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Fish physiology

EFFECT OF STARVATION ON NUTRITIVE VALUE OF CARP (*CYPRINUS CARPIO* L.) AND SELECTED BIOCHEMICAL COMPONENTS OF ITS BLOOD

WPŁYW GŁODZENIA NA WARTOŚĆ ODŻYWCZĄ I WYBRANE SKŁADNIKI BIOCHEMICZNE WE KRWI KARPIA (*CYPRINUS CARPIO* L.)

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The experiment, carried out in the aquarium room of the Faculty of Marine Fisheries and Food Technology, involved 72 carp juveniles. The fish were kept in 6 aquaria for 12 weeks without food. Levels of crude protein, AspAT and AlAT activities, levels of glucose, triacylglycerols, cholesterol and its fractions as well as body weight reduction and body composition were analysed.

Starvation was found to significantly affect the crude protein level and AspAT activity as well as the blood levels of glucose, triacylglycerols, cholesterol and its fraction and the body chemical composition. The significant changes occurred both in relation to the initial status of the fish and between successive stages of the experiment.

INTRODUCTION

Under natural conditions, many fish species are affected by prolonged periods of starvation, related predominantly to seasonal changes in food availability and to spawning migrations.

Starvation can take place in fish culture as well. At the final stage of rearing, fish farmers frequently stop administering food for a few days, subjecting the fish to the so-called depuration. At that time the fish regain their physiological equilibrium, cleanse their alimentary tracts of faeces, and get rid of skin and gill impurities. The properly depurated fish are regularly coloured, viable, and—importantly for the consumer—flavour qualities of their meat improve.

In this context, it was deemed interesting to find out how starvation would affect levels of selected biochemical components of blood, nutritive value-related chemical composition of the body, and changes in body weight reduction in carp, the most important cultured fish species in Poland.

MATERIAL AND METHODS

Materials

The experiment, conducted in the aquarium room of the Faculty of Marine Fisheries and Food Technology, involved 72 carp juveniles. The fish were kept in six 600 dm^3 working capacity, constantly aerated, aquaria. Each aquarium was stocked with 12 individuals. The experiment took 12 weeks during which time the fish were not fed. The fish were weighed at the onset of the experiment as well as after 4, 8, and 12 weeks.

Chemical analyses

Blood samples were taken from 12 individuals at the beginning of the experiment and after 2, 4, 6, 8, and 10 weeks. The blood was drawn from the caudal vein, between 7:30 and 8:30 hours, cooled down and centrifuged at 3500 U/min for 30 minutes at 4°C. The blood serum obtained was stored in polypropylene vials at –24°C. The samples of a given batch were assayed simultaneously.

The following assays were run on blood serum samples:

- crude protein level (biuret method, with POCh Gliwice reagents);
- AspAT and AlAT activities (enzymatically, with a Biotest Lachema Brno kit);
- glucose level (enzymatically, with a POCh Gliwice diagnostic kit);
- triacylglycerol level (enzymatically, with a Cormay TG-30 kit, on an Abbott biochemical analyser);
- total cholesterol and its HDL fraction (enzymatically, with a Human diagnostic kit, on an Abbott biochemical analyser);
- LDL cholesterol fraction was calculated from the formula:

$$\text{LDL} = \text{total cholesterol} - (\text{TG}/5+\text{HDL}).$$

The fish body chemical composition was assayed at 4-week intervals. In the bodies of 5 randomly selected individuals the per cent contribution of:

- crude protein (Kjeldahl technique);
- total lipids (Soxhlet technique);
- dry matter (12 h drying at 105°C);
- ash (weight loss on 10 h combustion at 550°C)

were determined.

During the experiment, water temperature was continuously measured (to 0.1°C) with an electronic thermometer.

Statistical treatment of data

The data tested for significant of differences with 1-way analysis of variance and the LSD test. The tests were run with the Statistica software.

RESULTS AND DISCUSSION

As shown by the data summarised in Table 1, starvation resulted in significant changes in levels of the carp blood serum components analysed.

The significant ($p < 0.01$) reduction of the blood glucose content was observed during the initial 6 weeks of starvation, following which the glucose content became stabilised—as of week 8—at a lower level and remained henceforth unchanged until the termination of the experiment. This effect may be indicative of an adaptive response of the fish body to new, nutritionally disadvantageous situation and can also evidence mobilisation of glycogen reserves. However, many studies have shown gluconeogenesis to be more important than glycolysis in maintaining a stable glucose level in the fish blood (Murat et al. 1978; Love 1980). Moreover, it is suggested that glucagon in fish, responsible for increasing the blood glucose level, enhances gluconeogenesis more than it does glycolysis (De Silva and Anderson 1995). That this effect does in fact takes place is supported by the observation that the hepatopancreatic glycogen was reduced by as little as 75 percentage points after 100 days of starvation (Nagai and Ikeda 1971) as well as by some reduction in the muscle glycogen in eel, *Anguilla anguilla* (cf. Dave et al. 1975) and in sockeye salmon, *Oncorhynchus nerka* (cf. French et al. 1975) subjected to prolonged starvation.

The significant ($p < 0.01$) and consistent decrease in the blood crude protein content may corroborate enhanced gluconeogenesis, particularly when it is remembered that the glucose level stabilisation in week 8-10 was accompanied by a significant reduction in the blood crude protein, without any concurrent change in the crude protein content in the body. A similar effect was observed by Heming and Paleczny (1987) in brook trout and by Shimeno et al. (1981) in carp. Cowey et al. (1977) and Love (1980) demonstrated that, during prolonged starvation, the fish used protein as an energy source via gluconeogenesis. Important in that process is alanine (Leech et al. 1979; Mommsen et al. 1980), not only that proteolytically released from muscles, but also that formed *de novo*.

Table 1

Changes in levels of selected blood serum components in starved carp ($\bar{x}$ ±SD), $n = 72$

Component	Beginning of the experiment (a)	Starvation time (weeks) 2 (b)	4 (c)	6 (d)	8 (e)	10 (f)	Significance of differences
Protein (g/l)	4.201 ±0.312	2.92 ±0.95	3.90 ±1.07	3.90 ±1.12	2.22 ±0.52	2.30 ±0.78	a-b**, a-d**, a-e**, a-f** b-c* c-d**, c-e**, c-f**
AspAT (IU/l)	44.86 ±11.4	32.69 ±15.49	40.77 ±15.12	31.02 ±4.05	30.29 ±7.40	28.95 ±13.81	a-e*, a-f* c-f*
AlAT (IU/l)	5.6 ±0.8	6.1 ±1.2	5.0 ±1.0	5.3 ±3.2	4.8 ±0.4	5.7 ±2.0	none
Glucose (mg/dl) (mmol/l)	 89.5 ±28.8 4.9 ±1.6	 79.4 ±13.5 4.4 ±0.7	 60.6 ±13.6 3.3 ±0.7	 49.9 ±18.5 2.7 ±1.0	 29.4 ±22.5 1.6 ±1.2	 27.9 ±7.2 1.5 ±0.4	a-c*, a-d**, a-e**, a-f** b-d**, b-e**, b-f** c-e*, c-f**
Triacyloglicerols (mg/dl) (mmol/l)	 374.1 ±95.7 4.3 ±1.1	 111.4 ±25.7 1.3 ±0.3	 107.0 ±48.7 1.2 ±0.5	 109.4 ±36.7 1.2 ±0.4	 104.2 ±37.2 1.2 ±0.4	 132.2 ±74.2 1.5 ±0.8	a-b**, a-c**, a-d**, a-e**, a-f**
Total cholesterol (mg/dl) (mmol/l)	 256.9 ±35.7 6.7 ±0.9	 265.6 ±58.9 6.9 ±1.5	 294.2 ±64.2 7.6 ±1.7	 227.4 ±41.4 5.9 ±1.1	 199.6 ±28.5 5.2 ±0.7	 229.4 ±34.5 5.9 ±0.9	a-e* b-e* c-d**, c-e**, c-f**
LDL-cholesterol (mg/dl) (mmol/l)	 109.9 ±39.5 2.9 ±1.0	 112.6 ±61.6 2.9 ±1.6	 137.5 ±58.3 3.6 ±1.5	 92.0 ±34.7 2.4 ±0.9	 86.5 ±19.8 2.2 ±0.5	 93.7 ±22.4 2.4 ±0.6	c-d*, c-e*
HDL-cholesterol (mg/dl) (mmol/l)	 72.1 ±14.1 1.9 ±0.4	 130.7 ±10.7 3.4 ±0.3	 135.3 ±22.6 3.5 ±0.6	 113.5 ±15.1 2.9 ±0.4	 92.3 ±26.7 2.4 ±0.7	 109.4 ±16.5 2.8 ±0.4	a-b**, a-c**, a-d**, a-f** b-e**, b-f* c-d*, c-e**, c-f* d-e*

*Difference significant ($p \leq 0.05$); **Difference highly significant ($p \leq 0.01$).

The decrease in the blood serum crude protein level was accompanied by a significant ($p \leq 0.05$) reduction in AspAT activity, which pointed out to a slowed-down rate of amino acid transformations via transamination. On the other hand, the lack of any significant changes in the activity of AlAT, an enzyme responsible for directing amino acids to catabolic pathways, indicates that starvation causes no damage either in the carp hepatopancreas or in the muscles, as evidenced by their increased activity (Friedrich and Stepanowska 2000).

In the experiment described, the most conspicuous effect of starvation was visible in the blood triacyglycerols. Their contents were observed to significantly ($p \leq 0.01$) decrease, to a level at which they stayed until the end of the experiment, as early as after 2 weeks of starvation. At that time, the triacylglycerol content was as low as 30% of the initial one.

Triacylglycerols are known to be lipolytically broken down to glycerol and free fatty acids. During starvation, the role of glycerols as glucose precursors becomes more important. The muscle metabolism changes as well. The muscles stop using glucose and restrict their ketone utilisation, the necessary energy being supplied via oxidation of fatty acids. The decrease in the blood triacylglycerol levels observed allows to presume that the lipolysis proceeding during starvation was the major source of energy (Shimeno et al. 1981; Heming and Paleczny 1987; Hung et al. 1997), particularly during the first two weeks of starvation. On the other hand, the significant ($p \leq 0.01$ and $p \leq 0.05$) reduction in the cholesterol content taking place as late as after 4 weeks could indicate that cholesterol metabolism did not change in any conspicuous way at the initial stage of starvation.

Not many significant changes were observed in LDL cholesterol, statistically significant ($p \leq 0.05$) differences being detectable between week 4 vs. week 6 and 8 only.

On the other hand, significant ($p \leq 0.01$ and $p \leq 0.05$) differences from one stage of the experiment to the next were recorded in HDL cholesterol content. The highest, significant ($p \leq 0.01$) increase took place as early as after 2 weeks of starvation. Unfortunately, the lack of any literature data does not allow to discuss the effect.

The prolonged starvation resulted also in substantial changes in the fish body composition (Table 2).

The significant ($p \leq 0.01$) reduction in the lipid level, observed after 12 weeks of starvation, coupled with the observed reduction in the blood serum triacylglycerol contents, lends support to the earlier conclusion that lipids constituted the major energy source for the starved fish. On the other hand, the absence of any body protein content reduction and, conversely, a significant increase in the protein level, allows to assume that a 12-week-long starvation was too short to induce any enhanced proteolysis. It should be, however, presumed that the observed increase in the tissue protein content was spurious and resulted from mathematical conversion based on the decreasing dry matter content, with no tissue

protein reduction. In the context of the reduced blood protein level, the lack of any visible proteolytic effects on the body proteins gives credibility to a conclusion that utilisation of protein in gluconeogenesis occurred in the blood only.

Table 2

Changes in body chemical composition of starved carp

Component (%)	Beginning of the experiment (a)	Starvation time (weeks)			Significance of differences
		4 (b)	8 (c)	12 (d)	
Dry matter	32.10 ±0.4	33.57 ±0.35	32.74 ±0.55	30.45 ±0.32	a-b**, a-d** b-c*, b-d** c-d**
Crude protein	12.44 ±0.46	13.40 ±0.38	13.11 ±0.39	13.08 ±0.11	a-b*, a-d*
Lipids	16.41 ±0.26	17.03 ±0.52	16.33 ±0.21	14.68 ±0.01	a-d** b-c*, b-d** c-d**
Ash	1.84 ±0.05	2.39 ±0.15	2.84 ±0.18	2.65 ±0.07	a-b**, a-c**, a-d** b-c**, b-d* c-d*

* Difference statistically significant ($p \leq 0.05$); ** Difference highly significant ($p \leq 0.01$).

No mortality was recorded during the experiment, but the fish body weight was constantly decreasing as a direct result of the lack of food (Table 3). The body weight decrease was not significant, but for a fish farmer any loss of body weight is disadvantageous.

Table 3

Changes in body weight (g) of the starved carp

Aquarium No.	Beginning of the experiment	Starvation time (weeks)		
		4	8	12
I	38.9	347.9	—	—
II	423.6	396.4	349.5	—
III	426.7	402.7	369.6	357.6
Mean	410.7	382.3	359.5	357.6

In addition to food availability and composition, the body chemical composition and weight are also affected by the physico-chemical conditions of water. In the experiment described, the mean water temperature was 16.7°C. It was lower than the thermal optimum of carp, i.e., a temperature at which the weight increments of ad lib fed fish are at their highest (Hokanson et al. 1977). The suboptimal water temperature resulted in slowed-down metabolism. Jauncey (1982) as well as Goolish and Adelman (1984) showed the carp to obtain maximum body weight gains at 25–30°C, metabolic activity decreasing above and below that range. Thus, considering that the water temperature in the experiment described was below the lower limit of the optimal range by an average of 8.3°C, it may be presumed

that the starvation-induced changes were not as extensive as those which could have been observed, had the fish been kept at a temperature optimal for their metabolic activity.

RECAPITULATION

The results obtained allow to conclude that 12-week-long starvation does not significantly affect the body functions, as indicated by the lack of mortality as well as by viability and condition of the fish. However, from the standpoint of nutritive qualities of fish meat, starvation should not take longer than 14 days.

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WPŁYW GŁODZENIA NA WARTOŚĆ ODŻYWCZĄ I WYBRANE SKŁADNIKI BIOCHEMICZNE WE KRWI KARPIA (*CYPRINUS CARPIO* L.)

STRESZCZENIE

Doświadczenie przeprowadzono w sali akwaryjnej Wydziału Rybactwa Morskiego i Technologii Żywności, na 72 kroczkach karpia. Ryby umieszczono w 6 akwariach o objętości użytkowej 600 dm^3, ze stale napowietrzaną wodą. Materiał obsadowy akwarium stanowiło 12 sztuk ryb. Badanie prowadzono przez 12 tygodni, w trakcie których ryby nie otrzymywały pożywienia. Na początku doświadczenia oraz po 2, 4, 6, 8 i 10 tygodniach jego trwania, każdorazowo od kolejnej partii ryb, była pobierana krew. Analizowano w niej poziomy białka całkowitego, aktywności AspAT, AlAT, poziomy glukozy, triacylogliceroli, cholesterolu i jego frakcji. Ponadto na początku doświadczenia oraz po 4, 8 i 12 tygodniach przeprowadzono ważenia kontrolne oraz dokonano analiz składu chemicznego ciała karpi.

Stwierdzono, że głodzenie ryb spowodowało statystycznie istotne zmiany w poziomie białka całkowitego i aktywności AspAT, poziomach glukozy, triacylogliceroli, cholesterolu i jego frakcji we krwi oraz w składzie chemicznym ciała, tak w stosunku do stanu przed rozpoczęciem doświadczenia jak i między kolejnymi etapami głodzenia.

Uzyskane wyniki pozwalają na stwierdzenie, że długotrwałe głodzenie karpia, chociaż nie zaburza istotnie funkcji jego organizmu na co wskazuje brak śnięć, żywotność i kondycja ryb, z punktu widzenia wartości odżywczych mięsa nie powinno trwać dłużej niż 14 dni.

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