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## SUPPLEMENTING THE FEED OF COMMON CARP (*CYPRINUS CARPIO* L.) JUVENILES WITH THE BIOSAF PROBIOTIC

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**ABSTRACT.** The studies determined the effect of carp feeds supplemented with the probiotic preparation BIOSAF, a concentrate of live yeast, *Saccharomyces cerevisiae* strain Sc 47, on the growth and food conversion ratio of carp juveniles. Four types of granulated experimental feeds were prepared; three contained different quantities of the BIOSAF probiotic (B1 – 0.5 g kg<sup>-1</sup>; B2 – 1.0 g kg<sup>-1</sup>; B3 – 1.5 g kg<sup>-1</sup>) and one was control feed K – without any probiotic. The added weight values of the probiotic were converted into colony forming units (CFU) of yeast, *Saccharomyces cerevisiae*, and were 4, 8, and 12 × 10<sup>9</sup> CFU, respectively, per kilogram of feed. During the 50-day growth test, the fish receiving feeds supplemented with the probiotic had significantly higher mean individual body weight ( $P \leq 0.05$ ) in comparison with the fish from the control group. The minimal value of specific growth rate throughout the test (1.98% d<sup>-1</sup>) was attained by fish fed control feed K, while the maximal value (2.45% d<sup>-1</sup>) was recorded in the B2 variant; the differences were statistically significant. The most favorable values of food conversion and protein efficiency ratio were noted in the B2 feed. The differences were statistically different in comparison with the remaining feed types. No fish losses were recorded during the growth test. The type of feed had an impact on the contents of protein and fat in the fish bodies, but it did not cause any changes in dry mass or ash.

Key words: PROBIOTIC, *SACCHAROMYCES CEREVISIAE*, BIOSAF, COMMON CARP (*CYPRINUS CARPIO*), FEEDING

## INTRODUCTION

In modern fish cultivation systems, products of biotechnology are gaining wider applications thanks to the development of production technologies that are environmentally friendly to water and ensure the well being of the animals. Feeds or feed organisms supplemented with probiotics containing live microorganisms or their products are beneficial to the host. In addition to their prophylactic impact (limiting the development of pathogens through food competition or by adhesion location in

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the alimentary tract and the production of inhibiting substances), probiotics also have a positive effect on digestive processes and the assimilation of food components (Irianto and Austin 2002). The main microorganism groups that exhibit a probiotic impact in fish are lactic acid bacteria (Ringo and Birkbeck 1999) and yeast (Andlid et al. 1994).

Yeast, most frequently brewer's yeast, is used as a protein source (about 45% of feed protein) in the production of full-portion fish feeds. It is also used as a binder in some feeds. The only factor limiting diet supplementation with yeast is its rather high price in relation to other proteinaceous components. Next to using yeast as a nutritive component, an alternative approach is to apply it in the feeding of animals as a functional component with probiotic properties. In such cases, selected strains of yeast are added to feeds as lyophilized live cells that are able to colonize the digestive tract in order to reproduce and generate metabolites.

No studies focusing on the practical probiotic effects of yeast supplementation in the feeding of cyprinid fish were found in the available literature. The current study identified the effect of supplementing the feed of carp, *Cyprinus carpio* L., juveniles with the BIOSAF probiotic, a thermostable concentrate of live yeast, *Saccharomyces cerevisiae*, strain Sc 47, on their growth rate (SGR) and food conversion ratio (FCR). According to data from the manufacturer of BIOSAF, S. I. Lesaffre (France), one gram of the preparation contains  $10 \times 10^9$  colony forming units (CFU) of yeast (the warranted minimum is  $8 \times 10^9$ ).

## MATERIAL AND METHODS

### EXPERIMENTAL FEEDS

The experimental feeds were made in the Feed Laboratory of the Experimental Plant of Feed Production Technology and Aquaculture in Muchocin, Poland. The basic composition of the feed variants was identical and consisted of the following components (by wet weight): fishmeal – 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%; monocalcium phosphate – 0.2%. The differentiating factor among the feeds was the amount of BIOSAF probiotic supplementation: B1 – 0.5 g kg<sup>-1</sup>; B2 – 1.0 g kg<sup>-1</sup>; B3 – 1.5 g kg<sup>-1</sup>. The quantity of yeast, *Saccharomyces cerevisiae*, expressed as colony forming units (CFU), added to each feed

variant was 4, 8, and  $12 \times 10^9$  CFU kg<sup>-1</sup>. The probiotic preparation was not added to the control feed (K). After conditioning with water vapour, the feeds were prepared with the 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 granulate was put through a cylinder crusher, and the pellets were segregated into two granulometric groups:

- 2.0 – 3.15 mm for carp with individual body weight up to 50 g;
- 3.15 – 4.5 mm for carp with individual body 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 pellet mass) with the spray method in a pelletizing drum.

## PHYSICAL AND CHEMICAL METHODS OF FEED EVALUATION

The water stability of the experimental feeds was evaluated with the Hastings-Hepher method (Hepher 1968) modified by Szumiec and Stanny (1975) based on the percentage of feed particle mass lost during a water bath that simulated water movements and after the sample was dried to a content mass at 105°C. The oxygen consumption by the water used for testing was determined in an alkaline environment applying the method described by Gomółka and Szypowski (1973).

The chemical analysis of the feeds was conducted according to Skulmowski (1974). The total protein content was determined in a Kjeld-Foss Automatic 16210 analyzer, while raw fat was identified with the Soxhlet method (ethyl ether extraction for 12 hours). The amount of raw fiber was determined with a Tecator Fibertec System M 1020 Hot Extractor. Ash content was determined through sample combustion at 550°C for 12 hours (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 with an ASS3 atomic absorption spectrophotometer (Carl Zeiss, Jena, Germany) according to the method in Gawęcki (1988). Total phosphorus was determined with the flame ionization technique. The feed protein amino acids were assayed in a Microtechna AAAT 339 analyzer after the sample (0.1 ml) had been hydrolyzed in 6N HCl at 106°C for 24 hours. Methionine and cystine were determined after 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 feeds was calculated from the chemical composition using the conversion factors of gross energy for fish: carbohydrates – 17.2 kJ g<sup>-1</sup>; protein – 23.6 kJ g<sup>-1</sup>; fat – 39.5 kJ g<sup>-1</sup> (Bureau et al. 2002).

## GROWTH TEST

The experiment was conducted under controlled conditions in an open supply system located in the aquarium hall of the Department of Inland Fisheries and Aquaculture, Agricultural University in Poznań, Poland. The water supply was drawn from the mains and was run through an active carbon filter to reduce the chlorine content. The principal element of the water system was the 2.4 m<sup>3</sup> equalizing tank where the water was heated to a constant temperature and aerated with a HIBLOW HP-60 blower. During the experiment, the physicochemical parameters of the water were maintained at the relatively constant, optimal levels for carp juveniles of 22-23°C temperature and oxygen saturation above 70% (Steffens 1986). These physicochemical parameters were monitored with an ELMETRON CO-315 oxymeter microcomputer.

Carp juveniles were placed in 60 dm<sup>3</sup> tanks in which a constant water flow was maintained at a total water exchange rate of five times per 24 hours. Every day at 08:00 the tanks were cleaned with a water siphon to remove excrement and unconsumed feed. The experimental feeds were supplied around the clock (24 hrs) using automatic belt feeders. The daily feed rations were calculated according to the feeding standards in Miyatake (1997) based on the actual fish body weight. The size of the rations was determined every tenth day based on weight monitoring which also served for determining the values of the other rearing indices.

The growth test lasted for 50 days from March 9 to April 29, 2003. The biological material consisted of carp bred at the facility with an average individual body weight of 40 g. The experiment was conducted in four variants (including a control group) of three replications each. Each tank was stocked with ten fish specimens.

Before the growth test began and following its conclusion, the fish were sampled randomly in order to determine the basic body chemical composition. The sampled carp were anesthetized using Propiscin (Kazuń and Siwicki 2001) and then decapitated. Subsequently, the bodies were ground and homogenized, and then the dry mass, total protein, raw fat, and ash were determined using the same analytic methods applied to the feed.

## STATISTICAL ANALYSIS

The fish stocking biomass obtained for the four experimental variants in each of the five terms was analyzed statistically. Furthermore, the fish stocking biomass and feed consumption were used to calculate the following zootechnical rearing indices:

- mean specific growth rate of fish (SGR, % d<sup>-1</sup>);
- mean absolute food conversion ratio (FCR);
- protein efficiency ratio (PER).

The Kolmogorov-Smirnov test (significance level  $P \leq 0.05$ ) revealed that the distribution of the stocking biomass and indices described above was normal. The homogeneity of variance for the same parameters was checked with the Bartlett test and the result was positive. Since the sets of data satisfied all the necessary assumptions, they were subjected to analysis of variance. The main effects included time and feed type. Interaction was also estimated. Following the analysis of variance, the post-hoc group of analyses was applied. Homogenous groups were determined with the T-Tukey's test.

## RESULTS

The water stability of the experimental feeds was satisfactory. All of the feeds obtained good evaluation marks on the loss of granule mass during the water bath, while feeds K, B1, and B2 obtained good evaluation marks for oxygen demand used in the testing and feed B3 was evaluated as very good (Table 1).

TABLE 1

Parameter	Water stability of feeds tested			
	Treatment			
	K	B1	B2	B3
Weight loss (after 40 min.) (%)	25.6	29.7	26.7	25.5
Score	good	good	good	good
Oxygen demand (mg O <sub>2</sub> dm <sup>-3</sup> )	50.2	58.6	55.2	48.9
Score	good	good	good	very good

The chemical composition determined for the tested feeds was as follows (%): total protein – 38.1; raw fat – 9.9; NFE – 33.1; raw fiber – 1.9; ash – 4.7; total phosphorus – 0.8; calcium – 1.23. The amounts of exogenous amino acids determined in the feeds (g 100 g<sup>-1</sup>

protein) were: arginine – 5.32; histidine – 3.89; lysine – 7.53; tryptophan – 2.84; phenylalanine with tyrosine – 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 a value of 76.93. The E/P relation in experimental feeds was 48.93 kJ g<sup>-1</sup> protein at a level of gross diet energy of 18.59 MJ kg<sup>-1</sup>.

Carp growth was stimulated significantly by the application of feeds supplemented with the BIOSAF probiotic starting on day 30 of the test (Table 2).

**TABLE 2**

Changes of mean individual fish body weight (g) during the growth test<sup>1</sup> (mean (±SD))

Days	Treatment			
	K	B1	B2	B3
1	39.06a (± 0.15)	39.37a (± 0.54)	39.44a (± 0.48)	39.32a (± 0.23)
10	49.15a (± 1.19)	50.66a (± 1.12)	52.03a (± 2.94)	52.70a (± 3.18)
20	62.37a (± 0.90)	66.63a (± 0.77)	71.37a (± 3.77)	71.28a (± 7.71)
30	74.99a (± 1.98)	82.31ab (± 1.34)	90.96b (± 4.66)	88.96b (± 4.72)
40	87.03a (± 2.70)	99.28b (± 4.30)	112.92c (± 4.07)	103.25bc (± 6.82)
50	105.03a (± 3.00)	116.55b (± 6.16)	134.00c (± 2.72)	118.99b (± 5.93)

<sup>1</sup>Values are means from triplicate groups of fish; means in each row with different superscript are significantly different ( $P \leq 0.05$ )

From that moment, the carp which received probiotic feeds attained significantly higher individual body weight ( $P \leq 0.05$ ) in comparison with those from the control group. This tendency was maintained until the end of the growth experiment.

The specific growth rate (SGR) of the fish depended on the type of feed. Throughout the test, the minimal value (1.98% d<sup>-1</sup>) was reached in variant K, while the maximal value (2.45% d<sup>-1</sup>) was achieved in variant B2; the differences in SGR values were also statistically significant (Table 3).

The indices of the utilization of the feed components used in the experiment were differentiated. The most favorable values of FCR and PER were noted for feed B2, and they differed significantly from the values obtained for the remaining feeds. During the growth test, no fish losses were recorded in any of the variants.

While the type of feed applied impacted the content of protein in the fish bodies, it did not cause any changes in the amount of dry mass, fat, or ash (Table 4). However, it did have some influence on protein and fat retention in the fish bodies (Table 3).

TABLE 3

Specific growth rate (SGR), survival rate (SR), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (aNPU) and apparent lipid retention (aLR) (mean ( $\pm$  SD)) in common carp juveniles fed the experimental diets<sup>1</sup>

	Treatment			
	K	B1	B2	B3
SGR (% d <sup>-1</sup> ) <sup>2</sup>	1.98a ( $\pm$ 0.05)	2.17b ( $\pm$ 0.13)	2.45c ( $\pm$ 0.06)	2.21b ( $\pm$ 0.10)
FCR <sup>3</sup>	1.58a ( $\pm$ 0.05)	1.38b ( $\pm$ 0.13)	1.17c ( $\pm$ 0.03)	1.34b ( $\pm$ 0.03)
PER <sup>4</sup>	1.66a ( $\pm$ 0.06)	1.91b ( $\pm$ 0.15)	2.25c ( $\pm$ 0.07)	1.96b ( $\pm$ 0.04)
aNPU <sup>5</sup> (%)	36.13 ( $\pm$ 1.22)	31.21 ( $\pm$ 1.03)	27.07 ( $\pm$ 1.55)	25.33 ( $\pm$ 2.0)
aLR <sup>6</sup> (%)	96.1 ( $\pm$ 3.36)	90.3 ( $\pm$ 5.21)	94.5 ( $\pm$ 4.21)	98.0 ( $\pm$ 2.38)
SR (%)	100a	100a	100a	100a

<sup>1</sup>Values are means from triplicate groups of fish; means in each row with different superscript are significantly different ( $P \leq 0.05$ )

<sup>2</sup>SGR =  $100 \times (\ln \text{ final body weight (g)} - \ln \text{ initial body weight (g)}) \text{ days}^{-1}$

<sup>3</sup>FCR =  $\text{dry feed intake (g)} \text{ weight gain}^{-1} \text{ (g)}$

<sup>4</sup>PER =  $\text{wet weight gain (g)} \text{ protein intake}^{-1} \text{ (g)}$

<sup>5</sup>aNPU =  $100 \times (\text{final protein content of fish body} - \text{initial protein content of fish body}) \text{ protein intake}^{-1}$

<sup>6</sup>aLR =  $100 \times (\text{final lipid content in fish body} - \text{initial lipid content in fish body}) \text{ lipid intake}^{-1}$

TABLE 4

Chemical composition (%) of fish body before and after the experiment<sup>1</sup>

Treatment	Dry weight	Ash	Crude protein	Crude fat
	Before the experiment			
	25.32a	15.81a	10.30a	2.34a
	After the experiment			
K	27.03a	15.67ab	12.44b	2.09a
B1	24.46a	15.61ab	13.79d	2.41a
B2	26.18a	16.03a	14.83e	2.71a
B3	26.72a	15.24b	12.92c	2.48a

<sup>1</sup>Values are means of analysis of three fishes from each experimental group; means in each column with different superscript are significantly different ( $P \leq 0.05$ )

## DISCUSSION

In Hastings-Hepher tests, all the feeds received satisfactory evaluations indicating that their water stability had no essential effect on the carp rearing results. The experimental feeds were correctly balanced regarding the content of total protein and raw 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), and energy level in the diet and in its relation to the amount of protein (Ohta and Watanabe 1996) for carp juveniles.

The results of other studies published to date confirmed the distinct probiotic effect of supplementing the diet of rainbow trout, *Oncorhynchus mykiss* (Walb.), with yeast, *S. cerevisiae* and *Debaromyces hansenii* (Andlid et al. 1995, 1998, 1999, Vazquezjuarez et al. 1997). This was evident in the ability of the yeast to quickly colonize the alimentary tract through strong adhesion to the intestine wall and by the lowered pH value of the food content. Furthermore, *in vitro* tests confirmed that both types of yeast produce inhibitors of the bacteria *Aeromonas salmonicida*. Growth tests conducted by Peng Li and Gatlin (2003) confirmed improved survival and growth rates in bass hybrids (*Morone chrysops* × *M. saxatilis*) fed feeds supplemented with yeast, *S. cerevisiae*.

In the present study, the carp fed experimental feeds (isoproteinaceous with 38% protein and isoenergetic with 18.59 MJ kg<sup>-1</sup>) supplemented with 0.5, 1.0, or 1.5 g kg<sup>-1</sup> of the BIOSAF probiotic had significantly improved zootechnical indices of SGR, FCR, and PER in comparison with those of the fish fed the control diet. The most favorable values of these indices were noted in the fish groups that received feeds supplemented with 1.0 g kg<sup>-1</sup> of the probiotic; the fish groups that received feeds supplemented with 0.5 or 1.5 g kg<sup>-1</sup> of the probiotic were characterized by poorer index values. The results of the growth test show explicitly that the optimal amount of BIOSAF probiotic in carp feed is 1 g kg<sup>-1</sup> of feed and that an increase of probiotic proportion to 1.5 g kg<sup>-1</sup> did not result in improved indices.

## CONCLUSIONS

1. Feeding carp juveniles feeds supplemented with the probiotic BIOSAF (*Sachcaromyces cerevisiae* SC47) improved rearing results.
2. The optimal supplementation of the BIOSAF probiotic in carp juvenile feed is 1 g of preparation per kilogram of feed ( $8 \times 10^9$  CFU of yeast in 1 kg of feed).

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## STRESZCZENIE

### ZASTOSOWANIE PROBIOTYKU BIOSAF W PASZACH DLA JUWENALNEGO KARPIA (*CYPRINUS CARPIO* L.)

Określono wpływ dodatku do upostaciowanych pasz probiotyku BIOSAF – termostabilnego koncentratu żywych drożdży *Saccharomyces cerevisiae* (szcep Sc 47) na tempo wzrostu i inne wskaźniki zootechniczne narybku karpia. Przygotowano cztery granulowane pasze doświadczalne, w których czynnikiem różnicującym był dodatek probiotyku BIOSAF: w paszy B1 – 0,5 g kg<sup>-1</sup>, w paszy B2 – 1,0 g kg<sup>-1</sup> i w paszy B3 – 1,5 g kg<sup>-1</sup>, co w przeliczeniu na ilość jednostek tworzących kolonie (jtk) drożdży *Sacharomyces cerevisiae* wynosiło odpowiednio: 4, 8 i 12 × 10<sup>9</sup> w kilogramie paszy. W paszy kontrolnej (K) nie zastosowano dodatku preparatu probiotycznego. W trwającym 50 dni teście wzrostowym ryby, którym podawano pasze probiotyczne osiągnęły istotnie wyższe średnie masy jednostkowe ( $P < 0,05$ ) w porównaniu z rybami z wariantu kontrolnego (tab. 2). Minimalna wartość względnego przyrostu masy ciała ryb (SGR) w czasie całego testu (1,98% d<sup>-1</sup>) została osiągnięta w wariancie K, natomiast maksymalna (2,45% d<sup>-1</sup>) w wariancie B2, a różnice były istotne statystycznie. Najkorzystniejsze wartości współczynników: pokarmowego (FCR) oraz wydajności wzrostowej białka (PER) odnotowano dla paszy B2 i różniły się one istotnie od wartości uzyskanych dla pozostałych pasz (tab. 3). W czasie testu wzrostowego w żadnym z wariantów nie odnotowano strat ryb. Rodzaj podawanej paszy miał wpływ na zawartość białka i tłuszczu w ciele ryb, natomiast nie powodował zmian w ilości suchej masy i popiołu (tab. 4). Żywienie narybku karpia paszami z dodatkiem probiotyku BIOSAF (*Sacharomyces cerevisiae* SC47) poprawia wyniki chowu, a optymalny dodatek probiotyku wynosi 1 g preparatu na kilogram paszy (8 × 10<sup>9</sup> jtk drożdży w 1 kg paszy).