Evaluation of prebiotic and probiotic dietary supplementation on growth performance and some blood parameters of *Cyprinus carpio* Frys.

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ABSTRACT

The objective of this study was to evaluate the effect of prebiotics and probiotics on the growth performance, non-specific immunity and chemical composition of *Cyprinus carpio*. A total of 250 fry of *Cyprinus carpio* with an average body weight and length were used in experiments 2.82 ± 0.12g and 3.12 ± 0.13cm were divided into five experimental groups fed the pelleted diets for 12week as follows: groups 1 (control group) fed diet (T1), groups 2, 3 fed on diets (T2, T3) which supplemented with 1.5 and 2.5 g kg^{-1} Organoferum dry prebiotic respectively. Groups 4, 5 fed on diets (T4, T5) which supplemented with 0.5 and 1 g kg^{-1} Biogreen E probiotic respectively. The results of this study revealed that fish in group 3 had significantly higher final body weight, weight gains and specific growth rate followed by fish in group 5. Length increments and survival rate in fish of group 3 and 5 were significantly higher ($P < 0.05$) than other groups. The highest crude protein and lipid content ($P < 0.05$) were found in the fish fed on diets T3 and T5. Total serum protein, albumin and globulin were significantly increased in fish fed the experimental diets T5 than other groups. While, lysozyme activity were significantly increased in fish fed on the experimental diet T3. The conclusion of present study reveals that a dietary supplementation 2.5 g/kg prebiotics was improved growth performance, and non-specific immunity of *Cyprinus carpio* Frys.

Keywords: *Cyprinus carpio*, prebiotics, probiotics, growth, body composition.

INTRODUCTION

Chemical additives, such as anabolic steroids, growth promoters and some antibiotics are commonly administered in feed to improve growth performance and to control the outbreak of diseases in aquaculture (Gaunt *et al*. 2010 and Defoirdt *et al.* 2011). In recent years, the research on pro- and prebiotics in fish nutrition is increasing with the demand for the consumer and environment-friendly aquaculture (Denev 2009).

A prebiotic is defined as a non-digestible dietary ingredient that beneficially affects the host by selectively stimulating the growth of and/or activating the metabolism of health-promoting bacteria in the gastrointestinal tract (Manning and Gibson 2004). Results from several studies have indicated that prebiotics can improve growth performance and feed utilization of various fish species (Burr *et al.* 2008; Grisdale-Helland *et al.* 2008; Yousefian and Amiri 2009), enhance their non-specific

immune responses and resistance to bacterial infections (Li and Gatlin 2005; Staykov *et al.* 2007; Buentello *et al.* 2010).

Probiotic is any live microbial supplement, which beneficially affects the host animal by improving its microbial balance (Gram *et al.* 1999). Since the first use of probiotics in aquaculture, growing number of studies have demonstrated their ability to increase the growth rate and welfare of farmed aquatic animals (Wang *et al.* 2005; Wang and Xu 2006; Wang 2007). 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).

Present study was planned to investigate the effect of probiotic and prebiotic on growth performance, body composition and nonspecific immune response of *Cyprinus carpio* fry.

MATERIALS AND METHODS

Measurements of water quality parameters:-

Chemical analysis of water temperature, pH, Dissolved oxygen and Ammonia were carried out by different methods according to Boyd (1984) and APHA (1985).

Diets and feed additives

Basal diet:

The control basal diet (T1) was without any additives and contained approximately 35% crude protein and 5.9% crude lipid (Table 1).

Table 1: Composition of basal diet.

Ingredients	Content (%)	Chemical analysis%	
Soya48	49.5	Dry matter %	91.7
Rice bran	24.5	Crude protein%	35.0
Corn	15.2	Ether extract%	8.9
Wheat middling	4.00	Ash%	5.8
Soya oil	1.8	Fiber %	6.0
Gelatin	1.5	[3] GE (Kcal/100 g)[4]	389.32
Salt	0.5		
[1]Minerals premix	1.5		
[2]Vitamins premix	1.5		
total	100		

1-Minerals mix.: Each kg contain manganese 60g, iron 80g, copper 5g, zinc 40g, selenium 0.15 and iodine 0.35g.

2- Vitamins mix. Provide (g, mg or l.U kg diet) Vit. A 5000 *I. U* ,D32.000 I.U, E 100mg, k3 10.0mg, C. 1.000mg. B1 10mg, B2 15.0mg, B6 7.5mg, B12 0.1mg, Biotin 0.2mg, Folicacid 0.4mg, cholin Hcl 1.0g inosit. 3000.0mg, patathemic acid 50.0mg, Nicotinic acid 100mg, P-Aminobenzonic acid 50.0mg.

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

Feed additives:

- Organoferm Dry® (A.T.C.O. Pharma Company –Tanta city) each 1 kg contained 9.2% MOS and 11.7% 1:3, 1:6 Beta-glucans.
- Biogreen E (Samu median Co-LTD) each 1 kg contain:

Bacillus subtilis	Not less than $1x10^{11}$	CFU
Streptococcus faecium	Not less than $1x10^{10}$	CFU
Asparagus oryzae	Not less than $1x10^{10}$	CFU
Lactobacillus casei	Not less than $1x10^{10}$	CFU
Cellulose	3000	Unit
Protease	125000	Unit
ά- amylase	250000	Unit
B- amylase	10000	Unit

The four experimental diets that manufactured consist of as follows: T2, T3 which supplemented with 1.5 and 2.5 g kg^{-1} Organoferum dry prebiotic respectively. T4, T5 which supplemented with 0.5 and 1 g kg^{-1} Biogreen E probiotic respectively.

Fish & Experimental design

A total number of 250 apparently healthy *Cyprinus carpio* collected from El-Abbassa Fish Hatchery-The General Authority for Fish Resources Development El-Abbassa Sharkia Governorate. The average body weight 2.82 ± 0.124 g and 3.12 ± 0.13cm of length were used in experiment. Fish were located in glass aquaria (40x30x250 cm) and filled with dechlorinated tap water provided with aerating devices and Thermostatic heaters. The fish were adapted to experimental condition for two weeks before the start of the experiment. Fish were divided into five equal groups, each with two replicate (25 fish replicate $^{-1}$) fed the pelleted diets as follows: groups 1 (G1) (control group) fed basal diet (T1), groups 2, 3 (G2, 3) fed on diets T2, T3. Groups 4, 5 (G4, 5) fed on diets T4, T5. All fish groups were fed on the diets at the rate of 5% from the body weight during 12 weeks of the experiment. The fish weight and length were taken at the start and every two weeks during the experiment.

Sampling and analytical methods

Growth performance

The fish were weighed every two weeks to assess growth performance. The final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and body length increment were determined according to Castle and Tiews (1980); Siddiqui *et al*. (1988) and De Silva and Anderson (1995).

Body composition

Five fish samples from each replicate were analyzed for dry matter, crud protein, ether extract and ash according to standard method (AOCA, 1990).

Blood analysis

Blood samples were collected at the end of the experimental period from the caudal vessels. Three fish per group randomly collected from each group, used for serum collection without adding anticoagulant. The blood sample was allowed to clot &centrifuged at 3000 r.p.m for 15 min, for obtaining nonhemolysed serum which used for determination of total protein (Henry and clim 1964), albumin (Wotton and freeman 1982). Globulin was determined by direct subtracting the values of the albumin from those of the total protein. Lysozyme activity of blood serum was determined as described by Anderson and Siwicki (1995).

Statistical analysis

The mean and standard error were calculated for each variable. The data were analyzed by analysis of variance (ANOVA) to identify the significantly different groups at ($P < 0.05$) using SPSS software statistical program (SPSS for windows ver.15.00, USA).

RESULTS AND DISCUSSION

Physicochemical parameters were measured along experimental period. Average temperature (ºC), pH, dissolved oxygen (mg/l) and ammonia (mg/l) were 21.4±0.6, 7.2±0.12, 7.8±0.12 and 0.017±0.013 respectively.

The growth performances and individual length increments of fish in different groups are shown in Table (2). Fish fed diet T3 was significantly higher final body weight, weight gain and specific growth rate followed by fish group fed T5. The lowest values were observed in the untreated control group. While feed conversion ratio was significantly lower in fish fed diet T3 compared to control group. Fry fed the diets T3 and T5 was significantly higher final body length ($P < 0.05$) followed by fry fed the experimental diets T2 and T4 than those of the control group. The fry fed the diet T5 had significantly higher ($P < 0.05$) mean individual length increments compared to the other groups. Similar results have been reported for *Saccharomyces cerevisiae* used in diets for carp (Noh *et al.* 1994), Nile tilapia Lara-Flores *et al*. (2003).

Table 2: Effect of dietary supplementation with prebiotic and probiotic on growth performance of *Cyprinus Carpio* fry.

parameters	Treatments				
	T1	T2	T3	T4	T5
Initial body weight (g)	2.69±0.094^{a}	2.62±0.14^{a}	2.52±0.12^{a}	2.94±0.12^{a}	3.35±0.13^{a}
Final body weight (g)	10.34±0.20^{d}	12.88±0.25^{c}	15.53±0.36^{a}	12.56±0.3^{c}	14.64±0.3^{b}
Weight gain (g/fish	7.65±0.16^{d}	10.26±0.13^{c}	12.98±0.21^{a}	9.56±0.19^{bc}	11.29±0.14^{b}
Specific growth rate (%)	1.42±0.27^{c}	1.64±0.20^{b}	1.86±0.28^{a}	1.45±0.34^{c}	1.58±0.25^{bc}
Feed intake (g)	23.1±0. 34^{d}	29.6±0.17^{b}	29.8±0.21^{b}	27.5±0.31^{c}	33.4±0.22^{a}
Feed conversion ratio	3.0±0.13^{a}	2.8±0.19^{c}	2.3±0.13^{d}	2.8±0.17^{c}	2.9±0.26^{b}
Initial body length(cm)	3.4±0.12^{a}	3.9±0.14^{a}	3.4±0.12^{a}	3.8±0.11^{a}	4.1±0.115^{a}
Final body length(cm)	8.7±12^{c}	9.1±20^{b}	9.8±24^{a}	9.1±12^{b}	9.6±18^{a}
length increment (cm)	5.3±0.14^{b}	5.20±0.18^{b}	6.40±0.11^{a}	5.3±0.17^{b}	5.5±0.17^{b}
Condition factor	1.57±0.03^{c}	1.71±0.03^{a}	1.65±0.02^{c}	1.66±0.08^{b}	1.65 ±0.02^{c}

Rengpipat *et al*. (1998) and Prabhu *et al*. (1999) reported that the probiotic treated group enhanced growth rate of shrimps. Survival of shrimps was significantly greater in treated group compared with the control group. Olvera *et al*. (2001) concluded that yeast had a positive effect on fish performance when cultured under stress condition of lowering dietary protein. Gibson *et al*. (2004) suggested that a prebiotic has to resist gastric acidity, hydrolysis by (mammalian) enzymes, GI absorption, fermented by the intestinal micro biota and stimulate selectively the growth and/or activity of intestinal bacteria associated with health and well-being. El-haroun *et al*. (2006) reported that with Biogen® as feed additive containing *B. subtilis* came to the conclusion that, this organism germinates in the intestine of fish, using a large numbers of sugar (carbohydrates) and produces a wide range of digestive enzymes (amylase, lipase and protease) which have a beneficial effects including higher growth rate and higher feed efficiency. Yanbo and Zirong (2006) found that for common carp, *Bacillus* spp can produce secondary metabolites which have been used industrially for production of antibiotics, bioinsecticides, fine chemicals and enzymes that readily hydrolyze carbohydrates, lipids and proteins into sugars, fatty acids, peptides and amino acids.

In contrast, Hidalgo *et al*., (2006) found that growth and feed conversion of juvenile dentex were not significantly influenced by probiotics. Shelby *et al*. (2006) found that the probiotic used with juvenile channel catfish diet had lack effect on specific growth promoting or immune stimulating aspects. Mazlum *et al*. (2011) found great growth in *Astacus leptodactylus* juveniles fed with MOS while the survival rate was not affected.

The survival rate was significantly higher ($P < 0.05$) in fry fed diets T3 and T5 followed by fry fed the diets T2 and T4 than those of the control group (Fig. 1). Also, the present result agree with Kennedy *et al.* (1998) who find that the addition of a gram-positive probiotic bacterium increased survival, and growth rate of marine fish larvae (snook, red drum, spotted sea trout and stripped mullet). Tovar-Ramírez *et al.* (2004) noticed that the growth of larvae of sea bass fed 1.1% live yeast as a probiotic was increased than control group. Also, survival rate of larvae was significantly higher than the control. Bagni *et al.* (2005) fed two groups of sea bass (*Dicentrarchuslabrax*) with a Macrogard diet containing 0.1% yeast B-glucans derived from the wall of *Saccaromyces cerevisiae*, they found that condition factor ranged from 0.82 to 1.35, and did not vary among the groups throughout the experiment. Kumar *et al.* (2006) found that an increased growth rate and survival rate which were observed in Indian major carp (*Labeo Rohit*) fed diet contained *Bacillus subtilis* compared with control. Wang and GU (2010) showed that increased the growth performance of grass carp fingerlings (*Ctenopharyngodon idella*) fed with diet contained of *Bacillus coagulans*, *Rhodopseudomonas palustris* and *Lactobacillus acidophilus*.

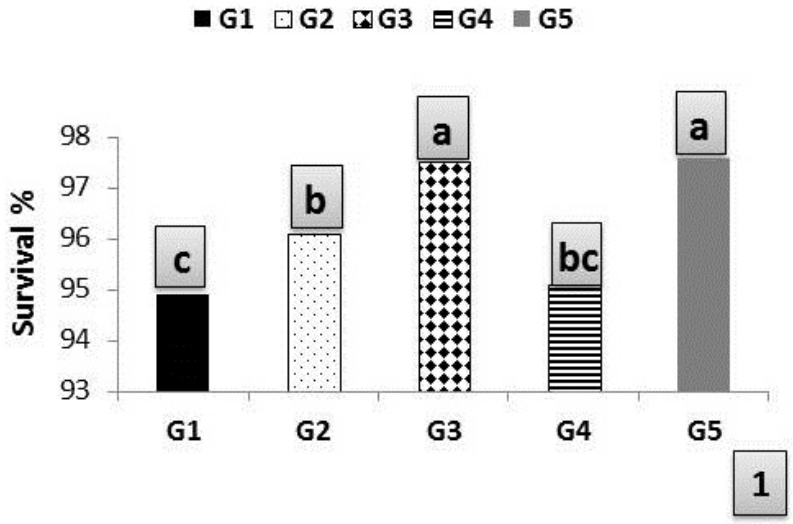

Fig. 1: Effect of dietary supplementation with prebiotic and probiotic on survival rate of *Cyprinus Carpio* fry.

The result of proximate analyses of whole body is present in (Fig.2). There was an increase the protein and fat content of fry fed diets T3 and T5 than other groups. These results go paralleled with the results obtained by Sealey *et al.* (2007) who used different levels of B-glucan on control diet for rainbow trout. Fish fed the wheat control diet or the high B-glucan barley diet had significantly higher moisture content than fish fed the low B-glucan barley diet following 3 weeks of feeding. Abdel-Tawwab *et al*. (2008) suggested that yeast supplementation played a role in enhancing feed intake with a subsequent enhancement of fish body composition. Similar results

have been observed in other fish species such as rainbow trout (Yilmaz *et al.* 2007) and hybrid tilapia (Genc *et al.* 2007a) when use Mannan Oligosaccharides (MOS).

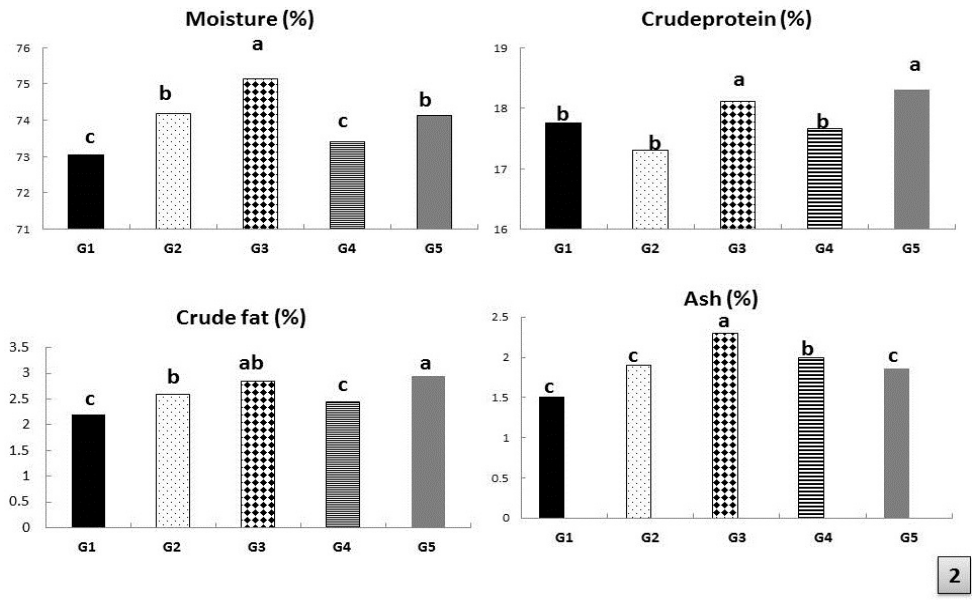

Fig. 2: Effect of dietary supplementation with prebiotic and probiotic on Body composition of *Cyprinus Carpio* fry.

Total serum protein, albumin and globulin were significantly increased in fish group fed the experimental diet T5 than other groups (Fig. 3). While, lysozyme activity were significantly increased in fish fed the diet T3 followed by fish fed the diet T5 then fish fed the diets T2 and T4 compared to control group.

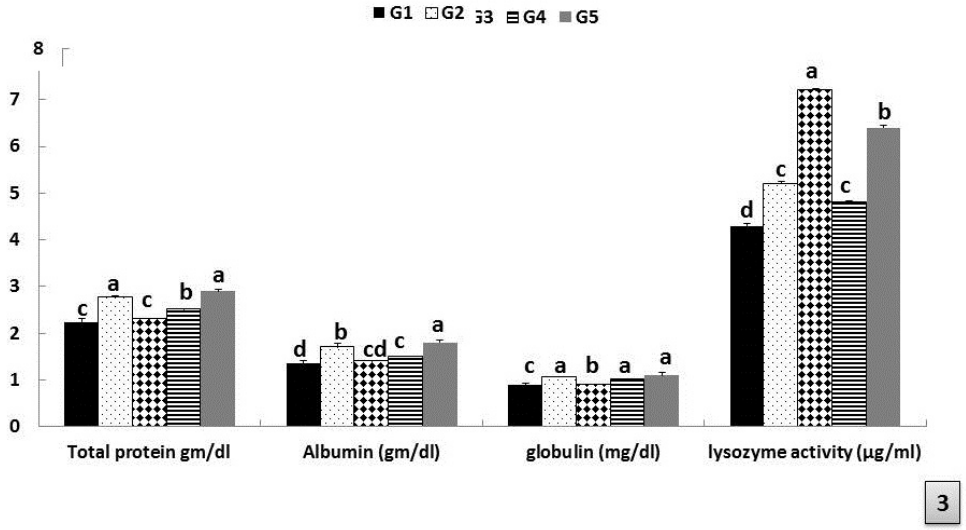

Fig. 3: Effect of dietary supplementation with prebiotic and probiotic on some immunological parameters of *Cyprinus Carpio* fry.

The use of probiotics improved the nutrition level of aquacultural and improves immunity to pathogenic microorganisms. Rengpipat *et al*. (2000) who observed that *Bacillus* sp. provided disease protection to shrimp by activating both cellular and

humoral immune defenses. Nayak *et al*. (2007) reported that glucan of yeast improved production antibody of carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. Tewary and Patra (2011) reported that the possible role of yeast as an imunostimulant may be attributed to its cell wall which composed of lipopolysaccharide such as glucan, which enhanced phagocytic activity of macrophages and globulin level as observed in the present experiment. Conversely, Kobeisy and Hussein (1995) studied effect of 0, 5, 10 and 20% dietary live yeast on the serum total protein and globulin of *Oreochromis niloticus* for 13 weeks. They found that both of serum total protein and globulin concentration was lower in treated groups than in control.

CONCLUSION

Considering the findings emphasized in the previous topics of the paper the fish diet supplemented with probiotic and prebiotic enhance their growth performance and nonspecific immune response.

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ARABIC SUMMRY

تقييم تاثير المكملات الغذائية من البريبيوتك والبروبيوتيك على اداء النمو وبعض مكونات الدم لزريعة المبروك العادى

وفاء العراقي[١] - محمد يحي[٢] - رشا محمد رضا[٣] - صلاح الاتربي[٤]

١- قسم التغذية و التغذية الاكلينكية كلية الطب البيطري - جامعة الزقازيق - مصر.

٢- قسم التلوث وحدة بحوث الاسماك المعهد القومي لعلوم البحار والمصايد فرع القناطر الخيرية – مصر.

٣- قسم أمراض و رعاية الاسماك كلية الطب البيطري - جامعة الزقازيق - مصر.

٤- قسم علوم البحار، كلية العلوم - جامعة قناة السويس - مصر.

الهدف من هذه الدراسة تقييم تأثير البريبيوتك والبروبيوتيك على أداء النموو بعض مكونات الدم (المناعة الغير محددة) والتركيب الكيميائى لزريعة المبروك العادى. تم اخذ عدد ٢٥٠ زريعة من المبروك العادى وقسمت على خمس مجموعات متوسط الوزن بين تلك المجوعات ٢,٨± ٠,١٢جم ومتوسط الطول ٣,١± ٠,١٣ سم وتم تغذيتها لمدة ١٢اسبوع. تم تغذية المجموعة الاولى على العليقة الاساسية محتوى البروتين بها ٣٥% بدون اى اضافات من البريبيوتك والبروبيوتك .المجموعة الثانية والثالثة تم تغذيتها بالعليقة الاساسية مضاف اليها ١,٥ و٢,٥ جم من البريبيوتك على التوالى. المجموعة الرابعة والخامسة بالعليقة الاساسية مضاف اليها ٠,٥ و١,٥جم من البروبيوتك على التوالى . دلت النتائج التى اخذت فى نهاية التجربة ان المجموعة الثالثة التى تم تغذيتها علي عليقة تحتوي ٢,٥جرام من البريبيوتك اعلي معنويا فى النموالنهائى للجسم والوزن المكتسب ومعدل النمو النوعى ويليها المجموعة الخامسة التى تم تغذيتها على عليقة تحتوي ١ جم من البروبيوتك. الزيادة المعنوية فى الطول ونسبة الاعاشة في كل من المجموعة الثالثة و الخامسة. و بتحليل التركيب الكيميائى للجسم كان محتوى البروتين والدهون اعلى معنويا فى المجموعة الثالثة ثم الخامسة. اما البروتين الكلي والالبيومين في مصل الدم كان اعلى معنويا فى المجموعة الخامسة عنه فى باقي المجموعات بينما نسبة نشاط الليزوزيم كان اعلى معنويا فى المجموعة الثالثة بالمقارنه بالمجموعات الاخرى. بذلك اظهرت هذة الدراسة ان المجموعة الثالثة المضاف اليها ٢,٥ جم من البريبيوتك كانت افضل من المجموعات الاخرى لزريعة المبروك العادى.