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UTTILIZATION OF LIPIDS AS SOURCE OF ENERGY DURING HIBERNATION OF RANA RIDIBUNDA PALLAS, 1771

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ABSTRACT

The aim of this study is to calculate the ene expenditure from fatty substance contents of the frog. Rana ridibunda during its hibernation. It was found that, almost, all frogs enter hibernation during the last week of December and emerge from hibernation during the first week of March. Hence, January and February are considered the hibernation period. December is the pre-hibernation period and March is the post-hibernation period. The reduction in percent of body lipid during the hibernation period was 4.8% in males and 7.7% in females. The reduction in percent of lipid of fat bodies during the hibernation period was 2.758% in males and 0.733% in females.

The calorific value of R. ridibunda lipid amounted to 12338.5 cal/gm. Therefore, energy loss from lipid content of body tissue was 10.04 cal/gm! day in males and 16.10 cal/gm! day in females. Energy loss from fat bodies during hibernation was 5.77 cal/gm of mass in males and 1.53 cal/gm of mass in females. The total energy losses during hibernation for R. ridibunda. in Baghdad. on dry weight basis, averaged 15.81 cal/gm! day in males and 17.63 cal/gm! day in females.

INTRODUCTION

It is now well established that amphibians utilize fat as a source of energy during dormancy (savage, 1961;Sherwin. 1965: Mazur. 1967: Brenner. 1969: Fitzpatrick. 1976). Several workers made Invetigations on changes in lipid weight of some organs in several species of anurans. It was found that fat bodies and other energy depots cover metabolic requirements before as well during and after hibernation. Kaloyianni et al. (1993) worked on the effect of adenosine on glucose metabolism of R. ridibunda erythrocytes. Seymour (1973) found that about half of the energy required during dormancy came from the fat bodies in spadefoot toads (Scaphiopus). Beurden (1980) has formulated an energy model to predict survival of frogs The pattern of utilization of energy depots varies greatly both between individuals within a population of frogs and between populations inhabiting varies localities. It also varies from year to year, probably depending upon prevailing feeding conditions (Jorgensen et al.. 1979). Behrisch & Rauch (1981) and Bradford (1983) gave good information regarding oxygen relation and energy metabolism of dormant amphibians. Storey & storey (1985) found that freezing exposure of the grey tree frog I-Iyla versicolor resulted in using of anaerobic glycolysis for energy production. It is widely believed that the body lipid and the most conspicuous site of fat storage in the fat bodies, in anurans, serve as an energy depot primarily during hibernation. Mazur (1967) has obtained the calorific values of the tissues and fat at different time of the year for Bufo bufo (L.) and Rana arvalis Nilss. This author found that energy loss during the winter-spring period due to disappearance of fatty and non-fatty substances.

The present study was undertaken to calculate the energy expenditure (cal/gm/dry body weight/day) from body tissue lipid and from the lipid of fat bodies during hibernation of male and female frog Rana ridibunda. It is hoped that this work should add useful informations for the adaptive physiology of amphibians.

MATERLALS AND METHODS

A total of 445 individuals (218 males and 227 females) of R. ridibunda were collected from Baghdad and its suburbs each month from October 1988 to the end of May 1989. During each sampling, water and air temperature was measured. Fat bodies were excised and weighed with accuracy of 0.001 gm. Animal were weighed. excluded fat bodies, with the stomachs empty. with an accuracy of 0.01 gm. Frogs and fat bodies were dried at 70 c to a constant weight, then they weighed to determine dry weight. Rose's (1967) method was used as a basis for extracting fat. Fat was extracted frOm the dry material h means of a hot mixture of chloroform and methanol until a clean extract was obtained. All calculation of fat content are presented as a percentage of the dry mass. The percentage of fat contents found for each sample and the mean value calculated. The caloric value (cal/gm/dry weight) of frog fat was obtained by means of burning in an oxygen Bomb calorimeter. The student 't" test was applied for ascertaining differences between mean figures for the content of fat in particular periods of investigations.

RESULTS

Table (1) and Fig. (1) show air and water mean temperature during the period from October 1988 till May 1989. The lowest mean temperature was in January (5.7 c° I- 6.5 of air and 4 c° + 6.5 of water). In February. the mean temperature was 9.7 c° +- 3.8 of air and 8.3 c° +- 4 of water. Then, the temperature was increased during March. April and May. Frogs entered hibernation at the end of the last week of December and emerged during the first week of March. Hence, January and February are considered as the period of hibernation. December is the per-hibernation period and March is the post-hibernation period.

Table (2) shows means of dry body weight of males and females and the percent lipid content of body tissue (December and March) and the variation in percent lipid content in the fat bodies (October-May). The percent lipid content of body tissue of R. ridibunda was high in both sexes during December (14.3% in males and 16.2% in females). In March, lipid content of body tissue amounted 9.5% in males and 8.5% in females. Table (2) and Fig. (2) show the cyclic changes in lipid content of bodies. In December. the content of fat body lipid was higher in males (3.73% +- 0.5073) than in females (1.56% +- 0.3436). The difference in means was highly significant ($p < 0.05$). In general, both in males and females, the lipid content of fat bodies decreased in February (0.730%+-0. 1924 in males and 0.177% +-0.627 in females). In March, however, on difference in fat body lipid weight was observed between the sexes (0.973% +- 0.3379 in males and 0.83 l%+-0.3975 in females). The mean value in both were decreased in April. In May, the content of fat body lipid in male was significantly higher than in females (2.380% +-0.7248 in males and l.076%+-0.3346 in females). The percentage of lipid contents consumed during hibernation was determined from the difference between and the amount of lipid calculated in March.

The calorific value of R. ridibunda lipid was 12,338.5 cal/gm. Table (3) shows energy losses from lipid content of body tissue during hibernation, the percent lipid consumed during this period was 4.8% gm in males and 7.7% gm in females. Therefore, the percent of lipid consumed from the lipid of body tissue per gm/day was 0.56% in males and 0.8 1% in females. Thus, the energy consumed during hibernation is 10.04% cal/gm! dry body weight! day for males and 16.10 cal/gm dry body weight! day for females.

Table (4) shows energy losses from lipid content of fat bodies during hibernation. It was 5.77 cal!gm dry body weight! day for males and 1.53 cal/gm dry body weight! day for females. Therefore, the total energy losses during hibernation for R. ridibunda. on dry weight basis. averaged 15.81 cal!gml day for males and 17.63 cal!gmlday for females.

Table (1): Air and water mean temperature (C°) in Baghdad during 1988 and 1989

Month	Air temperature	water temperature
October 1988	25.8+- 2.8	23.0 +-2.7
November	19.8+-1.3	16.6+-l.7
December	17.3+-5.1	l4.7+-3.2
January 1989	5.7+-6.5	4.0+-6.5
February	9.7+-3.8	8.3+-4.0
March	26.7+-9.1	23.7+-6.4
April	29.5+-3.5	24.0+-7.l
May	32.0+-l.4	29.0+-2.l

Table (2): Means of weights of dry body, percent body tissue lipid and percent fat body lipid of Rana ridibunda during 1988 and 1989.

Month	No.	Sex	Dry body weight(gm)	lipid content of body tissue (%gm dry body weight)	Lipid contentof fat bodies (%gm dry body weight)
October	25	M	6.25+-2.57		2.404+-0.4630
	21	F	9.33+-4.15		1.906+-0.552l
November	42	M	6.13+-2.l0		1.650+-0.2815
		F	7.95+-3.70		1.079+-+0.1755
December	20	M	7.I0+-2.00	14.3	3.731+-0.5073
	23	F	9.70+-3.16	16.2	1.564+-0.3436
January	6	M	4.27+-0.98		0.984+-0.3070
	8	F	6.12+-2.79		0.697+-0.2167
February	36	M	7.22+-2.29		0.730+-0.1924
	36	F	9.22+-5.21		0.l77+-0.0627
March	50	M	5.77+-1.91	9.5	0.973+-0.3379
	27	F	6.86+-3.37	8.5	0.83 l+-0.3975
April	22	M	6.88+-1.79		0.424+-0.1270
	20	F	10.57+-5.23		0.659+-0.2640
May	14	M	6.82+-l.35		2.380+-0.7248
	35	F	7.80+-2.82		1.076+-0.3346

Table (3): Energy losses from lipid content of body tissue (cal/gm/dry for body weight/day) of Rana ridibunda during hibernation.

Sex	Lipid content of Body dry body weigh)tissue (%gm		Lipid consumed during hibernation		Energy losses (cal/gm/ dry body weight! day)
	December		March		
M	14.3		9.5	4.8	10.004
F	16.2		8.5	7.7	16.10

Table (4): Energy losses from lipid content of fat bodies (cal/gm dry body weight/ day) of Rana ,-idibunda during hibernation.

Sex	Lipid content of Fat bodies (%gm dry body weight)		Lipid consumed during hibernaticth		Energy losses (cal/gm dry body weight/day)
	December	March			
M	3.731		0.973	2.758	5.77
F	1.564		0.831	0.733	1.53

DISCUSSION

Storage and utilization of body lipid as energy source during hibernation is influenced by the environmental temperature as well as physiological changes within the animal (Savage, 1961: Brenner, 1969). The lowest mean temperature of air and water (in Baghdad during 1988-1989) was recorded during January and February. Hence, these two months are considered as the period of hibernation for R. ridibunda (Table 1 and Fig. I). Table (2) shows the percent lipid content of body tissue (excluded the lipid of fat bodies). In December. it was 14.3% in males and 16.2% in females and the percent lipid content in March (after hibernation) was 9.5% in males and 8.5% in females. therefore, during both sexes utilization lipid, but the amount of lipid consumption per day was higher in females than in males. Previous worker showed that oogenesis continues throughout dormancy in amphibia and fat reserves are essential for normal egg development (Smith,1950: Bush. 1963: Mizell. 1964: Brenner. 1969). Rose (1967) showed in R. nigroniaculata relative weight of ovaries tripled during hibernation while no significant development was observed testes (Maruyama, 1979). Lipid content in fat bodies of R. ridibunda fluctuates in both sexes. In December. the content of fat body lipid was significantly higher in males than in females (Table 2 and Fig. 2). Fat bodies of both sexes were approximately equal in March and than decreased in April (breeding season). In May (after the breeding season), an increase in fat body weight is observed. Studies on annual cycles in amphibian fat bodies support the idea that the fat bodies in temperature zone anurans reach minimum size around the breeding time or after the spawning period and reach their maximum size in autumn or before hibernation (Jorgensen et al.. 1979). Smith (1950) found that fat body of R. temporaria attains maximum development in October (before hibernation). This being followed by a decrease through the winter and it fall to a minimum at the spawning season.

Tables (3 and 4) show the energy losses from lipid content of body tissue and of fat bodies. respectively. The total energy losses during hibernation averaged 15.8 cal/gm day in males and 17.63 cal/gm! day in females. From the investigation of Mazur (1967). concerning total energy losses during the winter and the breeding season for B. bufo and R. arvalis with wet weight averaged 47.99 cal/ 24 hours of B. bufo and in R. arvalis 14.00 cal/ 24 hours. However, it most be noted that Mazur (1967) used known caloricity of frog fat (9361.96 cal/gm). and he did not take in consideration the sex of the investigated amphibians.

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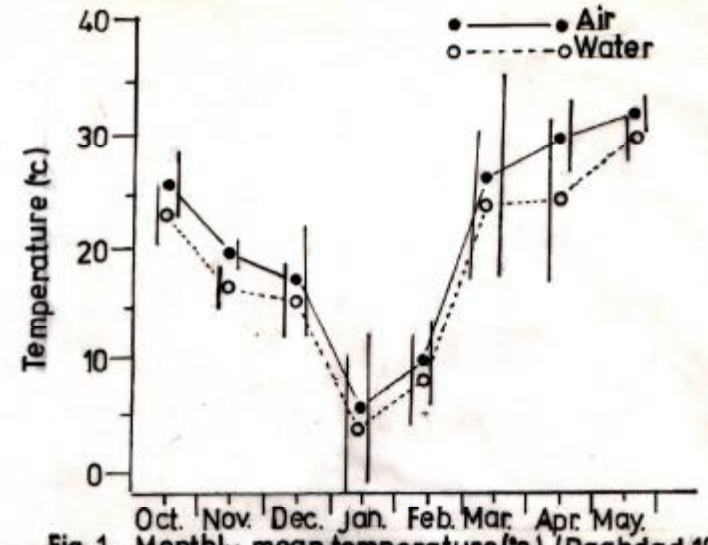

Fig. 1. Monthly mean temperature (°c.) (Baghdad 1989). Extended lines represent the range of value about the mean.

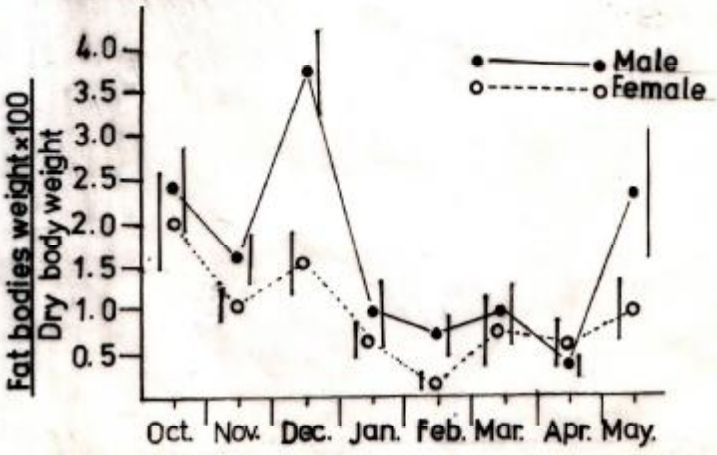

Fig.2. Percent lipid of fat bodies of Rana ridibunda. Vertical lines show the standard errors of the sample means.

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استهلاك الدهون كمصدر للطاقة خلال فترة سبات ضفادع المستنقعات ***Rana ridibunda***

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الخلاصة

ان الغرض من هذه الدراسة هو قياس مقدار الطاقة التي يستهلكها ضفدع المستنقعاتا نتيجة استهلاك الدهون الجسمية خلال فترة السبات. وقد اظهرت الدراسات بان معظم الضفادع تقريباً تدخل فترة السبات خلال الاسبوع الاخير لشهر آذار، وعلى ذلك اعتبر كل من شهري كانون الثاني وشباط فترة السبات لهذا الضفدع. كما اعتبر كانون الاول فترة ما قبل السبات وشهر آذار فترة ما بعد السبات.

بلغ انخفاض في النسبة المئوية في دهون الاجسام الدهنية خلال فترة السبات و ٧,٧% في الاناث. وبلغ انخفاض النسبة المئوية في دهون الاجسام خلال فترة السبات ٢,٧٥٨% في الذكور و ٠,٧٣٣ وبلغت كمية السعرات الحرارية لكل غرام دهني من دهون ضفدع المستنقعات ١٢٣٣٨,٥ سعره.

ومن هذا بلغت كمية الطاقة المفقودة من دهون انسجة الجسم ١٠,٠٤ سعره بذكور و ١٦,١ سعرة للاناث في اليوم الواحد. اما الطاقة المفقودة من دهون الاجسام الدهنية اثناء فترة السبات فبلغت ٥,٧٧ سعره للذكور و ١,٥٣ سعره للاناث في اليوم لكل غرام من وزن الحيوان الجاف. ومن هذا نستنتج ان مقدار الطاقة الكلية المفقودة اثناء فترة سبات ضفدع المستنقعات في بغداد، نسبة الى وزن الحيوان الجاف، بلغت ١٥,٨١ سعره للذكور و ١٧,٦٣ سعره للاناث في اليوم الواحد.