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IMPACT OF HIGH LEVEL OF INTENSIVE FEEDING OF TWO-YEAR-OLD CARP (*CYPRINUS CARPIO* L.)—WITH FEEDS DIFFERING IN FAT AND CARBOHYDRATE COMPOSITION —ON THE LEVELS OF CORTISOL, GLUCOSE, TRIGLYCERIDES IN THE BLOOD AND ON THE BODY WEIGHT INCREMENTS

WPŁYW INTENSYWNEGO CHOWU I ŻYWIENIA KROCZKA KARPIA (*CYPRINUS CARPIO* L.) PASZAMI Z RÓŻNYM UDZIAŁEM TŁUSZCZÓW I WĘGLOWODANÓW NA POZIOMY KORTYZOLU, GLUKOZY, TRIGLICERYDÓW WE KRWI ORAZ PRZYROSTY MASY CIAŁA

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Intensive culture of two-year-old carp, using a diet with elevated content of fats or carbohydrates causes highly statistically significant increase of cortisol and triglycerides in the blood of both feeding groups and statistically significant decrease in glucose level in the fish fed with high-carbohydrate diet.

INTRODUCTION

Post-cooling waters of power plants offer stable thermal condition, which are not dependable on the seasonal temperature fluctuations. Such waters not only enable continuous rearing of fishes, but also give effects substantially exceeding results of traditional aquaculture. It must be emphasized, however, that such high productivity is not only the cause of risen sensitivity of cultured fishes for environmental aggression factor (Friedrich 1996a, b), but also it can be a highly stressful factor. It has been known that the diet composition and also its modifications during the rearing act similarly to the other factors of environmental aggression (Barton et al. 1988; Scholz-Ahrens et al. 1991; Friedrich 1995).

Mechanism of stress reactions is considered to be one of most complex and not fully known regulation mechanisms in animal organisms. As it has been learned, one of the systems responsible for adaptive potential of the fish organism is

the hypothalamus-pituitary-interrenal system (Thomas 1990). During its stimulation the secretion of many hormones either increase or decrease—in this number ACTH and glucocorticoids, which in turn triggers a number of changes of biochemical and haematological blood components (Donaldson 1990; Thomas 1990; Friedrich 1996a, b).

The aim of the present study was to determine the impact of intensive rearing and feeding of two-year-old carp—with diets of the elevated content of fats or carbohydrates—on the concentrations of cortisol, glucose, and triglycerides in the blood.

MATERIAL AND METHODS

The experiment was carried out in July and August on clinically healthy two-year-old carp. They measured 237 ± 21 g and they were from a fish farm in Nowe Czarnowo. The carp were randomly divided into two groups, 90 specimens in each. Each cage (2.0 x 0.75 x 0.8 m) placed in the post-cooling water of the Dolna Odra power plant hold 30 fish.
The fish were fed accordingly to the following regime:

Composition of the standard diet before the beginning of the experiment:
protein—56 g = 938.6 kJ (60% of energy),
fat—11 g = 414 kJ (27% of energy),
carbohydrates—12 g = 201.1 kJ (13% of energy),
additives—1 g.
Total calorific value of 100 g of feed—554.5 kJ.

Group 1—fed with feed of elevated content of fats (supplement of 10 g of poultry fat for each 100 g of feed):
protein—35 g = 586.6 kJ (29% of energy),
fat—17.5 g = 659.9 kJ (33% of energy),
carbohydrates—45.5 g = 762.6 kJ (38 % of energy),
additives—2 g.
Total calorific value of 100 g of feed—2009.1 kJ.

Group 2—fed with feed of elevated content of carbohydrates (supplement of 10 g of wheat flour for 100 g of feed):
protein—35 g = 586.6 kJ(32% of energy),
fat—7.5 g = 282.8 kJ (16% of energy),
carbohydrates—55.5 g = 930.2 kJ (52% energy),
additives—2 g.
Total calorific value of 100 g of feed—1799.6 kJ.

The fish were fed daily from 8:00 to 17:00 every 60 minutes. The feed was served manually, on the water surface, in the amount of 2% of the metabolic weight ration (Filipiak et al. 1995). All the fish were weighed every 7 days to verify and correct the feedrations.

The feeds used, were manufactured in the Experimental Station of the Chair of Inland Fisheries, University of Agriculture in Poznań.

The blood for the studies was collected from the caudal blood vessels before the start of the experiment and after its end. The procedure was conducted within 7:30 and 8:00 and 20 carp of each group were sampled.

The concentration of the following components was determined in the blood serum:

cortisol—using radio-immunological method, with labelled 125J Cortisol of the set manufactured by Orion Diagnostica Finland,

glucose—using enzymatic method, with biotests POCh,

triglycerides—using enzymatic method, with biochemical analyser Abbott's Spectrum II.

The results obtained were subjected to statistical calculations. Significance of the differences was determined using t-Student test and the Duncan test.

RESULTS AND DISCUSSION

Intensive rearing of the two-year-old carp, using diets of the elevated (compare to the period before the experiment) levels of fats and carbohydrates, caused statistically significant changes ($P<0.01$; 0.05) in the levels of the analysed blood parameters (Tab. 1).

Table 1

Impact of intensive feeding of two-year-old carp with two-different diets on the levels of cortisol, glucose, triglycerides in the blood and body weight increments, ($\bar{x} \pm$ SD), Group I—elevated contend of fats; Group II— elevated contend of carbohydrates

Component	Before experiment (a)	After experiment		
		Group 1 (b)	Group 2 (c)	Significance of differences
Cortisol (nmol/dm^3)	188.2 ± 31.2	737.8 ± 372.5	1079.2 ± 534.6	a–b**, a–c**, b–c**
Glucose (mg/dl)	83.9 ± 18.7	85.5 ± 18.1	59.2 ± 11.7	a–c**, b–c*
Triglycerides (mg/dl)	126.3 ± 18.1	180.9 ± 24.9	204.3 ± 36.9	a–b**, a–c**, b–c*
Body weight increments (g)		326.1 ± 91.8	348.3 ± 64.6	

Statistically significant difference: * $p \leq 0.05$; ** $p \leq 0.01$

Statistically significant rise ($P<0.01$) of the cortisol level above the initial state, was observed in both nutritional groups. This growth was higher in group II,

fed with the feed of higher carbohydrate content. Compared to group I, fed with the feed of the elevated content of fat it was a statistically significant difference ($P<0.001$).

The changes were also observed in the levels of glucose. They were limited, however, to group II only, where statistically significant ($P<0.01$) drop of glucose level was observed compared to the initial value. In group I, the level of this compound was similar to the initial values after the end of the experiment.

Analysed levels of triglycerides, grew statistically significantly ($P<0.01$) after two months of the experiment in both groups. This growth was higher in group II and the difference between group I was statistically significant ($P<0.05$).

The weight increments estimated by the end of the experiment were comparable, although higher then in group II. The above difference was not statistically significant.

Change of the diet or its partial modification, through forceful change to another enzymatic and metabolic activity is perceived by the organism as an environmental aggression factor. It is manifested, among other things, by changes in glucocorticoid levels (Barton et al. 1988; Scholz-Ahrens et al. 1991; Friedrich 1995). It should be assumed, however, that the elapse of time allowing the organism to adjust its functions to new conditions would also normalise the changes observed. In the experiment conducted, however, the two-month period that elapsed from the change of diet, did not cause normalisation of the cortisol levels in the blood of the carp studied. It seems that the cause of this phenomenon can be not only the change of the diet, its new composition, but also—through weekly corrections of the feed amounts—also forced, intensive growth.

Analysing the impact of the diet composition on the studied blood parameters, it can be concluded—taking into account the level of cortisol—that the most burdening for an organism was the diet with elevated level of carbohydrates. It would be obvious, considering the nutritional biology of the carp (Jobling 1994), if such cortisol levels would be accompanied by respectively high concentration of glucose and—as a result of catabolic action of glucocorticoids—lower increment of the body weight.

Analysed fish of group II exhibited statistically significant ($P<0.01$) drop in glucose concentration. Using high-carbohydrate diet this effect, not only indicates good development of endocrine system of pancreatic islets and high sensitivity of carp tissues for insulin (Mackett et al. 1992; Nowak 1994) but can be also an effect of physiological mechanism, regulating removal of glucose from the blood after high-carbohydrate meal. This mechanism, well functioning in healthy humans, was discovered also in animals (Maćkowiak et al. 1993). It has been proven that its action depends on the amount of carbohydrates in the diet and when they are more abundant, the glucose is quicker evacuated to the blood. This mechanism

has not been learned completely. The explanation may be linked to the increase of insulin cell receptors, which are especially well developed in the carp (Nowak 1994), and to the increase of glucose transformation in the cells.

The role of insulin ant its receptors in the process of regulation of glucose level in the blood of carp fed with high-carbohydrate diet may be confirmed by statistically significant increase of triglyceride concentration, compared to the initial state ($P<0.01$) as well as to the fish fed with high-fat diet ($P<0.05$). It has been demonstrated that insulin stimulates, among others, also pentose pathway providing precursors for biosynthesis of fatty acids. It also enables penetration of glucose to fatty cells, where α-glycerophosphate is formed—a compound necessary for estrification of fatty acids (Ince and Thorpe 1974). Also the body weight increments—higher in the same experimental group, compared to the high-fat group, having calorific value by 50 kcal per 100 g of fed higher, would confirm lipogenic action of insulin. In this case the insulin release would be forced by the composition of high-carbohydrate diet. Numerous studies, however, proved that significant role in releasing insulin in fishes is played, beside glucose, also by amino acids (Plisetskaya 1989). Because in the experiment presently conducted the amounts of proteins were identical in both nutritional groups, it can be assumed, that changes that occurred, were most of all, connected with variable content of carbohydrates in the diets used.

Comparing the cortisol level in the blood of the carp of all nutritional groups it can be concluded that an intensive rearing using high-fat diet is less burdening for fish organism. The associated cortisol level, accompanied by correct glucose level is statistically significantly ($P<0.01$) lower, than that observed in the carp fed with high-carbohydrate diet. On the other hand it is as high as that associated with the other, very strongly active environmental aggression factors (Friedrich 1996a). The higher level of triglycerides in the blood of the carp of this nutritional group, however, seems to be a result of the composition of diet used (Filipiak et al. 1993).

Very high cortisol level in the blood of carp in both experimental groups, observed two months after the diet composition change, indicates constant influence of a stressful factor, which is associated with increased activity of the pituitary-interrenal system. The factor responsible for triggering the reaction seems to be the elevated content of fats and carbohydrates in the diet used as well as very intensive rearing process. The latter influenced the general metabolism so extensively, that within the two months, the carp increased their weight 2.5 times. The lack of catabolic effect, that could be expected to accompany such high concentration of cortisol in the blood, can be linked to the decreased sensitivity of steroid receptors under influence of high level of glucocorticoids, if such level is a result of chronic stress (de Kloet 1989). It would be confirmed by the total lack of diseases and

mortalities of the fish, expected to accompany the action of high concentrations of cortisol (Ślebodziński 1991). The most recent reports form various research centres in the world show that at the background of those phenomena can be the presence in the cells of specific proteins responsible for binding ACTH. The synthesis of these proteins rapidly increases during stress situations (Jamme 1989; Schlatter and Dokas 1989; King and Bearstschi 1990).

On the other hand, the observed changes in the levels of glucose and triglycerides in the blood of the experimental carp seem to suggest univocally the influence of the amounts of essential nutritional components in the diets used on certain metabolic pathways of organism. It was also confirmed by the results of the other authors studies (Alexis et al. 1986; El-Sayed and Garling 1988).

CONCLUSION

1. Intensive rearing of two-year-old carp, using feeds with risen content of fats or carbohydrates causes statistically significant increase of cortisol concentration in the blood of the fish.
2. The analysis of the values of cortisol in the blood of both nutritional groups, indicates that the more burdening was the high-carbohydrate diet.
3. The observed changes in the levels of glucose and triglycerides in the blood of both nutritional groups, point out at the influence of carbohydrates and fats in the diets, on the carbohydrate-lipid metabolism.

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STRESZCZENIE

Doświadczenie przeprowadzono na karpiach w drugim roku życia, hodowanych w kanale wody pochłodniczej Elektrowni Dolna Odra. Celem pracy jest określenie wpływu zmiany paszy i zastosowania intensywnego chowu przy użyciu pasz z podwyższonym udziałem tłuszczów – Grupa I lub węglowodanów – Grupa II, na poziomy kortyzolu, glukozy, triglicerydów oraz przyrosty masy ciała. Stwierdzono że: 1) zmiana składu paszy i intensywny chów karpia spowodował statystycznie istotny ($P < 0.01$) wzrost stężenia kortyzolu we krwi obu grup doświadczalnych w stosunku do stanu wyjściowego, przy czym wzrost ten był statystycznie istotnie ($P < 0.01$) wyższy w grupie żywionej dietą bogatą w tłuszcze w stosunku do grupy żywionej dietą bogatą w węglowodany; 2) wystąpił statystycznie istotny ($P < 0.01$) spadek poziomu glukozy u ryb żywionych dietą bogato węglowodanową; 3) wystąpił statystycznie istotny ($P < 0.01$) wzrost stężenia triglicerydów w obu grupach żywieniowych, oraz 4) zaobserwowano większe przyrosty masy ciała ryb żywionych paszą z wyższym udziałem węglowodanów.

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