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Alterations in Cholesterol, Glucose and Triglyceride Levels in Reproduction of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: The changes in glucose, cholesterol and Triglyceride (TG) levels were investigated during reproductive process stages of rainbow trout (*Onchorhynchus mykiss*). TG level decreased significantly from maturation to spawning in both sexes. Cholesterol levels fluctuated significantly during reproduction period. Glucose levels increased at maturation then decreased throughout the spawning. Low density level value increased to ovulation then fluctuated to post-spawning. High density lipoprotein values sharply decreased then increased and fluctuated significantly during the reproduction. Very low density lipoprotein values immediately increased then decreased and fluctuated significantly from pre-maturation to post spawning.

Key words: *Oncorhynchus mykiss*, cholesterol, triglyceride, HDL, VLDL, LDL, rainbow trout

INTRODUCTION

Among the most significant advancements in the field of aquaculture during recent decades is the development of techniques to induce reproduction in fish. These techniques have allowed farmers to profitably breed and raise species and to manipulate the timing of reproduction to suit production cycles. In fish, the reproductive process involves three basic steps namely: maturation-the development of the gametes (eggs and sperm) to a point where fertilization can occur; ovulation-the release of eggs from the ovary; and spawning-the deposition of eggs and sperm.

Fish in captivity may not always reproduce at the most advantageous time and alteration of the spawning cycle may be desirable. This allows a farmer to obtain fish outside of the normal spawning season either to lengthen time for grow-out or to produce hybrids with other species, to improve efficiency by getting fish to spawn on a predetermined date and to maximize survival by fertilizing and incubating eggs under hatchery conditions. Where successful, techniques for altering the spawning cycle of fish have become a valuable tool. In order to make good management plans, some physiological and biochemical properties of blood and body such as blood parameters, cholesterol, glucose and phospholipids should be monitored for the future usage as an inducement for the reproduction. Since, there are female specific lipoproteins, which appear in the hemolymph plasma during vitellogenesis, which are similar to vitellins, the egg lipoproteins that provide energy and nutrient for the developing embryo. During yolk synthesis primary oocytes increase their size by accumulating vitellus, which is composed of proteins, lipids and carbohydrates and represents the energy source for the developing embryo[1].

Peres *et al.*[2] reported that plasma triacylglyceride concentrations and liver glycogen content may be related to the mechanism of glucose regulation species. Nordrum *et al.*[3] reported that Medium Chain Triglycerides (MCT) have received much attention in recent years in animal and human nutrition because of their rapid absorption and oxidation without deposition in the body[4,5] and for their potential nitrogen-sparing effect[6]. MCT also appear to be potential stimulators of nutrient transport into enterocytes[7,8]. It appears that MCT may have the potential to enhance nutrient absorption, decrease amino acid oxidation, reduce body fat deposition and thereby allowing increased protein utilization. No information is available indicating whether this potential can be utilized in salmonids.

Phospholipids and sterols known as primarily structural lipids, as well as energy storage lipids such as triacylglycerols are insoluble in water. They are carried as lipoproteins, complexes of lipids bound to polypeptides in the blood of animals. Cholesterol, a white, waxy, fat-like substance produced naturally in the liver, triglyceride and other lipids are transported by the same way in body fluids by a series of lipoproteins[9]. In fact, a molecule of essential fat has to be hitched to a molecule of cholesterol to transport cholesterol in blood.

Lipoproteins are water soluble and can be separated into different classes, depending upon their hydrated densities. These classes include Very Low Density

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(VLDL), Low Density lipoprotein (LDL), High Density lipoprotein (HDL) and Very High-Density (VHDL) lipoproteins. The major egg yolk proteins are either called vitellins or lipovitellins, being the latter High Density Lipoproteins (HDL). The LDL is responsible for carrying approximately 60-70% of cholesterol through blood to tissues where it can be used in the body. Some carbohydrates have special functions in vital process such as ribose in the structure of nucleic acids, deoxyribose, galactose in some fats and glucose in blood[1].

Poli *et al.*[10] reported that the essentiality of phospholipid for aquatic animals is assumed to be due not only to its limited ability for phospholipid biosynthesis from fatty acid and or diglycerides, but also to its function as an emulsifier of triglycerides and cholesterol and as a constituent of lipoproteins essential for the transport of lipids. Levels within the normal ranges of plasma total protein, triacylglycerols and cholesterol indicate a good nutritional status in the fish. On the other hand, serum lipids and glucose levels are affected by several factors such as reproduction, feeding activity, food composition and environmental factors[11-13].

Wallaert and Babin[14] reported that environmental factors such as photoperiod or endocrine factors i.e. the concentration of steroid hormones can be correlated and/or involved in the regulation of basal plasma lipid and lipoprotein levels which were affected by two main factors, season and reproductive cycle in trout. The present study deals with the lipoproteins, cholesterol and glucose levels present in the plasma of rainbow trout to show the relationship between these parameters and reproduction period.

MATERIALS AND METHODS

Mature rainbow trout with 1600-2200 g mean weight reared in well water with 8.5°C constant temperature in our farm, located in Research and Extension Center in Atatürk University were kept in 3140 l circular (200 cm diameter and 100 cm depth) fiberglass tanks under natural light conditions with a constant flow (2 l min^{-1}) of aerated well water, 8-9°C temperature and with no recirculation. Dissolved oxygen, pH and total hardness of water were 7.6 ppm, 6.9 and 103 mg in $CaCO_3$ respectively. A commercial feed with 45% protein, 20% fat was offered two times daily (1% of live body weight) to brood stock during the study.

A total of 20 fish (10 male, 10 female in each tank) were separately stocked in two tanks during the study. Fish were tagged and secondary sex characters of them were monitored. The reproductive stages were determined by sacrificing the mature fish weekly and stripping with the gentle pressure to the abdominal cavity.

Fish were anesthetized with methanesulfonate (MS-222) (100 mg L^{-1})[15] and bled with a 5-mL syringe to collect blood samples in pre-maturation (no eggs), reproductive process stages namely maturation (small eggs present and not coming out by stripping), ovulation (eggs present and not coming out easily by gentle pressure to abdominal cavity), spawning (eggs coming out easily by gentle pressure to the abdominal cavity) and post spawning (no eggs). Research was started Pre-maturation and ended following Post-spawning. Blood samples were centrifuged at 4000 rpm for 15 min and plasma was subjected to analyses. Serum glucose, triglyceride, cholesterol, HDL, LDL and VLDL levels were determined using an automated system (Hitachi® 717).

Statistical analysis: Results are presented as Means±SD. Differences between parameters were analyzed by one-way analysis of variance (ANOVA), significant means subjected to a Multiple Comparison test (Duncan) at $\alpha = 0.05$ level[16].

RESULTS AND DISCUSSION

TG level decreased significantly from maturation to spawning in both sexes. Cholesterol levels fluctuated significantly during reproduction period. Glucose levels increased at maturation then decreased throughout the spawning. LDL value increased to ovulation then fluctuated to post-spawning. HDL values sharply decreased then increased and fluctuated significantly during the reproduction. VLDL values immediately increased then decreased and fluctuated significantly from pre-maturation to post spawning (Table 1).

Kocaman and Akyurt[17] reported that rainbow trout spawns annually from November to February in East Anatolia with cold climate. The highest triglyceride levels were determined in Pre-maturation in male and in Maturation in female. When ovulation started, a rapid significant decrease in triglyceride occurred in both sexes. Variations of serum triglyceride levels from both sexes of rainbow trout may suggest that the lipid deposition associated with the reproductive cycle. Therefore that may be used as an indication of reproduction stage. Many investigators also reported that fish deposit the source of nutrients in their tissue and liver. Then these deposited foods were used in reproduction and for other activities[18-21].

The peak values of glucose were observed in ovulation for male and in maturation for female, when

Table 1: Changes in plasma triglyceride, glucose, cholesterol, LDL, HDL and VLDL levels of rainbow trout during reproductive process. Means with different letters in a row represent significant differences between process stages. Each value is the Mean±SD of ten individual observations

	Sex	Pre Maturation	Maturation	Ovulation	Spawning	Post Spawning
Triglyceride (mg dl^{-1})	M	537.70±79.19a	504.90±38.45a	294.30±56.75b	255.30±46.03b	290.00±15.53b
	FM	446.10±54.62b	511.30±30.88a	318.20±44.32c	293.90±24.42cd	262.10±28.85d
Glucose (mg dl^{-1})	M	79.90±06.47b	81.30±10.12b	100.70±14.55a	84.40±12.40b	77.60±09.75b
	FM	72.40±09.79c	101.50±11.72a	96.60±07.14ab	79.00±06.46c	90.80±16.21b
Cholesterol (mg dl^{-1})	M	322.10±30.57b	282.00±51.03b	319.70±37.24b	212.20±47.53c	381.10±48.42a
	FM	222.10±28.90d	481.40±29.43a	326.20±35.29b	330.40±54.89b	272.40±34.98c
LDL (mg dl^{-1})	M	101.00±16.29b	157.80±40.64a	156.80±37.97a	97.90±19.67b	168.70±25.60a
	FM	82.90±16.41c	137.40±35.80b	167.10±20.63a	149.80±34.77ab	97.10±28.88c
HDL (mg dl^{-1})	M	115.50±08.55b	40.70±07.27d	142.10±28.90a	111.70±21.74b	79.60±11.20c
	FM	84.20±19.45b	60.60±15.06c	93.60±13.52b	116.40±27.49a	122.80±21.02a
VLDL (mg dl^{-1})	M	77.20±12.21b	104.50±12.48a	59.90±12.33c	40.40±04.74d	58.20±03.12c
	FM	81.50±12.44b	115.90±06.17a	64.50±09.54c	58.90±05.11c	62.80±05.37c

FM: Female M: Male

spawning started, a sudden significant decrease in glucose was observed in male. These variations in serum glucose levels from both sexes of rainbow trout suggest that glucose could be used for reproductive process. Some researchers reported that serum glucose levels elevated until early spawning, then rapid falling was observed associated with starting of reproduction[13,21,22]. While glucose values increased from pre-maturation to ovulation in male and decreased till post-spawning, these values increased but fluctuated from pre-maturation to post-spawning in female. Similar results were also reported[13,23,24].

It is known that while males produce milt during spawning season, females spawn only once[25]. Serum cholesterol levels of male fluctuated from pre-maturation to post-spawning, but it decreased from pre-maturation to maturation and increased up to post-spawning. It was significantly increased during reproduction process in female. It is estimated that deposited energy was used for reproduction activity. Low levels of cholesterol at the time of spawning have been confirmed by the studies of Dindo and MacGregor[20], Smith[21] and Cerda *et al.*[26].

The HDL levels diminished from pre-maturation to maturation in both sexes. The HDL levels regularly increased till post-spawning in female, but it increased from maturation to spawning and decreased in post-spawning in male. The LDL levels from Pre-maturation to ovulation increased in both sexes. This parameter reduced in spawning and then increased again in male and it decreased from spawning to post-spawning. Since significant changes occurred in spawning with respect to LDL, it may be used an indication of reproduction.

The values of VLDL in both sexes presented similar manner, increasing from pre-maturation to maturation. A significant change in VLDL was observed at maturation and then significantly reduced till post-spawning in both sexes. The similar results in LDL and VLDL and opposite HDL values were reported by Gjoen and Berg[27]. They concluded that differential HDL catabolism exists in lower vertebrates, where HDL is the dominant plasma lipoprotein. Furthermore, the VLDL and LDL concentrations in the plasma were closely correlated to the intake of dietary lipids. High levels of HDL in the carp and rainbow trout plasma were a result of blood collection before breeding and also due to the natural characteristics of fish.

The results of present study revealed that there were some changes in cholesterol, glucose and triglyceride levels in rainbow trout during reproduction, however there should be much available data from other fish species supporting the present study before deciding for the usage these parameters as inducement of reproduction time. Therefore; further studies should be conducted on the utilization of HDL, LDL and VLDL during reproduction stage.

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