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Fish physiology

**EFFECT OF DIET COMPOSITION ON THE LEVELS OF GLUCOSE,
LIPIDS, AND LIPOPROTEINS OF THE BLOOD
AND ON THE CHEMICAL COMPOSITION OF TWO-YEAR-OLD CARP
(*CYPRINUS CARPIO* L.) REARED IN COOLING WATERS**

**WPLYW SKŁADU DIETY NA POZIOMY GLUKOZY, LIPIDÓW
I LIPOPROTEIN KRWI ORAZ SKŁAD CHEMICZNY CIAŁA
KROCZKA KARPIA (*CYPRINUS CARPIO* L.) HODOWANEGO
W WODACH POCHŁODNICZYCH**

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The present study was conducted on two-year-old carp, reared in the production-scale conditions. The fish were kept for 62 days in cooling water and fed feeds differing in the fat content (35.9% of the energetic value of the diet) and carbohydrate content (35.6% of the energetic value of the diet). The experiment resulted in statistically significant differences in the levels of glucose, lipids, and blood lipoproteins as well as in the body composition of the fish in relation to the pre-experiment values and in relation to the other nutritional groups.

INTRODUCTION

The common carp is one of the principal fish species suitable for rearing in the controlled conditions. In addition, it constitutes a desirable food item for consumers aware of the need for rational nutrition. These two factors combined, oblige aquaculturists to deliver the fish that is not only of good weight and condition, but is also representing desirable taste- and nutritive values. The principal concern here are the pre-determined values of proteins and lipids in the muscle tissue of the fish. As for the lipids—it is not only their content, but also the quantitative composition. According to the literature data n-3 acids present in the fish lipids play a significant role in prophylaxis of the blood-circulation disorder-

ders (Gertig and Przysławski 1994) and they also can lower the frequency of breast cancer and tumors of colon (Ziemiański and Budzyńska-Topolowska 1992; Budzyńska-Topolowska and Ziemiański 1993). It has been demonstrated in a number of studies that the amounts and the composition of fish lipids are dependent on the feed components. It is not only the lipids, their quantities and the composition (Viola et al. 1981, 1982) but also carbohydrates (Shimeno et al. 1981). Direct and indirect impact of the lipids and carbohydrates present in the diet on the defined metabolic pathways of the fish organism is indicated by the levels of the components of the carbohydrate-lipid transformations. Among such components we can list: glucose, triacylglycerols, crude lipids, cholesterol and its LDL and HDL fractions (Friedrich 1997).

The aim of the present study was to determine a possible effect of various diets, differing in the fat content (35.9% of the energetic value of diet) and carbohydrate content (35.6% of the energetic value of diet), on the levels of glucose, lipids, and blood lipoproteins and the body composition of the two-year-old carp.

MATERIAL AND METHODS

The experiment was conducted in summer (July–August) on 180 two-year-old carp at the Fisheries Research Station (RSD) of the Agricultural University of Szczecin, situated at the premises of the Dolna Odra power plant at Nowe Czarnowo. The fish were kept in 6 cages ($2.0 \times 0.75 \times 0.8$ m) of the usable volume of 1.0 m^3 . Each cage was stocked with a total of 30 fish.

The fish were randomly divided into two groups and the differentiation factor was the diet composition. Each group was tested in three repetitions.

The fish were fed according to the following schemes:

The composition of the standard diet before the start of the experiment was as follows:

Protein—45 g = 754.2 kJ (44.6% of energy)

Fat—20 g = 670.4 kJ (39.6% of energy)

Carbohydrates—16 g = 268.2 kJ (15.8% of energy) + 2 g of other additives

Overall calorific value of 100 g of the feed—1692.8 kJ.

Group I—fed feed with elevated content of fat (addition of 10 g of poultry fat to 100 g of food)

Protein—38.7 g = 648.6 kJ (39.2% of energy)

Fat—17.7 g = 593.3 kJ (35.9% of energy)

Carbohydrates—24.6g = 412.3 kJ (24.9% of energy) + 2 g of other additives

Overall calorific value of 100 g of the feed—1654.2 kJ

Group II— fed feed with elevated content of carbohydrates (addition of 25 g of wheat flour to 100 g of food)

Protein—38.7 g = 648.6 kJ (42.6% of energy)

Fat—9.9 g = 331.8 kJ (35.9% of energy)

Carbohydrates—32.3 g = 541.3 kJ (35.6% of energy) + 2 g of other additives

Overall calorific value of 100 g of the feed—1521.7 kJ.

The fish were fed daily, every 60 minutes within 08.00–15.00 hours. The feeds were administered manually on the water surface in the cages. The daily feed rations, calculated in relation to the metabolic weight of the fish were 2%.

Blood for the analyses (from 20 carp) was collected from the caudal vessels between 07.30 and 08.30 hours before the start of the experiment and after its end from 10 carp of each group and from each repetition. The blood was chilled and centrifuged at 3500 RPM for 30 minutes at 4°C.

The content of the following components of the blood serum was determined:

- glucose, using an enzymatic method based on a diagnostic kit of POCh Gliwice,
- triacylglycerols (TG), using an enzymatic method based on a diagnostic kit of Cormay TG-30 and an Abbot biochemical analyzer,
- crude lipids, using an enzymatic method and an Abbot biochemical analyzer,
- crude cholesterol and its HDL (high-density lipoproteins) fraction, using an enzymatic method and an Abbot biochemical analyzer,
- cholesterol LDL (low-density lipoproteins) fraction, calculated based on the following formula: $LDL = \text{crude cholesterol} - (\text{TG}/5 + \text{HDL})$.

The analysis of the body composition of the fish body was conducted before the start of the experiment and after its end. It was based on 5 fish of each nutritional group, separately from each repetition. To obtain uniform samples the whole fish were ground and three times homogenized. The samples prepared in such way were analyzed for the content of:

- crude protein, using the Kjeldahl method,
- crude lipids, using the Soxhlet method,
- dry matter, drying the samples at 105°C for 12 hours,
- ash, roasting at 550°C for 10 hours.

During the experiment also the water temperature was monitored. It could be helpful in understanding possible disturbances that could have occurred at the time of the study.

The results obtained were processed statistically, using the Statgraphics® (version 6.0) computer program. Possible significance of differences was assessed using Student t-test and the Duncan test.

RESULTS AND DISCUSSION

The conclusion of the present study is that intensive rearing of two-year-old carp using the diets of elevated content of fat (35.9% of energy) (group I) or carbohydrates (35.6% of energy) (group II) caused significant differences in the levels of the analyzed components of the blood serum (Tab. 1) and in the body composition. The above differences were determined in relation to the pre-experiment status as well as between both nutritional groups (Tab. 2). In the course of the experiment a statistically significant ($p < 0.001$) drop in the glucose level was noted in the fish of group II in relation to the initial state and to the fish of group I. The latter fish had the level of this component, determined by the end of the experiment, similar to the initial values. Such phenomenon can be a confirmation of good development of the endocrine system of the pancreatic islets and a confirmation of high sensitivity of the carp tissues to insulin (Mackett et al. 1992; Nowak and Maćkowiak 1993; Nowak 1994). The observed decrease in the glucose concentration in this group of fish can be a result of a physiological mechanism regulating removal of glucose from the blood after a high-carbohydrate meal (Pelikanova and Kohout 1989). It has been demonstrated that the action of this mechanism depends on the amount of carbohydrates in the diet. The more carbohydrates are present—the more efficiently glucose is removed from the blood (Maćkowiak et al. 1993). The above mechanism has not been completely understood. Among the clues there can be an increase of the number of the cell receptors for insulin, which are especially well developed in carp (Nowak 1994) and / or escalation of the glucose transformation in the cells. A similar phenomenon of lowered levels of glucose in the fish fed diets of elevated content of carbohydrates was observed by Shimeno et al. (1981) and Alexis et al. (1986).

Table 1

Levels of selected components of the blood serum of the carp before and after the experiment
($\bar{x} \pm s$; $n = 80$)

Component	Before experiment (a)	After experiment		Significance of differences
		Group I (b)	Group II (c)	
Glucose (mg/dl)	83.9 ± 18.7	85.5 ± 18.1	59.2 ± 11.7	a-c**, b-c**
Triacylglycerols (mg/dl)	126.3 ± 18.1	180.9 ± 24.8	204.3 ± 36.9	a-b**, a-c**, b-c*
Cholesterol (mg/dl)	126.3 ± 10.2	167.4 ± 10.1	175.2 ± 16.1	a-b**, a-c**
LDL (mg/dl)	5.1 ± 2.3	22.1 ± 21.2	26.9 ± 18.2	a-b*, a-c*
HDL (mg/dl)	94.4 ± 8.8	109.0 ± 30.1	107.5 ± 27.0	no differences
Overall lipids (mg/dl)	491.3 ± 58.3	632.7 ± 31.8	673.7 ± 52.6	a-b**, a-c**, b-c**

* statistically significant difference ($p \leq 0.05$)

** statistically significant difference ($p \leq 0.01$)

Table 2

Chemical composition of the body of the carp before- and after the experiment (%)

Component	Before experiment (a)	After experiment		Significance of differences
		Group I (b)	Group II (c)	
Dry matter	28.80	29.67	27.41	a-b**, a-c*, b-c**
Crude protein	14.50	16.46	17.54	a-b**, a-c**, b-c**
Lipids	12.00	11.92	9.01	a-c**, b-c**
Ash	1.80	1.70	1.70	no differences

* statistically significant difference ($p \leq 0.05$)** statistically significant difference ($p \leq 0.01$)

Another fact in the present experiment that can confirm the role of insulin and its receptors in the regulation of the glucose level in the blood of the carp fed the high-carbohydrate diet can be a statistically significant increase of triacylglycerols concentration in this group of the fish in relation to the initial state ($p \leq 0.01$) as well as to the fish fed the high-fat diet ($p \leq 0.05$). It has been demonstrated that the diet-forced release of insulin stimulates, among others, the pentose cycle. The latter provides precursors to biosynthesis of fatty acids, and through facilitation of glucose penetration to adipocytes and through activation of glycerol 3-phosphate acetyltransferase it contributes to enhancement of their esterification (Pelikanova and Kohout 1989). Numerous other sources suggest, however, that a significant role in the insulin release can be played along with glucose, also by amino acids (Plisetskaja 1989). Because the amount of proteins in the present experiment in both nutritional groups was identical it can be assumed, that the changes observed, were related predominately to various levels of carbohydrates in the diets used. The observed changes in the levels of glucose and triacylglycerols in the blood of the carp fed high-carbohydrate diet seem to indicate, the presence of the defined metabolic pathways in the carp organism and a significant effect of the amount of carbohydrates present in the diet—on the functions of these pathways.

Studies conducted in the 1970s by Chapman et al. (1977; 1978) and Leger et al. (1979), related to the presence of lipoproteins in the blood serum of fish demonstrated that in fish, similarly as in humans, there are four major groups of lipoproteins of great physiological importance namely: chylomicrons, VLDLs (very low density lipoproteins), LDLs (low density lipoproteins), and HDLs (high density lipoproteins).

The present experiment resulted in a statistically significant ($p \leq 0.01$) increase in crude cholesterol in the blood of the fish of both nutritional groups. The increase was higher in the carp fed the high-carbohydrate diet than that in the fish fed high-fat diet. This phenomenon was accompanied by a statistically significant ($p \leq 0.05$) increase of LDL fraction of cholesterol. No significant changes were observed in the levels of HDL fraction. In gen-

eral, increase of the cholesterol levels and its LDL fraction is associated with consumption of fats in this number unsaturated fats. In the present experiment, higher increase of the analyzed components was observed in the fish with less fat in their diet. It had been demonstrated in a number of studies, that in humans (Kushi et al. 1985) and in higher animals (Barowicz et al. 1997) the presence saturated fatty acids and cholesterol in the diet can be a cause of increase of overall cholesterol and its LDL fraction. Unfortunately the accessible literature did not contain any data, that would indicate occurrence of a similar relationship in fishes. The results of the present experiment suggest that it does not occur. Possibly, the lower cholesterol level and its LDL fraction observed in the carp fed the high-fat diet was associated with the presence in their diet higher amounts of bran providing nutritive fiber. The role of fiber in lowering the levels of the analyzed components in the human blood has been well known (Kozłowska-Wojciechowska 1993). It has to be emphasized that a similar effect of high-carbohydrate diet was observed by Jeong et al. (1992). While studying the carp blood after 5 hours after feeding they observed, that along with the increase of extruded starch in the feed, the cholesterol level increased. On the other hand Henken et al. (1986), studying effect of diet on the cholesterol level in the blood of the African catfish, observed increase in cholesterol concentration along with the increase of the protein content in the diet.

The last of the analyzed biochemical components of the blood of two-year-old carp was the level of crude lipids. The present experiment yielded a statistically significant ($p \leq 0.01$) increase of crude lipids in the blood of both nutritional groups in relation to the pre-experiment status. The fish of group II also demonstrated a statistically significant ($p \leq 0.01$) difference of the levels of crude lipids compared to that of group I.

Robinson and Mead (1973), while studying absorption of lipids in trout, divided the lipids of serum into: phospholipids, esters of cholesterol, free cholesterol, triacylglycerols and free fatty acids. In view of the above, it can be presumed, that the existing differences in the levels of lipids in the blood of the carp studied are a consequence of existing and discussed earlier differences in the levels of triacylglycerols, cholesterol and its LDL fraction.

On the other hand, chemical composition of feeds, both qualitative and quantitative influences not only the levels of the analyzed blood components, but also on protein- and lipid content of the muscle tissue of fish and it also determines the effectiveness of the rearing process (Shimeno 1997; Steffens 1997).

The present study revealed also changes in the chemical composition of the carp body. Using the diets of the same content of proteins, a statistically significant difference ($p \leq 0.01$) in the content of crude protein between the bodies of group II fish (fed the diet of elevated content of carbohydrates) and the bodies of group I fish (fed the diet of elevated content of fat) as well as in relation to the pre-experimental state. It is likely, that the higher

protein content in the bodies of group II fish was caused directly by implicating the defined metabolic pathways by increased content of carbohydrates in the diet. It has been demonstrated, that insulin which release was forced by the composition of the diet used, causes in fish, among other things, incorporation of amino acids to the body proteins (Plisetskaja 1989).

The results of the present experiment indicate also a visible effect of the diets used to the lipid content of the bodies of the carp studied. A statistically significant difference ($p \leq 0.01$) in the levels of body lipids was noted between the fish fed the elevated content of fats and group II fish fed the diet of elevated content of carbohydrates, where lipid content was not only lower, but also it demonstrated a statistically significant ($p \leq 0.01$) difference compared to the pre-experimental state. It has been known that elevated fat content in the diet, in a significant way influences the lipid content in the fish body (El-Sayed and Garling 1988; Ellis and Reigh 1991; Filipiak 1995). The differences in the lipid levels observed in the present experiment, seem to be effects of the action of the defined metabolic pathways, stimulated by the components of the diet used.

It has been demonstrated that the lipid metabolism concerns chiefly fatty acids and cholesterol. The source of long-chain fatty acids is either *de novo* synthesis from acetyl-CoA provided by carbohydrate transformation or re-synthesis of fat from food (Kolanowski and Świdorski 1997; Steffens 1997). In the tissues, fatty acids can be oxidized to acetyl-CoA (β -oxidation) or can be a subject of esterification to acylglycerols. The latter in a form of triacylglycerols constitute the main energetic reserve of organism. It can be assumed that the principal source of fatty acids in the bodies of carp of group I was the poultry fat added to the feed. On the other hand, in group II, the lipids had to be bio-synthesized *de novo* from acetyl-CoA, being the final product of transformation of the carbohydrates delivered to the fish with the feed.

The source of lipids synthesized within the fish body is extremely important because of polyene fatty acids present in lipids. Polyene fatty acids of the n-3 and n-6 series, present in the fish lipids, play very important, physiological role in human nutrition (Ziemiański and Budzyńska-Topolowska 1992; Budzyńska-Topolowska and Ziemiański 1993). In the present experiment, the lipids present in the fish bodies nor the composition of the fatty acids were analyzed. Numerous studies, however, conducted on fishes indicate, that the kind of the diet used and in particular the kind of fat added to the feed and its content of higher fatty acids have effect on the kind and quantity of higher fatty acids present in the fat in the tissues of fishes (Viola et al. 1981, 1982; Kuczyński and Barska-Kłyta 1991; Steffens 1993). Observed in the present experiment lower level of fat in the bodies of carp fed high-carbohydrate diet can be disadvantageous from a point of view of the aquaculturist. How-

ever, from a perspective of human nutrition the kind and the composition of body fat of fish is more important than its overall content.

The present experiment revealed also statistically significant differences ($p \leq 0.01$) in the content of the dry mass between the nutritional groups. The higher content of the dry mass was noted in the fish fed the high-fat diet. It means that in the carp of the group fed high-carbohydrate diet, the observed higher increments of the body weight and higher protein content were accompanied by a higher water accumulation in the muscles. It has been known, that the fatty tissue in an organism is not only an energy reserve, but also a reserve of certain amount of water. The latter constitutes from 30 to 50% of the fatty tissue. In the consequence the lower level of the dry mass associated with the lower level of fat in the bodies of group II fish compared to that of the group I can be perceived as not very logical. Possibly, to some extent, this phenomenon was explained by Friedrich (1997), studying relationship between different diets and the release of cortisol, who concluded that feeding of a two-year-old carp the diet of elevated level of carbohydrates causes higher increase of cortisol level in the blood, than the feeding a standard diet or the diet with elevated level of fat. The effect of cortisol on changes in the volume of aqueous spaces of the organism in the extrarenal way on the level cell-intercellular fluid was demonstrated by Kolpakov et al. (1969). The effect of cortisol on increment of the serum volume was demonstrated by Swingle and Swingle (1966). It must be emphasized that the lower level of the dry mass associated with the higher body weight of the fish should be considered an undesirable phenomenon, especially when the fish are intended for a long frozen storage. Freezing the water causes certain damages to the structure of the cellular membrane and the partial release of structural proteins. In addition, the crystallization of water during freezing can damage hydrophobic-hydrophilic bindings causing denaturation of proteins (Sikorski 1980). It should be kept in mind, that the carp, being one of the most frequently consumed freshwater fishes is usually eaten fresh or processed without former freezing.

The content of individual components and their interrelationships in the body of fish undergoes constant changes. Not only the age and size of the fish, but also the chemical composition of the feeds administered to the fish have effect on that, but also the water temperature (Watanabe et al. 1987).

In the present experiment the temperature of cooling water changed within the range of 23.8–27.8°C. The average temperature for the entire experimental period was 26.3°C and it fits into the range assumed as optimal for the carp (Tab. 3).

Table 3

Average weakly temperatures of the cooling water during the experiment

Week of experiment	I	II	III	IV	V	VI	VII	VIII	IX
Average water temperature (°C)	23.8	25.8	24.3	26.9	27.8	27.4	27.2	26.7	27.4

CONCLUSIONS

Analysis of the acquired results permits to draw the following conclusions:

1. Statistically significant differences between the levels of glucose, lipids, and lipoproteins in the blood of fish representing both nutritional groups, indicate a direct and indirect effect of the diet components on the defined metabolic pathways of the organism.
2. Usage of the diets of the predetermined composition in the fish culture allows the aquaculturist to obtain, within certain limits, the fish of the chemical composition of the bodies consistent with the needs of the consumer.

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HODOWANEGO W WODACH POCHŁODNICZYCH

STRESZCZENIE

Doświadczenie przeprowadzono na karpach w drugim roku życia, w okresie letnim w Rybackiej Stacji Doświadczalnej, usytuowanej na terenie Elektrowni Dolna Odra. Celem badań było zaobserwowanie w jaki sposób u krocza karpia żywionego dietą z różnym udziałem tłuszczu (35,9% wartości energetycznej diety – grupa I) i węglowodanów (35,6% wartości energetycznej diety – grupa II) kształtują się poziomy glukozy, lipidów i lipoprotein krwi oraz skład chemiczny ciała. Stwierdzono: 1) statystycznie istotny ($p \leq 0,01$) spadek poziomu glukozy we krwi ryb grupy II w stosunku do stanu wyjściowego, jak również w stosunku do grupy I, w której poziom tego składnika po zakończeniu doświadczenia był zbliżony do wartości wyjściowych; 2) statystycznie istotny wzrost stężenia triacylogliceroli we krwi ryb grupy II, tak w stosunku do stanu wyjściowego ($p \leq 0,01$) jak i w stosunku do ryb grupy I ($p \leq 0,05$); 3) statystycznie istotny ($p \leq 0,01$) wzrost poziomu lipidów całkowitych we krwi karpia obu grup żywieniowych w stosunku do stanu przed doświadczeniem, przy czym wzrost ten był wyższy w grupie II, a różnica w stosunku do grupy I statystycznie istotna ($p \leq 0,01$); 4) statystycznie istotny ($p \leq 0,01$) wzrost zawartości białka ogólnego w ciele ryb obu grup żywieniowych w stosunku do stanu przed doświadczeniem, przy czym zawartość ta była wyższa w grupie II, a różnica w stosunku do grupy I statystycznie istotna ($p \leq 0,01$); 5) statystycznie istotny ($p \leq 0,01$) spadek zawartości lipidów w ciele karpia grupy II, w stosunku do stanu wyjściowego i ryb grupy I.

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