SERUM BIOCHEMISTRY OF AFRICAN LUNG FISH (*Protepterus annectens)* DURING ACTIVE STATE AND HIBERNATION PERIOD AT WALAI, ITAS GADAU LOCAL GOVERNMENT AREA, BAUCHI STATE, NIGERIA

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Abstract: The African lung fish, *Protepterus annectens* (*P annectens)* is a highly priced food fish in Nigeria. The main objective of this study was to examine the fish and to provide the knowledge of its serum biochemical parameters during active state and hibernation period. The results of biochemical profile of *P. annectens* in this research showed higher values during active state except serum protein that showed higher value during hibernation period. This information may perhaps be very vital for effective exploitation, sustainable management and conservation of *P. annectens*.
Keywords: Bauchi State, biochemistry, Itas-Gadau, lung fish, Nigeria, *Protepterus annectens*, serum.

Introduction

African lungfish (*P. annectens*) (Fig. 1) is an obligate air breather and can undergo hibernation in subterranean mud cocoons for as long as four years during drought (Smith, 1930; Forey, 1986; Kees and Chapman, 2002; Ballantyne and Frick, 2010; Lederoun *et al*., 2020). Aestivation is a state of torpor often associated with arid conditions at high environmental temperature and without food or water intake for an extended period. It is characterized mainly by physical inactivity and low metabolic rate (Pinder *et al.*, 1992; Withers and Cooper, 2010). In nature, aestivation can occur inside a subterranean mud cocoon. In the laboratory, African lungfishes can be induced to hibernate in dried mucus cocoons in air (Chew *et al*., 2004; Loong *et al*., 2005; 2007, 2008a, b; 2012). There are three phases of aestivation: induction, maintenance and arousal. During the induction phase, the aestivating fish detects environmental cues, turns them into internal signals, and makes the required biochemical, physiological, structural and behavioural changes for aestivation. It hyperventilates and secretes plenty of mucus. The mucus will turn into a cocoon after 6 - 8 days. The fish enters into the maintenance phase of aestivation when it is encased in a dried mucus cocoon, with a complete cessation of feeding and locomotors activities. The aestivating lungfish must prevent cell death, preserve biological fuels and structures, and sustain a slow rate of waste production during the maintenance phase of aestivation. The lungfish can be aroused from aestivation with the addition of water. After struggling out of the cocoon, it swims sluggishly to the water surface to gulp air. Upon arousal, it re-hydrates, excretes the accumulated waste products and begins to feed after 7 - 10 days (Hiong *et al.*, 2015). It is found in a wide range of freshwater habitats in West and Middle Africa, as well as the northern half of Southern Africa. It is widespread, being found in Sierra Leone, Guinea, Togo, Ivory coast, Cameroon, Niger, Nigeria, Burkina Faso, Gambia, Ghana, Central African Republic, Chad, Benin, Senegal, Kenya, Mali and Sudan (Froese and Pauly, 2021). *P. annectens* is a highly prized food fish in Nigeria (Otuogbai, 2001; Ovuru and Ekweozor, 2004). It is distributed in shallow parts of rivers and lakes of some West African countries ranging from Senegal to Cameroon where it contributes to a relatively high percentage of artisanal fisheries (Otuogbai, 2001; Okafor 2004; Oniye *et al*., 2006).
Blood biochemical and hematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural freshwater resources in the tropics. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes (Afolabi *et al*., 2011; Fazio *et al*., 2013; Faggio, 2014; Knowles, 2006)). Biochemical parameter can be used to detect health of fish. Determination of reference values of these parameters in *P. annectens* is of utmost importance since so many factors including species, age, sex, season and management system has been reported to have effect on the haemato-clinic chemistry values in fishes. This study was carried out to examine the serum biochemistry of the *P. annectens* during both active state and hibernation period.

Fig. 1: *Protepterus annectens* (Adapted from Froese and Pauly, 2021)

Materials and Methods

Study Area

The study was conducted in Itas Gadau Local Government Area of Bauchi state, Nigeria (12º08’ 12.33’N and 10º17’10.28 3’E) with a total area of 1,436 km^2. The study sites were selected from the study area. These comprised of Rafin Dada (site A), Rafin Mai Roba (site B) and Kai da Jini (site C) (Fig. 2).

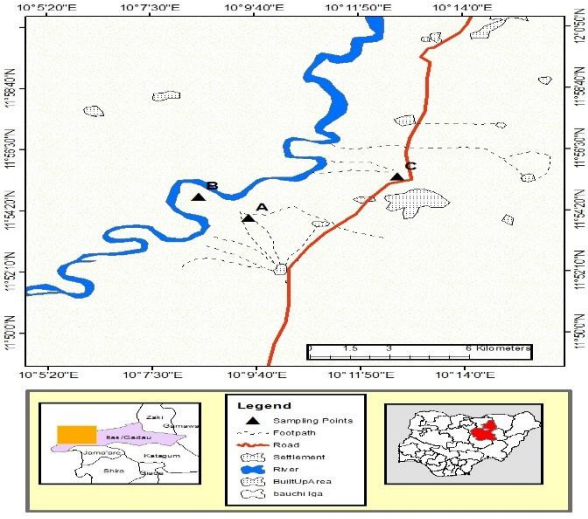

Fig. 2: Map of the Study Area showing the Sampling Sites (A, B, and C)

Sample collection

Live fresh samples of *P. annectens* irrespective of their length, weight and sexes were obtained from the fishermen of the study area during active state. The fishermen mostly use nets and traps to catch the fish. While during hibernation period the fishes were obtained by digging the ground to some certain distance from their known behavior during that period.

Blood Collection and Analyses

Blood samples were collected in the sampling field using syringe. Samples from the caudal vein of each fish were taken using syringe and put into containers which do not contain EDTA (anticoagulant) for serum analysis, where serum protein, serum cholesterol, serum glucose and serum urea were determined using an Olympus AU 400 biochemical analyzer (Olympus medical system Tokyo, Japan).

Data Analyses

Student T-test was used to compare biochemical parameters of fish samples during active state and hibernation period. One way ANOVA was used to determine mean differences among the various sites. All statistical analyses were done using excel and the statistical package for social sciences (SPSS) version 25.0.

Results

A total of ninety fish samples were collected for the research. Sixty were collected during the active state and thirty during hibernation period. Summary of the body measurements is presented in Table 1.

Table 1: Summary of body measurements of *P. annectens* used for the study

	N	Variable	Mean	SE	SD	Min	Max
Active State	60	Total Length (cm)	40.97	0.83	6.41	32.10	53.90
		Weight (g)	307.40	17.80	138.30	130.00	620.00
Hibernation Period	30	Total Length (cm)	47.38	1.78	9.77	32.20	74.30
		Weight (g)	594.00	89.60	490.70	180.00	2400.00
Total	90						

The mean±SD of serum chemistry parameter of *P. annectens* during active state and hibernation period in Rafin Dada is contained in Table 2. Serum protein was found to decrease in active state than hibernation period. Statistically there was no significant difference (P>0.05). So also serum chemistry, serum glucose and serum urea show relative increase in active state than hibernation period and statistically there was significant difference (P>0.05). Table 3 showed the mean±SD of serum chemistry parameters of *P. annectens* during active state and hibernation period in Rafin Mai Roba. Serum protein showed decrease in active state than hibernation period, statistically there was significant difference (P<0.001). Serum cholesterol and serum glucose showed relative increase in active state than hibernation period (P<0.001). Serum urea showed relative increase in active state than hibernation period and statistically there was significant difference (P<0.01). Table 4 contained the mean±SD serum chemistry parameter of *P. annectens* during active state of hibernation period in Kai da Jini. Serum protein showed a decrease in active state than hibernation period, statistically there was no significant difference (P>0.05). Serum cholesterol and serum glucose showed relative increase in active state than hibernation period, statistically there was significant difference (P<0.001). Serum urea showed relative increase in active state than hibernation period, statistically there was no significant difference (P>0.05). Whereas, Table 5 indicated the mean±SD of serum chemistry of *P. annectens* during active state and hibernation period of sites A, B and C combined. Serum protein indicated relative decrease in active state than hibernation period, statistically there is significant difference (P<0.01). Serum glucose, serum cholesterol and serum urea revealed a relative increase in active state than hibernation period, statistically there was significant difference (P<0.001).

Table 2: Mean±SD of serum chemistry of P. annectens during active and hibernation period of Rafin Dada (site A)

Parameters	Active State	Hibernation Period	P-value
Protein (mg/dl)	15.71±58.68	55.70±5.81	0.230
Cholesterol (mg/dl)	59.35±70.71	4.51±0.58	**<0.001**
Glucose (mg/dl)	95.79±88.64	7.62±3.30	**<0.001**
Urea (mg/dl)	37.16±17.30	17.19±3.37	**<0.001**

ns= not significant (P>0.05), *significant at P<0.05, **highly significant P<0.001

Table 3: Mean±SD of serum chemistry of *P. annectens* during active and hibernation period of Rafin Mai Roba (site B)

Parameters	Active State	Hibernation Period	P-value
Protein (mg/dl)	5.27±1.16	49.10±6.06	**<0.001**
Cholesterol (mg/dl)	191.49±178.94	4.40±0.64	**<0.001**
Glucose (mg/dl)	83.81±55.05	4.04±0.74	**<0.001**
Urea (mg/dl)	20.04±13.44	9.64±3.61	**0.003**

ns= not significant (P>0.05), *significant at P<0.05, **highly significant P<0.001

Table 4: Mean±SD of serum chemistry of P. annectens during active state and hibernation period of Kai da Jini (site C)

Parameters	Active State	Hibernation Period	P- value
Protein (mg/dl)	13.2 ± 7.48	60.33±21.40	0.057
Cholesterol (mg/dl)	243.63±144.79	4.59±1.16	**<0.001**
Glucose (mg/dl)	120.67±73.06	7.35±1.78	**<0.001**
Urea (mg/dl)	23.4 ±6.21	12.83±5.27	0.394

ns= not significant (P>0.05), *significant at P<0.05, **highly significant P<0.001

Discussions

Blood is an active transport medium in higher animals especially in the vertebrates and it is explained to be a medium that constantly bathes all the organs and tissues of the body, enabling the exchange of material between the internal and external environment of the organ and tissue (Tiamiyu *et al*., 2019). Blood biochemistry is the most economical and authentic tool to assess the health state of fishes (Samina *et al.*, 2013). The results of the biochemical profile of *P. annectens* can be used to assess the health state of the fish, these biochemical parameters can change with the fish species, age and cycle of sexual maturity and health condition (Okafor and Chukwu, 2010). Serum protein gives an index of the health status of the fish and indicator of nutritional status. The present study showed serum protein levels during active have lower values than hibernation period, no significant differences (P>0.05). Serum protein is the protein component of the blood and is vulnerable to increase with starvation or any other stress (Knowles, 2006). In the present study, serum protein concentration in *P. annectens* ranged from 5.27±1.16 to 15.71±58.68 during active state and 49.10±6.06 to 60.33±21.40 during hibernation period. This is in line with the findings of Ebeh *et al*. (2017). The level of total protein, cholesterol and triglycine are considered to be major indices of the health status of fish, increased concentration of serum protein can be caused by structural liver alterations reducing amino-transferase activity, with concurrent reduced deamination capacity (Burtis and Ashwood, 1998) and impaired control of fluid balance (Cozrakobac *et al.*, 2005). Increase in serum protein is also caused by starvation or any other stress (Knowles, 2006).

Blood glucose is an important source of energy for many cells. Blood glucose is normally maintained by the breakdown of dietary carbohydrate. Generally glucose is continuously required as an energy source by all body cells and must be maintained at adequate level in the plasma (Percin and Konyalioglu, 2008). In this study glucose concentration was found to be lower during hibernaton ranging from 4.04±0.74 to 7.62±3.30 and significant (P<0.05) and higher during active state ranging from 120.67±73.06 to 83.81±55.05 the concentration of glucose in *P. annectens* during active state may be due to higher growth rate and higher food conversion efficiency. This contradicts with the findings of Bittencourt *et al*. (2003) and who obtained the value of glucose to be 60.32 mg/dl for Nile Tilapia, when comparing with the values obtained in this study, it is higher than the values obtained during hibernation period and lower than the value obtained during active state. This finding also contradict with the findings of Oluwalola *et al.* (2016) who obtained the value of glucose (0.90±0.8 and 0.53±0.13 mg/dl for male and female *O. niloticus* respectively), which is lower than the values obtained in this study. According to Bittencourt *et al.* (2003) some fishes become hypoglycemic under same condition. Other environmental condition such as seasonal changes and physiological factors may contribute to the variation observed. Blood glucose level may vary according to season and water temperature, and glucose level in fish decreased with age and size of fishes (Bridges *et al.*, 1976). In this research the age and size of fishes obtained during hibernation period were higher than that obtained during active state.

Cholesterol is an important component of cell membrane and functions as precursors for the synthesis of sexual hormones. A number of fish species have seasonal variation in cholesterol with increased level during spawning. Total cholesterol level is found to be associated with disease resistance in fish. Cholesterol concentration varies both among and within fish species because of variation in diet activity and sexual development. In this study the values of cholesterol of *P. annectens* was found to be higher during active state ranging from 59.35±70.71 to 243.67±144.79 and lower during hibernation period ranging from 4.40±0.64 to 4.59±1.16. The cholesterol value was high during active state this may be due to chemical composition of their food. The cholesterol level differs according to age, general health condition and feeding diet. This is in line with the findings of Kulkarni and Pruthviraj (2016) who reported the value of cholesterol level ranging from 150.6-263.7 mg/dl.

Urea is a nitrogenous end product of metabolism. Urea is the primary metabolites derived from dietary protein and tissue protein turnover. The urea is roughly one-half of the blood urea. Blood urea nitrogen is waste product produced from breakdown of protein. Blood urea is removed from the body through urine, so blood urea nitrogen levels increase as kidney function decrease. Blood urea nitrogen is major nitrogen containing metabolic product of protein catabolism and act as a major osmolite, therefore it can be used as a sensitive tool to predict the gills and kidney dysfunction in fish. Any increase in protein introduced into the intestine to be digested can increase urea in the blood. Dehydration also increased urea level. Increase in blood urea nitrogen level suggests impaired kidney function. This may be acute or chronic kidney disease, damage or failure. It may also be due to conditions that result in decreased blood flow to the kidney. In Coho salmon affected by kidney disease, elevation of serum urea nitrogen indicates the loss of renal function (Wedemeyer and Ross, 1973). In this research revealed the values of blood urea ranges from 20.04±13.44 to 37.16±17.30 during active state and 9.64±3.61 to 17.19±3.37 during hibernation period. This indicated the serum urea level was higher during active state, which may due to presence of all metabolic activities in active state.

Conclusions and Recommendation

Conclusively, the main objective of this study was to examine the African lung fish (*P. annectens)* and to provide the knowledge of the characteristics of biochemical parameters of the research fish during active state and hibernation period. The result of biochemical profile of *p. annectens* in this research revealed serum protein level during active state has lower values than hibernation period. The concentration of glucose was higher during active state than hibernation period. It also revealed that cholesterol was higher during active state and lower during hibernation period Furthermore; the research revealed that the blood urea level was higher during active state. • Further studies on serum biochemical parameters of *P. annectens* from the same sites and probably other areas would be of great help since to the best of my knowledge this is represents the first information on its present form from the study area.

Conflict of Interest

Authors declare there is no conflict of interest related to this research.

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