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Effect of brine salt rich diets on growth performances and survival of Asian seabass (*Lates calcarifer*) juveniles reared in freshwater systems

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Abstract. The present study was conducted to study the effect of salt added diets on growth performance and survival of Asian sea bass juveniles reared in freshwater. The indoor experimental setup consisted of fifteen rectangular rearing tanks each of a 100-L capacity. The tanks were grouped into five separate systems. Fifty four-day-old juveniles of Asian sea bass were used for the 56 day experiment. Seventy fish were randomly stocked in each tank. The experimental salt added diets were prepared by adding salt to a commercial pelleted fish. Fish were hand-fed ad libitum three times daily. Survival and feed conversion ratio were monitored daily. Fish fed the 8% salt added diet attained the highest growth rate ($P<0.05$) followed by the fish group fed the 10% salt added diet and by the control group. Diets with 12% and 14% added salt promoted the lowest growth rates. An inverse relationship was noticed in survival, the higher the salt incorporated in the diet, the lower the survival rate. Based on the results of this study, it is concluded that the addition of 8% salt to the pelleted fish feed of Asian sea bass *Lates calcarifer* juveniles reared in freshwater is advantageous towards their growth and survival rates. But this study needs further research to find out the optimum level of dietary salt requirement for the juveniles Asian seabass.
Key Words: Asian seabass, barramundi, salt diet, brine salt, growth and survival.

Zusammenfassung. Die vorliegende Studie wurde durchgeführt, um die Wirkung von Salz-Studie hat Ernährung auf Wachstum und Überleben der asiatischen Seebarsch Jugendlichen in Süßwasser gehalten werden. Die Indoor-Versuchsaufbau aus fünfzehn rechteckigen Hälterungsbecken jeweils aus einem 100-L Kapazität. Die Behälter wurden in 5 getrennten Systemen zusammengefasst. Fünfzig vier Tage alten jungen asiatischen Seebarsch wurden für die 56 Tage Versuch eingesetzt werden. Siebzig Fische wurden nach dem Zufallsprinzip in den einzelnen Tanks lagernd. Die experimentelle Salz hinzugefügt Diäten wurden durch Zusatz von Salz zu einem kommerziellen pelletiert Fisch zubereitet. Die Fische wurden Hand-fed ad libitum dreimal täglich. Überleben und die Futterverwertung wurden täglich überwacht. Fische verfüttert werden die 8% Salz hinzugefügt, Ernährung erreicht die höchste Wachstumsrate auf ($p <0,05$), gefolgt von der Gruppe Fische fütterte die 10% Salz hinzugefügt, Ernährung und von der Kontrollgruppe. Diäten mit 12% und 14% Salz gefördert hat den niedrigsten Wachstumsraten. Ein umgekehrtes Verhältnis wurde im Überleben bemerkt, desto höher ist das Salz in der Nahrung aufgenommen, desto geringer ist die Überlebensrate. Basierend auf den Ergebnissen dieser Studie wird der Schluss gezogen, dass der Zusatz von 8% Salz des umhüllten Fischfutter der asiatischen Seebarsch Pleuronectiformes Jugendlichen in Süßwasser gehalten ist auf ihre Wachstums-und Überlebensraten von Vorteil. Aber dieser Studie bedarf noch weiterer Forschung, um herauszufinden, die optimale Verteilung der Nahrung Salz Voraussetzung für die jungen asiatischen Seebarsch.
Schluesselworte: Asian Seebarsch, Barramundi, Salz Ernährung, Sole-Salz, das Wachstum und Überleben.

Rezumat. Studiul de față a fost realizat pentru a observa efectul adaosului de sare în dietă asupra performanțelor de creștere și asupra supraviețuirii la juvenilii de barramundi crescuți în apă dulce. Experimentul s-a desfășurat în cincisprezece bazine rectangulare de creștere amplasate în spațiu închis, fiecare având o capacitate de 100 L. Bazinele au fost grupate în cinci sisteme separate. Pești juvenili de barramundi în vârstă de 54 zile au fost folosiți pentru experimentul de 56 zile. Șaptezeci de pești au fost distribuiți randomic în fiecare bazin. Adăugarea experimentală a sării în mâncare s-a făcut prin amestecarea cu granulele de furaj. Furajarea s-a făcut manual și ad libitum, de trei ori pe zi. Supraviețuirea și rata de conversie au fost monitorizate zilnic. Peștii hrăniți cu dietă îmbogățită cu sare

8% au atins cea mai mare rată de creștere (P<0.05), urmați fiind de grupul furajat cu furaj și 10% sare, iar apoi de grupul martor. Grupurile furajate cu 12% și 14% sare au crescut cel mai puțin. O relație inversă a fost observată în cazul supraviețuirii, cu cât concentația de sare adăugată a fost mai mare, cu atât a fost mai mică rata supraviețuirii. Pe baza acestor rezultate, concluzionăm că adaosul de sare 8% în furajul granulat al juvenililor de barramundi (*Lates calcarifer*) crescuți în apă dulce este avantajoasă sub aspectul creșterii și ratei de supraviețuire. Se impun studii în continuare pentru identificarea nivelului optim de sare adăugată în furajul peștilor juvenili de barramundi.
Cuvinte cheie: specii asiatice, barramundi, sare adăugată, sare de mare, creștere și supraviețuire.

Introduction. The Asian sea bass, *Lates calcarifer* (Bloch, 1790), is a carnivorous, euryhaline marine species which is reared under intensive conditions in seawater, brackish water and freshwater in re-circulating systems and earthen ponds. It is an important species for aquaculture in Asian and Australian continents. Catacutan & Coloso (1997) reported that this carnivorous Asian seabass has a high market demand in Southeast Asia, and thus the production is increasing in this region and the need to develop an appropriate seabass feed exists. Currently, the culture of seabass relies heavily on trash fish, however, this traditional feed does not often adequately meet the nutritional requirements of the fish. The traditional feed chopped or minced trash fish is difficult to store, has variable nutritional quality, and may be unavailable. Critical to formulating effective economical feed is a knowledge of the nutritional requirements of the animal. Thus, a cost-effective practical feed has to be developed for seabass cultured in ponds or cages.

Zaugg et al (1983) and Gatlin et al (1992) stated that incorporation of minerals (salts) in the fish feed is an importance source of energy and that compensate all the nutritional requirements of fish. But the mineral requirements in fish appear to be very complicated since metabolism and nutrition of minerals are closely related not only to those present in the diet but also to those dissolved in the surrounding water (Ogino & Kamizino 1974). The beneficial effects of dietary salt have been reported in several fish species: chinook salmon, carp, eel, channel catfish, red sea bream and yellow tail (Ogino & Kamizino 1974), common carp and mrigal (Nandeesha et al 2000), rohu (Gangadhara et al 2004) and gilthead sea bream (Appelbaum et al 2008). Ogino & Kamizino (1974) reported that dietary salts have enormous effects on not only growth and survival but also helped the fishes from deformity and malformation.

In freshwater or low saline water adopted euryhaline fishes, passive ion losses must be overcome by active uptake of ions from the water (Marshall 1988; Smith et al 1989). The role of gills is crucial in ion absorption, and it is reasonable to expect that diet is an important source of salts that could satisfy the osmoregulatory requirements of the fish in fresh water or low saline water. The supplementation of diet with salt might spare energy used in osmoregulation, thereby leaving more energy available for growth (Zaugg et al 1983; Gatlin et al 1992).

The Asian seabass is a euryhaline teleost capable of living in both freshwater and salt water environments, including freshwater indoor recirculation systems (Appelbaum & Arockiaraj 2008). Improved growth, survival and feed conversion ratio in Asian sea bass was found by adding 4% salt to the diet compared to no salt added diets (Harpaz et al 2005). Appelbaum & Arockiaraj (2009) found that gilthead sea bream (*Sparus aurata*) fed a diet with 12% salt added had the highest growth and survival. Since sea bream grew best with 12% salt inclusion in the diet, the present study examined if dietary salt levels greater than 4% (Harpaz et al 2005) resulted in increased growth performance and survival of Asian sea bass juveniles reared in freshwater.

Material and Method

Experimental setup. The indoor experimental setup consisted of fifteen rectangular rearing tanks each of a 100-L capacity. The tanks were grouped into 5 separate systems each consisting of three rearing tanks connected to one common water-cleaning unit (a 100-L tank filled with volcanic gravel and strongly aerated which acted as a both biological and mechanical filter). All systems were filled with dechlorinated freshwater. In each system the water recirculated at the rate of 3-L per minute. Thirty percent of the

dechlorinated freshwater was replaced daily in each system to compensate for losses from evaporation and sludge removal. Oxygen levels in the rearing tanks were maintained above 4 ppm. Water temperature was maintained at 28±1ºC using thermostatically controlled electric heaters.

Experimental fish. Fifty four-day-old juveniles (average initial weight = 0.18g) of Asian sea bass, produced via induced spawning ("Maagan Michael Hatchery", Israel), were used for the 56 day experiment. Seventy fish were randomly stocked in each of the 15 rearing tanks.

Experimental diets. The experimental salt rich diets were prepared by adding salt to a commercial pelleted fish feed (protein 45%, lipid 14%, fiber 2.4% and ash 9.5%; "Rannan" Company, Israel) that was ground and repelleted after adding the salt. This salt was obtained by evaporation of the brine (53.7‰ total dissolved salts; Table 1) produced during desalination of brackish geothermal water from a deep well located in the Israeli Negev desert. The prepared diets were oven-dried at 60ºC for 6 hours and stored in an airtight container. Fish were hand-fed ad libitum three times daily. Fish in the different systems were fed as described in Table 2.

Table 1

Composition of the brine used to obtain the salt added in the experimental diets

Salts	*Units*	*Other parameters*	*Units*
Chloride	40880 ppm	Alkalinity (measured as calcium carbonate)	22 ppm
Sodium	22600 ppm	Electric conductivity (micromhos/cm/25)	98200 mmho
Sulfate	7481 ppm	Hardness (measured as calcium carbonate)	14700 mg/l
Calcium	2339 ppm	Bicarbonate (measured as HCO_3)	26 ppm
Magnesium	2152 mg/l	Ammonia	24 ppm
Potassium	540 ppm	Nitrite	2 ppm
Boron	33 ppm	pH	7.6
Fluoride	32 ppm	Total dissolved matter	79020 ppm

Table 2

Different salt added diets provided to the fish in various systems

Different systems	*No. of tanks*	*Types of feed*
System 1	3	Control diet (no salt added)
System 2	3	8% salt added diet
System 3	3	10% salt added diet
System 4	3	12% salt added diet
System 5	3	14% salt added diet

Observation and statistics. Representative fish samples (N=30) from all experimental groups were weighed on a digital weighing balance every two weeks for monitoring growth performance. Survival and feed conversion ratio were monitored daily. Survival (%), weight gain (g), average weight gain (%), specific growth rate (SGR %d-1), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated as follows: survival (%) = no. of fish stocked – no. of mortalities/ no. of fish stocked x 100; weight gain (g) = final mean weight (g) – initial mean weight (g); average weight gain (%) = final mean weight (g) – initial mean weight (g)/ initial mean weight (g) x 100; specific growth rate (% d-1) = ln final mean weight – ln initial mean weight/ duration of the experiment (days) x 100; feed conversion ratio = amount of feed provided (g)/ weight gain (g) and protein efficiency ratio = weight gain (g)/ protein absorbed (g). The growth and survival data were analyzed using one-way ANOVA and Tukey's Multiple Range Test (Zar 1984).

Results. All experimental diets were willingly accepted by all fish. Fish fed the 8% salt added diet attained the highest growth rate ($P<0.05$) followed by the fish group fed the 10% salt added diet and by the control group. Diets with 12% and 14% added salt promoted the lowest growth rates (Fig 1 & Table 3). Fish receiving the diet with 8% added salt also showed a better specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) compared to all other experimental groups. An inverse relationship was noticed in survival, the higher the salt incorporated in the diet, the lower the survival rate (Fig 2).

Table 3

Growth performances of Asian sea bass juveniles reared in freshwater and fed salt added diets for 8 weeks

	Control diet	*8% salt diet*	*10% salt diet*	*12% salt diet*	*14% salt diet*
Initial weight (gr.)	0.18±0.05	0.18±0.05	0.18±0.05	0.18±0.05	0.18±0.05
Final weight (gr.)	3.62^{b}±0.42	4.96^{a}±0.29	3.65^{b}±0.51	2.30^{c}±0.17	1.97^{c}±0.45
Weight gain (gr.)	3.44^{b}±0.21	4.78^{a}±0.38	3.47^{b}±0.24	2.12^{c}±0.18	1.79^{c}±0.21
Average weight gain (%)	172.0^{b}±10.9	239.2^{a}±12.6	173.9^{b}±9.5	106.1^{c}±12.6	89.5^{c}±11.8
FCR	1.98^{b}±0.15	1.45^{a}±0.23	1.96^{b}±0.13	3.12^{c}±0.26	3.65^{c}±0.29
PER	1.25^{b}±0.32	1.72^{a}±0.31	1.27^{b}±0.21	0.79^{c}±0.26	0.68^{c}±0.15
SGR (% d^{-1})	0.99^{b}±0.12	1.24^{a}±0.18	1.0^{b}±0.11	0.64^{c}±0.14	0.52^{d}±0.13

* In a row, a, b and c denote significantly different values at $P < 0.05$ level by one-way ANOVA and Tukey's Multiple Range Test

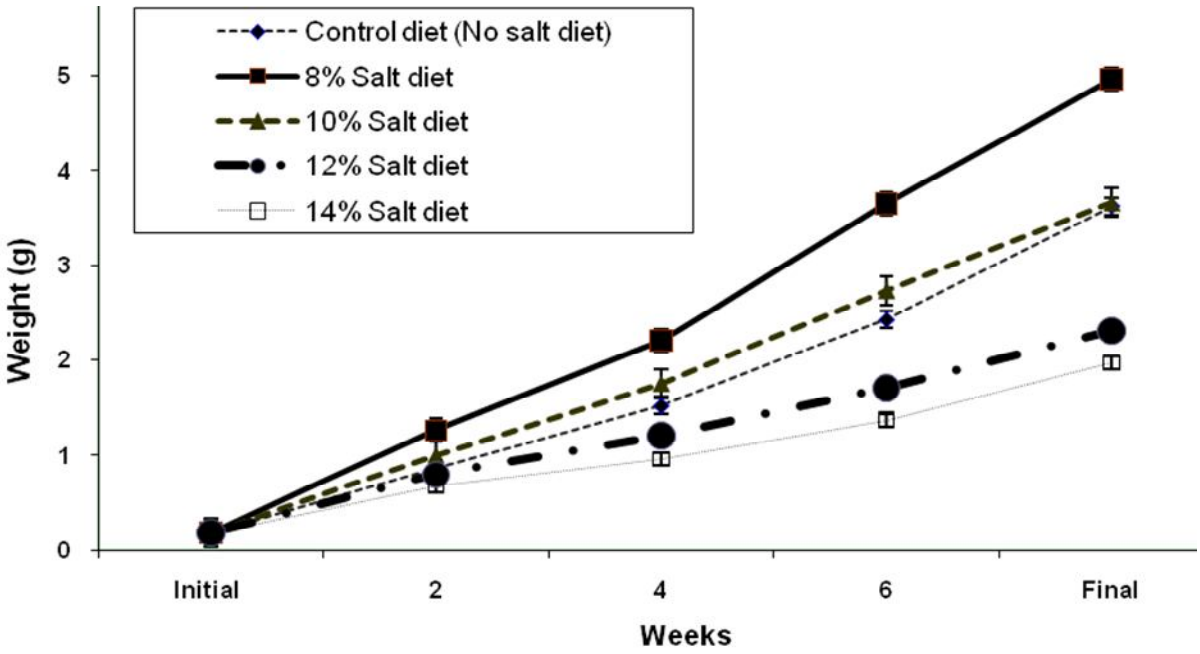

Figure 1. Growth (g) of Asian sea bass reared in freshwater and fed salt added diets for 8 weeks

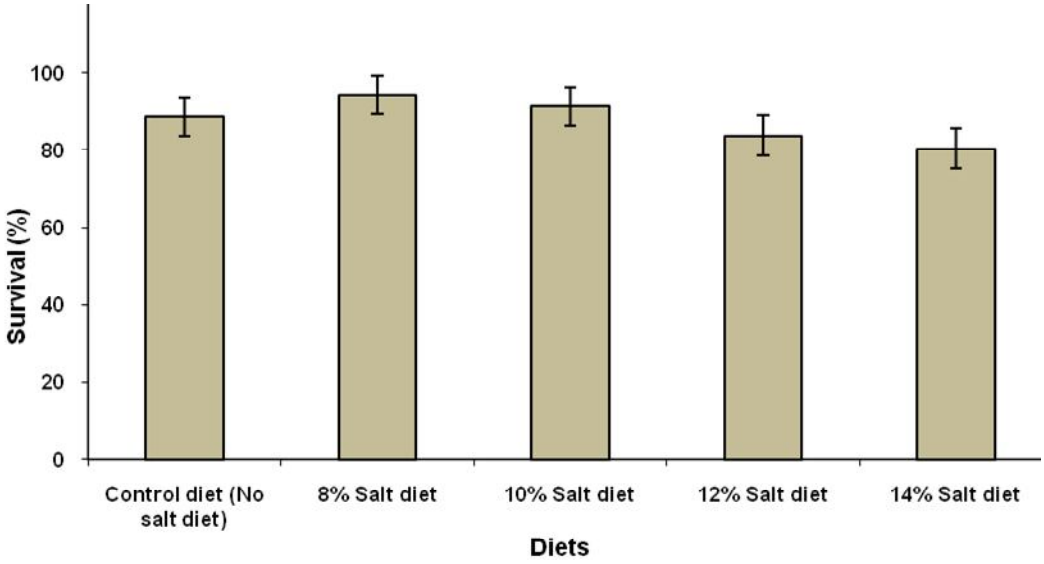

Figure 2. Survival (%) of Asian sea bass reared in freshwater and fed salt rich added for 8 weeks

Discussion. Dietary salt has been found to improve fish growth and feed conversion efficiency in several species: the Japanese eel (Arai et al 1974), rainbow trout (MacLeod 1978), common carp and mrigal (Nandeesha et al 2000), rohu (Gangadhara et al 2004) and gilthead sea bream (Appelbaum et al 2008). Harpaz et al (2005) reported that adding 4% salt to the diet of *L. calcarifer* resulted in significant improvement in their feed conversion ratio and enhanced the activity of the brush border enzyme activity. Fontainhas-Fernandes et al (2000) reported that adding salt to fish diet increases appetite and digestibility. Salman & Eddy (1988) reported that the food intake and appetite of the rainbow trout were not adversely affected by adding salt to the diet, but did enhance growth. Gatlin et al (1992) reported that juvenile red drum exhibited greater feed efficiency and significant weight gain when 2% NaCl was added to their diet. In this study, a significant improvement in growth, FCR and higher survival rate were recorded in Asian sea bass fed the 8% salt added diet, but diets with higher salt incorporation caused a reduction both in growth and in survival rates.

MacLeod (1978) reported that the addition of excessive salt to the diet could have an adverse effect on food intake, digestion and/or absorption, because of the change in the gastric/intestinal environment and may even have pathological effects, ultimately leading to growth reduction. No such effects were detected with any of the tested diets in this experiment. Ogino & Kamizono (1974) and Tacon & De Silva (1983) reported that a 4% or 5% salt inclusion in the diet of trout and carp produced better growth and feed utilization. Smith et al (1995) observed that 12% salt-enriched diets enhanced the feed uptake and benefited the growth of rainbow trout. But in this study the control diet (no salt added) better than 12% and 14% salt added diets in terms of survival, growth and feed conversion ratio. Eroldogan et al (2005) found that 5% salt supplementation in the diet of European sea bass enhanced growth and feed utilization. All these findings show that salt addition to fish diet improve growth and survival rates.

Conclusion. Based on the results of this study, it is concluded that the addition of 8% salt to the pelleted fish feed of Asian sea bass *L. calcarifer* juveniles reared in freshwater is advantageous towards their growth and survival rates. But this study needs further research to find out the optimum level of dietary salt requirement for the juveniles Asian seabass. Because in this study we achieved better growth and survival rates of Asian seabass fed at 8% salt added diet, but the earlier study of Harpaz et al (2005) results showed that the Asian seabass produced highest growth rate when feeding with 4% NaCl diet. Eventhough the composition of the salt which is incorporated in the diets of these

two studies are entirely different, it needs further research to find out the optimum dietary salt requirement for the Asian seabass juveniles.

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