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Effects of low temperature and starvation on plasma cortisol, triiodothyronine, thyroxine, thyroid-stimulating hormone and prolactin levels of juvenile common carp (*Cyprinus carpio*).

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ABSTRACT

In this study, the hormonal responses such as Plasma Cortisol, Triiodothyronine (T3), Thyroxine (T4), Thyroid-stimulating Hormone (TSH) and Prolactin of juvenile carp induced by low temperature and starvation as stressors have been investigated. Four treatments were conducted under the following condition: the first group was fed and kept at a temperature of 18°C (18F); the second group was fed and kept at 6°C (6F); the third group was not fed and kept at 18°C (18FD) and the fourth group was not fed and kept at 6°C (6FD). Hormonal responses of fish were measured in every 15th day of 45 days trial. According to the results, it was determined that cortisol level of fish in the group 18FD significantly increased while T3, T4 and TSH levels of fish increased in the group 6FD ($P < 0.05$). No significant differences were obtained between the treatments in terms of Prolactin. To conclude up, the temperature and total food deprivation and their interactions had a significant effect on the plasma hormone levels of common carp juveniles.

Introduction

In nature, fish are exposed to varying factors of stress. Similarly, stress is also present in aquaculture systems which are getting more intensive and known as one of the major issues which may cause significant losses in fish farms in terms of fish population and growth, thus economy. These adverse effects of stress on the organism are defined as the response of the cell, or organism to a physiological cascade of event that occurs when the organism is attempting to resist death or reestablish homeostatic norms in the face of an insult (Schreck et al. 2001).

Grading, transportation, and vaccination are the components of modern intensive fish culture which may have negative effects on fish (Iwama 1998). Stocking density, temperature and starvation, can also be listed as other several major stressors encountered in fish farms (Küçükgül and Şahan 2008). Moreover, diet and feeding technique both have strong effects on stress responses, subsequent stress tolerance, health and the occurrence of aggressive behavior over fish (Ashley 2006).

Although the aquacultural industry has many tools available for monitoring and preventing stress, exact solutions for stress issues still have not been entirely found. Therefore, many researches have been carried out in order to prevent cultured organism from stress factors. Therefore, strategies to enhance culture conditions while minimizing stress responses and avoiding aggression is vital (Ashley 2006).

To define the effects of stressors on fish, hormonal responses are widely used in studies. It is accepted that chronic stress affects metabolism and growth of fish through the action of cortisol and therefore, cortisol is widely used as a model to simulate chronic stress (Van Weerd and Komen 1998). Many studies have shown rapid rises in plasma cortisol levels in response to a variety of acute stressors in fish (Balm and Pottinger 1995; Barton and

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Iwama 1991; Rotllant et al. 2003; Ruane et al. 2001; Vijayan et al. 1997; Waring et al. 1996). Moreover, thyroid hormones (THs) are known for their plethora of physiological actions which spread their effects on development, growth and reproduction of fishes (Gorbman 1969; Eales 1979; Eales 1985; Grau 1988; Leatherland 1994; Power et al. 2001; Peter 2007; Blanton and Specker 2007; Peter 2011). The sensitivity of thyroid axis and the involvement of thyroid hormones (THs) therefore, support a role for THs in stress response of fish (Peter 2011). Many other hormones have also been found to play a role in stress response in fish including prolactin, Melanocyte-stimulating hormone (MSH) and testosterone (Pottinger et al. 1992; Wendelaar Bonga 1997; Sumpter 1997; Ismail-Beigi et al. 1986; Peter 2007; Peter 2011).

Production of freshwater fishes has always been dominated by carps (71.9 percent, 24.2 million tons; FAO, 2012). One of the major species over carps in aquaculture is common carp (*Cyprinus carpio* L.) which have an important economic value and trade potential for aquaculture over the world and it is also used extensively in experimental studies as model fish (Bongers et al. 1998). Despite, being one of the most cultured fish species over the world, the effects of several stressors on common carp (*Cyprinus carpio*) have been poorly documented.

The aim of this study is to investigate the effects of temperature and food deprivation as stressors on hormonal responses of juvenile common carp. For this purpose, changes on the level of hormones such as Plasma Cortisol, Triiodothyronine (T3), Thyroxine (T4), Thyroid-stimulating Hormone (TSH) and Prolactin have been determined.

Material and methods

Fish and Experimental Design

Cyprinus carpio juveniles (240) with an average body weight of 21.8±0.6 g obtained from broodstocks held at Aquaculture Research Center of Atatürk University. Water at a constant temperature of 9°C was obtained from a well adjacent to experimental unit. A concrete degassing pool (10 x 8 x 1.5 m) was used in order to counteract the potential harmful gases and to enhance the oxygen level (Oxygen level was measured approximately 8.4 ppm after degassing pool). After degassing, water was distributed into 12 cylindrical fiberglass tanks each were containing 70 L of water. Fish were placed at a density of 20 juveniles per each tank. 1 L/min water flow was set for each kg of fish. A commercial feed was used to feed the fish during the experiment (Table 1).

Fish were distributed into each tank according to randomized blocks (Yıldız and Bircan 1994). The temperature was set to 12°C in all tanks for a 15 days adaptation period and fish were fed with commercial feed during that period. 12 h light:12 h dark photoperiod was maintained. After acclimatization, four different treatment groups were created as follows: the temperature was set to 18°C for the groups 18F and 18FD, while Group 18F was fed with commercial feed, Group 18FD was food-deprived; the temperature was set to 6°C for the Group 6F and 6FD, while Group 6F was fed with commercial feed, Group 6FD was food-deprived. All treatments were run in triplicate and samples were periodically taken in every 15th day for biochemical analysis.

Table 1. Nutritional value of the commercial fish feed

Nutrients	Values	Units
Moisture	12	(%)
Crude Protein	45	(%)
Crude Fat	12	(%)
Crude Cellulose	2.5	(%)
Crude Ash	12	(%)
Starch	8	(%)
P	1.5	(%)
Ca	1-2	(%)
W3 HUFA	15	(mg/g)
Vitamin A	12000	(IU/kg)
Vitamin C	200	(mg/kg)
Vitamin D3	2500	(IU/kg)
Vitamin E	200	(mg/kg)
Vitamin K3	5	(IU/kg)

Biochemical and Hormonal Analysis

3 fishes were randomly sampled from each triplicate and anesthetized in 10 L of water including 3g Tricaine Methanesulfonate (MS-222) and 6g NaHCO3. They were well wiped and cleaned in order to avoid mucus mixing into the blood, then blood was taken from the fish through the caudal vein by a heparinized syringe and was placed in heparinized tubes. After the blood was coagulated, the tubes were centrifuged at 6000xg for plasma separation and plasma was stored below -20°C (Ruane et al. 2001). Plasma cortisol, thyroid stimulating hormone (TSH), free thyroid hormones (T3 and T4) and Prolactin levels were determined by using ELISA kit (Neogen, Lexington, KY).

Statistical Analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test in order to compare the differences between mean values among groups. Differences between feeding and food-deprived groups in each temperature were tested using Student's t-test. Statistical significance was accepted at $P < 0.05$.

Results

Results and statistical data of cortisol, TSH, T3 and T4 levels obtained from treatment groups were given in Table 2. There were no significant differences obtained among the groups in terms of Prolactin levels, therefore those findings were not placed in Table 2.

According to multiple comparison test it has been observed that, raising the temperature from 12°C to 18°C increased the cortisol levels for the group "18F" at first period while cortisol levels for the group "18FD" increased at second period ($P < 0.05$). Differences between groups on cortisol levels were shown in Figure 1.

Table 2. Cortisol, TSH, T3 and T4 levels obtained from treatment groups.

Hormones	Trial Periods	Treatments			
		18°C + Feeding (18F)	18°C + Food Depriving (18FD)	6°C + Feeding (6F)	6°C + Food Depriving (6FD)
Cortisol	1	21.97 ± 13.08[a]	1.03 ± 0.06[b]	1.07 ± 0.12[b]	1.03 ± 0.06[b]
	2	25.87 ± 2.35[a]	29.23 ± 2.00[b]	1.70 ± 0.70[c]	2.23 ± 0.80[c]
	3	7.13 ± 4.12[a]	1.07 ± 0.12[b]	1.07 ± 0.12[b]	1.10 ± 0.17[b]
TSH	1	0.071 ± 0.077[a]	0.010 ± 0.005[a]	0.033 ± 0.048[a]	0.198 ± 0.099[b]
	2	0.006 ± 0.001	0.011 ± 0.008	0.007 ± 0.002	0.006 ± 0.001
	3	0.011 ± 0.006	0.007 ± 0.002	0.035 ± 0.047	0.015 ± 0.007
T3	1	9.14 ± 2.53[a]	10.25 ± 0.92[a]	7.42 ± 0.89[a]	3.11 ± 0.99[b]
	2	7.22 ± 1.78	8.86 ± 4.90	5.63 ± 2.91	1.65 ± 0.92
	3	5.78 ± 3.57	12.81 ± 4.19	5.22 ± 2.04	6.49 ± 2.10
T4	1	0.378 ± 0.059[a]	0.224 ± 0.098[b]	0.124 ± 0.014[bc]	0.079 ± 0.041[c]
	2	0.086 ± 0.059	0.117 ± 0.090	0.150 ± 0.080	0.024 ± 0.001
	3	0.439 ± 0.215	0.376 ± 0.124	0.194 ± 0.014	0.143 ± 0.011

Asterisks indicate significant differences between treatments (P < 0.05).

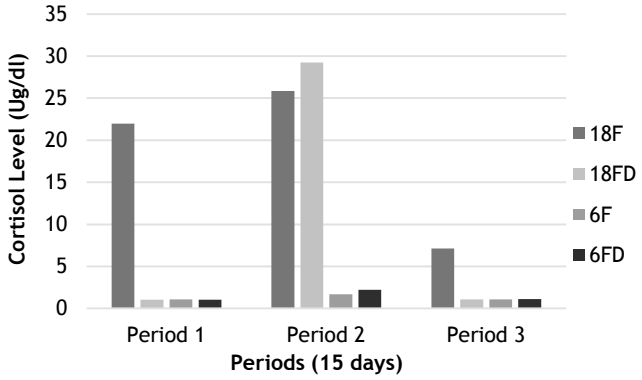

Figure 1. Cortisol level graphic for all treatments.

Thyroid hormone levels showed significant differences among the groups. According to the results, TSH and T3 levels significantly decreased in the group "6FD" for the first period ($P < 0.05$); while there were no differences obtained for the following periods ($P > 0.05$). Results for T3 level were similar for other treatments ($P > 0.05$). Graphic demonstrations of the TSH and T3 levels were shown in Figure 2 and Figure 3.

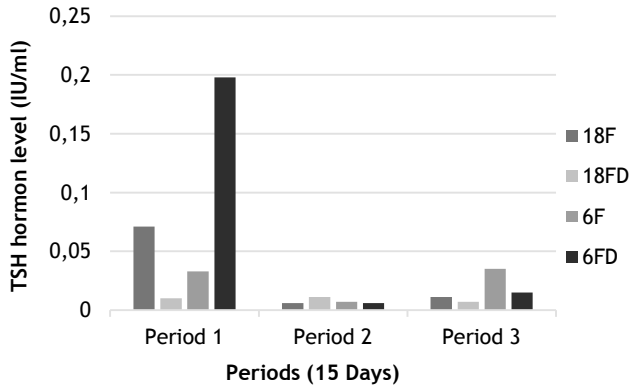

Figure 2. TSH level graphic for all treatments.

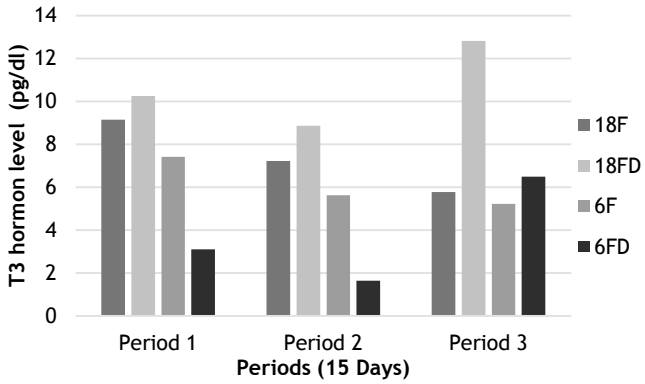

Figure 3. T3 level graphic for all treatments.

Significant differences were obtained in terms of T4 level among treatments at first period; while the highest decrease was occurred in the group "6F" and "6FD", the lowest decrease was occurred in the group "18F" ($P < 0.05$). There were no differences obtained for T4 levels for the following periods ($P > 0.05$) (Figure 4).

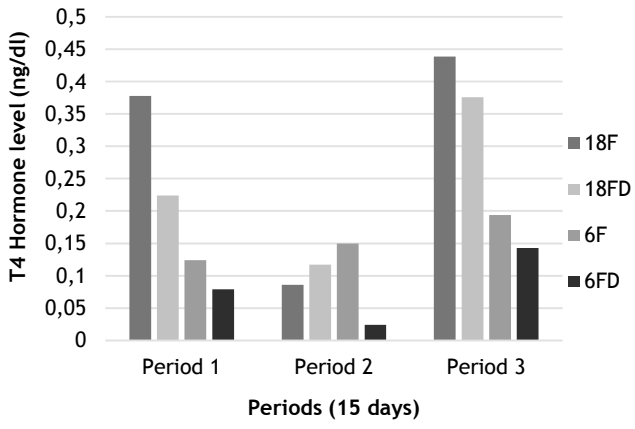

Figure 4. T4 hormone level graphic for all treatments.

The statistical differences were found to be important only for the first period of the trial for TSH, T3 and T4 levels.

Discussion

An immediate physiological response is generated by fish when a stress factor is perceived (Mazeaud et al. 1977). Food deprivation is one of the most effective techniques for the examination of endocrine-nutrient interactions. Since periods of fasting are often a natural part of the annual cycle, many fish species are highly tolerant of prolonged food deprivation. This tolerance provides an opportunity to test the dynamics of endocrine response to the cessation of nutrient intake as well as the role of hormones in the mobilization of nutrient from storage (Navarro and Gutiérrez 1995).

Plasma cortisol level in many different fish species normally varies between 2 and 42 ng/mL (Gamperl et al. 1994) and these levels can rise up from 20 to 500 ng/mL in different conditions. Despite being fairly domesticated, cyprinid fish can also produce very high cortisol responses against stress factors (Pottinger et al. 2000). Due to importance of the cortisol for determination of stress level, both high temperature and food deprivation were assumed as important factors for this trial. Because cortisol levels increased at first period for the group "18F" while cortisol levels increased only at second period for the group"18FD". However, it has been thought that fish were adapted to stress conditions at the end of the 45 days because of reverting back on cortisol levels.

Fish are also rarely affected by several combined stressors at the same time and a cumulative response is occurred against stressors. For instance, when a rainbow trout subjected to an acidic environment in conjunction with a handling manipulation, glucose and plasma cortisol levels increase while sodium concentration decreases (Barton et al. 1985). It has been understood from this trial that, the temperature and food-deprivation stressors are not effective independently, while significantly effective when the stressors are simultaneous.

Stress also has effects on Thyroid (TSH, T3, and T4) and prolactin levels (Brown et al. 1978; Spieler and Meier 1976). There were no significant differences obtained in terms of Prolactin levels in this experiment however Thyroid levels changed among the treatments. Inhibition of thyroid function appears to be one of the most consistent endocrine responses to food deprivation. Circulating levels of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) decline associated with the diminished growth induced by food deprivation (reviewed in Eales 1988; Leatherland 1994). It has been proposed that starvation influences the hypothalamic –pituitary-thyroid axis at multiple levels, reducing the sensitivity of the thyroid to thyrotropin stimulation as well as the peripheral conversion of T4 to T3 (Leatherland and Farbridge 1992), resulting in differential effects on circulating T3 and T4 (MacKenzie et al. 1993).

In general, it has been thought that feeding of the mirror carp is stopped at temperatures below 10°C therefore feeding is not done at those temperatures. However, it has been convinced that at least a trace of feeding found to be useful for maintaining the hormonal balance of fish when sudden temperature decreases occurred in winter.

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