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STUDY THE EFFECT OF DIFFERENT LEVEL OF FRUCTOOLIGOSACCHARIDE (FOS) ON SOME BLOOD INDICES IN YOUNG COMMON CARP (*CYPRINUS CARPIO L.*)

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ABSTRACT

The present study was conducted to investigate the effects of dietary fructooligosaccharide (FOS) on some blood indices of common carp *Cyprinus carpio*. The fingerlings were adopted for 2 weeks and then reared in triplicate groups in 9 tanks (four fish per tank with average initial weights of (40 ± 3 g). Fish fed experimental diets containing different levels of fructooligosaccharide (FOS); (7.5 and 10 g FOS/kg diet) to apparent satiation twice a day for 10 weeks. The results showed significantly differences in Red blood cell count (10^{12} cells/dl) in T2. Hemoglobin (g/dl) data was 11.597 in the control. The mean corpuscular hemoglobin (pg/cell) was 63.333 in control, 56.033 in T21 and 52.100 in T2; mean corpuscular hemoglobin concentration (g/l) was 28.100 in the T1; mean corpuscular volume were (fL) 215.950 in control. White blood cell (10^9 cells/dl) data was (112.350) for control; granulocyte % was (15.050) in control; Lymphocyte % was (82.750) for control; Platelets % was (56.500) in T2 and monocyte % was (13.300) in T1.

INTRODUCTION

Aquatic animal production is the industry sector with the highest growth among animal production sectors. Between 1970 and 2010 it had an average annual growth rate of 2.9%, while total terrestrial meat production had an average annual growth rate of 2.7% [25].

Common carp *Cyprinus carpio* L. is one of the most important farmed species in world aqua culture, especially in Asia. It was ranked in the third place (3,043,712 tonne) among all freshwater species worldwide in 2005, more than 90% of which comes from Asia [11]. Improving the health conditions and growth performance in commonly farmed fish such as

common carp is a topic of strenuous interest. Hence, such components as antibiotics, hormones, ionophores and some salts have been frequently used to enhance fish health and growth [16].

Prebiotics mainly consist of oligosaccharides promoting beneficial bacterial growth within the gastrointestinal tract of higher vertebrates [13]. In different studies from last ten years, many substances have been investigated as prebiotics. Based on the studies of [18] any food stuff that reaches the colon (e.g. non-digestible carbohydrates, some peptides and proteins, as well as certain lipids) is a candidate prebiotic [27].

However, most studies have focused on non-digestible carbohydrates, mainly oligosaccharides. According to [12] only three oligosaccharides were classified as prebiotics: inulin, transgalactooligosaccharide (TOS) and lactulose. A more recent study includes fructooligosaccharides (FOS) in the list of prebiotics [21]. Mannanoligosaccharides (MOS) and fuctooligosaccharides (FOS, or oligofructose) are perhaps the most well studied oligosaccharides in fish but some information regarding applications of galactooligosaccharides, xylooligosaccharides, rabinoxylooligosaccharides and maltooligosaccharides in aquatic animals is also available [9].

Among prebiotics, fructooligosaccharides (FOS) is nondigestible carbohydrates, which selectively stimulate the growth and metabolism of health promoting bacteria present in the host gut. It is reported that FOS could overcome the limitations and side effects of antibiotics and other drugs, leading to high production through enhanced growth, stimulated immune response and increased resistance to pathogens of fish [14]. Previous studies also demonstrated the efficacy of FOS to increase the growth performance and the non-specific immunity of fish [28]. However, some researchers have proved that continuous administration of high levels of immunostimulants has no beneficial effects on growth or/ and immunity [5]. Others also suggested that discontinuous administration of immune stimulants may solve those problems [7]. Unfortunately, up to date, there were no report concerning the feeding modes of FOS on growth, immunity and disease resistant in fish. So the main objectives of the present studies evaluate the effect of adding FOS as prebiotic on common carp blood indices such as RBC (Red Blood Cell; 10 12 cells/l); WBC (White Blood Cell; 109cells/l); Hb (Hemoglobin; g/l);

MCH (Mean Corpuscular Hemoglobin; pg); MCHC (Mean Corpuscular Hemoglobin Concentration; g/l); MCV (Mean Corpuscular Volume; fL), white blood cell (WBC) , granulocyte %, Lymphocyte %, Platelets %, monocyte %.

MATERIALS AND METHODS

The experiment was conducted for 70 days we used 36 young common carp *C. carpio* L. The fish were distributed among experimental tanks with mean initial weight of (40 ± 3 g). They were pre-acclimated to laboratory conditions for 14 days prior to the feeding trials. Each tank was provided with a proper continuous aeration. Each tank was stocked with four fish. In control fish were fed an experimental diet without FOS, While in T1, fish were fed a diet 7.5 g/kg FOS, T2 represents the third treatment, in which fish were fed on a diet 10 g/kg FOS.

Diet formulation: Experimental diets were prepared with Animal concentrate Soya meal, Yellow corn, Wheat bran, Barely and Premix, and the composition of the different diet 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. The fish were fed 2 times a day, once was at 9:00 am and another time at 2:00 pm. Feeding rate started with 4% of biomass then the accurate feeding rate was determined to be 3% by third week depending on satiation level. Fish were individually weighed bimonthly. The feeding amount was then recalculated according to new weights.

Table 1: percentage of the feed ingredients in fish diets:

Feed ingredients	Percent
Animal concentrate	10
Soya meal	40
Yellow corn	15
Wheat bran	18
Barely	15
Premix	2
Total	100
Chemical Composition	
Crude protein %	27.351
Crude fat %	2.584
Crude fiber %	6.155
Energy KGal/ kg	2235.2
Ash %	87.61

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 analyser BC-2800 is a compact, fully automatic hematology analyser with 19 parameters for complete blood count (CBC) test. RBC (Red Blood Cell; 10 12 cells/l); WBC (White Blood Cell; 10^9cells/dl); Hb (Hemoglobin; g/dl); MCH (Mean Corpuscular Hemoglobin; pg); MCHC (Mean Corpuscular Hemoglobin Concentration; g/l); MCV (Mean Corpuscular

Volume; fL), (WBC) white blood cell(10^9 cells/dl) , Granulocyte %, Lymphocyte %, Platelets %, Monocyte %.

Statistical analysis: The experimental was conducted using the (CRD) design and the general linear models (GLM) procedure of XLSTAT. Pro. 7.5 one way (ANOVA). Duncan test's was used to compare between means of the control and experiment treatments. The mode of analysis was as follows: [9]

$Yij = \mu + Ti + Eij$ μ = The overall mean.

Ti = The effect of treatment. *Eij*= The random error.

RESULTS AND DISCUSSION

Table (2) summarizes the blood parameters results for the date variety used in this study. The data indicate that FOS affects significantly the Red Blood Cell (RBC) in control (1.633), Hemoglobin (g/dl) was 11.133, 10.633 and 9.735 for the T1, T2 and control respectively, observed that control significantly (P≤0.05) differ. The Mean Corpuscular Hemoglobin (pg/cell) data in Table (2) were 63.333, 56.033 and 52.100 for control, T1 and T3 and the significant (P≤0.05) different among treatments appeared in control which was significantly higher than other treatments. The Mean Corpuscular Hemoglobin Concentration (g/l) were 28.100, 25.900 and 25.350 for the T1, control and T2 respectively, and the T1 was higher than control and T2 significantly (P>0.05). Mean corpuscular volume (MCV) ranged 213.800, 210.900 and 208.867 for the control, T2 and T1 respectively.

Table 2: Effect of FOS on some blood parameters of common carp during 70 day of rearing:

T	RBC (10^{12} cells/dl)	Hb (g/dl)	MCH (pg/cell)	MCHC (g/l)	MCV (fL)
Control	**$1.633^{c} \pm 0.024$**	**$9.735^{c} \pm 0.145$**	**$63.333^{a} \pm 1.615$**	**$25.900^{b} \pm 0.200$**	**$215.950^{a} \pm 0.150$**
T1	**$1.757^{b} \pm 0.013$**	**$11.133^{a} \pm 0.133$**	**$56.033^{b} \pm 0.825$**	**$28.100^{a} \pm 0.600$**	**$208.867^{b} \pm 0.561$**
T2	**$1.947^{a} \pm 0.003$**	**$10.633^{a} \pm 0.517$**	**$52.100^{c} \pm 0.252$**	**$25.350^{b} \pm 0.350$**	**$210.900^{c} \pm 0.503$**

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

The present results agree with that of [4] they used prebiotic Immunogen (supplementation at concentrations of 5 and 10 g kg^{-1} prebiotic) in RBC, and with [1], in which the level of 2.5 g/kg Fructooligosaccharide affect significantly the RBC, WBC and Hemoglobin. Their results, together with ours, indicate that research in this area should continue and causal relationships should be sought between dietary prebiotics and some haematological and serum plasma biochemical parameters of fish [20], in this study, RBC and MCH did not change after feeding with inulin.

The adding of FOS has different stimulation in present 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 [24]. 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 defence for the host [17]. The results of the study of [23] 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. [22], which possess cell wall components such as lipopolysaccharides which have immunostimulatory properties [26]. However, [6] 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 [19].

White blood cell (WBC) in Table (3) presented 112.350, 109.000 and 105.650 for T1, T2 and control respectively, where control was significantly differ than other treatments. The data as presented in Table (3) the granulocyte percentage showed 15.050, 12.450 and 7.750 for T12, T2 and control respectively, and observed that T1 was significantly higher than control and T2 ($P>0.05$). In Table (3) Lymphocyte percentage were 82.750, 75.450 and 73.980 for control, T2 and T3 respectively, observed control had significant ($P\leq0.05$) different among other treatments. In the same table monocyte percentage showed 13.300, 12.200 and 11.850 for T1, T2 and control respectively, control was significantly lower than other treatment. Table (3) showed and the platelets percentages were 56.500, 53.000 and 31.000 for the T2, T1 and control respectively.

Table 3: Effect of FOS on on differential WBC count of common carp during 70 day of rearing

T	WBC (10^9cells/l)	GRAN %	Lymph%	Mon%	PLT%
contro	$105.650^{c} \pm 0.800$	$7.750^{c} \pm 0.350$	$82.750^{a} \pm 1.050$	$11.850^{b} \pm 0.450$	$31.000^{B} \pm 1.00$
T1	$112.350^{a} \pm 0.150$	$15.050^{a} \pm 0.050$	$73.950^{b} \pm 0.350$	$13.300^{a} \pm 0.100$	$53.000^{a} \pm 2.00$
T2	$109.000^{b} \pm 0.100$	$12.450^{b} \pm 0.250$	$75.450^{b} \pm 0.450$	$12.200^{b} \pm 0.208$	$56.500^{a} \pm 0.500$

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

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 defences to adverse condition [2]. 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 [8], the increase in WBC count might be due to stress suffered by fish as a result of daily feeding on β-glucan.

[15] reported an increase of WBC counts in *C. carpio* after herbal treatment with *Azardicha indica*, 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, the improved health condition in *C. carpio* fingerlings is probably due to the β-glucan and MOS components of the Immunogen [3].

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دراسة التأثير مستوى مختلف من السابق الحيوي (Fructooligosaccharide) في بعض معايير دم صغار أسماك الكارب الاعتيادي (*Cyprinus carpio* L.)

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الخلاصة

أجريت هذه الدراسة لتبيان تأثير (فركتوليكوسكرايد) كسابق حيوي كمصدر للتكافل. اجريت التجربة في مختبرات كلية الزراعة، جامعة السليمانية. استغرقت التجربة أشهرين بعد مدة أقلمة استمرت 15يوم. استعمل 63سمكة كارب اعتيادي بمعدل وزن 3±40غم لاختبار تأثير مستويات مختلفة من السابق الحيوي في بعض معايير دم صغار أسماك الكارب الاعتيادي *Cyprinus carpio* L. في المعاملة السيطرة (control)غذيت الأسماك بعليقة خالية من السابق الحيوي، والأسماك في المعاملة الاولى غذيت بالسابق الحيوي 7.5غم/ كغم، غذيت أسماك المعاملة الثانية بعليقة 10غم/ كغم من السابق الحيوي تتكون كل المعاملات من ثلاثة مكررات وفي كل مكرر خمس أسماك كارب اعتيادي والتي رُبيت 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 المعاملة الثانية (1.947)، سجلت بيانات الهيموغلوبين (11.133غم/ دسم) في المعاملة السيطرة، وكان متوسط هيموغلوبين الكرية (63.333) بايكوغرام/ كرية في المعاملة السيطرة ، كان متوسط تركيز الكرية 28.100غم/ لتر في المعاملة الاولى، وكان متوسط حجم الكرية 215.950 فيمتولتر في المعاملة السيطرة ، تفوق المعاملة الاولى معنويا ($P\leq0.05$)في عدد خلايا الدم البيض(خلية لكل لتر10^{12}) (112.350) ، ونسبة خلايا الدم البيض الحبيبية كانت (15.050%) في المعاملة الاولى ، وكذلك كان نسبة الخلايا اللمفاوية (82.750 %)في السيطرة ، الصفائح الدموية كانت (56.500 %) في المعاملة الثانية ، و كان نسبة خلايا الدم الوحيدة (13.300 %) في الاول.

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