Clinical and Experimental Health Sciences

Effect of dietary n-3 series fatty acids on sperm motility duration of rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792)

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ABSTRACT

Objective: This study was performed to determine the effect of different ratios of n-3 series fatty acids added to the diets of rainbow trout (*Oncorhynchus mykiss*) on sperm motility duration.

Methods: A total of 48 male rainbow trouts about 3 years of age were used in the study and 4 groups were formed. The one that n-3 series fatty acids were not added to the diet was used as control group. n-3 series fatty acids were added to the experimental groups diets at the ratios of 1% (E1), 2% (E2) and 3% (E3), respectively. Semen was collected by abdominal massage. The time-dependent sperm motility change was detected under the microscope at x400 magnification.

Results: A significant decrease was detected in the motility only at 360th hour in E3 group compared to the control group ($p<0.05$).

Conclusion: As a result, it was concluded that feeding with diets containing different ratios of n-3 series fatty acids had no effect on the motility of rainbow trout semen under in vitro condition.

Keywords: Diet, duration of motility, fatty acid, rainbow trout, sperm motility.

1.INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*), which is a fresh-water fish, farming increases year by year in our country depending on its existing potential. Rainbow trout belongs to the Salmonidae family. It matures in 2-3 years and spawns between December and May (1).

The fast growth of the world population leads to a rapid decline in animal protein sources. Therefore, the production of animal proteins should be accelerated. The current biotechnological research contributes to the increase in fish farming worldwide. Recently, studies focused on the yield increased significantly. As in the hatcheries in our country, farmers aim to increase the number of tiddlers, knowledge about the evaluation criteria of the yield characteristics in the brood fishes, which will be used in farming, is critical. Sperm motility is one of the most important sperm parameters. To increase the yield, the use of sperm with good sperm quality is essential (2-4). The duration spermatogenesis is usually shorter in fish than in mammals. In principle, spermatogenesis can be divided, from a morpho-functional point of view, in three different phases: the mitotic or spermatogonial phase with the different generations of spermatogonia, the meiotic phase with the primary and secondary spermatocytes, and the spermiogenic phase with the haploid spermatids emerging from meiosis and differentiating, without further proliferation, into flagellated spermatozoa (5). Like other living creatures, also fishes need energy for growth, breeding, living, and their physiological activities. Lipids are their main source of energy. Lipids consist of fatty acids and are not water-soluble. Fatty acids are divided into two groups depending on the number of bonds. Fatty acids with one bond are called saturated and with double bonds are called unsaturated fatty acids. In addition, fatty acids with carbon atom number between 18-20 and double bonds between 2-4 are called polyunsaturated fatty acids (PUFA) and fatty acids with more than 20 carbon atom number and more than 4 double bonds are called highly unsaturated fatty acids (HUFA) (6). Like in other fishes, the rainbow trout needs also n-3 and n-6 fatty acids (7). Furthermore, PUFAs like linolenic, linoleic and α-linolenic acid are essential fatty acids and disorders related to growth, development, and proliferation may emerge if their need for these fatty acids is not met (6).

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In this study, our objective was to investigate the effect of n-3 series fatty acids, which were added to the compound feed of rainbow trout in different proportions, on the duration of the sperm motility.

2. METHODS

This study was conducted in the Cip Fish Breeding Farming of the Firat University Aquaculture Faculty. A total of 48 male rainbow trouts at age 3 were included in the study. The study was conducted in 4 cement ponds (dimensions: 2x1x0.8m). The study sample was divided into 4 groups. 12 male broodstocks were placed in each pond. The fishes were adapted to the experimental conditions for one month. They were fed with control feed during this period. Then broodstocks were fed for three months three times daily according to the free feeding technique. They fastened for 24 hours before the sperm yield. They were anesthetized before the stripping process (5 mL phenoxyethanol/L) (8). The abdomen of the dried male broodstocks was manually massaged from front to the back and sperm was yielded (9).

2.1. Organization and preparation of the study feed

Soybean meal, corn gluten, anchovy meal, wheat starch, n-3 fatty acid concentrated from the anchovy oil, unrefined sunflower oil, antioxidants, vitamin mixture, mineral mixture, and wheat bran were purchased from a commercial company for the preparation of the study feed.

In the control group, fishes received feed with no added n-3 fatty acids. The experiment groups (E) E1, E2, and E3 received feed with 45% concentrated raw protein, 3619 kcal/kg metabolized energy and anchovy oil with an n-3 fatty acid concentration of 1%, 2%, and 3% respectively. Besides, we used corn gluten, soybean meal, anchovy meal, and fatty acids as the protein source and unrefined sunflower oil (as a source of n-6 fatty acids) n-3 fatty acids as the energy source (Table 1) (6, 10).

Table 1. *Formulation of the experimental diets*

Feed items (%)	Experimental Diets			
	Control	Experiment 1	Experiment 2	Experiment 3
Anchovy flour (%56,9)	35	35	35	35
Soybean meal (%42,2)	30	30	30	30
Corn gluten (%52,6)	5	5	5	5
Wheat starch (%10,2)	5	5	5	5
N-3 series fatty acid [1]	-	1	2	3
Sunflower oil	15.2	14.2	13.2	12.2
Antioxidant [2]	0.1	0.1	0.1	0.1
Vitamin Mix [3]	1	1	1	1
Mineral mix [4]	1	1	1	1
Wheat bran	7.7	7.7	7.7	7.7

[1] n-3 series fatty acid (Solgar OMEGA-3 700) was concentrated from anchovy oil; and containing 54.3% eicosapentaenoic acid (EPA), 37.1% docosahexaenoic acid (DHA) and 8.6% other n-3 series fatty acids (docosapentaenoic, linolenic, stearidonic acid).

[2] Butylene Hydroxy Toluene (BHT); 125.000 mg/kg.

[3] Vitamin Mix (as active ingredient per 1 kg Rovimix 107); Vitamin A 250.000 IU, vitamin D3 240.000 IU, vitamin E 10.000 IU, vitamin K 3.000 mg, vitamin B1 1.000 mg, vitamin B2 3.000 mg, vitamin B6 2.000 mg, vitamin B12 4 mg, choline chloride 100.000 mg, vitamin C 6.000 mg, niacin 30.000 mg, calcium d-pantothenate 10.000 mg, folic acid 600 mg, d-biotin 200 mg.

[4] Mineral Mix (mg/kg); Manganese 1.300, zinc 3.000, iron 6.000, copper 300, iodine 110, potassium 70, phosphorus 60, selenium 30, cobalt 20, magnesium 5.

2.2. Determination of the sperm motility and sperm duration

After semen collection, semen was stored in the cooling cabinet at 4 °C. Sperm motility determination was done at certain intervals until motility was exhausted. In order to determine the motility of the semen samples obtained from the fishes, 2 ml of the 119 mmol NaCl solution was poured in a tube. Then 1 drop semen was added and mixed. One drop of this mixture put on a slide glass and covered with a cover glass. The motility rate (%) was determined under a mirror microscope with a 400x magnification. The time-dependent change in this rate was investigated.

The study data were expressed in mean values and standard error of means (±SEM) after the statistical analysis. The software package SPSS (22.0, Chicago, IL, USA) was used for the comparative statistical analysis. For all analyses, $p<0.05$ was considered statistically significant.

Regarding the motility, non-parametric Kruskal-Wallis variance analysis was used for the intergroup comparisons and non-parametric Mann-Whitney U test for the paired comparisons.

3. RESULTS

The mean motility rate (%) according to time (min) in rainbow trout in the control and experimental groups, which were fed with diets containing different n-3 series fatty acid concentrations, were summarized in Table 2.

The sperm motility was numerically higher in the experimental groups than the control group during first 30th minute. At the 60th minute, while the motility value in the E3 group decreased numerically compared to the other groups, E1, E2 remained the same with the control. The numerical decrease in sperm motility of the experimental groups continued after 120th minute compared to the control group.

A significant decrease was detected in the motility only at 360th hour in E3 group compared to the control group ($p<0.05$).

Table 2. *Average (±SEM) motility (%) values of control and experimental groups according to time (minute)*

	Begining	5th min	10th min	15th min	20th min	25th min	30th min	60th min	120th min	180th min	240th min	300th min	360th min	420th min
Control	80.00±5.77	76.66±3.33	76.66±3.33	73.33±3.33	70.00±0.00	70.00±0.00	70.00±0.00	70.00±0.00	60.00±0.00	60.00±0.00	53.33±3.33	40.00±5.77	26.66±3.33^{a}	3.33±3.33
E1	88.75±3.14	80.00±0.00	77.50±2.50	75.00±2.88	72.50±2.50	72.50±2.50	70.00±0.00	70.00±0.00	65.00±2.88	55.00±2.88	55.00±2.88	40.00±4.08	20.00±4.08^{a}	0.0±0.00
E2	88.33±4.40	86.66±3.33	86.66±3.33	86.66±3.33	80.00±5.77	80.00±5.77	73.33±12.0	70.00±10.0	56.66±8.81	53.33±6.66	53.33±6.66	33.33±6.66	20.00±5.77^{a}	3.33±3.33
E3	88.33±4.40	80.00±5.77	80.00±5.77	76.66±6.66	76.66±6.66	76.66±6.66	73.33±3.33	63.33±3.33	53.33±3.33	43.33±8.81	43.33±8.81	26.66±6.66	3.33±1.66^{b}	0.0±0.00

a and b : Differents letters within a column showed significant differences between groups ($p<0.05$).

4. DISCUSSION

The yield of good quality sperm is one of the main goals of fish farming. Several biotic and abiotic factors affect the yield and quality of the sperm. The nutrition of the broodstocks has a direct effect on sperm quality. Although nutrition has an important effect on reproductive physiology, there is only limited evidence that these changes can affect sperm quality. Fatty acids especially PUFAs, n-3 series fatty acids and their derivatives can affect the number of eggs (11, 12).

In bony fish species, which are reproduced via external fertilization, the spermatozoon activity is short and reaches the maximum speed after the dilution. Besides, their speed declines during motion. The very short duration of sperm motility is the main reason for the low fertilization rate in salmonids (13). The mean duration of the sperm motility is 20-25 seconds in trouts and more than 1 minute in carps (14). The duration of spermatozoon motility in the active rainbow trouts is 30-35 seconds during the spawning season. The duration of motility drops up to 15 seconds at the end of the season (15).

In our study, the duration of motility was very long compared to the previous studies and the vitality of the rainbow trout spermatozoon was preserved up to 360 minutes. We believe that the high extracellular K^+ concentration inhibited the spermatozoon motility in the previous studies. Therefore, we believe that the spermatozoon motility can be activated with the extracellular K^+ diluent. This diluent causes membrane hyperplasia, which triggers the activation (16). Besides, osmotic pressure is one of the factors determining sperm activation and is commonly considered as a triggering factor for the initiation of sperm motility. Motility starts at a higher osmotic pressure in saltwater fishes compared to the freshwater species (17). Furthermore, as the duration of motility is short in the trout spermatozoon, the motility measurement should be performed quickly. The difference in the motile lifespans between the studies may depend in general on the environmental factors and the temperature in the laboratory.

The lipid and fatty acid composition of the feed are defined as the main factor for successful reproduction of fishes and their survival (18). As the lipid composition is closely related to the spermatozoon quality, its quantity in the feed is important. Lahnsteiner et al. (11) investigated the compositions of the total fatty acids in the seminal plasma and sperm in trouts and found saturated fatty acids such as myristic acid, palmitic acid, and stearic acid and unsaturated fatty acids like oleic acid, vaccenic acid, and linolenic acid. Another study reported that free fatty acids and sterols were the main lipid components in the seminal fluid and stated that the fatty acid composition of the sperm was affected by the nutrition (19). The investigators found out that the n-3 PUFA deficiency in the feed affected the sperm motility and the motility was lower compared to the controls. In our study, we determined that in the group, in which 3% of fatty acid was added to the diet, the sperm motility impaired the sperm lifespan only at 360th minute.

In salmonids, lipids are the main energy source for sperms and therefore they are important for the preservation of sperm vitality (11). Fatty acid oxidation occurs as a result of adenosine triphosphate production, which is stored in the sperm cell membrane, mitochondria, middle segment, and tail of the sperms. Therefore, the increase of the fatty acids in the testicular cells stimulates the sperm production capacity in testicles and thus the rate of the sperm survival and sperm motility increase (20). In our study, a significant decrease was detected in the motility from only at 360th hour in E3 group compared to the control group. Along with several factors affecting sperm survival, a high concentration of fatty acids increases the metabolism in sperms and leads to an early decline of the motility, which may be considered as an additional factor.

In a study conducted with the European seabass (*Dicentrarchus labrax*), Asturiano et al. (21) observed that diets containing PUFA had a positive effect on the reproduction parameters including sperm volume and density. In another study

conducted with *Barbus barbus* species, Alavi et al. (22) fed the fishes with PUFA-containing diet during the breeding season. However, their investigation on the sperm quality demonstrated that different diets did not affect sperm volume, total sperm quantity, concentration, and motility. In addition, in another study focused on *Carassius auratus*, it was determined that in vitro essential fatty acids stimulated the testicular testosterone production and affected theprostaglandins (18). Thus, the production of the steroid hormones declines in fatty acid deficiency, spermiation time is delayed and consequently, the fertilization rate may decline Lahnsteiner et al. (11) investigated the fatty acids during the short time preservation of sperm in rainbow trout and reported that the sperm survival prolonged and had a positive effect on sperm motility and consequently on fertility.

5. CONCLUSION

As a result, it was concluded that feeding with diet including different concentrations of n-3 series fatty acids has no effect on motility in rainbow trout semen under in vitro conditions.

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