

Increased reproductive performance of rainbow trout, *Oncorhynchus mykiss* with moderate food restriction

Kohsari H.^{1*}

Received: November 2022 Accepted: February 2023

Abstract

Producing high-quality eggs is one of the aspects of rainbow trout breeding. Diets used vary among rainbow trout breeders and are usually not designed to optimize egg production. Although, the reduction of feeding is important due to the reduction of costs of production. The aim of the recent study was to investigate the effect of moderate food restriction on the reproductive performance of female rainbow trout. For this purpose, two feeding strategies were compared during the four months before the second reproduction. Females in the control group were fed ad libitum with a commercial trout diet. In the experimental group, females were fed 75% of ad libitum (restricted feeding). An artificial photoperiod program was carried out in summer to trigger reproduction. At the end of the experiment, the weight of the females in the restricted feeding group was lower than females in the ad libitum group (p=0.04) whereas gonadosomatic index (GSI) and hatching rate of eggs in the females in the restricted feeding group was significantly higher than females in the ad libitum group. Females in the restricted feeding group produced larger eggs than females in the ad libitum group. They produced a lower number of non-viable eggs compared to the females in the ad libitum group too. The results of the study showed that feed intake of female rainbow trout broodstock can be reduced by up to 25% without having a negative effect on egg production and quality.

Keywords: Food restriction, Reproductive performance, Rainbow trout

¹⁻ Department of Veterinary, Agriculture Faculty, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran.

^{*}Corresponding autor's Email: kohsari.hesam@yahoo.com

Introduction

The reproductive cycle of rainbow trout (Oncorhynchus mykiss) is affected by photoperiod and environmental temperature. The recruitment of oocyte and early vitellogenesis are stimulated by the gradual increase of photoperiod, while the synchronization of late vitellogenesis and ovulation are depended on the shortness of the photoperiod (Whitehead et al., 1978). In order to produce eggs throughout the year, out of the spawning season, photoperiod can be manipulated. Using a long photoperiod in winter induces early summer spawning. Out-of-season spawning sometimes causes the heterogeneity response of female trouts and suboptimal egg quality (Bonnet et al., 2007).

Various factors affect the reproductive performance and quality of the eggs of the female trout. The quality of nutrition of broodstock is one of the factors that affect the quality of eggs and larvae (Migaud et al., 2013). During oocyte development, nutrients are transported from the blood to the oocyte and provide sufficient nutrients to support the growth and development of the embryo until exogenous feeding (Izquierdo et al., 2001).

The hypothesis of the recent study was that a 25% reduction in the diet does not have a negative effect on the quality of eggs and reproductive performance of broodfish rainbow trout. The aim of the recent study was to investigate the effect of moderate food restriction (i.e., 25%) on the reproductive performance in female rainbow trout.

Materials and methods

The recent study was carried out in the research department of one of the ponds for the rainbow trout breeding near the city of Kermanshah, Iran. The research was conducted in accordance with the principles of local Bioethics Committee of Veterinary Faculty of Kermanshah University. Female rainbow trouts from autumn-spawning strain were reared under natural photoperiod conditions for two years until their first reproduction. Two months after reproduction, 72 female rainbow trouts were weighed, tagged, and randomly transferred to one of the 4 outdoor $2m^2$ tanks. The weight of the rainbow trouts was 1523±289 g. Female rainbow trouts were fed with a special commercial rainbow trout broodstock diet (11% crude fat, 1.7% phosphorus, 50% crude protein, 11.5% ash, 18 MJ/kg gross energy, VIT.D3 2800 IU, VIT.E 400 mg kg-1, VIT.A 16000 IU, VIT.C 1000 mg kg-1, Omega-6 0.5%, Omega-3 2.7%; Le Gouessant, Lamballe, France) for 5 months, then two different nutritional protocols were implemented. Half of the female rainbow trouts (n=36) were fed with free feeding (ad libitum (AL)) and the other half (n=36) were given restricted food (R). In the R group, females were fed 75% of ad libitum. The rainbow trouts were fed five times a day. The fish were considered satiation when only a few pellets remained at the bottom of the tank. During the 4 months of the experiment, an artificial photoperiod program was carried out to obtain the secondary reproductive period during the summer. The water renewal rate was 50% per hour. The temperature was about 10°C and it was checked twice a day. During the spawning season, female fish were checked for ovulation once a week by manual pressure on the abdomen. Feeding of the fish was stopped when the first ovulation was detected. Two days after detection of ovulation, the fish were sacrificed and weighed. Ovarian fluid and eggs were sampled and weighed for each fish. The GSI was calculated from the following equation: (egg+ovary) weight/total body weight for each fish. Then approximately 600 eggs were fertilized with sperm collected from male fish fed AL with a commercial diet, then the hatching rate was evaluated for each fish. After that, the eggs were transferred to the individual incubator. Dead eggs and embryos were removed periodically and the survival rates were evaluated according to the total number of eggs in the hatching stages. Furthermore, the of percentage morphological abnormalities in embryos was evaluated after resorption the yolk sac. An image of egg sample was obtained by using the VisEGG system: a dedicated shooting system including of digital SLR camera (canon EOS 1000D, resolution: 10.1M pixels) equipment and light tablet. A picture processing algorithm was next developed and coded as a VBA macro with Visilog 7.3 software (Thermo Scientific). This software allows the automatic detection and individual of the

automatic eggs until make measurements for each egg (number, size, white egg percentage (non-viable eggs)). The eved eggs were reared in the pool under normal photoperiod and water temperature of about 17 °C. To evaluate the growth of the fish, they were weighed every 2 weeks. The juvenile fish were fed with a commercial diet (61% crude protein, 17% crude fat, 23.8 MJ/kg gross energy; Skretting, Stavenger, Norway) 5 times a day. The survival and growth rates of fish were calculated 80 days after rearing. Data analysis was done using a SAS (SAS Institute Inc. 2014. SAS® OnDemand for Academics: User's Guide. Cary, NC: SAS Institute Inc.) statistical software.

Results

The comparison of various parameters between treatment and control groups is shown in the Table 1.

Linear regression showed that egg diameter and absolute fecundity have a significant and positive relationship with GSI. (Pearson coefficient=0.53 and 0.65 respectively, *p*-value<0.01 for both parameters).

Table 1: Comparison of parameters between treatment and control groups.				
Parameter	Ad libium	Restricted feed	<i>P</i> -value	n (per treatment)
Survival value (%)	96.93±2.92	96.87±2.83	0.7	36 females
Fish weight (g)	3050.01±560.37	2713.23±439.2	0.04	36 females
Fish length (mm)	489.46±31.96	482.05 ± 29.49	0.2	36 females
GSI	12.12±3.40	13.85 ± 3.20	0.04	36 females
Egg spawn weight (g)	353.53±11.12	333.12±14.13	0.6	36 spawns
Ovarian fluid weight (g)	62.86±9.78	75.12±9.33	0.4	36 spawns
Absolute fecundity	6952.38±832.12	6236.33±713.4	0.5	36 spawns
Egg diameter (mm)	4.42±0.37	4.53±0.29	< 0.001	16730 eggs
Egg white 24h after spawning (%)	5.30±0.42	1.53±0.29	0.02	36 spawns
Hatching rate (%)	55.54±8.10	70.12±7.40	0.03	36 spawns
Fingerling survival 80 dph (%)	92.15±2.58	81.12±4.13	0.6	1022 fingerlings
Fingerling weight 80 dph (g)	10.53±0.5	11.09 ± 1.2	0.5	1022 fingerlings

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GSI: Gonadosomatic index. Absolute fecundity: Average number of eggs per fish. dph: days post-hatching.

Discussion

As expected, the weight of fish at the end of the experiment was lower in R group than AL group. It has been demonstrated that feed restriction reduces the growth rate of adipose tissue in fish (Salmeron *et al.*, 2015).

Gonadosomatic index in R group was significantly higher than AL group, and this phenomenon indicates the positive effect of moderate feed restriction on reproductive performance of the female rainbow trout in the recent study. This finding is in contrast with the results obtained from food restriction on salmonids. It has been shown that feed restriction, about 40% of ad labium, has negative effects on maturation and GSI in salmonids (Cleveland et al., 2012; Caldwell et al., 2013). It has been shown that fish modulate allocation of reserves under moderate feed restriction (Cardona et al., 2019). In fish that experience feed restrictions, fat viscera reserves are mobilized to be used in reproduction (Aussanasuwannakul et al., 2011). The results of the recent study indicate that a compensatory physiological mechanism is activated in cases of feed restriction that maintains reproductive activity. The lower GSI in the fish in the AL group compared to the R group in the recent study shows that the excess amounts of fat and protein in the body were not transferred into the eggs in the late phase of vitellogenesis and were stored in other tissues of the body.

Absolute fecundity did not differ between the groups in the recent study. It can be considered that the higher GSI in the fish of R group compared to the AL group is related to the larger diameter of the eggs in the R group. The recent study showed that there is a positive and significant relationship between GSI and egg diameter (p<0.01). This finding is in accordance with the results obtained from another study (Cardona *et al.*, 2019, Weber *et al.*, 2022). It has been demonstrated that high-quality eggs have a higher survival rate during development and produce healthier fry with a higher growth rate (Bobe and Labbe, 2010).

In the recent study, feed restriction led to the production of fewer white eggs (non-viable eggs) and on the other hand, also significantly increased the it hatching rate. In one study, rainbow trouts that tolerated feed restriction compared to rainbow trouts that were fed ad libitum produced eggs with higher amounts of essential fatty acids which necessary for the are normal development of the egg (Izquierdo et al., 2001).

It is known that the aging of the oocyte post ovulation, 7 days or more, has a negative effect on the quality of the egg (Aegerter and Jalabert, 2004). In the recent study, ovulations were checked every 4 days and the eggs were removed 2 days later, so the quality of the eggs did not decrease in any of the groups. In the recent study fry viability 80 days posthatching, was not different between R and AL groups, and this result is in accordance with the results obtained by other researchers (Carrillo et al., 2000; Cardona et al., 2019). It was shown in the recent study that the effect of management on survival and growth rates of fingerlings is greater than the effects which are exerted by the mother.

In conclusion, the results of the recent study showed that feed intake of female rainbow trout broodstock can be reduced by up to 25% without having a negative effect on egg production, egg quality, or survival rate of fingerling. In addition, quality and size of the eggs were increased in feed-restricted fish. The difference in the allocation of food reserves in the body can explain this difference. The female rainbow trouts that were fed ad libitum, stored nutrients in the body or use them for growth, while in restricted fed fish, nutrients were used to develop gonads and increase the quality of the egg.

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