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Variation in energy reserve in *Rana tigrina* and *Bufo melanostictus* during annual seasonal cycle

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ABSTRACT

Blood glucose, liver and muscle glycogen total serum protein and fat body are estimated during four phases of their annual. Liver glycogen was high during period of dormancy but low during breeding. Fat body weight was high during hibernation but low during post breeding season. Serum protein was high during hibernation and aestivation but low during breeding and post breeding seasons.

Key words: Energy reserve, *Rana tigrina, Bufo melanostictus,* seasonal cycle.

INTRODUCTION

Amphibians are ectothermal vertebrates and like other poikilotherms are markedly influenced by seasonal change in temperature and water availability. Rana Tigrina and Bufo Melanostictus are two common Indian anurans. They under go four different phases during their annual biological cycle. Hibernation or winter sleep (Dec.-Feb.) and aestivation or summer torpidity (April-June) are periods of dormancy. During these periods they maintain minimum metabolic activities, practically starve and rely on energy reserves. The spawning seasons coincides with the monsoon rain from June to September (Mukherjee and Deb, 1960). Post breeding (Oct.-Nov.) is a hyperphagic phase during which large amount of food is consumed.

Glycogen in muscle and liver, fat in the fat bodies and serum proteins are major energy reserves in these vertebrates. There are some other less important energy reserves that could not be taken into account. Very few investigators have studied the metabolism of these energy reserves in anurans (Chaudhary 1984, Scapin *et al.,* 1994).

In the present investigation, liver and muscle glycogen, Fat and fat bodies, serum protein and blood glucose has been investigated in both Rana tigrina and toad Bufo melanostictus in order to explore the role of these energy reserves in their physiological activities during the annual cycle.

MATERIAL AND METHODS

Normal healthy adult frog Rana tigrina and toad Bufo melanostictus were captured from Khagaul near Patna. Each toad wighed an average 150 gm. and a mean tip of snout to vent measurement of 90 mm. Each frog weighed an average of about 300 gm. and had a mean snout to vent measurement of 120 mm.

Collection where made in all the four season and each experiment sample consists of ten animals of each were taken for investigation for each seasons or phase. Quantitative estimation of total serum protein was made by the method of Kingsley (1942) followed by Mehl (1945) and Weichselbaum (1948). Blood glucose was estimated quantitatively by O-toluidine method of Cooper and Mc Danile (1970).

The quantitative estimation of glycogen from liver and muscle was done according to a modified method of kemp and Andrien (1954). Fat body was weighed by usual method.

RESULT AND DISCUSSION

In both Rana tigrina and Bufo melanostictus, blood glucose level in low during hibernation and aestivation, but liver glycogen is higher during these period, low level of blood glucose may be related to its relatively inactive nature of these anurans during these periods and the surplus of glucose is converted into glycogen in the liver and serves as energy reserves. Wintering in ectothermal vertebrates causes low blood glucose and high lives glycogen (Bonnet 1994, Scapin *et al.,* 1994 Silvia *et al.,* 2004). The high liver glycogen found in the present study may also be due to the fact that during these period both the animals practically starve. The high glycogen content of the liver is associated with significant drop in feeding. Liver glycogen content is grater during hibernation than summer. Freeze Tolerence in various amphibians promote production of glucose in the form of cryoprotectant by glycogenolysis in hepatocytes (Holden *et al.,* 2000, Mommsen *et al.,* 1992). The view supports utilization of liver glycogen during the period between hibernation and aestivation.

In both Rana and Bufo blood glucose and liver glycogen content is low during breeding season. It is due to increased utilization of this energy reserve in spawning for energy need. A higher level of liver glycogen content inBufo than in Rana is indicative of a grater dependence of the former on liver glycogen than the later.

In both the anurans studied there is a slight increase muscle glycogen during summer as compared hibernation. The muscle glycogen store probably increased at the cost of liver glycogen because liver glycogen is lower and blood glucose

Table 1: R. Tigrina

Season	Total Serum Protein gm/100 mL	Glucose mg/100mL	Liver Glycogen mg/gm	Muscle Glycogen mg/gm	Fat Body weight gm.
Aestivation	3.52±0.02	52.2±3.78	39.4±3.97	37.2±3.19	0.69±0.04
Breeding	2.28±0.03	51.0±3.53	29.8±3.32	59.2±2.86	2.0±0.03
Post Breeding	2.94±0.04	70.2±3.72	22.0±2.91	67.0±5.83	0.45±0.02
Hibernation	3.40±0.03	40.2±3.19	46.2±5.02	37.6±4.15	3.41±0.03

Table 2: B. melanostictus

Season	Total Serum Protein gm/100 mL	Glucose mg/100mL	Liver Glycogen mg/gm	Muscle Glycogen mg/gm	Fat Body weight gm.
Aestivation	4.6± 5.22	69.4± 4.15	69.4± 4.15	5.83±1.54	1.60±0.03
Breeding	3.0±0.33	64.4± 3.32	66.4± 2.73	4.09± 1.02	1.10± 0.02
Post Breeding	3.1± 0.31	80.2± 3.81	58.0±2.39	6.04± 1.15	0.35± 0.03
Hibernation	4.06± 0.14	41.0± 4.63	89.2± 2.86	4.84± 0.23	2.29± 0.02

is higher during summer than winter. The increase in muscle glycogen during this period may be seem as an adaptation towards increased nee in locomotor activity during the following seasons i.e. spawning season. Muscles glycogen decreases during hibernation although liver glycogen and fat bodies are high. It is possible that muscle glycogen rather than liver glycogenm is the major source of blood glucose during hibernation and also because locomotor activity considerably dropped muscle glycogen is transported from muscle to liver and get stored in liver.

In poikilotherms, fat bodies are the main depot for lipid (Dickerson 1976, Lela *et al.,* 1979). In both Rana and Bufo fat body weight is highest during hibernation. It is due to dormancy and low BMR (Flanengan 1993, Tocque 1994). In both the amphibians fat body weight is very low immediately after breeding. There is a rapid rise in the size and weight of the fat body from post breeding to hibernation. The rapid increase in fat body weight is obviously related to intensity of feeding during post breeding season. In Rana there is a marked decrease in fat body weight. In the period between hibernation and aestivation but the slope in Bufo is less steeper. Although metabolism in hibernating amphibians are low but synthetic activities continue. This reflects the difference in extent of individual reserves found during hibernation and aestivation. In tegu lizard during dormancy fatty acids from fat is converted into β – hydroxyl butyric acid in liver to compensate for glucose as source of energy (Stuart and Vallentyne 1997). This view also support utilization of fat during these periods by poikilitherms. In both Rana and Bufo serum protein level is higher during aestivation and hibernation and there is light increase from hibernation to aestivation. This indicates that during period of torpidity, these two amphibians utilize mainly lipid and glycogen as energy reserves and maintain a high level of protein. Epigian and hypogian amphibian show variation in dergree of utilization of individual energy reserves (Harvent *et al.,* 2001). From the preset study it is revealed that in Rana there is not much difference in liver glycogen during hibernation and aestivation but fat body weight is much lower in summer than winter. In Bufo liver glycogen content is much less in aestivation than hibernation and there is not much difference in weight of fat bodies. This indicates that Rana primarily utilizes fat store for obtaining energy needed during winter and summer and most of the glucose in blood is of gluconeogenic origin where as Befo mostly utilizes glycogen as source of blood glucose during these periods. In Rana there is accretion of fat bodies from aestivation to breeding but in Bufo, there is a slight decrease in fat bodies during this period. Glycogen content and serum protein decreases from aestivation to breeding but the slope of glycogen level is more steeper in frog than in toad. This indicates that Rana primarily utilizes glycogen and protein for immediate energy needs and accumulates fat for their utilization during breeding season. Whereas Bufo utilizes protein, glycogen and fat for energy requirement during this period.

The fat body weight is minimum in both Rana and Bufo during post breeding period. It is related to the fact that the fat bodies are utilized primarily for reproductive activities, gametogenesis etc. during breeding season (Selvia *et al.,* 2004). The range of variation in fat body weight is markedly higher in Rana than in Bufo which probably suggests a grater dependence on fat for energy by Rana than Bufo.

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