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The level of Haemoglobin in wild-caught females of the Emballonurid bat, *Taphozous kachhensis* (Dobson) during reproductive cycle

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Abstract
Haemoglobin level in female *Taphozous kachhensis* was investigated to find out the significant differences in haemoglobin level during different stages of reproductive cycle. Monthly changes in haemoglobin level was analysed for twelve months representing entire reproductive cycle. During lactation and quiescence the mean haemoglobin level was found to be 14.52 ± 0.25 and 13.86 ± 0.31 gm/dl respectively. Significant increase ($P < 0.01$) in haemoglobin level was noted during recrudescent stage. However significant decline ($P< 0.01$) in haemoglobin level was observed during all stages of pregnancy. During entire reproductive cycle in female, haemoglobin level was found in the range of 11.9 to 17.6 gm/dl.

Keywords: *Taphozous kachhensis*, bat, haemoglobin, anaemia, deficiency of iron

1. Introduction
Taphozous kachhensis is an emballonurid insectivorous bat. The reproductive cycle of *Taphozous kachhensis* was identified into seven stages viz. quiescence, recrudescent, oestrous, early pregnancy, mid pregnancy, advanced pregnancy and lactation. Insectivorous bats plays a key role in keeping the night flying insects in balance which are among the agricultural pests and vectors of diseases that may cause considerable damage to economy. Haemoglobin is a conjugated protein containing iron and globin molecule. Haemoglobin level in animals is directly proportional to iron content in the diet. Normal haemoglobin level is a sign of good health status in animals. Decreased haemoglobin level is termed as anaemia. Anaemia condition in animals may occur due to deficiency of iron in the diet of animals. In females, during pregnancy haemoglobin variability may be observed, because of increased demand of iron for placental growth, foetal development and uterine growth [1]. Haemodilution also occur during pregnancy which leads to physiological anaemia in females. Bats have higher respiration and metabolic rate as compared to terrestrial mammals [2]. To meet the oxygen demand for higher respiration, bats have higher haemoglobin level. Many studies on variability of haemoglobin level in different species of bats exist in the literature [3, 4, 5, 2, 6, 7, 8, 9, 10]. Thus in the present study, attempt was made to observe the variations in haemoglobin level in wild caught female *Taphozous kachhensis* during different stages of reproductive cycle.

2. Materials and methods
A. Collection of Specimen: In present study, specimens of *Taphozous kachhensis* were collected
on monthly basis to represent all the stages of reproductive cycle in females. Six female bats were collected during every month for this study. Overall 72 female bats were collected during entire reproductive cycle throughout the year from Ambai Nimbai caves which is 45 kilometers away from Bramhapuri (M.S.) with the help of mist net of the mesh size (10 mm). Identification of the specimen was done using standard monograph [11]. After collecting, these were brought to the laboratory. These female bats were anesthetized with ether and weighed on the electronic weighing balance.
B. Collection of Blood Sample: From these bats, blood samples were collected from the pectoral and subclavian veins in EDTA thoroughly mixed or double oxalated anticoagulated Eppendorf tube, without hurting the animal. All the specimens were released back to the nature after recovery from the anesthesia. Haemoglobin estimation of each sample was done by using Sahli's acid hematin method. N/10 HCl was added to the tube with markings of Hb% upto 2gm% and 20 µl of blood sample was added to this tube with the help of micropipette. Solution was kept undisturbed for 10 minutes. This results in the conversion of haemoglobin into brown coloured acid hematin. This solution was mixed with glass rod and then was diluted with distilled water till the colour of the solution matches with the brown coloured comparator box which is present on both the sides of hemocytometer graduated tube. Stirrer was removed from the tube and readings were noted directly from the tube and expressed in gm/dl.
C. Statastical Analysis: All the observations were analysed to get mean, standard error, standard deviation and variance. One way ANOVA with post – hoc Tucky HSD was calculated to observe the significant differences in haemoglobin level by using Statistical Package for Social Sciences (SPSS 10.0).

3. Observations and results
The observed values for haemoglobin concentrations in females during every month are presented in the table 1. The ANOVA with post-hoc Tucky HSD for comparison of significant changes in haemoglobin percentage in *Taphozous kachhensis* during different stages of reproductive cycle is presented in table 2 and the P-value

corresponding to F-statistic of one way ANOVA (P =1.2135e-12) which is lower than 0.05 suggesting significant differences in the means of seven groups is shown in table 3. Histograph showing mean haemoglobin concentration level in female *Taphozous Kachhensis* during different stages of reproductive cycle is presented in figure 1.

In the present investigation mean haemoglobin concentration found to be 14.52 ± 0.25 gm/dl during the lactation stage. During the sexually quiescence stage the non significant decrease was observed in the haemoglobin concentration in females. The mean haemoglobin concentration during quiescence stage was found to be 13.86 ± 0.31 gm/dl. During recrudescent stage the haemoglobin concentration was found to be 17.11 ± 0.45 gm/dl. Significant increase at $P < 0.01$ in the haemoglobin concentration was observed during recrudescent stage, when compared with lactation, quiescence, early, mid and advanced pregnancy stages. The mean haemoglobin concentration during the oestrous stage was found to be 16.41 ± 0.53 gm/dl. Non-significant decrease in haemoglobin level during oestrous stage was observed when compared with recrudescent stage. Haemoglobin concentrations reported during early pregnancy, mid pregnancy and advanced pregnancy were 15.50 ± 0.15 gm/dl, 13.85 ± 0.12 gm/dl and 13.05 ±0.20 gm/dl respectively. During early pregnancy, non-significant decrease in haemoglobin level was observed when compared to oestrous stage. However significant decrease at $P < 0.05$ in the haemoglobin level was noticed when compared with the haemoglobin level of recrudescent stage. At the mid pregnancy, the haemoglobin concentration significantly decreases at $P < 0.01$ when compared to early pregnancy and recrudescent stage. During advanced pregnancy, no significant differences were observed in the mean haemoglobin level when compared with haemoglobin values of mid pregnancy and lactation. Pooled total mean haemoglobin in females during entire reproductive cycle was reported to be 14.71 ± 1.17 gm/dl.

Table 1: Comparison of Haemoglobin (grams/dl) in female *Taphozous kachhensis* during different stages of life cycle.

Month of Collection	Reproductive status	No. of Bats	Hb Range in (grams/dl)	Mean ± S.E.	Variance	Standard Deviation
August	Lactation	06	12.3 to 14.5	13.63 ± 0.32	0.64	0.80
September	Quiescence stage	06	12.7 to 15.6	14.03 ± 0.42	1.07	1.03
October	Quiescence stage	06	11.9 to 15.1	13.66 ± 0.49	1.46	1.21
November	Recrudescent	06	15.2 to 18.3	17.11 ± 0.45	1.22	1.10
December	Oestrous	06	14.7 to 18.0	16.41 ± 0.53	1.68	1.29
January	Early Pregnancy	06	14.7 to 16.3	15.4 ± 0.24	0.35	0.59
February	Early Pregnancy	06	14.9 to 16.4	15.6 ± 0.21	0.28	0.53
March	Mid Pregnancy	06	13.5 to 14.6	14.08 ± 0.15	0.14	0.38
April	Mid Pregnancy	06	12.9 to 13.9	13.61 ± 0.16	0.15	0.39
May	Advanced Pregnancy	06	12.5 to 13.9	13.05 ± 0.20	0.26	0.51
June	Lactation	06	13.8 to 15.7	14.33 ± 0.28	0.49	0.70
July	Lactation	06	14.8 to 16.3	15.6 ± 0.24	0.36	0.60

Table 2: One-way ANOVA with post- hoc Tukey HSD showing comparison of haemoglobin (grams/dl) in female *Taphozous kachhensis* during reproductive cycle.

Reproductive status	No. of Bats	Haemoglobin Range in (grams/dl)	Mean ± S.E.	Variance	Standard Deviation
Lactation	18	12.3 to 16.3	14.52 ± 0.25^{a}	1.14	1.06
Quiescence	12	11.9 to 15.6	13.86 ± 0.31^{ab}	1.19	1.19
Recrudescent	06	15.2 to 18.3	17.11 ± 0.45^{c}	1.22	1.22
Oestrous	06	14.7 to 18.0	16.41 ± 0.53^{acd}	1.68	1.68
Early preganacy	12	14.7 to 16.4	15.50 ± 0.15^{ad}	0.30	0.30
Mid preganacy	12	12.9 to 14.6	13.85 ± 0.12^{ab}	0.19	0.19
Advanced Pregnancy	06	12.5 to 13.9	13.05 ± 0.20^{abe}	0.26	0.26
Pooled Total	72		14.71 ± 1.17	2.09	1.44

Table 3: One-way ANOVA of seven independent groups showing p- value corresponding to F- statistic.

source	sum of squares SS	degrees of freedom NN	mean square MS	F statistic	p-value
treatment	94.6360	6	15.7727	19.0058	1.2135e-12
error	53.9428	65	0.8299		
total	148.5787	71			

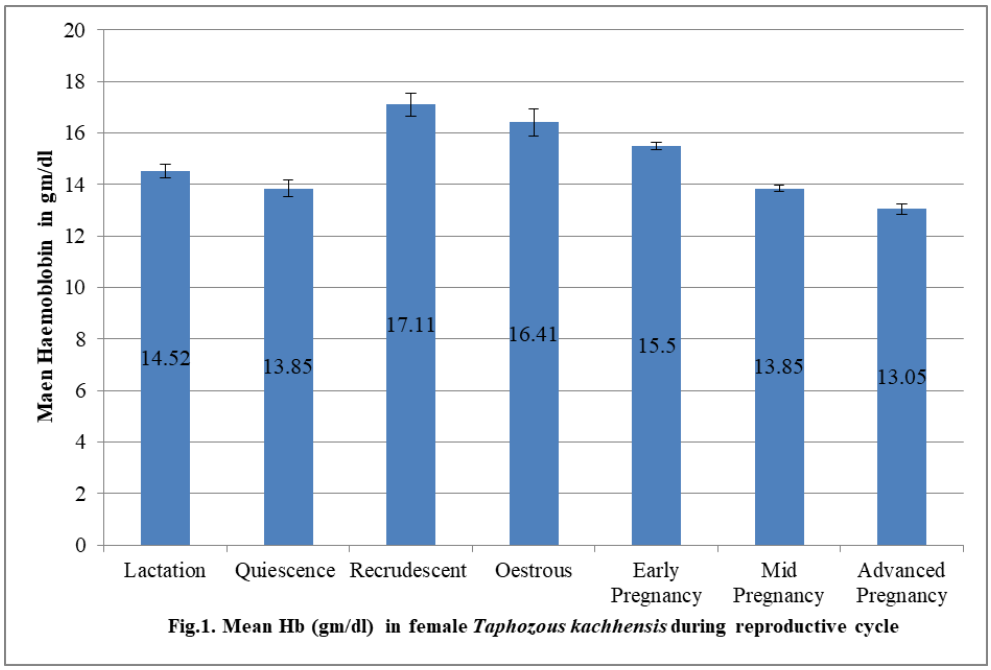

Fig 1: Mean Hb (gm/dl) in female *Taphozous kachhensis* during reproductive cycle

4. Discussion

Haemoglobin is a respiratory pigment in mammals. Functionally it is important for the transport of respiratory gases that are O_2 as well as CO_2, which is required by living organisms for survival [12]. Biochemically haemoglobin is a metalloprotein containing globin molecule with Fe^{2+} ion. During binding of oxygen Fe^{2+} ion gets oxidized into Fe^{3+} ion. Increased level of haemoglobin increases the oxygen binding capacity in an organism. Bats are the only mammals capable of flight which help them to exploit diffuse resources and diverse array of feeding habits. Flight adaptations in bats results in increased requirement of oxygen to cope with increased metabolic rate in them. These all adaptive features for flying and diverse feeding habits, affects the haematological parameters in bats [13, 14]. Hematological parameters are affected by season, availability of food, quality of food, different metabolites, age, gender and reproductive stage in animals [15, 16]. Bats have significant higher levels of haemoglobin than the terrestrial mammals [3, 4, 17, 2]. There is a correlation between body size and rate of metabolism and the haemoglobin levels in animals. Small sized mammals have higher metabolic rate and haemoglobin concentration [18]. Many studies have been conducted on haemoglobin level in bats, which indicates the usefulness of this parameter to decide the general health status of the bats and phylogenetic analysis [19]. Schinnerl *et.al*. [7] have studied the haematological parameters in 26 species of bats belonging to five families that are, Emballonuridae, Molossidae, Mormopidae, Phyllostomidae and Vespertilionidae. Heard and whittier [5], in three species of *Pteropus*, Viljoen et. al., [2] in *Miniopterus schrebersii natalensis*, Arevalo *et.al*., [3, 4] in Vespertilionidae bats and in *Rhinolophus ferrumequinum* and *Miniopterus schrebersii* respectively, Ratnasooriya *et.al*., [6], in *Miniopterus schrebersii and Taphozous melanopogon*, Bhatkulkar and Sastry [20] in *Rousettus leschenaulti*, Hossain *et.al*., [8] in *Pteropus giganteus*, Abdel- Rachied *et.al*., [21] in *Rousettus aegyptiacus*, Rashid *et.al*., [9] in *Scotophilus heathii* and *Pipistrellus pipistrellus*, Rahma *et.al*., [10] in *Cynopterus tithaecheilus* has described the haemoglobin level from 8.0 to 22.22gm/dl in different species of bats. Abdel- Rachied *et.al*. [21] has shown the effect of flying stress on haematological parameters in *Rousettus aegyptiacus* and reported the significant increase in haemoglobin level in bats under flying stress when compared with non-stressed bats. During present investigation observed mean haemoglobin range in females was 13.05±0.20 to 17.11±0.45 gm/dl. Bhatkulkar and Sastry, [20] in *Rousettus leschenaulti* and Rashid *et.al*, [9] in *Scotophilus heathii* and *Pipistrellus pipistrellus* had not observed the significant differences in the mean haemoglobin level between male and females. However Sealander [18] had reported the significant differences in haemoglobin concentration in *Eutamias minimus* and *Clethrionomys gapperi*. Bhatkulkar and Sastry, [20] had reported the mean haemoglobin concentration in the range of 8.8 to 13.4 gm/dl in males and 8.0 to 14.9 gm/dl in females of *Rousettus leschenaulti*. Rashid *et.al*., [9] had studied the haematology of two bats, *Scotophilus heathii* and *Pipistrellus pipistrellus* belonging to family Vespertilionidae and observed the mean haemoglobin concentration 15.83 gm/dl during spring and 19.44 ± 0.5 gm/dl during summer in *S. heathii*. However haemoglobin concentration reported in *P. pipistrellus* were 18.17 ± 0.40 gm/dl in spring and 22.22 ± 0.52 gm/dl during autumn. Non-significant differences were observed by them with respect to gender and season. Our reported concentrations of haemoglobin in emballonurid bat, *Taphozous kachhensis* are less than the vespertilionid bats. Ratnasooriya *et.al*, [6] had studied the haemoglobin concentration in microchiropteran bats *Miniopterus schrebersii* and *Taphozous melanopogon* and reported the haemoglobin concentration in the range of 16.5 to 19.0 gm/dl and 14.5 to 18.0 gm/dl. respectively. The reported haemoglobin concentration range in *Taphozous melanopogon* is similar with our study. Rahma *et.al*. [10] had studied the mean haemoglobin concentration in *Cynopterus tithaecheilus* and observed the haemoglobin concentration as 15.36 ± 1.16 gm/dl in males and 15.08 ± 1.13 gm/dl in females. A higher level of haemoglobin fulfils the energy demand of *Cynopterus tithaecheilus* to fly.

5. References

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