World Journal of Zoology 5 (4): 278-281, 2010
ISSN 1817-3098

The Correlation Between Blood Biochemical Factors with Some Biological Characteristics of Gonad, Fertilization Success, Hatching Rate and Larval Size in Caspian Kutum, *Rutilus frisii kutum*

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Abstract: Correlation between some biochemical factors of blood (sodium, potassium, magnesium, calcium, cholesterol, total protein and glucose) and biological characteristics of gonad (biological characteristics of egg, hydrated egg and gonad's weight), fertilization success, hatching rate and larval size was Evaluated among 80 female of the migratory population of Caspian Kutum in Tajan river of Iran in spring of the 2007. There were a significant correlation between sodium and perivitelline space ($P<0.05$); total protein and ratio of yolk sphere to perivitelline space ($P<0.01$). On the other hand, there was an invert correlation between total protein and perivitelline space ($P<0.05$). With increasing of glucose, yolk sphere to perivitelline space ratio increased ($P<0.01$). But correlation between glucose and perivitelline space ($P<0.01$) was reverse.

Key words: Caspian · Kutum · Blood · Egg

INTRODUCTION

Low level of blood total protein (ToPr), potassium (K^+), calcium (Ca^{+2}) and transient elevating in magnesium (Mg^{+2}) of blood happen when temperature is low or rapid fluctuation occurs. Glucose is elevated as well. On the other hand, continuous decreasing in temperature does not influence on the given parameters. This shows that fish is unable to control protein level and ions [1].

Caspian kutum, *Rutilus frisii kutum* Kamenskii (1901) is an endemic fish of Caspian sea and its populations generally recorded along near the coast, from the Trek river the north to the southern part [2]. It consists more than 70% of fishermen catch in Iran coastal of the Caspian sea [3]. Kutum is a high market demand fish [4] and due to the dwindling of its natural resources by overfishing, dam construction in migration path and destruction of its natural reproduction environments, studies on this valuable species reproduction assist the aquaculture industry in meeting the ever increasing demand for kutum, by improving protocols for higher efficiency of egg production and enhanced survival of progeny.

The current work aimed to evaluate different parameters related to reproductive biology and finding the important blood factor for breeding of Caspian Kutum.

MATERIALS AND METHODS

Broadstock Preparation and Fertilization: Our investigation was carried out on 80 migratory sexually mature Caspian Kutum (*Rutilus frisii kutum*). The body average weight was 977±229g (without the weight of gonad) and body length was 49.62±1.2 cm. Each female stripped separately to attain ovum for fertilization experiments. For establishing equal condition for fertilization and lowering the effects of male characteristics on the fertilization results, 15 mature male with the weight of 735.7±241.7g and length of 44.2±4.9Cm was cached and after spermiating, equal part of attaining sperm from all male specimens mixed [5]. Then, Fertilization success was determined under a loop equipped with ocular micrometer (with the accuracy of 100micro meter) at second cell fusion stage.

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Gonad Biological Characteristics Experiment: Before fertilization some ovum was taken and after fertilization some hydrated eggs was taken and placed in acetic acid 5% for 10 minutes in order to evaluate the diameter of ovum, hydrated eggs and yolk under a loop equipped with ocular micrometer (with the accuracy of 100micro meter). The surface-to-volume ratio, S/V, was calculated with the following formula:

$$S = 4 \Pi r^2, V = 4/3 \Pi r^3 [3]$$

In the formula, S is surface, V is volume and r is ovum and hydrated diameter. The volume (mm^3) of the yolk space was calculated using the formula [6], Ps = V - Ys where Ps is the perivitelline space, V is the egg volume and Ys is the yolk space; the Ys/Ps ratio was calculated by dividing the yolk space by the perivitelline space.

At the end of the experiment, fertilized eggs were transferred to the hatchery located in shahid Rajaee, Sari. Upon the hatching, hatching rate and larvae size was measured. In order to determine the hatching rate of each broodstock, the number of fertilized eggs which transferred to each incubator as well as the number of larvae from each incubator, belonging to each female, calculated with weight method using the following formula: number of egg (or larvae)= number of egg (or larvae) in gram × the weight of all attaining eggs in gram. Hatching success is calculated by dividing the number of larvae by the ovum number, according to the following formula: Hatching success= number of larvae / number of ovum×100.

For determining the larvae size, after immobilizing them with formalin solution (5%), observed under oculometer loop and measured the size and continued this process until yolk sack disappeared.

Biochemical Compounds of Blood: Blood sampling was done from the caudal vasculature with cutting peduncle [1]. The blood placed in tube containing heparin sodium, then transferred into a 2 ml microcentrifuge tubes and centrifuged for 15 minutes at 3000g at 4°C. The plasma was removed and transferred to another microtube and stored frozen at -80°C until subsequent analysis for ion concentration.

Sodium (Na^+) and potassium (K^+) were measured by flame photometer (JENWAY-PF P7). Magnesium (Mg^{+2}), Calcium (Ca^{+2}) and total protein (ToPr), cholesterol (Chole) and glucose (Glu) were measured by spectrophotometer (WPA-S2000).

Statistical Analysis: The correlation between biochemical factors of blood and blood biochemical compounds and biological characteristics of gonad, fertilization success, hatching rate and larval size were analyzed using the bivariate correlation coefficients of Pearson (SPSS, ver. 10.05; SPSS, Chicago, IL).

RESULTS

There was significantly direct correlation between Na^+ and perivitellin space ($P<0.05$). With increasing in blood ToPr, the ratio of Ys/Ps increased significantly

Table 1: Correlation between evaluated parameters

	Na^+	K^+	Ca^{+2}	Mg^{+2}	Na^+/K^+	Ca^{+2}/K^+	ToPr	Chole	Glu	pH	Hematocrite (%)
Ovum diameter	-0.152	-0.215	0.385	0.170	0.028	0.196	0.347	0.178	0.536*	-0.177	-0.258
Ovum surface	-0.175	-0.204	0.400	0.095	0.023	0.192	0.352	0.181	0.554*	-0.175	-0.258
Ovum volume	-0.196	-0.195	0.415	0.088	0.018	0.189	0.357	0.184	0.571*	-0.174	-0.258
S/V	0.107	0.237	-0.351	-0.117	-0.041	-0.206	-0.334	-0.171	-0.499*	0.181	0.255
Yolk diameter	-0.161	-0.252	0.321	0.061	0.023	0.164	0.340	0.148	0.563*	-0.145	-0.295
Yolk surface	-0.182	-0.239	0.340	0.056	0.016	0.192	0.347	0.153	0.58*	-0.147	-0.293
Yolk volume	-0.204	-0.227	0.359	0.051	0.011	0.188	0.355	0.158	0.596*	-0.149	-0.291
Perivitellin space	0.539*	0.089	-0.216	0.472	0.245	0.021	-0.621*	0.051	-0.732**	-0.189	0.299
Ys/Ps	-0.401	-0.124	0.449	-0.183	-0.127	0.091	0.722**	0.213	0.775**	-0.072	-0.337
Fertilization success	-0.204	0.306	0.234	-0.059	-0.295	-0.253	0.034	-0.263	-0.059	0.102	-0.343
Hatching rate	0.449	-0.163	-0.434	0.109	0.255	-0.303	0.047	0.178	-0.274	-0.094	0.501
Larvae size (first biometery)	0.548*	-0.133	-0.355	0.439	0.288	0.295	-0.035	0.366	-0.258	-0.490	0.180
Larvae size (second biometery)	0.068	-0.147	-0.158	-0.019	-0.103	0.095	0.220	0.352	0.383	-0.179	-0.246

*P<0.05, **P<0.01

Na^+: sodium, K^+: potassium, Ca^{+2}: Calcium, Mg^{+2}: Magnesium, ToPr: Total Protein, Chole: Cholesterol, Glu: Glucose; S/V: surface-to-volume ratio, Ys/Ps: yolk sphere-to- perivitelline space ratio

Table 2: Regression equation and coefficient between some evaluated factors

r^2	Regression equation	Coeficient
Na^+ and Ps	$Y= 4.6405X^2-31.665X+396.02$	0.34
Glu and Ps	$Y=6576.9e^{0.3622X}$	0.62
ToPr and Ps	$Y= 5.1632X^2-60.245X+208.39$	0.54
Glu and Ys/Ps	$Y= -406.11X^2+2180.9X-422.03$	0.62
ToPr and Ys/Ps	$Y = 28.742X^2-47.924X+57.639$	0.64

Na^+: sodium, Ps: perivitellin space, Ys: yolk space, Glu:glucose, ToPr: total protein

($P<0.05$), but perivitellin space significantly decreased ($P<0.01$). Between blood glucose and diameter of ovum as well as yolk a significantly direct correlation existed ($P<0.05$). This correlation followed by correlation between blood glucose and the surface and volume of ovum and yolk ($P<0.05$).

The correlation between blood glucose and Ys/Ps ratio was significantly direct ($P<0.01$). On the contrast, the correlation between blood glucose and perivitellin space ($P<0.01$) as well as the ratio of S/V ovum was significantly reverse ($P<0.05$).

DISCUSSION

With increasing in blood Na^+, perivitellin space gets larger. This phenomenon could be explained that high level of blood Na^+ and probably ovum prenucleus space (future perivitellin) during ovulation result in higher osmotic pressure difference between perivitellin space and egg surrounding water during fertilization, which lead to higher hydration by egg. Increase in perivitellin space followed by higher volume and wet weight of eggs would result in more eyed eggs [5]. Therefore, it can be claimed that higher Na^+ and probably other ions, which lead to higher osmotic pressure of eggs and higher egg hydration and perivitellin space, cause to more eyed eggs and higher hatching rate in progress.

High Total protein of blood results in low perivitellin space and consequently higher Ys/Ps ratio. The reason for this phenomenon related to the ovulation stages within broad stock. Increasing of total protein in blood plasma and probably perinucleus space of ovum (future perivitellin), due to the blood correlation of future ovum during ovulation, lead to the lower osmotic pressure differences between perivitellin space and coelomic fluid or to some extent egg surrounding water during fertilization. The result of the latter phenomenon is lower hydration of egg and perivitellin space. This has both positive and negative consequences. Negative aspect includes with decreasing of perivitellin space as well as egg volume and wet weight, decreases the number of eyed eggs [5]. Therefore, we could claim that with increasing of total protein and other factors which decrease the difference of osmotic pressure between egg and its surrounding (coelomic fluid and water) followed by low egg hydration and low perivitellin space, result in decreasing of eyed eggs and later on hatching rate. Other aspect is with increasing of total protein followed by low perivitellin space, increases Ys/Ps ratio, which is accompanying with increasing of embryo respiratory capacity [7]. What's more, larvae from these eggs are relatively larger, because progressing in this process lead to the increasing of occupation of yolk space volume within egg [7-9].

Correlation between blood glucose and perivitellin as well as Ys/Ps ratio is similar to total protein. Blood glucose has a direct correlation with ovum as well as yolk diameter followed by surface and volume of ovum and yolk. Reverse correlation existed between blood glucose and ovum S/V ratio and its reason related to the ovulation stages.

In conclusion, each mentioned factors can be effective in broadstock selection programs and larvae growth in culture condition.

ACKNOWLEDGMENT

We are grateful to the administrator and staff of the Shahid Rajaee teleost and sturgeon breeding and culturing center and Tajan River capturing of Sari. We also thank for cooperation of central laboratory of Gorgan university of Agricultural Sciences and Natural resources.

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