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SEASONAL AND SEX VARIATION IN CARBOHYDRATE LEVELS OF THE COMMON AFRICAN TOAD *BUFO REGULARIS*

*Isehunwa O. Grace and Alada AR Akinola
Department of Physiology, College of Medicine, University of Ibadan, Nigeria

ABSTRACT

Most of the studies on carbohydrate levels in amphibians have been limited to frogs. The study was designed to investigate the effect of rainy and dry seasons of the year on blood glucose and glycogen levels of the Common African toad *Bufo regularis*. A total of 156 adult toads of either sex were used for the study. Each toad was fasted and anesthetized with sodium pentobarbitone (3mg/100g i.p). Blood samples were collected from the truncus arteriosus to estimate the blood glucose using modified glucose oxidase, while the glycogen level was determined using anthrone reagents method. The fasting levels of blood glucose during rainy season was significantly higher ($p< 0.05$) than the fasting values of blood glucose during the dry season. The fasting levels of liver and muscle glycogen during rainy season were significantly lower ($p< 0.01$) than the fasting liver and muscle glycogen levels during the dry season. The normal mean blood glucose of female toads was significantly ($p<0.01$) higher than the normal mean glucose of the male toads in rainy season while during the dry season the normal mean glucose of the male toads was not statistically higher than the mean blood glucose of the female toads. The results showed that seasonal changes produced significant effect on the carbohydrate levels and caused sexual variation in the blood glucose of the Common African toad *Bufo regularis*.

Keywords: *Bufo regularis*, glucose, glycogen, rainy season, dry season, sex.

INTRODUCTION

Various factors have been reported to affect the carbohydrate levels in amphibians. The factors include season, nutrition, analytical procedure, and geographical location (Farrar and Fyre, 1979). Seasonal variations in the glucose levels have been reported in the frogs (Smith, 1950, 1954; Mizell, 1965; Farrar, 1972; Hanke and Neuman, 1972; Byrne and White, 1975; Coppo *et al*., 2004; Peterson and Gleeson, 2007; Varadaraju, 2013) and toads (Hermansen and Jorgensen, 1969; Varadaraju, 2013). These studies revealed that the glucose levels are higher during breeding and summer than during winter while liver and muscle glycogen are higher in winter than summer. Lagerspetz (1977) reported seasonal changes in liver glycogen of *Rana temporaria* with latitude. The studies of (Mattew and Zaentz, 1963; Rocha and Branco, 1998) showed that the plasma glucose levels decreased with temperature and that the effect was greater during summer than autum in *Rana pipiens and Rana catesbeiana* respectively. Most of the studies on seasonal effects on carbohydrate levels in amphibians have been limited to frog species and the studies carried out in the temperate regions of the world. The available information on carbohydrate levels on toads found in the tropics is scarce and limited.

Similar seasonal variation in carbohydrate levels have been reported in mammals and humans. For instance, in small mammals that go into hibernation low blood sugar and high liver and muscle glycogen levels have been reported during hibernating period (Lyman and Chatfield, 1955). While in humans, the fasting glucose levels was found higher in the fall and winter than in spring and summer. The fasting glucose levels was found to correlate directly with percentage possible sunshine and inversely with temperature (Suarez and Barret-Connor, 1982; Jarrett *et al*., 1984; Behall *et al*., 1984).

There are also reports of sexual variation in the glucose levels of frogs. For instance, the early study of Scott and Kleitman (1921) showed the blood sugar of the female *Rana pipiens* was higher than that of the male. Smith (1950) reported higher blood sugar in the female *Rana temporaria* than the male between June and September but, from September to January, the male blood sugar was higher than the female. This was attributed to different rates of gonadal generation in the two sexes. Recently, Varadaraju (2013) reported sex difference in the blood glucose of aquatic frog *Hoplobatrachus trigerinus* and terrestrial toad *Duttaphrynus melanostictus* with the male blood glucose higher throughout the seasons. However,

*Corresponding author e-mail: funmisehunwa@yahoo.com

the studies of (Bosman and Zwarenstein, 1930; Coppo *et al*., 2004) found no sex difference in the blood sugar of *Xenopus laevis* and *Rana catesbeiana*, respectively. Alnagdy *et al*. (1995) found no sex difference in the blood sugar of Egyptian toad *Bufo regularis* although their study was carried out between June and August.

The common African toad *Bufo regularis* is commonly found in Nigeria especially during the rainy season. Toads and frogs show different behavioral activities during the different seasons of the year. For instance, they undergo period of hibernation during which the energy demand may be low. There has been no reported study on the effects of seasons and sex on carbohydrate levels in the common African toad *Bufo regularis*. This study was therefore designed to investigate the effects of rainy season (May-October) and dry season (November-April) and sex on the glucose and glycogen levels of the common African toad *Bufo regularis.*

MATERIALS AND METHODS

A total of 156 adult toads of either sex weighing between 70-100g were randomly picked and used for the study. The toads collected from Botanical garden environment of the University of Ibadan were brought into the laboratory after capture and kept in a plastic wire-gauged cage. Insects were kept away from the cage. Each animal had free access to drinking water. Each animal was fasted 24hrs before the start of the experiment. The animal was weighed and anaesthetized with sodium pentobarbitone 3mg/100g body weight given intra peritoneally (i.p). The thorax was opened and the truncus arteriosus was dissected free from the surrounding connective tissue for blood sample collection. Each animal was allowed 30mins to stabilize after surgery. After stabilization, blood sample was taken from the truncus arterious to determine the blood glucose. The blood glucose was determined immediately blood sample was collected by modified glucose oxidase method (Trinder, 1969). 156 toads were collected and used for the study during two rainy seasons (May-October) and two dry seasons (November-April).The glycogen content was determined using anthrone reagents method and as previously described by Isehunwa *et al*. (2013). The ambient temperature was measured throughout the period of the study. The ambient temperature was 28^0C (26^0C- 28^0C) during the rainy season and 32^0 C (30^0 C- 32^0C) during the dry season.

STATISTICAL ANALYSIS

The mean and standard error of all values were calculated. Significance was assessed by student's t-test for two independent variables. P values of 0.05 or less were taken as statistically significant.

RESULTS

The results of the study are shown in Tables 1-6 below. Tables 1 and 2 show the fasting glucose levels of the toads in different groups during rainy and dry seasons. In dry season, sixteen toads had no measurable blood glucose while during the rainy season only four toads did not have measurable blood glucose (Tables 1 and 2). The toad with highest blood glucose (Table 1) was found during the rainy season. The results of the study showed that the mean fasting blood glucose levels of *Bufo regularis* during the rainy was higher ($p< 0.05$) than the mean fasting blood glucose levels during the dry season (Table 3). However, the mean fasting liver and muscle glycogen levels during the rainy season were significantly ($p< 0.01$) lower than the mean fasting liver and muscle glycogen levels during the dry season (Table 4).

The results also showed that the mean fasting glucose level of the female toads was significantly ($P< 0.001$) higher than the mean fasting blood glucose of male toads during the rainy season (Table 5) while during the dry season the mean fasting blood glucose level of male toads was higher than that of the female toads although not statistically significant (Table 6).

Table 1. Fasting blood glucose levels of *Bufo regularis* during rainy season.

Season	Blood glucose mg/dl, (Range)	Number of Toads (N)
Rainy	0-20	4
	21-40	6
	41-60	32
	61-80	14
	81-100	4
	101-120	1
	121-140	2
	141-160	1

Table 2. Fasting blood glucose levels of *Bufo regularis* during dry season.

Season	Blood glucose (mg/dl), (Range)	Number of toads (N)
Dry	0-20	16
	21-40	17
	41-60	25
	61-80	11
	81-100	5
	101-120	1
	121-140	1
	141-160	0

DISCUSSION

The normal mean fasting glucose levels of the common African toad *Bufo regularis* observed in the present study is consistent with those reported in other species of amphibians (Smith, 1950; Mattew and Zaentz, 1963; Mizell, 1965; Hermansen and Jorgensen, 1969; Oyebola *et al*., 1998). The values of blood glucose are lower than those reported for mammals. It has been reported that the normal fasting levels of blood glucose in amphibians is lower than other vertebrates (Umminger, 1977). The low blood sugar levels probably suggest that amphibian's metabolism does not depend on closely regulated blood glucose levels but on another source than glucose for energy (Wright, 1959; Miller, 1961; Copeland and Deroos, 1971, 2005).

The observation during the present study in which some of the toads did not have measurable blood glucose agrees with the findings in frogs (Wearn and Richard, 1924; Wright, 1959; Copeland and Deroos, 1971; Herman, 1977; Copeland and Deroos, 2005). The absence of measurable circulating blood glucose probably suggests that glucose is not a substrate for energy in amphibians and may depend on other sources for energy (Wright, 1959; Miller, 1961; Copeland and Deroos, 1971, 2005).

Table 3. Mean fasting blood glucose levels during rainy and dry seasons.

Seasons	Rainy Season (n=60)	Dry Season (n=60)
Mean fasting glucose levels (mg/dl)	62 ± 3.0	51.4 ± 2.7

Student´s t-test: Significant (P <0.05)

Table 4. Mean fasting liver and muscle glycogen levels during rainy and dry seasons.

Seasons	Liver glycogen (mg/100g tissue), Mean ± S.E.M, (Range)	Muscle glycogen (mg/100g tissue), Mean ± S.E.M, (Range)	Number of toads (n)
Rainy	325.3 ± 27.3 (64.4 - 443.3)	165.5 ± 13.7 (13.6-216.3)	8
Dry	**757.5 ± 89.2 (585-1187.5)	*265.3 ± 36.5 (10.7-440)	8

Student's t-test: significant *(p< 0.05); ** (p< 0.01)

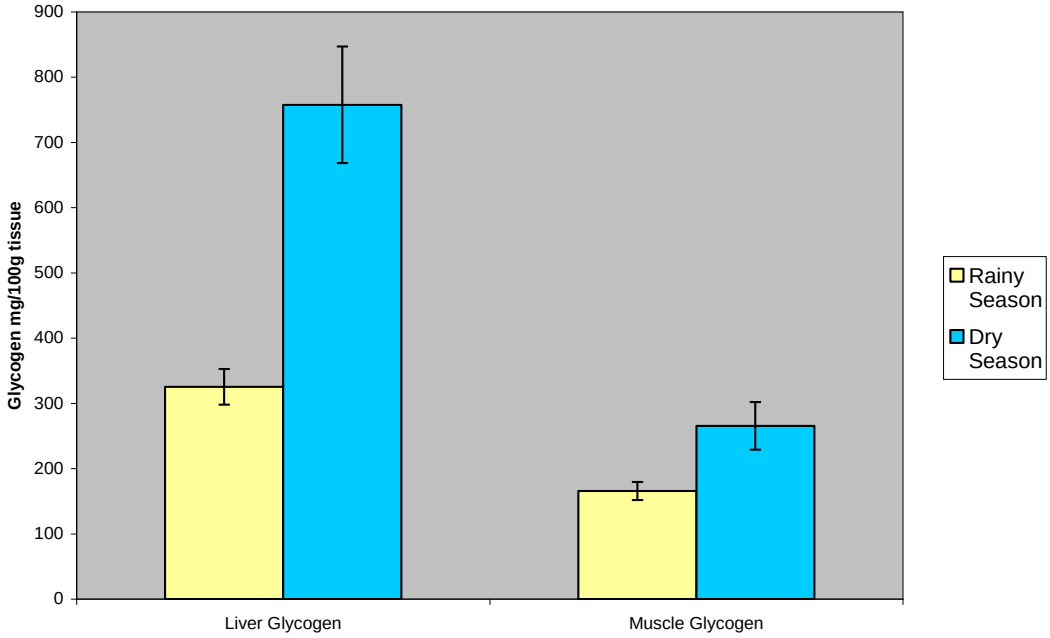

Fig. 1. Liver and Muscle Glycogen Levels during Rainy and Dry Seasons.

The mean fasting liver and muscle glycogen levels during the dry season were significantly higher than during the rainy season ($p<0.05$) (n=8 for each season).

Table 5. Fasting blood glucose levels of male and female toads during rainy seasons.

Sex	Male toads (n=24)	Female toads (n=24)
Blood glucose levels mg/dl	57.3 ± 2.8	66 ± 5.5

The mean fasting blood glucose level (66.0 ± 5.5mg/dl) of female toads was significantly ($p< 0.001$) higher than the mean fasting glucose level (57.3 ± 2.8 mg/dl) of male toads during the rainy season.

Table 6. Fasting blood glucose levels of male and female toads during dry seasons.

Sex	Male toads (n=24)	Female toads (n=24)
Blood glucose levels mg/dl	63.9 ± 5.0	57.6 ± 5.2

During the dry season, the mean fasting glucose levels (63.9 ± 5.0mg/dl) of male toads was higher than the mean fasting blood glucose levels (57.6 ± 5.2mg/dl) of female toads although not statistically significant.

In the present study, the higher glucose levels observed during the rainy season compared with dry season may be due to increased availability of food and greater food intake in rainy season. During rainy season, and at ambient temperature of 28^0C, the animal is involved in a lot of physical activities which include jumping around and reproductive activities. Smith (1954) had shown that increased activity could cause increase in circulating levels of adrenergic compounds which in turn cause transformation and mobilization of glucose from glycogen stores. The higher blood glucose levels observed during the rainy season could also be due to reproductive and thyroid hormones. Thyroxine has been shown to facilitate emotional hyperglycemia by inhibiting destruction of sympathomimetic amines (Spinks and Burn, 1952; Smith, 1954). This may also explain the corresponding low fasting liver and muscle glycogen levels observed during rainy season in the present study. Due to increased activities in rainy season, most of the food reserves are used up even though food intake is increased. The low fasting liver and muscle glycogen and corresponding high glucose levels observed during rainy season suggest that liver and muscle glycogen were probably the sources of glucose production. On the other hand in dry season, due to increased environmental temperature (32^oC), and harsh environmental conditions the toads hibernate. As a result, activities both physical and reproductive are at low levels. Hence, food reserve is conserved while natural foods are scarce and limited. This probably accounts for the low glucose level and high liver and muscle glycogen levels observed in the present study during the dry season. Further studies on the levels of hormones like catecholamine and thyroxine during the two seasons not measured in this study will provide better understanding of the effects of seasons on carbohydrate levels in the toads.

The observed differences in the fasting levels of glucose and glycogen during the two seasons of the year is an indication that seasonal changes affect the carbohydrate levels of the common African toad *Bufo regularis*. This is consistent with the studies in frogs (Smith, 1954; Mizell, 1965; Farrar, 1972; Hermansen and Jorgensen, 1969; Byrne and White, 1975; Petersen and Gleeson, 2007; Varadaraju, 2013) in mammals (Layman and Chartifield 1954) and in humans (Suarez and Barrett-Connor, 1982; Behall *et al*., 1984; Jarrett *et al*., 1984).

The differences in the glucose levels of the female and male bufo regularis observed during the present study agrees with the findings of (Scott and Kleitman, 1921; Smith, 1950; Varadaraju, 2013) but contradicts the observations of (Bosman and Zwarenstein, 1930; Alnagdy *et al*., 1995) who observed no sex difference in the blood sugar of Xenopus Laevis and Egyptian toad *Bufo regularis*, respectively. The observed sexual difference in the glucose levels of the Common African toad *Bufo regularis* in the present study may be due to different activities which include reproduction that the toad engages in during the two seasons. During the rainy season, the female toad is involved in both physical and reproductive activities. Smith (1954) showed that increase in activity could cause increased circulating levels of adrenergic compounds and in turn cause transformation and mobilization of glucose from glycogen stores. However, Varadaraju (2013) in his study observed higher blood glucose in the male animals throughout the seasons. Since we did not measure hormone levels in this study it is recommended for further study.

The present study shows that seasonal changes produced significant effects on the blood glucose and glycogen levels of the Common African toad *Bufo regularis*. This is

similar to the reports in frogs, mammals and humans. We have also shown in this study that seasonal changes caused sexual variation in the blood glucose of the toad *Bufo regularis*.

CONCLUSION

In conclusion, this study showed that the fasting levels of glucose and glycogen in the common African toad *Bufo regularis* varied significantly with seasons. A significant sex by season interaction was also observed in the carbohydrate levels. These results also suggest that seasonal variation should be considered when evaluating carbohydrate levels in the common African toad. Further study on the levels of hormones will help our understanding of seasonal changes and carbohydrate levels in the common African toad.

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