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Effect of Starvation and Refeeding on Gamete Quality and Fertilization in Rainbow Trout (*Oncorhynchus mykiss*) Broodstock

Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Anaçlarında Açlık Döngüsü ve Yeniden Beslemenin Gamet Kalitesi ve Döllenme Üzerine Etkisi

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ABSTRACT

In this study, the impact of starvation and refeeding on broodstocks was observed by examining quality parameters of gametes and fertilization. While the control group fish were fed every day, the male and female members of the group were fed one week apart and placed in starvation after a week of feeding. Weight gain in the control group was the highest. The feed conversion ratio was normal in all groups (0.9-1.3%). Relative fecundity was (935±62 eggs/kg) in control group females; it was found to be (1317±241 eggs/kg) in starving females and statistically different in the groups ($p < .05$). The egg diameter of the group receiving intermittent feeding for a week (3.36±0.2 mm) was found to be the lowest. All spermatological parameters were similar between groups, except for sperm volume. The most sperm count was seen in the group that received one-week intermittent feeding (46.9± 20 ml). The results of fertilization with the control female in the fertilization study based on male individuals showed similarity for the male individuals of the trial group, the highest fertilization rate was seen in fertilization using the control female and control male.

Keywords: Broodfish feeding, Egg quality, Reproductive performance

ÖZ

Bu çalışmada, açlık ve yeniden besleme rejimi uygulanan anaçlarda, bu besleme düzeninin gamet kalitesi ve döllenme üzerindeki etkisi incelenmiştir. Kontrol grubu balıkları her gün beslenirken, deneme grubunun erkek ve dişi bireyleri bir hafta beslenmiş, bir hafta aç bırakılmıştır. Ağırlık artışı en yüksek kontrol grubunda görülmüşken; yemden yararlanma oranı tüm gruplarda normal düzeyde bulunmuştur (%0,9-1,3). Relatif fekondite kontrol grubu dişilerinde 935±62 yumurta/kg; bir hafta açlık rejimi uygulanan balıklarda ise 1317±241 yumurta/kg olarak bulunmuştur ($p < ,05$). En düşük yumurta çapı bir hafta aç bırakılan grupta elde edilmiştir (3,36±0,2 mm). Sperm hacmi dışında tüm spermatolojik parametreler gruplar arasında benzerlik göstermiştir. Sperm miktarı bir hafta açlık uygulanan balıklarda (46,9±20 ml) kontrol grubuna nazaran daha yüksek bulunmuştur. Erkek bireylere dayalı döllenme çalışmasında kontrol dişi ile yapılan döllenme sonuçları deneme grubunun erkek bireyleri için benzerlik göstermiş, en yüksek döllenme oranı kontrol dişisi ve kontrol erkeği kullanılarak yapılan döllenmede görülmüştür.

Anahtar Kelimeler: Damızlık balık besleme, Yumurta kalitesi, Üreme performansı

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Introduction

Rainbow trout's reproductive process is controlled by both photoperiod and temperature. However, the rainbow trout can perceive nutritional status and regulate its reproductive activities accordingly. In individuals whose gonads have reached maturity before the reproductive period, body growth slows down, with most of the energy and nutrients going to the production of vitollogenin, which is necessary for gonad growth (Reading et al., 2018). The broodstock's nutrients during oocyte growth are transferred to the developing eggs. These reserves, taken from the female by mobilization from the yolk sac are used for the development of the embryo until external feeding. Therefore, the feeding regime, ratio and nutritional content of the feed applied before the breeding period have a direct effect on gametes to be obtained from the broodstock fish and on the larval life (Carrillo et al., 2000).

In aquaculture, especially in broodstock fish, the establishment of a species-specific feeding protocol is important both in terms of quality parameters in the gametes to be obtained and in terms of the expenses of the enterprises. Considering the feed expenses included in the production cost with 60-80% of the operating expenses, the establishment of a feeding scheme that will not affect the quality of gametes and larvae in broodstock brings about a cost-effective strategy for fish farmers. Fish are fed daily under culture conditions. To reduce the cost of current feed in broodstock management, it may be beneficial to understand how feed restriction and refeeding affect gonad and gamete development (Izquierdo et al., 2001). The impact of feed restriction on fish reproductive performance is still a matter of contention. Testicular development in zebra cichlid (*Cichlasoma nigrofasciatum*) fish is not affected by feed restriction as stated by Townshend and Wooton (1984). Jobling et al. (1993) report that feed restriction in Arctic charr (*Salvelinus alpinus*) does not influence the number of mature males. The GSI ratio of tilipia fish (*Tilapia zillii*) remained unchanged despite the fed restriction (Coward & Bromage, 1999). It has been shown that feed restriction application in rainbow trout has no effect on GSI and egg quality (Ridelman et al., 1984). However, gonadal maturation in fish species, such as goldfish (*Carassius auratus*, Sohn et al., 1998), European seabass (*Dicentrachus labrax*, Cerda et al., 1994), and Salmonid (Thorpe et al., 1990; Reimers et al., 1993; Hopkins and Unwin 1997) has been observed to be hindered by a decrease in feeding rate. Feed restriction before breeding period has been found to have an impact on gametes, particularly egg productivity, in rainbow trout through previous studies (Imsland & Gunnarsson, 2011; Caldwell et al., 2014; Cleveland et al., 2012).

By sensing the nutritional status of teleost fish, they can regulate their reproductive development and activity accordingly. The quality of gamete may be affected by feed restriction, but it's unclear how. This research aimed in part to address these problems by subjecting rainbow trout to starvation regimes. A feeding trial with two treatments was conducted to assess the impact of starvation periods on growth efficiency and reproductive success before the breeding period. During the control group's daily feeding, the experimental group was given food for seven days and then fasted for the next seven days. The success of fertilization with gametes from both sexes and their quality were investigated using this six-month feeding pattern.

Materials and Methods

Experimental Fish and rearing conditions

This study was carried out between 01.07.2022-15.01.2023 at Istanbul University Faculty of Aquatic Sciences, Sapanca Inland Aquaculture Production Research and Application Unit. A total of 40 females (mean weight 1601±231 g; mean total length 49.7±3.1 cm) and 40 males rainbow trout (*Oncorhynchus mykiss*) (mean weight 670±127 g; mean length 36.7±2.31 cm) aged 2^{+} were used. During the study, the fish were taken care of in four round fiberglass tanks with a diameter of 3 m, a height of 80 cm and a water volume of 3 m^3. The well water with an average temperature of 11.2-13.5 °C was supplied to the system. The experiment was conducted with broodfish raised under natural photoperiod conditions.

Experimental diet

During the study, 8 mm diameter commercial trout broodstock feed supplied from a private company was used to feed the fish (proximate compositions: %48.2 crude protein; %19.3 lipid; %9.6 ash; %1.3 crude cellulose; %92.2 dry matter).

Feeding programme

The male (CM) and female (CF) fish that make up the control group were fed every day during the study (180 days). Male (OWM) and female (OWF) individuals constituting the experimental group were fed every day for a week and starved for a week. All trial groups were fed fish as *ad libitum* by hand twice a daily (09:00; 16:00). The amount of feed delivered to each group was recorded. The tanks were cleaned after evening feeding three days a week. On the days when the gamete maturation would be checked, the fish were not given feed.

Growth Performance

The calculation of feed conversion ratio and growth performance was done using Ricker's formulas (1979).

Weight gain (g) = [final weight (g) – initial weight (g)]
Feed conversion ratio (FCR) = [total feed supplied (g) / weight gain (g)]

Gamete quality analysis

Sperm quality

The spawning activity of male broodfish was monitored every three days during gamete maturation (September to December) by gently pressing the abdomen to check for any sperm. Sperm were obtained by applying abdominal pressure without the use of anaesthetics and stored in clean glass beakers labelled and heated in polystyrene boxes at 4°C until they were analyzed in the lab. Following the measurement of sperm amount (ml), the hemocytometry method was employed to determine sperm density. In brief, a Microcentrifuge tube was used to mix a sperm sample with 0.7% NaCl solution at a ratio of 1:1.000 and analyse it using a Thoma slide (0.00025 mm^3) under a light microscope (Nikon Eclipse E100, <unk> 40). The following equations (ANSCI 2017) were used to determine sperm density (Ekici et al., 2012);
Concentration/ml = (dilution factor) x (count in five squares) x ($0.05x10^6$)

At 12°C, motility parameters were assessed using CEROS II (Hamilton-Thorne) connected to CX41 microscope (Olympus). Images were captured at 60 frames per second using the rainbow trout variables determined in the Hamilton configuration using a digital camera (U-TV1X-2 Tokyo). Sperm motility (Mot,%) and velocity of curvilinear (VCL, <unk> m/sec) were measured in every sperm sample. Sperm with a velocity of less than 20 m/s were classified as immotile. Motility and kinematic parameters were established by using Leja 2 cell chambers with a 20-µl deep chamber (Leja Products). With a dilution rate of 1:500, hatchery water was employed as an activator. All sperm samples were conducted in triplicate by the same operator to minimize errors.

Egg quality

Female individuals were monitored every week for their egg maturation. To observe egg production, stripping was carried out on individuals. Using a towel, the abdomen was dried and gently massaged. Eggs were extracted from clean and labeled stripping jars and than weighed. To prevent eggs from direct sunlight, a sheet was put on the jars. The eggs in each jar were counted by taking a 10-gram sample. Absolute fertility was determined using the following formulas. The determination of relative fecundity was made by dividing absolute fecundity with the total weight (g) of fish (Hunter et al., 1985).
Absolute fecundity (amount⁄Σkg) = [Number of eggs insub−sample×total egg weight (g)] / [Weight of the sub-sample (g)]

The Leica stereo microscope with 20 magnification was used to analyze egg samples (n:10) from each female in the groups. The microscope system software was used to measure egg diameters after photographing all eggs.

Fertilization experiment

Fertilization studies were carried out in a controlled manner for each trial group. To reveal the productivity of female individuals in trial groups; eggs from experimental group was fertilized with the same sperm samples from control group male fish. Again, to reveal the efficiency of male individuals, sperm samples taken from trial group was used for fertilization with eggs belonging to the same female from the control group. The groups in which the fertilization study was performed are given in Table 1.

Table 1.
Fertilization experimental in groups

Groups	Control male	Male (OWM)*
Control female	X	X
Female (OWF)*	X	

*OWF: weekly feeding cycle and a week of fasting female, OWM: weekly feeding cycle and a week of fasting male

Fertilizing the eggs (300 per female) taken from an individual in the trial group was achieved by mixing them with the sperm of the male individual whose spermatologic characteristics were analyzed. Separate incubation trays (30 x 40 cm^2) were used for placing the eggs after fertilization. The incubation trays were filled with water, which was flown continuously (at a rate of 1.5 liters per minute and 10°C). Dead and unfertilized eggs were collected daily from the incubator. The success of fertilization was measured by analyzing the percentage of eyed eggs 16 days after insemination and calculating it based on (number of eyed eggs x initial egg number - 1*100%) (Ekici et al., 2014).

Statistical Analysis

The data obtained at the end of the study are presented with their mean values and standard deviations. The data obtained was analyzed using an ANOVA and then compared by using the Tukey's ($p < .05$) multiple range test in STATISTICA v. 8 program.

Results

The weight gain, calculated according to the date determined as a result of the weighing made at the beginning of the trial and at the end of a total of 6 months, was measured individually, and the values were given with mean and standard deviations. Since the total length of the fish did not differ at the beginning and the end of the experiment, the results were not evaluated.

Growth performance of rainbow trout broodstock

Growth and survival parameters are given in Table 2. Mortality was not recorded in any of the groups. The fasting group had a statistical difference in weight gain ($p < .05$) compared to the control group, which experienced the highest weight gain.

Table 2.
Growth parameters of fish in experimental groups

	CF	CM	OWF	OWM
Initial weight (g)	1588±269	673.5±126	1643.5±257	681±153
Final weight (g)	4352±270[a]	1847±403[A]	3365±540[b]	1145±165[B]
Weight gain	2763	1173	1721	464
FCR	0.9	1.4	1	1.4
Survival (%)	100	100	100	100

CF: control female, CM: control male, OWF: weekly feeding cycle and a week of fasting female, OWM: weekly feeding cycle and a week of fasting male. Each treatment's mean±S.D. value are included in the results. Different superscripts in the same row led to significant differences in values ($p < .05$).

Reproductive performance

Female

In Table 3, the spawning rates of female individuals are shown. Spawning occurred within 177-187 days after the start of experimental feeding.

Table 3.
Spawning parameters of female fish in experimental groups (n:20)

Parameters	CF	OWF
Spawning (days)	180±3	181±3
Spawning females (%)	100	100
Absolute fecundity[2]	4029±432	4389±804
Relative fecundity[3]	935±62[a]	1317±241[b]
Egg diameter (mm)	3.5±0.16	3.36±0.24

CF: control female, OWF: weekly feeding cycle and a week of fasting female, TWF: two week feeding cycle and two weeks of fasting female. 2Mean number of eggs per fish; 3Mean number of eggs per kg body weight. Values in the same row with different superscripts were significantly different ($p < .05$).

In the statistical study conducted on the relative fecundity the control group and the trial group were found to be significant ($p < .05$). However, there was no significant difference between the groups in total fecundity values ($p > .05$). Eggs from broodfish in experimental groups appeared normal, and were similar in shape, and color. However, egg diameters were higher in the control group (3.5±0.16 mm) and lower in the group fed one week apart (3.36±0.24 mm). The egg diameter values of the trial group fed one week apart were compared with the control group and a significant difference was found ($p < .05$).

Male

Table 4 displays the spawning performance of male rainbow trout broodfish. Between 177-187 days after the onset of experimental feeding, spawning occurred.

Table 4.
Spawning parameters of male fish in experimental groups

Parameters	CM	OWM
Spawning (days)	180±3	181±3
Volume (ml/individual)	21.8±7[a]	46.9±20[b]
Spermatozoa density (x10^9/ml)	4.04±0.7[a]	6.5±0.8[b]
Total Motility rate (%)	94.2±2.4	93.42±1.5
Motility duration (s)	24.8±2.9	23.6±1.4
Total Curvilinear velocity, VCL (µm/s)	63.1±9.1	65.7±6.9

CM: control male, OWM: weekly feeding cycle and a week of fasting male. Values in the same row with different superscripts were significantly different ($p < .05$).

In the statistical analysis with the results of sperm density; the group that was fed intermittent for one week was differed from the control group ($p < .05$). The motility rate ranged from 90 to 94 and there was no significant difference between the total motility values of sperm cells between the groups ($p > .05$). In addition, no significant difference was found between the groups in the motility duration of sperm cells ($p > .05$). The total VCL value of sperm cells were 63.1±9.1 µm/sec in the control group, 65.7±6.9 µm/sec in the group fed one week apart. Total VCL values were highest in the one-week intermittent feeding group, but no significant difference was found between the groups in terms of total VCL values ($p > .05$).

Fertilization

In the study, fertilization studies were carried out in a controlled manner for each trial group. To reveal the productivity of female individuals in trial group; eggs from trial group were fertilized with the same sperm samples from control male fish. Again, to reveal the efficiency of male individuals, sperm samples taken from trial group were used for fertilization with eggs belonging to the same female from the control group. In the fertilization study conducted based on female individuals, the highest fertilization rate was observed between control group female and control group male individuals (97.75±1.2%). There was statistically

significant difference between the data obtained in the fertilization study using one week-intermittent female*control male (%. 43±7) ($p > .05$) (Figure 1).

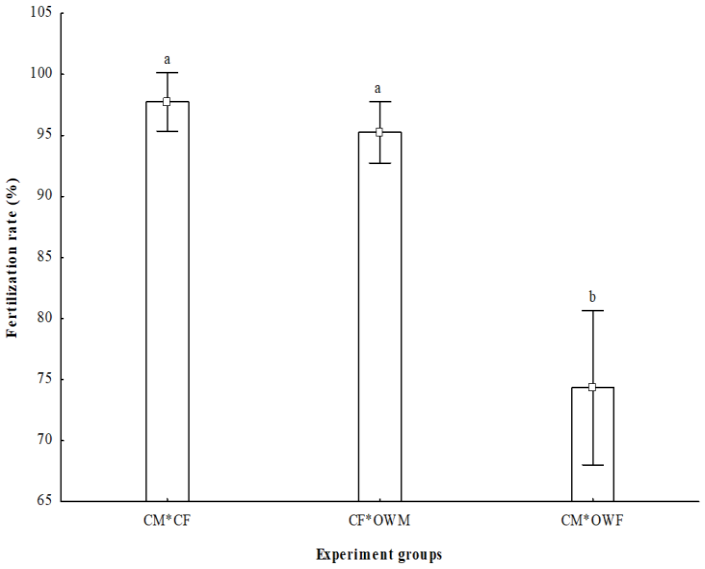

Figure 1.
The percentage (%) of fertilization obtained in the fertilization study based on male and female individuals in the trial groups (Mean±SD). CF: control female, CM: control male, OWM: weekly feeding cycle and a week of fasting male, OWF: weekly feeding cycle and a week of fasting female, the statistical difference between the values indicated by the different letters was found to be significant at the 95% accuracy level according to the Tukey test.

Discussion and Conclusion

By utilizing feed restriction and refeeding cycles in broodstock management of rainbow trout during the 6 months prior to spawning, we were able to achieve a 25% decrease in feed consumption. Live weight gain was observed in both groups, but the control groups of female (2763 kg) and male (1737 kg) fish showed the highest levels. In the study where the growth and reproductive performance of feeding with full ration (100%) and half rations (50%) in Alpine trout (*Salvelinus alpinus*) broodfish was examined; weight gain was achieved in groups fed with a full ration; it was found to be greater than the weight gain in half-ration-fed groups (Imsland and Gunnarsson, 2011). In a study with sea bass (*Dicentrarchus labrax*), broodfish fish were fed with two different feeding rates (0.45% and 1.04 days). Although the starvation cycle is not studied in the study conducted with sea bass, it is thought that in this thesis study, the feeding pattern is subject to a similar restriction as the feed restriction it inevitably brings (Chatzifotis et al., 2011). Before the breeding period in turbot fish (*Scophthalmus maximus*), broodstock fish were fed for 12 months in such a way that the amount of feed increased or decreased according to the months but the total amount of constant feed was determined. At the end of the study, the researchers reported that feeding with low feed rations covering 4 months immediately preceding spawning reduced fish weight by 70% (Bromley et al., 2000). In *Tilapia zillii* fish, more growth was observed in female fish that became broodstocks in high and low feed rationing for about 17 months from the first feeding transition stage compared to fish fed with high feed ration (Coward & Bromage, 1999). In the rainbow trout, the weight gain was found to be less in the feed-restricted groups than in the control group in the results obtained by feeding the broodstock fish regularly as ad-libitum 5 months before the breeding period and feeding by giving 80% of the feed given to the control group by going to feed restriction (Cardona et al., 2019). In contrast to these studies, in the study conducted only with male chinook salmon (*Oncorhynchus tshawytscha*), the fish were fed at intervals of one week for 10-12 months and continued to be fed regularly every day after 10-12 months. Researchers reported that there was no difference between the groups in weight gain in fish (Hopkins & Unwin, 1997). The results obtained in our study were similar to other studies except for the study with chinook salmon. The highest weight gain was seen in the control group of individuals fed regularly daily. Regular feeding has been shown to have a significant effect on weight gain, but this will be re-evaluated by gamete quality.

The feed conversion rate (FCR) may vary between species and within species. FCR, which varies according to the life stage of the fish, the breeding conditions and the feed material, is ideally expressed as 0.5-2 in trout (Davis, 2022). In this study, the FCR value ranged from 0.9 to 1.4. The FCR of this feed determined in each group are similar to the ideal values that should be in fish farming. These results show that the feed given in groups was consumed in a healthy way for use in both weight gain and gamete maturation.

In present the study, although feed restriction did not have a temporal effect on the gonadal maturation of females, it was determined that it caused a change in egg quality. The relative fecundity was found in the control group (935±62 n/kg) and the highest was found in the female group fed one week apart (1317±241 n/kg) and this group statistically different from the control group. When the total amount of eggs taken per individual was evaluated, the highest total fecundity was found in the trial group with one-week interval feeding (4389±804 n/individual). For the control group, 4029±432 n/individuals were found, but there was no significant difference in total fecundity values between the groups. It has been found that changing the normal feeding pattern in rainbow trout and giving 25 and 50% of the daily feed amount decreases in total fecundity (Bromage 1995). In a study where the broodstock fish of the Black Sea trout were fed with commercial feed as ad libitum, the egg

amounts were found as 1476±1043 n/individual (Erbaş & Başçınar, 2013). Erbaş et al. (2013) stated that the fed Black Sea trout once and twice a day without altering the feed ration, and the broodstock group fed once a day produced higher-quality eggs. However, according to studies, decreasing feed intake in rainbow trout can have no negative effect on egg production or egg quality (Cardona et al., 2019). The fecundity values obtained in our study are similar to those of other studies. Even though the relative fecundity results are different, the fact that there was no difference in total fecundity is believed to be linked to the fish's live weight. The lowest relative fecundity in the control group females, which saw the greatest live weight gain, shows that these fish used in the feed consumed more for body weight gain in addition to egg formation. The fact that the total fecundity is similar to the groups in the control group with high kilogram weight gain can be explained by this weight gain. It is thought that intermittent feeding of fish increased relative fecundity, but the reason why fish in this feeding pattern used the feed they received directly to the egg formation did not affect total fecundity because they gained less weight.

In addition to fecundity, one of the quality parameters of female gametes is the egg diameter of the eggs obtained. It is stated that the size of the pre-larva at the stage of hatching is related to the diameter of the egg, and the size of the pre-larvae emerging from large eggs is large (Bromage, 1995). In order for the egg diameter, which is affected by factors such as reproductive period, individual age, genetic structure and nutrition, to be at the desired level, female individuals should be fed with high-quality and adequate nutrition in the period between the two reproductive periods, especially in completing the gametogenesis process (Mananos et al., 2009). For the family Salmonidae, the egg diameter is expressed in the range of 4.9-7.2 mm (Bromage, 1995). Egg diameter in brown trout is 5.2 mm (Gunnes & Gjedrem, 1978); 5-5.3 mm in Black Sea trout (Sonay, 2008); 4.55-5.12 mm (Erbaş & Başçınar, 2013); 4.3±1.8 mm (Gelinçek & Yamaner, 2020); in rainbow trout, 5.4±0.1 mm (Yıldız et al., 2020); has been reported. In this study, egg diameters were found to be 3.5±0.16 mm in the control group; and 3.36±0.24 mm in the one-week intermittent feeding group. The egg diameter data obtained in the study were found to be lower when evaluated intra-species and inter-species compared to other studies. There are studies reporting that feed restriction in rainbow trout leads to larger egg diameters in females and less mortality in eggs (Cardona et al., 2019). Restriction of nutrition in fish during oocyte formation and especially vitellogenesis is known to slow down oocyte growth (Bromley et al., 2000). However, in this study, it is thought that the egg diameters of the eggs in the control group are similar to the results in the fasted fish, and the reason why both results are different from other studies is due to reasons such as different origins of the fish, care conditions, etc. In addition, the highest relative fecundity and the lowest egg diameters obtained in the one-week intermittent feeding group seem to be related to each other. The small egg diameter but the large number of egg production in this group revealed the effect of one-week intermittent feeding.

The amount of sperm differs between individuals of the same species, as well as between each species. In the studies conducted to date, the amount of sperm without any manipulation has been reported as 5-20 ml/individual in the Salmonidae family (Alavi et al., 2008; Dziewulska et al., 2008; Tekin et al., 2007; Yıldız et al., 2021).

In this study, the amount of sperm in the one-week intermittent group differed with control group ($p < .05$). The amount of sperm obtained in the one-week intermittent feeding group was found to be much higher than control group. It was found that the amount of sperm increased significantly in the application of the feeding regime with an interval of one week in rainbow trout broodstock. There are studies showing that the amount of sperm changes with the change in feed amount or feed content (Izquierdo et al., 2001; Davis, 2022). The reason for obtaining more sperm amount in the one-week intermittent feeding group, where the feed content does not change but the feed amount is applied less due to the feeding pattern, is thought to be the fact that the fish spend the feed they consume as soon as they leave the starvation cycle on testicular maturation. The fact that the weight gain was greater in the control group than in the group fed intermittently for one week, but the amount of sperm was less than in this group supports this idea.

The spermatozoa density can vary between 2.42-23.40x10^9 cells/ml in the family Salmonidae (Dziewulska et al., 2008; Lahnsteiner, 2013; Erbaş & Kocabaş, 2013; Gelinçek & Yamaner, 2020; Yıldız et al., 2021). In this study, sperm cell density was highest in the group fed at intervals of one week (6.5±0.8x10^9 cells/ml). The fact that the sperm cell density, which is similar to the sperm amount results, is found to be the highest in the group fed intermittently for one week is thought to be due to the high amount of sperm produced in this group. Although the sperm cell density obtained in the one-week intermittent feeding group was similar to that of Salmonidae family species, the sperm cell densities obtained in all groups were found to be low for rainbow trout. These low results of sperm cell density can be related to the age of the fish and the spawning time. It has been determined that one-week intermittent feeding regime in rainbow trout broodstock also leads to an increase in sperm cell count depending on the amount of sperm.

Sperm motility is one of the most important sperm quality parameters that reveal the fertilization of sperm cells. Sperm motility, which differs within and between species like other gamete quality parameters, is under the influence of many abiotic and biotic factors. The accepted motility in breeding conditions for a successful fertilization study is reported as 70% and above (Mananos et al., 2009). When the motility results were compared, no difference was detected between the groups and it was concluded that the cells in all groups were capable of fertilization with values above 70% and that the intermittent and regular feeding regimen had no effect on the motility values of sperm cells.

When the results obtained from the fertilization studies were evaluated, it was found that there was no difference in the fertilization percentages of the male individuals in the trial group with the control group of female individuals. However, intermittent and regular feeding has been found to affect fertilization in eggs from female fish that have undergone this feeding pattern. The highest percentage of fertilization was seen in gametes obtained from male and female individuals of the control group, and this percentage was statistically different from the other trial group.

Feed used in aquaculture is effective in the performance of vital functions such as survival and growth as well as in gonad development, maturation, reproductive performance and quality parameters of the gametes obtained after maturity (Bromage, 1995). Furthermore, it is also known that the starvation cycle affects gonad and gamete maturation in both sexes (Chatzifotis et al., 2011).

In conclusion, it was determined that feeding the fish regularly every day led to more weight gain in the broodstock fish, but this feeding pattern led to less egg production per kg in the fish, more eggs per kg were produced by feeding the fish one week apart, but the egg diameters produced were smaller and also contributed to the production of more sperm and sperm cells in male individuals. The results obtained with the motility and kinematic parameters of the sperm cells and the results of fertilization were in parallel, and it was concluded that intermittent feeding did not make a difference except for sperm volume and cell density in male individuals, but that the fertilization percentages were low in female individuals, and that although more eggs were produced, the eggs obtained by intermittent feeding did not fully mature. As a result of this study, it is thought that this change in feeding regime, which is made 6 months before the breeding period, should be started earlier than the period applied in this study. Gonadal maturation is influenced by starvation during vitellogenesis, but further research is needed to establish the duration and period of the year during which the starvation re-feeding regime should be implemented.

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Ethical Statement: This study was carried out after the animal experiment was approved by İstanbul University Local Ethics Committee (Decision number: 2022/15).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – GY; Design – NN, GY; Supervision - GY; Resources – NN, GY; Materials – NN, GY; Data Collection and/or Processing – NN, GY; Analysis and/or Interpretation - GY; Literature Search - NN; Writing Manuscript - GY; Critical Review - GY

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