RESEARCH ARTICLE (Open Access)

Biochemical and hormonal changes during prolonged starvation in common carp (*Cyprinus carpio*)

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Abstract

Long-term starvation can greatly disrupt homeostasis and leads to oxidative stress. In the present study, a comparison was made between stress hormone, glucose and acute phase protein levels in starvation. To evaluate the effect of starvation on plasma cortisol, glucose and ceruloplasmin and fibrinogen levels of carp, fishes were starved for a period of seven months. Blood samples were obtained from the vena caudalis after electrical stunning. The collected blood samples (approximately 2.0 ml) were centrifuged at 2500 rpm for 5 min at room temperature and plasma was immediately separated and stored at -20°C until analysis. Plasma cortisol was assayed by laser fluorescence reader (i-chroma TM Reader Boditech Med Incorporated). Plasma glucose levels were analysed with commercial diagnostic kits. The concentrations of acute phase proteins were estimated by nephelometric and colorimetrically methods. The results revealed that plasma cortisol level were not affected by 150-day starvation (17.31±0.37). However, after 180 (17.81±0.90) and 210 days (18.42±0.22) in fish previously starved values increased (P>0.05) and remained high until the end of the experimental period. The release of cortisol was accompanied with reduction in glucose activity in the 210 day (2.15±0.46) but significant changes were not detected (P>0.05). An increment in the levels of ceruloplasmin (31.93±4.21) of starved fishes was recorded, whereas a decline in the fibrinogen values was observed (0.83±0.16).

Keywords: Acute phase protein, carp, cortisol, glucose, starvation.

1. Introduction

The health of fish can be affected by environmental factors (stress), pathogens as well as by nutrition. Under natural and production conditions, many fish species are affected by prolonged periods of starvation. Starvation, as a stress factor may affect biochemical processes of the fish e.g. increase catecholamines and cortisol excretion, which has a direct effect on carbohydrate (glucose) metabilism leading to glycogenolysis [17]. Acute phase proteins (APPs) can be used as diagnostic tool in many diseases even starvation and thus provide an alternative means of monitoring animal health [7]. APPs are more useful for monitoring health than cytokines, because the latter are cleared from the circulation within a few hours, whereas APP levels after a single stimulus remain unchanged for 48 h or longer period [5]. The aim of this study was to find out how prolonged starvation would affect levels of certain serum biochemical parameters and hormones in carp.

2. Material and Methods

Twelve immature, starved 5 months common carp (initial weight 462.04±2.13 g*)* were provided by fish farm Tundzha 73, Bulgaria and transported alive to the aquaculture experimental base at Trakia University. The fish were acclimatized for 2 weeks and were randomly assigned in 3 tanks with a capacity of 1.000 L. The experiment was performed according to the Guidelines of the European Union (2010/63/UE) and Animal Welfare Act and approved by the Committee on Animal Experimentation at the Trakia University, Stara Zagora, Bulgaria. The

fish were electro-anesthetized by mobile device. The anesthesia process in this and subsequent procedures were completed in less than 3 sec. Blood samples for analysis werecollected monthly, for 3 month in containers (2.0-2.5 ml) with heparin as anticoagulant reagent. Samples were centrifuged at 2500 rpm for 10 min (Ohaus FC5515, Ohaus Corp., Germany) and plasma was immediately separated and stored at -20°C until analysis. The concentrations of glucose was analysed using a Semi-Auto Chemistry Analyzer (Mindray BA-88, Mindray Bio-Medical Electronics, China) and commercial kits (Giesse Diagnostics, Italy). Plasma cortisol was assayed by laser fluorescence reader (i-chroma TM Reader, Boditech Med Incorporated, Germany). The concentration of fibrinogen and ceruloplasmin was estimated by nephelometric and spectrophotometric (λ=530 nm, Carl-Zeiss Jena, Germany) determination respectively. Statistical analysis was performed using one-way analysis of variance (ANOVA). The results were processed with software Statistica v.10 (StatSoft Inc., USA, 2002). All results were presented as mean and standard deviation of the mean (Mean±SD).

3. Results and Discussion

As shown in Table 1, long-term starvation resulted in insignificant changes in the levels of analysed plasma components. It is known that long-term starvation can greatly disrupt homeostasis and may induce different responses on hormonal status. The secretion of corticoids in response to environmental stressors is present in teleostean and other bony fishes [11]. It was found that the plasma cortisol levels have wide variation range and can rise up during starvation in some cyprinid fish such common carp (*Cyprinus carpio*) 50-450 mg/ml-1 [3; 13], gold fish (*Carassius auratus*) 5-300 mg/ml-1 [16], *Labeo rohita* 4.75 μg/ml (4750 ng/ml), *Cirrhanus mrigala* 5.75 μg/ml (5750 ng/ml), [9], *Garra gotyla gotyla*, 115.0-210.5 ng/ml. Generally, on the onset of stress, the release of cortisol in fish is delayed and a period of active secretion is followed by concentration increase. Our results revealed that plasma cortisol levels were not affected by 150-day starvation (Table 1). However, prolonged starvation lead to increased values of the hormone (P>0.05) – 180 days (17.81±0.90) and 210 days (18.42±0.22), and remained high until the end of the experimental period. The findings of the current experiment were similar to that shown in cyprinid fish (*Garra gotyla gotyla*) after fasting for period of 9 weeks [10]. Similar findings have also been observed in relation between temperature and food deprivation as stressors in juvenile common carp [1].

Table 1. Changes in levels of selected blood serum components in carp exposed to starvation (χ±SD), n = 12

Parameteres	Starvation time (days)		
	150	**180**	**210**
Cortisol (μg/dL)	17.31±0.37	17.81±0.90	18.42±0.22
Glucose (mM/L)	2.76±0.55	2.48±0.31	2.15±0.46
Ceruloplasmin (mg/L)	26.27±5.22	32.50±5.87	31.93±4.21
Fibrinogen (g/L)	1.17±0.86	0.96±0.28	0.83±0.16

Hyperglycaemia in higher vertebrates, is an effect of some stressors (e.g temperature, diet) acting upon the cortisol as the hormone considered in controlling carbohydrate metabolism. The increased release of cortisol, accelerates the degradation of proteins to amino acids and their relocation from body tissues to blood. Immediately thereafter, in the liver cells they are converted to glucose by gluconeogenesis [2]. In the study described, the most conspicuous effect of starvation was visible in the plasma glucose levels - a decrease in starvation fish (2.15±0.46) was observed. This might be result of adaptive response to food shortage. Some authors suggest that glucose reduction in long-term starvation may be attributed to a hepatopancreas and muscle glycogen depletion [12; 15]. A similar significant reduction in glucose activity was observed in carp after fasting for a period of 4 weeks [12; 13] and 12 weeks [4]. Acute phase proteins are a nonspecific response to inflammation or tissue damage and include positive proteins (fibrinogen, ceruloplasmin and others) which are produced by the liver in response to cytokines released. The acute phase reaction can be used for assessment of general health, including starvation. It was found that ceruloplasmin concentrations were lower within the first five months (26.27±5.22), comparing to the last month (31.93±4.21) of starvation. The highest concentration was

observed in the 6th month (32.50±5.87) of the experiment. Similar increment and decline in the levels of ceruloplasmin was observed in starving fish during wintering [8].

The presented data do not exhibit significant changes in the median values of fibrinogen between month 5 (1.17±0.86) vs. month 7 (0.83±0.16). During long-term starvation or anorexia, general depression of hepatic protein synthesis occurs and response of positive acute phase variables may be less evident. Hypofibrinogenemia can occur in liver failure and cachexia [6].

4. Conclusions

The results of this study showed insignificant changes in some serum parameters responsible for stress in carp after feed deprivation that are indicative of the relation between starvation effects and fasting period. The absence of mortality and the good condition of the fish indicate that common carp can survive long periods of food shortage.

5. Acknowledgements

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6. References

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