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Effects of pre-sampling fasting on serum characteristics of common carp (*Cyprinus carpio* L.)

Seyyed Morteza Hoseini[1]*, Melika Ghelichpour[1]

[1]Department of Fisheries, Faculty of Fisheries and Environment, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Abstract: Common carp, *Cyprinus carpio* (L.) were blood-sampled after 0, 2, 6, 12, 24, 48 and 72 h fasting to find serum baseline levels. There was a significant difference in serum glucose, lactate, triglyceride and total protein but not cholesterol, albumin and calcium levels among the treatments. Glucose levels increased with fasting tine and reached to a peak after 6 h. Then, the glucose level decreased to the lowest level after 12 h. Changes of glucose and lactate had reverse trends, as lactate levels decreased with fasting time and reached to the dip point at 6 h and, thereafter, the levels increased to peak point at 12 h. Serum glucose and lactate levels showed stable values during 24-72 h of fasting, compared with the values measured at 0 h. Triglyceride levels showed an increasing trend parallel to that of the fasting period and reached to a peak point after 6 h. The levels reached 0 h values at 24 h and showed further decrement at 48 and 72h. Total protein showed elevation while fasting progressed and reached the peak point at 6 h and remained stable during 24-48 h fasting; However, it decreased after 72 h fasting. According to the results, cholesterol, calcium and albumin baseline levels were not affected by 0-72 h fasting. Glucose and lactate baseline could be determined after 24-72 h fasting. Total protein baseline could be determined after 24-48 h fasting. Triglyceride levels are significantly affected by fasting period which should be taken into account when it is measured. Possible mechanisms involving in common carp serum fluctuation over 0-72 h fasting period are discussed.

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Introduction

Blood sampling is necessary for monitoring of fish physiological condition in aquaculture and researches. Period of pre-sampling fasting may alter the serum characteristics, thus choosing the correct sampling procedure guarantees precise scientific outputs. Several researchers (Schurmann and Steffensen, 1997; Stillwell and Benfey, 1997; Wagner et al., 2003) starved the fish prior to sampling to ensure the fish are in post absorptive state. During fasting period, the level of metabolites changes under the modulation of the hormones, mainly pancreatics (Blasco et al., 1992). On the other hand, the metabolite levels are indicator of some other phenomenon like as stress and nutritional condition. Thus, when the results of different studies are compared, the fasting period might mask or magnify the differences.

To date, there are no studies on the effect of pre-sampling fating period on the fish blood biochemistry exception of the study by Shi et al. (2010) on Amur sturgeon, *Acipenser schrenckii*. Researchers chose different fasting periods prior to blood collection (Iversen et al., 2003; Holloway et al., 2004; Roubach et al., 2005; Bystriansky et al., 2006; Hoseini, 2010; Hoseini and Hosseini, 2010, Hoseini et al., 2010). Most studies have used a 24-h fasting period (Cho and Heath, 2000; Roubach et al., 2005; Bystriansky et al., 2006; Hoseini, 2010; Hoseini and Hosseini, 2010, Hoseini et al., 2010). However, Cataldi et al. (1998) and Wagner et al. (2003) chose 48-h pre-sampling fasting to study the

* Corresponding author: Seyyed Morteza Hoseini
E-mail address: seyyedmorteza.hoseini@gmail.com
Tel: +989112750713

stress response in Adriatic sturgeon, *Acipenser naccarii* and Atlantic salmon, *Salmo salar* (L). The others chose other periods, for example, 72 h or no fasting period (Iversen et al., 2003; Hyvarinen et al., 2004). On the other hand, other researchers investigated the effect of different factors on serum biochemistry of fish without mentioning the fasting period (Ortuno et al., 2002a, b; Holloway et al., 2004).

Common carp, *C. carpio* (L.) is a species with long term fasting tolerance (Créach, 1972) during which the muscle protein mobilizes substantially. Blasco et al. (1992) found decrease in common carp hepatosomatic index after 8 days fasting, mainly due to glycogen mobilization. Blasco et al. (1992) reported short (2, 5 and 10 days) and long (20 and 50 days) term change in carp serum metabolites, insulin and glucagon levels. However the changes in serum glucose, lactate, triglyceride, cholesterol and total protein was not monitored during the first three days (the period which the researchers used as pre-sampling fasting). Thus it might be of interest to determine the effect of fasting period on serum biochemistry in common carp. In this case, there is not a common procedure. For example, while Ruane et al. (2002a, b) and Velisek et al. (2009) did not have a pre-sampling fasting period in their studies, Ruane et al. (2001), Sudova et al. (2009), Hoseini (2010), Hoseini and Hosseini (2010) and Hoseini et al. (2010) starved their specimens for 24 h prior to blood collection. Thus, the aim of the present study was to investigate the changes in serum biochemistry in common carp starved for 0, 2, 6, 12, 24, 48 and 72 h.

Materials and methods

Fish were obtained from the artificial propagation of wild-caught brood stocks from Caspian Sea. The larvae were reared to juvenile (120 g) in the earthen ponds over 10 months (April 2010 - January 2011). Fish fed on natural food of the ponds as well as on artificial diet (34% protein and 8.5% lipid). Total of 150 fish were transported to the laboratory using fish transporting tanks equipped to a pure oxygen supplier.

A total of 90 fish were stocked in 15 rectangular glass tanks (0.8×0.55×0.55 m). All tanks were filled with 170 L dechlorinated tap water. Continuous aeration was provided to all tanks and fish were fed on artificial diet (34% protein and 8.5% lipid) based on 1.4% of fish body weight, daily. Fish were fed twice (08:00 am and 20:00 pm) a day. Water exchange was performed daily corresponding to 85% of the tanks' water volume. Water quality was as follow: temperature= 20.2 ± 1.1 ºC, dissolved oxygen = 6.74 ± 0.61 ppm, pH = 7.5 ± 0.18 and N-nitrite = 0.001 ppm. After 15 days acclimation, fish were blood-sampled at 0, 2, 6, 12, 24, 48 and 72 h after the last feeding (treatments 0, 2, 6, 12, 24, 48 and 72 h). Blood sampling was conducted at 08:00; thus, to avoid the potential effect of daily rhythm on fish physiological status, fasting was begun at different time. Accordingly, feeding was ceased at 08:00 in treatments 0, 24, 48 and 72 h, while the last meal of the treatment 12, 6 and 2 was on 20:00, 02:00 and 06:00, respectively. Each treatment was constituted of three tanks. Two fish were sampled from each tank to attain 6 samples per treatment. To avoid catch-born stress, fish were netted by a large dip net to allow 2 fish capture in a single effort. Fish were immediately placed in anesthetic bath (eugenol 100 ppm) over 40 s, prior to caudal puncture. After blood-sampling, the specimens were placed in a freshwater tank to recover and their condition was monitored over a 3-day period.

Blood samples were collected in non-heparinized 1.5-ml tubes and centrifuged for 7 min at 5000 rpm to separate serum. Serum samples were stored at -80 °C until further analyses. Serum lactate levels were determined enzymatically using a commercial kit (Pars Azmun Co. Ltd, Tehran, Iran). Serum levels of glucose, cholesterol, triglyceride, albumin and calcium were determined by glucose-oxidase, cholesterol oxidase, glycerol-3-phosphate oxidase, bromocresol green and cresolphthalein complexone methods, respectively (Pars Azmun Co. Ltd, Tehran, Iran). Serum total protein was determined using the biuret reagent (Pars Azmun Co. Ltd, Tehran, Iran).

Samples were analyzed three times and the averages over times were used for statistical analyses.

Normality of data and homogeneity of variance were examined using Kolmogorove-Smirnov and Levene's tests, respectively. Data were analyzed using a one-way ANOVA followed by LSD test, using the statistical software SPSS version 16. All data are presented as mean ± SD.

Results

Serum levels of glucose and lactate are shown in figures 1 and 2, respectively. Glucose and lactate levels were significantly affected by fasting period (P<0.05). There was a reverse trend in glucose and lactate levels as high levels of glucose were accompanied with low levels of lactate and vice versa. Glucose levels increased with increase of fasting duration and reached to a peak at 6 h, when it decreased until the hour 12. Then it increased again and, at 24-72 h, it reached to the concentrations recorded at the 0 h levels. In the contrary, lactate levels decreased in line with fasting period and reached its dip point at 6 h, thereafter it showed an increase and reached its peak at 12 h. Lactate levels at 24, 48 and 72 h decreased again and reached the level of 0 h.

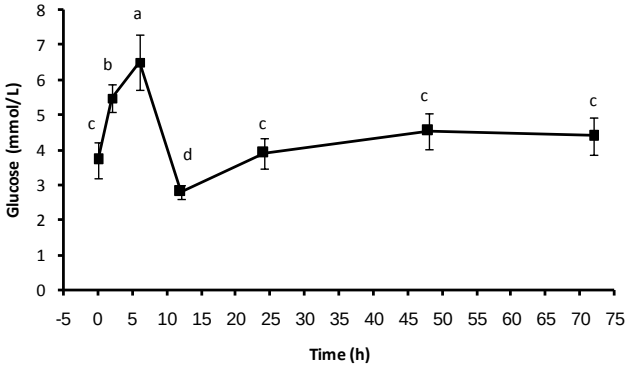

Figure 1. common carp serum glucose levels during fasting. Different letters show significant difference (P< 0.05).

Serum levels of triglyceride are shown in figures 3. Serum triglyceride levels were significantly different among the fasting points (P<0.05). The levels showed increase and reached the peak at 6 h. The levels, then, started to fall and reached the 0 h level at 24 h. The levels continued the decreasing pattern and reached the lowest levels at 72 h.

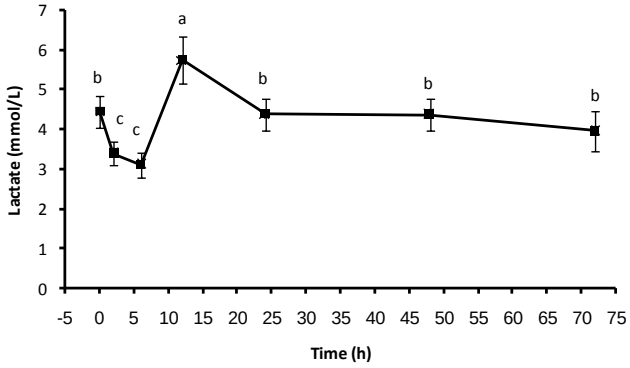

Figure 2. common carp serum lactate levels during fasting. Different letters show significant difference (P< 0.05). n = 6.

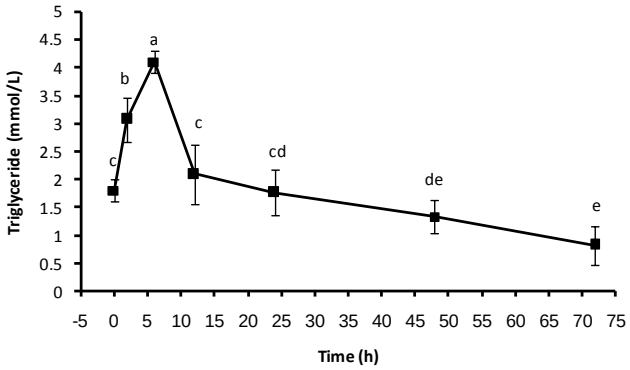

Figure 3. common carp serum triglyceride levels during fasting. Different letters show significant difference (P< 0.05). n = 6.

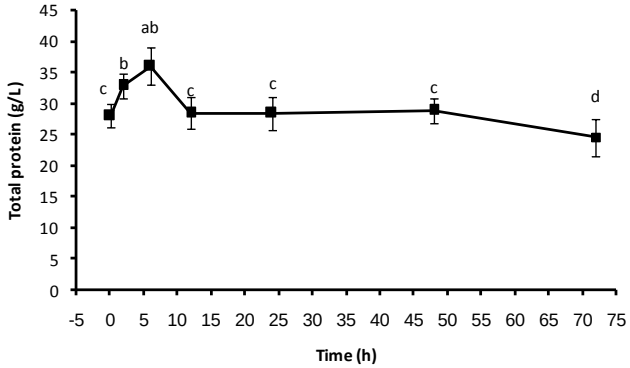

Figure 4. common carp serum total protein levels during fasting. Different letters show significant difference (P< 0.05). n = 6.

Changes in serum total protein are shown in figure 4. Total protein values were significantly affected by fasting period (P<0.05). The levels increased and reached the peak point at 6 h which was followed by a sharp decrease at 12 h. The levels remained

unchanged until 48 h, however, it showed a significant decrease at 72 h.

Table 1. serum cholesterol (mmol L^{-1}), calcium (mmol L^{-1}) and albumin (g L^{-1}) levels during fasting period in common carp. No significant difference was detected. n = 6.

Fasting duration (h)	Cholesterol	Calcium	Albumin
0	3.92 ± 0.60	1.75 ± 0.12	9.00 ± 1.15
2	4.73 ± 0.60	1.96 ± 0.11	9.92 ± 1.62
6	4.99 ± 0.40	1.90 ± 1.14	9.80 ± 1.51
12	4.00 ± 0.77	1.82 ± 0.19	8.00 ± 0.60
24	3.87 ± 0.63	1.81 ± 0.14	9.11 ± 0.90
48	4.38 ± 1.08	1.75 ± 0.18	9.35 ± 1.75
72	4.21 ± 1.19	1.88 ± 0.17	8.17 ± 2.16

Serum cholesterol, calcium and albumin levels are shown in table 1. There was no significant difference among the fasting points.

Discussion

Homeostasis of energy in fish during food deprivation is directly related to mobilization of energy reserves such as lipids, activation of hepatic gluconeogenesis and reduction in the rate of glucose utilization (Sheridan and Mommsen, 1991; Navarro and Gutierrez, 1995). Protein catabolism (Andenen et al., 1991), glycogenolysis (Vijayan and Moon, 1992), and tissue storage release and uptake are the factors affecting metabolite level of serum. On the other hand, serum levels of metabolite are affected by stressors (reviewed by Wendelaar Bonga, 1997). However, as the fish in this study were from the same origin and sampled following a constant protocol, any changes in the serum biochemistry are supposed to be as a result of fasting period.

There was no significant difference in the tested parameters between 0 and 24 h fasting. The reason seems be due to short time between feeding and blood collection at 0 h. In fact, fish of 0 h groups were sampled immediately after last meal, which did not allow nutrient absorption from the gut.

There is only one study (Shi et al., 2010) focusing on the effect of short-term fasting on fish serum biochemistry, nevertheless, there are some studies on the effect of long term starvation or feeding rate on carp serum biochemistry (Blasco et al., 1992; Shimeno et al., 1997; Ruane et al., 2002). However, results of these studies could not be used to determine the effect of pre-sampling fasting period of carp serum biochemistry, due to methodology and fasting period.

Glucose is one of the most important metabolites in fish serum which its level fluctuates over the fasting period (Blasco et al., 1992; Sala-Rabanal et al., 2003). At the present study, a high level of glucose at 2 and 6 h stems from absorption of glucose from gut, introduction to blood stream and decrease at 12 h which is a result of uptake by tissues and energy production. Recovery of glucose levels at 24-72 h suggests that glucose was produced via gluconeogenic and glycogenolysis (Blasco et al., 1992). Serum lactate levels showed reverse trend compared to glucose. Lactate is back transported to hepatic cells for glucose production under hypoglycemic condition. This explains reverse trends in glucose and lactate over fasting periods, where the highest levels of one item was accompanied by the lowest levels of the other one, which was reported by other researchers (Figueroa et al., 2000). Increase in lactate levels at 12 h might be as a result of activation of gluconeogenesis pathway due to fall in glucose circulating levels. Further decrement in lactate levels might be related to its utilization for glucose production and/or serum glucose elevation as a result of gluconeogenesis. At the present study, serum glucose level was statistically stable during 24 -72 h fasting, which is in agreement with the results on rainbow trout, *O. mykiss* (Figueroa et al., 2000; Congleton and Wagner, 2006) and common carp, *C. carpio* (Blasco et al., 1992). Thus it is suggested that, for common carp serum glucose and lactate analyses, fish should be fasted 24-72 h to allow serum glucose and lactate to reach the baseline level. On the other hand, Shi et al. (2010) found no change in serum glucose levels after 0, 12 and 48 h fasting and significant decrease at 24 and 72 h fasting in Amur sturgeon, *A. schrenckii*. Difference between this study and the present one, might be due to species differences, as common carp is different in some aspects of

metabolism compared to the other species, i.e. glycogen and lipid metabolism over food deprivation (Navarro and Gutierrez, 1995). On the other hand, Navarro and Gutierrez (1995) suggested that the sluggish (like carp) and active (like sturgeon) species are difference in serum glucose maintenance.

Lipids are stored as triglycerides in different tissues. Lipid mobilization occurs during fasting, with or after carbohydrate mobilization (Navarro and Gutierrez, 1995). In our study, the increase of serum triglyceride at early fasting might be as a result of absorption from the gut. Previous study by Ruane et al. (2002b) showed a decrease in triglyceride and no changes in cholesterol due to the low ration in common carp. However, the decrease of triglyceride might be as a result of supplying energy demand together with carbohydrate mobilization, in common carp (Navarro and Gutierrez, 1995). Also, decrease of triglyceride levels may come from low food intake. Lack of significant changes in cholesterol levels might be due to short term of fasting, as mentioned above. Shimeno et al. (1997) showed decrease in the both triglyceride and cholesterol levels as a result of low ration or fasting in common carp, after 30 d. Congleton and Wagner (2006) failed to detect any significant change in cholesterol levels in rainbow trout, *O. mykiss* and Chinook salmon, *Oncorhynchus tshawytscha* after 1 and 3 d fasting. There is no study on the effect of fasting on serum triglyceride and cholesterol levels in common carp, to make a precise comparison. However, Shi et al. (2010) found no significant changes in triglyceride and cholesterol levels in Amur sturgeon, *A. schrenckii*, after 0, 0.5, 1 and 2 d fasting; however, significant increase occurred at the third day. The difference in triglyceride and cholesterol patterns between the two studies might be due to species differences (sluggish carp vs. active sturgeon), diet (low lipid for carp vs. high lipid for sturgeon) and different metabolic pathways, as there are wide differences in basal levels of triglyceride (3.06 vs. 4.97 mmol l^{-1}), cholesterol (4.71 vs. 1.99 mmol l^{-1}) between the two species. Based on the results, common carp serum levels of cholesterol are not related to pre sampling fasting period, however, serum triglyceride level is significantly affected by fasting which should be taken into account when it is measured and compared with the other data.

Total protein of Serum is known as an indicator of the nutritional status which provides information on fish metabolism. There is a clear tendency for plasma proteins to decrease in fasting fish (Navarro and Gutierrez, 1995). Increase in serum total protein might be due to absorption from the last meal and increase of liver protein synthesis. At the present study, total protein of serum remained stable during the 24-48 h fasting. Thus to determine total protein baseline level in common carp, fish should be fasted for 24-48 h. Significant decrease after 72 h fasting comes from decrease in hepatic protein synthesis as well as protein catabolism for energy production.

Serum albumin did not change along with the decrease of serum total protein, suggesting the physiological importance of this protein. Measurement of the different serum proteins can help better understanding of protein catabolism over the fasting period. Change in serum total protein levels are related to the change of serum globulin levels. According to the results, common carp serum albumin baseline level is not affected by 0-72 h fasting.

In this study serum calcium were determined over the fasting period, because its linkage with serum proteins (Bjornsson et al., 1989). About half of total plasma calcium is ionized and the rest is bound to serum proteins (Andreasen, 1985; Bjornsson et al., 1989), thus decline in proteins in the fasted fish might also lower plasma calcium concentrations. However, such results were not observed at the present study. Similarly, Andreasen (1985) failed to detect any correlation between plasma protein and total calcium concentrations. Congleton and Wagner (2006) did not find any changes in plasma calcium after 1 and 3 d fasting and even after extended periods of fasting (14 and 24 d, when plasma total protein decreased) in rainbow trout, *O. mykiss* and Chinook salmon, *O. tshawytscha*. Results of the

present study showed that serum calcium level is not sensitive to ore sampling fasting period.

It is concluded that pre-sampling fasting period affects serum levels of glucose, lactate, triglyceride and total protein, in common carp. Accordingly, serum glucose and lactate levels could be determined after 24-72 h fasting, while serum baseline level of total protein should be determined after 24-48 h fasting. The results, also, show serum triglyceride fluctuates over the fasting period which should be taken into account when it is measured. Likewise, common carp serum cholesterol, albumin and calcium levels are not sensitive to fasting.

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