I.J.S.N., VOL. 2(3) 2011: 653- 658 ISSN 2229 – 6441

INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

VARIABILITY OF HAEMOGLOBIN CONCENTRATION DURING THE MALE AND FEMALE REPRODUCTIVE CYCLE OF A MEGACHIROPTERAN BAT *Rousettus leschenaulti* (DESMEREST)

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ABSTRACT
The Indian fruit bat, *Rousettus leschenaulti* shows a peculiar breeding cycle. Adult males show double peaks in their testicular weight corresponding to the two pregnancy cycles of the female. The first peak occurs during October-November and the second during February-March. Females show I-pregnancy cycle from December to April and IInd cycle from April to July. The variations in haemoglobin percentage in male and female has been studied throughout the reproductive cycle 2008-2009. Capillary blood or EDTA thoroughly mixed or double oxalated anticoagulated venous blood was used for the determination of hemoglobin by Sahli hemoglobinometer where the hemoglobin is converted to brown coloured acid hematin. The average mean in the male ranges from (8.8 – 13.4%, gram/100 ml) and in the female (8.0 – 14.4%, gram/100ml), suggestive of high haemoglobin percentage as compared to their body size. The means for haemoglobin values varied from month to month and in the same direction but did not differ significantly. Morphological abnormalities in the RBCs observed in some samples supported the variability of Hb concentrations. The objective of this study is to investigate relationship between the reproductive status and difference attributable to sex.

KEYWORDS:- bats, haemoglobin, blood.

INTRODUCTION
Certain hematological values, such as haemoglobine (Hb) concentration show a large amount of variation throughout the adult life of an individual mammal, one of them is Hb concentration, this labile blood value is affected by various factors such as age, gender and reproductive state, by endogenic rhythms of various metabolities as well as external factors such as season, time of the day, food availability and quality (Westhuyzen, 1978; Minematsu *et al*., 1995; Korine *et al*., 1999; Smucny *et al*., 2001; Hassimoto *et al*., 2004; Asadi *et al*., 2007 and Kinoti, 2008). Haemoglobin is an indicator of both individual and population condition and useful in phylogenetic analysis.

MATERIAL AND METHODS
Female *Rousettus leschenaulti* breeds twice in a year in quick succession, similarly the adult males show two peaks of activity once during October-November and second during February-March. The specimens of *Rousettus leschenaulti* were collected with the help of mist net placed at the entrance of Mansar / Kandri mines near Nagpur once every calender month throughout the complete reproductive cycle. Immediately the bats were brought to the laboratory for the sampling of blood. For each sampling, three bats of both the sexes were used. Blood samples (2ml) were collected either in Eppendorf tubes or into 6-8 heparinized capillary tubes after puncturing a wing vein. This capillary blood or thoroughly EDTA mixed or double oxalated venous blood was used for the determination of hemoglobin % (gram/100 ml.) by Sahli hemoglobinometer where the hemoglobin is converted to brown coloured acid hematin (Godkar and Godkar, 2003). After blood sampling each bat was released.

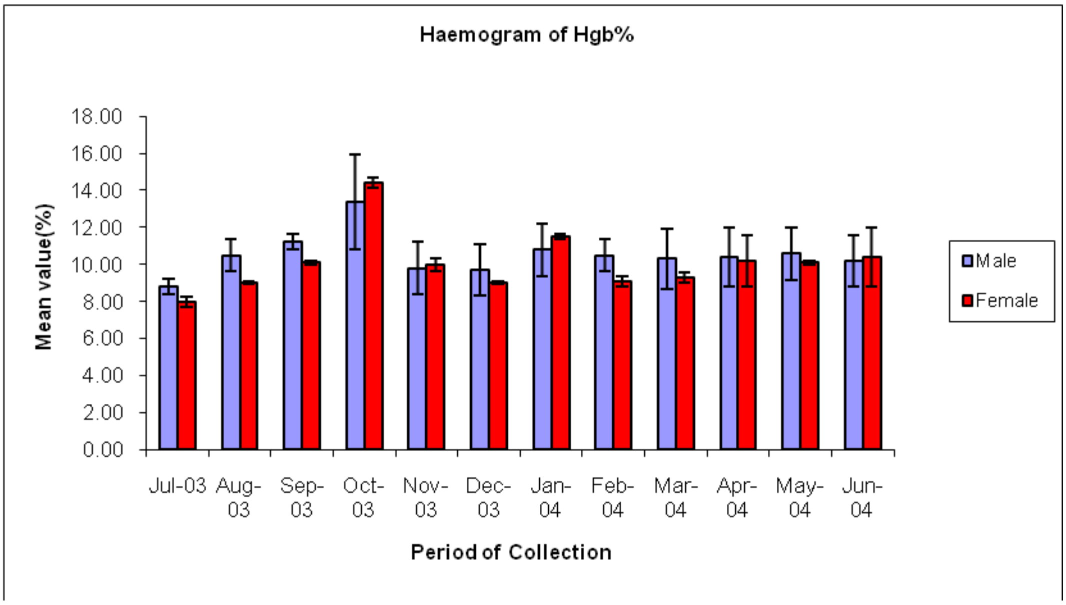
RESULTS
The mean range was found to be in the male (8.8 – 13.4%) and in the female (8.0 – 14.4%). In the female there was considerably more variance in the measurements being significantly highest during October (Recrudescent female, 14.4 gms%); January (mid-pregnancy, 11.5%) but then values tended to stabilize (in the range 10-10.5%) during theser months, September (anoestrous female); November (oestrous female); February (Advance pregnancy); December (Ovulation time and early pregnancy); August (Phase-II lactation), March (just delivered, post-partum estrous); April (lactation / early pregnancy / abortion); May (mid-pregnancy) and June (advance pregnancy / abortion). The haemoglobin values significantly regressed during July (phase-II delivery, 8.0 gms%), this aberrant value was of importance, in that they represent an unstable, severely stressed animal in the population, which probably does not survive adverse environment. There was considerably more variance in the measurements during the months of October (active male, highest values 13.4%) and September, (inactive male 11.2 gms/100ml) but then the values tended to stabilize (10%) again in January (active male), February (active male), August (inactive male), November (active male) and December (active male), March (active male), April (active male), May (regressed male) and June (regressed male). The haemoglobin values were regressed during July (inactive male). No significant sex difference was observed. (Table-1).

To support the haemoglobin percentage calculated by haemoglobinometer in both the sexes during the reproductive cycles, a morphological study of erythrocytes and associated changes in their haemoglobin content such as the occurrence of large, small, rectangular central pallor, central slit-like pallor and purple coloured granules of iron or pappen heimer bodies has been illustrated in the male (figs. 1 – 12) and female (figs. 13-24). The above observations have also been supported either by the increase or decrease in the number of erythrocyte count.

TABLE 1. Haemoglobin values for male & female *Rousettus leschenaulti* during reproductive cycle (2008 – 2009)

Date of Collection	Male				Female			
	Reproductive Status (n=3)	Mean value (%gm/100ml.)	S.E.	P Value	Reproductive Status(n=3)	Mean value	S.E.	P Value
31/07/08	Inactive	8.8	±0.41	0.01	Late pregnancy/ just delivered	8.0	±0.27	0.01
29/08/08	Inactive	10.5	±0.85	0.01	Lactating female	9.0	±0.07	0.01
29/09/08	Recrudescent	11.2	±0.41	0.01	Anaestrous female	10.1	±0.10	0.01
21/10/08	Recrudescent	13.4	±2.55	0.01	Recrudescent female	14.4	±0.27	0.01
20/11/08	Active	9.8	±1.40	0.01	Female at Oestrous	10.0	±0.33	0.01
29/12/08	Active	9.7	±1.40	0.01	Ovulation / Early pregnancy	9.0	±0.07	0.01
25/01/09	Active	10.8	±1.42	0.01	Mid- Pregnancy	11.5	±0.16	0.01
28/02/09	Active	10.5	±0.85	0.01	Advanced pregnancy	9.1	±0.25	0.01
25/03/09	Active	10.3	±1.63	0.01	Just delivered / post partum oestrous	9.3	±0.25	0.01
25/04/09	Active	10.4	±1.61	0.01	Lactation / early pregnancy/ abortion	10.2	±1.39	0.01
27/05/09	Regressed	10.6	±1.41	0.01	Mid- Pregnancy	10.1	±0.10	0.01
24/06/09	Regressed	10.2	±1.39	0.01	Advanced pregnancy / abortion	10.4	±1.61	0.01

(Values are mean ± SE, n in paranthesis indicate number of animals used).



DISCUSSION

A perusal of literature on haematology of bats reveals that haemoglobin mean concentration range was found to be from 8-19%. Our values are less compared to other frugivorous bats and are near to insectivorous bats. However, Kinoti (2008) described high haemoglobine values, with means of 20-70 and 18-15 per 100ml. of blood in a tropical, cave-dwelling bat, *Otomops martinsseni*. Previous studies on the mammals and bats have been with regard to diet / nutrition, temperature,

season, ecological, general health of the species, diseases or infections and hibernation. Even though no attempt has been done to correlate the Hb values to the reproductive cycle, few earlier workers have considered some phases of reproductive cycle such as oestrous, pregnancy, lactation and parturition in the female and rut period in the male (Sealander, 1964; McEwan and Whitehead, 1969; Valdivieso and Tamsitt, 1971; O'Brien and Endean, 2001).

PLATE 1. Blood smear from male

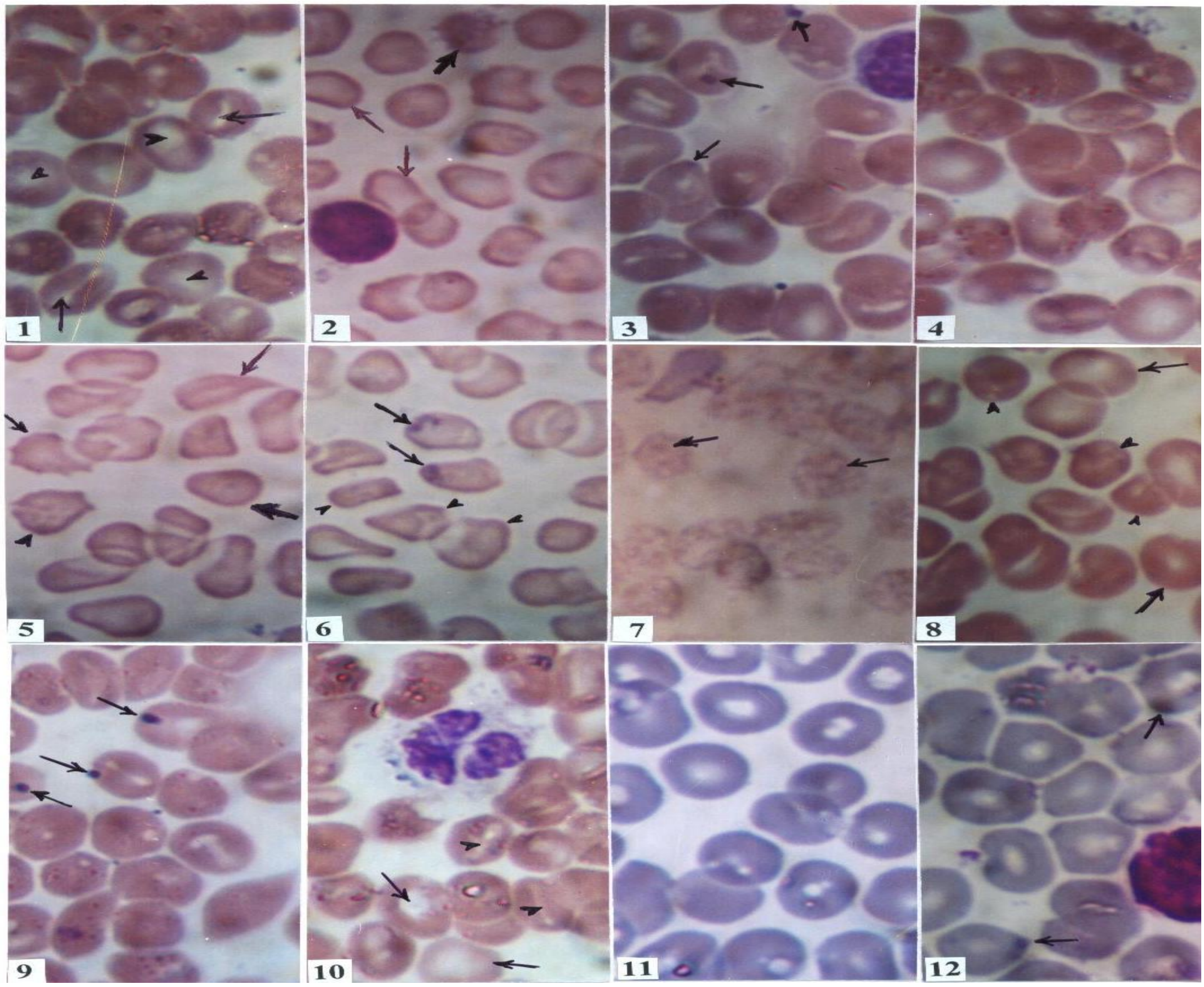


Fig. 1. Note hypochromic macrocyte (RBC) with a large area of central-pallor due to decreased concentration of haemoglobin. Also note central-slit of pallor (arrow), oval or rectangular area of central pallor (arrow head) from active male (25/1/08) x 500.

Fig. 2 The abnormalities of erythrocytes are burr cells (arrow), oat-shaped (thin arrow) from active male (28/2/08) x 500.

Fig. 3 Note anisocytosis-some RBC with central slit of pallor, spherocytes, ovalocytes and elliptocyte from active male (25/3/09). Some RBC show presence of round purple stained nuclear fragments–Howell-Jolly body (arrow) x 500.

Fig. 4 Note anisocytosis, spherocytes with no central area of pallor, but others with central – slit of pallor from active male (25/04/09) x 500.

Fig. 5 Note decrease in size of RBC. The abnormalities of erythrocytes are burr cells (arrow); pear-shaped (thick arrow); tear shaped (thin arrow); crenated (arrow head) from regressed male (27/5/09) x 500.

Fig. 6 Occurrence of siderocytes (Pappen heimer bodies/ purple coloured granules of iron-arrow). Note fragments of erythrocytes or schistocyte (arrow head) from regressed male (24/06/09) x 500.

Fig. 7 Blood smear from inactive male *Rousettus leschenaulti* collected on (31/7/08). Erythrocytes appear shrunken showing iron and stroma. Haemoglobin appears hypochromic (arrow) x 500.

Fig. 8 Moderate anisocytosis and poikilocytosis. Normoblasts (arrow) and microcytic hypochromic RBC (arrow head) from inactive male (29/8/08) x 500.

Fig. 9 Erythrocytes showing Heinz bodies (arrow); decrease in the presence of central slit from recrudescent male (29/9/08) x 500.

Fig. 10 Hypochromic macrocyte with large area of central-pallor due to decreased concentration of haemoglobin (arrow). RBC with central slit of pallor (arrow head) from recrudescent male (20/11/08) x 500

Fig. 11 Erythrocytes appear biconvex. Iron deficiency is predominent as evident by large area of central pallor from active male (20/11/08) x 500.

Fig. 12 Iron deficiency is due to central pallor and dark Heinz bodies or denaturated globin (arrow) are of common sight from active male (29/12/08) x 500.

PLATE 2. Blood smear from Female

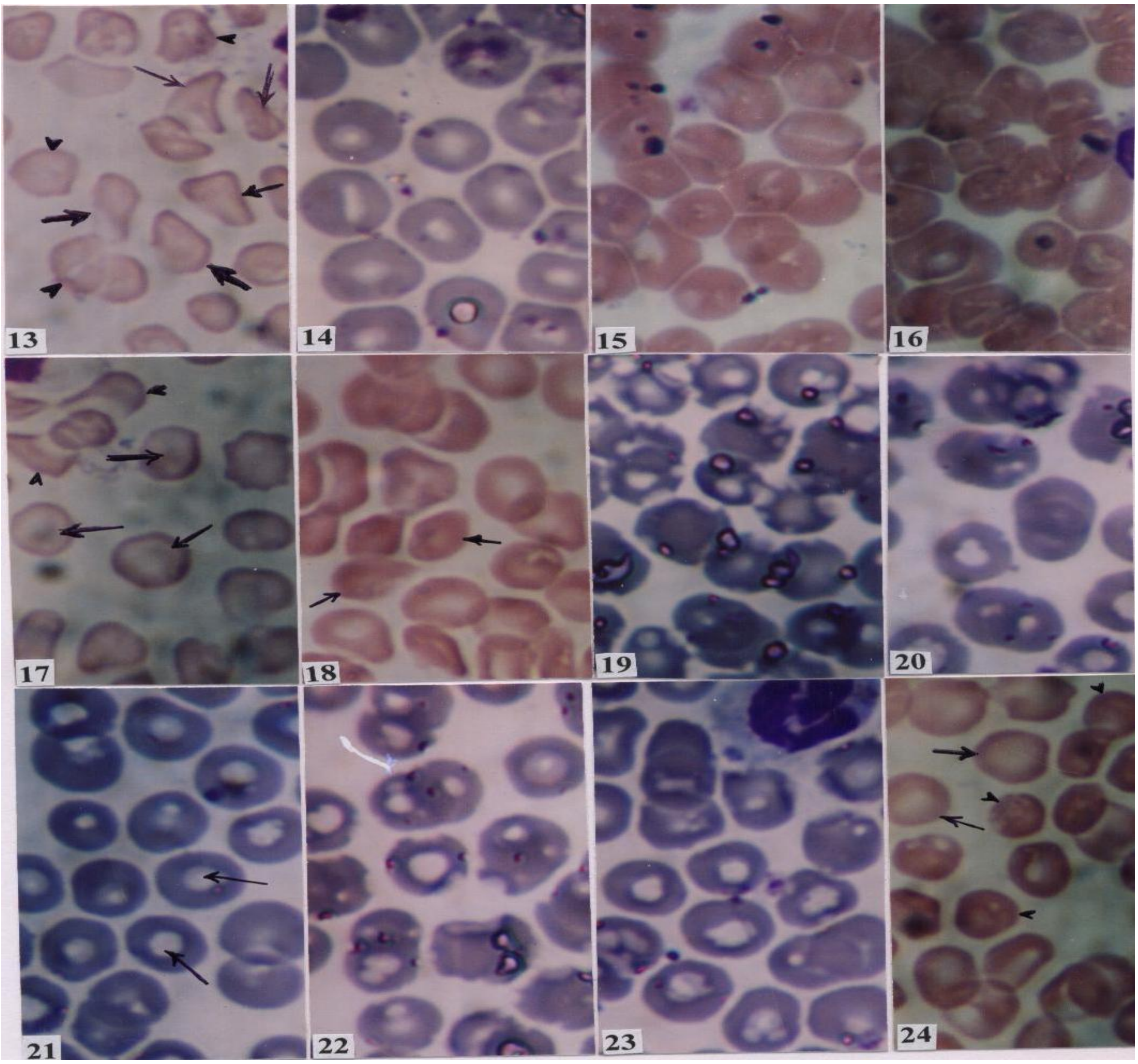


Fig. 13 Note decrease in sizes of erythrocytes. The abnormal erythrocytes include pear-shaped (thick arrow), tear-shaped (thin arrow) and crenated (arrow head) from mid-pregnant female (25/01/08) x 500.

Fig. 14 Note anisocytic and poikilocytic and hypochromic RBCs. Dark Heinz bodies or denaturated globin are of common sight from advanced pregnant female (28/2/08) x 500.

Fig. 15 RBC's showing large area of central and slit-pallor due to decreased concentration of haemoglobin from just delivered or post-partum oestrous females (25/03/09). Dark Heinz bodies are of common sight x 500.

Fig. 16 Note many erythrocytes with central and slit-pallor due to decreased concentration of haemoglobin from lactating females (25/4/09). Dark Heinz bodies are of common sight x 500.

Fig. 17 Note occurrence of siderocytes (Pappen heimer bodies/ purple coloured granules of iron in red cells) are common (arrow). Fragments of erythrocytes or schistocyte are also a common feature (arrow head) from mid-pregnant (27/05/09) x 500.

Fig. 18 Note moderate anisocytosis and poikilocytosis. Note normoblast and microcytic hypochromic erythrocytes (arrow) from females undergone abortion (24/06/09) x 500.

Fig. 19 Note high percentage of erythrocyte abnormalities-thorn cell; burr cells and spur cells (acanthocytosis) and hypochromic RBCs from late pregnancy (31/7/08) x 500.

Fig. 20 Note different hypochromic abnormal cells (burr cells, crenated cells, siderocytes, spur cells) from lactating females (20/8/08) x 500.

Fig. 21 Most of the erythrocyte appear biconvex but others show poikilocytosis. Iron deficiency is predominant as evident by large area of central pallor (arrow). The percentage of normal red cells, round cells having a small area of central pallor is higher from anoestrous females (29/9/08) x 500.

Fig. 22 Higher percentage of hypochromic abnormal cells (Burr cells, crenated cells, siderocytes, spur cells) from recrudescent females (21/10/08) x 500.

Fig. 23 Iron deficiency is predominent as evident by large area of central pallor but the degree of abnormalities is moderate (oestrous-20/11/08) x 500.

No significant difference between male and female were noticed but in females the values were slightly greater. On the contrary a significant difference between sexes for haemoglobin concentration was noted in *Eutamias minimus* and *Clethrionomys gapperi* (Sealander, 1964). The absence of any significant variation in hemoglobin values of *Rousettus leschenaulti* suggest that metabolism does not undergo any change throughout the year. Therefore, physiological mechanisms other than an increased blood oxygen capacity may be employed to meet intermittent or emergency increases in the demand of the tissues for oxygen but in the female significant decrease was noticed during July (Phase-II delivery). This aberrant value is of importance, in that they represent an unstable, severely stressed animal in the population, which probably does not survive adverse environment. Similar regression in the haemoglobin values during July (inactive male) has been noticed in severely stressed male population.

The monthly samples in the present study showed evidences of pathological anemia in both the male and female and particularly during pregnancy (hypochromic, microcytic, macrocytic, sideroblastic, spherocytic, anisocytic, acathocytic, poikilocytic) as evidenced by hypocromicity in RBCs (occurrence of both central and slit-pallor). Such a condition has been described in human (Miale, 1962; Sood, 1996; O'Brien and Endean, 2001; Godkar and Godkar, 2003) and other mammals. For such abnormalities the iron deficiency may be the reason. Similarly Krutzsch and Hughes (1959) described macropolycystic pernicious anaemia in the bats, *Tadarida brasiliensis mexicana* and *Myotis velifer incantis*. The hemoglobin levels of *Rousettus leschenaulti* remained relatively constant in the male during activity (January / February / March / April) and during regression (May and June) and in the female during September (anestrous), November, (Oestrous), April (early pregnancy), May (mid-pregnancy), June (advance pregnancy). This apparent stability may indicate a fairly stable metabolism, which in turn, reflected the more or less uniform thermal characteristics of the microenvironment inhabited by these bats.

Bats are characterized by high haemoglobin levels when compared with terrestrial mammals (Lewis, 1977; Noll, 1979; Jurgens *et al*., 1981; Arevalo *et al*., 1987; 1992; Wightman *et al*., 1987; Viljoen *et al*., 1997; Korine *et al*., 1999; O'Brein and Endean, 2001; Kinoti, 2008). The high Hb levels in bats may be due to changes in vascular permeability (Arad and Korine, 1993), due to temperature (Arevalo *et al*., 1991) or due to heightened activity used for flights as high haemoglobin concentrations increases oxygen delivery to tissues in species with small body size and high metabolic rates (Arevalo *et al*., 1992) but our results in some cases do not agree as we have often noticed occurrence of slit-like central or rectangular pallor areas comparable to hypochromicity or loss of haemoglobin.

The association of animal health condition with haemoglobin has been observed by a number of workers working on wild animals. Korine *et al*., 1999 during winter and early spring noticed *Rousettus aegyptiacus* in a poor physical and physiological state, at the end of the resting phase *Rousettus* showed mild state of dehydration and therefore increased hemoglobin levels. The low Hb values observed in *Rousettus leschenaulti* may be a mechanism for avoiding the effects of abrupt body temperature or due to immobilization (Seal *et al*., 1972) or a means for increasing oxygen delivery to the tissues of small species with high metabolic rates (Pearson, 1948a; Schmidt-Neilson and Larimer, 1959).

The intersexual and seasonal high Hb concentration in two bats, *Rhinolophus ferrum equinum* and *Miniopterus schreibersii* was due to formation of many small erythrocytes (Arevalo *et al*., 1992). An increase in Hb value in the present work may be due to capture restrain, manual or chemical. Similarly we noticed an increase in the population of small erythrocytes. High Hb during pregnancy also noticed by Arevalo *et al*., 1987 in *Pipistrellus pipistrellus* but our results disagree with them.

An increased circulation rate and probably breathing rate as well might have the same effect as an increase in oxygen capacity of the blood in supplying oxygen to the tissues. During winter an increase in the muscle tissue of the female *Rousettus leschenaulti* indicates an increase in myoglobin concentration for the facilitation of oxygen transfer from the blood to the tissues as described by Sealander, 1964. According to him the most important adaptation of small mammals is the behavioural one of avoiding the full impact of low temperatures as the body to air temperature gradient is greatly reduced and survival is possible if the food supply is adequate. Similarly high Hb may be characteristics of wild animals and of animals having high levels of muscular activity as the bats (Wilson and Hoskin, 1975) or due to physical exertion when release of more and larger erythrocytes with no change in haemoglobin concentration but absolutely more heamoglobin in the blood (Brannon, 1985).

From the foregoing it is concluded that for high Hb% in *Rousettus* only reproductive status is not responsible but there are mulitude of factors and the results of the present study suggest that the blood profile for *Rousettus leschenaulti* serve as a good indicator of their physiological state.

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