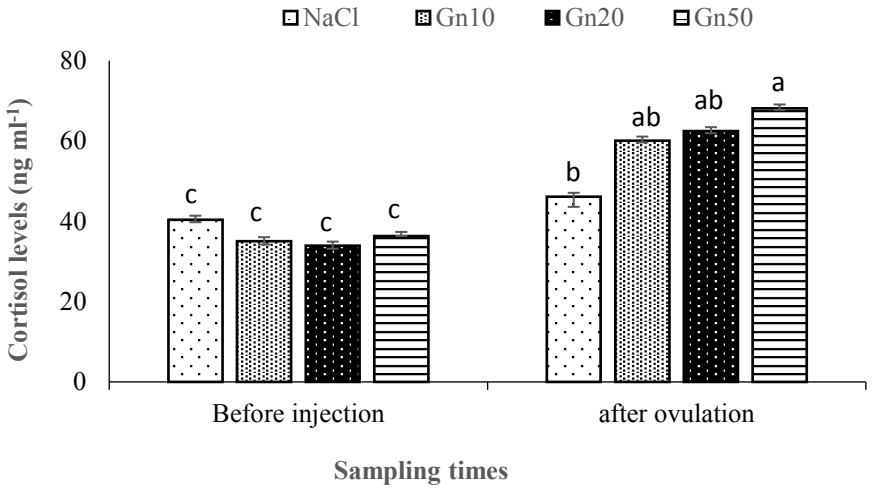
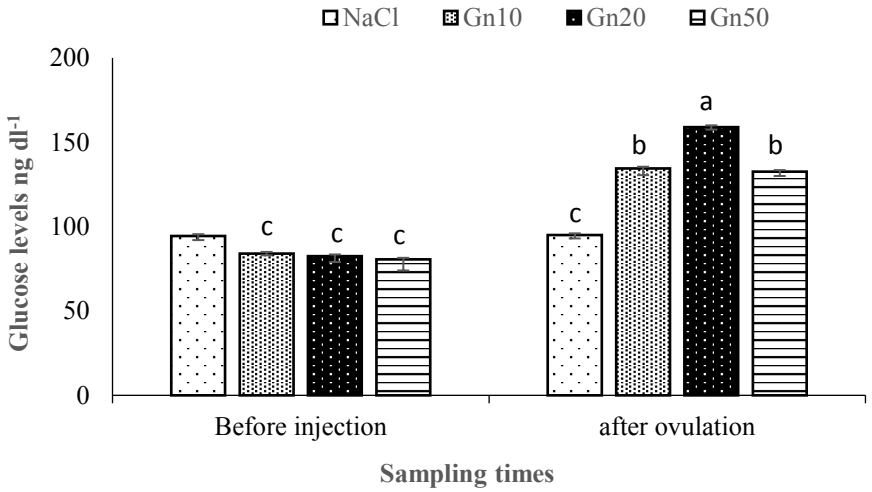
A



B



C

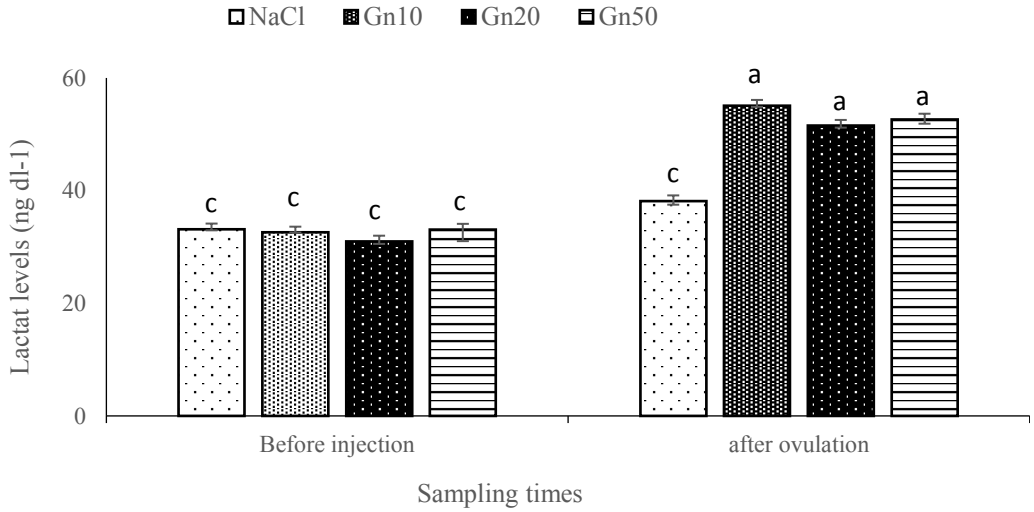


Figure 1. Plasma cortisol levels (A), glucose (B), and lactate (C) in koi carp (*Cyprinus carpio*) broodstock. Mean ± SD; n = 6 for each treatment. Different letters designate significant differences as determined by Tukey's post-hoc tests.

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Discussion

The current study displayed that koi carp brood stocks treated with GnRH has more efficient spawning rate than control group. Consistent with the current result, the injection with GnRHa improved reproductive performances in tench (*Tinca Tinca*) females (Podhorec *et al*. 2016). GnRH could increase final oocyte maturation and spawning rate in fish species (Drori *et al*. 1994; Targonska and Kucharczyk, 2011). The most abundant spawned broodstock was observed in the Gn20 treatment. GnRH higher dose may decline egg quality (Garcia, 1989) while GnRH lower doses can decrease spawning frequency (Mohammadzadeh *et al*. 2021). Suggesting that GnRH 10 and 50 μg kg^{-1} showed less output compared to GnRH 20 μg kg^{-1} to induce spawning.

Stress can play a large role in necessary life functions, including reproduction biology, invertebrates (Schreck, 2010). The results of the present study showed that cortisol, lactate, and glucose levels were affected by hormone injection in females koi carp.

In this study, cortisol concentration increased after ovulation compared to the baseline measures both in hormonal treatments and the control group. These results indicated that brood stocks are delicate to handling. This sensitivity may be due to physiological conditions during the reproduction period (Falahatkar and Poursaied, 2013). Females carry a massive volume of oocytes in the ovary during ovulation and their body cavity is swollen. In this term, Falahatkar and Poursaied (2013) suggested that this swelling may make stress response in fish. Kusakabe *et al*. (2003) reported that an increase in cortisol level during ovulation period in rainbow trout (*Oncorhynchus mykiss*). These results showed that cortisol levels in treatments receiving different doses of GnRH were higher than the control group after ovulation. Consistent with the current result, the treatment of CPE and LHRHa for final maturation enhanced cortisol level (Bayunova *et al*. 2002; Semenkova *et al*. 2002). Cortisol levels increase with increasing dose of GnRH and the highest amount is seen in fish injected with a dose of 50 μg kg^{-1} BW.

Glucose levels increased after ovulation in the Gn10, Gn20, and Gn50 treatments. This result agreed to a study in which glucose levels rised in brood pikeperch after hormonal injection (Falahatkar and Poursaied, 2013). Catecholamines and corticosteroids has a directly role during stressful conditions to motivate glycogenolysis, which in turn relase glucose (Mommsen *et al*. 1999). In acute stress conditions, glucose and corticosteroid levels occur at the same time (Wendelaar Bonga 1997; Barton, 2002; Falahatkar *et al*. 2012). Stress is an energy-demanding process and it increases metabolic demands so for providing this energy by elevating of plasma glucose may be one of the ways.

In the current study, lactate levels were significantly higher in Gn10, Gn20, and Gn50 treatments 24 h post-injection than before injection. A similar result for lactate level was found in sterlet sturgeon (*Acipenser ruthenus*) after injected with LHRH-A2 (Falahatkar *et al*.

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2017). The increase in plasma lactate is due to the anaerobic consumption of glucose as the source of energy during the final maturation (Falahatkar *et al*., 2016). Former studies have proved a rapid increase in lactate levels (30 min to 2 h) after hormone injection and stress (Falahatkar and Poursaied, 2013).

Lower cortisol, lactate, and glucose levels in the control group (physiological serum) in comparison with other treatments may be concerened to the spawning status. No ovulated females were observed in control group (physiological serum); therefore, it proves that ovulation instils stress responses in female koi carp.

In conclusion, it has been shown that different doses of GnRH affect reproductive success in Koi carp and the best dose is 20 µg kg^{-1} body weight. These results showed that hormonal inductions can affect primary and secondary stress responses. Due to alters of plasma cortisol and glucose levels in koi carp, females, are very sensitive to stress and thus must be held with minimal disorder especially during artificial reproduction process.

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Conflicts of interest

Authors have no conflict of interest on this work.

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