Comparative effect of probiotic (*Saccharomyces cerevisiae*), prebiotic (Fructooligosaccharide) and their combination on some blood indices in young common carp (*Cyprinus carpio L.*)

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Accepted: 17/6/2015

Summary

This study was carried out to examine the effect of Fructooligosaccharide as a source of prebiotic, and commercial dry yeast (*Saccharomyces cerevisiae*) as a source of probiotic and their combination in different level as a source of synbiotic. The experiment was conducted in the fish laboratory of Animal Production Department, Faculty of Agricultural Sciences of Sulaimani University. The trials lasted for three months after 21 days of adapting period 120 common carp fingerlings with an average weight of 20 ±2 gm, were used to test the effect of different levels of the Fructooligosaccharide, yeast and their combination. In T1 fish were fed a diet with 2.5 gm/kg Fructooligosaccharide, in T2, fish were fed a diet 2.5 gm/kg yeast, T3 represents the third treatment, in which fish were fed on a diet 5 gm/kg Fructooligosaccharide. While, in T4 fish were fed a diet 5 gm/kg yeast, and T5 2.5 Fructooligosaccharide: 2.5 Yeast (gm/kg), T6 was 5 Fructooligosaccharide: 5 yeast (gm/kg), T7 2.5 Fructooligosaccharide: 5 Yeast gm/kg), while T8 5 Fructooligosaccharide, 2.5 (gm/kg) Yeast. Each treatment in three replicates in which five fingerlings common carp were stocked in plastic tanks, which fed the experimental diets twice daily. Blood parameters of tested fish showed significant differences in Red blood cell count (10^{12} cells/l) in T5 and T7 by 1.235 and 1.260, respectively. Hemoglobin (g/dl) data were 117.000 in the fifth treatment. The mean corpuscular hemoglobin (pg/cell) was 121.400 in T2, 137.850 in T3, 121.050 in T4, and 135.300 in T6; mean corpuscular hemoglobin concentration (g/l) was 924.000, in the seventh treatment, mean corpuscular volume were (fL) 232.500 and 233.050 in T4 and T8 respectively. There were different effects of the treatment in the studied blood parameters in which the level of 2.5 g/kg in both Fructooligosaccharide and dry yeast affect significantly the Red blood cell, White blood cell and Hemoglobin.

Keywords: Probiotic, Prebiotic, Synbiotic, Blood indices, Common carp.

Introduction

Numerous studies have demonstrated the benefit effects of probiotics for aquatic animals, such as the stimulation of growth to improve feed digestion, immune responses and water quality control (1). A number of probiotic products have been researched as evidenced by their efficacy in aquaculture, beneficial bacterial inocula that were species-specific probiotics have become widely available to the aquaculture industry, these preparations have been refined to have more effective function as applied probiotics (1). The application of new analysis methods, including molecular methods, for the evaluation of probiotic products and for *in vivo* validation, was expected to significantly improve both the quality and functional properties of probiotics (2). Yeasts were a rich source of protein and B-complex vitamins; they have been used successfully as a complementary protein source in fish diet, in addition, they have been used as a supplement in animals feed to compensate for the amino acid and vitamin deficiencies of cereals, and are recommended as a substitute for soybean oil in diets for fowl, in addition, they were considered a cheaper dietary supplement as they were easily produced on an industrial level from a number of carbon-rich substrate by-products (3). Therefor the nutritive value of yeast products differs according to its type, *Candida* sp., *Hansenula* sp., *Pichia* sp. *and Saccharomyces* sp. were special importance as components in fish feeds, and however, the presence of high percentage of non-protein nitrogen sets some limitation against yeast consumption (4). A prebiotic was first defined as a 'non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (5). However, a prebiotic effect has been attributed to many food components, sometimes without due consideration to the criteria required, in particular, many food oligosaccharides and polysaccharides (including dietary fibre) have been claimed to have prebiotic activity, but not all dietary carbohydrates were prebiotics, there was, therefore, a need to establish clear criteria for classifying a food ingredient as a prebiotic.

Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a form of synergism, hence synbiotics, enhancing their isolated beneficial effects, when two nutritional ingredients or supplements were given together the resulting positive effect generally follows one of three patterns: additives, synergism or potentiation, additive effect occurs when the effect of two ingredients used together approximates to the sum of the individual ingredient effects, in case of synergism, it is said to occur when the combined effect of the two products is significantly greater than the sum of the effects of each agent administered alone, the term potentiation was used differently (6). The Aim of this study was to compare the effects of adding different levels of dry yeast (as probiotic), FRUCTOOLIGOSACCHARIDE (as prebiotic) and their combination (as synbiotic) to carp diet on some blood indices. The specific objectives include examining immune response of the fish by measuring blood cells and other blood parameters.

Materials and Methods

The experiment was conducted at the fish laboratory of Animal Production Department, Faculty of Agricultural Sciences of Sulaimani University, Sulaimani, Kurdistan Region, Iraq, for 105 days on 120 young common carp *C. carpio* L. which were brought from a local aquarium fish supplier located in daqoq Middle of Iraq. The weight of fish varied between (19-21g). The fish were distributed among experimental aquaria with mean initial weight of 20.26g. They were pre-acclimated to laboratory conditions and fed with commercial pellets, their chemical composition is shown in (Table, 1) for 21 days prior to the feeding trials.

Each tank was provided with a proper continuous aeration. Each aquarium was stocked with five fish. In T1 fish were fed a diet with 2.5 g/kg FOS, While in T2, fish were fed a diet 2.5 g/kg yeast, T3 represents the third treatment, in which fish were fed on a diet 5 g/kg FOS, While, in T4 fish were fed a diet 5 g/kg yeast, and T5 2.5 g/kg FOS: 2.5 g/kg yeast , T6 was 5 g/kg FOS: 5 g/kg yeast, the T7 2.5 g/kg FOS: 5 g/kg yeast, while T8 5 g/kg FOS: 2.5 g/kg yeast. Experimental diets composed of a standard commercial diet type found in Kurdistan markets, enriched with probiotic (yeast), prebiotic (FOS) and their combination (synbiotic), the chemical composition of the different diets are shown in (Table, 1).

Table, 1: The chemical composition of experimental diet.

Treatment	Protein %	Fat %	Ash %	Moisture %
T1 (2.5g/kg FOS)	21.87	3.53	10.48	8.4
T2 (2.5g/kg yeast)	25.37	3.92	11.66	7.56
T3 (5g/kg FOS)	24	3.97	11.58	7.56
T4 (5g/kg yeast)	28	3.13	11.64	7.58
T5 (2.5g /kg FOS +2.5 g/kgyeast)	23.62	3.04	11.81	7.50
T6 (5g kg FOS + 5g kg yeast)	25.81	2.80	11.99	7.34
T7 (2.5g/kg FOS + 5g/kg yeast)	26.25	2.63	11.13	7.92
T8 (5g/kg FOS+ 2.5g/kg yeast)	24.50	2.93	11.26	7.82
Used yeast	36.39	1.53	8.43	5.50

The ingredients were mixed with water to obtain dough. Then, the dough was passed through an electrical mincer for pelleting by using Kenwood Multi-processors. The pellets were dried at room temperature for a few days and crushed to yield fine particles. Fish were fed twice a day at 9:00 AM and 2:00 PM with a ratio of 5% of body weight but after a week adjusted to 3%. Fish were individually weighed weekly. The feeding amount was then recalculated according to weekly weights. The feeding trial continued for 12 weeks.

At the end of the experimental period, three fish were randomly taken from each experimental group. All fish samples were weighed individually. The blood samples from each fish of the different groups were collected by cutting of the caudal peduncle. Whole blood samples were collected in small plastic vials containing heparin for determination of some blood parameter and the concentrations were determined by using the hematology analyzer BC-2800 is a compact, fully automatic hematology analyzer with 19 parameters for complete blood count (CBC) test. RBC (Red Blood Cell; 10^{12} cells/l); WBC (White Blood Cell; 10^9cells/l); Hb (Hemoglobin; g/l); MCH (Mean Corpuscular Hemoglobin; pg); MCHC (Mean Corpuscular Hemoglobin Concentration; g/l); MCV (Mean Corpuscular Volume; fL).

Results and Discussion

Hematological parameters are shown in (Table, 2). RBC data were 1.260 x 10^{12} cells/l and 1.235 10^{12} cells/l for synbiotic treatments T7, T5, showing that they were significantly differ from other treatment. The data regarded of Hb 117 g/dl, for the T5 which was higher than other treatments significantly (P<0.05). The results for mean corpuscular hemoglobin (MCH) were 137, 135, 121, 121, 95, 85, 81 and 78 pg/cell for the T3, T6, T2, T4, T5, T1, T8 and T7 respectively, that there were significant (P<0.05) differences among treatments. The data for mean corpuscular hemoglobin concentration (MCHC) revealed that T7 (924 g/l) had significantly higher values (P≤0.05) than other dietary treatments. Mean corpuscular volume (MCV) values observed in (Table, 2) showed that T8 (233 fL) and T4, (232 fL) had significant (P<0.05) differences among treatments.

The results in (Table, 2) indicated significant differences among the treatments especially the yeast addition. According to the results of (7), a positive effect represented by significant increase in RBCs count, PCV%, Hb and differential leukocytes count, these could be attributed to the fact that, the probiotics used increased the blood parameter values as a result of hemopiotic stimulation. These results supported the results of other researchers (8), also the results in this study was confirmed by the histological pictures of *Oreochromis niloticus* groups received diets supplemented with probiotics in which the histological structure of both liver and spleen were normal and showed hyperactivity of Kupffer cells and melenomacrophage centers with intensity of melanin pigment and the oval individual cells of *S. cerevisiae* approved to be colonized to the intact intestinal epithelial cells and scattered in the intestinal lumen and with the study of (9) in hybrid tilapia (*Oreochromis niloticus* female × *Oreochromis aureus* male).

Table, 2: Effect of adding dry yeast, FOS and their combination in carp diet for 12 weeks on fish blood parameters.

Treatment	RBC (10^{12} cells/l)	Hb (g/dl)	MCH (pg/cell)	MCHC (g/l)	MCV (fL)
T1	0.750^{c}	113^{ab}	85^{c}	364^{c}	210^{a}
T2	0.795^{bc}	110^{abc}	121^{ab}	351^{c}	214^{a}
T3	0.275^{d}	98^{abc}	137^{a}	$231.^{c}$	215^{a}
T4	0.730^{c}	95^{bc}	121^{ab}	525^{b}	232^{a}
T5	1.235^{a}	117^{a}	95^{bc}	265^{c}	220^{a}
T6	0.465^{d}	105^{abc}	135^{a}	641^{b}	182^{b}
T7	1.260^{a}	92^{c}	78^{c}	924^{a}	211^{a}
T8	1.010^{b}	113^{ab}	81^{c}	359^{c}	233^{a}

Mean values with different superscripts within a column differ significantly (P≤0.05).

In spite of all these advantages, there is little information on the use of whole yeast in fish diets concerning the hypothesis that in vivo administration of whole yeast could enhance the fish immune system (10) as it was observed in the different significance among yeast addition in WBC of our results, these results agree with that obtained with mrigal Carp (11), Catla carp (12), hybrid striped bass (13,14 and 15), Japanese flounder (16), Rainbow trout and *O. mykiss* (17 and 18) (under publishing). Three components in brewer's yeast may have immunomodulating effects: β-glucans (19) have been shown to enhance immune responses and disease resistance of several fish species (20). Nucleotides recently have been reported to improve disease resistance of Atlantic salmon (21) and common carp (22) and Chitin has been reported to have immunomodulatory effects in gilthead seabream (23) and these is might be the reason of present results.

In the study of researchers (24) the yeast cell wall β-glucans did not affect appetite, but was followed by bettered feed utilization and faster growth, showing that gut health is an important production parameter for Atlantic salmon. Yeasts can stimulate the immune response in fish, ß-glucans is likely the most important compound in this regard, but some other cell-wall components or soluble factors may also play a role, both cellular and humoral responses have been induced by dietary yeast, depending on the experimental conditions, other benefits may be expected for the host, especially the intestinal colonization of early feeding fry with yeast, which may have some effect on development, e.g. by accelerating the maturation of the digestive system (25). The adding of FOS has different stimulation in our results as shown in (Table, 2). This means that a stimulation of the immune response of fish through dietary supplements is possible and is of high interest for commercial aquaculture as stated by (26). The innate immune system is very important in this regard because aquatic animals are continually vulnerable to numerous opportunistic pathogens and this part of immune response provides the first line of defense for the host (27).

The results of the study by (28) showed that dietary FOS could modulate the innate immune responses of Caspian roach fry. The immunostimulatory nature of prebiotics may be attributed to stimulation of the growth of beneficial bacteria such as lactic acid bacteria and *Bacillus* spp. (29 and 30), which possess cell wall components such as lipopoly -saccharides which have immunostimulatory properties (31). However, (32) reported that dietary inulin (5 or 10 g inulin /kg) had no effect on the innate immune response of gilthead seabream (*Sparus aurata* L.) compared to the control group (0 g /kg). This contradictory result may be attributable to the low dosage, different duration of prebiotic administration, life stage and/or different fish species (10). The use of natural immunostimulants is a promising area in aquaculture because they are biodegradable, biocompatible and safe for both the environment and human health (33). Consequently, several substances, including vitamins, chitin, glucans and different animals and plants components, as well as yeast cells have been tested as immunostimulants in fish (32). In the prebiotics research programed, the knowledge gap has to be filled, as to the effect of prebiotics on physiological state evaluated according to the hematological and bio -chemical parameters of peripheral blood, the first study of some hematological and serum biochemical parameters of juvenile beluga (*H. huso*) fed oligofructose at varying levels (1, 2 or 3%) was performed by (34). They found significant differences not only in comparison with the control group (HCT values, proportion of lymphocytes, cholesterol level) but also between experimental groups with different oligofructose levels agree with the results obtained in our study. Their results, together with ours, indicate that research in this area should continue and causal relationships should be sought between dietary prebiotics and some hematological and serum plasma biochemical parameters of fish (35), in this study, RBC and MCH did not change after feeding with inulin.

The common carp fingerlings responded to the dietary prebiotic levels with significant differences ($P<0.05$) in blood constituents when fed diets containing 0.5–2.5 g Immunogen/kg, one of the most distinct effects was a rise in total protein and leucocyte levels; generally accepted that in all vertebrates including fish, stressors elicit a stress response in leucocytes (36). It can be seen from the data of (Table, 2) that there was no obvious trend in blood parameters in response to synbiotic addition of pre- and probiotic. However, Treatment 5, 7 and 8 showed an elevation in number of red blood cells and haemoglobin value, as indicator of healthy condition.

Also (6) found that hematocrit value was higher in the EM and EMP groups than the C, E, M, P, and EP groups, and significant higher hematocrit value was recorder in the E and M groups than the C and P groups. Dietary administration of a commercial synbiotic (37) has demonstrates an increase in the serum protein and albumin content in rainbow trout. However, this study does not evaluate the capacity of pre and probiotic contained in synbiotic when administered single, so not allow consider the synergic properties of both ingredients.

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التأثير المقارن لكل من المعزز الحيوي (*Saccharomyces cerevisiae*) والسابق الحيوي Fructooligosaccharide والتداخل بينهما في بعض معايير دم صغار أسماك الكارب الاعتيادي (*Cyprinus carpio* L.)

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الخلاصة

أجريت هذه الدراسة لتبيان تأثير (فركتوليكوسكرايد) كسابق حيوي، والخميرة الجافة التجارية كمعزز حيوي والتداخل بينهما كمصدر للتكافل. اجريت التجربة في مختبرات كلية الزراعة، جامعة السليمانية. استغرقت التجربة ثلاثة أشهر بعد مدّة أقلمة استمرت 21 يوم. استعمل 120 سمكة كارب اعتيادي بمعدل وزن 20±2 غم لاختبار تأثير مستويات مختلفة من السابق الحيوي والخميرة والتداخل بينهما. في المعاملة الأولى غُذّيت الأسماك بعليقة تحتوي على 2.5 غم/ كغم من السابق الحيوي، والأسماك في المعاملة الثانية غُذّيت بالخميرة 2.5 غم/ كغم، غُذّيت أسماك المعاملة الثالثة بعليقة 5 غم/ كغم من السابق الحيوي والمعاملة الرابعة بـخميرة 5 غم/ كغم والمعاملة الخامسة بـخميرة 2.5: وسابق حيوي 2.5 غم/ كغم وغُذّيت أسماك المعاملة السادسة بـخميرة 5: وسابق حيوي 5 غم/ كغم والمعاملة السابعة بخميرة 5 : 2.5 غم/ كغم من السابق الحيوي في حين غُذّيت أسماك المعاملة الثامنة بخميرة 5 : 2.5 غم/ كغم سابق حيوي. تتكون كل المعاملات من ثلاثة مكررات وفي كل مكرر خمس أسماك كارب اعتيادي والتي رُبّيت في أحواض بلاستيكية وتغذيتها مرتين باليوم. أظهرت قياسات دم الأسماك المفحوصة فروقا معنوية تحت مستوى ($P<0.05$) في عدد كريات الدم الحمر (10^{12}) كرية/ لتر في كل من المعاملة الخامسة والسابعة 1.260 و 1.235 على التوالي. سجلت بيانات الهيموغلوبين 117.000 غم/ دسم في المعاملة الخامسة، وكان متوسط هيموغلوبين الكرية 121.400 بايكوغرام/ كرية في المعاملة الثانية، 137.850 في المعاملة الثالثة و 121.050 في المعاملة الرابعة و 135.300 في المعاملة السادسة. كان متوسط تركيز الكرية 924.000 غم/ لتر في المعاملة السابعة، وكان متوسط حجم الكرية 232.500 و 233.050 فيمتولتر في المعاملة الرابعة والثامنة على التوالى. تستنتج الدراسة أن هناك تأثيرات مختلفة للمعاملات قيد الدراسة حيث كان لمستوى 2.5 غم/ كغم في كل من السابق الحيوي والخميرة الجافة تأثيرا معنويا في كل من عدد كريات الدم الحمر والبيض والهيموغلوبين.

الكلمات المفتاحية: المعزز الحيوي، السابق الحيوي، تكافل، قياسات الدم، الكارب الاعتيادي.