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Effect of Different Supplementary Diets on Growing Rate, Fat and Protein Contents of Flesh Common Carp (*Cyprinus carpio*) Fingerlings

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Abstract

The effect of different supplementary diets containing humic substances and probiotics for common carp, based on growth rate, specific growth rate %, protein and fat content was investigated. Humic acid was mixed with different mineral elements and the gram-positive bacteria combined as probiotics supplementary diets. These supplements were added to the experimental basal diet (control) at a rate of 0.5%, 1% and 2%. Four groups of fingerling with an average mass of 0.5 g were fed on the experimental diets, three times per day for 11 weeks. 30 fish per tank were weighed randomly every week to measure their growth rate and at the end of the experiment, 5 fish from each treatment were weighed and their fat and protein content was analyzed individually. The results showed that 1% and 2% probiotics increased the growth rate while all treatments have less specific growth rate % than control. In relation to fat and protein contents, the results showed that fish fed with probiotics 1% had high fat and protein contents compared to other groups in general. All fish fed with different levels of supplemented diets showed a decrease in fat in compare with control group. However, protein contents in fish fed with HS 0.5% and probiotics 1% were significantly higher than control and other group of fish fed with different level of supplemented diets. These results suggest that humic acid substances cannot improve fish growth rate, but fat and protein contents could be changed slightly, while probiotic supplementary diets can improve growing rates, having a significant effect on protein content of fish meat.

administration of different dietary supplements to affect growth performance in common carp (*Cyprinus carpio*) has been reported. If the type and amount of feed supplements are optimized they can have a considerable impact on fish growth but in some studies no positive effects have been observed [1-5]. The use of feed additives in the aquaculture industry has received considerable attention in recent years [6]. However, a search for additives that offer effective growth stimulation are required in farmed fish, but without affecting the environment [7,8]. The poor growth performance in some fish species could be attributed to the low palatability of the diet [9]. For example, palatability is linked to poor growth performance of some fish species, which were fed a diet containing meat and bone meal [10].

Probiotics is a generic term, products can contain yeast cells, bacterial cultures or both, that stimulate microorganisms capable of modifying the gastrointestinal environment to favor health status and improve feed efficiency and is also one of the feed additives that can improve intestinal microbial balance, food absorption, digestive enzyme activities and reduced pathogenic problems. The application of probiotics in aquaculture as an environmentally friendly treatment is also increasing rapidly [11]. There are several studies that have demonstrated growth promotion in pigs, poultry and fish [11-13].

Humates are derived from plant matter decomposed by bacteria and contain humus, humic acid, fulvic acid and some microelements. Humates promote growth by altering the partitioning of nutrients' metabolism and reducing mortality and improving feed conversion efficiency [12]. Humic substances (HS) can control a variety of processes in freshwater ecosystems. They can impact bioavailability or the release of trace elements and may have an influence on enzyme activities in fish, invertebrates and macrophytes. It has been indicated that the supplementation of diets with humic acid substances is an effective way to increase growth performance, reduce backfat thickness and improve meat quality in growing-finishing pigs [14,15]. Thus the aim of this study was to evaluate the use of fish meal as a major source of protein and some additive

Keywords: Humic acid; Probiotics; Growing rate; SGR%

Introduction

Many studies have been conducted to distinguish the effect of different diets on growth rate in different species of fish. The growth rate in fish is influenced by the effect of two notable factors: water temperature and food availability. The

supplements like probiotics by providing nutrients and enzymatic contribution of digestion, improving water quality and enhancing growth; and humic substances, which make vitamins and elemental minerals more absorbable by transforming them into complex organic and ionic forms that are easily transported into and through the cell membrane in the diets of common carp (*Cyprinus carpio*) and a comparison of their effects on weight, fat and protein contents in common carp, which is one of the most popular freshwater fish species in the central and eastern European countries, especially in Hungary.

Materials and Methods

Animals and housing

Eight hundred and forty healthy common carp (*Cyprinus carpio*) fingerlings provided by Bocskai Fisheries Ltd. (Hajdúszoboszló, Hungary), were acclimatized in three concrete tanks for 2 weeks and fed with basal feed three times daily. At the end of the second week, fingerlings with average initial weights of 0.5 g were distributed into 14 tanks with stocking density of 60 common carp per tank. Tanks were equipped with a recirculation system. The water temperature in the tanks was kept in the range of 21°C-24°C, while lighting varied according to the natural photoperiod. The water quality was monitored and analyzed for total ammonium (0.00 mg/l), nitrite (0.15-0.5 mg/l), nitrate (10-50 mg/l) and phosphate (3 mg/l) using the MColortest™ (Merck KGaA, Darmstadt, Germany). pH (9.5), temperature (21°C-24°C) and conductivity (0.57-0.69 mS) were measured using Combo pH/EC Multiparameter tester model HI98130 (HANNA instruments®, USA). Tanks were totally cleaned of faeces and the water was partially changed. The tanks were supplied with biologically and mechanically filtered fresh tap water after aeration. The water volume of the tanks was recirculated in every 30 minutes. Diets were formed in granulate with 2-3 mm diameter and then kept at room temperature until used.

Treatments

Seven treatments (two tanks per treatment) were formed, including the control group. The control group consumed a fish meal based on a basal diet. The basal diet was supplemented either with 0.5%, 1% or 2% of humic substances (Vita pulvis) (HS0.5, HS1 and HS2) or 0.5%, 1% or 2% of probiotic product (PB0.5, PB1 and PB2). The humic substance (Organit Ltd., Hungary) contained 40% of humic acids and the following amount of minerals, Ca 5.5%, Mg 0.45%, P 0.1%, Na 0.1%, Fe 7000 mg/kg, Mn 130 mg/kg, Cu 10 mg/kg, Mo 8 mg/kg, Co 3 mg/kg and Se 2 mg/kg. The probiotic product contained combinations of *Bifidobacterium* sp., *Bacillus* sp. and *Lactobacillus* sp., which was prepared in the Department of Feed and Food Biotechnology, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Hungary. The diets were pelleted to 3mm in diameter and 2-3 mm in length and were kept at room temperature until used. The Weende analysis was done in Central Laboratory of Agricultural and Food Products, Faculty of Agricultural and Food Sciences and Environmental Management, by determining the 1/Crude protein, (ISO 5983-2:2000): that specifies a methods for the determination of nitrogen content of animal feeding stuffs according to the Kjeldahl method, and a method for the calculation of the crude protein content. It is suitable for use as a semi-micro rapid routine method using digestion, copper catalyst, and steam distillation into boric acid. 2/Crude fat, (ISO 11085:2015): a specific procedure for the determination of the fat content of cereals, cereal-based products, and animal feeding stuffs. 3/Crude ash (ISO 5984:2002): that is specified for the determination of crude ash of animal feeding stuffs. 4/Fiber (ISO 6865:2000): using for determination of crude content with intermediate filtration. 5/Dry matter, (ISO 6496:1999): determination of moisture and other volatile matter content and also the gross energy of supplementary diets were calculated (Table 1). Carps were fed three times daily at 08:30, 12:30 and 16:30. The daily feed allowance was 2% the estimated total body weight.

Table 1: Chemical composition and gross energy content of the experimental diets.

Treatment	Dry matter	Protein	Fat	Fiber	Ash	Energy
Control	91.1	31.7	2.5	8.2	6.4	12.9
HS0.5	95	34.2	5.2	2.4	8.4	13.9
PB0.5	93.7	34.3	5.1	2	8.4	13.9
HS1	93.6	34.4	4.7	2.2	8.6	13.8
PB1	96.7	34.4	5.1	2.3	8.3	13.9
HS2	93.9	34.3	5.9	2.7	8.8	13.9
PB2	93.1	35.5	5.2	2.9	8.4	13.8

All amounts are in percentage %, except energy (MJ kg⁻¹).

Measurements

Fingerlings were randomly chosen and weighed for growth rate analysis weekly during the 11-week experimental period.

The survival rate of fish was recorded for each tank. On the last day of the 11th week, five fingerlings per tank were randomly selected to be weighed individually, killed and flesh samples were taken and homogenized after removal of skin and bone.

Dry matter, crude protein and crude fat contents of fish meat were analyzed using the standard methods including ISO1442:2000 for determination of moisture content, ISO937:1978 as a reference method for determination of nitrogen content, consists in digesting of test protein with concentrated sulphuric acid to convert organic nitrogen to ammonia ions, using copper (II) sulphate as a catalyst. Then alkalization, distillation of the liberated ammonia into an excess of boric acid solution, titration with hydrochloric acid to determine the ammonia bound by the boric acid, and calculation of nitrogen content of the sample from the amount of ammonia produced and ISO1443:1973 to determine fat content of fish meat, that method consists in boiling of a test portion with hydrochloric acid to free the occluded and bound lipid fractions, filtrating of the resulting mass, drying, and extracting with n-hexane or light petroleum, of the fat retained on the filter.

Statistical analysis

Data was analyzed using the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). In case of significant treatment effect differences between means, they were tested using Tukey's Studentized Range (HSD) test.

Results

Means of weight, fat and protein contents affected by different diets including basal diets, Vita Pulvis and probiotics in range of 0.5% to 2% were analyzed and the results of proximate analysis of Common carp fed at week 11 of growing period are presented in **Table 2**.

Table 2: Fat, weight gain and SGR% of common carp fed different level of supplemented diets.

Treatments	Control	HS			PB			RMSE
	0.00%	0.50%	1.00%	2.00%	0.50%	1.00%	2.00%	
Initial weight (g)	2.80 ^a	2.90 ^a	3.10 ^b	3.10 ^b	3.10 ^b	3.40 ^c	3.30 ^c	0.83
Final weight (g)	8.40 ^b	8.12 ^b	8.72 ^b	8.65 ^b	8.95 ^c	9.64 ^c	9.20 ^c	1.36
Weight gain (g)	5.46 ^b	5.22 ^a	5.65 ^b	5.58 ^b	5.81 ^b	6.25 ^c	6.01 ^c	1.36
SGR%	1.41 ^c	1.33 ^a	1.35 ^b	1.34 ^a	1.36 ^b	1.36 ^b	1.34 ^a	0.2
Fat (%)	44.30 ^c	30.50 ^a	35.60 ^b	39.90 ^b	31.00 ^a	41.80 ^b	40.00 ^b	1.26
Protein (%)	46.62 ^b	48.15 ^c	44.80 ^a	46.79 ^b	45.56 ^a	48.79 ^c	45.31 ^a	0.52

Means with the same letter are not significantly different (P<0.05). SGR%: Specific Growth Rate%, HS: Humic Substances, PB: Probiotic, RMSE: Root-Mean-Square Error.

Throughout the feeding period fish in all experimental groups were in good health and the survival ratio was 100%. All experimental diets were readily accepted by common carp fingerlings during the experimental period. While humic acid did not increase the weight gain, the probiotic feed had an effect (P<0.05) at 1% and 2% content. All treatment decreased the fat in the body mass and these treatments could have a favorable effect on the protein content. It is also shown that, the lowest significant (P<0.05) value of fat contents obtained in fish fed Vita pulvis 0.5% and probiotics 0.5%, compared to and fish fed with Vita pulvis 0.5% and probiotics 1% showed the highest significant (P<0.05) protein contents compared to control group. Weight gain results show that fish fed with probiotics 1% with highest fat and protein contents have significantly high weight gain among the treated groups. SGR% results shows control fish had the highest percentage of growing rate in comparison with other groups.

Discussion

The results of this present study indicated that the supply of probiotic supplementary diets including *Bifidobacterium* sp., *Bacillus* sp. and *Lactobacillus* sp. could improve the growth rate (weight gain) of common carp (*Cyprinus carpio*) fingerlings as an

omnivorous fish, while it has also been demonstrated that the normal flora colonized in the intestine of herbivorous fish therefore the probiotics are being good for the fish species [16]. In relation to this, Lara-Flores et al. [17] concluded that the use of *Saccharomyces cerevisiae* as probiotic for fry of Nile tilapia resulted in better growth and food efficiency, Similar results from previous studies have proved that using probiotics improved the growth performance of Israeli carp [11,18]. Increase of weight gain and survival was observed for turbot larvae fed rotifera enriched with acid-lactic bacteria [19]. In 1998, Moriarty demonstrated that prawn survival rate increased in ponds where probiotics were used as a supplementary diet including some strains of *Bacillus* sp. Gupta et al. [13], has proved that the supplementation of different probiotic strains in the diet resulted in survival improvements and growth performance of common carp fingerlings. The result of this study also demonstrated high weight gain in fish fed probiotics as a supplementary diet. There are many similar experiments related to the improving effects of probiotics on different species of fish including Indian carp (*Cyprinus mrigala*) [18], Nile tilapia (*Oreochromis niloticus*) [17], Chinese carp (*Cyprinus. Carpio*) [20], Persian sturgeon (*Acipenser persicus*), Faramarzi et al. [12] and giant freshwater prawn (*Macrobrachium rosenbergii*) [21], Gupta et al. [13] determined *Paenibacillus*

polymyxa is a potential probiotic species to improve growth of fry common carp, *Cyprinus carpio*. In contrast, Gunther and Jimenez-Montealegre [22] revealed that diets including probiotics did not improve growth and feed utilization of tilapia, *O. niloticus*, and freshwater prawn *M. rosenbergii*, under laboratory conditions.

Carlsson et al. [23] has reported that the humic substances addition could stimulate the growth and nitrogen utilization of *Prorocentrum minimum*. It has been shown that humic substances added to animal feed increased growth rate and product quality [14,24] Karaoglu et al. [25] has demonstrated that humates added to the feed of poultry promote growth. Yasar et al. [26] concluded that HS induced an increase in weight gain in rats and they suggested that the increasing of weight gain was associated with improving the feed intake and gain: feed ratio. The results of using humic acid substances in this study showed that there are no significant differences in growth rates ($P>0.05$) compared with the control group. Also experimental diets for piglets showed that humic substances had beneficial effects on general health status and reduced the mortality rate, which could be due to the effect of humic acid on the metabolism of proteins and carbohydrates by microbe's metabolism.

Similar to our results, in which HS supplemented diets presented the lowest fat content in fish when compared to the control, whilst protein content was higher than the control in fish fed 0.5% HS [27-31]. It has been also reported that with the same amount of HS backfat thickness in pigs has been reduced, regardless of their [15,32-34].

According to the results of the current study, it is concluded that dietary supplementation with humic acid substances cannot improve fish growth rate, but fat and protein contents could be changed slightly, while probiotic supplementary diets can improve growing rates and having a significant effect on protein content of fish meat, however there are no significant differences between fish fed with basal fish meal and supplemented probiotic diets.

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