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[Short Communication]

The effects of different levels of biogen probiotic additives on growth indices and body composition of juvenile common carp *(Cyprinus carpio* L.*)*

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ABSTRACT

An experiment was conducted to evaluate the effects of biogen probiotic additives on growth performance of common carp, *Cyprinus carpio*. Four practical diets containing 0 (control) , 0.1, 0.2 and 0.3 % Biogen probiotic were used to feed fish. Seventy-two juvenile common carp (65 ± 3.5g) were randomly distributed between 12 fiberglass tanks of 500 L capacity. During 60 days of feeding trial, the fish receiving probiotic feeds showed significantly higher growth performance in terms of weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), food conversion ratio (FCR) and survival rate (SR) in comparison with those fed the control diet ($P<0.05$). The best results in food efficiency and growth performance were observed in fish fed diets containing 0.3% of Biogen probiotic that were significantly different with that in other treatments ($P < 0.05$). Growth performance did not differ significantly ($P>0.05$) in fish fed diets containing 0.1 and 0.2 percent of probiotic. Fish carcass composition in the diets receiving probiotic improved from initial values i.e. higher levels of protein and lipid with lower levels of moisture and ash content and showed significant difference ($P< 0.05$) only with the basal control diet. There were no significant differences ($P > 0.05$) in body composition between the treatments which received probiotic.

Feeding juvenile carp with diets containing Biogen probiotic improves growth performances and feeding efficiency in them; the optimum addition of probiotic is 0.3 g/100gof diet.

Keywords: *Biogen probiotic, Cyprinus carpio, Growth performance, Fish carcass.*

INTRODUCTION

Studies on practical application of the positive effects of some probiotics in the feeding of aquatic animals have been recently developed. Akrami *et al*., (2008) observed that growth, survival and feeding efficiency of juvenile *Huso huso* was improved when it received probiotic in its diet. Gatesoupe, (1991) found best growth and survival for turbot larvae (*Scopthalmus maximus*) when they were fed probiotic strains of *Lactic bacteria*; Ziemer and Gibson (1998) overviewed the useful effects of probiotics when they was incorporated in animal diets. Eid and Mohamed (2008) found that probiotics acted as growth promoters in Nile Tilapia (*Oreochromis niloticus*). Use of feed additives give a bacteriostatic effect, or stimulate the populations of bacteria living in alimentary tract, e.g. acids, herbs and herb extracts, prebiotics, probiotics and synbiotics actually represent substances contained in food (or added to food) which activate growth or activity of beneficial strains of bacteria occurring in the alimentary tract (Freitag., *et.al*, 1999). The probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response to host species; and enhancement of nutrition of host species through the production of supplementary digestive enzymes (Thompson *et al*., 1999; Carnevali *et al*., 2006). Supplementary feeding in the form of additives in diet increase the carrying capacity of culture systems and enhance fish production specially in warm water fishes like carp by many folds (Hepher,

1975) and also offers the best means of production within shortest time in the pond (Devaraj, 1986).

One of the most important factors for carnivorous and omnivorous fishes like common carp is digestibility and food absorption of artificial pelleted feeds; non-conventional additions like fiber and probiotic in diets will improve food digestibility and its absorption (Piva 1998; Noh, et.al 1994). In respect to commerical aspects for manufacturing of aquatic animals, food constitute more than 50% of recurring expenses for fish farming management. Considering the economical value of carp farming and also the limited studies of probiotic in the diet of juvenile carp, this survey was done in order to determine the optimum dose for probiotic use and its effect on growth performances and feeding efficiency of juvenile carp.

MATERIALS and METHODS

Experimental setup

Seventy-two juvenile common carp with an average body weight of 65± 3.5g were obtained from a private carp farm in (Somehsera city, Guilan provice, Iran) and brought to wet-lab of Faculty of Natural Resources, University of Guilan. Initially, all fish were stocked in 5 m^3 tanks without feeding for 48 hrs. Then prior to the experiment, fish were randomly transferred to 12 tanks of 500 L with 6 fish per tank. Based on observations and feeding behavior of juvenile common carp during the trial, they were feed up to satiation 3 times a day (8 h, 12 h, and 18 h). Feeds were adjusted at 2% of body weight. .

Fish were weighed once every week and daily rations were adjusted accordingly. The duration of the experiment was sixty days.

The growth trials were conducted in June and July. The trials were conducted on a 12L:12D photoperiod. Water parameters like temperature, pH, DO were 24.2 ± 2 ^oC (well water), 7.5 ± 0.6 and 5.8 ± 0.71 mg/l, respectively.

Diet design

Four iso-nitrogenous diets were formulated from practical ingredients (Table 1). The control basal diet was without feed additives (Biogen) *, while the other diets were supplemented with 0.1, 0.2 and 0.3 % Biogen. To prepare the experimental diets each component was individually weighed and the premixes of vitamin, mineral and other additives were thoroughly mixed together and then added to the main components together with oil and again mixed for 15 min. Water was added (300 ml kg^{-1}) until the mixtures became suitable for making strands (dough). The wet mixture or dough was slow cooked (steamed without pressure) for 15 – 20 min. To obtain stiff dough, this dough was extruded through a kitchen noodle maker with 2 mm diameter to obtain strand or pellets of 2 mm × 2 mm; the strand were oven dried at 60 ^oC for 24 hour to reduce the moisture content to less than 10 % (Lovell, 1991).

* Manufactured by China Way Corporation 16-4 No. 424 Chung Ming Road. Taichung Taiwan.

Growth and proximate chemical composition

The experiment was carried out in four variants (including a control variant), each with three replicates. Each tank (500L) was stocked with 6 pcs of fish with an average initial weight of 65±3.5g. Fish were weighed once every week and mortality was recorded daily. Rations were adjusted every week according to weight of biomass and mortality. All ingredients, test diets and whole body composition were analyzed at the nutritional laboratory (Faculty of Natural Resources, University of Guilan). Chemical analysis was carried out according to procedures described by A.O.A.C 1995); the water content (moisture) was determined by method of drying samples at 105C^O to a constant weight, total crude protein by Kjeldahls method with the multiplier 6.25; lipid content (E.E) by Soxhlet method, with petroleum ether as solvent for 8hours; total ash content was determined by the mineralization of sample at temperature of 550C^O for 8 hours (Linn Electo-therm Furnace). The amount of Nitrogen-Free extract was estimated by differences between dry mass and sum of remaining components.

For body composition analysis, three fish were randomly sampled from each treatment at the end of experiment, killed with over dose of MS222, pooled, passed through a meat grinder, freeze-dried for 64 hour, and then body crude protein, lipid (E.E) and ash content were determined for final body analysis (as prescribed before).

Also six fish were sampled from the stock in the beginning for the initial body composition.

The growth parameters were calculated as follows:

Weight gain (WG; g) = Ave. Final wt – Ave. Initial wt (Baker, 1985)

Specific growth rate (SGR; % / day) =

Ln. Final wt – Ln. Initial body wt × 100 / experimental periods (day) (Helland, *et.al*. 1996)

Food conversion ratio (FCR) = Dry Feed intake (g)/wet weight gain (g) (Hevroy, 2005)

Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g) × 100 (Helland, *et.al*. 1996)

Survival Rate (%) = No. Final Fish/No. Initial Fish× 100 (Baker, 1985)

Statistical analysis

Initially, data were analyzed for normal distribution using Kruskal Wallis test. If the data were normal, then the mean comparison between treatments was carried out by Duncan-multiple range test in one-way analysis of variance (ANOVA). In case of non normal distribution we activated them to normal data and in the case of being not normal again, we used the non-parametric test of Kruskal-Wallis. Mean comparison of data for being significant or non-significant between treatments was conducted by using software package and Excel at 5% level.

Table 1. Percentage composition of ingredients of experimental diets and their proximate analysis (% as feed basis)(Practical Diet

Feeding ingredients	Experimental Diets			
	control	0.1	0.2	0.3
Fish meal (60 % cp)	15	15	15	15
Soya bean meal (42 % cp)	20	20	20	20
Wheat powder (18 % cp)	20	20	20	20
Corn powder (25 % cp)	15	15	15	15
Wheat brand (10 % cp)	11	11	11	11
Rice brand (7 % cp)	8.73	8.63	8.53	8.43
Sunflower oil	2	2	2	2
Fish oil	2	2	2	2
Vitamin premixes[1]	3	3	3	3
Mineral premixes[2]	2	2	2	2
Binder	1	1	1	1
Anti-fungi[3]	0.25	0.25	0.25	0.25
Antioxidant[3]	0.02	0.02	0.02	0.02
Biogen[4]	0	0.1	0.2	0.3
Proximate analysis n=? (%) ± SD				
Moisture	11± 1	9.8 ± 0.8	10 ± 0.4	9.6 ± 0.32
Crude protein (cp)	25.3 ± 0.26	25.1 ± 0.66	25.2 ± 0.71	25.7 ± 0.66
Ether Extract (lipid)	6.3 ± 0.38	6.4 ± 0.38	6.5 ± 0.46	6.8 ± 0.39
Crude fiber (cp)	7.4 ± 0.22	7.1 ± 0.32	6.9 ± 0.42	6.6 ± 0.47
Ash	10.3± 0.18	10.6 ± 0.19	10.1 ± 0.38	10.5 ± 0.73
Nitrogen Free Extract (NFE)	40.3± 2.2	40.5 ± 2.6	40 ± 2.8	40.8 ± 2.3
Gross Energy[4] (kcal / 100g)	390.8 ± 13	390.4 ± 15	390.6 ± 14	390.8 ± 12

* Danesh, *et.al* 1994

1, 2. NRC 1993 and Noverian, *et.al* 2009

3. Anti-fungi,make the food free from fungus, while Anti-oxidant prevent it from being rancid during trial.

4. Each kg Biogen® contain: Allicin 0.247 micromil/gm, high-unit hydrolytic enzyme 3690 units/gm, (proteolytic-lipolytic-amylolytic and cell separating enzymes), *Bacillus subtills* Nato 6× 107 cells/gm, Glinsen extract. Manufactured by China Way Corporation 16-4 No. 424 Chung Ming Road. Taichung Taiwan.

4. Gross energy-Based on 5.65 Kcal/g protein, 9.45 Kcal/g fat and 4.1 carbohydrate Kcal/g (NRC, 1993)

RESULTS and DISCUSSION

The growth performance parameters of juvenile common carp fed diets supplemented with feed additives of Biogen probiotic are shown in table 2. At the end of the experimental period the group of fish fed with supplemented diets of Biogen showed significantly ($P < 0.05$) better growth performance and feed efficiency than the fishes fed the control diet. Growth performance like WG, SGR, FCR and PER in fish fed with 0.1 and 0.2 %

Biogen was not significantly different (P>0.05); however, fish fed the supplemented diets of Biogen at 0.3 % level had significantly higher (P < 0.05) values in growth performance as compared with that with other diets. The survival rate of fish fed with supplemented Biogen showed no significant difference (100 %). These results are in agreement with the results of Mehrim (2001) and Diab, *et al.,* (2002) for warm water fishes like Nile Tilapia (*Oreochromis niloticus*). Khattab, *et al* (2004) and Mohamed *et al.,* (2007) reported that Nile tilapia fingerlings fed on diets supplemented with probiotics exhibited greater growth performance and feed efficiency than those fed with control diet (0 % probiotic); these results are comparable with our results for common carp. Similar results were reported by Gafarian *et al* 2007, (*Huso huso*) using bacteria as probitics which promote growth and feed efficiency.

Table 2. Growth performance and feed efficiency of Juvenile common carp fed experimental diets (X ± SD)

Parameters	Experimental diets			
	control	0.1	0.2	0.3
Initial ave. wt (g)	65 ± 2.29	65.2 ± 3.1	65.4 ± 2.1	65.3 ± 2.6
Final ave. wt (g)	89 ± 1.9^{a}	103 ± 2.6^{b}	106.2 ± 3.7^{b}	131.3 ± 3.3^{c}
WG (g)	24 ±101^{a}	37.8 ± 2.8^{b}	40.8 ± 2.3^{b}	66 ±3.6^{c}
SGR (% / day)	0.40 ± 0.02^{a}	0.63 ± 0.06^{b}	0.68 ± 0.03^{b}	1.1 ± 0.08^{c}
FCR	1.91 ± 0.06^{a}	1.63 ± 0.05^{b}	1.60 ± 0.04^{b}	1.30 ± 0.05^{c}
PER	0.47 ± 0.07^{a}	0.63 ± 0.03^{b}	0.64 ± 0.03^{b}	0.97 ± 0.26^{c}
Survival Rate (%)	85 ^{a}	100 ^{b}	100 ^{b}	100 ^{b}

Value in the some row with a different superscript are statistical different (P < 0.05).

Table 3. Chemical composition (wet weight basis) of whole body of Juvenile common carp fed experimental diets X ± SD

Chemical composition (%)	Experimental diets(Biogen probiotic)				
	initial	control	0.1	0.2	0.3
Moisture	67.3 ± 0.22	65.7 ± 0.21	59.4 ± 0.46	59.3 ± 0.21	59.1 ± 0.33
Crude protein	15.56 ± 0.61	16.3 ± 0.33	18.7 ± 0.51	18.6 ± 0.34	18.7 ± 0.26
Ether Extract	4.60 ± 0.44	4.90 ± 0.38	6.8 ± 0.28	6.86 ± 0.35	6.88 ± 0.39
Ash	6.70 ± 0.32	5.99 ± 0.18	4.3 ± 0.23	4.20 ± 0.31	4.10 ± 0.28

The mean differences were not observed between treatments (P > 0.05).

CONCLUSION

In addition Carnevali *et al.,* (2006) for sea bass juveniles (*Dicentrarchus labrax*), and Decamp and Moriarty (2006) for shrimps (*Litopenaeus vannamei* and *Penoeus monodon*) found significant effects of supplemented addition of probiotic on growth performance. Similar trends were found in this respect with Noh *et al.,* (1994) and Bogut, *et al.,* (1998) who studied the effect of supplementing common carp feeds with different additives, including antibiotics, yeast and bacteria; they observed better growth with bacteria as probiotic supplemented diets and also best survival rate (100 %) which is comparable with our results. Average of whole body composition including crude protein, lipid (ether extract), ash and moisture estimated on wet weight basis are presented in Table 3. No statistical differences were observed in body composition in fish groups that received Biogen probiotic (P>0.05) although they improved in body composition from their initial values i.e. higher deposition of protein and fat with lower deposition of moisture and ash. The fishes not receiving Biogen probiotic (control) showed lower values in body composition compared with other diets (P < 0.05). These results are in close agreement with results of Diab *et al.,* (2002); Lara-Flores *et al.,* (2003) and Mohamed *et al.,* (2007).

It could be concluded that the addition of Biogen probiotic showed a positive influence on growth performances of juvenile common carp. From feed utilization data and from the economical point of view, the diet supplemented with 0.3 % of Biogen probiotic was the best treatment.

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REFERENCES

Akrami, R., Hajimoradlo, A. M., Matinfar, A., Abadian, A.N., & Alimohmmadi, S. A., (2008). The effect of Inulin probiotic on growth indices, feed efficiency, survival rate and body composition of juvenile Beluga (*Huso huso*). J. Agri. Sci. Natur. Resour. 55 – 64. (in Persian).

A. O. A. C. (1995)Association of Official Analytical Chemists, Official methods of analysis. 16th edition, AOAC, Arlington, VA. 1832 pp.

Baker, D.H., (1985). Critical revive problems and pitfall in animals experiment designed to establish dietary requirement of essential nutrients. J. Natur. 116: pp 2339-2349.

Bougut, I., Milakovic, Z., Bukvic, Z., brkic, S., Zimmer, R., (1998). Influence of probiotic (*Streptococcus faecium* M74) on growth and content of intestinal microflora in carp (*Cyprinus carpio*). Czech. J. Anim. Sci. 231 – 235.

Carnevali, O., De Vivo. L., Sulpizio. R., Gioacchin. G., Olivotto, I., Silvi.S & Cresci. A., (2006). Growth improvement by probiotic European sea bass juveniles (*Dicentrarchus labrax*), with particular attention to IGF-1, myostatin and cortisol gene expression. Aquaculture. 430 – 438.

Danesh, K.A., (1994). Final project report of polyculture *frisii kutum* with Chinese carp, research center of Guilan province. pp 28.(in Persian).

Decamp, O., Moriarty, D.J.W., (2006). Probiotics as alternative to antimicrobials: Limitation and pontial. World Aquaculture. 60 – 62.

Devaraj, K. V., Keshavappa G.Y., and Manissery J.K., 1986. Growth of grass carp, *Ctenopharynogodon idella*. (val.) fed on two terrestrial fodder plants. Aquaculture and fisheries Management, 17: 123-128.

Diab, A.S., EL-Nagar, O.G & Abd-El-Hady, M.Y., (2002). Evaluation of *Nigella sativa* L. (black seeds; baraka), *Allium sativum* (garlic) & Biogen as a feed additives on growth performance of *Oreochromis niloticus* fingerlings. Vet. Med., Sues canal university V2. 753 – 754.

Eid, A. H., and Mohamed, K. A., 2008. Effect of using probiotic as growth promoters in commercial diets for monosex nile Tilapia (*Oreochromis niloticus*) fingerlings

Freitag, M., Hensche, H.U., Schulte-Sienbeck,H., Reichelt, B., (1999). Biological effects of conventional and alternative performance enhancers. Feed Magazine. 50 – 57

Gafarian, H., Soltani, M., & Abedian, A. M., (2007). The influence of some of the probiotic bacillus on feeding efficiency and nutrient body composition of Beluga (*Huso huso*) larvae. J. Agri. Sci. Natur. Resour. 35 – 47. (in Persian).

Gatesoupe, F.J., (1991).The effect of three strains of lactic bacteria on the production rate of rotifers, *Brachionus plicatillis*, and their dietary value for larval turbot, *Scophthalmus maximus*. Aquacalture. 335 – 342.

Helland, S.J., GrisdaleH. B., and Nerland, S., (1996). A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture. 139 pp 157-163.

Hevroy E.M., Espe, M., Waagbo, R., Sandness, K., Rund, M., and Hemre, G.I., (2005). Nutrition utilization in Atlantic Salmon (*Salmo salar* L) fed increased level of fish protein hydrolysate during a period of fast growth. Aquaculture Nutrition. 11pp301-313.

Hepher, B., 1975. Supplementary feeding in fish culture. In: Proc. 9th Intl. Conger. Of Nature Vol. 3. Karger, Baselm, Mexico, pp: 183-198.

Khattab, Y.A.E., Shalaby, A.M.E., Sharaf Saffa, M., El-Marakby, H. & RiziAlla, E.H., (2004).The physiological changes and growth performance of the Nile Tilapia *Oreochromis niloticus* after feeding with Biogen® as growth promoter. Egypt. J. Aquqt. Biol. And Fish. 145 – 158.

Lara-Flores, M., Olvera-Novoa, M.A., Guzma n-Me ndez, B.E. & Lo pes-Madrid, W., (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquacalture. 193 – 201.

Lovell, R. T. 1991**.** Nutrition of aquaculture species, Journal of Animal Science,

Vol 69, Issue 10 pp: 4193-4200.

Mehrim, A.I.M., (2001).Effect of some chemical pollutants on growth performance, feed and nutrient utilization of Nile Tilapia (*Oreochromis niloticus*). M.Sc. thesis, Saba. Basha. Alex. University. 215 pp.

Mohamed, K.A., Badia Abdel Fattah & Eid, A.M.S., (2007). Evaluation of using some feed additives on growth performance and feed utilization of Monosex Nile Tilapia (*Oreochromis niloticus*) fingerlings. Agricultural Research Journal, Sues Canal University. 49 – 54.

Noh, H., Han, K.I., Won, T.H. & Choi, Y.J. (1994). Effect of antibiotics, enzymes yeast culture and probiotics on the growth performance of Israeli carp. Korean J. Anim. Sci. 480 – 486.

Noverian, H. A., Zahmatkesh, A., Zamani, H. A., Ghanat Parest, A., (2009). The effect of different levels of Multivitamin on growth indices of Caspian *frisii kutum*. Pajouhsh and Sazandegi. Vol. 21, 163 – 173. (in Persian).

NRC. (1993). Nutrient requirement of fish. National Academy press, Washington DC.

Piva, A., (1998). Non- conventional feed additives. J. Fees Sci. 143 – 154

Thompson, F. L., Abreu, P.C., & Cavalli, R., (1999). The use of microorganisms as food source for *Penaeus paulensis* larvae. Aquaculture. 139 – 153

Ziemer, C. J., Gibson, G. R., (1998). An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. Int. Dairy J. 473 – 479.

اثر سطوح مختلف بیوژن پروبیوتیک بر شاخص‌های رشد و ترکیب بدن کپور معمولی جوان (*Cyprinus carpio*. L)

ح. ع. نویریان، ا. نصرالله زاده

چکیده

یک آزمایش جهت ارزیابی سطوح مختلف بیوژن پروبیوتیک (بعنوان مکمل و اضافه کننده) بر عوامل رشد و کارآیی در کپور معمولی جوان (*Cyprinus carpio*)، انجام گرفت. چهار جیره پایه (تجاری) حاوی 0 (شاهد)، 0.1، 0.2و 0.3 درصد بیوژن پروبیوتیک به جیره کپور اضافه شد و مورذ تغذیه سه گروه از ماهیان (سه تکرار) قرار گرفت. تعداد 72 قطعه کپور جوان (3.5 ± 65 گرم) بصورت کاملا تصادفی بین 12 مخزن فایبر گلاسی 500 لیتری توزیع شدند. بعد از 60 روز آزمایش، گروه ماهیانی که بیوژن پروبیوتیک دریافت کردند، عوامل رشد و راندمان غذایی آنها مانند افزایش وزن (WG)، نرخ رشد ویژه (SGR)، کارآیی پروتئین (PER)، ضریب تبدیل غذا (FCR) و درصد بقا سطح بالاتر و مطلوبتری را نسبت به گروه ماهیانی که جیره آنها فاقد بیوژن پروبیوتیک (شاهد) بودند، نشان دادند ($P < 0.05$). بهترین عوامل رشد و راندمان غذایی در گروه ماهیانی که با جیره 0.3 درصد بیوژن پروبیوتیک تغذیه شدند، بدست آمد و به لحاظ آماری با سایر تیمار ها دارای اختلاف معنا داری بودند ($P < 0.05$). عوامل رشد و کارآیی غذا در جیره های 0.1 و 0.2 درصد بیوژن پروبیوتیک به لحاظ آماری اختلاف معنا داری را نشان ندادند ($P > 0.05$). ترکیبات شیمیایی لاشه در آن گروه از ماهیانی که پروبیوتیک دریافت کردند، بهبود یافتند، به عبارتی میزان پروتئین و چربی لاشه افزایش یافته در حالیکه میزان رطوبت و خاکستر نسبت به ترکیبات اولیه (قبل از تغذیه) کاهش را نشان دادند و با جیره پایه فاقد پروبیوتیک (شاهد) دارای اختلاف معنا دار آماری بودند ($P < 0.05$). ترکیبات شیمیایی لاشه در تیمار های مختلف حاوی بیوژن پروبیوتیک به لحاظ آماری اختلاف معنا داری را نشان ندادند ($P > 0.05$). عوامل رشد و کارآیی غذا در کپور ماهیان جوان که با جیره حاوی بیوژن پروبیوتیک بعنوان مکمل و اضافه کننده مورد تغذیه قرار گرفتند بطور قابل ملاحظه ای بهبود یافته که سطح مطلوب آن در این آزمایش 0.3 گرم در 100 گرم جیره می باشد.