

Mariola *FRIEDRICH*, Katarzyna *STEPANOWSKA*

Fish physiology

**EFFECTS OF INTENSIVE CULTURE AND FEEDING ISOPROTEIN
DIETS WITH DIFFERENT FAT AND CARBOHYDRATE CONTENTS
ON CORTISOL, TOTAL PROTEIN AND PROTEIN FRACTIONS
CONTENTS, ASPAT AND ALAT ACTIVITIES, AND ON BODY
COMPOSITION AND WEIGHT INCREMENTS IN CARP
(*CYPRINUS CARPIO* L.) FINGERLINGS**

**WPŁYW INTENSYWNEGO CHOWU I ŻYWIENIA KROCZKA KARPIA
(*CYPRINUS CARPIO* L.) IZOBIAŁKOWYMI DIETAMI Z RÓŻNYM
UDZIAŁEM TŁUSZCZÓW I WĘGLOWODANÓW, NA POZIOMY:
KORTYZOLU, BIAŁKA CAŁKOWITEGO I JEGO FRAKCJI,
AKTYWNOŚĆ ASPAT, ALAT ORAZ PRZYROSTY MASY
I SKŁAD CIAŁA**

Department of Human Nutrition Physiology, Agricultural University of Szczecin, Poland

The experiment was run on carp individuals in their second year of life. The fish were randomly divided into two groups fed two different isoprotein diets, one with increased fat level (Group I) and the other with increased carbohydrate level (Group II). Cortisol, total protein and protein fraction contents, AspAT and AlAT activities, and body composition and weight increments were monitored. Statistically significant differences between the two experimental groups were found in cortisol content, albumin level, blood AspAT and AlAT activities as well as in dry weight, total protein, and fat contents of the fish body.

INTRODUCTION

Culturing the fish in heated water reservoirs in which the elevated temperature is only slightly dependent on seasonal variations in ambient temperature, makes it possible not only to feed the fish continuously, but also produces effects by far superior to those obtained in traditional culture systems. It should be, however, borne in mind that this forced high productivity brings about an increase in the cultured fish susceptibility to environmental stress (Friedrich 1996a; Friedrich 1996b; Friedrich 1998) and may in itself be

a highly stressing factor. Composition of a diet and its modification during the culture period were found to exert an effect similar to those produced by other components of environmental aggression (Barton et al. 1988; Scholz-Ahrens et al. 1991; Friedrich 1995).

Stress response belongs to the most complex and not fully understood mechanisms in animal bodies. It is, however, known, that the hypothalamus-hypophysis-interrenal coupling is one of those systems responsible for the adaptive potential in fish (Thomas 1990). Stimulation of the system leads to intensified or reduced secretion of numerous hormones, the haematological blood components being always increased (Donaldson 1990; Thomas 1990; Friedrich 1996a; Friedrich 1996b; Friedrich 1998; Friedrich and Stepanowska 1998).

This work was aimed at following effects of intensive culture and isoprotein diets with elevated fat or carbohydrate levels on contents of cortisol, total protein and protein fractions, activities of AspAT and AlAT, and body chemical composition and weight increments in carp fingerlings.

MATERIAL AND METHODS

Table 1

Ingredients of experimental diets (%)

Ingredients	Daily feeding rate 2%	
	I Group	II Group
Poultry by-product meal	45.0	45.0
Poultry fat	10.0	0.0
Wheat bran	25.0	10.0
Wheat meal	0.0	25.0
Lupine meal	17.7	17.7
Rapessed lecithin	0.5	0.5
Polfamix W (Vitamin mixture)	1.5	1.5
Vitasol (Vitamin mixture)	0.1	0.1
Choline chloride	0.2	0.2

Table 2

Chemical composition of experimental diets (%)

Components		Diet before experiment	Diet I	Diet II
Total protein		45.0	38.7	38.7
Crude fat		18.0	17.7	9.9
Ash content		8.0	7.6	7.3
Carbohydrates		20.2	24.6	32.3
Dry weight content		81.0	88.6	88.2
Energy brutto	(kcal/g)	4.07	4.87	4.45
	(kJ/g)	17.05	20.40	18.65

Experimental diets

The fish were fed daily, at 60-min. intervals between 8:00 and 15:00 hours. The feed was applied manually, by throwing rations amounting to 2% metabolic weight (Filipiak et al. 1995) onto the water surface. To adjust the ration, the fish were weighed at 7-d intervals.

The feeds used in the experiment were manufactured at the Experimental Station of Poznań Agricultural University's Chair of Inland Fisheries (Tabs. 1–2).

Culture

The experiment involved 180 carp individuals at the second year of life and 235 ± 20 g initial individual weight. The

experiment was run for 9 weeks in summer (July-August) at the Szczecin Agricultural University's Fisheries Experimental Station situated in the vicinity of the Dolna Odra Power Station. The fish were randomly divided into two groups; one group was fed a fat-enriched diet, while the other received a carbohydrate-enriched feed. The fish were kept in 6 cages measuring $2.0 \times 0.75 \times 0.8$ m and having 1.0 m^3 working capacity.

Chemical assays

The blood to be assayed was collected before the experiment and on its termination, within 7:30 – 8:00 hours, from caudal vessels of 30 individuals picked out from each experimental group, each cage yielding 5 individuals.

The following assays were made in the blood serum:

- cortisol level, radioimmunologically, with 125J Cortisol (Orion Diagnostica, Finland);
- total protein, albumin, and globulin contents and AspAT and AlAT activities, enzymatically on Abbott Spectrum II biochemical analyser.

The body chemical composition was analysed before the experiment and on its termination. Five individuals picked out from each cage in each experimental group were used. The following assays were made on the homogenous material obtained by grinding the fish and homogenising the ground tissues three times:

- total protein, Kjeldahl method;
- crude fat, Soxhlet method;
- dry weight content, by drying for 12 h at 105°C ;
- ash content, by combustion for 10 h at 550°C .

During the experiment, water temperature, pH, and dissolved oxygen content were monitored with a continuous recorder.

Statistical treatment

Significance of differences in the data was tested for with Student's t-test and Duncan's multiple range tests. The tests were run with the aid of Statgraphics® version 6.0 computer package.

RESULTS AND DISCUSSION

As a result of feeding the fish isoprotein feeds differing in fat and carbohydrate contents, statistically significant ($P \leq 0.01$ or $P \leq 0.05$) differences in the levels of the blood components studied and in fish weight increments (Tab. 3) as well as in the body chemical composition (Tab. 4) were revealed on termination of the experiment. The differences were recorded both between the two groups and in relation to the initial state in each. Statistically significant increase in the cortisol level was observed in both groups. The increase was, however, significantly higher in Group II (carbohydrate-rich feed) than in Group I

(fat-rich diet). Statistically significant changes were observed in the albumin content, both with respect to the initial level and between the groups. The albumin level increased significantly after two months of feeding, the increase being significantly higher in Group II than in Group I.

Table 3

Concentrations of some component in serum of carp before and after experiment $\bar{x} \pm \text{SD}$; $n = 90$)

Component	Before experiment (a)	After experiment		Significance of differences
		Diet I (b)	Diet II (c)	
Cortisol (nmol/dm ³)	188.20 \pm 31.2	737.80 \pm 372.5	1079.20 \pm 534.1	a-b**, a-c**, b-c**
Total protein (g/dm ³)	25.25 \pm 2.04	27.75 \pm 5.72	29.24 \pm 3.62	a-b*, a-c*
Albumin (g/dm ³)	7.49 \pm 0.62	11.40 \pm 0.84	12.81 \pm 1.50	a-b**, a-c**, b-c*
Globulin (g/dm ³)	18.76 \pm 0.76	17.53 \pm 0.84	16.33 \pm 3.41	—
AspAT (IU/dm ³)	57.20 \pm 8.41	56.55 \pm 12.12	41.80 \pm 9.35	a-c**, b-c*
AlAT (IU/dm ³)	6.80 \pm 1.42	6.65 \pm 1.69	4.61 \pm 2.94	a-c*, b-c*

* - significance of differences – $P \leq 0.05$

** - significance of differences – $P \leq 0.01$

Table 4

Body weight increments (g) and chemical composition (%) of carp before and after experiment

	Before experiment (a)	After experiment		Significance of differences
		Diet I (b)	Diet II (c)	
Body weight increments	235.0 \pm 20.0	558.9 \pm 91.8	589.4 \pm 64.6	a-b**, a-c**
Dry weight content	28.80	29.67	27.41	a-b**, a-c*, b-c**
Total protein	14.50	16.46	17.54	a-b**, a-c**, b-c**
Crude fat	12.00	11.92	9.01	a-c**, b-c**
Ash content	1.80	1.70	1.70	—

* - significance of differences – $P \leq 0.05$

** - significance of differences – $P \leq 0.01$

A diet effect was also observed in the AspAT and AlAT activities. The effect was significant in Group II only. A statistically significant reduction in activities of the two enzymes was recorded in Group II with respect to both the initial level and that in Group I in which the activities were close to those recorded at the beginning of the experiment.

The fish weight increments recorded on termination of the experiment were comparable in the two groups; although Group II attained slightly higher increments than Group I, the differences were not significant.

The diets used in the experiment were found to affect the chemical composition of the fish body. A statistically higher dry matter content in Group I, with respect to both the

initial level and that in Group II as well as a statistically higher fat content in Group I than in Group II, but comparable to the initial level, were revealed. On the other hand, the two groups showed a significantly increased protein level, compared to the initial state. In spite of the diets having identical protein contents, the final protein level was significantly higher in Group II receiving carbohydrate-rich diet.

By forcing alteration in enzymatic and metabolic activity, a change in diet composition or a partial modification of the diet is perceived by the organism affected as an environmental stress, which is visible, i.e., as changes in the blood glycocorticoid level (Barton et al. 1988; Scholz-Ahrens et al. 1991; Friedrich 1995). One would presume, however, that the changes should level off with time as the body adapts to the new set of conditions. As shown by the experiment, the blood cortisol level did not revert to its initial level during the two months elapsing from the moment the diet was changed. It seems that the lack of normalisation could result not only from the dietary change, but also from the fact that the feed ration was increased every week, as required by the intensive culture of the fish.

The analysis of diet composition effects on the blood constituents examined allows to infer that, with respect to cortisol levels found, the carbohydrate-rich diet was more stressing to the fish organism than the other diet. Considering carp biology (Jobling 1994), that conclusion would be obvious, if the high cortisol level were accompanied by reduced body weight increments resulting from catabolic activity of glycocorticoids.

Generally, glycocorticoids are regarded as those hormones that greatly intensify body protein catabolism. However, no adverse effects of glycocorticoids either on blood protein synthesis or on protein incorporation into body tissues were observed in the experiment discussed. The total blood protein level at the end of the experiment was in both groups significantly higher than the initial level and was typical of carp of the given age. A similar trend was recorded in the albumin content, the increase observed during the experiment being significantly higher in Group II (carbohydrate-rich diet) than in Group I. At the same time, a slight reduction in the globulin fraction was recorded. The analysis of changes in total blood protein and protein fractions prompts a conclusion that the changes were more related to fish age and season of blood sampling (Stosik 1996) than to the type of diet applied. However, one cannot exclude a possibility that a higher albumin content in Group II was related to the albumin function to bind different ligands in blood, including free fatty acids (FFA). Earlier studies demonstrated the high triacylglycerol levels in fish fed carbohydrate-rich diet to enhance the blood FFA levels (Friedrich and Stepanowska 1999).

On the other hand, the significant decrease in AspAT and AlAT activities in Group II, in relation to both the initial state and to Group I, can be without doubt ascribed to the diet used. Although glycocorticoids function to, i.e., provide a pool of free amino acids to be used in adaptive protein synthesis, which is associated with inducing the synthesis of

enzymes necessary in different amino acid cycles, the effect was not observed in the experiment described. The lower activity of the enzymes studied was recorded in those fish having a higher cortisol level. The differences in enzymatic activities observed in this study were perhaps related to a higher (the high fat diet) or lower (the high carbohydrate diet) demand on the hepatopancreas as an organ involved in indirect metabolism of the diets' components.

The experiment showed also the dietary effect on the fish body composition. At identical protein levels, the carbohydrate-rich diet (Group II) produced a total protein level that was significantly higher than both the initial content and that in Group I. Presumably, the effect was directly, by implying certain metabolic pathways, related to the high carbohydrate content. Insulin, the secretion of which was induced by the composition of the diet (Mackett et al. 1992), had been demonstrated to increase amino acid incorporation into body protein in fish (Plisetskaya 1989). This mechanism seems to be confirmed by body weight increments, higher in Group II fish than in those fed the fat-rich diet the caloric content of which was by 42 kcal/100 g higher than that in the Group II diet.

Judging by the composition of the diets used, the significantly lower fat content in the Group II fish could have been expected. Numerous studies have shown that increased dietary fat level substantially enhances the body fat content (El-Sayed and Garling 1988; Ellis and Reigh 1991). Lipid metabolism involves primarily fatty acids and cholesterol. The long-chain fatty acids are derived either by resynthesis from the dietary fat or by more energy-consuming *de novo* synthesis from acetyl-CoA obtained from metabolism of carbohydrates supplied in the diet (Steffens 1997).

The two groups of fish showed statistically significant differences in their body dry matter contents. That effect could have been produced by the cortisol level. Non-renal effects of cortisol on changes in water content, at the cell-extracellular fluid level, and on the increase in blood plasma volume were demonstrated by Kolpakov et al. (1969) and Swingle and Swingle (1966), respectively. Water-holding capacity could have been enhanced also by the significantly higher albumin level in Group II. The higher water content could have been the cause of higher body weight increments recorded in the fish of that group.

Numerous studies have shown that contents of different components and their ratios in the fish body are subject to continuous changes, induced not only by age, size, or diet composition, but also by the physical and chemical regime of the culture. The average water temperature during the experiment described was 26.3°C and stayed within the range regarded as optimal for carp. The average dissolved oxygen content was 7.5 mg O₂/dm³. The average water pH (8.4), too, remained within the safe range. One can therefore conclude that the culture regime was adequate for the young carp, as indicated by appropriate

body weight increments, 100% survival rate, and the very good condition of the fish studied.

RECAPITULATION

Analysis of the acquired results indicate a different effect of isoprotein diets with elevated fat or carbohydrate levels on contents of cortisol, total protein and protein fractions, activities of AspAT and AlAT, and body chemical composition.

In Group II receiving carbohydrate-rich diet was observed increase cortisol content and decrease AspAT and AlAT activities with increase total protein and albumin fraction in the blood and also protein level higher of the bodies of the carp compared to the Group I receiving fat-rich diet.

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TŁUSZCZÓW I WĘGLOWODANÓW, NA POZIOMY: KORTYZOLU, BIAŁKA
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STRESZCZENIE

Doświadczenie przeprowadzono na 180 karpkach, w drugim roku życia. Karpki podzielono losowo na dwie grupy, żywiono izobiałkowymi dietami z podwyższonym udziałem tłuszczu – grupa I lub węglowodanów – grupa II. Analizowano poziomy kortyzolu, białka całkowitego i jego frakcji, aktywności Aspart, AlAT oraz przyrosty masy ciała i jego skład chemiczny.

Stwierdzono, że dieta z podwyższonym udziałem węglowodanów w stosunku do diety z podwyższonym udziałem tłuszczu, spowodowała, przy obniżeniu aktywności Aspart i AlAT, statystycznie istotny wzrost zawartości białka całkowitego i jego frakcji albumin w surowicy krwi oraz zawartości białka w ciele badanych karpki. Towarzyszył temu statystycznie istotnie wyższy poziom kortyzolu.

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Authors' address:

Mariola Friedrich, PhD DSc Prof
Department of Human Nutrition Physiology
Agricultural University of Szczecin
Papieża Pawła VI 3, 71-439 Szczecin, Poland