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USABILITY OF FERMACTO PREBIOTIC IN FEEDS FOR COMMON CARP (*CYPRINUS CARPIO* L.) FRY

Summary. The studies have determined the effect of Fermacto prebiotic addition in feeds, on the growth and food conversion ratio in common carp fry. Four types of granulated experimental feeds were prepared, from which three feeds contained different amounts of Fermacto prebiotic (F1 – 1.0 g/kg, F2 – 2.0 g/kg, F3 – 3.0 g/kg) and a control feed K – without a prebiotic. During a 50-day growth test, the fish receiving prebiotic feeds showed significantly higher mean individual body weight ($p \leq 0.05$) in comparison with the control group. The best effects have been obtained in the variant F3, where the specific growth rate (SGR) was 2.44%, feed conversion ratio (FCR) was 1.21, protein efficiency ratio (PER) was 2.18, and the values significantly differed from the remaining variants. The values of the coefficients of feed protein retention were contained in the interval: 2.80-4.53%, while the values of fat retention coefficients ranged from 8.97 to 15.07%. During the growth test, no fish losses were recorded. Feeding of carp fry with feeds containing an addition of Fermacto prebiotic improves the rearing results; the optimal addition of prebiotic is 3 g of the preparation per 1 kg of feed.

Key words: prebiotic, Fermacto, common carp, *Cyprinus carpio* L., feeding

Introduction

Studies on practical application of the positive effects of some microorganisms in the feeding of water animals including fish have been recently intensively developed because of the search for new biotechnologies which could be friendly to water environment. This interest follows directly from the necessity to resign from anti-microbial growth promotors (AMGP) and to limit antibacterial therapy in animal breeding. In turn, the above restrictions stimulate the development of alternative conceptions of animal feeding. One of such conceptions is the use of feed additions which give a bacteriostatic or bacteriocidal effect, or they stimulate the populations of bacteria living in the alimentary tract, like e.g. acids, herbs and herb extracts, prebiotics, probiotics, synbiotics (PIVA 1998, FREITAG et AL. 1999).

Prebiotics represent substances contained in food (or added to food) which activate growth or the activity of beneficial strains of bacteria occurring in the alimentary tract. Confirmed prebiotic properties are shown by oligosaccharides: derivatives of fructose – inuline, oligofructose, fructooligosaccharides and derivatives of glucose – maltooligosaccharides. Inulin and oligofructose occur in many plants, e.g. in asparagus, artiichoke, peanuts and particularly in chicory which is the source of obtaining these substances in industrial way.

Criteria which must be met by prebiotic substances are the following:

- they cannot be hydrolized or aspired in the upper sections of the alimentary tract,
- they must be subjected to a selective fermentation by potentially beneficial bacteria strains existing in the intestine,
- they must favourably modify the microflora system in the intestine,
- the obtained effect must be adventageous for the host's health.

Prebiotics bind water in the intestinal lumen increasing the volume of intestine content and excrements (similarly as the soluble and not soluble food fibre with which they are frequently identified) constituting at the same time an adequate habitat (loose structure, big surface area) and fermentation substrate for probiotics (ZIEMER and GIBSON 1998, FOOKS et AL. 1999).

Our present studies aimed at the determination of the effect of the addition of Fermacto prebiotic on the growth and food conversion by carp fry. Fermacto is an American product of PET-AG company localized in Elgin, Illinois. It is an addition to feeds foreseen for all monogastric animals including fish. The composition of this preparation remains a secret of the producer.

Material and methods

Fish, rearing conditions and calculations

The experiment was carried out in controlled conditions (aquarium hall of the Department of Inland Fisheries and Aquaculture, Poznań University of Life Sciences) in an open system of supply. Water was supplied from water-pipe network, and subsequently, in order to reduce chlorine content, it was conditioned by active carbon filter. The main element of the water system installation was an equalizing tank of 2.4 m^3 capacity in which water was heated to constant temperature and it was aerated with a Hiblow HP-60 blower. During the experiment, the physico-chemical parameters of water were maintained on relatively constant optimal levels for carp fry: temperature 22-23°C, oxygen saturation above 70% (STEFFENS 1986). Control of water physico-chemical parameters (water temperature and content of oxygen solved in water) was carried out with the use of microcomputer oxymeter Elmetron CO-315.

The growth test lasted 50 days, in the period: 9.03-29.04.2003. The biological material consisted of carp from own breeding with average individual weight of 40 g. The experiment was carried out in four variants (including a control variant), each in three replications. Each basin was stocked with 10 pcs of fish.

Carp juveniles was placed in basins of 60 dm^3 capacity where a constant water flow was maintained (total water exchange in the basin took place five times per 24 h). Every day (at 08:00) basins were cleaned by removing excrements and the not consumed feed using a water syphon. Experimental feeds were supplied around the clock (24 h) using automatic belt feeders for fish with a clock drive. Daily feed rations were calculated according to the feeding standards given by MIYATAKE (1997) taking into consideration the actual fish body weight. The size of rations were determined every 10th day on the basis of weight control which at the same time served also for the determination of the values of other rearing indices.

From the data, the following parameters were calculated: specific growth rate (SGR = $100 \cdot (\ln w_t - \ln w_o) / t$), food conversion ratio (FCR = $F / (w_t - w_o)$, protein efficiency ratio (PER = $(w_t - w_o) \cdot (F \cdot P)$; symbols in the equations are as the follows: w_o and w_t – the initial and the final average weight, t – number of days of feeding trial, F – the total amount of feed per fish consumed during growth test, P – the protein content of the diets. The survival rate SR = (final number of fish · 100) / initial number of fish. Protein retention PR = (final protein content in fish body – initial protein content in fish body) · 100 / protein intake and lipid retention LR = (final lipid content in fish body – initial lipid content in fish body) · 100 / lipid intake.

Before the commencement and after the termination of the growth test, fish samples were randomly taken in order to determine the basic chemical composition of fish bodies. The sampled carps were put sleep using Propiscin (KAZUŃ and SIWICKI 2001) anaesthetic and they were decapitated. Subsequently, fish bodies were ground and homogenized, and in the obtained material, the following determination were made: dry mass, total protein, raw fat and ash (using the same analytical methods as for feeds).

Experimental feeds

Experimental feeds were made in the Feed Laboratory of the Experimental Plant of Feed Production Technology and Aquaculture in Muchocin. The basic composition of feeds was identical and it consisted of the following components (by weight): fish meal – 23.4%, blood meal – 10.0%, soya meal – 15.0%, rape meal – 7.0%, wheat flour – 34.1%, albuminous binder – 2.0%, rape oil – 6.5%, soya lecithin – 0.5%, premix – 1.1%, choline chloride – 0.2% and monocalcium phosphate – 0.2%. The differentiating factor in the feeds was the added amount of Fermacto prebiotic applied in the particular feeds: F1 – 1.0 g/kg, F2 – 2.0 g/kg and F3 – 3.0 g/kg. In the control feed (K), no addition of probiotic preparation was applied. After conditioning with water vapour, the feeds were prepared by high pressure method in a laboratory granulator (from Richard Sizer Co. England). The diameter of the matrix openings was 6.0 mm.

After drying, the obtained granules were crushed on a cylinder crusher and the pellets were segregated into two granulometric groups:

- 2.0-3.15 mm for carp of individual weight up to 50 g,
- 3.15-4.5 mm for carp of individual weight above 50 g.

The pellets were covered with a film of rape oil heated to 70°C (in the amount of 2.0% of the pellet mass). It was done by spraying method in a pelletizing drum.

Chemical analysis of feeds was carried out according to AOAC (OFFICIAL METHODS... 1996). The content of total protein was determined on Kjel-Foss Automatic

16210 analyser; raw fat was identified by Soxhlet method (ethyl ether extraction for 12 h). The amount of raw fibre was determined on Tecator Fibertec System M 1020 Hot Extractor. Ash content was found by sample combustion at 550°C for 12 h (Linn Electro-Therm furnace). The amount of N-free extract was estimated as the difference between the dry mass and the sum of the remaining components. Total calcium was determined in the feed by atomic absorption spectrophotometer, type ASS3 (Carl Zeiss, Jena) according to the method given by GAWĘCKI (1988). Total phosphorus was determined by flame ionization technique. Aminoacids of the feeds protein were assayed in a Microtechna AAAT 339 analyser after hydrolysis of a sample (0.1 ml) in 6n HCl at 106°C for 24 h. Methionine and cystine were determined after previous oxidation in formic acid. Tryptophan was determined by colorimetric method (VOTISKY and GUNKEL 1989). On the basis of the results of aminoacidic analyses of protein, the chemical value of experimental diets was defined by calculating the chemical score (CS) and the indispensable amino-acids index (IAAI) (HARDY and BARROWS 2002).

Gross energy of the diets was calculated from the chemical composition using the conversion factors of gross energy for fish: carbohydrates – 17.2, protein – 23.6 and fat – 39.5 kJ/g (BUREAU et AL. 2002).

The determined chemical composition of tested feeds was the following one (%): total protein – 38.1, raw fat – 9.9, NFE – 33.1, raw fibre – 1.9, ash – 4.7, total phosphorus – 0.8 and calcium – 1.23. The determined amounts of exogenous amino acids in the feeds (g per 100 g of protein) were: arginine – 5.32, histidina – 3.89, lysine – 7.53, tryptophan – 2.84, phenyloalanine with thyrosine – 7.18, methionine with cystine – 2.62, threonine – 3.98, leucine – 8.59, isoleucine – 3.51, valine – 5.57. The limiting amino acid was methionine with cystine (Cs – 45.28) and the IAAI index reached the value of 76.93. The E/P relation in experimental feeds was 48.93 kJ/g protein at the level of gross diet energy equal to 18.59 MJ/kg.

Statistical analysis

The results were analysed using the Statistica 5 PL Program. Mean results per tank were subjected to one-way analysis of variance (ANOVA). Homogeneous groups were determined with the Duncan multiple range test. The level of significance $p \leq 0.05$, and the results are presented as mean ± standard deviation of the mean (SD).

Results

The feeding on feeds with an addition of Fermacto prebiotic starting from 30-ieth day of the test significantly stimulated the growth of carp fry (Table 1). From that moment carps which received prebiotic feeds reached significantly higher individual weights ($p \leq 0.05$) in comparison with fish from the control variant. This tendency was maintained until the end of the growth experiment.

The final result of the growth test presented in Table 2 indicates that the best effects have been obtained in the variant F3, where the specific growth rate (SGR) was 2.44% and the value of this indicator differed significantly from the values obtained in the other variants. The worst results were recorded in variant K which is confirmed by

Table 1. Changes of mean individual fish body mass during the growth test (g)
Tabela 1. Zmiany średnich mas jednostkowych ryb w czasie testu wzrostowego (g)

Day	Variant			
	K	F1	F2	F3
1	39.06 a (±0.15)	39.68 a (±0.52)	39.68 a (±0.40)	39.64 a (±0.32)
10	50.09 a (±0.32)	52.18 a (±2.41)	51.93 a (±2.08)	52.03 a (±2.94)
20	63.42 a (±2.33)	70.02 a (±3.26)	69.21 a (±2.44)	71.37 a (±3.77)
30	75.21 a (±2.97)	84.06 ab (±2.58)	84.44 b (±2.80)	90.96 b (±4.66)
40	86.80 a (±3.81)	97.06 b (±4.36)	98.90 b (±2.74)	112.92 c (±4.07)
50	101.48 a (±4.05)	112.93 ab (±5.32)	114.99 b (±3.67)	134.00 c (±2.72)

Values are means from triplicate groups of fish, and the means in each row with different superscript are significantly different ($p \leq 0.05$).

Table 2. Specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR) and lipid retention (LR) in common carp fry fed the experimental diets
Tabela 2. Dobowe przyrosty średnich mas jednostkowych ryb (SGR), przeżywalność ryb (SR), współczynniki pokarmowe pasz (FCR), wskaźniki wydajności wzrostowej białka pasz (PER), wskaźniki retencji białka paszowego (PR) oraz wskaźniki retencji tłuszczu paszowego (LR) narybku karpia żywionego paszami doświadczalnymi

Parameter	Variant			
	K	F1	F2	F3
SGR (%)	1.91 a (±0.07)	2.09 b (±0.07)	2.13 b (±0.06)	2.44 c (±0.05)
FCR	1.61 a (±0.07)	1.41 b (±0.06)	1.40 b (±0.03)	1.21 c (±0.04)
PER	1.63 a (±0.07)	1.86 b (±0.07)	1.88 b (±0.04)	2.18 c (±0.08)
PR (%)	2.80 a (±0.07)	4.39 b (±0.18)	4.11 b (±0.22)	4.53 b (±0.19)
LR (%)	8.97 a (±0.41)	15.07 b (±0.99)	13.82 b (±0.78)	14.29 b (±0.88)
SR (%)	100	100	100	100

Values are means from triplicate groups of fish, and the means in each row with different superscript are significantly different ($p \leq 0.05$).

changes in the mean individual body weights of fish in the particular variants and the SGR coefficients during fish breeding. The lowest value of feed conversion ratio (FCR) was recorded in variant F3 and its value was 1.21, whereby it differed significantly from the results obtained in other variants. The highest value of protein efficiency ratio (PER) was obtained also in F3 variant, while the lowest PER value was shown in variant K, whereby both values significantly differed from the remaining variants. The values

of the coefficients of feed protein retention were contained in the interval 2.80-4.53%, while the values of fat retention coefficients ranged from 8.97 to 15.07%.

Fish survival rate in all variants during the test was 100%.

Comparison of the chemical composition of carp body at the beginning and at the end of experiment (Table 3) indicates significant changes which took place in the raw fat content, where the values in variants Fl and F2 significantly increased. Changes took place also in the content of total protein in variant F3, the protein content significantly decreased. For the remaining body components, like dry matter and raw ash, no significant changes were recorded.

Table 3. Chemical composition of fish body before and after the experiment (%)
Tabela 3. Skład chemiczny ciała karpi na początku i na końcu doświadczenia (%)

	Dry weigt	Ash	Crude protein	Crude fat
Before the experiment	25.32 a	15.81 a	10.30 a	2.34 a
After the experiment				
K	27.03 a	15.67 ab	12.44 b	2.09 a
F1	24.46 a	15.61 ab	13.79 d	2.41 a
F2	26.18 a	16.03 a	14.83 e	2.71 a
F3	26.72 a	15.24 b	12.92 c	2.48 a

Values are means of analysis of three fishes from each experimental group, and the means in each column with different superscript are significantly different ($p \leq 0.05$).

Disscusion

Experimental feeds were correctly balanced regarding the content of total protein and raw fat (OGINO 1980 a, JAUNCEY 1982, WATANABE 1982, 1988), mineral components (SATOH et AL. 1991, NUTRIENT REQUIREMENTS... 1993, KIM et AL. 1998), exogeneous amino acids (NOSE 1979, OGINO 1980 b) and energy level in the diet and in its relation to the amount of protein (OHTA and WATANABE 1996) for carp fry.

The idea of prebiotics utilization in the feeding originated on the basis of observations that inulin and oligofructose stimulate the selective growth of bifidobacteria exerting a positive effect also on human organism (BLAUT 2002, TEITELBAUM and WALKER 2002). Other substances such as mannose, oligosaccharides and lactose are also regarded as substances which possess the properties of prebiotics for humans and animals. However, information about prebiotics for water organisms is limited. OLSEN et AL. (2001) observed that a diet with the content of 15% inulin caused negative effects in the feeding of Arctic charr. Earlier studies (RINGO et AL. 1998, RINGO and OLSEN 1999) showed that fatty acids and carbohydrates differ in the bacterial flora of different fish species. In the studies on fish feeding, the commercial prebiotic Grobiotic was used which in a significant way increased the feeding effectiveness, improved the survival rate, immunological reactions and the resistance in striped bass which was experimen-

tally infected with *Streptococcus hiriae* bacteria. The positive effect of Grobiotic prebiotic on the weight increase resulted from changes in the internal microflora through the influence of mannose, oligofructose, lactose and other carbohydrates contained in dairy produce components. Partially, this was also influenced by autolyzed brewery yeasts or dried fermentation products (PENG and GATLIN III 2003).

Lactic acid bacteria are regarded as being beneficial for organisms living in the alimentary tract of fish because they produce bacteriocins which in turn inhibit the development of some pathogens in fish and thereby they have a positive effect on the microflora of fish. Some authors (SZILAGYI 2002) indicate that lactose, similarly as other prebiotics, can increase the maintaining of an adequate level of bacteria producing lactic acid and they can prevent the development of potential pathogens connected with human diseases. However, these hypotheses require still a confirmation by studies carried out on fish.

Studies performed so far, referring to the application of natural growth stimulators in the breeding of water organisms, have shown great possibilities of an improvement in the obtained results through the introduction to the diets of prebiotics. The results obtained in the presented experiment indicate a distinct improvement of the breeding effects of juvenile forms of carps fed on feeds with an addition of a prebiotic. This confirms the directions of the new feeding strategies giving high possibilities for health improvement of fish and their resistance to infections (KESARCODI-WATSON et AL. 2008).

Conclusions

1. Feeding of carp fry on feeds with an addition of the Fermacto prebiotic improved rearing results.

2. The optimal addition of Fermacto prebiotic in the feeds for carp fry is 3.0 g of the preparation per 1 kg of feed.

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ZASTOSOWANIE PREBIOTYKU FERMACTO W PASZACH DLA NARYBKU KARPIA (*CYPRINUS CARPIO* L.)

Streszczenie. Określono wpływ dodatku prebiotyku Fermacto na tempo wzrostu i przewartościowanie składników pokarmowych pasz w żywieniu narybku karpia. Przygotowano cztery granulowane pasze, z których trzy zawierały dodatek prebiotyku Fermacto w następujących ilościach: pasza F1 – 1,0 g/kg, pasza F2 – 2,0 g/kg, pasza F3 – 3,0 g/kg. Pasza kontrolna K nie zawierała dodatku prebiotyku. Ryby żywione paszami prebiotycznymi osiągnęły w 50-dniowym teście wzrostowym statystycznie istotnie większe ($p \leq 0,05$) średnie masy jednostkowe w porównaniu z karpiami z grupy kontrolnej. Najlepsze wyniki odnotowano w wariancie F3, w którym średni dobowy przyrost masy jednostkowej (SGR) wyniósł 2,44%, współczynnik pokarmowy paszy (FCR) – 1,21 oraz współczynnik wydajności wzrostowej białka paszowego (PER) – 2,18, a istotność różnic została potwierdzona statystycznie. Wartości współczynnika retencji białka mieściły się w przedziale od 2,80 do 4,53%, a wartości współczynnika retencji tłuszczu – w przedziale od 8,97 to 15,07%. Podczas testu wzrostowego nie odnotowano śnięć ryb. Żywienie narybku karpia paszami zawierającymi prebiotyk Fermacto wpłynęło na polepszenie efektów chowu; najlepsze wyniki osiągnięto, skarmiając pasze z dodatkiem prebiotyku w ilości 3 g na 1 kg paszy.

Słowa kluczowe: prebiotyk, Fermacto, karp, *Cyprinus carpio* L., żywienie

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