



Assessing indices of growth of Oscar fry (*Astronotus ocellatus*) fed up with Nauplius of Artemia enriched with MOS extracted from yeast cell wall (*Saccharomyces cerevisiae*)

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ABSTRACT: This research has been done at biochemistry laboratory at Islamic Azad university of Lahijan for 8 weeks, with the aim of determining the effect of different levels of Mannan Oligosaccharides prebiotic (MOS) and prebiotic of yeast cell wall on index of growth survival rate (SR), of Oscar fry (*Astronotus ocellatus*). After a week of adaptation with culture condition, one hundred Oscar fries with average weight of 0.35gr were randomly fed up into 4 aquariums of 60 liters regarding water temperature of 10% of biomass. In this experiment by separating MOS from prebiotic of yeast cell wall and enriching Nauplius of Artemia with prebiotic MOS at 4 levels of 0 (control group), 250, 500, 750 mg /l (with three replicate) equal to 21 days the Oscar fries were fed up, and from the beginning of the fourth week till the end of eighth week by adding prebiotic of yeast cell wall at four levels of 0 (control group), 1%, 2% and 3% (with three replicate) to the first food of Oscar fry (Biomar) was designed. At the end of a period indices of growth such as weight gain average (WG), indices of specific growth rate (SGR), final biomass (FB) and final length (FL) at 2 and 3 % levels was more than the 0% (control group) and 1% levels, and significant statistical difference has been observed ($p < 0.05$) but primary biomass of fries between treatments and the control group at the end of a period didn't show significant statistical differences ($p > 0.05$). Average daily growth and percent of weight gain (WG) between treatments was more than the control group and at 3% level was more than the control group and other treatments were shown significant between the treatments ($p < 0.05$). Food conversion rate (FCR) of fries at 3 and 2% levels was less than 0% (control group) and 1% treatment (better performance) and statistically significant reduction has been observed ($p < 0.05$). Average rate of obesity between treatments and control group didn't show significant statistical difference ($p > 0.05$). Survival rate at all treatments was more than the control group and has significant difference with the control group ($p < 0.05$).

Key words: Fish (*Astronotus ocellatus*), prebiotic of yeast cell wall, MOS, Artemia, indices of growth

INTRODUCTION

Food is one of the most expensive parts of aqua culturing and its optimization can have important function at reducing production expenses. In this direction different reports have been presented about using prebiotics at food ration at aqua culturing on criteria of growth and survival. Suitable and sufficient food is one important and effective factor at pisciculture. Natural food that is so-called as live food has high importance at culturing and saving ornamental fries (Shaterian, 2011). Agh reported in 2001 that Artemia due to having 55% of protein, 4-20% of oil, all main Amino acids and most fatty acids at favorable

level are counted as the best food of aquatics. In this state, live foods provide enzymes for digesting food eaten by new-born fries. Two important actions for aquacultures are supplying creatures proper with the size of fry's mouth during primary steps of feeding and then supplying many of these creatures for culturing new-born fries. Artemia or Brine shrimp is a tiny creature from Branchiopoda species that is part of fresh water aquatics that for its survival and potential has gone to the brine water and finally very brine water, on the other hand because this aquatic doesn't have any defensive tool against other aquatics, it will rapidly be defeated by kinds of predators.

Therefore it has high adaptability power against different brines (Azari Takami, 2009). One important cases of use of infant *Artemia* and adult *Artemia* are as carriers of materials that are directly used by different farmed fishes and crustaceans, through bio-encapsulation *Artemia* are fed up with some of vital materials like necessary nutrition, vitamins, vaccine, pigments and kinds of necessary drugs that are needed at aqua culturing and then these *Artemias* as carriers are eaten by farmed aquatics. This action that is called enrichment causes weight gain, survival increase, increase of resistance against kinds of stress like salinity, temperature, transportation, density at farmed aquatics. Based on the kind of using, enrichment can be done by various kinds of required materials. For example enrichment with proteins, oils, vitamins, vaccines and antibiotics (Sorgeloos & Lavens 1996). The basis of proper enrichment are the maximum enrichment (reaching the highest degree) at the shortest time, this time depends on the duration of reaching Nauplius to the first nutritional step (Instar2) and is related to the characteristics of hatching rate and hatching coordination of cysts. As the duration of hatching coordination is shorter that is cysts could hatch with each other at a more limited time, better result is obtained at enrichment. As the duration of hatching coordination is longer, during the accessibility to enrichment ration a number of Nauplius hasn't still started feeding and this factor reduces the total absorption of nutrient at napoleons mass (Azaritakami, 2007). The influence of prebiotics on criteria of growth and survival of aquatics, accessing procedures that can increase efficiencies of growth and survival of aquatics is the important goal of constant aquaculture. Prebiotics are in fact cell wall extracted from the yeast *Saccharomyces cerevisiae* that are produced by the company named Bioncy Orange Natural Company in Austria is supplied from Parsiyan Shafagh darou company that is the formal delegacy of the company in Iran. For doing the research cell wall of the yeast is the origin of 2 important materials of Immunostimulant called β -Glucan (1-3) and MOS, MOS and β -Glucan are the main materials mostly affecting prebiotics (Huang, 2008). Cell wall of the yeast is composed of 30-60% of polysaccharide (MOS & β -Glucan), 15-30% of protein, 5-20% of lipid and a little cetin (Huang *et al*, 2004, 2005). MOS has also an active prebiotic and can be preserved as nutrition for growth of beneficial bacteria in colon of intestine. MOS are indigestible Glucoma and protein that provide places of establishment of Mannose at Velvet pile of intestine and prevent connecting of pathogenic bacteria to enterocytes cell (absorptive epithelial cells) of intestine, also prevent formation of bacterial colony and infection of host cells that it leads to the increase of cohesion of intestine velvet pile in order to improve and increase

the efficiency of intestine and leads to more and better beneficiary of nutrients (Pryor *et al*, 2003; Newman, 2007). MOS is a suitable nutritional source for growth and activity of bacterial flora at gastrointestinal tract such as bacteria of lactic acid, lactobacillus and bifidobacters. Lactic acid bacteria by producing Bacteriocin prevents pathogens growth. (Ringo, *et al*, 1998). Mos as a moderate source of energy is counted by lactic acid bacteria (Miles, 1993). Oscar fry was firstly named as *labotes ocellatus* by Baron Kovieh in early 1800 but nowadays this fry with a scientific name of (*Astronotus ocellatus*) belongs to cichlidae family. Regarding the positive effects of prebiotic of yeast cell wall it considers the mentioned fish with the aim of increasing growth and survival rate.

MATERIALS AND METHODS

Extracting MOS from prebiotics of yeast cell wall:

The first step of this research has been done at chemistry lab of Islamic Azad University of Lahijan in field and experimental forms for a month. At separating and extracting prebiotics MOS from prebiotic of yeast cell wall in any step of separation that has been done at the first step 20gr of prebiotic of yeast cell wall was measured by digital scales. at the second step 2gr of 5% sodium hydroxide was dissolved at 200ml distilled water into a Beaker and 20gr of powder of prebiotic of yeast cell wall was added to it. It is necessary to mention that inside a Beaker one or two magnets have been placed in order to keep the ingredients suspending then the ingredients of the Beaker was put on a heater at the temperature of 100 C for two hours at the second step beaker was taken from heater to become cool and after making the ingredients cool 5% HCl was added slowly and with PH-meter it was measured until its pH reaches neutral ph that is pH = 7. At the third step the ingredients of beaker was purified by filtration system. At the fourth step 80ml of pure ethanol was added to the ingredients of beaker at this step MOS sediment this sediment was separated from ethanol by centrifuge machine with 5000rpm turns for two minutes and at the last step this sediment was washed by diethyl ether (Huang *et al*, 2010).

Preparation of incubator for hatching Artemia:

Constructing incubation for hatching cyst Artemia:

For this purpose incubators were made by mineral water bottles for hatching cyst *Artemia* with 4 levels at 0 (control group), 250, 500 and 750mg/l and (with three replicate).

Firstly 5cm of bottom of each bottle was cut then at the center of cap of bottles a hole was made for passing air tube in order to do the action of delivering oxygen and suspension of cysts constantly then the bottles were put in an inverted form as its cap was placed downward and the action of delivering oxygen was done in a bottom-up direction and placed in aquarium.

At the first step, 2gr of cyst Artemia and 28-35gr salt rock for each bottle were measured by digital scale and were transferred into each bottle, mild and constant aeration action and permanent light, fixing temperature at 30°C during 4 hours has been done very well (for fixing temperature a 150w heater was placed in aquariums).

Enriching Artemia:

After 36 hours, Nauplius of artemia hatched from cysts and 12 hours after hatching, while Nauplius entered in instar step and have started active feeding from outside environment, enrichment has been started and prebiotic MOS at 4 levels of 0, 250, 500 and 750mg/l was enriched to Nauplius of Artemia and finally fed up to fishes. One hundred Oscar fries fishes (*Astronotus ocellatus*) were sent to each four 60 liters aquariums after biometry (assessing weight and length) and determining biomass with average weight of 35%gr and minimum length of 1.25cm and maximum 2 ± 0.5 and density of 25 ones. Then the act of making fishes compatible with basic ration (that the standard food was Biomar) based on 8-10% of their body weight has been done at three turns (at 10a.m, 12 o'clock and 3p.m) for a week (Pourali *et al*, Mohseni *et al*, 2006, 2003). After a week of compatibility of Oscar fries and bioassay of all studying population of the research for 21 days (3 weeks) in 4 treatment groups (with three replicated) consisting fishes fed up with Nauplius of Artemia without entering with MOS (control group) fishes fed up with Nauplius of Artemia enriched with 250mg/l of MOS (first treatment) and fishes that fed up with Nauplius enriched with 500mg/l of MOS (second treatment) and fishes that fed up with Nauplius enriched with 750mg/l of MOS (3rd treatment). Treatments of fourth week till the end of eighth week were as below, fishes fed up with Biomar food 0% (control group), 1% prebiotic of yeast cell wall, 2% prebiotic of yeast cell wall and fishes fed up with mixture of Biomar and 3% prebiotic of yeast cell wall. At the end of third week, 60 pieces of fishes were measured with digital scale with the accuracy of 1% of their weight and ruler with precision of millimeter of the total length of these fishes. Before doing bioassay, fishes felt hungry in order to empty their digestive tubes (Ebrahimi *et al* 2004, Hosseini *et al* 2010). From fourth week till the end of eighth week, fishes were fed up with 1%, 2% and 3% prebiotic of yeast cell wall in combination with biomar food four times (10a.m, 12 o'clock, 3p.m) and control group that was only fed up with biomar food. During this period biometry (assessing length and weight) has been done twice and in each biometry 60 pieces of fishes has been used. Three minutes after the first period of daily feeding the act of siphoning remained food and removing waste was done. Washing filters and stones of aquarium has also been done once a week. Measuring physical and chemical factors

including temperature with thermometer, water pH with digital pH meter of Eco model and the amount of soluble oxygen has been done by Eutech digital Oxymeter daily and data were recorded. Average temperature was 28-30°C, average oxygen 6.62ppm and average pH 8-8.7, during the culturing period that mentioned physical and chemical factors during culturing period didn't have significant difference ($p>0.05$).

Statistical analysis:

At the end of a period growth indices were calculated based on available resources of mathematical equations for considering normal distribution of data in groups and repeats them in order to conform treatment the test Shapiro-Wilk and Histogram drawing was used. In case of normality of data for statistical comparison between groups in treatments, the one-way ANOVA test was used and for considering the reciprocal effect, 2-way ANOVA and after doing test of Homogeneity of variances for comparing groups, Duncan test was used. All statistical analysis was done by using SPSS software version 17 and for drawing chart the software Excel 2003 was used.

RESULTS

The result indicated that growth factors except obesity coefficient and average length and primary biomass for treating all factors such as average percent of weight gain, food conversion rate, specific growth rate (SGR), average weight gain (WG) and survival of fishes at the end of a period between treatments and control group showed significant statistical difference (to the certainty level of $p<0.05$). Also the result showed that a treatment that fed up with Nauplius enriched with 500mg/l MOS has devoted the highest amount to himself at the most of growth factors such as average percent of weight gain, average specific growth rate and average daily growth fishes at the end of a period, the degree of food conversion rate of fishes at 20 treatment was less than 500 and 700 treatments and statistically significant reduction has been observed ($p<0.05$).

And this is the symbol of better performance; survival at each 3 treatments was more than control treatment. Primary biomass of fishes between treatments didn't have significant statistical difference with control group. Applying different level of prebiotic MOS has had high effectiveness on increasing performance of growth and survival at Oscar fries and totally positive correlation was obtained between parameters of growth and nutrition with moderate levels of prebiotics MOS of nutrients in comparison with different levels, prebiotic MOS of the levels 500mg/l has had higher efficiency at growth performance of Oscar fries.

The effect of different levels of MOS and yeast cell wall on parameters of growth and survival rate of Oscar fries has been presented in Table 1.

Table 1. Comparing average rate of growth, survival of Oscar fries fed up with MOS and yeast cell wall during 60 days of culturing.

Treatment	Control	250mg/l of MOS- and 1% prebiotic of yeast cell wall	500mg/l of MOS- and 2% prebiotic of yeast cell wall	750mg/l of MOS- and 3% prebiotic of yeast cell wall
Average initial weight (gr)	1/89 ±0/031	1/15 ±0/024	1/32 ±0/049	1/001 ± 0/020
Average initial Length (cm)	4/36 ±0/15	4/6 ±0/1	4/83 ±0/06	4/63 ± 0/03
Average final weight (gr)	16.77± 0.5 ^a	17.66 ± 1.63 ^a	21.97 ± 0.39 ^b	23.52 ± 1.5 ^b
Average final Length (cm)	9.2 ± 0.35 ^a	9.06 ± 0.20 ^a	10.1 ± 0.25 ^b	10.33 ± 0.31 ^b
Average initial Biomass	27/23 ±0/059	28/86±0/62	33/03±1/22	25/03±0/51
percent of weight gain	1439.77 ± 44.28 ^b	1433.29 ± 161.4 ^b	1565.80 ± 48.27 ^a	2246.93 ± 20.73 ^b
Average Factor obesity	2/17±0/14	2/35±0/23	2/14±0/074	2/13±0/033
food conversion rate	0.91 ± 0.04 ^b	0.79 ± 0.07 ^b	0.62 ± 0.02 ^a	0.58 ± 0.03 ^a
Average specific growth rate	0.28 ± 0.16 ^a	0.28 ± 0.03 ^a	0.37 ± 0.006 ^b	0.4 ± 0.03 ^b
average daily growth	4.59 ± 0.07 ^a	4.75 ± 0.016 ^a	4.92 ± 0.08 ^a	5.53 ± 0.08 ^a
Period average weight gain	15.68 ± 0.49 ^a	16.50 ± 1.64 ^a	20.64 ± 0.35 ^b	22.52 ± 1.15 ^b
survival	85.3 ± 1.33 ^a	94.67 ± 1.33 ^b	94.67 ± 1.33 ^b	94.67 ± 1.33 ^b

DISCUSSION

Applying different levels of prebiotic MOS and yeast cell wall has high effectiveness on increasing growth and survival performance, at Oscar fries and totally positive correlation has been obtained between growth, parameters with levels of prebiotic MOS and yeast cell wall at ration. In comparison between different levels of prebiotic MOS and yeast cell wall at ration, 2% and 3% levels have had higher efficiency at growth performance of Oscar fries.

Taati 2010 announced that using prebiotic Immunowall stimulant (compound having MOS and Glucan) at 3% level causes significant increase at obesity rate of cultured Beluga that doesn't adapt with the findings of this research.

In a research the effect of 3 prebiotics of Ferocto oligosaccharide(FOS), Galacto oligosaccharide (GOS) and Bio-MOS containing MOS extracted from yeast at dose of 1% for a period of 8 weeks on *ocellatus sciaenops* showed that food conversion rate at the whole period of culturing and controlling didn't have significant difference where as in our findings it has

significant statistical reduction that it correspond to this research that its reason can be known as better digestion of the 1% nutrient and control group than ration having prebiotic MOS or yeast cell wall because there is a little chitin at yeast cell wall (Huang *et al*, 2004). Factors such as environmental factors especially due to being poikilotherm(season, salinity, light period, temperature, density) physical factors (aquatic species, reproductive cycle and maturity situation, age, gender and nutritional condition) time of sampling, the way of supplying sample, accuracy and sensitivity of methods of measuring on activity of indices of growth and survival can affect it and causes difference at interpretation of the result of the research (Verdegem *et al*, 1997).

Tukmechi *et al* in 2011 considered the effect of yeast cell wall on growth factors at rainbow trout for a period of 30 days and observed that at obesity coefficient, significant statistical difference hasn't been observed that correspond to our findings also he observed that the percent of weight gain, specific growth coefficient has had significant difference with control group that corresponds to the findings of our research.

But survival rate at this research didn't have significant difference with control group that doesn't correspond to our findings that this difference can be due to difference of species, length of culturing period, type of consumable ration, qualitative condition of water and resistance of Oscar froes and initial feeding with MOS. Ashourpour, 2011 claimed that using prebiotic of yeast cell wall at high dose (that is 2% and 3% treatment), survival to the amount of 1% against control treatment. (88.33% and 1% treatment) that is compatible with the findings of our research and its reason is probably due to eradicating harmful bacteria by fermentation of this prebiotic at intestine and so production of beneficial bacteria such as lactic acid bacteria that produces compounds such as bacteriosin and prevent growth of harmful bacteria at intestine (Roofchayi, 2011).

Using prebiotic MOS to the amount of 3 gr per kilogram ration at Gulf sturgeon species didn't lead to the appearance of significant difference with control treatment at growth and nutrition that is incompatible with our finding and perhaps the reason of this difference is at the kind of species and qualitative and quantitative conditions of water of culturing environment.

Gatlin and Li in 2004 by adding 1 and 2 percent prebiotic of the kind of probiotic I-A and 1 and 2 percent prebiotic of Brewer's yeast o ration of striped hybrid bass observed that growth performance, efficiency of nutrition and these supplement in comparison with control treatment has had significant increase that is compatible with our findings.

Daniels in a research in 2006 has considered the effect of enrichment of Artemia with business culture environment of DHASECCO and different levels of PPt 2, 20, 200MOS at *Gammarus hamarus* and reported that adding MOS at the level of 2 and ppt20 increases the degree of survival rate and growth that is compatible with our findings.

Culjak *et al*, in 2006 added prebiotic Bio-Mos at the level of 0.6% to the food of young Cyprinids that leads to the significant increase at growth parameters and significant reduction of losses and food conversion rate in comparison to the control group that is compatible with our findings.

Suxsue *et al* (2009) by adding 2 levels of 0.2 and 0.4 percent of business matter DVAQQA resulted from fermentation of *Saccharomyces Cerevisiae* to ration of hybrid tilapia (*Oreochromis niloticusoareus*) at system of culturing in cage found that beside concentration of beneficial intestinal bacteria, improvement of performance at growth, FCR and survival. Totally the present difference at the result reported by different researchers for applying kinds of prebiotics at various species of cultured aquatics should be probably related

to the type of cultured species, environmental condition, nutritional behavior and physiological features of aquaculture. Also the effect of different prebiotic can be evaluated based on quantity and quality of ration, kind of consumable prebiotic, purity degree and its consumable degree at ration and probably especial microbial population being able to use different kinds of prebiotics. Factors such as environmental factors especially cppl-bloodedness of fishes seasons, salinity, light period, temperature, density) physiological factors (aquatic species, reproduction cycle and maturity situation, age, gender and nutritional condition) time of sampling, the way of supplying sample, accuracy and sensitivity of method of measuring affect activity of indices of growth and survival and causes different interpretation of the result (Verdegem *et al*, 1997).

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