ISBN 978-93-86878-04-5
7th International Conference on Chemical, Agriculture, Environment and Natural Sciences (CAENS-2017)
Kuala Lumpur (Malaysia) Nov. 20-21, 2017

Dietary Ergosan as a Supplemental Nutrient on Growth Performance, and Stress in Zebrafish (*Danio Rerio*)

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***Abstract*:** *In this study, the effects of different levels of Ergosan (control group (0), 2, 4 and 6 gr Ergosan per Kg diet) as nutritional supplement were investigated on growth indices and stress in Zebra fish for 3 months. Larvae (4-day-old after hatching) were fed with experimental diet from the beginning of feeding until adult (adolescence) (average weight: 69.3 g, length: 5.1 cm). Different levels of Ergosan had no significant effect on rate survival (P<0.05). The results showed that diet containing 6 gr Ergosan significantly caused the best FCR in Zebra fish (P<0.05). By increasing the Ergosan diet, specific growth rate increased. Body weight gain and Condition factor had significant differences (P<0.05) as the highest and the lowest were observed in treatment 3 gr of Ergosan and control, respectively. The results showed that fish fed with experimental diet, had the highest resistance to environmental stresses compared to control, and the test temperature, oxygen, salinity and alkalinity samples containing 6 gr/kg, was significantly more resistance compared to the other treatments (P<0.05). Overall, to achieve high resistance to environmental stress and increase final biomass using 6 gr/kg Ergosan in diet fish Zebrafish.*

Keywords: *Ergosan, Stress, Growth Performanceb, Danio rerio*

1. Introduction

Globally, aquaculture is expanding into new directions, intensifying and diversifying. A persistent goal of global aquaculture is to maximize the efficiency of production to optimize profitability. In recent years, the study of probiotics and prebiotics in fish nutrition is increasing with the demand for consumer and environment – friendly aquaculture (Denev, 2008). Aquavac Ergosan is made of two brown sea weeds, *Laminaria digitata* and *Ascophyllum nodosum*. The attitude constituents are registered food additives: Algines and alginic acids. Moreover, ago experiments has shown that higher levels of lysozyme, complement and heat-shock-protein can be detected in sea bass fed with AquaVac Ergosan (Bagni et al 2005). Both in vivo and in vitro researches have mainly been focused on the effect of Ergosan on fish growth, stress and survival rate (Gioacchini et al., 2008; Jalali et al., 2009). Ergosan is an algal extract composed of 0.002% unspecified plant extract, 1% alginic acid from Laminaria digitata, and 98.998% algal based carrier. Ergosan can function as antibiotic, antioxidant, and plant-growth promoting substances (Duy et al., 2010). Therefore, use of this immune stimulator can benefit the fish farmers via increasing the growth performance and immune system of fish.

Algines have been used for a range of commercial applications including thickening agents, gelling agents and dispersion stabilizers (Peddie et al., 2002). There are several studies available related to the subjects including the effects of ergosan on the growth indices, immune system in fish (Peddie, et al., 2002; Bagni et al., 2005). There are not many studies using the Ergosan on Zebra fish in the literature.

2. Materials and Methods

In this trial, Zebrafish were cultivated in the tank services of SADRA center of ornamental fish, IRAN. To feeding the fish, used Biomar Feed (Made in French) and. Fish were reserved at a rearing temperature of 24.2±0.8°C, 12 D: 12 L photoperiod and pH 7.4±0.1. The number of fish per aquarium was used to 40 individuals in each glass aquariums. The experiment was directed in 12 glass tank at 30 fish/ tank in triplicate. These aquariums were randomly divided to four experimental groups. For each aquarium, 50% of water was changed and the tank bottom was siphoned three times a week throughout the exposure period. To prepare the diets, a marketable pellet diet was crushed, mixed with the appropriate extract concentration and gelatin added to water. The pelleted diets were air-dried, ground and sieved to products a proper crumble mm). The fish were weighed individually using an electronic balance. For each treatment, to quantify the weight gain percent (%), specific growth ratio (SGR), condition factor (CF) and survival (%) all the fish were considered. The foregoing were calculated as follows: WG = (final body weight− initial body weight) × 100 /initial body weight, SGR = [Ln (final weight) − Ln (initial weight)] / experimental period duration (in days) × 100 and condition factor CF = Weight of fish(g) / (fish length) ^{3}(cm)^{3} × 100 and Survival (%) =100 × final number of fish / initial number of fish (Imanpour, et al., 2011).

TABLE I: Analysis of the Ergosan presented by Schering-Plough Company.

Analysis	Dry matter	*Crude* protein	Crude fat	Crude ashes	Crude fiber
Not less than (%)	52.45	12.44	2.1	11.35	8.12

TABLE II: Chemical composition of the basal diet of *Danio rerio*

Proximate analysis	**Protein**	**Lipid**	**Fiber**	**Ash**	**Moisture**	**Energy (Cal/gr)**
%	52.45	12.44	2.1	11.35	8.12	4598.56

2.1. Stress test:

Later 60 days fish feeding with diets enclosing different levels of Ergosan, 12 hours before the test feeding fish was interrupted in order to determine their resistance to stress. Fish were exposed to salinity stress tests of each treatment in three replications high and low pH (acidic stress) groups. It is worth mentioning that the fish were exposed to stress once when the last fish has completely killed in the conditions mentioned, were recorded (Rahnama et al., 2013).

2.2. Statistical Analysis:

In the current test, a wholly randomized design with 3 repeats was used. Data were tested for the homogeneity of variance and then statistically examined using one-way ANOVA, followed by evaluation of means by Duncan's multiple range test (α=0.05). The statistical analysis was conducted using SPSS22 for Windows. Values are expressed as the mean ± standard deviation.

3. Results

3.1. Effects of Ergosan on Growth Indices:

The results obtained of this trial indicated that survival rate had not significant difference (P>0.05) among treatments. Specific growth rate (SGR), body weight gain (BWG), food conversion rate (FCR) and condition factor (CF) were affected significantly by Ergosan (P<0.05). Regarding the findings of mean comparison of these parameters, it was observed that these parameters were significantly (P<0.05) higher at T3 treatment (Table 3).

TABLE III: Growth Indices of *Danio Rerio* Feed with Different Levels of Ergosan for 90 Days

Treatments	SR (%)	SGR	FCR	CF	BWG
T0 (Control)	97.3±2.6[a]	0.022±0.001[a]	0.098±0.03[a]	3.688±0.021[a]	298.321±14.26[a]
T1 (5g/kg)	99.1±1.4[a]	0.030±0.002[b]	0.097±0.04[b]	3.723±0.019[b]	419.235±16.34[b]
T2 (10 g/kg)	100±2.5	0.035±0.004[b]	0.086±0.04[c]	4.187±0.022[c]	536.308±25.47[c]
T3 (15 g/kg)	100±1.3[a]	0.044±0.007[b]	0.077±0.03[d]	4.246±0.017[c]	569.429±26.19[c]

TABLE IV: Survival Time for Danio Rerio Exposed to Acidic pH Stress After 60 days of Feeding Diets Containing Ergosan

Treatments	T0 (Control)	T1 (5 g/kg)	T2 (10 g/kg)	T3 (15 g/kg)
Salinity	[a] 102.34±3215.6	3428.7±114.26 [b]	3662.8±126.2[c]	3859.7±128.9 [d]
Alkalinity (Second)	385.4±14.8 [a]	624.2±11.8 [b]	597.8±25.1[c]	723.6±24.8 [d]
Acidity (Second)	1346.8±257.1 [a]	1421.2±195.4 [b]	1498.9±185.6[c]	1562.3±236.1 [d]
Temperature (Second)	[a] 7.9±100.84	104.65± 7.28 [b]	108.59± 7.32 [c]	110.3± 7.5 [c]
Oxygen (Second)	80.3±3.22 [a]	95.4±2.31 [b]	110.3± 3.14 [c]	132.4±2.1 [c]

4. Discussion and Conclusion

Fish feed supplementation with Ergosan was obviously beneficial, as showed by the increased growth performance. In this study, all treatment groups presented higher growth indices (BWG, SGR, and CF) and lower FCR than the control group after a period of 90 days feeding. These findings are in agreement with the on rainbow trout that received fermented *S. cerevisiae* (Barnes et al. 2006) and Ergosan (Gioacchini et al. 2010). In this study, Ergosan enhanced growth indices and feed intake. These results are in agreement with those achieved with the administration of alginic acid in rainbow trout (Gioacchini et al. 2010) and Beluga sturgeon *Huso huso* (Jalali et al. 2009). Conversely, alginic acid had no effects on tilapia (*Oreochromis niloticus*) (Merrifield et al. 2011) and sea bass (*Dicentrarchus labrax*) (Bagni et al. 2005). In this case, the results of Peddie et al (2002) showed that fish fed with AquaVac Ergosan had lower overall mortality rates than those fed on a normal production diet.

Ahmadifar et al (2011) investigated the effects of thymol-carvacrol on growth performance of juvenile rainbow trout (*Oncorhynchus mykiss*). Their results indicated that dietary administration of thymol-carvacrol can influence some growth parameters in rainbow trout juveniles, that is in agreement with our results.

Akbari et al (2014) suggested the potential of Ergosan to activate growth performance and immunological parameters in rainbow trout (*Oncorhynchus mykiss*) that it is in agreement with our results. Also Heidarieh et al (2012) reported that Ergosan effectively promotes growth performance, lipase activity and gastrointestinal structure in rainbow trout (*Oncorhynchus mykiss*).

Also, Heidarieh et al. (2014b) were noted higher significant growth performance in fish fed irradiated Ergosan and significant changes in FCR were shown, compared with control group (basal diet without Ergosan). Sheikhzadeh et al (2016) expressed that Ergosan at 0.33 g/kg was found to improve growth performance and mucus biological components in rainbow trout (*Oncorhynchus mykiss*) significantly in comparison with the control group (basal diet without Ergosan) that it was supported by the results of the present study.

Differences between fish species concerning their physiology may be a reason of different results in growth performance. Other aspects such as age and health station of animals used, differences in dietary enclosure levels, variations in the types and purity of Ergosan evaluated might be responsible for variable results.

Sheikhzadeh et al (2016) that short-duration administration of Ergosan resulted in non-significant differences in growth performance compared with control group, but confirms Heidarieh et al (2012b), which showed that after 45 days' continuous feeding, Ergosan had positive effects on rainbow trout growth parameters. The results of the present study showed the better performance and growth parameters in fish fed with experimental diets than control group.

Rahnama et al (2013) investigated the resistance of goldfish about environmental stress (temperature, pH and salinity). They founded that goldfish fed with prebiotic, had higher resistance on environmental stress than control group. These results were supported by our results in zebrafish.

The positive growth performance in this study may be due to a number of reasons. First, the improved performance may be attributable to a reduction in pathogenic bacteria and an increase in positive bacteria in the gut of the Ergosan-fed fish. Second, Ergosan may influence nutrient especially protein digestibility by maintaining the function and structure of the small intestine, leading to an increased digestive capacity of the gut.

5. References

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