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Correlations Between Biochemical Factors of Coelomic Fluid with Biological Characteristics of Gonad, Fertilization Success, Hatching Rate and Larval Size in Caspian Kutum, *Rutilus frisii Kutum*

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Abstract: A number of biochemical factors of coelomic fluid (sodium,potassium, magnesium, calcium, cholesterol, total protein and glucose) were determined with biological characteristics of gonad (biological characteristics of egg, hydrated egg and gonad's weight), fertilization success, hatching rate and larval size in 80 female of the migratory population of Caspian Kutum in Tajan river of Iran in Spring of the 2007. There was significant correlation between potassium and perivitelline space ($P<0.05$). In contrast, there was an invert correlation between potassium and yolk sphere to perivitelline space ratio ($P<0.05$). With increasing of magnesium, gonad's weight increased. There was an invert correlation between sodium to potassium ratio with number of egg in one gram of ovulated gonad ($P<0.05$). A significant correlation between sodium to potassium ratio and calcium to potassium ratio with larval size existed in second biometry ($P<0.05$). Correlation between total protein with hydrated egg's diameter, surface and volume was direct ($P<0.05$), but it was contrast for surface to volume ratio. There was a direct correlation between cholesterol and volume of hydrated egg ($P<0.05$). Correlation between glucose with hydrated egg's diameter, surface and volume and perivitelline space, contrary to surface to volume ratio, was invert ($P<0.05$).

Key words: Caspian Kutum % Egg % Coelomic fluid % Fertilization % Quality

INTRODUCTION

Egg quality assessment is a determinative factor in fish growth and development and it is possible that coelomic fluid induce final oocyte maturation before spawning [1]. Egg quality traits could be helpful for fish breeders in order to assess fingerling production, hatching management, improving cultural technique and production quality [2, 3].

Variation in diameter of Egg, yolk and previtellin space as well as egg genetic factors affect on larval survival, size, nutrition, resistance to starvation and predator avoiding which result in diversity in length, weight and life stage in progress [4].

Fish eggs are probably released electrolytes which establish buffering capacity and is able to regulate its environmental pH [5]. Coelomic fluid acidity and total protein are significantly related to the percentage of eyed eggs, which is an index for egg quality evaluation. Survival is more among the eggs with higher ability in hydration stage than the others. Eyed egg amounts is also related directly to increasing in hydrating egg wet weight, which is influenced by osmotic pressure of egg and coelomic fluid [6]. Egg survival in fish extremely depends on coelomic fluid and egg traits. With increasing in maintaining time of egg in coelomic fluid, its quality decreased due to the overrippening [7, 8]. With overrippening of egg in coelomic fluid, coelomic pH is significantly decreased. In contrast, total protein and steroidal fatty acids (cholesterol) is significantly increased [7, 9]. What's more, unhydrated egg wet weight increased, but the rate of increasing in wet weight in hydrating stage decreased. Yolk and previtellin space in newly attained eggs are more consistent and smaller than overripen eggs [7]. Coelomic fluid protein reversly related to the eyed eggs percentage [10].

Coelomic fluid influence on sperm traits such as motility pattern, motility lifespan and the percentage of motile sperm cells [11, 12]. Despite many measurements for assessment of sperm quality, sperm motility is the most determinative factors in fertilization [13].

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Approximately ten to thirty percent of total volume of eggs (in salmon) consists of coelomic fluid and affect on some sperm traits such as speed movements, motility pattern, total duration of movement. The percentage of motile sperm, lifespan and the duration of motility is related to coelomic fluid pH. Considering to low buffering capacity of semen plasma, sperm motility rate is highly influenced by surrounding pH (water and coelomic fluid). Because of the diversity among fish species, there are many variations in coelomic fluid quality. How ever, the differences may be even among the same species breeders. Variations in coelomic fluid can affect on inducing the males for spermiating in natural conditions. These variations are effective in selection programs. If no water added to the eggs, sperm movement duration and egg fertility would increase, which is due to the existing protein and minerals compounds in coelomic fluid. It is the reason why the fertilization percentage of eggs with no contact with water is more than which contacts with water.

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 [14]. It consists more than 70% of fishermen catch in Iran coastal of the Caspian sea [15]. Kutum is a high market demand fish [16] 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.

With regard to the importance of kutum in inland waters and its breeding, coelomic fluid was considered in its reproduction. The relationship between Biochemical compounds of coelomic fluid with biological traits of gonad, fertility success, hatching rate and larvae size in Kutum was evaluated in order to give an insight to the basic knowledge about selection programs for broodstock and improving the larvae quality and production.

MATERIALS AND METHODS

Broodstock Preparation and Fertilization: Our investigation of a possible correlation between some biochemical compounds of coelomic fluid (e.g. sodium, potassium, magnesium, calcium, cholesterol, total protein and glucose) and a number of biological characteristics of the kutum gonad (e.g. ovum, hydrated egg and gonad weight) and fertility success, hatching rate and larvae size 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±1.2Cm. For fertilization experiment 15 mature male with the weight of 735.7±241.7g and length of 44.2±4.9 Cm was used. For establishing equal condition for fertilization and lowering the effects of male characteristics on the results, equal part of attaining sperm from all male specimens mixed [17].

After each female stripped separately, attaining ovum fertilized with mixed sperm and the fertilization success was determined at second cell fusion stage. For determination of fertilization success, due to the thick chorionic layer, fertilized eggs placed in acetic acid for 10 minutes. Then, under a loop equipped with ocular micrometer (with the accuracy of 100micro meter), the amounts of fertilized eggs and the stage of cell division determined. before fertilization, some ovum was taken and after fertilization some hydrated eggs was taken for evaluating 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:

$$S = 4 B r^2, V = 4/3 B r^3$$

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 [18], 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 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 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. To determine the larvae size, they immobilized with formalin solution (5%) and measured under oculometer loop and this operation continued until yolk sack disappeared.

Biochemical Experiments of Coelomic Fluid: For sampling coelomic fluid, after stripping, eggs with coelomic fluid placed within a clean cloths and coelomic fluid transferred to the vials [17].

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 coelomic fluid and egg size, fertilization success, hatching rate and larvae size were analyzed using the bivariate correlation coefficients of Pearson (SPSS, ver. 10.05; SPSS, Chicago, IL).

RESULTS

Overall results is presented in table1. There was a direct significantly relation between K^+ and perivitelline space ($p<0.05$).

The relationship between K^+ and Ys/Ps was reverse ($p<0.05$). With increasing in K^+ level, gonad fluid weight was increased ($p<0.01$). The relation between Na^+/K^+ and the number of eggs per one gram of gonad was reverse ($p<0.05$). In contrary, there was directly significantly relationship between Na^+/K^+ and Larvae size in second biometry ($p<0.05$). These relationship was the same between Ca^{+2}/K^+ and Larvae size in second biometry ($p<0.05$). The relationship between total protein and egg diameter, surface and volume was direct ($p<0.05$). It was reverse between total protein and S/V ratio. Relationship between cholesterol and egg volume was also direct ($p<0.05$). The relationship between glucose and egg diameter, surface, volume as well as perivitelline space was reverse ($p<0.05$). It was in contrast between glucose and S/V ratio, which was direct ($p<0.05$).

DISCUSSION AND CONCLUSION

With increasing in K^+ concentration of coelomic fluid, privitellin space increased. It could be related to the difference in osmotic pressure of privitellin space and water surrounding egg as a consequence of high level of K^+ of coelomic fluid and possibly egg prenucleus space (future privitellin) during ovulation which leads in more water absorption of egg. It could have both positive and negative consequences: for positive case, with increasing in privitellin space and followed by increasing in volume and wet weight of eggs, the amounts of eyed eggs will be increased [17]. For negative case, decreasing in ratio of perivitelline space-to- yolk space could be followed by high level of K^+ and increasing in previtellin space, which is accompanying with low respiratory potential of embryo [19].

K^+ and Ca^{+2} are respectively sperm inducing and inhibiting motility factors [12]. High levels of K^+ result in low Ca^{+2}/K^+ ratio. Regard to the role of these two ions in sperm motility and their existence in coelomic fluid, as an effective compounds in fertilization, they should have a suitable balance [12]. There was a significantly relationship between Ca^{+2}/K^+ ratio of coelomic fluid and larvae size in second biometery. If this case confirmed by future research, it could be used as a measure for female selection programs [11]. High Na^+/K^+ ratio lead in low number of eggs per one gram of gonad and higher size of larvae in second biometery. It could be related to the high size of egg due to the high osmotic pressure followed by high ratio of Na^+/K^+ within egg (due to the vicinity to the coelomic fluid), which consequences in high water absorption from coelomic fluid. Therefore, it could be a positive factor in broodstock selection [11].

High total protein level of coelomic fluid leads in increasing of egg diameter as well as egg surface and volume. How ever, S/V ratio and perivitellin space

Table 1: Reciprocal correlation between factors

	Na^+	K^+	Ca^{+2}	Mg^{+2}	Na^+/K^+	Ca^{+2}/K^+	ToPr	Chole	Glu	pH
Gonad weight	0.426	-0.126	0.494	0.776**	0.484	0.344	0.194	0.403	-0.303	-0.105
the number of eggs per gram	-0.484	0.327	-0.03	-0.193	-0.634*	-0.317	0.337	0.381	-0.15	-0.627*
egg diameter	-0.072	0.205	0.153	0.097	-0.173	0.017	0.565*	0.486	-0.618*	-0.137
S/V	0.081	-0.196	-0.152	-0.112	0.172	-0.03	-0.528*	-0.456	0.613*	0.114
perivitelline space	-0.144	0.613*	0.038	-0.046	-0.312	-0.349	-0.621*	0.426	-0.623*	-0.108
Ys/Ps	0.11	-0.623*	0.031	0.16	0.23	0.442	-0.144	-0.155	0.223	0.013
Fertilization success	0.149	0.056	-0.439	0.18	0.148	-0.084	0.339	0.289	-0.57	0.013
Hatching rate	-0.095	0.238	0.121	0.501	-0.049	0.1	-0.489	-0.263	0.181	0.395
Larvea size(First biometry)	0.278	0.219	0.263	0.417	0.222	0.155	-0.092	-0.038	-0.238	0.43
Larvea size(second biometry)	0.395	-0.46	0.394	0.399	0.541*	0.609*	-0.015	-0.065	0.155	0.282

*P<0.05, **P<0.01

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

Table 2: Regression equation and R^2 between some parameters.

Correlation	Regression equation	R^2
perivitelline space and K^+	$Y= 0.4614X^2-2.3413X+8.6086$	0.39
Gonad fluid weight and Mg^{+2}	$Y= -0.5X^2+0.0004X+0.9417$	0.63
egg diameter and ToPr	Y= 164.96X2-841.8X+1074.8	0.73
perivitelline space and ToPr	$Y=2.149X^2-20.036X+47.713$	0.44
S/V and Glu	$Y=123.83 X^{2.3543}$	0.4

decreased. It means that increasing of egg diameter, due to the increasing in perivitellin space followed by hydration, is more affected by yolk space. As already described, high time duration of maintaining the eggs within coelomic fluid result in secretion of some egg protein into coelomic fluid [6, 7, 12]. This secretion, which is probably accompanying with low hydration by eggs due to the osmotic pressure differences between egg and coelomic fluid, results in low perivitellin space. Because of the increasing of egg diameter followed by low S/V ratio of egg, oxygen diffusion into embryo within the egg (and consequences hatchability) decrease [10]. Increase in egg diameter has direct relationship with yolk diameter (as an oxygen consumption part) and reverse relationship with perivitellin space. As a result, total protein level of coelomic fluid can be a negative index for egg quality. How ever, finding of some previous researchers did not show this factor as a suitable index for determining egg maturation and quality [10].

Glucose has a reverse relationship with egg diameter, surface, volume and perivitellin space, but direct relationship with S/V ratio. Glucose in coelomic fluid can be a positive index for egg quality determination and to some extent in hatching rate [19].

In conclusion, each evaluated factor can be effectively used in broodstock selection and possibly these ratio could affect larvae growth in cultural systems.

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