

The effects of oxygen supplementation on growth and survival of rainbow trout (*Oncorhynchus mykiss*) in different stocking densities

Bilen S.^{1*}; Bilen A.M.¹; Önal U.²

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Abstract

In this study, the effects of oxygen supplementation in different stocking densities of rainbow trout (*Oncorhynchus mykiss*) on the specific growth rates (SGR), feed conversion ratios (FCR) and survival rates were investigated in a commercial-scale culture system. High density cultures were maintained in a total of nine (12 m³) flow-through systems supplemented with O₂. Initial stocking rates for 3 different treatments with three replicates were 26.88±0.30 (low density), 36.72±0.26 (medium density) and 55.03±0.14kg m⁻³ (high density), corresponding to a total of 5800, 8000 and 12000 fish/tank, respectively. Water flow rate for each tank was maintained at 11±1.1 L sec⁻¹ (660 L min⁻¹) over the study period for each raceway, and the temperature was 12.3±0.8°C throughout the experimental period. At the end of the study, there were no significant differences in the survival rates and SGR among treatments ($p>0.05$). However, FCR was negatively affected in the high density treatment ($p<0.05$). Overall, our results indicated that oxygen supplementation supported fish growth and survival in high stocking densities. However, high production cost is prohibitive and further studies are required to reduce costs.

Keywords: Rainbow trout, Intensive culture, Stocking density, Oxygen supplementation

1-Department of Basic Sciences, Faculty of Fisheries, Kastamonu University, Kastamonu 37200, Turkey

2-Department of Aquaculture, Faculty of Marine Science and Technology, ÇanakkaleOnsekiz Mart University, Çanakkale 17100, Turkey

*Corresponding author's email: soner_bilen@yahoo.com

Introduction

Aquaculture is one of the fastest-growing sectors of the food production industry in the world. The main reasons for the rapid development include over-fishing of wild populations (Musick *et al.*, 2001), increased demand for fish (Delgado *et al.*, 2003), replacement of fish meal with other ingredients (Rumsey, 1993; Carter and Hauler, 2000; Hu *et al.*, 2008) and the development of new technologies enabling high density aquaculture (Colt *et al.*, 1991, Ellis *et al.*, 2002).

Rainbow trout (*O. mykiss*) is the most popular aquaculture species in Turkey with a production of 108,000 tons (TUIK, 2012). Traditionally, rainbow trout are cultured in raceways but recently, netpen culture in freshwater lakes and coastal areas along the Black Sea coast is gaining popularity. However, in lakes and coastal areas, seasonal high water temperature in summer is a major bottleneck for rearing trout throughout the year. Another problem on raceway production in flow-through systems is limitations due to limited water supply and problems associated with the increasing stocking densities.

Increasing demand for cultured trout and restrictions on availability of suitable areas for raceway culture have forced the industry to adopt new technologies to enable higher stocking densities.

Commercial farmers in Australasia, Europe and North America usually operate within a density range of 15 to 40 kg m⁻³, with 60 kg m⁻³ being seen as a maximum (Ellis *et al.*, 2002). It is known that, under high stocking densities and no support, growth is affected negatively (Boujard *et al.*, 2002). Oxygen supplementation is one

such support and an effective method for achieving higher biomass in commercial finfish culture and recommended oxygen level for salmonids must be >5mg L⁻¹ (Foss *et al.*, 2002). Various methods have been developed for oxygen supplementation including paddle wheel aerators, jet aerators, pressurized packed columns and oxygen diffusing systems. In the present study, we investigated the effects of oxygen supplementation on the survival, growth and feed conversion rates of rainbow trout at three different stocking rates. Oxygen supplementation was provided through oxygen diffusers which is the most extensively used method for Salmonid production in raceways in Turkey. Increased stocking densities through oxygen supplementation without compromising growth and survival will be an effective way towards developing high density Salmonid aquaculture.

Materials and methods

Experimental animals and research area

The present study was conducted at Filiz Su Ürünleri LTD., Kırklareli Province, Turkey, with a batch of Trout lodge Inc. strain rainbow trout. Nine raceways (8X2X0.75 m) were used. Each raceway had a water volume of 12 m³. The raceways were supplied with spring water (pH 7.5, oxygen 9.4 ± 0.6 mg/L, 12.1±0.8°C), and water flow was adjusted to 11 L sec⁻¹ (660 L min⁻¹). Photoperiod was natural. During the experiment, fish were fed with a commercial trout diet (Sibal LTD.; 48 % protein, 18% lipid).

Experimental design

Treatments consisted of low stocking density group (L), medium stocking density group (M) and high stocking density group (H), and designed as triplicate. Total number of fish stocked in each raceway was 5800, 8000 and 12000 fishes for treatments L, M and H, respectively. Rainbow trout were randomly allocated into three different treatments with three replicates each. Weights of the groups were recorded every 30 days until the end of the experiment (90 days). Oxygen levels and temperature were measured regularly (three times a day) one hour after feeding, from the inflow and outflow using an oxygen meter (Oxyguard Hand Polaris) three times in a day long. Water temperature remained constant ($12.3 \pm 0.8^\circ\text{C}$) during experiment in all experimental raceways.

Oxygen Diffuser System

Oxygen was supplied to raceways using an oxygen generator (Oxymat O-230; $20 \text{ kg h}^{-1} \text{ O}_2$ with 89% purity) and delivered to raceways through diffusers (Akuamaks; 30 cm in length) for maximum efficiency. In order to keep oxygen levels identical ($6.4\text{--}8.9 \text{ mg L}^{-1}$) in each treatment with different

stocking densities, 4, 6 and 8 diffusers/raceway were used in treatment L, M and H, respectively.

Growth

Total food consumption (FC) was calculated as the difference between food supplied and food wasted for the whole experimental period. The specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated as follows:

$$\text{SGR (\% day}^{-1}\text{)} = 100 \times [(\ln \text{ final fish weight}) - (\ln \text{ initial fish weight})] / \text{days fed.}$$

$$\text{FCR} = \text{feed intake (g)} / \text{weight gain (g)} \times 100$$

$$\text{PER} = \text{wet body mass gain} / \text{crude protein intake}$$

Data analysis

Each value was expressed as mean \pm standard error of mean (SEM) for each parameter measured. Two-way ANOVA was used to determine the interaction between density and time. Statistical significance ($p < 0.05$) was determined by one-way analysis of variance (ANOVA) followed by a Tukey's multi comparison test (SPSS 17.0) (Table 1).

Table 1: Results of two-way analysis of variance (ANOVA). (Significance levels are $p < 0.05$. NS, $p > 0.05$).

	Density	Time	Density x Time
Average weight	*	*	*
Feed consumption	*	*	*
SGR	NS	*	*
FCR	NS	*	NS
PER	*	*	NS

SGR: Specific Growth Rate, FCR: Feed Conversion Ratio, PER: Protein Efficiency Ratio

Results

One-way analysis of variance (density \times time) showed significant interaction for average weight, feed consumption and SGR values but not for FCR and PER (Table 2).

Oxygen contents of inflow and outflow for each raceway were similar during the experimental period, and varied between $6.8 \text{ mg}^{-1}\text{L}$ and $8.9 \text{ mg}^{-1}\text{L}$ in the inlets, and between $6.4 \text{ mg}^{-1}\text{L}$ and $8.9 \text{ mg}^{-1}\text{L}$ in the

outlets. The amount of oxygen in raceways did not change significantly in each treatment ($p>0.05$).

Table 2: Fish performance and feed utilization for trout containing different stocking densities for 90 days. (Different letters in the same line indicate significant differences within groups ($p<0.05$). Values are mean \pm SEM (n=3)).

	Stocking density		
	Low	Medium	High
Average initial fish weight (g)	55.62 \pm 1.07	55.08 \pm 0.67	55.03 \pm 0.24
Average final fish weight (g)	195.83 \pm 5.45 ^a	190.10 \pm 10.65 ^a	183.62 \pm 3.46 ^a
Weight gain (%)	252.14 \pm 5.73 ^a	245.07 \pm 9.40 ^a	233.71 \pm 4.44 ^a
Feed consumption (kg)	691.00 \pm 10.69 ^a	1022.67 \pm 78.16 ^b	1543.00 \pm 104.53 ^c
FCR	0.83 \pm 0.04 ^a	0.94 \pm 0.08 ^{ab}	1.10 \pm 0.02 ^b
SGR	1.45 \pm 0.03 ^a	1.30 \pm 0.08 ^a	1.48 \pm 0.06 ^a
PER	0.50 \pm 0.02 ^a	0.34 \pm 0.02 ^b	0.19 \pm 0.02 ^c
Survival (%)	98.19 \pm 1.14 ^a	97.79 \pm 0.51 ^a	97.79 \pm 0.73 ^a

SGR: Specific Growth Rate, FCR: Feed Conversion Ratio, PER: Protein Efficiency Ratio

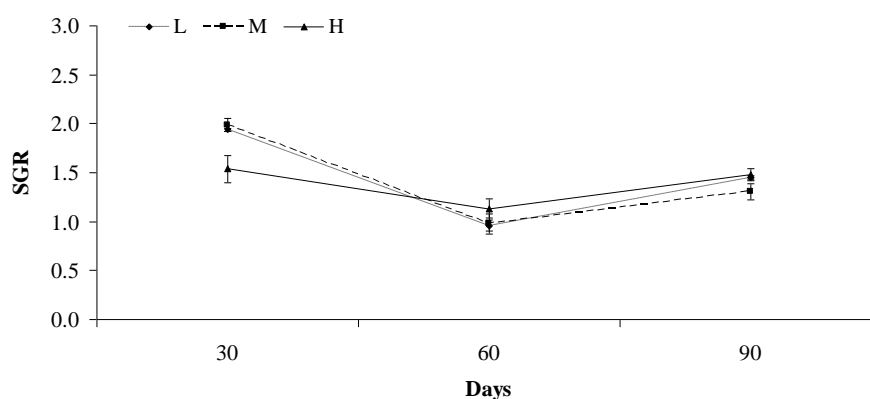


Figure 1: SGR of trout different stocking density over a 90-day period.

L: low stocking density group, M: medium stocking density group, H: high stocking density group

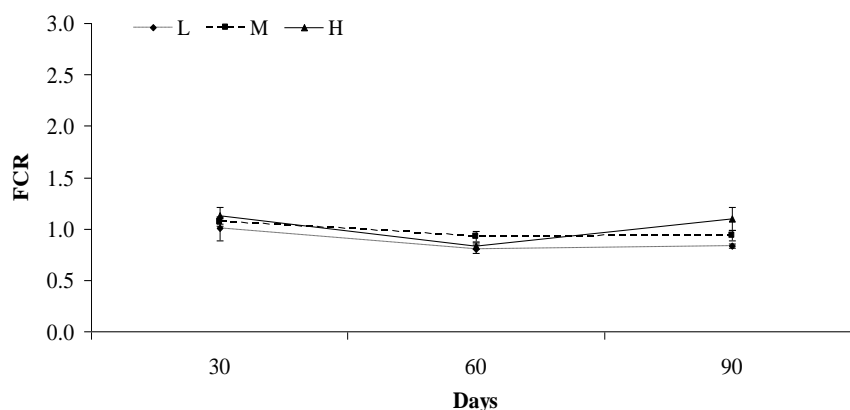


Figure 2: FCR of trout different stocking densities over a 90-day period.

L: low stocking density group, M: medium stocking density group, H: high stocking density group

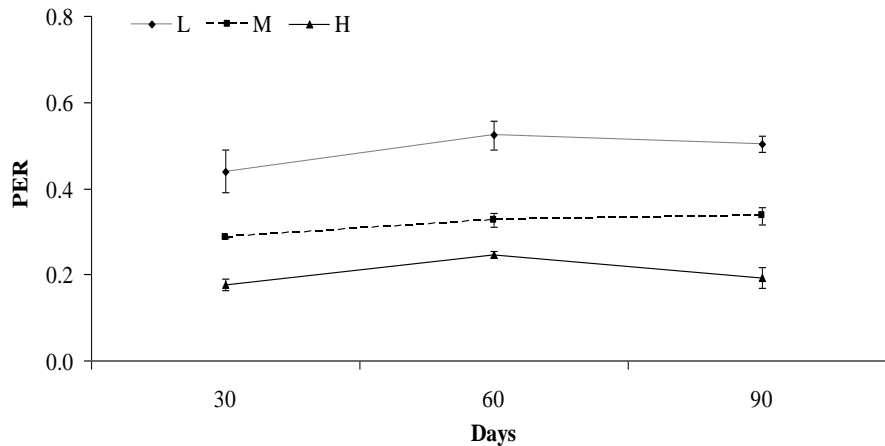


Figure 3. PER of trout different stocking densities over a 90-day period.

L: low stocking density group, M: medium stocking density group, H: high stocking density group

No significant differences were observed in average fish weight (Fig. 1) among treatments ($p>0.05$). Mortality was low for

all experimental groups during the study and did not change among treatments ($p>0.05$).

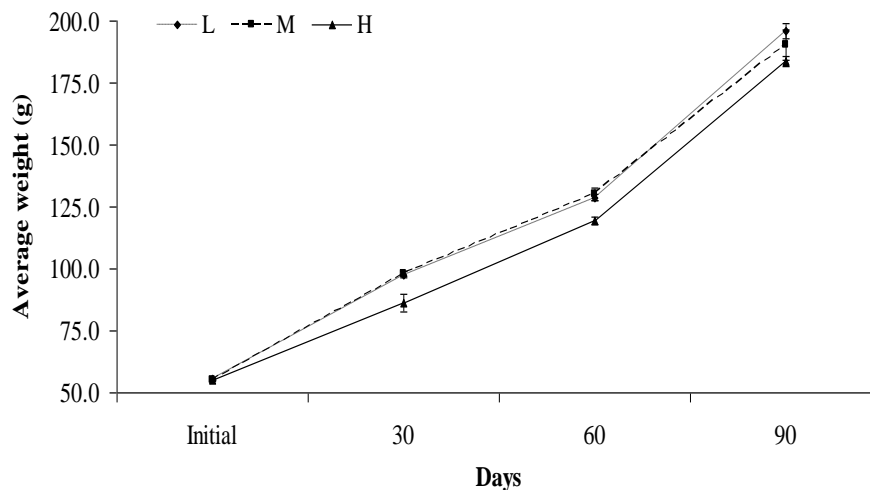


Figure 4: Weight of trout different stocking densities over a 90-day period.

L: low stocking density group, M: medium stocking density group, H: high stocking density group

Average densities and total number of fish for each raceway are given in Table 3. Stocking densities of different treatments increased to 92.92 ± 1.16 in L, 127.16 ± 2.77 in M and 179.55 ± 2.45 in H. SGR did not show significant differences among

treatments (Figs. 2, 3). FCR was negatively affected when stocking density was high especially in treatment H ($p<0.05$). However, the lowest PER (Fig. 4) was observed in treatment H and the highest value was observed in treatment L.

Table 3: Quantity and density for trout containing for 90 days. (Data at the same experimental time with different lower case is significantly different among treatments ($p < 0.05$)).

Treatment	Experimental periods							
	Initial		30 th day		60 th day		90 th day	
	Quantity	Density	Quantity	Density	Quantity	Density	Quantity	Density
L	5800	26.88±0.30	5701.00±65.00 ^c	46.37±0.97 ^c	5699.67±65.34 ^c	61.14±0.32 ^c	5695.00±66.00 ^c	92.92±1.16 ^c
M	8000	36.72±0.26	7916.33±71.67 ^b	64.66±1.29 ^b	7908.33±67.67 ^b	85.75±1.44 ^b	7822.67±41.01 ^b	127.16±2.77 ^b
H	12000	55.03±0.14	11975.33±12.67 ^a	85.93±3.53 ^a	11866.33±59.33 ^a	118.08±1.87 ^a	11734.00±88.07 ^a	179.55±2.45 ^a

L: low stocking density group, M: medium stocking density group, H: high stocking density group

Discussion

In salmonid culture, stocking density varies depending on the size of fish, culture area and culture methods (North *et al.*, 2006; Larsen *et al.*, 2012). At high stocking densities carbon dioxide and ammonia have negative effects on fish health and growth performance without additional oxygen supplementations (Cooke *et al.*, 2000; Boujard *et al.*, 2002; North *et al.*, 2006). In addition, Vijayan and Leatherland (1988) mentioned that poor growth in brook charr, *Salvelinus fontinalis* reared at high densities were associated with increased activities of key metabolic enzymes in liver and muscle. High density reduces specific growth rate and growth performance in the rainbow trout, even when water quality is high (Larsen *et al.*, 2012; McKenzie *et al.*, 2012). Similarly, Ruyet *et al.* (2008) observed negative results on growth when stocking density was high (120 kg m⁻³). However, in our study results showed no significant differences on growth performance among different treatment. Foss *et al.* (2003) determined an interacting effect of oxygen saturation and ammonia

level on growth and reported that growth rate was significantly higher in the hyperoxic (14.5 mgL⁻¹) NH₃ group compared to that of the normoxic/NH₃ group. In the present study, oxygen contents in experimental raceways were always kept at high levels (6.6-8 mg/L).

FCR is another parameter which was affected negatively at high densities (Ellis *et al.*, 2002; Larsen *et al.*, 2012; McKenzie *et al.*, 2012). In the present study, no differences in FCR's were observed in the L and M treatments. However, FCR in treatment M was significantly higher than H and L treatments. SGR and FCR showed negative correlation when the stocking density was high; suggesting that oxygen content has beneficial effects on SGR but not on FCR. Survival rates in all treatments were very high with no significant differences. In contrast to our findings, some reports showed that increasing stocking density had negative effects on fish health, especially on fin erosion (Winfrey *et al.*, 1998), reduced immunity (Miller *et al.*, 1995; North *et al.*, 2006) and

losses. In this study, we did not observe any significant differences in mortality.

In this study, oxygen levels and temperature were similar in all treatments. In terms of growth performance, SGR did not differ among treatments while FCR values showed significant differences. Oxygen supplementation via oxygen diffusers did not have any positive or negative effect on growth performance at higher stocking densities. However, the use of oxygen generator has an additional cost. In addition, increased FCR value is associated with higher feeding cost compared to that of the normal stocking densities. Further work is required to lower costs for oxygen supplementation and feeding. The present study may offer fish farmers an effective method to enhance O₂ concentrations in the water column using oxygen diffuser systems at high stocking densities. Such an approach may prove useful when farmers are limited with culture area or freshwater supply.

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References

- Boujard, T., Labbe, L. and Auperin, B., 2002.** Feeding behaviour, energy expenditure and growth of rainbow in relation to stocking density and food accessibility. *Aquaculture Research*, 33, 1233-1242.
- Carter, C.G. and Hauler, R.C., 2000.** Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture*, 185, 299–311.
- Colt, J.E., Orwicz, K. and Bouck, G., 1991.** Water quality considerations and criteria for high-density fish culture with supplemental oxygen. In: J. Colt, R.J. White (Eds.), Presented at the Fisheries Bioengineering Symposium, 10th edition, American Fisheries Society, Bethesda, MD: pp.372–385.
- Cooke, S.J., Chandroo, K.P., Beddow, T.A., Moccia, R.D. and McKinley, R.S., 2000.** Swimming activity and energetic expenditure of captive rainbow trout *Oncorhynchus mykiss* (walbaum) estimated by electromyogram telemetry. *Aquaculture Research*, 31, 495–505.
- Delgado, C.L., Wada, N., Rosegrant, M.W., Meijer, S. and Ahmed, M., 2003.** Fish to 2020: Supply and demand in a changing World. IFPRI, Washington, DC.
- Ellis, T., North, B., Scott, A.P., Bromage, N.R., Porter, M. and Gadd, D., 2002.** The relationships between stocking density and welfare in farmed rainbow trout. *Journal of Fish Biology*, 61, 493-531.
- Foss, A., Evensen, T.H. and Øiestad, V., 2002.** Effects of hypoxia and hyperoxia on growth and food conversion efficiency in the spotted wolffish *Anarhichas minor* (Olafsen). *Aquaculture Research*, 33, 437–444.
- Foss, A., Vollen, T. and Øiestad, V., 2003.** Growth and oxygen consumption in normal and O₂ supersaturated water, and interactive effects of O₂ saturation and ammonia on growth in spotted

- wolffish (*Anarhichas minor* Olafsen). *Aquaculture*, 224, 105-116.
- Hu, M., Wang, Y., Wang, Q., Zhao, M., Xiong, B., Qian, X., Zhao, Y. and Luo, Z., 2008.** Replacement of fishmeal by rendered animal protein ingredients with lysine and methionine supplementation to practical diets for gibel carp, *Carassius auratus gibelio*. *Aquaculture*, 275, 260–265.
- Larsen, B.K., Skov P.V., McKenzie, D.J. and Jokumsen, A., 2012.** The effects of stocking density and low level sustained exercise on the energetic efficiency of rainbow trout (*Oncorhynchus mykiss*) reared at 19°C. *Aquaculture*, pp.324-325, 226-233.
- McKenzie, D.J., Höglund, E., Dupont-Prinet, A., Larsen, B.K., Skov, P.V., Pedersen, P.B. and Jokumsen, A., 2012.** Effects of stocking density and sustained aerobic exercise on growth, energetics and welfare of rainbow trout. *Aquaculture*, pp. 338-341, 216-222.
- Miller, S.A., Wagner, E.J. and Bosakowski, T., 1995.** Performance and oxygen consumption of rainbow trout reared at two densities in raceways with oxygen supplementation. *Progressive Fish-Culturist*, 57, 206–212.
- Musick, J.A., Harbin, M.M., Berkeley, S.A., Burgess, G.H., Eklund, A.M., Findley, L., Gilmore, R.G., Golden, J.T., Ha, D.S., Huntsman, G.R., McGovern, J.C., Parker, S.J., Poss, S.G., Sala, E., Schmidt, T.W., Sedberry, G.R., Weeks, H. and Wright, S.G., 2001.** Marine, estuarine, and diadromous fish stocks at risk of extinction in North America (exclusive of Pacific salmonids). *Fisheries*, 25, 6-30.
- North, B.P., Ellis, T., Turnbull, J.F., Davis, J. and Bromage, N.R., 2006.** Stocking density practices of commercial UK rainbow trout farms. *Aquaculture*, 259, 260-267.
- Rumsey, G.L., 1993.** Fish meal and alternative sources of protein. *Fisheries*, 18, 14-19.
- Ruyet, J.P.L., Delliou, H.L., Bayon, N.L., Severe, A., Roux, A.L., Le, H., Quemener, L., 2008.** Combined effects of water quality and stocking density on welfare and growth of rainbow trout (*Oncorhynchus mykiss*). *Aquatic Living Resource*, 21, 185–195.
- TÜİK, 2010.** Fishery Statistics. Turkish statistical Institute, Ankara, 71P.
- Vijayan, M.M. and Leatherland, J.F., 1988.** Effect of stocking density on growth and the stress-response in brook charr, *Salvelinus fontinalis*. *Aquaculture*, 75, 159–170.
- Winfrey, R.A., Kindschi, G.A. and Shaw, H.T., 1998.** Elevated water temperature, crowding, and food deprivation accelerate fin erosion in juvenile steelhead. *Progressive Fish Culturist*, 60, 192-199.