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Comparison of Some Elements in Sperm Seminal Plasma of Rainbow Trout (*Oncorhynchus mykiss*) and Brown trout (*Salmo trutta fario*)

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Abstract
This research was carried out major and trace elements (Ca^{++}, Mg^{++}, Na^{+}, K^{+}, Fe^{++} and Zn^{++}) in seminal plasma and quality parameters of sperm (Volume, pH and spermatocrit of sperm, motility, duration and density of spermatozoa) of two different trout (*Oncorhynchus mykiss* and *Salmo trutta fario*) in a fish farm. As results, the levels of Ca^{++}, Fe^{++} and volume of sperm between two species of trout were significant difference as statistically ($p<0.05$). We aimed to investigate performance and management status of male brood stocks in this fish farm.

Finally, male brood stocks of this fish farm had very low quality parameters of sperm as normal standards. Additionally, all the results showed that this farm had not a management of brood stocks.

Key words: Seminal plasma elements, sperm quality parameters, *Oncorhynchus mykiss, Salmo trutta fario.*

INTRODUCTION

It is very important to control and examine performances of brood stocks in hatchery stations of fish farms for their quality and productivity of gametes will enter into aquaculture systems. It determined that reproduction performance has been affected on level of stocking, quality, amount and rate of feeding, quality of physicochemical of water, method of spawning and stripping, age and size of salmonid brood stocks [1]. The determining quality of sperm is necessary to define its quality. Motility, duration of motility and density of active spermatozoa, pH, spermatocrit and seminal plasma contents of sperm has been used to determine quality of sperm [2, 3].

The sperm constituents have very important roles which are metabolism, function, survival and spermatozoa motility. Especially, Na, K and Cl in the seminal plasma of sperm have created to osmotic balance and these essential trace elements are also acted into many important enzymes. So, the biochemical evaluation of sperm seminal plasma is very important about assessment of sperm quality [4].

Some studies showed that seminal plasma contents of oviparous fish have been vary in different species of fish. Generally, the upper and lower quantities of ionic contents of seminal plasma have included as 3-2.6 mM Ca, 0.8-3.6 mM Mg, 20-66 mM K and 103-140 mM Na in salmonids [5]. However, in general, motility of spermatozoa has closely related with rates of K and Na ions of sperm seminal plasma in fish [6]. Recently, some researchers refer to some studies of spermatozoa structure which provide both some data about reproductive biology and an useful tool on taxonomic and systematic researches [7].

In this research was aimed to obtain the presence of performance by determined levels of elements of sperm seminal plasma and sperm quality parameters with comparison in *Oncorhynchus mykiss* and *Salmo trutta fario* in a fish farm.

MATERIALS AND METHODS

Brood stocks care

Male brood stocks of rainbow and brown trout were obtained from a fish farm in Sürgü town of Malatya province, Turkey. In this farm, brood stocks (male and female) were being maintained at a density of 170 per fish in a concrete pond of 18 m^3 without natural photoperiod (indoor) until reproduction period. For this research, 10 males fish took out and stocked into 300-L fiberglass tank at reproduction term in this farm. Tank was supplied flow of water. Fish of experiment adapted in this tank for 2 weeks. Feeding stopped ago 2 days from stripping and then sperms were collected from males.

In this study, weight and length of the brood stocks and the water quality parameters of tanks which are pH, temperature and dissolved oxygen were measured according to standards [8]. Sperms were supplied by massage from the front to back of fish abdomen.

Spermatozoa density, motilty and spermatocrit

Spermatozoa concentration was measured through a spectrophotometric method after dilution (2µl sperm:1998µl NaCl, 0.7%) by 605 nm [9]. Sperm pH was measured using standard pH-electrodes. Spermatocrit of sperm was determined using sperm collected into microhaematocrit tubes without heparin (75 mm length, 1.1–1.2 mm inner diameter) and centrifuged at 10000 rpm for 5 minutes.

Spermatozoa motility in each sample was evaluated within five minutes following sperm collection. Sperm samples were kept at approximately +4 C throughout the motility tests. Motility of spermatozoa was evaluated at 400X magnification with Olympus BX 41 model microscope according to Steyn et al.1989 [10].

Seminal plasma composition

Seminal plasma was collected after centrifugation of the sperm at 3400 g for 10 min in Beckman L-8- 70M ultracentrifuge (Rotor SW-28; Beckman L8-70M Ultracentrifuge, Munchen, Germany). Seminal plasma was centrifuged twice to avoid possible contamination with spermatozoa and stored at -20°C until the beginning of analysis. The samples of semen were digested in the ultrasonic bath for 10 min by using 1 mL HNO_3 3 g of sample. Digested solutions were diluted with deionised water and the final volume of the solution was adjusted to 10 mL. After dilution, the digests were analyzed for Na, K, Ca, Mg, Fe and Zn [11, 12].

Metal concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES VARIAN 725-ES, CCD). Inductively coupled plasma is widely recognized as a suitable technique for the determination of trace elements, the particular advantages being the multi element capability, large dynamic range, and effective background correction. High-purity water from a Milli-Q system (Millipore, Molsheim, France) was used in the preparation of all the solutions and samples. All standard solutions used (0.01, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and 100 mg/L) were prepared by diluting 1 mg/ml stock multielement standard solutions for ICP-OES. ICP OES instrumental parameters also showed in Table 1.

Table 1. ICP OES instrumental parameters.

Radiofrequency power	1.2 kW
Plasma gas flow rate	15.0 L min^{-1}
Auxiliary gas flow rate	1.50 L min^{-1}
Nebulizer flow rate	0.75 L min^{-1}
Replicate read time	3 s
Delay	15 s
Sample uptake delay	30 s
Pump rate	15 rpm
Rinse time	20 s
Replicates	3
Torch	Quartz for vertical view

Statistical analysis

Student's t-test was used on independent groups for unpaired comparisons. Statistical difference was indicated when the *p* value was less than 0.05. Normality test was done by sample K-S as nonparametric test.

RESULTS AND DISCUSSION

In this study, element contents of sperm seminal plasma in Rainbow trout and Brown trout have been showed in Table 2 and Figure 1. It determined elements of Ca, Mg, K, Fe and Zn for each fish species. Also Table 3 has been showed sperm quality parameters (volume, spermatocrit and pH of sperm, motility, density and duration of motility of spermatozoa) both Rainbow and Brown trout.

The level of K determined 26.823 mmol/L (rainbow trout) and 20.454 mmol/L (Brown trout) in this study (Table 2). As results of authors in Table 4, our K results were very low. Also Billard and Cosson [13] said that a high percentage of sperm motility become with the presence of high K concentrations (40-80 mmol). The level of Na observed 84.992 mmol/L (rainbow trout) and 66.294 mmol/L (Brown trout). Authors in Alavi and Cosson [14] showed that levels of Na are between 103-159 mmol/L (Table 4). It showed that our results of Na were determined very lower level than other results.

Some studies showed that seminal plasma contents of oviparous fish have been vary in different species of fish. Generally, the upper and lower quantities of ionic contents of seminal plasma have included as 3-2.6 mM Ca, 0.8-3.6 mM Mg, 20-66 mM K and 103-140 mM Na in salmonids [5]. However, in general, motility of spermatozoa has closely related with rates of K and Na ions of sperm seminal plasma in fish [6]. Therefore, we can understand why motility of spermatozoa decreased because the ions of Na and K were few levels in this study.

Density of spermatozoa, motility and duration of motility are the most commonly used parameters to evaluate sperm quality. From comparison between species, sperm production can be expressed in 10^9 sperm per g body weight: it was 7 for rainbow trout, 4 for carp, 2.7 for guppy, 0.6 for pike and 0.1 for *Leporinus*. Measurements of beat frequency on three species show that the duration of motility is very short in trout (20-25s) and lasts slightly longer than 1 minute in carp and halibut. The beat frequency of the majority of sperm declines progressively within 20-25s (trout) and 80-90s (carp) [4].

In this study, density and duration of motility in spermatozoa determined 7.60 $x10^9$ per/ml and 13.70s (rainbow trout) and 7.60 $x10^9$ per/ml and 14.66s (Brown trout), respectively. However, if we think density of spermatozoa in rainbow trout as Billard et al. [4], our results obtained closely their results. But other authors reported for rainbow trout density of spermatozoa 11.8 [9], 8.9 [15], for Atlantic salmon, *Salmo salar*, 3.5 [16], 12 [17]. Other hand, our results of duration of motility (13-14s) determined lower than Billard et al. [4] (20-25s). According to several studies, the differences in sperm production can be related to many factors which are age and weight of the male, ecology and spawning behavior of brood stock and sampling period and method [18, 19, 20]. However we think that reason of lower our results are that male brood stocks reared by stress as such as more stock rate in a little pond (170 per adult fish/18 square meter).

Kime and Nash, 1999 [21] said that the quality of fish gametes depends on the suitable hormonal environment during their development but they may be affected negative by stress. However, Campbell et al., 1999 [22] reported that the repeated acute stress during reproductive development in rainbow trout caused to significantly delayed ovulation and reduced egg size and also they observed significantly decreased sperm counts and decreased survival rates for progeny between stressed and unstressed controls. Okumuş [23] said that reproduction is very sensitive to stress and especially important for the males. The main sources of stress are handling, water quality parameters and stocking density which they should be watched carefully. However, while stock density depends on the water temperature, dissolved oxygen level and flow conditions, it should be lower than for grow-out in brood stocks and Ingram [24] suggested 8-10 kg/m^3 as maximum for rainbow trout during non-spawning season.

In this study, brood stocks (male and female) were being maintained at a density of 425 kg in a concrete pond of 12 m^3 without natural photoperiod (indoor) until reproduction period. Videlicet, the brood stocks were stocked 35 kg/m^3 as more density stocking rate. It means that especially males were very stressful from stocking rates and we think that all spermatological parameters affected on this status.

Finally, in this study aimed to determined seminal plasma constitutes and sperm quality parameters in Rainbow trout and Brown trout in a fish farm. As results, especially the levels of K and Na of seminal plasma of sperm determined very low such as normal standards. Sperm quality parameters which are motility and duration of spermatozoa showed highly low levels. All of these results mean that this farm has not a good brood stock management. By these results, we can suggest that farmer has to manage firstly owned brood stocks such as density rate and feeding practices and urgently density rate of mature males should be attenuate.

Table 2. Element contents in seminal plasma of sperm.

Elements (mmol/L)	Rainbow trout	Brown trout	Wavelength in ICP (nm)	Independent t-test Level of Sign. (N=6)
	Mean ±S.D.	Mean ±S.D.		
Ca	1.768±0.193	1.289±0.252	396.487	0.004*
Mg	1.113±0.150	0.986±0.329	280.270	0.411
Na	84.992±8.495	66.294±19.769	588.892	0.059
K	26.823±3.567	20.454±6.836	769.892	0.071
Fe	0.003±0.001	0.015±0.010	259.940	0.021*
Zn	0.006±0.005	0.007±0.005	213.857	0.687

* Significant difference as statistically, $p<0.05$.

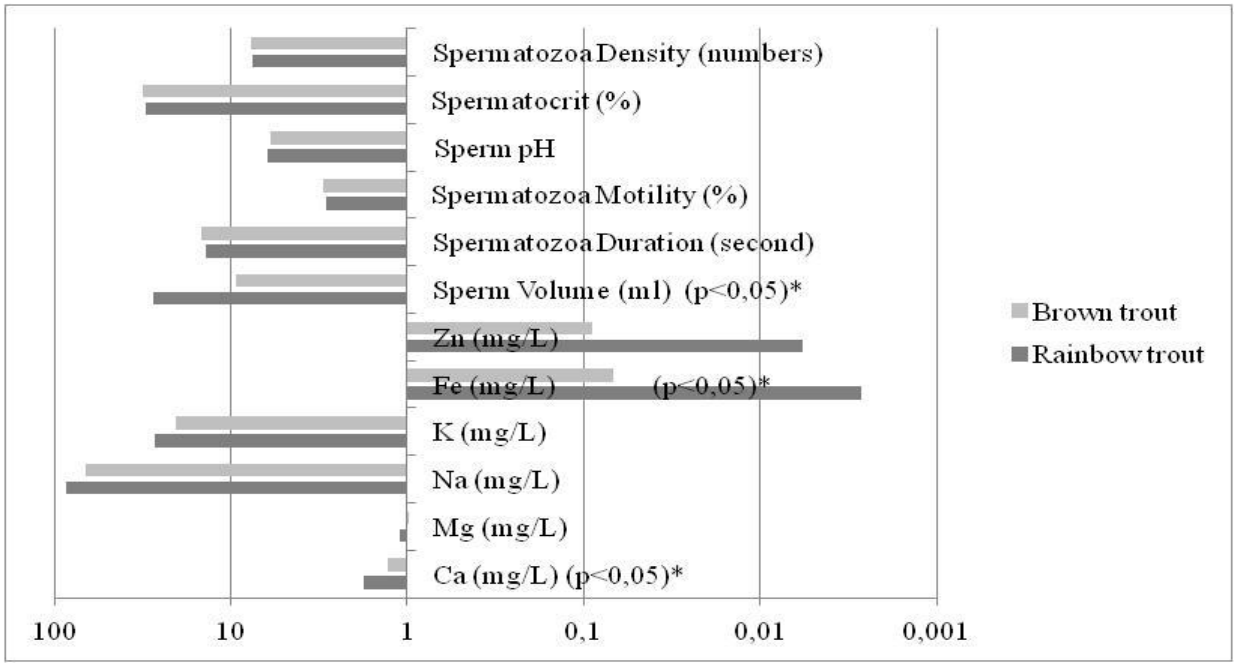

Figure 1. Some sperm quality parameters of Rainbow and Brown trout.

Table 3. Sperm quality parameters of male brood stocks**.**

Parameters	Rainbow trout	Brown trout	Independent t-test Level of Sign. (N=6)
	Mean ±S.D.	Mean ±S.D.	
Volume of sperm, ml	27.29±16.65	9.33±4.84	0.028*
Duration of spermatozoa, second	13.70±3.7081	14.66±4.29	0.674
Motility of Spermatozoa, %	65.00±0.50	60.00±0.50	0.780
pH of sperm	7.16±0.23	6.92±0.73	0.427
Spermatocrit , %	30.51±6.94	31.22±5.53	0.843
Density of spermatozoa, $x10^9$ per/ml	7.60±1.35	7.60±2.39	0.997
Weight of male brood stocks, g	1371.43±154.53	1750.00±581.55	0.124
Length of male brood stocks, cm	46.43±1.27	48.83±5.42	0.276

* Significant difference as statistically, $p<0.05$

Table 4. Ionic contents of the seminal plasma and sperm of Salmonidae [14].

K^+ (mmol/L)	Na^+	Ca^{2+}	Mg^{2+}	Authors
Oncorhynchus mykiss				
30.4±4.5	122±14.2	1.10±0.26	0.85±0.12	Glogowski et al., 2000
25.7±4.1	159.8±30.8	1.2±0.3		Lahnsteiner et al., 1996
23.5	104	1.4	1.1	Holtz et al., 1977
25.8	107	2.6	0.8	
20	133			Schlenk and Kahman, 1938
Salmo salar				
22	103	1.3	0.9	Hwang and Idler, 1969
Salmo clarki				
38.6	107	0.3	1.5	Cruea, 1969
Salmonids				
20-66	103-140	0.3-2.6	0.8-3.6	Billard et al., 1995a,b

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