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Utilization of lipids during aestivation of the African lungfish, *Protopterus annectens*

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Abstract

Sequential alterations of body weights as well as total lipids, triglycerides, cholesterol and ketone body levels in the blood of *Protopterus annectens* during twelve month duration of aestivation were investigated. The results revealed that after the first trimester of dormancy, there was significant body weight reduction ($p<0.05$) coupled with significant hypolipodaemia, hypotriacylgly-cerolaemia and hypocholesterolaemia respectively ($p<0.05$) but without significant ketonaemia ($p<0.05$). The total lipid, triglyceride, cholesterol and body weight reductions continued through the second, third and fourth trimesters of aestivation respectively ($p<0.05$) but with serum ketone body levels remaining unaltered ($p<0.05$). Thus, the utilization of lipids as a source of energy during aestivation of P. annectens does not lead to ketone body accumulation.

Key words

Total lipids, Triglycerides, Cholesterol, Ketone bodies

Introduction

Aestivation is a natural phenomenon of ecological significance because it enables certain animals to become dormant in response to unfavourable environmental conditions such as extreme hot weather or water shortage (Ndukuba, *et al.,* 2007b; Okafor, 2008a). During the wet season of April to October, the African lungfish, *P. annectens* lives in the swampy, oxygen-poor and weed covered edges of some Nigerian rivers and lakes such as Oguta lake (Ndukuba, 2007a). But in the dry season of November to March, *P. annectens* digs a tunnel at the lake side (or river banks) and dwells there, though in an inactive state probably until the next wet season (Okafor, 2008a,2009).

As it stays in this tunnel in the form of aestivation, the fish emits a mucus – like secretion from its skin which soon hardens to form a cocoon that wraps very properly the whole body of the fish, leaving only a small aperture at the head region to admit air into the lungs (Okafor and Odiete, 2002). It stays in this subterranean nest for several months with no food or water, undergoing series of physiological/behavioural adjustments in order to avoid desiccation and survive (Okafor *et al.,* 2003; Ndukuba *et al.,* 2007b). Previous reports by Okafor *et al.,* 2003; Okafor and Chukwu 2005; Ndukuba *et al.* (2007b) and Okafor (2008a,b) discussed some excretory, metabolic and haematological adjustments that go along with this state of inactivity in *P. annectens*. Okafor and Odiete (2002) had reported that prior to aestivation of *P. annectens* of Anambra river, fats were accumulated in some body organs such as gonads, liver, kidneys etc which would serve as energy source during the period of aestivation. Okafor (2008c) also reported that after a twelve month duration of aestivation of *P. annectens* there was significant reduction of total lipids and neutral fat levels of the blood but without a significant change in plasma ketone body levels.

The present report therefore was aimed at providing information on sequential changes in the level of total lipids, cholesterol, triacylglycerol and ketone bodies in the blood of *P. annectens* when subjected to aestivation for twelve months.

Materials and Methods

The African lungfish, *P. annectens* used for this study were obtained from Agulu lake, Anambra State, Nigeria and brought to laboratory. The specimens were authenticated using the standard methods of Sterba (1962), Boulenger (1964) and Gosline (1971) to ensure that they were only *P. annectens* and there were no intrusion of other species of *Protopterus*, especially *P. dolloi* of Congo river that had been reported to occasionally migrate into

Gabon and Cameroun rivers (Mbini, 2007) since Cameroun shares a common boundary with Nigeria. The specimens were acclimated for four weeks at room temperature ($26^0C \pm 5^0C$) inside twenty four plastic tanks (0.54 x 0.38 x 0.30m) each of which was filled with six litres of dechlorinated tap water at a stocking rate of five specimens per tank. All tanks were aerated with air pumps (Cosmo LMH 11,000) but left uncovered. The fish were fed daily on pelleted fish feed *ad libitum* until used for the experiment. The water in all tanks was renewed thrice weekly. After acclimation, 36 of the healthy survivors were selected, weighed and their total lengths (TL) determined, after which they were divided into four groups on basis of TL. Group 1 (TL 31.1 – 34.0cm) had 8 specimens. Group 2 (TL 34.1-37.0cm) had 12 specimens. Group 3 (TL 37.1 – 40.0cm) and Group 4 (TL 40.1 – 43.0cm) had 12 and 4 specimens respectively. This was to estimate weight loss during aestivation and for analysis of total lipid, triacylglycerol, cholesterol and ketone bodies at all the intervals: 0, 3, 6, 9, 12 months of aestivation respectively. Blood (0.2 ml) was drawn from the tail blood vessels of each selected fish using 1.0 ml heparinized syringe and transferred into 1.0 ml heparinized test tube. After the blood extraction, the 36 fish were aestivated for 12 months inside 18 laboratory tanks. The remaining survivors, about 66 in number were still kept in tanks and maintained in the laboratory for 12 months while their counterparts were being aestivated. The aestivation was induced with some modifications using the method of Okafor and Odiete (2002). Briefly each of the 18 tanks was filled to the brim with 25 kg of pulverized muddy soil collected from the bush beside the Works Department of Abia State University, Uturu Campus, Nigeria. Fifteen litres of dechlorinated water were poured inside each tank in order to soften the soil and facilitate burrow excavation by the lungfish. The 36 specimens were now placed on the soil surface at a stocking rate of two specimens per tank, each fish giving the other a gap of not less than 0.25 m. After less than two hours, all the fish had already dug their respective aestivation chambers, went to reside in them and so were completely absent at the soil surface. Throughout the twelve month duration of aestivation, the fish were neither provided with food nor water, nor were the tanks aerated or covered. However, they were observed weekly until the end of the experiment. The temperature of the aestivating room was 26.6 ± 8^oC.

The total lipid, triglyceride, cholesterol and ketone body levels of collected blood were estimated. Total lipid level was estimated by following the method of Folch *et al.* (1957). The triacylglycerol level was determined by the method of Gottferied and Rosenberg (1973). Cholesterol level was estimated by following the method of Stadam (1975) while the ketone body level was estimated following the method of Sumner and Somers (1949).

The means, standard deviation and regression analysis were carried out using appropriate statistical tools (Zar, 1984). Statistical comparison between aestivated and unaestivated (control) groups were made by the student's t-test and analysis of variance (ANOVA). Results were considered to be statistically significant at $p<0.05$.

Results and Discussion

The changes in body weights of specimens of *P. annectens* after every three months of aestivation showed gradual body weight deplition. Fish with total length (TL) (31.0-34.0 cm) had a mean percentage weight loss of 27.63, 41.02, 47.27 and 49.18 after 3, 6, 9 and 12 months of aestivation respectively. The fish of TL (34.1- 37.0cm) had a mean percentage weight loss of 26.91, 38.74, 43.62 and 45.88 after 3, 6, 9 and 12 months of aestivation respectively. Fish of TL (37.1-40.0 cm) had a mean percentage weight loss of 26.72, 40.82, 44.25 and 46.11 after 3, 6, 9 and 12 months of aestivation respectively. Fish of TL (41.1-3.0 cm) had a mean percentage weight loss of 25.83, 37.61, 43.22 and 44.37 after 3, 6, 9 and 12 months of aestivation respectively.

The mean plasma level of total lipids, triacylglycerol, cholesterol and ketone bodies in the four groups of TL specimens of *P. annectens* before aestivation and after every trimester of aestivation are also presented in Table 1. There was significant hypolipodaemia, hypotriacylglycerolaemia and hypocholesterolaemia respectively after the first trimester of aestivation when compared to their non-aestivating, or aquatic counterparts ($p<0.05$). The hypolipodaemia, hypotriacylgly-cerolaemia and hypocholesterolaemia continued through the second, third and fourth trimesters of aestivation respectively. Excess glucose that cannot be immediately catabolised is converted to glycogen and stored in the liver and muscles (Ganong, 2005). However, the quantity of glucose stored as glycogen is insufficient to meet the body's energy requirements for over twelve hours (Cunningham, 1978). Consequently, after some of the excess glucose had been converted to glycogen, the rest is converted into fats and stored in adipose tissues beneath the skin and abdominal cavities (Guyton and Hall, 2006). Over a dozen kg of fats can be stored in this manner and that may be sufficient to take care of the body's energy requirements for so many months (Cunningham, 1978; Guyton and Hall, 2006).

However prior to aestivation, *P. annectens* stored quite a large quantity of fats (Okafor and Odiete, 2002). The fats were gradually being broken down to provide energy for maintenance of life processes during aestivation as the fish was starving (Okafor and Odiete, 2002; Okafor, 2008c).

In this study, aestivation of *P. annectens* caused the progressive depletion of total lipid, triglyceride and cholesterol levels in all the four groups ($p<0.05$) but that of ketone bodies remained significantly unaltered ($p<0.05$). It could be possible that after the ketone bodies had been synthesized in the fish's liver, they entered the blood stream from where they were conveyed to other tissues and there became catabolised by joining the normal citric acid cycle to give rise to CO_2, H_2O and energy. As a result, there was no accumulation of ketone bodies in the blood (ketonaemia) especially

Table - 1: The mean levels of total lipid, triacylglycerol, cholesterol and ketone body in plasma of *Protopterus annectens* during aestivation

Parameter	TL group	Months of aestivation				
		0	**3**	**6**	**9**	**12**
Total lipids (mg dL^{-1})	x	368	178	88	72	60
	y	395	195	92	74	65
	z	448	240	118	94	72
	f	455	265	124	93	80
	TMV	416.5±43.2	219.5±43.5	105.5±18.4	83.3±11.1	69.3±10.0
Triacylglycerol (mg dL^{-1})	x	230	120	65	51	45
	y	232	110	58	44	39
	z	235	115	72	47	40
	f	240	125	54	48	45
	TMV	234.3±5.2	117.5±7.5	62.3±9.3	47.5±3.5	42.3±2.9
Cholesterol (mg dL^{-1})	x	168	84	40	35	32
	y	165	91	46	34	31
	z	169	79	51	47	42
	f	173	89	47	44	40
	TMV	168.8±3.9	85.8±6.1	46.0±5.5	40.0±6.6	36.3±5.4
Ketone bodies (mg dL^{-1})	x	0.9	0.9	1.0	1.1	1.1
	y	1.1	1.0	1.2	1.4	1.4
	z	1.3	1.3	1.1	1.7	1.6
	f	1.2	1.2	1.2	1.5	1.8
	TMV	1.1±0.2	1.1±0.2	1.1±0.1	1.4±0.3	1.5±0.4

TL = Total length (cm), x = TL (31.1-34.0 cm), y = TL (34.1-37.0cm), z = TL (37.1-40.0cm), f = TL (40.1-43.0cm), TMV = Total mean value

in the group 1 specimens ($p<0.05$) and no depletion of alkali reserve in the body that might cause metabolic acidosis. The paper therefore revealed that during aestivation of *P. annectens*, the rate of ketone body catabolism was higher in the smaller sized specimens ($p<0.05$).

The present work agrees to a large extent with earlier reports of Bezerra and Kemper (1999), Cowan *et al.* (2000) and Kabine *et al.* (2003). Bezerra and Kemper (1999) for instance, reported that during aestivation of the snail, *Biomphalaria glabrata*, the intermediate host of the blood fluke, *Schistosoma mansoni*, the ketone bodies did not accumulate, but their concentrations in tissues increased for a while before decreasing to normal values. Cowan *et al.* (2000) reported that during aestivation of Spadefoot toads, *Scaphiopus couchii*, there was increased ketone body and amino acid catabolism. Kabine *et al.* (2003) gave evidence that during hibernation of a small rodent, Jerboa (*Jaculus orientalis*) native to the sub-desert highlands of Morocco in North Africa, there was significant reduction of plasma neutral fat levels, but accelerated catabolism of ketone bodies. Pinder *et al.* (1992) reported that stored lipids in many amphibians during aestivation or hibernation were easily depleted due to accelerated ketolysis but without ketonaemia. Land and Bernier (1995) also reported that during aestivation of many fish species, there was a reorganization of some metabolic pathways to prevent the ketone bodies from accumulating in the blood.

In conclusion, this paper reports that during the one year aestivation of *P. annectens* there was a continuous catabolism of total lipids, triglycerides and cholesterol which was coupled with gradual body weight depletion, but without ketone body accumulation.

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