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Effect of Dietary Vegetable Wastes as Prebiotics on Serum Biochemical Parameters and Intestinal Microflora in Juveniles of the Common Carp *Cyprinus carpio*

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Abstract: Effects of replacing wheat flour in fish feed with vegetable wastes on some biochemical parameters and normal flora of juvenile common carp *Cyprinus carpio* were studied in laboratory. Three replacing ratio (5, 7.5 and 10%) were investigated. The total protein and albumin concentration in blood serum increased significantly ($p<0.05$) in all feeding treatments compared with control treatment. Highest concentrations were achieved by fish of feed group T3 (94.63 and 5.72 mg/100 ml respectively). Levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein and low-density lipoprotein (LDL) were decreased significantly ($p<0.05$) in fish with 10% replacing ratio (being 130.86, 46.10, 85.46, 3.26 and 9.22 mg/100 ml, respectively) compared with control diet. Population of the lactic acid bacteria (intestinal microflora) increased significantly ($p<0.05$) after feeding experiment in all treatments and reached the highest in T3 (2.93 Log CFU/g). The results reveal that supplementation of dietary vegetable wastes have most positive effects on improving the beneficial intestinal microbiota and on some haemato-serological parameters of juveniles of *C carpio*. Future studies are needed to focus on disease challenge and stress response as vegetable wastes have been known as a good prebiotic candidate for applications with common carps.

Keywords: Fishes, Prebiotics, Vegetable Wastes**,** Serum Biochemistry, Intestinal Microflora.

Introduction

During the last few decades, the intensification of aquaculture production, diseases and deterioration of environmental conditions are major problems in fish farming and face massive economic losses in prevention and control of diseases, antibiotics used as traditional strategy and also in fish growth as well as efficacy of feed conversion (Ahmadifar et al., 2011). Development of antimicrobial resistant pathogens were recognized but there is a huge risk of transmission of resistance bacteria from aquatic environment to humans (Ringø et al., 2014).

Using antibiotics is harmful for aquatic animals as they kill beneficial microbiota in their gastrointestinal system and also they accumulate in fish

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products which become unsafe for human consumption. Considering these factors, the improvements of non-antibiotic agents are more suitable for health management in aquacultures (Ringø et al., 2010a).

Dietary supplement such as prebiotics provide nonspecific disease protection and also act as growth promoting factors. The use of prebiotics, nondigestible dietary ingredients beneficially affects the host by selectively stimulating the growth of and/or activating the metabolism of health-promoting bacteria in the intestinal tract is a novel concept in aquaculture (Momeni-Moghaddam et al., 2015; Forsatkar et al, 2017). The dietary administration of prebiotics can exert some effect on the intestinal microbiota, digestive enzyme activities, morphology and volatile short chain fatty acids (VSCFAs) production in various aquatic animal species (Ringø et al., 2014).

This study was carried out to investigate the effect of replacing ratio (feed diets) of vegetable wastes on biochemical condition of blood serum and normal flora in common carp juveniles with different concentrations of replacements.

Materials and Methods

The vegetable wastes of lettuce (*Lactuca sativa*), green onion (*Allium cepa*), garlic (*Allium sativum*) and kale (*Brassica oleracea*) were collected from a local market in Basrah city, Southern Iraq. All vegetable wastes were dried in room temperature for 72 hours and ground by using electric grinder. Ground material was sieved through a 0.4 mm mesh for homogenization and kept in marked plastic bags.

Juveniles of the Common carp were obtained from the University of Basrah fish farm. A total of 144 specimens with initial mean weights of 13.71±0.57 g were randomly distributed among 12 aquarium tanks (12 fish per tank, three replicates per treatment). Water temperature, dissolved oxygen, pH and salinity (‰) were measured before and after each experiment.

The experimental diets used in fish feed were manufactured by the Marine Science Center, University of Basrah. The diets were formulated from fishmeal, soybean meal, corn oil, barley flour and wheat flour. The feed replacement which included vegetable wastes was added at percentage of 5%, 7.5% and 10% replacing wheat flour in fish diets (Table 1). Fishes were fed 5% body weight per day. Proximate composition of the experimental diet is shown in Table (2).

Blood samples from each fish in the different groups were collected by suction of the caudal peduncle. Blood serum was then separated by centrifugation for three minutes at 1500 rpm (Yang & Chen, 2003). The levels of serum biochemical parameters were assayed according to the instructions provided with the corresponding biochemical kits. These parameters were total protein (TP), albumin (AL), total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL).

Globulin concentration was calculated directly according to Wolf & Darlington (1971) as:

$$\text{Globulin} = \text{Total protein} - \text{albumin}$$

Low density lipoprotein (LDL) cholesterol concentration was calculated according to Taherpour et al. (2009) as:

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL} - \text{VLDL})$$

One-way analysis of variance (ANOVA) was made by using SPSS software (version 18) and the difference between the means and the significant differences was verified by using RLSD test.

The bacterial counts (TBC) and lactic acid bacteria (LAB) were assayed according to Andrews et al. (1992).

Table 1. Fish experimental diet composition (%).

Ingredients	Feed group			
	Control (T1)	5% (T2)	7.5% (T3)	10% (T4)
Fishmeal	20	20	20	20
Soybean meal	15	15	15	15
Barely flour	20	20	20	20
Corn oil	5	5	5	5
Wheat flour	40	35	32.5	30
Vegetable wastes	0	5	7.5	10

Table 2. Proximate composition of the experimental diet.

Ingredients	Feed group			
	Diet (% dry weight)			
	Control (T1)	5% (T2)	7.5% (T3)	10% (T4)
Protein	32.56	31.8	30.84	31.24
Lipid	9.11	9.65	10.02	9.23
Carbohydrates	49.52	49.36	50.31	50.68
Moisture	2.88	3.17	3.24	2.96
Ash	5.93	6.02	5.59	5.89

Results

Values of water quality parameters are shown in Table (3). They were adequately stable; temperature (27.1-27.7 Cº), pH (8.8-8.9), dissolved oxygen (8.8-9.4 mg/l) and salinity (2.1-2.3‰).

Table 3. Water quality parameters of rearing water for common carp juvenile during the experiment.

Parameter	Replacing ratio (%) feed group (Mean ± S.D)			
	Control (T1)	5% (T2)	7.5% (T3)	10% (T4)
Temperature (C^0)	27.6±0.24	27.1±0.24	27.7±0.30	27.7±0.20
pH	8.85±0.02	8.8±0.03	8.9±0.04	8.9±0.04
Dissolved oxygen (mg/l)	8.8±0.2	8.8±0.4	9.4±0.2	9.4±0.2
Salinity (‰)	2.3±0.20	2.3±0.10	2.2±0.20	2.1±0.04

The general biochemical indices of juveniles of the common carp before and after feeding on experimental feeds which contain different inclusion replacing ratios are presented in Table (4).

Table 4. Blood serum biochemical (mg/100 ml) of common carp juvenile before and after feeding on experimental feeds.

Parameter mg/100 ml	Replacing ratio (%) feed group (Mean ± S.D)			
	Control (T1)	5% (T2)	7.5% (T3)	10% (T4)
Total protein	$3.86±0.58^a$	$4.39±0.67^a$	$4.68±0.06^b$	$5.72±0.75^c$
Albumin	$2.89±0.45^a$	$3.26±0.67^b$	$3.70±0.21^b$	$4.63±0.12^c$
Globulin	$1.10±0.13^a$	$1.13±0.23^a$	$0.98±0.27^a$	$1.08±0.65^a$
Total cholesterol	$208.10±30.11^a$	$189.30±1.27^{ab}$	$169.96±9.60^b$	$139.86±4.3^c$
Triglycerides	$123.42±20.86^a$	$87.25±2.57^{ab}$	$84.95±4.83^b$	$46.10±5.26^c$
HDL	$110.34±21.66^a$	$92.31±7.10^{ab}$	$72.67±5.96^{bc}$	$85.46±2.23^c$
LDL	$91.51±3.27^a$	$79.48±2.03^b$	$67.17±2.92^{bc}$	$63.26±1.67^c$

Values in the same raw with different superscript letters are significantly ($p<0.05$) different.

The total bacterial counts (TBC) and lactic acid bacterial counts (LAB) were significantly ($P<0.05$) increased by the addition of 10% and 7.5% replacing ratios of vegetable wastes than in control fishes (Table 5).

Table 5. Bacterial counts in the intestine of common carp juvenile during the experiment.

Intestinal Microflora (Log CFU/g)	Replacing ratio (%) feed group (Mean ± S.D)			
	Control (T1)	5% (T2)	7.5% (T3)	10% (T4)
TBC	$5.31±0.05^b$	$5.53±0.06^b$	$5.70±0.06^c$	$5.87±0.02^c$
LAB	$2.20±0.17^a$	$2.41±0.09^a$	$2.71±0.05^b$	$2.93±0.02^b$

Values in the same raw which carry different superscript letters are significantly ($p<0.05$) different.

Discussion

Prebiotics are functional dietary supplements with documented positive applications in a range of fish and crustacean species (Ringø et al. 2010a). They can change the composition of the normal flora by changing the types of substrates available to bacteria and make them more appropriate to the micro-ecophysiological conditions of the resident intestinal microflora which allow them to grow rapidly (Ringø et al. 2010b; Carbone & Faggio, 2016). Haematological and biochemical parameters are considered as useful measuring instruments in order to check the quality of fish health and physiological responses, nutrient absorption and animal surroundings that affect fishes (Hoseinifar et al., 2011)

Values of water quality parameters indicated in Table 1 are within the suitable ranges for culture of the common carp according to Marković et al. (2009).

The results of the present study show that supplementation with prebiotic derived from a vegetable wastes powder: fructo-oligosaccharides (FOS), mannan-oligosaccharides (MOS) and xylo-oligosaccharides (XOS) have positive effects on the bacterial populations in the intestine of the experimental fishes (Eldahshan & Singab, 2013; Gebreyohannes & Gebreyohannes, 2013; Mageney et al., 2017). The degree of the positive effects on the bacterial populations in the intestine was affected by the prebiotic dose (Ringø et al., 2010b; Forsatkar et al., 2017).

The results in Table (4) indicated that the total proteins, albumin and globulin increased. The total proteins in the serum include albumin and globulin. Globulin is made up of fractions of a1, a2, b and c globulins that made immunoglobulin (Ig), the increase in the levels of serum protein, albumin and globulins in fishes is thought to be associated with a stronger innate immunity response (Ringø et al., 2014).

The present study also showed that there were significant differences in the serum biochemical parameters such as cholesterol and triglyceride, between treatments (Table 4). Andrews et al. (2009) observed a significant improvement in fed MOS supplemented diet in comparison with those fed on the control diet. Ye et al. (2011) showed that dietary FOS and MOS decreased flounder cholesterol and triglyceride compared to the control. Ahmed (2014) and Abdulrahman et al. (2016) observed significant differences of different levels of FOS on some blood indices in young common carp.

Total bacterial count (Table 5) increased with increasing prebiotic doses. This indicates that the doses of prebiotics provided a substrate for the growth of bacteria in the intestine, which led to larger bacterial populations in the treated groups than in the control (Ye et al., 2011). In the present study, significant increase in lactic acid bacteria (LAB), especially in the 10%

replacing ratio was noticed. Lactic acid bacteria are able to produce bacteriocins, lactic acid and other antagonistic compounds, which inhibit the growth of certain fish pathogens and hence they improve the fish intestinal ecosystem (Ringø et al. 2006). They are not generally considered to be the dominant species in the gut, but prebiotics shift the normal flora to them and increase their population (Ringø et al. 2010a, b).

The results of the present study indicate that prebiotic supplementation (vegetable wastes) influenced fish biochemical parameters and normal flora. Such results are in agreement with earlier studies (Akrami et al., 2012; 2013, Hoseinifar et al., 2014 a, b; Amani et al., 2015; Hoseinifar et al., 2016).

Conclusions

In conclusion, the results observed in this trial reveal that supplementation of dietary vegetable wastes appears to be most positive for beneficial intestinal microbiota and some biochemical parameters of juveniles of the common carp. It is necessary that future studies would be focused on disease challenge aspects and stress response as vegetable wastes are known as a good prebiotic candidate for applications with common carps.

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