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THE EFFECT OF FEEDING METHOD ON BODY WEIGHT GAINS, CONCENTRATIONS OF SELECTED COMPONENTS IN THE BLOOD, AND PEROXIDATION PROCESSES OF CARP, *CYPRINUS CARPIO* L.

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Background. Fish are the principal source of n-3 and n-6 fatty acids for humans. Traditionally, these fatty acids have been supplied in the form of captured marine and freshwater fishes. Recently, freshwater cultured species have been increasing their share in the fish volume on the market. Therefore it is crucial for fish farmers to rear fish under proper condition and with proper feeding in order to receive good quality fish flesh at harvest. The presently reported study was aimed at determining the effect of diet with the increased level of lipids or carbohydrates on selected blood indices, reduced glutathione content, lipid peroxidation products concentration in the liver, and body weight gains in carp fingerlings reared in post-cooling waters.

Materials and Methods. The experiment was carried out in spring (2004) on 300 carp divided into two feeding groups. Group I was fed high-fat feed, whereas group II high-carbohydrate feed. After 7 weeks, fish blood and livers were collected for further analyses.

Results. Significantly higher body weight gains were found in the fish fed high-fat feed as well as significant increase of haemoglobin (Hb) concentration in the blood of both feeding groups, which was accompanied by the increase of haematocrit (Ht) index. Furthermore, an increase in the concentration of lipid peroxidation products was observed as well as significantly lower content of reduced glutathione (GSH) in the livers of fish examined.

Conclusion. The observed MDA values and reduced glutathione (GSH) concentration in the fish liver may indicate enhanced peroxidation processes in the organisms of the fish from both experimental groups, caused by their intensive feeding with extruded feeds. The peroxidation processes were more intensive in fish fed high-fat feeds.

Keywords: fish, carp, *Cyprinus carpio*, haemoglobin (Hb), haematocrit (Ht), reduced glutathione (GSH), lipid peroxidation products (MDA), breeding, nutrition

INTRODUCTION

Recent years marked a major shift in human understanding of the role of food products in maintaining their proper health status. A particular interest was focused on the role of foodstuffs on prophylaxis and prevention of so called diseases of civilization such as: obesity, non-insulin-dependant diabetes mellitus, circulatory diseases, and neoplastic diseases. It has been explicitly demonstrated in extensive epidemiological studies that n-3 and n-6 fatty acids show remarkable physiological and health-oriented properties (Ziemlański and Budzyńska-Topolowska 1992, Budzyńska-Topolowska and Ziemlański 1993, Kolanowski and Świderski 1997, Ziemlański 1997, Arts et al. 2001,). The excellent source of these acids are fish, either free-living ones, acquired from the sea, or increasingly important cultured ones (Steffens and Wirth 2005). This imposes an obligation on fish-farmers to produce fish of, not only, adequate body weight and gustatory values but also of good condition and having specific customer-demanded nutrients, in this number proper lipid levels, of proper quality.

The presently reported study was aimed at determining the effect of lipid- and carbohydrate-enhanced diets on selected blood parameters, reduced glutathione (GSH) content, lipid peroxidation products (MDA) concentrations in the liver, as well as on body weight gains in carp fry reared intensively in post-cooling waters.

MATERIALS AND METHODS

The experiment was carried out in spring of 2004 at the Experimental Fisheries Station at Nowe Czarnowo on 300 carp, weighing on average 170 ± 10 g. The fish were rea-

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red in 6 cages of 1.0 m^3 production volume each, suspended on self-supporting platforms positioned in the post-cooling water discharge canal of the Dolna Odra Power Plant. Stocking material consisted of 50 fish in each cage. The fish were randomly divided into two groups and the differentiation factor was the diet composition. Each group was tested in three repetitions. The experiment lasted for 7 weeks.

The fish were fed different commercial feeds, available at the domestic market, and featuring high levels of lipids or carbohydrates (group I and II, respectively). The detailed composition of the feeds is presented in Table 1.

The fish were fed according to the following schemes.

- The standard diet before the start of the experiment: 45 g of protein (=754.2 kJ = 44.6% of energy); 20 g of fat (= 670.4 kJ = 39.6% of energy); 16 g of carbohydrates (= 268.2 kJ = 15.8% of energy); overall calorific value of the feed = 1692.8 kJ.
- Group I (fed high-fat feed): 45.3 g of protein (= 729.1 kJ = 38.9% of energy); 28.6 g of fat (= 958.7 kJ = 51.1% of energy); 11.7 g of carbohydrates (= 187.7 kJ = 10.0% of energy); overall calorific value of 100 g of the feed = 1875.5 kJ.
- Group II (feed high-carbohydrate feed): 43.7 g of protein (=732.4 kJ = 44.6% of energy); 14.7 g of fat (=492.7 kJ = 29.9% of energy); 24.9 g overall calorific value of the feed = 1642.4 kJ.

The fish were fed daily, every 60 minutes (0900–1500 h). The feed was thrown manually on the water surface in the cages. The daily feed ration, calculated with relation to fish metabolic body weight (Sadowski 1998), amounted to 2%. In order to control fish body weight and correct feed ration, the weights of all fish were monitored every 7 days.

Blood for analyses was collected from the caudal vessels of 12 carp before starting the experiment and of 12 carp after completing the experiment. The procedure was performed for each feeding group and it was carried out between 0730 h and 0830 h. The blood for haemoglobin and haematocrit readings was collected on heparin whereas that for protein assessments—on coagulum.

Furthermore, the content of reduced glutathione (GSH) (Ellaman's method) and the concentration of lipid peroxidation products (MDA) (reaction with thiobarbituric acid) were analysed in homogenates of fish livers before the experiment and after its completion. Basic physico-chemical indices of the water, such as temperature, oxygen level, and pH, were also recorded during the experiment with the aid of automatic analyzer and subsequently analysed (STATISTICA for Windows). Significance of differences was determined basing on one-factor analysis of variance and LSD test.

The experimental protocol had been approved by the Local Ethical Committee for Animal Research (Szczecin, Poland)

RESULTS

The physico-chemical indices of post-cooling water during the experiment were as follows:

- mean water temperature: 20.3°C (range of 19.3–21.2°C)
- mean water oxygen content: 8.4 mg $O_2 \cdot L^{-1}$ (range of 7.3–8.9 mg $O_2 \cdot L^{-1}$)
- mean water pH: 8.3 (range of 8.0–8.6).

It was determined that intensive rearing of carp fingerlings which involved using a diet containing increased level of lipids (group I) or carbohydrates (group II) induced significant changes in levels of the analysed blood parameters, liver content of reduced glutathione (GSH), and liver concentration of lipid peroxidation products (MDA), as well as in body weight gains of the experimental fish (Tables 2, 3, 4).

Intensive body weight gains were stated in the carp of both feeding groups, with those being significantly higher ($P \leq 0.01$) in the fish fed high-fat feed when compared to carp fed high-carbohydrate feed.

Also the increase of haemoglobin concentration in the blood of fish of both feeding groups was highly significant ($P \leq 0.01$) when compared to the pre-experiment state.

This was accompanied by an increase in the value of haematocrit, which was highly significant ($P \leq 0.01$) in the fish of group I, fed high-fat feed and significant ($P \leq 0.05$) in those of group II fed high-carbohydrate feed when compared to the initial state.

On the other hand, no changes were observed in the content of total protein in fish blood, both between the feeding groups and when compared to pre-experiment state.

Table 1

Composition of feed mixes used in fish feeding (according to manufacturer's data)

Components [%]		Group I feed	Group II feed
Protein		44.0	45.0
Fat		31.0	15.0
Carbohydrates		11.0	21.0
Total ash		8.0	8.0
Vegetable fibre		1.0	2.5
Gross energy	[kcal · g^{-1}]	5.9	4.9
	[kJ · g^{-1}]	24.5	20.4

In the fish of both feeding groups, a highly significant ($P \leq 0.01$) increase in the weight of livers was stated when compared to pre-experiment state, with that being significantly higher in the fish fed high-fat feed.

Also the concentration of lipid peroxidation products increased significantly ($P \leq 0.01$), being the highest ($P \leq 0.01$) in the fish fed high-fat feed.

However, the content of reduced glutathione decreased significantly ($P \leq 0.01$), with that being the lowest ($P \leq 0.01$) in the fish of group I.

DISCUSSION

Environmental factors, such as water temperature, oxygen level, and pH, have a direct effect on fish metabolism

Table 2

Mean unit body weight of carp

	Before experiment	After experiment		
		Group I	Group II	Significant differences
	(a)	(b)	(c)	
Mean unit body weight [g]	170 ± 10	439 ± 15	407 ± 15	a – b**, a – c**, b – c**

(b) fed high-fat feed; (c) fed high-carbohydrate feed; * statistically significant difference at $P \leq 0.05$; ** statistically significant difference at $P \leq 0.01$; $\bar{x} \pm s$, $n = 150$

Table 3

Values of selected carp blood components before and after the experiment

Component	Before experiment	After experiment		
		Group I	Group II	Significant differences
	(a)	(b)	(c)	
Protein				
[mg · dL^{-1}]	31.6 ± 2.6	31.2 ± 4.1	32.6 ± 2.2	none
Haematocrit				
[%]	30.33 ± 2.27	36.50 ± 2.34	35.67 ± 3.01	a – b**, a – c*
[g · dL^{-1})	5.02 ± 0.49	6.98 ± 1.17	7.41 ± 3.01	a – b**, a – c**
Haemoglobin				
[mmol · L^{-1}]	3.11 ± 0.30	4.32 ± 0.72	4.60 ± 0.54	a – b**, a – c**(b)

(b) fed high-fat feed; (c) fed high-carbohydrate feed; * statistically significant difference at P ≤ 0.05; ** statistically significant difference at $P \leq 0.01$; $\bar{x} \pm s$, $n = 36$

Table 4

Content of reduced glutathione (GSH) and concentration of lipid peroxidation products (MDA) in carp livers before- and after the experiment

Parameters	Before experiment	After experiment		
		Group I	Group II	Significant differences
	(a)	(b)	(c)	
Weight of livers	0.62 ± 0.10	1.34 ± 0.36	1.05 ± 0.23	a – b**, a – c**,
MDA nmol · g^{-1} · tissue	12.99 ± 1.64	126.66 ± 28.19	77.7 8 ± 16.15	a – b**, a – c**, b – c*
GSH nmol · g^{-1} · tissue	2366.1± 359.0	1205.5 ± 317.9	1555.7 ± 323.1	a – b**, a – c**, b – c* b – c**

(b) fed high-fat feed; (c) fed high-carbohydrate feed; *statistically significant difference at $P \leq 0.05$; **statistically significant difference at $P \leq 0.01$; $\bar{x} \pm s$, $n = 36$

and their health state, and the same on rearing efficiency. The analyses of the physico-chemical conditions of post-cooling water, carried out during the whole experiment, permit the concussion that all analysed water parameters were within the ranges suitable for carp (Jaunacey 1982), which was evidenced indirectly by the high body weight gains.

The control weighing revealed intensive body weight gains of fish representing both feeding groups. The increments, however, were significantly higher in fish of group I, fed high fat feed, which, could have been expected considering high energy value of this type of feed. Nevertheless, relatively large gains of body weight in the fish of group II fed high-carbohydrate feed may constitutes an evidence that carp is also able to digest and assimilate excessive amounts of carbohydrates (Wilson 1994, Keshavanath et al. 2002).

Significant increase of haemoglobin (Hb) and haematocrit (Ht) value was observed in the blood of fish of both feeding groups, which undoubtedly resulted from intensive consuption of extruded feeds of increased levels of lipids or carbohydrates. The effect of the feed composition on the blood parameters was also observed by Sobecka (1986). She found an increase of Hb- and Ht levels in carp fed high-protein feeds. On the other hand, Hilge (1978), examining the effect of feed composition on specific blood parameters in carp fry, noticed an increase in Ht value in the fish fed high-protein- but also high-fat diets. Also Klinger et al. (1996) observed the effect of lipids and their different types in fish diets on the levels of Ht, red blood cells (RBC) and white blood cells (WBC). Lihačeva et al. (2000), however, observed also the effect of not only feed composition but also the feed ration size on Hb concentration.

A different conclusion reached Serpunin et al. (2002) who was not able to observe any effect of the feeding method on fish blood parameters (total protein, Hb, MHC, RBC, WBC).

On the other hand, Sobecka (1985) noticed that Hb level in the blood of carp may depend on physico-chemical conditions of the water. The Hb concentration increases along with the increase of water temperature, which undoubtedly results from the organism's adaptation to changing environmental conditions.

In the presently reported experiment, no significant changes were observed in the concentration of total protein in the blood serum of carp, either between the feeding groups or in relation to pre-experiment situation. Nevertheless, these concentrations were characteristic both for the season in which the experiment was carried out and for the age of fish (Stosik 1996, Szerow et al. 1996).

The increased level of lipid oxidation products (MDA) and the decreased concentration of reduced glutathione (GSH) in the liver tissue may be indicators of disturbances of organism functioning, resulting from organism pro- and anti-oxidation unbalance with the predominance of pro-oxidation state, i.e. oxygen stress. It was demonstrated in many studies that oxygen stress is a very important mechanism in the pathophysiology of a number of diseases. Numerous authors point to the influence of environmental conditions and feeding method on fish anti-oxidation status. Hai et al. (1997) showed an increase in the activity of anti-oxidation enzymes and oxidation processes in the tissue homogenates of carp and catfish fed organophosphorus compounds. Gul et al. (2004) observed an increase in the activity of catalase (CAT), superoxide dismutase (SOD), and lipid oxidation products (MDA) concentration in the livers of fish from the areas with high water pollution levels. Enhanced oxidation processes occur also in fish under the influence of heavy metals (Romeo and Gnassia-Barelli 1997, Žikić et al. 2001) as well as during malnutrition and limited access to feed (Pascual et al. 2003).

On the other hand, physiologically higher concentration of antioxidants, both enzymatic and non-enzymatic ones, can be found in Antarctic fishes as an expression of adaptation to constant low temperatures and high oxygen concentration (Ansaldo et al. 2000).

The values of lipid oxidation products (MDA) and reduced glutathione (GSH) concentrations in the fish livers, observed during the experiment, indicate an enhanced oxidation processes in animal organisms of both feeding groups caused by intensive feeding with extruded feeds. At the same time, they suggest that oxidation processes were more intense in the fish of group I, fed high-fat feed.

In this group of fish, larger livers weights were also observed, which could be related to larger gains of unit weight in fish fed high-fat feeds.

It can be concluded that carp utilised high-fat feed slightly better for unit body weight gains. However, it should be emphasised that apart from high body weight gains it is also important to know that fish in the final stage of rearing are a food product which should meet specific nutritive requirements, expressed by its chemical composition. This is particularly related to the content of protein and body lipids of specified quality and nutritive value, which are particularly targeted by perioxidation.

RECAPITULATION

In modern intensive fish rearing it should be stipulated that production environment would serve natural needs and well-being of cultured fish and would not surpass physiological abilities of their organism; this is also valid for the fact that fish in the final stage of rearing should be a high-value food product for humans, both in respect of specific gustatory qualities and nutritive values.

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