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Effects of yeast cell wall (*Saccharomyces cerevisiae*var. *ellipsoideus*) on growth, survival and intestinal microbiota of cultured Persian sturgeon (*Acipenserpersicus*)

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ABSTRACT

Current study has been done for evaluating the effect of additive prebiotic of yeast cell wall (*Saccharomyces cerevisiae* var. *ellipsoideus*) in brief (YCW) in diet of cultured Persian sturgeon (*Acipenserpersicus*). 360 Persian sturgeon with average weight of 27.80±0.20 gram were fed up for 8 weeks by 3 different concentrations of YCW including 1%, 1.5% and 2% YCW per kilogram food and a control group (without adding YCW). Treatments were distributed randomly into 12 fiber glass tanks with density of 30 ones in each tank in the form of 4 treatments with 3 repeats. At the end of period the results showed that the factor of final length has had no significant difference(P>0.05). Final weight and body weight increase showed significant difference in 2% YCW treatment (P≤0.05). In factors of condition factor, significant difference has been observed between 1% YCW and 2% YCW treatments (P>0.05) and 2% YCW treatment showed higher condition factor than other treatments. Average daily growth, percent body weight increase and specific growth rate had significant difference between control and 2% YCW treatments of (P≤0.05). Food conversion ratio in 1% YCW treatment was higher than other treatments and it had significant difference (P≤0.05). Survival was equal in all treatments and was 100% and significant difference wasn't observed (P>0.05). The results of intestinal microbiota of fishes fed up with different levels of YCW and basic diet showed that there was significant difference in the number of total viable bacteria and Lactic Acid bacteria between control and 2% YCW treatment (P≤0.05).
KEY WORDS: yeast cell wall, growth indices, intestinal microbiota, Persian sturgeon.

INTRODUCTION

Sturgeons are national, regional and international valuable resources that are very important in the world ecologically, biologically and economically. Persian sturgeon is one of the most important specious in sturgeons that its meat and caviar is valuable [1]. Using dietary supplements, like prebiotics is an important idea introduced in industrial aquaculture for increasing growth and efficiency of dietary consumption[2]. Prebiotics as indigestive dietary ingredients that had positive effects on growth or health of fishes has shown positive result in dietary of aquatics [3,4]. Yeast cell wall (*Saccharomyces cerevisiae*) in brief(YCW) is the origin of two important materials of immune stimulant named (1→3)-β-d-Glucanand Mannan oligosaccharides or MOS. Mannan oligosaccharide and β-d-Glucan are the most effective materials of probiotics [5]. Different researchers have reported useful effects of yeast cell wall *Saccharomyces cerevisiae*, MOS and β-d-Glucan on growth and survival of different species of fishes [6,7,8,9,10]. Although some cases confirm no significant effect of YCW [11] and Mannan oligosaccharide[12] on growth of different species of fishes. However there is no information in the field of effects of YCW on growth of Persian sturgeon based on information of writer. The goal of this study is considering the effect of probiotic yeast cell wall *saccharomyces cerevisiae*var. *ellipsoideus*on indices of growth, survival, intestinemicrobiota of cultured young Persian sturgeon (*Acipenserpersicus*).

MATERIALS AND METHODS

This research was done at the center of Propagation and Culturing Sturgeons of Shadid Marjani in Gorgan located in 45 kilometers northeast of Gorganin August to December 2013. 360young Persian sturgeons (*Acipenserpersicus*) were chosen after biometry (measuring weight and length) with average weight of 27.80±0.20 gr and after two weeks of adaptation with basic diet, they were introduced to the 12 tanks with density of 30 randomly. Biomass in all tanks was equal and average weight didn't have significant difference (P>0.05).

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Experimental groups are 3 treatments of YCW (*saccharomyces cerevisiae*) of 1%, 1.5% and 2% YCW per kilogram food and a control group with 3 repeats. Feeding of fishes was done based on 2% of biomass weight in each tank [13] for 8 weeks of culturing period and at 2 turns (at 8:00 AM and 8:00 PM).Temperature and pH was measured by (WTW pH 330i, Weilheim Germany) and dissolved oxygen was measured by (WTW oxi 330o, Weilheim Germany) each week during culturing period. Applied dietary in this research was Biomar® standard formulated dietary produced by France, with diameter and length of 1.9mm.After adding YCW it has been analyzed based on standard method [14](Table1). Impenetrable plastic bags were used for packing and preserving foods at 3˚c. After weighting and temperature balancing of food with environment, regarding considered treatments they were given to fishes. Tanks cleaned after feeding every day to exit unconsumed probable food and wastes from culturing environment. Weight and length of all fishes of tanks were measured and counted. Growth indices such as average daily growth, body weight increase, percent of body weight increase, specific growth rate, feed conversion ratio and condition factor were calculated. For analyzing food, crude protein was measured by using standard Macro-kjeldahl method(model BAP40).For Nitrogen determination and by considering proper protein coefficient of the sample, the amount of protein percent was measured by the relation $Cp = \%N \times 6.25$. Crude fat was measured by using Soxhlet fat measuring device (model BOHER) and through solving fat in Ether and determining its amount through soxhlet method. Percent of ash was measure through putting samples in Electrical furnaces (Muffle furnances) and by the device (Heraeus) made in Germany at 550˚c for 4 hours [15]. Applied YCW in this research was analyzed separately as percent of crude protein, crude fat, moisture, dry matter and ash, that was 33.81%, 0.63%, 5.56%, 94.44%, 5.05% and crude energy was 311.87 kcal/kg respectively. For considering the number of Lactic Acid bacteria and also total viable bacteria available in intestine of Persian sturgeon fed up with different levels of YCW at the end of period, 3 fishes were caught from each repeat randomly and transferred to the lab.After anesthesia of fishes by Ms222(200ppm) and hitting to the head, abdominal side of fishes were sterilized by 70% alcohol with a piece of cotton [16,17]. In sterile condition abdominal side of fishes were cut with Scalpel blade and intestine were ejected. After exiting the ingredients of intestine, it was washed three times by sterile physiology serum and weighed. Then for homogenization they were transferred to sterile urine bottle. After preparing homogeny fluid by using sterile salty solution(0.87% w/V NaCl) dilution of 10^1 to 10^{10} were provided from intestine. The volume of 0.1ml was taken from provided dilution under condition of aseptic and transferred to the culturing field (Tryptic Soy Agar or TSA for determining total count of viable bacteria of intestine and special culturing field of Lactic Acid bacteria or MRS for determining the count of lactic acid bacteria) and distributed on the surface of plates[18,19].Incubation of plates was done inaerobic condition for TSA and anaerobic condition in the jar for MRS at 30˚c. After incubation of bacteria of each plate based on colony forming unit(CFU) per gram intestine they were considered based on phenotype characteristics such as color, shape, size[20].

Table1. Proximate composition of experimental diets

Diet composition	Basic dietary with 0% YCW	Basic dietary with 1% YCW	Basic dietary with 1.5% YCW	Basic dietary with 2% YCW
Crude protein (%)	50.22	50.08	48.23	48.01
Crude fat (%)	19.26	19.92	21.16	21.49
Moisture (%)	4.55	3.34	4.27	4.11
Dry matter (%)	95.45	96.66	95.73	95.89
Ash (%)	9.00	9.05	9.05	9.80
Crude energy (kcal/kg)	4539.71	4612.89	4693.87	4712.42

This research has been done in the form of completely random design. Firstly normality of data was examined by kolmogorov-Smirnov test and homogeneity test of group was investigated by Levene's test. In case of homogeneity of data, for comparing means between dietary treatments one-way ANOVA analyze and for separating homogeneous groups Duncan test at the level of 5% significance was used. For heterogeneous data non-parametric kruskal-Vallis test was used and meaningfulness of groups was distinguished by using Mann-Whitney test at the level of 5% probability. Statistical software of SPSS version 19 for data analysis and Excel 2007 was used for design charts.

RESULTS

Temperature, oxygen level and pH during cultivation period was 24˚c,5.6ppm and 7.8 respectively. The results of growth indices has been shown in table (2). Initial weight and length had no significant difference between treatments (p>0.05). According to multiple-range test of Duncan for pair wise comparisons between means of treatments and control considering indices of growth at the end of period showed that the final length had no significant difference (P>0.05). Final weight in 2% YCW treatment was higher than other treatments (138.86±1.30) and showed significant difference (P≤0.05).Result of body weight increase (BWI) showed significant difference in 2% YCW treatment(P≤0.05) and it was higher in 2% YCW (111.11±1.08) than others. In comparing average daily growth (ADG), percent of body increase (PBWI) and specific growth rate (SGR) means there was no significant difference between control and 2%YCW treatment and also between 1% and 1.5% YCW treatment (P>0.05).Based on this test the result of feed conversion ratio (FCR) showed significant difference in1% YCW and it was higher than others(p≤0.05) and the minimum FCR was seen in1.5% and 2% treatment. Significant difference has been observed in comparison of condition factor (k) between 1% YCW and 2% YCW treatments (P≤0.05) and the highest and lowest condition factor was observed in2% YCW treatment(0.43±0.00) and 1% YCW treatment(0.36±0.04). Survival at the end of period was equal in all treatments and 100% and there wasn't significant difference between them (P>0.05). As it has been shown in table 3 in considering intestinal microbiota of fishes fed up with different levels of YCW there was significant difference (P≤0.05) between control and 2% YCW treatments in total viable bacteria and also number of Lactic Acid bacteria Based on log CFU/ gr intestine, the total viable bacteria in intestine of fishes was high in control (6.69±0.16) and the lowest was in 2% YCW treatment (4.93±0.71). Number of Lactic Acid bacteria was high in 2% YCW(2.00±0.00) and also control showed lower number of Lactic Acid bacteria (0.67±0.67) between treatments.

Table 2. Growth performance of Persian sturgeon in the 8-week feeding trial with different levels of YCW (N=90 per treatment)

	control	1% YCW	1.5% YCW	2% YCW
Initial weight(g)	26.44±1.64	28.30±1.02	27.21±0.76	27.74±0.52
Initial length(cm)	19.98±0.28	20.28±0.20	19.94±0.42	20.18±0.06
Final weight(g)	134.17±1.77^{ab}	133.07±3.53^{ab}	133.53±1.52^{ab}	138.86±1.30^{a}
Final lenghth(cm)	32.04±0.26	33.49±1.23	30.06±0.21	31.93±0.23
ADG(%)	6.80±0.19^{a}	6.17±0.07^{ab}	6.51±0.05^{ab}	6.68±0.06^{a}
BWI(g)	107.72±0.84^{ab}	104.76±2.97^{ab}	106.31±1.09^{ab}	111.11±1.08^{a}
PBWI(%)	408.17±11.51^{a}	369.98±3.96^{ab}	390.69±2.84^{ab}	400.51±3.50^{a}
SGR(% day^{-1})	2.71±0.04^{a}	2.58±0.01^{ab}	2.65±0.01^{ab}	2.68±0.01^{a}
FCR	0.80±0.01^{ab}	0.82±0.01^{a}	0.79±0.00^{ab}	0.79±0.01^{ab}
K	0.41±0.00^{ab}	0.36±0.04^{a}	0.41±0.00^{ab}	0.43±0.00^{b}

Data in the same column with different letters are significant differ (P≤0.05) among different treatments. Values are mean ± S.D

Table 3.total number of viable bacteria (TVC) and Lactic acid bacteria (based on log CFU/gr intestine) in intestinal microbiota of Persian sturgeons 8-week feeding trial with different levels of YCW

	control	1% YCW	1.5% YCW	2% YCW
Total viable bacteria	6.69±0.16^{a}	5.33±0.63^{ab}	5.30±0.66^{ab}	4.93±0.71^{b}
Lactic Acid bacteria	0.67±0.67^{a}	1.33±0.67^{ab}	1.33±0.67^{ab}	2.00±0.00^{b}

Data in the same column with different letters are significant differ (P≤0.05) among different treatments. Values are mean ± S.D

Totally, the final weight, body weight increase and condition factor were higher in 2% YCW treatment than others. Adding YCW to Persian sturgeon food was not effective on other mentioned growth factors. But adding 2% YCW to food increase the count of intestinal Lactic Acid Bacteria and improve LAB count and decrease the count of total viable anaerobic bacteria of intestine.

DISCUSSION

By the developing of intensive aquaculture many antibiotics are used to prevent occurrence of disease and over dosing leads to appearance of resistance pathogens against them. Therefore difficult rules were made about using antibiotics [21]. Prebiotic industry are useful additive dietary that influences stimulating growth or activating Lactic Acid bacteria, decrease harmful effects of infective elements and increase the rate of survival in confrontation with pathogens [22]. MOS available in YCW causes increase of survival and efficiency of dietary, improvement of growth performance, increase of count of useful bacteria of intestine and stimulation of specific and non-specific

immune system against pathogens in different species[3,4]. β-d-Glucan available in YCW increases growth of Lactic Acid bacteria and improves health and immune situation of the host [22]. Intestinal microbiota of fishes includes indigenous and transmit bacteria that are not counted as part of fixed bacterial flora[23]. Lactic Acid bacteria have been known as gram positive and useful intestinal bacteria in many fishes like Great sturgeon (*Husohuso*), Common carp (*Cyprinuscarpio*) and rainbow trout (*Oncorhynchusmykiss*) that had the ability of using them as prebiotic in aquaculture but are not part of dominate microbiota of intestine [24, 25]. Different researchers have reported useful effects of the YCW (*saccharomyces cerevisiae*) on different species of fishes and it was distinguished that this prebiotic cause increase of growth performance of fish and increase of specific and non-specific immunity of fishes. In this study final length of fishes showed no significant difference between treatments and control but final weight, body weight increase and condition factor was higher in 2% YCW than other treatments and showed significant difference ($P \leq 0.05$).Hosseinifar *et al*. [2] showed that average final weight of young *Husohuso* fed up with dietary having 1% and 2% levels of YCW have had significant difference than control and also average final length in 1% YCW treatment had higher significant difference than control treatment. Alsoin their research BWI between yeast treatments (1% and 2%) and control treatment didn't have significant difference that is not similar with the result of current research. Differences in results can be due to differences of species, duration of culturing and type of consumption nutrition. In this study BWI factor in 2% YCW treatment was higher than other treatments. In other growth factors that includes final length, ADG, PBWI,SGR , FCR and survival there was no improvement by adding YCW to food on mentioned growth factors.

According to the results of this study adding 2% YCW to food of Persian sturgeon affected on final weight, body weight increase and condition factor and also improve the count of intestinal LAB and decrease the number of total anaerobic bacteria of Persian sturgeon intestine.

Therefore the various results of researchers can be related to environmental condition, physiological and genetic factors, the time of sampling, formulation of dietary, type of probiotic, its purity degree, the degree of using it in diet ,different methods of adding to diet and probably special intestinal microbiota in fishes[27].

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