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# Alterations in serum metabolites and enzymes of juvenile common carp (Cyprinus carpio) during long-term starvation

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***ABSTRACT***

*The objective of the present study was to determine the changes of some serum parameters in common carp (Cyprinus carpio) during 12-weeks-long starvation. Results indicate that in case of totally food restriction an increased mobilization of glycogen and lipids occurs in common carp. Fish replace the lacking energy in a complex way, main processes is the oxidation of body lipids, which constituted the major energy source for starved carp.*
(Keywords: common carp, starvation, metabolism, blood serum composition)

## INTRODUCTION

Most of the fish species are exposed to short-term or long-term starvation during their lifespan both under natural and artificial conditions. Blood chemical parameters change significantly during long-term starvation. Alterations of blood plasma lipid and amino acid content are in context of the extent of the glyconeogenesis and lipid mobilization (*Friedrich and Stepanowska*, 2001; *Stepanowska et al*., 2006; *Hung et al*., 1997; *Figueiredo-Garutti et al*., 2002). Level of plasma glucose generally remains unchanged during starvation in most fish species (*Gillis and Ballantyne*, 1996), because it is a homeostatic parameter. The glucose, total lipid and protein level of serum have been investigated in carp related to starvation (*Friedrich and Stepanowska*, 2001) but there is a lack of information regarding other metabolites and enzymes.

Therefore, the aim of our recent study was to analyse the changes of some serum parameters (protein, albumin, cholesterol, triacylglycerol, LDH and ALP) in common carp (*Cyprinus carpio*) during 12-week-long starvation. The changes of proximate body composition and somatic indices of the same population during 12-week-long starvation were reported previously (*Varga et al*., 2014).

## MATERIAL AND METHODS

Common carp (*Cyprinus carpio*) fingerlings (4 g) were introduced into a recirculation system in the Fish Laboratory of the Kaposvár University (Hungary). Fish were reared in fish tanks till they were reached 25 g live-weight. Feeding of carps during this period was ad libitum with a commercial fish feed (Aller Aqua). Fish were adapted to the artificial conditions and feed, so stress and disturbing environmental circumstances were excluded.

For the experiment 200 idividuals were used (20 replications x 10 fish). They were stocked into 60 l individually aerated fish tanks of a small recirculation system with a simple biofilter unit. Feeding was totally withdrawn for 12 weeks. Water temperature was measured daily (n=84), the average water temperature was 18.3 ± 1.5 ˚C during the experiment.

Sample collection was carried out initially and every two weeks during the experiment. In every time-point 10 randomly selected individuals were sampled (Ntotal = 7 x 10 = 70). Blood samples were taken from tail vein (vena caudalis). After withdrawal into Eppendorf tubes the blood was immediately placed on ice, left to clot, centrifuged (1500 g / 10 min) and serum were stored frozen (-70 $^{o}$C) until analysis. Clinical chemical analysis (serum protein, albumin, triacylglycerol, cholesterol concentration, LDH and ALP activity) was performed on an automated equipment (Hitachi 917) in a single analytical run.

Acquired data were tested for normality (Shapiro-Wilk test). For the analysis of the effect of starvation on blood parameters one-way ANOVA was used (time as fix factor), followed by Tukey post hoc test. SPSS 10 for Windows (1999) was used for the statistical analyses.

The experiment was approved by the Animal Experimentation Ethics Committee of the University of Kaposvár, as allowed by the Somogy County Animal Health and Food Control Authority (allowance no.: XV-I-31/446-10/2012).

## RESULTS AND DISCUSSION

During the starvation period no mortality was recorded. The basic serum clinical chemical results are summarized in *Table 1*. 12 week long starvation led to a significant decrease of total protein, triacylglycerol concentration and LDH activity. Serum albumin and cholesterol concentration and ALP activity did not indicate the effect of starvation.

While the total body protein content remained unchanged (*Varga et al.*, 2014), the concentration of blood total protein significantly decreased. This decrease in the blood total protein content may refer to the enhanced gluconeogenesis. Several studies have shown gluconeogenesis to be more important than glycolysis in maintaining the glucose level in the starving fish blood (*Murat et al*., 1978; *Love*, 1980). A similar effect was also observed in carp during starvation. Serum protein content decreased, without the decrease of body protein content (Shimeno et al., 1981). Moreover, we may add that this process happens based on the degradation of globulins, since albumin concentration was unaltered (*Thrall*, 2004). Most probably the albumin concentration was fully maintained since albumin is responsible for keeping constant colloid-oncotic pressure of the blood (*Michelis et al*., 2010)

It was stated, that during long term starvation fish species use serum protein as an energy source via gluconeogenesis (*Cowey et al*., 1977; *Love*, 1980). Blood triacylglycerol content was observed to significantly decrease. It was declined by 43 percent in the first two weeks as a direct result of food withdrawal. In case of starvation the role of glycerols turns into more important as a glucose precursor (*Friedrich and Stepanowska et al*, 2001). Starving muscles replace the utilization of glucose by the oxidation of lipids. The observed decrease of blood TG concentration (coupled with the significant decrease of total body fat content) confirms that lipolysis of intramuscular

lipids was the major energy source for locomotion during starvation in juvenile carps. It was supported by other authors (*Shimeno et al*., 1990; *Hung et al*., 1997).

**Table 1.**

**The serum basic clinical chemical results of the starved carp**

| | Weeks | | | | | | | Sig |
|---|---|---|---|---|---|---|---|---|
| | **0** | **2** | **4** | **6** | **8** | **10** | **12** | |
| | Mean ± St. Dev. | Mean ± St. Dev. | Mean ± St. Dev. | Mean ± St. Dev. | Mean ± St. Dev. | Mean ± St. Dev. | Mean ± St. Dev. | P |
| **Total protein (g/L)** | 24.4 ± 3.57$^{ab}$ | 27.0 ± 5.87$^{a}$ | 21.78 ± 3.38$^{b}$ | 22.9 ± 1.69$^{ab}$ | 21.11 ± 2.42$^{b}$ | 21.1 ± 1.59$^{b}$ | 20.88 ± 1.25$^{b}$ | <0. 001 |
| **Albumin (g/L)** | 6.88 ± 0.64$^{a}$ | 6.29 ± 1.7$^{a}$ | 6.22 ± 0.97$^{a}$ | 8.0 ± 0.87$^{a}$ | 5.78 ± 2.22$^{a}$ | 6.8 ± 1.03$^{a}$ | 6.5 ± 1.41$^{a}$ | NS |
| **Triacylglycerol (mmol/L)** | 1.97 ± 0.36$^{a}$ | 1.12 ± 0,18$^{b}$ | 1.05 ± 0.17$^{b}$ | 1.19 ± 0,18$^{b}$ | 1.02 ± 0.2$^{b}$ | 1.09 ± 0.15$^{b}$ | 1.06 ± 0.12$^{b}$ | <0. 001 |
| **Cholesterol (mmol/L)** | 3.59 ± 0.82$^{a}$ | 3.75 ± 0.99$^{a}$ | 3.4 ± 0.8$^{a}$ | 4.13 ± 0.65$^{a}$ | 3.38 ± 0.88$^{a}$ | 3.71 ± 0.61$^{a}$ | 4.24 ± 0.55$^{a}$ | NS |
| **LDH (IU/L)** | 1378.2 ± 731.91$^{a}$ | 1535.4 ± 597.52$^{a}$ | 882.0 ± 409.64$^{ab}$ | 752.44 ± 313.88$^{b}$ | 778.67 ± 466.51$^{b}$ | 747.2 ± 421.78$^{b}$ | 475.29 ± 243.68$^{b}$ | <0. 001 |
| **ALP (IU/L)** | 35.6 ± 12.29$^{ab}$ | 48.7 ±29.47$^{ab}$ | 34.0 ± 36.21$^{a}$ | 49.67 ± 44.33$^{ab}$ | 28.22 ± 11.63$^{ab}$ | 37.1 ± 18.2$^{ab}$ | 51.88 ± 40.83$^{b}$ | NS |

Means bearing different small superscript letters are significantly different (P<0.05).

Lactate dehydrogenase (LDH) was showing a decreasing activity during the starvation period in carps. After two weeks LDH concentration decreased significantly. It is thus supposed that glycolytic activities occur only in the first two weeks, till stored glycogen was depleted. After glycogen-lactate transition lactate enters to the glyconeogenesis (Cory cycle). Lactate is a known glycogenic substrate of fish muscles (*Pagnotta and Milligan*, 1991).

Alkaline phosphatise has two sources in the blood. One isoform is connected to the osteoblast activity, while the other is of intestinal origin. The alterations may be connected with the fact during prolonged starvation the gastrointestinal tract undergoes a marked dystrophy, as underscored by the VSI as well (*Varga et al.*, 2014).

Summarized, results indicate that in case of totally food restriction an increased mobilization of glycogen and lipids occurs in common carp. After 2–4 weeks animals were adapted to the lack of external energy supply and slight intramuscular lipid oxidation was maintained (*Varga et al*., 2014). Fish replace the lacking energy in a complex way in case of long term starvation period. Main processes are glyconeogenesis and oxidation of body lipids, which constituted the major energy source for starved carp.

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