ADVANCES IN EARLY LIFE HISTORY
STUDY OF FISH
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Salt incorporated diets for enhancing growth performance and survival in gilthead sea bream *Sparus aurata* L. juveniles reared in low saline brackish water

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SUMMARY: Following previous observations, this study was undertaken to determine the level of salt required in the diet of gilthead sea bream (*Sparus aurata* L.) juveniles in order to enhance growth performances and survival when rearing in inland brackish water with a salinity of 2.9‰ TDS (Total Dissolved Salts). The juveniles were reared for 10 weeks in twelve rearing tanks, each stocked with 65 fish. Four different test diets were prepared: a control diet without added salt, and three diets with 12%, 14% and 16% added salt. Fish were fed by hand *ad libitum* three times daily. All diets were accepted equally well. The highest growth rate ($p<0.05$) was achieved in fish fed the 12% diet followed by the 14% diet, the control diet, and finally the 16% diet. The 12% diet produced the best specific growth rate, feed conversion ratio and protein efficiency ratio. The highest survival was achieved in fish fed the 12% diet (88%) and the lowest in fish fed the 16% diet (80%) and the control diet (80%). This study demonstrates that growth performance and survival in gilthead sea bream reared in water of low salinity can be significantly improved by adding 12% salt to their diet.

Keywords: gilthead sea bream, salt-added diet, growth and survival, low salinity, brackish water, geothermal water, arid land aquaculture.

RESUMEN: DIETAS CON SAL INCORPORADA PARA MEJORAR EL RENDIMIENTO EN EL CRECIMIENTO Y SUPERVIVENCIA DE JUVENILES DE DORADA *SPARUS AURATA* L. CRIADOS EN AGUAS SALOBRES DE BAJA SALINIDAD. – A raíz de las observaciones anteriores, este estudio se realizó para determinar el nivel de sal requerido en la dieta de la dorada (*Sparus aurata* L.) con el propósito de incrementar el crecimiento y la supervivencia en aguas salobres con salinidad de 2.9‰ TDS. Los juveniles ($X = 0.58$ g) fueron criados durante 10 semanas en doce tanques con 65 organismos en cada uno. Cuatro dietas fueron probadas: control sin adición de sal y adicionadas con el 12, 14 y 16% de sal. Los peces fueron alimentados *ad libitum* manualmente tres veces al día. Todas las dietas fueron bien aceptadas, sin embargo la mayor tasa de crecimiento se obtuvo con la dieta que adicionaba el 12%, seguida por la del 14%, la dieta control y la de 16% de sal agregada ($p<0.05$). La mayor tasa de crecimiento específico, conversión alimenticia y relación de eficiencia proteica se observó con la dieta del 12%. La mayor supervivencia (88%) se encontró con la dieta del 12% y la menor con 16% y en el grupo control (80%). Este estudio demuestra que el crecimiento en la dorada criada en aguas de baja salinidad puede mejorarse significativamente mediante la adición del 12% de sal a su dieta.

Palabras clave: dorada, sal dieta, crecimiento, supervivencia, baja salinidad, agua salobre, agua geotérmica, acuicultura de las tierras áridas.

INTRODUCTION

Gilthead sea bream is a euryhaline teleost capable of living in environments with salinities ranging from 2‰ to 60‰. Its natural habitat stretches from the Mediterranean and Black Sea to the eastern Atlantic Ocean from Senegal to the United Kingdom. It is commonly found in shallow lagoons along the coast, but migrates into deeper water to spawn after late autumn. Fish in low-saline water show a passive outward flux of ions such as Na^+ and Cl^- to the water via the gills, faeces and renal system, which must be compensated by the active uptake of ions from the water and/or from the diet. Gills, the major osmoregulatory organ in fish, undergo large morphological changes at low water salinities. Euryhaline fish in different environmental salinities induce activation of their ion transport mechanism, usually accompanied by changes in oxygen consumption, causing variations in the energy demands for osmoregulation.

Since minerals absorbed from the water do not always meet the total metabolic requirements in fish, their supplementation through the diet promotes growth (Hepher, 1988). Fish diet is therefore an important source of salts not only to satisfy the needs for growth, but also for osmoregulation. Providing a sufficient amount of salt through feeds can spare energy that is used for osmoregulation, thereby reducing stress and allowing more energy for growth. Dietary salt has been found to be beneficial for growth in rainbow trout (MacLeod, 1978), common carp and mrigal (Nandeesha *et al.,* 2000), Asian sea bass (Harpaz *et al.,* 2005), European sea bass (Eroldogan, 2003; Eroldogan *et al.,* 2005) and gilthead sea bream (Appelbaum *et al.,* 2008a, b; Appelbaum and Arockiaraj, 2008b, 2009). Salman and Eddy (1988) and Smith *et al.* (1995) found that feeding rainbow trout with a 12% salt-added diet promoted better growth, better survival and a better feed conversion ratio, but the inclusion of over 12% salt resulted in a deterioration in all growth performances. Yakupitiage (1993) reported that the use of salt as a supplement in fish feed is a normal practice in China and India. Veerina *et al.* (1993) reported that about 44% of the freshwater fish farmers in the southern state of India use salt as an additive to fish diets.

Recent studies by Appelbaum *et al.* (2008a, b) showed that gilthead sea bream juveniles reared in brackish water (3‰ TDS) and fed a diet supplemented with 1.5% salt grew significantly ($p<0.05$) better than those fed the control diet (no added salt). Appelbaum and Arockiaraj (2008a) reported that rearing the juveniles of gilthead sea bream in low salinity brackish water (2.9-3.6‰ TDS) significantly improved their growth and survival rates when they were fed 6% salt-added diets**.** A further study (Appelbaum and Arockiaraj, 2008b) using diets supplemented with higher levels of salt (8%, 10% and 12%) showed that gilthead sea bream juveniles reared in brackish water of 2.9‰ (TDS) salinity grew better and had the highest survival rate when fed a diet consisting of 12% added salt. Following these previous observations, the present study was undertaken to determine the required levels of salt incorporated in the diet for enhancing growth performances and survival of gilthead sea bream (*Sparus aurata* L.) juveniles reared in low-saline inland brackish water.

MATERIALS AND METHODS

Experimental system

Twelve rectangular plastic rearing tanks of 60-L capacity were used for the study. Each tank was filled with water of 2.9‰ (TDS) salinity originating from a deep brackish water well in the southern region of the Israeli Negev desert. The rearing tanks were grouped into 4 separate systems. Each system consisted of three rearing tanks connected to a 100-L water-cleaning unit filled with volcanic gravel and strongly aerated water acting as a mechanical and biological filter. Water from the cleaning unit entered each rearing tank at the rate of 3 L per minute. Thirty percent of the rearing water was renewed daily in each system. Continuous aeration was provided by a blower with diffused air stones keeping oxygen levels above 4 ppm. The water temperature was maintained at 28±1°C using thermostatically controlled electric heaters. Ammonia and nitrite were kept at recommended levels for gilthead sea bream.

Experimental fish

Gilthead sea bream juveniles with an average initial wet weight of 0.58 g were used for the 10-week feeding trial. Each rearing tank was randomly stocked with 65 juveniles.

Experimental diets

Four different test diets were prepared by grinding a commercial sea bream feed (protein 45%, lipid 14%, fibre 2.4% and ash 9.5%; "Zemach" Feed Mills, Israel), into which 5% commercial krill meal and 5% baker's yeast were added. Before re-pelleting, salt was added to the ingredients as follows: Diet 1, without salt, served as the control diet; Diet 2, 12% salt added; Diet 3, 14% salt added; and Diet 4, 16% salt added (Table 1). The salt incorporated into the diets was obtained by evaporating brackish geothermal water from the same source as the rearing water in the present experiment. After re-pelleting, the

TABLE 1. – Diet compositions (% dry weight basis).

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4
Commercial sea bream feed*	90	78	76	74
Krill meal	5	5	5	5
Baker's yeast	5	5	5	5
Salt added	0	12	14	16

* Protein 45%, lipid 14%, fibre 2.4%, ash 9.5%, calcium 2.4%, phosphorous 1%, copper 5 mg/kg, selenium 0.3 mg/kg, lysine 2.7%, methionine 1%, vitamin A 1200IU, vitamin C 150 mg/kg, vitamin D3 1750IU and vitamin E 150 mg/kg.

diets were oven-dried at 60ºC for 6 hours and stored in an airtight container. Fish in all tanks were fed by hand *ad libitum* three times daily. Fish in system 1 were fed the control diet, fish in system 2 were fed the 12% salt-added diet, fish in system 3 were fed the 14% salt-added diet and fish in system 4 were fed the 16% salt-added diet.

Observations

Growth, survival and feeding rate were monitored and calculated as follows: survival (%) = [(no. of fish stocked - no. of mortalities)/ no. of fish stocked] x 100; weight gain (g) = final mean weight – initial mean weight; weight gain (%) = [(final mean weight – initial mean weight)/ initial mean weight] x 100; specific growth rate (SGR; % d^{-1}) = [(In final mean weight – In initial mean weight)/ duration of the experiment] x 100; feed conversion ratio (FCR) = amount of feed provided (g)/ weight gain (g); and protein efficiency ratio (PER) = weight gain (g)/ protein intake.

Statistical analyses

The growth data were analysed using one-way ANOVA and Tukey's multiple range test (Zar, 1984).

RESULTS

Fish growth performance and survival are presented in Table 2 and Figures 1 and 2. All experimental diets were accepted by the fish equally well, the highest growth rate ($p<0.05$), however, being achieved in the fish groups fed the 12% salt-added diet, followed by those fed the 14% salt-added diet,

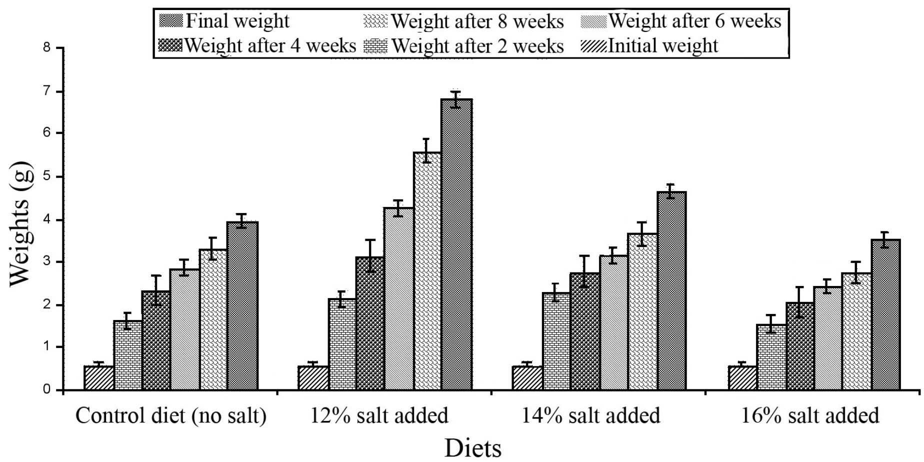

FIG. 1. – Weight gain (g) of gilthead sea bream (*Sparus aurata* L.) juveniles reared and fed different salt-added diets for 10 weeks. Data shown are means and standard deviations.

TABLE 2. – Growth performance of gilthead sea bream (*S. aurata* L.) juveniles fed different salt-added diets for 10 weeks (values given are the mean of three replicates ± standard deviation)

Growth performances	Diet 1	Diet 2	Diet 3	Diet 4
Initial weight (g)	0.58 ± 0.08	0.58 ± 0.08	0.58 ± 0.08	0.58 ± 0.08
Final weight (g)	3.94^{a} ± 0.17	6.81^{b} ± 0.29	4.66^{c} ± 0.25	3.52^{a} ± 0.26
Weight gain (g)	3.36^{a} ± 0.58	6.23^{b} ± 0.67	4.08^{c} ± 0.34	2.94^{a} ± 0.41
Weight gain (%)	579.3^{a} ± 26.5	1074.1^{b} ± 48.3	703.4^{c} ± 37.8	506.8^{a} ± 36.1
Specific growth rate (%d^{-1})	1.18^{a} ± 0.14	1.96^{b} ± 0.23	1.42^{c} ± 0.33	1.05^{a} ± 0.18
Feed conversion ratio	2.13^{a} ± 0.46	1.04^{b} ± 0.68	1.63^{c} ± 0.41	2.44^{a} ± 0.52
Protein efficiency ratio	1.04^{a} ± 0.18	2.12^{b} ± 0.37	1.36^{c} ± 0.25	0.91^{a} ± 0.09

a, b and c denote significant differences at $p<0.05$ level by one-way ANOVA and Tukey's multiple range test

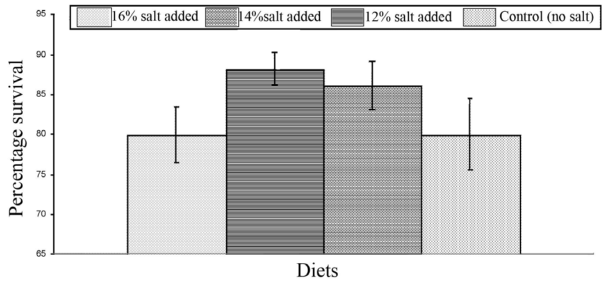

FIG. 2. – Survival (%) of gilthead sea bream (*Sparus aurata* L.) juveniles reared and fed different salt-added diets for 10 weeks. Data shown are means and standard deviations.

the control diet, and finally the 16% salt-added diet, which produced the lowest growth rate. Significantly ($p<0.05$), the highest specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were also observed in the groups fed the 12% salt-added diet (Table 2 and Fig. 1). The highest survival (88%) was achieved in fish groups fed the 12% salt-added diet and the lowest (80%) in fish groups fed the 16% salt-added diet and the control diet (Fig. 2).

DISCUSSION

The results of the present study demonstrate that the growth of gilthead sea bream juveniles cultured in inland low-salinity brackish geothermal water can be significantly improved by incorporating 12% salt into the fish diet. Recent publications on euryhaline fish have indicated that growth and feed conversion ratio are improved by feeding diets with added salt (sodium chloride; NaCl) (Nandeesha *et al.,* 2000; Harpaz *et al.,* 2005; Eroldogan *et al.,* 2005). Salman and Eddy (1988) and Pelletier and Besner (1992) reported that the inclusion of more than 12% salt in salmon diets has no positive but rather negative effects on growth and feed conversion ratio. In this study, incorporating 12% salt (obtained from evaporating brackish geothermal water) into the diet significantly improved fish growth performance, with no visible detrimental effect. However, the 14% or 16% salt-added diets lowered the growth and survival rates compared with the 12% salt-added diet and the control diet (no salt added).

Many studies (Schmidt-Nielsen, 1997; Sangiao-Alvarellos *et al.,* 2003; Laiz-Carrion *et al.,* 2005) have indicated that fish development, growth and survival are influenced by various physiological factors, one of which is water salinity. Various developmental stages during fish embryogenesis depend on water salinity. Salinity plays a key role in the regulation of growth, growth rate, metabolic rate, feed intake and feed conversion. In summary, the present observations indicate that for rearing gilthead sea bream juveniles in brackish water of 2.9‰ salinity, supplementing the fish diet with 12% salt is recommended in order to achieve a better growth performance, feed conversion ratio and survival.

CONCLUSION

This study demonstrates that when gilthead sea bream juveniles are reared in low saline brackish water, fish growth performance, feed conversion ratio and survival can be significantly enhanced by feeding a salt added diet. A 12% salt supplement was found to be the best of the tested diets for sea bream juveniles reared in brackish water with a salinity of 2.9‰ (TDS). This study shows that when a salt-supplemented diet is used, inland brackish water of low salinity provides a promising realistic opportunity for farming gilthead sea bream which is traditionally farmed in sea water.

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