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EFFECT OF *CARNOBACTERIUM DIVERGENS* AND *ENTEROCOCCUS HIRAE* AS PROBIOTIC BACTERIA IN FEED FOR COMMON CARP, *CYPRINUS CARPIO* L.

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ABSTRACT. Studies were conducted on the effect the addition of a probiotic preparation containing *Carnobacterium divergens* or *Enterococcus hirae* bacteria isolated from the alimentary tract of common carp, *Cyprinus carpio* L. had on the growth and food conversion ratio in the rearing of two-year-old carp. The use of feeds with the addition of probiotic bacteria did not have any significant effect on the specific growth rate (SGR), food conversion ratio (FCR), or protein efficiency ratio (PER). The type of feed had no impact on the protein level in the fish bodies but the water content decreased. The highest changes were observed in the content of fat and mineral components in the fish bodies. The experimental results did not confirm any production advantages when *Carnobacterium divergens* or *Enterococcus hirae* bacteria were added to carp feeds.

Key words: PROBIOTIC BACTERIA, GROWTH, CHEMICAL COMPOSITION OF FISH BODY, COMMON CARP, FEEDING

INTRODUCTION

Probiotics are favorable live microorganisms or their metabolites that are used commonly as feed additives in the rearing of farm animals. They are used for two purposes in the rearing of aquatic organisms (mollusks, crustaceans, fish): (1) to improve the quality of the aquatic environment, and (2) to introduce useful microflora to the alimentary tract (Gatesoupe 1999).

In aquaculture, useful bacteria can be introduced to the alimentary tract of fish in three ways: (1) by introducing bacteria into the alimentary tract of organisms that are live food for fish; (2) by adding lyophilized bacteria to live food for fish; (3) by adding

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lyophilized bacteria to feeds. The use of balanced diets supplemented with the addition of probiotics permits meeting the total requirement of fish for nutritive components and energy, and at the same time it influences the micro flora composition in the alimentary tract (Irianto and Austin 2002).

From a nutritional point of view, the most important mechanism of probiotic microflora in fish alimentary tracts is the improvement of feed digestion by the production of extracellular enzymes (Gatesoupe et al. 1997) and vitamins (Sugita et al. 1991). The other beneficial effects of probiotics in fish include the reduced incidence and duration of diseases, immune system modulation, and antiviral action (Balcazar et al. 2006).

The first attempts of probiotic use in common carp culture were described by Noh et al. (1994). A commercial preparation of *Streptococcus faecium* was added to the feed, and after two weeks of feeding, *Escherichia coli* had been entirely eliminated from the alimentary tract. Fish growth and feed utilization were also considerably improved. In the study carried out by Yanbo and Zirong (2006), the addition of *Bacillus* sp. and lyophilized photosynthetic bacteria to feed significantly improved carp, *Cyprinus carpio* L. growth performance and feed conversion ratio. After 60 days of the feeding trial, changes in enzymatic activity in the fish intestine were also observed.

The objective of the present study was to determine the effect of *Carnobacterium divergens* or *Enterococcus hirae* bacteria added to feeds on production results in the rearing of 2-year-old carp.

MATERIALS AND METHODS

EXPERIMENTAL FEEDS

The recipes of the experimental feeds were calculated with a specially written program using the Simplex method in Turbo Pascal 5.0. The feeds included the following components (by weight): fish meal – 13.5%, erythrocyte meal – 10.0%, soy meal – 15.0%, rape meal – 10.0%, triticale meal – 42.7%, fish oil – 5.0%, soy lecithin – 0.5%, Polfamix W – 1.0%, Vitazol AD₃EC – 0.1%, choline chloride – 0.2%, monocalcium phosphate – 0.5% and feed chalk – 1.5%.

The probiotic preparations were made using bacteria from the Collection of Pure Cultures of the Department of Biotechnology and Food Microbiology at the Agricul-

tural University in Poznań. All species of bacteria were isolated from the alimentary tracts of common carp. No probiotic preparation was added to control feed A. The experimental feeds were supplemented with probiotic preparations containing the following: feed B – *Enterococcus hirae* bacteria at a concentration of 10^9 CFU (colony forming units) kg^{-1} feed; feed C – *Carnobacterium divergens* at a concentration of 10^7 CFU kg^{-1} feed; feed D – *C. divergens* at a concentration of 10^9 CFU kg^{-1} feed.

Feeds were prepared with the high pressure method in a laboratory granulator (Richard Sizer Co. England). The best experimental feed granulation conditions were obtained with the following technological parameters: feed mix moisture – 10%; granulator head temperature – 50°C ; time of mix passage through granulator – 55 s; nozzle diameter – 6.0 mm.

The granules passing through the matrix were cut with a rotary knife into 9 mm segments. Then they were dried on sieves in a stream of heated air (40°C), and after drying, the average diameter of the pellets was about 7 mm. The granules were covered with a film of fish oil not exceeding 1.5% of the granule mass. The oil was heated to 70°C and the procedure was done with the spray method in a pelletizing drum.

The dry matter of tested feeds contained 32.00% total protein, 7.63% total fat, 39.62% nitrogen-free extractives (NFE), 3.10% raw fiber, 5.72% raw ash, 1.49% calcium, and 0.74% phosphorus. The calculated level of digestive energy was 17.38 MJ kg^{-1} and the E/P (energy to protein) ratio was 54.31 kJ g^{-1} protein. The content of exogenous amino acids in the feed proteins (in $\text{g } 100 \text{ g}^{-1}$ of protein) was as follows: arginine – 5.14; histidine – 3.71; lysine – 6.94; tryptophan – 2.78; phenylalanine with tyrosine – 6.21; methionine with cystine – 2.88; threonine – 3.68; leucine – 8.19; isoleucine – 3.34; valine – 5.77. The index of exogenous amino acids calculated based on amino acid analysis (EAAI) was 74.28 and the first limiting amino acid was methionine with cystine – 49.28.

EXPERIMENTAL FISH AND FEEDING TRIAL

The 6-week growth test was conducted in twelve ponds (each 40 m^2 in area and 1.2 m deep) in triplicate. Each of the 12 experimental groups consisted of 12 individuals; the initial average weight was $403 \pm 5 \text{ g}$ (mean $\pm$ SD). Temperature ($^\circ\text{C}$) and dissolved oxygen ($\text{mg O}_2 \text{ dm}^{-3}$) were monitored daily at 08:00 with an Elmetron CO-315 electronic oxygen meter. The average daily water temperature during the experiment

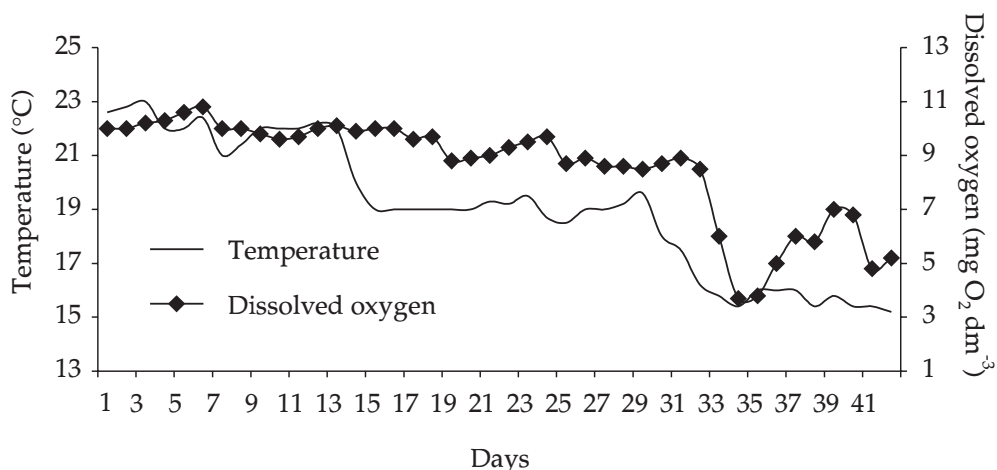


Fig. 1. Diel changes of water temperature and dissolved oxygen during the growth test.

ranged from 14.2 to 23.0°C. The content of dissolved oxygen was highly variable from 3.70 to 10.8 mg O₂ dm⁻³ (Fig. 1).

The daily feed rations were calculated according to the daily feeding rate in Miyatake (1997) and in consideration of water temperature and fish biomass. Experimental feeds were supplied for 12 hours (09:00 – 21:00) using band feeders equipped with a clock drive (FIAP Fischtechnik GmbH). The dose size was changed every 14 days based on weight monitoring, from which fish growth rate and other rearing results (SGR, FCR, PER) were determined.

SAMPLE COLLECTION AND ANALYSIS

Nutrient composition of the experimental diets was determined according to AOAC (1996). The feeds were tested to determine the content of the following: dry mass (at 105°C for 12 hours); crude protein (Kjel-Foss Automatic 16210); raw fat (Soxhlet method; drying at 60°C, 12 hours of extraction with paraffin ether); crude fiber (Tecator Fibertec System M 1020 Hot Extractor); ash (combustion at 550°C for 12 hours, Linn Electro-Therm). The content 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 with an ASS3 atomic absorption spectrophotometer (Carl Zeiss, Jena). Total phosphorus was determined with the flame ionization technique.

The amino acid contents of the feed protein were assayed in a Microtechna AAAT 339 analyzer after the sample had been hydrolyzed (0.1 ml) in 6n HCl at 106°C for 24 hours. Methionine and cystine were determined following oxidation in formic acid. Tryptophan was determined with the colorimetric method (Votisky and Gunkel, 1989). Based on the results of the amino acid analyses of the protein, the chemical value of the experimental diets was defined by calculating the chemical score (CS) and the indispensable amino-acids index (IAAI) (Hardy and Barrows 2002).

The gross energy of the model diets was calculated from the chemical composition using the conversion factors of gross energy for fish: carbohydrates 17.2; protein 23.6; fat 39.5 kJ g⁻¹ (Bureau et al. 2002).

Prior to and following the growth test, fish samples were taken at random to determine their proximal composition. The fish were anesthetized using Propiscin, ground and homogenized, and then the dry mass, total protein, raw fat, and raw ash were determined (according to feed analysis).

STATISTICAL ANALYSIS OF EXPERIMENTAL RESULTS

The data obtained were used to calculate the following parameters: specific growth rate (SGR, % d⁻¹); protein retention ratio (PR, %); food conversion ratio (FCR); protein efficiency ratio (PER); survival rate (SR, %). In order to detect statistically significant differences between the variants, data were analyzed using the Statistica 5 PL Program. The significance among means of treatments at 0.05 was determined with the t-Tukey multiple range test.

RESULTS

WEIGHT GAINS AND FEED UTILIZATION

The use of feeds with the addition of probiotic bacteria did not exert any significant effect on the growth rate of carp in the particular stages of the growth test (Table 1). After six weeks of the test, the increment of the mean body mass ranged from 302 g (variant A) to 350 g (variant D). The values obtained for fish rearing indices (specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER)) did not show any statistically significant differences between the particular variants of the experiment. No fish losses were recorded during the growth test.

TABLE 1

Individual fish body mass (BM, g), specific growth rate (SGR, % d⁻¹), feed conversion ratio (FCR), protein efficiency ratio (PER), in common carp fed experimental diets
(mean values $\pm$ SD from triplicate groups of fish)

Variants	Parameter			
	BM	SGR	FCR	PER
After 14 days of the growth test				
A	494.17 $\pm$ 23.20	1.42 $\pm$ 0.27	1.99 $\pm$ 0.44	1.47 $\pm$ 0.30
B	498.61 $\pm$ 26.16	1.57 $\pm$ 0.35	1.80 $\pm$ 0.45	1.65 $\pm$ 0.41
C	478.33 $\pm$ 29.93	1.31 $\pm$ 0.40	2.08 $\pm$ 0.49	1.43 $\pm$ 0.39
D	517.50 $\pm$ 28.17	1.77 $\pm$ 0.40	1.91 $\pm$ 0.22	1.50 $\pm$ 0.17
After 28 days of the growth test				
A	579.09 $\pm$ 28.27	1.71 $\pm$ 0.13	1.65 $\pm$ 0.16	1.73 $\pm$ 0.17
B	595.76 $\pm$ 16.50	1.90 $\pm$ 0.26	1.52 $\pm$ 0.20	1.90 $\pm$ 0.26
C	574.55 $\pm$ 35.50	1.83 $\pm$ 0.09	1.53 $\pm$ 0.13	1.87 $\pm$ 0.15
D	591.21 $\pm$ 2.29	1.78 $\pm$ 0.05	1.53 $\pm$ 0.15	1.87 $\pm$ 0.19
After 42 days of the growth test				
A	704.81 $\pm$ 44.75	2.75 $\pm$ 0.02	1.92 $\pm$ 0.25	1.50 $\pm$ 0.20
B	739.63 $\pm$ 24.15	2.77 $\pm$ 0.11	1.88 $\pm$ 0.18	1.52 $\pm$ 0.14
C	738.89 $\pm$ 14.57	2.93 $\pm$ 0.25	1.81 $\pm$ 0.26	1.59 $\pm$ 0.25
D	751.11 $\pm$ 8.39	3.04 $\pm$ 0.08	1.82 $\pm$ 0.16	1.57 $\pm$ 0.13

$SGR = 100 \times (\ln \text{final weight (g)} - \ln \text{initial weight (g)}) / \text{days}$

$FCR = \text{dry feed intake (g)} / \text{wet weight gain (g)}$

$PER = \text{wet weight gain} / \text{protein intake}$

FISH PROXIMAL COMPOSITION

During the six-week experiment, the water content in the fish bodies decreased but the protein level did not change (Table 2). A statistically significant increase ($P \leq 0.05$) in fat content was noted in the carp from groups C and D, while a decrease of this component was recorded in group A. The largest changes took place in the content of mineral components. In all variants, a decreased level of ash was noted, and in groups B, C, and D the decrease was statistically significant in comparison with the values determined at the beginning of the growth test.

TABLE 2

Proximal composition of fish bodies before and after the growth test (%)
(mean values $\pm$ SD from triplicate groups of fish)

	Dry weight	Ash	Crude protein	Crude fat
Start of the growth test	20.20 ^a $\pm$ 0.81	2.61 ^c $\pm$ 0.12	13.60 ^a $\pm$ 0.51	8.51 ^b $\pm$ 0.75
End of the growth test				
A	26.23 ^b $\pm$ 0.52	2.22 ^{bc} $\pm$ 0.41	14.05 ^a $\pm$ 0.31	7.37 ^a $\pm$ 1.06
B	27.54 ^b $\pm$ 0.03	2.03 ^b $\pm$ 0.28	13.89 ^a $\pm$ 0.66	9.62 ^b $\pm$ 0.96
C	29.11 ^b $\pm$ 1.93	1.72 ^a $\pm$ 0.38	14.31 ^a $\pm$ 1.29	11.64 ^c $\pm$ 0.62
D	28.98 ^b $\pm$ 1.35	1.73 ^a $\pm$ 0.17	13.54 ^a $\pm$ 0.01	11.54 ^c $\pm$ 0.74

Results with the same letters are not significantly different ($P > 0.05$)

DISCUSSION

During the experiment, the water temperature in the ponds was within the range favorable for carp. In the final phase of the test, the water temperature decreased to the lowest limit, which permits effective fish growth (14°C maximum) (Szumiec 1998). However, this parameter had no significant effect on the rearing results because decreased water temperature did not affect the carp growth rate. The dissolved oxygen content in the water was within the optimal range for carp (min. 3.7 mg O₂ dm⁻³).

The feeds tested were correctly balanced regarding the content of nutritive components for carp such as total protein and fat (Ogino 1980a, Jauncey 1982, Watanabe 1982, 1988), mineral components (Satoh et al. 1991, NRC 1993, Kim et al. 1998), exogenous amino acids (Nose 1979, Ogino 1980b), as well as the amount of energy and its relation to the content of total protein (Ohta and Watanabe 1996).

In studies conducted thus far that focus on applying different types of probiotics in order to modify the microflora of the alimentary tract of fish, explicitly positive effects were obtained with juvenile stages in many farmed fish species, including common carp, (Bogut et al. 1998a, Przybył et al. 2006), European wels, *Silurus glanis* L. (Adamek 1994, Bogut et al. 1998b), Atlantic cod, *Gadus morhua* L. (Gildberg and Mikkelsen 1997), Mozambique tilapia, *Oreochromis mossambicus* (Peters) (Naik et al. 1999), Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Robertson et al. 2000).

In older fish, where the alimentary tract is anatomically and functionally fully developed, the addition of probiotics to feed does not always result in positive effects. Metaillier and Hollocou (1991), who analyzed the results of studies on the use of different probiotics in the feeding of European seabass, *Dicentrarchus labrax* (L.), indicated that distinct effects on the growth rate and feed conversion ratio can be produced in younger fish populations.

In the present studies, the fish mass increments and food conversion indices obtained must be regarded as satisfactory; however, the anticipated favorable effect of feed supplementation with the addition of *Carnobacterium divergens* or *Enterococcus hirae* bacteria on the production results in carp farming was not confirmed. Feeding 2-year-old carp on feeds containing the addition of the tested bacteria did not show any significant differences in the growth of fish mass or in the values of rearing indices in comparison with fish fed the control feed (no addition of any probiotic preparation).

CONCLUSIONS

The addition of *Carnobacterium divergens* or *Enterococcus hirae* bacteria to the feeds of 2-year old carp did not improve the production results in fish rearing.

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STRESZCZENIE

ZASTOSOWANIE BAKTERII PROBIOTYCZNYCH *CARNOBACTERIUM DIVERGENS* I *ENTEROCOCCUS HIRAE* W PASZACH DLA KARPIA, *CYPRINUS CARPIO* L.

Przeprowadzone badania miały na celu określenie wpływu na wykorzystanie składników pokarmowych paszy oraz wzrost ryb i podstawowy skład ich ciała dodatku probiotyku zawierającego bakterie *Carnobacterium divergens* lub *Enterococcus hirae* do pasz stosowanych w żywieniu handłówki karpia *Cyprinus carpio* L. Preparaty probiotyczne zostały przygotowane na bazie bakterii wyizolowanych z przewodów pokarmowych dorosłych karpia. Rybom podawano cztery pasze (wszystkie o zawartości 32% białka ogólnego i 17,38 MJ kg⁻¹ energii brutto): pasza A kontrolna, bez dodatku probiotyku; pasza B z dodatkiem bakterii *Enterococcus hirae* w koncentracji 10⁹ jtk (jednostki tworzące kolonie) w kg paszy; pasza C z dodatkiem bakterii *Carnobacterium divergens* w koncentracji 10⁷ jtk kg⁻¹ paszy; pasza D z dodatkiem bakterii *C. divergens* w koncentracji 10⁹ jtk kg⁻¹ paszy. Skarmianie pasz zawierających bakterie probiotyczne nie miało istotnego wpływu ($P \leq 0,05$) na przyrosty masy ciała karpia podczas trwającego sześć tygodni testu wzrostowego. Uzyskane wartości wskaźnika dobowego tempa wzrostu masy ryb (SGR), współczynnika pokarmowego (FCR) oraz wskaźnika wydajności wzrostowej białka pasz (PER) nie różniły się istotnie (tab. 1). Odnotowano zmiany w składzie ciała karpia przed rozpoczęciem i po zakończeniu doświadczenia: zawartość wody w ciele uległa obniżeniu przy jednoczesnym wzroście ilości białka. Odnotowano statystycznie istotny ($P \leq 0,05$) wzrost zawartości tłuszczu w ciele karpia z grup B, C i D, a spadek ilości tego składnika w ciele ryb z grupy A. We wszystkich wariantach doświadczenia obniżeniu uległa zawartość popiołu; u ryb z grup B, C i D różnice były istotne statystycznie w porównaniu z rozpoczęciem testu wzrostowego (tab. 2). Podawanie karpom pasz z dodatkiem bakterii probiotycznych *Carnobacterium divergens* lub *Enterococcus hirae* nie miało wpływu na uzyskane wyniki chowu.