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THE EFFECT OF EXPERIMENTAL STARTERS ON MORPHOLOGICAL CHANGES IN THE INTESTINE AND LIVER OF COMMON CARP (*CYPRINUS CARPIO* L.) LARVAE REARED UNDER CONTROLLED CONDITIONS

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ABSTRACT. The aim of present study was to evaluate the effects of five experimental starters on the growth rate, survival, development, and morphological changes in the digestive systems of common carp, *Cyprinus carpio* L., larvae reared under controlled conditions. The starters tested were based on fish hydrolysate and blood meal (diets K, KL, and KLP), fish meal and blood meal (diet KR), and fish meal, blood meal, and fish hydrolysate (diet KHR). Starters KL and KLP were supplemented with intestine lyophilisate obtained from market-sized carp (as a source of digestive enzymes) while a probiotic (mixture of lyophilized lactic acid bacteria) was added to starter KLP. Start-feeding common carp larvae of an average body mass of 2.32 mg and total length (LT) of 6.58 mm were used for the test. The stocking density per tank (volume of 20 dm^3) was 100 fish. After 14 days of feeding, the highest average survival (62.9%) was obtained on the KLP diet, while the lowest (11%) was noted on the KR feed. The body total length of carp larvae ranged from 7.83 mm (KR diet) to 8.77 mm (KL diet). In the K, KL, and KLP groups, the final body mass of carp larvae was 48-49 mg, while in the KR and KHR it was 22 mg. Normal digestive tract development was observed in the carp fed the KL and KLP diets.

Key words: COMMON CARP (*CYPRINUS CARPIO*), STARTER DIETS, PROTEIN SOURCES, LARVAL DEVELOPMENT, INTESTINE, HEPATOCYTES

INTRODUCTION

The development of commercial rearing technology of cyprinid fishes is limited mainly by the lack of appropriate artificial feeds that meet the nutritional requirements of larval and juvenile stages of these fish. Rearing common carp, *Cyprinus carpio* L., larvae using starters from the very beginning of exogenous feeding seems particularly difficult (Appelbaum and Dor 1978, Dąbrowski et al. 1983, Charlon and Bergot 1984, Szlamińska 2003, Wolnicki 2005). The first attempts to use Ewos commercial carp

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starters C10 and C20 (Dąbrowski 1982, Szlamińska et al. 1993) or the Ekvizo starter formulated by Ostroumova et al. (1980) were unsuccessful. Common carp larvae fed these diets exhibited slow growth, low survival, as well as frequently various developmental disturbances.

The ability to uptake, digest, and absorb exogenous food by fish larvae depends on the development of the digestive tract. Histological methods permit evaluating its functional status as well as detecting diet-related morphological changes. The latter is very useful for understanding the causes of the success or failure of larval pre-rearing under controlled conditions.

The present study was undertaken to evaluate the effects of five experimental starters on the growth rate, survival, development, and morphological changes in the digestive system of common carp larvae under controlled conditions.

MATERIALS AND METHODS

EXPERIMENTAL FEEDS

The experimental starters were formulated in the Experimental Station of Feed Production Technology and Aquaculture in Muchocin (Poland) using various methods including barothermal preparation. Five starters with different main protein sources were prepared: K, KL, and KLP – with fish hydrolysate and blood meal; KR – with fish meal and blood meal; KHR – with fish meal, blood meal, and fish hydrolysate (Table 1). Starters KL and KLP were supplemented with intestine lyophilisate obtained from market-sized carp (as a source of digestive enzymes). Starter KLP was also fortified with a probiotic (a mixture of lyophilized lactic bacteria *Carnobacterium piscicola* and *Carnobacterium divergens*) at concentrations 10×10^5 CFU (colony forming units) kg^{-1} feed (Table 1). Carp intestine homogenate and bacteria were freeze-dried using the Christ Ewert 1825 lyophilizer at – 40°C.

The feeds were prepared as microgranules of two fractions:

- “00” grain diameter 0.071 – 0.16 mm;
- “0” grain diameter 0.10 – 0.20 mm.

The chemical analysis of the feeds was performed according to the methods described by Skulmowski (1974). Protein levels were evaluated using the Kjel-Foss Automatic 16210 analyzer, crude fat with the Soxlet method, and crude fiber with the

Tecator Fibertec System M 1020 Hot Extractor analyzer. Ash content was measured in samples that had been mineralized at 550°C for 12 hours (Linn Elektro Therm stove). The amount of nitrogen-free extract compounds was calculated as the difference between dry mass content and the sum of the remaining nutrients. Calcium concentration was measured using the AAS technique (ASS3 by Carl Zeiss Jena, Germany), according to Gawęcki (1988), and phosphorus content with the flame ionization method.

TABLE 1

Composition of experimental starters

Specification	Feed				
	K	KR	KHR	KL	KLP
Fish meal	-	44.0	20.0	-	-
Blood meal	7.2	12.5	8.9	7.2	7.2
CPSP 90[1]	9.5	-	-	9.5	9.5
CPSP Special G[2]	35.0	-	25.0	35.0	35.0
Estrilvo 70[3]	8.0	8.0	8.0	8.0	8.0
Sodium caseinate	10.0	10.0	10.0	10.0	10.0
Wheat flour	18.5	9.1	14.9	18.5	18.5
Cod liver oil	5.5	10.1	6.9	5.5	5.5
Soybean lecithin	0.5	0.5	0.5	0.5	0.5
Fodder chalk	2.0	2.0	2.0	2.0	2.0
Calcium monophosphate	2.0	2.0	2.0	2.0	2.0
Polfamix W[4]	1.5	1.5	1.5	1.5	1.5
Vitazol AD_3EC	0.1	0.1	0.1	0.1	0.1
Choline chloride	0.2	0.2	0.2	0.2	0.2
Threonine	0.1	-	-	0.1	0.1
Carp intestine lyophylisate	-	-	-	+	+
Probiotic	-	-	-	-	+

[1]*CPSP 90 – fish hydrolysate, Sopropeche, Boulogne-Sur-Mer, France*
[2] *CPSP Special G – fish hydrolysate, Sopropeche, Boulogne-Sur-Mer, France*
[3]*Estrilvo 70 soya protein concentrate, Sopropeche, Boulogne-Sur-Mer, France*
[4]*Polfamix W, BASF Polska S.A. Kutno – containing per 1 kg: vitamin A – 1 000 000 IU, vitamin D_3 – 200 000 IU, vitamin E – 1.5 g, vitamin K – 0.2 g, vitamin B_1 – 0.05 g, vitamin B_2 – 0.4 g, vitamin B_{12} – 0.001 g, nicotinic acid – 2.5 g, D-calcium panthotenate – 1.0 g, choline chloride – 7.5 g, folic acid – 0.1 g, methionine – 150.0 g, lysine – 150.0 g, Fe – 2.5 g, Mn – 6.5 g, Cu – 0.8 g, Co – 0.04 g, Zn – 4.0 g, J – 0.008 g, carrier > 1000.0 g*

The separation of amino acids in the dietary protein was done after the samples had been hydrolyzed in 3n HCL at 106°C for 24 hours, using a Microtechna AAT 339 analyzer. Tryptophan content was measured using the colorimetric method (Votisky and Gunkel 1989). The results of amino acid analysis were used to determine the chemical

value of the experimental diets by calculating the chemical score (CS) and indispensable amino acids index (IAAI) (Hardy and Barrows 2002).

Gross energy was calculated from the chemical composition of diets using the coefficients for fish: carbohydrate – 17.18 kJ g^{-1}; protein – 23.46 kJ g^{-1}; fat – 39.38 kJ g^{-1} (Bureau et al. 2002). The chemical composition and nutritive value of the diets are presented in Table 2.

TABLE 2

Chemical composition, amino acid content, chemical nutritive value of protein, and calorific value of experimental starters

	Feed				
Component (% of dry mass)	K	KR	KHR	KL	KLP
Total protein	55.02	54.99	55.07	55.02	55.02
Crude fat	14.68	14.67	14.64	14.68	14.68
N-free extract	16.69	11.08	14.56	16.69	16.69
Crude fiber	0.37	0.33	0.35	0.37	0.37
Crude ash	7.36	8.85	7.97	7.36	7.36
Total phosphorus	0.83	1.52	1.14	0.83	0.83
Calcium	1.45	3.23	2.26	1.45	1.45
Amino acid (g $100g^{-1}$ of protein)					
Arginine	5.850	5.611	5.775	5.850	5.850
Histidine	2.643	4.217	3.334	2.643	2.643
Lysine	7.139	8.598	7.801	7.139	7.139
Tryptophan	0.943	4.091	2.380	0.943	0.943
Phenyloalanine + tyrosine	6.518	7.137	6.808	6.518	6.518
Methionine + cystine	3.154	2.657	2.909	3.154	3.154
Treonine	3.749	4.002	3.828	3.749	3.749
Leucine	7.635	8.844	8.207	7.635	7.635
Isoleucine	3.945	3.742	3.872	3.945	3.945
Valine	5.181	5.759	5.440	5.181	5.181
Parameter*					
CS	Tyr 48.78	Met+cys 45.81	Tyr 48.18	Tyr 48.78	Tyr 48.78
IAAI	72.64	76.11	77.02	72.64	72.64
Gross energy (MJ kg^{-1})	21.55	20.58	21.18	21.55	21.55
E/P (kJ g^{-1})	39.18	37.42	38.47	39.18	39.18

**see Materials and Methods*

Nitrogen concentration in the feeds was similar (protein level 54.99-55.07%) as were energy levels and the energy to protein ratios (from 37.42 to 39.18 MJ kg^{-1}). The levels of essential amino acids were similar in all the starters. The first limiting amino acid in diets K, KHR, KL, and KLP was tyrosine and for the KR diet – methionine with cystine. Starter KR, which contained fish and blood meal, had the highest IAAI level, while the K, KL, and KLP diets made of fish protein hydrolysate and soybean protein concentrate contained the lowest IAAI levels.

FISH AND REARING FACILITIES

The growth test was carried out in the Aquarium Hall of the Department of Inland Fisheries and Aquaculture of the Agricultural University of Poznań (Poland). The water in the flow-through system was aerated using a HIBLOW HP-60 air pump (Hiblow, Japan). Water temperature and oxygen level were measured daily using an ELMETRON CO-315 device (Merazet, Poland). During the experiment, water temperature ranged from 23.7 to 25.3°C, and dissolved oxygen saturation from 78.9 to 88.4%, which were within the optimum ranges for common carp larvae (Steffens 1986).

Start-feeding common carp larvae, which were bred by the researchers and had an average body mass of 2.32 mg and total length (LT) of 6.58 mm, were used for the test. They were stocked at a density of 100 fish $aquarium^{-1}$. The carp were reared in glass 20 dm^3 flow-through aquaria (the entire volume of water was renewed five times per day). Every day (at 08:00 and 20:00) the tanks were cleaned by removing feces and food remains. The fish were fed *ad libitum* for 24 hours using automatic feeders. The experiment involved five feeding groups (each in three replicates) and lasted for 14 days.

The nutritional value of the starters was evaluated using the indices of fish survival, final body mass, and body length. Dead larvae were registered daily and the number of sampled larvae was recorded to calculate survival. The final fish biomass was determined by weighing the entire tank population to the nearest 0.001 g with a Medicat 160M scale (PAG OERLIKON AG Zürich, Switzerland). Mean individual fish body mass was calculated by dividing fish biomass by the number of fish. Ten larvae per tank were measured to the nearest 0.01 mm with a Helios electronic slide caliper (Helios Messtechnik GmbH & Co. KG, Germany). These measurements were used to calculate the mean total length (LT).

HISTOLOGICAL AND MORPHOMETRIC ANALYSES

The histological and morphometric analyses were performed at the Laboratory of Ichthyobiology and Fisheries of the Warsaw Agriculture University (Poland). For histological studies, five larvae from each tank were sampled on days 0, 3, 6, 9, 12, and 14 of the experiment (15 larvae from each feeding group). The fish were anesthetized using MS-222 (tricaine methanesulphonate). Then they were measured (TL) to the nearest 0.01 mm (using a stereoscopic microscope), weighed to the nearest 0.01 mg, and preserved in 10% buffered formaldehyde and Bouin's solution.

The samples obtained were subjected to standard histological procedure; whole fish were embedded in paraffin, cut into transverse and longitudinal sections 4-6 μm thick with a microtome, and stained with hematoxylin and eosin, Masson's trichrome and alcian blue, periodic acid-Schiff reagent (AB/PAS) pH 2.5, 1.0, 0.5. Periodic acid-Schiff's was used to stain for glycogen and mucins (carbohydrate compounds) with diastase as the control (Martoja and Martoja-Pierson 1970, Pearse 1985). Cell and tissue measurements were done using a Nikon-Alphaphot-2YS2 microscope with a Nikon 4300 digital camera (Nikon Corporation, Tokyo, Japan) and the image analysis systems MicroScan (v. 1.5) and Lucia 4.21 (Laboratory Imaging, Prague, Czech Republic).

The histological evaluation of morphological changes in the digestive systems of fish fed various diets was performed, and the following measurements were taken: the height of intestine folds and enterocytes of the middle and posterior intestine sections; the diameter of absorptive vacuoles in the supranuclear regions of enterocytes in the posterior intestine (to the nearest 0.01 μm). Hepatocyte diameter was used as an indicator of liver activity. Morphometric evaluation was done on days 0, 3, and 14 of the experiment. For morphometric evaluation, 50 samples of 5 fish from each tank were analyzed at 400 x magnification (for each feeding group $n = 50 \times 15 = 750$).

STATISTICAL ANALYSIS

The means and standard deviations were calculated for larval survival, body length, and mass within the feeding groups. The differences among the groups were tested using one-way ANOVA ($P \leq 0.05$). All of the statistical analyses were carried out using the SPSS 12 statistical package for Windows.

The morphometric data were analyzed using one-way hierarchical ANOVA taking into consideration the effect of feeding and variability within the feeding groups.

RESULTS

The highest average survival (62.9%) was obtained on the KLP diet and the lowest (11%) on KR feed (Table 3). The total length of carp larvae after 14 days of rearing ranged from 7.83 mm (KR) to 8.77 mm (KL). In the K, KL, and KLP groups, the final mean body mass of carp larvae was 48-49 mg, while in the KR and KHR groups it was 22 mg (Table 3).

TABLE 3

Final results of the growth test and morphometric data of the common carp intestine and liver

	Treatment				
Growth test results	K	KR	KHR	KL	KLP
Survival (%)	54.9 ± 3.8c	11 ± 1.9a	13 ± 2.0a	47.4 ± 4.1b	62.9 ± 6.7d
Total length (mm)	8.59 ± 0.41b	7.83 ± 0.28a	7.93 ± 0.21a	8.77 ± 0.15c	8.71 ± 0.31c
Body mass (mg)	48 ± 0.13b	22 ± 0.11a	22 ± 0.09a	49 ± 0.12b	49± 0.11b
Morphometric data – 3 days-post-hatch					
Height of middle intestine mucosal folds (µm)	53.23 ± 1.52a	77.05 ± 3.00c	62.38 ± 2.62 b	85.63 ± 9.27d	74.37± 2.46c
Enterocyte height in middle intestine (µm)	24.91 ± 2.77b	37.82 ± 1.71c	22.17 ± 3.06a	21.63 ± 2.52a	23.85 ± 3.29ab
Lipid vacuole diameter (µm)	5.13 ± 1.31ab	8.71 ± 3.12c	6.61 ± 1.71b	3.63 ± 0.92a	5.514 ± 1.63b
Height of posterior intestine mucosal folds (µm)	74.31 ± 3.87c	58.34 ± 13.39a	57.62 ± 1.14a	67.20 ± 3.41b	87.4 ± 2.81d
Enterocyte height in posterior intestine (µm)	30.70 ± 2.95b	26 ± 4.52a	28.75 ± 3.43ab	29.13 ± 6.13ab	29.08 ± 2.35ab
Hepatocyte diameter (µm)	11.03 ± 11.49	9.98 ± 1.23	10.43 ± 1.50	10.66 ± 0.68	10.74 ± 0,76
Morphometric data – 14 days-post-hatch					
Height of middle intestine mucosal folds (µm)	71.50 ± 4.4c	43.23 ± 9.62a	49.52 ± 2.33b	93.43 ± 3.49d	88.5 ± 4.57d
Enterocyte height in middle intestine (µm)	28.50 ± 1.90b	24.2 ± 1.91a	24.17 ± 2.76a	25.88 ± 2.28a	32.06 ± 2.67c
Height of posterior intestine mucosal folds (µm)	92.97 ± 6.68c	52.25 ± 3.53b	40.85 ± 2.40a	94.29 ± 2.91c	112.23 ± 5.91d
Enterocyte height in posterior intestine (µm)	20.80 ± 2.18b	24.14 ± 2.37c	14.63 ± 0.98a	25.96 ± 2.22cd	27.18 ± 2.83d
Hepatocyte diameter (µm)	11.71 ± 0.54b	8.98 ± 1.19a	8.78 ± 1.49a	11.45 ± 0.56b	11.90 ± 1.50b

Values in the same row with different letters are significantly different (P < 0.05).

At the beginning of the experiment, the larval intestine was lined by a unilayered cylindrical epithelium with a brush border. The cytoplasm of the intestinal epithelium was basophilic (H-E). Among the enterocytes, mucous cells occurred producing acidic carboxylated and sulfated mucins (AB – positive at pH 2.5, 1.0, 0.5). The anterior and central intestine sections were lined by a slightly folded mucosa. The nuclei of enterocytes were located centrally, and supranuclear regions did not contain absorptive vacuoles (Fig. 1A). The epithelial cells of the posterior intestine section were more diversified (Fig. 1B).

The supranuclear regions of the posterior intestine enterocytes showed small absorptive vacuoles. The average height of the epithelium in the anterior intestine sec-

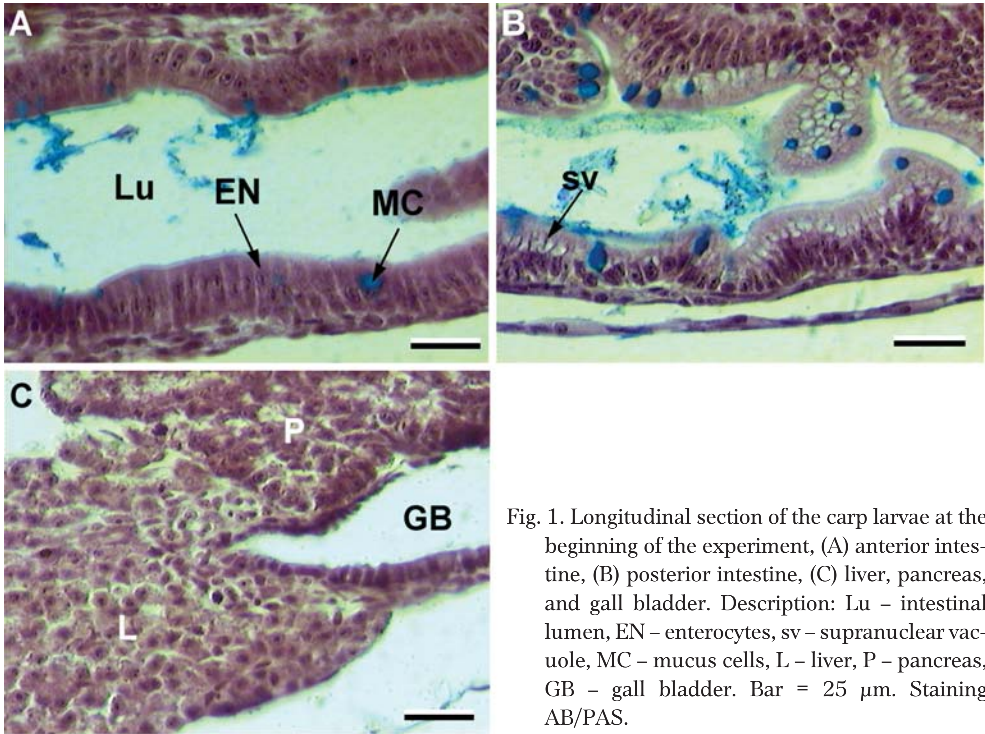

Fig. 1. Longitudinal section of the carp larvae at the beginning of the experiment, (A) anterior intestine, (B) posterior intestine, (C) liver, pancreas, and gall bladder. Description: Lu – intestinal lumen, EN – enterocytes, sv – supranuclear vacuole, MC – mucus cells, L – liver, P – pancreas, GB – gall bladder. Bar = 25 μm. Staining AB/PAS.

tion was 23.39 ± 0.68 μm, while in the posterior part it was 22.13 ± 1.49 μm. The liver surrounded the anterior intestine. The hepatocytes were oval, with centrally located nuclei and a small amount of basophilic cytoplasm. At this developmental stage, no lipid or glycogen storage occurred in the hepatocyte cytoplasm. The average diameter was 7.01 ± 0.74 μm. The pancreas was situated under the swim bladder. Its basophilic cells contained the first granules of proenzyme (H-E and PAS-positively stained). The gall bladder occurred between the liver and pancreas, and its mucosa consisted of unilayered cubical cells (Fig. 1C).

Histological analysis on day 3 of the experiment revealed that the lipid vacuoles appeared in the supranuclear regions of middle intestine enterocytes in all experimental groups (Fig. 2). The enterocytes of the posterior intestine showed absorptive vacuoles containing PAS-positive inclusions (protein) (Fig. 3). The intestine length increased, and its surface showed folds. The longest folds occurred in the intestines of fish fed KL, while the shortest were in those fed the control diet. The smallest

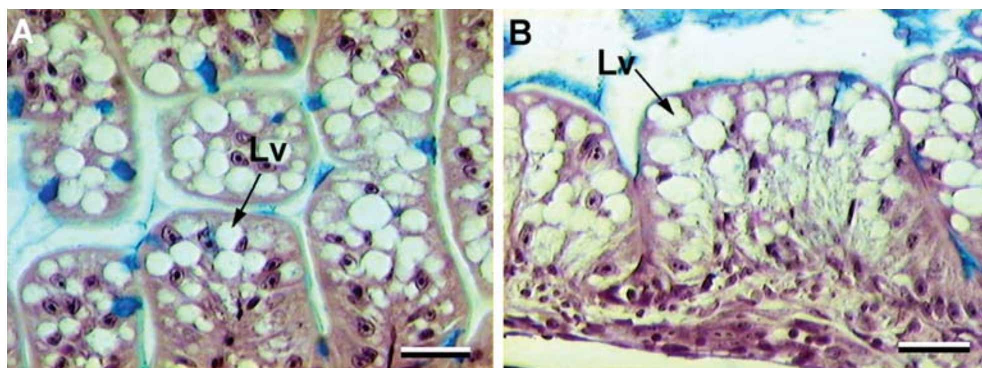

Fig. 2. Section of enterocytes of the middle intestine of carp larvae fed KL (A), KR diets (B). Lv – lipid vacuole. Bar = 25 μm. Staining AB/PAS.

enterocytes and lipid vacuoles were observed in the fish fed the KL diet (Fig. 2A, Table 3) while the largest were noted in the fish fed KR (Fig. 2B, Table 3).

The larvae fed KL, KLP, and K showed higher mucosal folds in the posterior intestine in comparison with those fed the KR and KHR diets (Fig. 3, Table 3). The hepatocytes of 3-day-old common carp larvae of all feeding groups contained glycogen (PAS-positive) and lipids. The largest hepatocytes were observed in the larvae from the control group (Table 3), and these fish also showed higher lipid storage in comparison with glycogen (Fig. 4A). The hepatocytes of fish fed the KR diet had larger glycogen areas in comparison with the other experimental groups (Fig. 4B).

Histological observations done on days 6, 9, and 12 of the experiment did not reveal considerable differences in the development of larvae fed the K, KL, and KLP

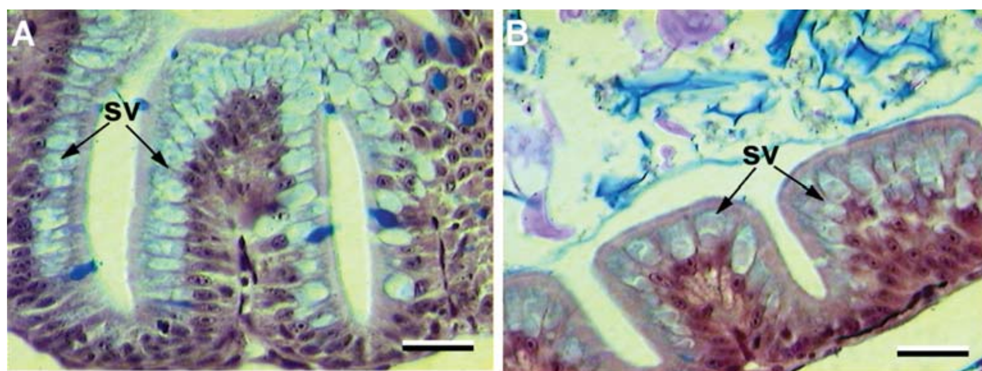

Fig. 3. Section of enterocytes of the posterior intestine of carp larvae fed KLP (A) and KHR diets (B). Sv – supranuclear vacuole. Bar = 25 μm. Staining AB/PAS.

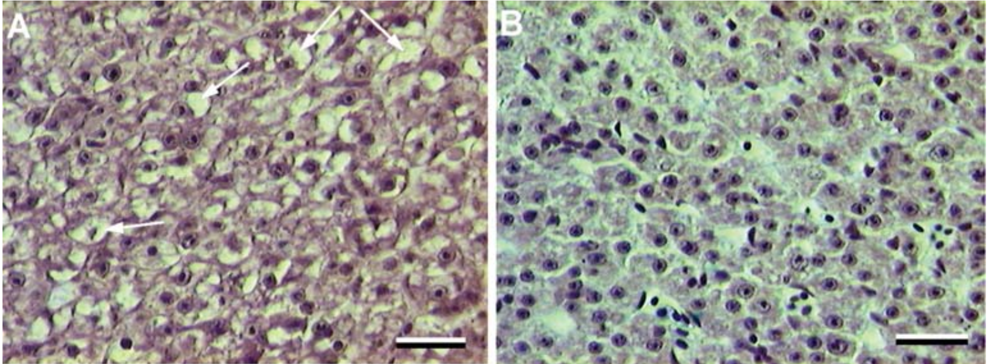

Fig. 4. Section of liver of common carp larvae fed the control diet (A), and KR diet (B). Lipid vacuole (arrows). Bar = 25 μm. Staining AB/PAS.

diets. Large lipid vacuoles in the supranuclear regions of the middle intestine enterocytes were strongly reduced (2.38 ± 0.66 μm) (Fig. 5A), except for the fish fed the KR diet, in which the size of these vacuoles decreased after day 9 of the experiment. The PAS-positive absorptive vacuoles in the supranuclear regions of the enterocytes of the posterior intestine were observed in fish from all the feeding groups. The height of intestinal mucosa folds gradually decreased in the fish fed the KR and KHR diets (Table 3).

The results of histological observations on day 14 of the experiment showed lower mucosal folds along the entire intestine of the fish fed the KR and KHR diets (compare to Fig. 5C – the KLP diet) and hyaline inclusions were visible (Fig. 5B and D). The size and number of lipid (middle intestine) and protein (posterior intestine) vacuoles decreased (Fig. 5B and D). The hepatocytes of the carp larvae fed K, KL, and KLP diets showed similar levels of glycogen and lipid storage, while in the larvae fed the KR and KHR feeds, the hepatocyte diameter was strongly reduced with a small amount of cytoplasm around the nuclei. The nuclei themselves were pyknotic, and the hepatic sinuses were enlarged (Fig. 6).

DISCUSSION

The development of cyprinid starters has involved attempts to enrich feed with proteolytic enzymes (Dąbrowska et al. 1979) or lyophilized livers with yeast (Charlon et al. 1986, Szlamińska and Przybył 1986); these resulted in a fish body mass that

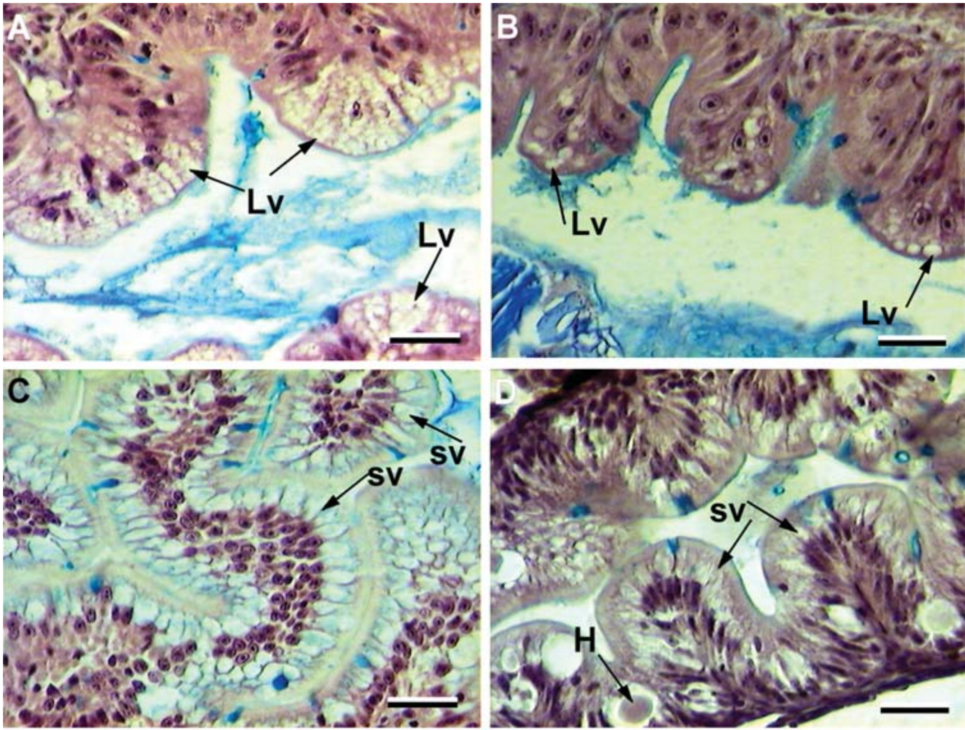

Fig. 5. Section of middle intestine enterocytes of common carp larvae fed KLP diet (A), KR diet (B). Section of posterior intestine enterocytes of common carp larvae fed KLP diet (C), KR diet (D). Lv – lipid vacuole, Sv – supranuclear vacuole, H – hyaline inclusions. Bar = 25μm. Staining AB/PAS.

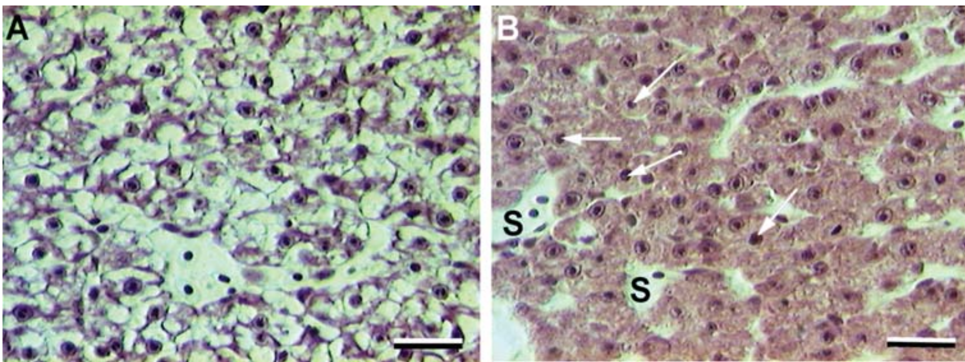

Fig. 6. Section of liver of common carp larvae fed control diet (A), KHR diet (B). S – sinuses, pyknotic nuclei (arrows). Bar = 25 μm. Staining AB/PAS.

exceeded 100 mg (over a 3-4 week period) and survival of more than 90%. Przybył and Madziar (1993) obtained 303.43 mg fish and 83.2% survival at the end of a 28-day test in which common carp larvae were fed a micropelleted formula (Przybył et al. 1992) containing ground Torula yeast and lyophilized pork pancreas and liver.

In the present study, the best results were obtained on the KLP starter containing hydrolyzed fish protein and lyophilized carp intestine homogenate supplemented with the probiotic lactic bacteria. After 14 days of feeding, the fish reared on this starter exhibited the highest survival and growth rates. The KLP starter was also beneficial for fish development. The results of various studies confirmed the positive effect of probiotics on survival, growth, and the health of early stages of fish with undeveloped digestive tracts, such as common carp larvae or summer fry (Bogut et al. 1998). The experimental starters KR and KHR, which contained fish and blood meal as the source of animal protein, reduced fish survival and growth, and caused pathological changes in the digestive tract of the first feeding carp.

At the beginning of the experiment, the larvae were able to take up, digest, and absorb exogenous food, as was indicated by the physiological states of the liver and pancreas, and by the normal development of the intestine and its epithelium. The large lipid droplets in the cytosol of the enterocytes of the central part of the carp intestine could have played the role of temporary cellular lipid storage and might have resulted from the inability to mobilize fat or excessive dietary fat content. The excessive vacuolization of the posterior intestine epithelial cells observed at the beginning of the experiment in carp fed experimental diets indicated that intracellular enterocytic digestion and nutrient absorption into the blood were very slow. Lipids are transported by the blood to the liver as protein-lipid chylomicron complexes and small vacuoles containing VLDL (very low density lipoprotein). The lipid vacuoles in the enterocytes are considered to be a temporary product of fatty acid re-esterification and are accumulated when fatty acid content exceeds the export abilities of the enterocytes (Fontagné et al. 1998). The changes of intestinal cells in response to diet change have been observed in various fish species (Olsen et al. 1999, Fontagné et al. 1998, Caballero et al. 2003). The inhibition of lipoprotein synthesis in carp larvae probably resulted from the underdevelopment of their enterocytes at this developmental stage and was related to larval age since the number and size of fat drops was reduced in the older larvae. The disappearance or reduction of lipid vacuoles is the result of the increased lipoprotein synthesis ability of the enterocytes (Deplano et. al. 1991a, Perńa et. al. 2003). The larger size of

excessively vacuolated enterocytes was a mechanical consequence of fat deposition or the inhibition of fatty acid re-esterification. Lipid accumulation in the supranuclear regions of the enterocytes was caused by a reduced reacylation and lipoprotein synthesis rate (Caballero et al. 2003). The largest lipid drops occurred in the larvae fed the KR diet. The term "intestinal steatosis" is used when the diameter of the lipid vacuoles exceeds 5 μm (Fontagné et al. 1998). The larger enterocyte size in the larvae exhibiting epithelial steatosis was probably related to the temporary storage of lipids in the cells.

In all the feeding groups the supranuclear regions of the posterior intestine section contained acidophylic, PAS-positive inclusions. The enterocytes of other fish species larvae also contained supranuclear protein vacuoles (Deplano et al. 1991b, Bisbal and Bengston 1995, Ostaszewska et al. 2003). During larval development, the absorptive cells of the posterior intestine section are able to take up dietary protein by pinocytosis. The absorption of macromolecules and the presence of supranuclear vacuoles in the enterocytes may be related to the lack of a stomach and inefficient protein digestion (Moyano et al. 1996) and, in some species, to the very low activity of extracellular proteases (Rombout et al. 1985). The lack of absorptive vacuoles in the cells of the posterior intestine indicates starvation (Crespo et al. 2001). On the other hand, excessive vacuolation suggests an inhibition of intracellular digestion in the enterocytes, and reduced transport of digestion products into the blood circulation (Ostaszewska et al. 2005). The absorptive vacuoles in carp enterocytes were visible as early as at the beginning of feeding and persisted throughout feeding with the control diet and the KL and KLP feeds, and their presence indicated normal food digestion and nutrient absorption. No excessive vacuolation was observed in any group of fish, but the enterocytes of the larvae fed the KR and KHR diets exhibited a reduced number of vacuoles.

The reduced hepatocyte diameter in fish fed the KR diet, in comparison with the remaining fish groups, resulted from lower lipid storage in the hepatocyte cytoplasm, which was probably related to lower chylomicron and VLDL transport from the intestine to the liver. In the final phase of the experiment, hepatocyte size was also reduced in the fish fed the KHR diet.

Histological observations of digestive system structures are considered a good indicator of the nutritional condition of fish larvae (Green and McCormick 1999). The poor condition in these larvae was also indicated by lower and less frequent intestinal folds, reduced enterocyte height, and reduced supranuclear vacuole size. The small size of the hepatocytes indicates reduced lipid and glycogen storage ability. According to

Margulies (1993), changes in hepatocyte structure such as the reduced number and size of lipid vacuoles are important indicators of the inappropriate feeding or starvation of fish. Morphological changes in the liver and intestinal epithelium observed in the carp fed the KR and KHR diets and accompanied by high mortality were similar to those found in other marine and freshwater fish species fed various experimental diets (Segner and Braunbeck 1988, Deplano et al. 1991a, Díaz et al. 1999, Olsen et al. 2000, Caballero et al. 2002). Developmental disturbances were related to diet composition, dietary fat imbalances, the deficiency of fatty acids, and digestive physiology.

The form of protein used in dry fish diets is another important issue. Normal digestive tract development and high survival were observed in carp fed the KL and KLP diets. It was proven that the incorporation of a moderate level of protein hydrolysate to larval fish diets promotes the secretion of pancreatic and intestinal membranous enzymes (Cahu et al. 1999) and increases larval survival (Carvalho et al. 2004). In the feeding of cyprinid larvae, the use of native protein and a protein hydrolysate mixture produced better results than the addition of each form separately (Carvalho et al. 1997).

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STRESZCZENIE

WPŁYW STARTERÓW DOŚWIADCZALNYCH NA ZMIANY MORFOLOGICZNE JELITA I WĄTROBY U LARW KARPIA (*CYPRINUS CARPIO* L.) PODCHOWYWANYCH W WARUNKACH KONTROLOWANYCH

Celem prezentowanych badań było określenie wpływu skarmiana pięciu starterów doświadczalnych na wzrost, przeżywalność oraz rozwój i zmiany morfologiczne przewodu pokarmowego u larw karpia, *Cyprinus carpio* L., podchowywanych w warunkach kontrolowanych. Startery podawane rybom w teście wzrostowym zostały wykonane na bazie hydrolizatu białka rybnego i mączki z krwi (diety K, KL oraz KLP), mączek rybnej i z krwi (dieta KR), mączek rybnej i z krwi oraz hydrolizatu białka rybnego (dieta KHR). Ponadto startery KL i KLP wzbogacono dodatkiem liofilizatu jelit dorosłych karpi (jako źródła enzymów trawiennych), a do starteru KLP dodano także probiotyk (mieszaninę liofilizowanych bakterii kwasu mlekowego) (tab. 1.). Podstawowy skład chemiczny i skład aminokwasowy diet doświadczalnych był wyrównany – 55% białka ogólnego, 14,6% tłuszczu (tab. 2). W teście wzrostowym wykorzystano larwy karpia w stadium przechodzenia na odżywianie pokarmem egzogennym o średniej masie 2,32 mg i długości ciała (l.t.) 6,58 mm, przy obsadzie 100 sztuk na zbiornik o objętości 20 dm^3. Po 14 dniach podchowu najwyższą średnią przeżywalność (62,9%) odnotowano w grupie żywionej starterem KLP, natomiast najniższą (11%) u ryb karmionych starterem KR. Długość ciała larw mieściła się w zakresie od 7,83 mm (ryby żywione dietą KR) do 8,77 mm (dieta KL). W grupach otrzymujących startery K, KL i KLP końcowa masa larw karpia wynosiła 48-49 mg, podczas gdy w grupach KR i KHR tylko 22 mg (tab. 3).

W momencie rozpoczęcia eksperymentu jelito larw karpia wyścielone było przez pojedynczy nabłonek walcowaty (rys. 1A, 1B). W trakcie rozwoju larw jelito powiększyło swą długość i uległo znacznemu pofałdowaniu. Najdłuższe fałdy błony śluzowej środkowego odcinka jelita występowały u larw żywionych dietą KL, a najkrótsze u larw żywionych dietą K (tab. 3, rys. 2). U larw żywionych dietami KL, KLP oraz K obserwowano wyższe fałdy nabłonka śluzówki w tylnym odcinku jelita w porównani z wysokością fałd nabłonka śluzówki larw karpia żywionych dietami KR i KHR (tab. 3, rys. 3). W hepatocytach wątroby larw żywionych dietą KR obserwowano większe obszary cytoplazmy magazynujące glikogen w porównaniu z pozostałymi grupami żywieniowymi (rys. 4). Na podstawie obserwacji histologicznych w 14 dniu eksperymentu stwierdzono znacznie mniejszą wysokość fałd błony śluzowej na całej długości jelita u larw żywionych dietami KR i KHR (rys. 5B, 5D). Ponadto zmniejszyła się wielkość i liczba wakuol w nadjądrowych powierzchniach enterocytów zarówno wskazujących na absorpcję lipidów (enterocyty środkowej części jelita), jak i białek (entrocyty tylnej części jelita) (rys. 5B, 5D). W hepatocytach larw żywionych dietami K, KL i KLP magazynowanie glikogenu i lipidów pozostawało na podobnym poziomie, natomiast u larw żywionych dietami KR i KHR znacznie zmniejszyła się średnica hepatocytów (tab. 3), w których obserwowano wokół jąder niewielką ilość cytoplazmy (rys. 6).

Wyniki przeprowadzonych badań wskazują, że startery wykonane na bazie hydrolizowanego białka rybnego wzbogacone liofilizatem przewodu pokarmowego karpi oraz probiotykiem wpływają korzystnie na rozwój, przeżywalność oraz tempo wzrostu larw karpi.