www.roavs.com EISSN: 2223-0343

RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Research article DOI: 10.20490/ROAVS/16-020

Effects of stocking density on growth performance, survival rate, and haematological parameters in rainbow trout (*Oncorhynchus mykiss*) fingerlings

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Article history
Received: 29 Nov, 2015
Revised: 14 May, 2016
Accepted: 18 May, 2016

Abstract

The present study evaluated the effects of stocking density on growth performance, survival rate, and haematological parameters rainbow trout (*Oncorhynchus mykiss*) fingerlings. An experiment was conducted for 8 weeks with different stocking densities including T1: 10 kg/m^3, T2: 15 kg/m^3, T3: 25 kg/m^3 and T4: 35 kg/m^3. After 8 weeks, the juveniles were randomly sampled and analyzed for growth indices including weight gain, survival, specific growth rate (SGR), feed conversion rate (FCR), and condition factor (CF) as well as haematological parameters including haemoglobin (Hb), haematocrit (Hct), mean crepuscular haematocrit (MCH), mean crepuscular haematocrit concentration (MCHC), and red blood cells (RBC). Treatments with higher density showed lower growth rates (SGR and weight gain) compared to those of lower densities. Highest survival rate was observed in T2 and the lowest in T4. Based on the results, the stocking density significantly affected Hct, Hb, and RBC (P<0.05), while MCH and MCHC were not significantly affected (P<0.05). The present study demonstrated that there is a significant negative effect of increasing rearing density on juvenile rainbow trout. Further, it is suggested 15 kg/m^3 as an appropriate stocking density for rainbow trout fingerlings.

Keywords: Stocking density; growth; haematological parameter; fish

To cite this article: Eagderi S, M Rahimi, N Rostamian and SA Habibi, 2016. Effects of stocking density on growth performance, survival rate, and haematological parameters in rainbow trout (*Oncorhynchus mykiss*) fingerlings. Res. Opin. Anim. Vet. Sci., 6(4): 115-120.

Introduction

Environmental stressors are important factors limiting fish performance under aquaculture conditions (Pickering, 1992; Wedemeyer, 1997; Ellis et al., 2002). When fish are subjected to adverse environmental conditions, some of their endocrine and physiological properties alter, often resulting in changes the ability of the fish to survive, grow and reproduce (Pickering, 1992; Barton and Iwama, 1991; Trenzado et al., 2008). In intensive fish culture systems, the increasing density is applied to intense the production (Hasanalipour et al., 2013). However, high stocking density has been considered as an aquaculture-related chronic stressor

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(Vijayan and Leatherhead, 1988; Wedemeyer, 1997; Barton and Iwama, 1991) and poor growth and increasing diseases are often observed at high density. An important factor influencing the growth of fish is the development of hierarchies, mediated by intraspecific competition for food, and this is greatly affected by stocking density (Sirakov and Ivanchev, 2008). Jobling and Baardvik (1994) showed that the manipulation of stocking densities may inhibit or reduce the effects of such hierarchies in Arctic charr, *Salvelinus alpinus.*

Many works have been focused on evaluating the effects of rearing density on growth (Holm et al., 1990; Bjørnsson, 1994), survival (Hasanalipour et al., 2013), food intake (Jørgensen et al., 1993) and hormonal related changes (EL-Khalid, 2010). Stocking density has been shown to affect behavioural interactions in several fish species (Brown et al., 1992; Christianssen et al., 1992; Irwin et al., 1999; Trenzado et al., 2006) and may ultimately affect growth rates. The effect of stocking density on growth has been reported for a range of cultured fish species such as gilthead seabream, *Sparus aurata* (Montero et al., 1999), juvenile turbot, *Scophthalmus maximus* (Irwin et al., 1999), rainbow trout *Oncorhynchus mykiss* (North et al., 2006), Japanese flounder, *Paralichthys olivaceus* (Sergio et al., 2006), rainbow trout *Oncorhynchus mykiss* (Trenzado et al., 2008), silver perch *Bidyanus bidyanus* (Rowland et al., 2006), halibut *Hippoglossus hippoglossus* (Bjørnsson, 1994), Arctic charr, *Salvelinus alpinus* (Jørgensen et al., 1993), rainbow trout *Oncorhy-nchus mykiss* (Zoccarato et al., 1992), and Atlantic salmon *Salmo salar* (Liu et al., 2014).

Both positive and negative relationships between stocking density (SD) and growth performance have been reported, and the pattern of this interaction appears to be species specific. In addition, intraspecific competition may influence the growth of reared fish (Sirakov and Ivanchev, 2008). This has been supported by observations on a number of aquatic species, including chum salmon *Oncorhynchus keta* (Davis and Olla, 1987) and freshwater prawns *Macrobrachium rosenbergii* (Ra'anan and Cohen, 1984). It has also been reported that high stocking density affects some haematological parameters. Though the results of haematological parameters are widely variable and not always conclusive, many authors reported alternations of the haematological parameters as a common effect of fish crowding (Trenzado et al., 2006, 2007). Therefore, this study was conducted to investigate the effect of density on growth performance, survival rate, and haematological indicators in rainbow trout, *Oncorhynchus mykiss* fingerlings.

Materials and Methods

Experimental design and procedures

A total of 12 similar canals (10×1×1) were used as four triplicate treatments with different stocking densities. The stocking densities were 10 kg/m^3 (T1 as control), 15 kg/m^3 (T2), 25 kg/m^3 (T3), and 35 kg/m^3 (T4), respectively. Rainbow trout fingerlings had an average initial weight of 2.1 ± 0.15 g. Well-water with a flow rate of 30 l/sec, after passing through a spiral channel was divided equally among the 12 rearing canals. Mean recorded pH and dissolved oxygen (DO), temperature and photoperiod were 7.8, 8.5 ppm, 10-13°C and 10L: 14D, respectively, during experiment. Juveniles were fed at a rate of 3-5% of body weight five times a day (8, 11, 13, 15 and18 hrs). Diet formulations and its proximate compositions are shown in Table 1.

Sample collection and growth index

A total of 30 juveniles were randomly sampled from each canal every ten days. They were anesthetized using 1% clove powder solution and their weights and lengths were measured by digital scale to the nearest 0.01 g and a digital calliper to the nearest 1 mm, respectively. At the end of the experiment, the fish of each pond (n= 40) were analyzed for growth indices, including weight gain, survival, Specific Growth Rate (SGR), Feed Conversion Rate (FCR), and Condition Factor (CF) based on the following formula (Das and Ray, 1989):

Weight gain (g) = Final weight - Initial weight
FCR = Dry feed intake / Wet weight gain
SGR = 100 × (Ln final weight - Ln initial weight) / 56 days
Survival (%) = 100 × (initial fish number − dead fish number) / initial fish number
CF = (W/ L^3) × 100

Haematological analysis

At the end of the experiment, fish were sampled from each group (n = 15) and anesthetized immediately by MS-222 (100 mg/l) and then, the blood samples were taken from their heart using a heparinized syringe. The changes in the haematological parameters, including haematocrit, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cells (WBC), and Red Blood Cells (RBC)] were measures based on method of Haghighi (2009). Haemoglobin was measured using a test kit (Pars Azmun CO., Tehran, Iran) with colorimetric method on a spectrophotometer using 540 nm (Haghighi, 2009).

Statistical analysis

For statistical analysis, normality of the data was analyzed by one-sample Kolmogorov-Smirnov test. In the case of normal data, two-way ANOVA was used to analyze the data by the use of SPSS statistical software. The data were compared using Duncan's test at a significance level of 5%.

Results

After 8 weeks of the experiment, treated fish with lower density i.e., 10 and 15 kg/m^3, significantly ($P<0.05$) showed greater body weight and SGR but lower FCR than the higher density (Table 2, Figure 1). Greater weight gain was found in T2 (17.86 ± 0.4 g) and the lowest weight gain was observed in T4 (14.3±1.4 g). The estimated SGR in T2 was significantly ($P<0.05$) higher than those in T1, T3, and T4 ($P<0.05$), although T3 and T4 were not significantly different ($P>0.05$). Low FCR was observed in T2, that was no significantly different from T1, whereas T2 showed a significant difference with T3 and T4 ($P<0.05$). The CF had no significant difference between treatments ($P>0.05$). Highest survival rate was observed in T2 and the lowest rate in T4. There was a significant difference ($P>0.05$) in the survival rates of fingerlings among treatments. Survival rate was significantly ($P<0.05$) lower in T4 compared to the control.

The stocking density had a significant effects on Hct, Hb, and RBC ($P<0.05$), whereas other haematological parameters i.e., MCH and MCHC were not significantly affected ($P>0.05$; Table 3). Values of Hb, Hct, and erythrocytes in fish with high stocking density i.e. 25 and 35 kg/m^3, were significantly higher than those treatments with low stocking density i.e. 10 and 15 kg/m^3 (Table 3).

Discussion

In this study, the juveniles reared at the higher stocking densities (T3, T4) showed significantly slower growth rates than those treatments with lower stocking densities (T2, T1). The highest growth was recorded in a density of 15 kg/m^3 (T2) with an increased weight of 17.86 g and the lowest growth in a density of 35 kg/m^3 (T4) with an increased weight of 14.3 g. Growth, feed efficacy and feed consumption of fishes are governed by environmental factors (Brett, 1979). Many authors have suggested that increasing density has a negative effect on growth factors (Zoccarato et al., 1992; Ross and Watten, 1998; Irwin et al., 1999; Montero et al., 1999; Trenzado et al., 2006; Gholipour et al., 2006; Trenzado et al., 2007; Trenzado et al., 2008;) i.e. overcrowding is a common chronic stressor in aquaculture (Zoccarato et al., 1992). High stocking density suppresses growth (Ross and Watten, 1998; Irwin et al., 1999; Rowland et al., 2006), which has been attributed to several factors such as decreased food consumption. In addition, high stocking density imposes increased energy demands that require fish to cope with metabolic adjustments such as changes of gluconeogenic and glucolytic enzyme activities. Under such a condition, food consumption is reduced and the extra expenditure of energy has to be met by the body reserves, resulting in reduced growth (Trenzado et al., 2007, 2008; Garcia et al., 2007). Also, the negative impact of the stocking density on growth may be due to increased social interactions between individuals. These interactions are led to intraspecific size variation in groups resulting from the suppression of growth of subordinate individuals by larger conspecifics (Yamagishi et al., 1974; Jobling, 1995; Koebele, 1985).

In some works, numbers of fish are reduced as the fish grow to maintain constant biomass stocking densities (Papst et al., 1992); whereas, in this study, numbers of fish were kept constant during the experiment and, therefore, biomass density was allowed to increase due to the growth of the fish and based on the results the maximum biomass was observed in treatment with a density of 15 kg/m^3 (T2). There is also evidence that removing fish may disrupt hierarchies, and the resulting high levels of interactions between individuals following changes in group structure may affect growth rates (Jobling and Baardvik, 1990).

Growth in terms of weight, weight gain and SGR of fingerlings was significantly higher in T2 where the stocking density was low compared to those of T3 and T4. The low growth rate of fry and fingerling in treatments T3 and T4 appears to be related to higher densities and increased competition for food and space (Haque et al., 1994; Islam et al., 1999; Islam, 2002; Islam et al., 2002; Rahman and Rahman, 2003; Chakraborty and Mirza, 2005).

The FCR values of T2 and T1 were significantly lower than those of T3 and T4, respectively. The FCR values are lower than those reported by Das and Ray (1989), Islam (2002), and Islam et al. (2002). De Silva and Davy (1992) stated that digestibility plays an important role in lowering the FCR value by efficient utilization of food. Digestibility, in turn, depends on daily feeding rate, frequency of feeding, and type of used food (Chiu et al., 1987). However, low FCR value in the treatment T2 of the present study indicates better food utilization efficiency, despite this values increased with increasing the stocking densities.

The fingerlings had significantly lower survival rate in highest stocking density i.e. T4. The reason for reduced survival rate in this treatment was probably due to higher stocking density of fry as well as competition for food and space. Similar results were obtained by Tripathi et al. (1979), Uddin et al. (1988), Haque et al. (1994), Kohinoor et al. (1994), Rahman and Rahman (2003) and Chakraborty and Mirza (2005).

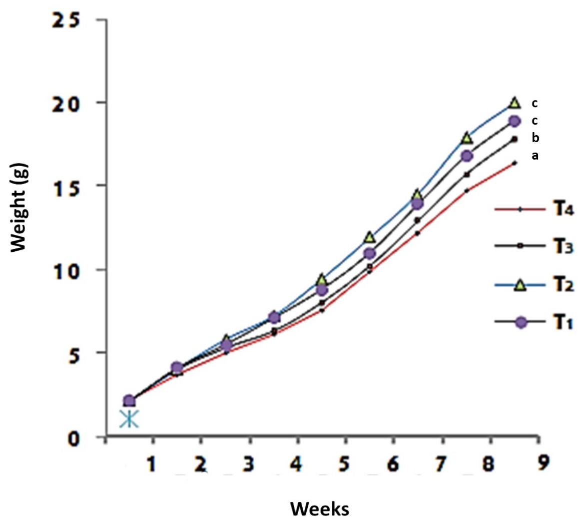

Fig. 1: Changes in the weight of the reared rainbow trout fingerlings at different stocking densities during experiment (T1:10 kg/m^3, T2:15kg/m^3, T3: 25 kg/m^3 and T4: 35kg/m^3).

Table 1: Diet formulations and its proximate compositions

Ingredient	Amount
Fish meal	58
Wheat flour	14
Meat flour	12
Dextrin	5
Fish oil	6
Vegetable Oil	2.2
Filler (sawdust)	0.8
Mineral mixturec [1]	1
Vitamin mixture (vitamin E free)[2]	1
Proximate composition (analyzed)	
Moistur	10 ±0.8
Crude protein	49±1
Crude lipid	15.1±0.8
NFE	16.5±.92
Energy(Kcal/gr)	380

Stocking density and growth rates are often reported to be related; however, the relationships between these two may not be uniformly positively or negatively linear for a given species. In some species e.g. Arctic charr (*Salvelinus alpinus*) however, a positive effect of density on growth is reported (Papst et al., 1992). For example, Baker and Ayles (1990) suggested that growth of Arctic charr increased with stocking density up to a threshold of 40-50 kg/m^3 and then declined at higher densities. Bjornsson (1994) also reported that stocking density affects the growth of halibut (*Hippoglossus hippoglossus*) only above a certain threshold level corresponding to approximately 100% coverage of the tank bottom.

It has also been reported that high stocking density affects some haematological parameters. The results are, however, widely variable and not always conclusive. Therefore, interpreting the effect of stocking density on haematocrit is difficult; the haematocrit may have either elevated in T4 compared with the other treatments, possibly indicating an acute stress response (Barton and Iwama, 1991; North et al., 2006) or, alternatively, it may have reduced in T3 as opposed to T2, possibly suggesting anaemia (North et al., 2006). Previous studies have measured the effect of stocking density on haematocrit in rainbow trout (reviewed by Ellis et al., 2002) suggesting higher haematocrit levels in response to increased stocking density (Papst et al., 1992; Wagner et al., 1996). This response may be a strategy for increasing the oxygen carrying capacity of blood under high energy demand situations such as chronic stress (Montero et al., 1999; Trenzado et al., 2006; Trenzado et al., 2008).

In conclusion, the results of the present study demonstrated that there is a significant negative effect of increasing rearing density in juvenile rainbow trout population; increasing densities also resulted in heterogeneous growth rates and the depression of growth of some individuals.

Table 2: Growth performance and survival of rainbow trout fingerlings after 8 weeks of rearing at different densities

Density(kg/m^3)	Weight gain (g)	SGR	FCR	CF	Survival (%)
10 kg/m^3 (T1)	16.9±0.5 ^{c}	3.44±0.09 ^{b}	1.1±0.02 ^{a}	1.16±0.14^a	89.6±2.6 ^{c}
15 kg/m^3 (T2)	17.86±0.4 ^{c}	3.78±0.06 ^{c}	1.09±0.01 ^{a}	1.15±0.12 ^{a}	91.2±4.04^c
25 kg/m^3 (T3)	15.76±1.1^b	3.28±0.08 ^{a}	1.21±0.08 ^{b}	1.13±0.14^a	86.8±3.5 ^{bc}
35 kg/m^3 (T4)	14.3±1.4^a	3.22±0.04 ^{a}	1.32±0.04 ^{c}	1.14±0.15 ^{a}	79±2.6 ^{a}
The effect of variable					
Density	0.00*	0.04*	0.03 *	0.26	0.00*

Values in the same row having the same superscript are not significantly different (P>0.05); * mean of expression at the level of 0.05.

Table 3: Haematological parameters of rainbow trout fingerlings after 8 weeks of rearing in different stocking densities

Density(kg/m^3)	MCH (pg)	Hct (%)	Hb (g/l)	RBC (10^6/l)	MCHC (g/l)
10 kg/m^3 (T1)	97.1±3.6 ^{a}	29.73±6.8 ^{a}	6±0.36 ^{a}	0.51±0.06^a	18.1±1.87 ^{a}
15 kg/m^3 (T2)	98.1±8.9^a	36.5±4.7 ^{ab}	6.23±0.3 ^{ab}	0.65±0.06 ^{b}	18.25±1.7 ^{a}
25 kg/m^3 (T3)	100.7±6.9 ^{a}	34.9±5.7 ^{ab}	6.9±0.4 ^{bc}	0.69±0.1^b	18.3±1.6 ^{a}
35 kg/m^3 (T4)	100.9±5.4 ^{a}	41.33±6.1 ^{b}	7.5±0.3 ^{c}	0.73±0.1 ^{c}	17.6±1.8^a
The effect of variable					
Density	0.29	0.01 *	0.014 *	0.00 *	0.86

Values in the same row having the same superscript are not significantly different (P>0.05); * mean of expression at the level of 0.05.

Acknowledgments

The kind assistance of the management of Tehran Ghezel Ala Co. is appreciated.

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