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RESEARCH ARTICLE

COMPARATIVE EFFECT OF PROBIOTIC (*SACCHAROMYCES CEREVISIAE*), PREBIOTIC (FRUCTOOLIGOSACCHARIDE FOS) AND THEIR COMBINATION ON SOME DIFFERENTIAL WHITE BLOOD CELLS IN YOUNG COMMON CARP (*CYPRINUS CARPIO* L.)

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ABSTRACT

This study was carried out to study the effect of Fructooligosaccharide (FOS) as a source of prebiotic, commercial dry yeast (*Saccharomycescerevisiae*) as a source of probiotic and their combination in different level as a source of symbiotic. The experiment was conducted in the fish laboratory of Animal Production Department, Faculty of Agricultural Sciences of Sulaimani University, Sulaimani, Kurdistan Region, Iraq. The trials lasted for three months after an adaptation period of 21 days.120 common carp fingerlings with an average weight of 20 ±2 gm, were used to test the effect of different levels of the FOS, yeast and their combination. 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. Each treatments 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 WBC (10^9cells/l) differ significantly 234.850 for the fifth treatment and 230.500 for T8; Significant differences were in granulocytes count was 74.800 in T3 while the lymphocytes differ among treatments 15.500, for T8, Monocytes differ significantly was 34.150 in T5. Platelets were 34.000 in the first treatment and 38.000 in T2.

INTRODUCTION

Yeasts are 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 are considered a cheaper dietary supplement as they are easily produced on an industrial level from a number of carbon-rich substrate by-products (Lee and Kim, 2001). There for the nutritive value of yeast products differs according to its type, *Candida* sp., *Hansenula* sp., *Pichia* sp. *and Saccharomyces* sp. are special importance as components in fish feeds, and however, the presence of high percentage of non-protein nitrogen sets some limitation against yeast consumption (Ebrahim and Abou-seif, 2008). 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 (Gibson and Roberfroid, 1995).

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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 are prebiotics, there is, 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 are 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 is used differently, (Rodriguez-Estrada *et al*., 2009). The Aim of this study was to compare the effects of adding different levels of dry yeast (as probiotic), FOS (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 differential WBC.

MATERIALS AND METHODS

The experiment was conducted 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.

Diet formulation: 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). 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.

Blood parameters: 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, WBC (White Blood Cell; 10^9cells/l); GRAN (Granulocyte; %); Lymph (Lymphocyte; %); Mon (Monocyte; %); PLT (Platelet; 10^9 cells/l).

RESULTS AND DISCUSSION

The data presented in the Table (2) showed that the granulocyte percent obtained for T3 was 74.800, and that T3 was significantly higher than other dietary treatments ($P<0.05$). Lymphocyte percent was 15.500, in T8, that also was significantly different ($P\leq0.05$) from other dietary treatments. The platelets percentages in Table (2) were 38.000 and 34.000, for the T2 and T1 respectively. T1 and T2 were significantly different ($P\leq0.05$) from other dietary treatments. The white blood cells (WBC) in Table (8) were 234.850, and 230.750, for T5, T8, and were significantly different from other treatment.

Probiotic

The results in Table 2 indicated significant differences among the treatments especially the yeast addition. According to the results of Marzouk *et al*. (2008), a positive effect represented by significant increase in differential leukocytes count, these could be attributed to the fact that, the probiotics used increased the blood parameter values because of hemopiotic stimulation. These results supported the results of Rajesh *et al*. (2006), also the obtained results in this study was confirmed by the histological pictures of *O. 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 He *et al*., (2012) in hybrid tilapia (*Oreochromis niloticus* female × *Oreochromis aureus* male) .

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 (Ortuno *et al.,* 2002) as it observed in the different significance among yeast addition in WBC of our results, these results agree with that obtained with mrigal Carp (Swain *et al.,* 1996), Catla carp (Mohanty *et al.,*1996), hybrid striped bass (Li and Galtin, 2003, 2004 and 2005) , Japanese flounder (Taoka *et al.,* 2006), Rainbow trout, *O. mykiss* (Kamgar *et al.,* 2013, and Abdul Rahman and Al-Jader, (2013) (under publishing). Three components in brewer's yeast may have immunomodulating effects: β-glucans (Rayes, 2013) have been shown to enhance immune responses and disease resistance of several fish species (Gatlin, 2002), Nucleotides recently have been reported to improve disease resistance of Atlantic salmon (Burrell *et al*., 2001) and common carp (Sakai *et al*. 2001) and Chitin has been reported to have immunomodulatory effects in gilthead seabream (Esteban *et al*., 2001) and these is may be the reason of our results.

In the study of Refstie *et al*. (2010) 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 (Gatesoupe, 2007).

Prebiotic

The adding of FOS has different stimulation in our results as shown in Table (2). The data presented in the Table (2) also showed that the granulocyte percent obtained for T3 was 74.800, and that T3 was significantly higher than other dietary treatments ($P<0.05$).

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 +2.5g/kg yeast)	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

Table 2. Effect of adding dry yeast, FOS and their combination in young carp diet for 12 weeks on differential WBC count

Treatment	GRAN %	Lymph%	Mon%	PLT%	WBC (10^9cells/l)
T1	58.050^{bcd}	6.350^{c}	28.950^{b}	34.000^{a}	186.500^{cd}
T2	59.550^{bc}	6.750^{c}	33.700^{a}	38.000^{a}	159.800^{d}
T3	74.800^{a}	1.600^{d}	23.600^{c}	15.000^{b}	192.000^{bcd}
T4	62.150^{b}	6.700^{c}	31.150^{ab}	19.000^{b}	200.850^{abc}
T5	53.600^{d}	12.100^{b}	34.150^{a}	11.500^{bc}	234.850^{a}
T6	56.650^{cd}	8.000^{c}	32.050^{a}	6.000^{c}	153.500^{d}
T7	55.850^{cd}	6.550^{c}	33.200^{a}	11.500^{bc}	213.200^{abc}
T8	47.150^{e}	15.500^{a}	33.150^{a}	13.000^{bc}	230.500^{ab}

Mean values with different superscripts within a column differ significantly ($P \leq 0.05$)

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 Staykov *et al*. (2007). 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 (Magnadóttir, 2006). The results of the study of Soleimani *et al*. (2012) 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. (Sang *et al*., 2011; Zhang *et al*., 2011), which possess cell wall components such as lipopolysaccharides which have immunostimulatory properties (Van Hai and Fotedar, 2009). However, Cerezuela *et al*., (2008) 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 (Ibrahem *et al*., 2010).

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 (Ortun˜o *et al*., 2002). 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 (Cerezuela *et al*., 2008). 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 biochemical 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 Hoseinifar *et al*. (2010). 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 (Řehulka *et al*., 2011). Lymphocytes are one of the most important cells that can affect immune response of fish, these cells produce antibodies by specific immunity and increase in macrophages, an increase of such immunity cells can promote fish defenses to adverse condition (Ahmdifar *et al*., 2011). 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 (Davis *et al*., 2008), the increase in WBC count might be due to stress suffered by fish as a result of daily feeding on β-glucan. Harikrishnan *et al*. (2003) also reported increased WBC counts in *C. carpio* after herbal treatment with *Azardicha indica*, the observed increases in the leucocyte and total protein levels as well as lower mortalities resulting from the pathogenic *A. hydrophila* infection appear to be signs of enhanced health status of the prebiotic-fed fish, moreover, high concentrations of serum proteins including humoral elements of the non-specific immune system are likely to be results of an enhancement in the non-specific immune response of the fish, the improved health condition in the *C. carpio* fingerlings is probably due to the β-glucan and MOS components of the Immunogen (Al-Jammoor, 2012).

Synbiotic

It can be seen from the data of Table s 2, that. The white blood cells (WBC) in Table (2) were 234.850, and 230.750, for T5, T8, and were significantly different from other treatment.

Lymphocyte percent was 15.500, in T8, that also was significantly different ($P \leq 0.05$) from other dietary treatments. Rodriguez-Estrada *et al*. (2009), also 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 in Mehrabi *et al*. (2011) 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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