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Research Note

Hematological and Biochemical Parameters in Female Black King Fish Cobia (*Rachycentron canadum)*

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Black King Fish Cobia (*Rachycentron canadum*) is a potential candidate fish for aquaculture. In the present investigation an attempt was made to understand the biochemical and hematological parameters in different stages of maturity in females. Blood samples were collected from different stages of maturity and subjected to hematological parameters such as hemoglobin (Hb), Total Erythrocyte Counts (RBC), Total Leucocytes Count (WBC) and hematocrite value (Ht) and biochemical parameters such as blood glucose, total serum protein, cholesterol and triglycerides. The hemoglobin level was 15.77±0.15 gm/dl in immature female, 14.57±0.92 gm/dl in matured female and 11.63±0.12 gm/dl in spent fish. The hematocrite value also show similar trend with 46.6±1.15, 45.34±3.45 and 34.67±0.58 in immature, mature and spent fish respectively. The total RBC count was 5.40±0.1ml/cm^3 in immature, 5.2±0.24 ml/cm^3 in mature and 3.83±0.12 ml/cm^3 in spent fish. The WBC count also showed a similar trend of 16.30±0.1 cells/mm^3, 15.83±0.34 cells/mm^3 and 11.57±0.12 cells/mm^3 in immature, mature and spent fish respectively. Significant change was observed in the blood glucose level in all the stages of maturity. The serum cholesterol level was significantly higher in mature fish (291.67±1.00 mg/dl) than immature (204.0±0.89 mg/dl) and spent (203.67±1.0 mg/dl) fish. The results of the present investigations indicated higher metabolic activity in immature fish than matured and spent fishes and also indicated gradual mobilization of vital blood parameters during the process of maturation.

Keywords: Cobia, hematology, biochemical, maturation

The black king fish, Cobia is the sole representative of the family Rachycentridae. It is a pelagic fish distributed worldwide; in tropical, sub-tropical and temperate warm waters regions with the exception of the eastern pacific ocean (Briggs, 1960). The black king fish is highly carnivorous in nature and mostly prefers crustaceans especially crab hence also known as crab eater. It is a gonochoristic, having either a fixed male or female sex. External differences between sexes are limited. The exact size and age at which cobia are sexually mature varies with location however, male and female fish generally under go first spawning period at 1 to 2 and 2 to 3 years respectively. It is a multiple batch spawner, with spawning season extending from April to September. Its fecundity ranges from 0.37 million to 1.9 million (Burns et al., 1998). It is considered as a potential species for aquaculture due to its faster growth rates and it grows 6 to 8 kg per year (Lu et al., 2022; Sakthivel et al., 2019; Killekar et al., 2017). It can tolerate wide range of temperature (16.5-32 ^oC) and salinity (22.5- 44.5 ppt) (Kaiser & Holt, 2005). As per published reports, the haematological variations appear to be correlated to the reproductive cycle in several fish species

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(Colgrove, 1966; Einsporn-Orecka, 1970; Pickering, 1986; Suzuki, 1986).

Data on the hematological and biochemical parameters are very scanty for cobia fish. These parameters are very much useful and acts as biomarker to assess the health condition of brood stock of cobia fish. The present study was aimed at generating the data related to hematological and biochemical indices of cobia fish at different gonadal maturation to assess the health status of cobia fish.

The fishes were caught in stretches of Kovalam (12^0 49′ N and 80^0 15′ E) located 35 km south of Chennai and run parallel to the sea coast. Approximately 4 to 5 animals were caught each month with hook-line, between March 2010 and April 2011. After capture, the animals were acclimated in tanks containing 250 L of water, with constant aeration and transported immediately to the hatchery located close to the bank of Kovalam backwater. The time needed to transport the fish from site of capture to the hatchery was approximately 30 min. In the hatchery fish were acclimated for 48 h in tanks containing 8000 L of water, under flow through system with constant aeration to recuperate from the effect of capture, handling and transportation stress. Studies have shown that after 4 h of stress in fish, the levels of hematological parameters are similar to those observed at time zero (Espelid et al., 1996; Frisch & Anderson, 2000). In this study, the animals captured were selected after careful examination for assuring their health status, where animals excluded were those with clinical signs of disease and anatomical and pathological alterations after post-mortem examination (Lucký, 1982; Rehulka et al., 2004) and significant parasitic infestations.

The animals were anesthetized with 3 % phenoxy ethanol, measured (TL=total length in cm) and weighed (Wt=weight in kg), and cannulated. Gonadal samples collected were examined under microscope to find out the size and stage of maturity. Immature, matured and spent cobia gonadal sample were having tissue mass with small oocyte, free eggs (700 micron) and only tissue mass respectively. Based on canulation three maturity stages, i.e. immature (4 to 6 Kg), matured (8 to 12 Kg) and spent (14 Kg) were selected for haematological and biochemical study.

Blood was collected from the caudal vein using a syringe (no.23), previously rinsed with 2.7 % EDTA solution. Blood collected was transferred immediately to a test tube containing a small amount of EDTA powder (as an anticoagulant) and shaken gently to prevent haemolysis of blood cells. The blood samples were used for determination of haemoglobin content, total erythrocyte count, total leucocyte count and hematocrit. For serum, blood was collected without anticoagulant, allowed to clot for 2 h and then centrifuged at 5000 g for 5 min followed by collection of serum with a micropipette. Serum was stored at -20 ^oC until use.

Glucose was estimated by the method of Nelson & Somogii (1945). The haemoglobin percentage was determined by estimating Cyanmethemoglobin using Drabkin's Fluid (Qualigens, India). The hematocrit value (Ht) was determined by the micro-hematocrit method (Goldenfarb et al., 1971). Red blood cell diluting fluid and WBC diluting fluid (Qualligens Fine Chemicals, India) were used for total erythrocyte count and total leucocyte count respectively. Twenty microlitres of blood was mixed with 3980 μL of corresponding diluting fluid in a clean test tube and shaken well to suspend the cells uniformly in the solution. Cell counts were done using a Neubauer's counting chamber. Cell numbers were calculated according to the following formula: No. of cells (cu. mm $^{-}1$) = (no. of cells counted x dilution) / (area counted x depth of fluid). Blood samples were centrifuged for 5 mins at 12,000 rpm. Hematocrit or packed cell volume (PCV) was measured by using the microhematocrit pipette.

Serum protein was estimated by biuret method (Reinhold, 1953) using kit (Qualigen Fine Chemicals, India). Cholesterol was estimated by following CHOD/ PAP method (Medichem Kit) and triglyceride was estimated by GPO/PAP method (Medichem Kit)

Statistical significance of different variables was analyzed using one-way analysis of variance (ANOVA) via SPSS 16.0 for Windows. Duncan's multiple range test was used for post hoc comparison of mean (P<0.05) between different maturity stages. All data presented are means ± standard error and statistical significance for all statistical tests was set at P<0.05.

The hematological and biochemical results related to gonadal maturation stage provide important information on physiological conditions, health status and defence system of cobia fish. The values of haematological parameters (RBC, Ht, Hb) did not show any significant difference (P>0.05) between

Table 1. Hematological and biochemical parameters of female black king fish cobia under different gonadal maturation stages

Parameters	Immature	Matured	Spent
Hematocrit (%)	$46.60^{a} \pm 1.15$	$45.34^{a} \pm 3.45$	$34.67^{b} \pm 0.58$
RBC (cu. mm $^{-1}$)	$5.40^{a} \pm 0.10$	$5.20^{a} \pm 0.24$	$3.83^{b} \pm 0.12$
WBC (cu. mm $^{-1}$)	$16.30^{a} \pm 0.10$	$15.83^{b} \pm 0.34$	$11.57^{c} \pm 0.12$
Hemoglobin (mg/dL)	$15.77^{a} \pm 0.15$	$14.57^{a} \pm 0.92$	$11.63^{b} \pm 0.12$
Glucose (mg/dL)	$294.00^{a} \pm 2.08$	$197.60^{b} \pm 0.67$	$31.33^{c} \pm 1.33$
Cholesterol (mg/dL)	$204.00^{b} \pm 0.89$	$291.67^{a} \pm 1.00$	$203.60^{b} \pm 1.00$
Triglyceride (mg/dL)	$373.00^{a} \pm 2.00$	$342.00^{b} \pm 0.58$	$222.30^{c} \pm 0.88$
Total protein (g/dL)	$7.66^{a} \pm 0.03$	$6.60^{b} \pm 0.15$	$5.33^{c} \pm 0.33$

Values are expressed as mean ± SE. Different values in column with different superscript differ significantly ($P<0.05$)

immature and matured groups. However, significant ($P<0.05$) decrease was observed in spent stage of maturity (Table 1). It is well established that in teleost, the pre-spawning period or stage of maturing, fishes release large amounts of steroids into the blood stream, especially the steroid C_{21}, a hormone that controls final maturation of the oocytes in females (Luskova, 1998; Pinillos et al., 2003; Kavadias et al., 2004). Generally, spent fish have reduced hormonal levels and these hormones influence the sexual maturation and the haematological parameters (Burgos-Aceves et al., 2019; Malinovskyi et al., 2022). Another probable reason for the same could be lower metabolic rate and reduction in daily feed consumption at spent stage. In the case of WBC, gradual decrease in the mean values was observed from immature to spent stage. It indicates the gradual reduction of immune system as maturity advances. Similar observation was reported by Einszporn-Orecka (1970) in Tench and Santos et al. (2009) in *Centropomus parallelus*. The serum biochemistry vary from species to species and can be affected by many biotic and abiotic factors like water temperature, food, seasonal pattern, age and sex of the fish. In present study, biochemical parameters such as blood glucose, cholesterol, triglyceride and total serum protein were significantly different ($P<0.05$) in immature, matured and spent fish (Table 1). Significantly higher blood glucose levels were observed in immature and matured stage compare to spent stage. Glucose is a primary source of energy for fish and the higher blood glucose levels in these stages may reflect increased glucose uptake and utilization to support growth and development. In addition to this, during the mature stage, fish may also have higher blood glucose levels as they prepare for reproduction. Reproduction requires a significant amount of energy and higher glucose levels observed in the study could be to support the development of eggs and spermin in fish (Antomagesh et al., 2023; Qian et al., 2019). Concentration of total protein in blood serum was used as a basic index of condition and health status of fish (Rehulka, 1996 & 1998). Higher total serum protein was noticed in immature and mature stage. Significant decrease in serum total protein was observed as maturity progresses. Shell 1961 reported that a substantial decrease in protein levels in female fish during spawning season likely signifies considerable use of body protein in the development of eggs. In addition, a cyclic reversal of protein was noticed in small mouth bass, *Micropterus dolomieui* (Malinovskyi et al., 2022). The high content of protein observed in the immature fishes suggests that in the maturing phase, the fish needs a high protein diet. Higher concentration of triglycerides were present in immature and mature stage in which synthesis of vitellogenin take place during pre-spawnig period. The process of vitellogenesis is highly energy demanding and fats (triglycerides) are preferred source of energy (Bon et al., 1997). Cholesterol, a component of all eukaryotic plasma membranes is essential for the growth and viability of cells in higher organisms. It is also the precursor of steroid hormones such as progesterone, testosterone, estradiol and cortisol (Stryer, 1975). Cholesterol is incorporated in membrane and endogenous structure of eggs and thus its concentration in blood serum decreases at the time of reproduction (Diwan & Krishnan, 1986). In the present study the serum cholesterol is higher in maturing stage and lower in spent and immature

stage. The reason for the high serum cholesterol could be for utilization of blood cholesterol for egg development during maturation. From the present study it can be summarized that the immature and matured cobia are physiologically active compared to spent one.

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