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Studies on Blood Glucose Level in Emballonurid Bat *Taphozous kachhensis* during Reproductive Cycle

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Abstract: *Taphozous kachhensis* is an emballonurid insectivorous bat that plays a crucial role in agricultural ecosystems by regulating populations of nocturnal flying insects, which are primarily composed of proteins and lipids. Food generally consists of carbohydrates, proteins, and lipids, all of which ultimately break down into simple sugars like glucose, which plays a central role in metabolic pathways. Hence, understanding the role of glucose in homeostasis is of great importance. The objective of the present study was to determine glucose levels in female *Taphozous kachhensis* throughout their reproductive cycle. Specimens were collected year-round for this investigation. During the entire reproductive cycle of female *Taphozous kachhensis*, the mean glucose level ranged from 77–160 mg/dl. The mean glucose level during lactation and quiescence stages was 107.0 ± 3.05 mg/dl and 97.50 ± 3.66 mg/dl, respectively, with no significant differences observed between these stages. During recrudescence, oestrous, and early pregnancy, the mean glucose levels were found to be 140.0 ± 2.82 mg/dl, 126.0 ± 6.32 mg/dl, and 128.50 ± 6.90 mg/dl, respectively, with no significant differences among these stages. However, a significant increase in glucose levels was observed during recrudescence, oestrous, and early pregnancy compared to lactation, quiescence, mid-pregnancy, and advanced pregnancy. The pooled total glucose level across all reproductive stages was 113.66 ± 2.40 mg/dl. The findings of this study reveal that significant variations in glucose levels occur with changes in reproductive status.

Keywords: *Taphozous kachhensis*, Emballonurid, Bat, Glucose, Reproductive cycle

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Introduction

Bats are an important representative of the thriving order Chiroptera, which is the second largest order in terms of diversity after Rodentia within the Mammalia class, comprising more than 1,421 species grouped into 21 families (Nurfitrianto *et al*., 2013; Uzenbaeva *et al*., 2019; Simmons and Cirranello, 2020). Chiroptera is divided into two suborders: Microchiroptera and

Megachiroptera (Hill and Smith, 1984; Heard and Whittier, 1997; Murphy *et al*., 2001; Teeling *et al*., 2002, 2003, 2005). Bats are capable of flight, but unlike birds, they do not have air sacs. Flying requires significant energy, which leads to high respiration and metabolic rates in bats. Their heart size is larger than that of other mammals (Canals *et al*., 2005). Bats serve as indicators of the ecological system's richness. They have adapted to a wide range of ecological niches and exhibit the largest variety of diets among mammals, including frugivorous, insectivorous, and hematophagous species (Ben-Hamo *et al*., 2012).

Taphozous kachhensis, an emballonurid insectivorous bat, plays a crucial role in agricultural ecosystems by helping control night-flying insect populations. Insects, which make up the primary diet of insectivorous bats, are rich in proteins and lipids (Altringham, 1996; Kunz and Fenton, 2003). Although carbohydrates, proteins, and lipids ultimately break down into glucose, which serves as a universal energy fuel, it is important to understand the glucose profile throughout the reproductive cycle of bats. Glucose is central to the metabolic pathway, making it essential to study its fluctuations during reproduction (Nakrani *et al*., 2023). The reproductive cycle of female *Taphozous kachhensis* has been identified in seven stages: quiescence, recrudescence, estrus, early pregnancy, mid-pregnancy, advanced pregnancy, and lactation. This study aims to investigate changes in the glucose profile of female *T. kachhensis* throughout its entire reproductive cycle.

Materials and Methods

The present study was conducted on female *Taphozous kachhensis*. Bats were collected from the caves of Ambai Nimbai, located 45 km from Bramhapuri (M.S.), India. The bats were captured using a mist net with a mesh size of 10 mm, then transported to the laboratory. The animals were anesthetized with ether and weighed using an electronic weighing balance. Blood was collected from the subclavian and pectoral veins without causing harm to the animals. After blood collection, the samples were centrifuged to separate the serum. All specimens were released back into their natural habitat after recovery. Bats were identified using the standard monograph (Bates and Harrison, 1997).

The glucose estimation was carried out using the AutoSpan Liquid Gold Glucose Test Kit, which employs the Glucose Oxidase - Peroxidase (GOD-POD) method. In this method, Glucose Oxidase oxidizes glucose to gluconic acid and hydrogen peroxide. In the presence of the Peroxidase enzyme, hydrogen peroxide reacts with phenol and 4-Aminoantipyrine (4-AAP) to form a coloured Quinoneimine dye. The absorbance of the dye is measured at 505 nm and glucose is calculated as:

Glucose in mg/dl = (Optical density of Test / Optical density of Standard) × 100 mg/dl.

Statistical Analysis

Mean, standard error, and significance were calculated using the Statistical Package for Social Sciences (SPSS 10.0). All graphs in this study were created using Microsoft Excel Software.

Results

The glucose levels in female *Taphozous kachhensis* were analyzed throughout different stages of their reproductive cycle. The statistical analysis, performed using one-way ANOVA followed by post hoc Tukey HSD, assessed significant variations in glucose levels across these reproductive stages. The summary of these statistical comparisons is provided in Table 1, while Table 2 presents the P-value (P = 1.9726e-07) corresponding to the F-statistic, confirming significant differences in glucose levels across the reproductive phases. Additionally, Figure 1 illustrates the mean glucose levels in female *Taphozous kachhensis* during different reproductive stages.

During the entire reproductive cycle, the glucose levels in females ranged between 77 mg/dl and 160 mg/dl, with notable fluctuations observed at different stages. The mean glucose

Table 1: One-way ANOVA with post- hoc Tukey HSD showing comparison of glucose (mg/dl) in female *Taphozous kachhensis* during reproductive cycle

Reproductive status	No. of Bats	Glucose Range in (mg/dl)	Mean ± S.E.	Variance	Standard Deviation
Lactation	18	85 – 128	107.00 ± 3.05[a]	168.11	12.96
Quiescence	12	77 – 120	97.50 ± 3.66[a]	160.81	12.68
Recrudescent	06	130 – 150	140.00 ± 2.82[b]	48	6.92
Oestrous	06	98 – 142	126.00 ± 6.32[abc]	240	15.50
Early Pregnancy	12	91 – 160	128.50 ± 6.90[b]	572.63	23.92
Mid Pregnancy	12	85 – 134	115.00 ± 4.78[ae]	274.72	16.57
Advanced Pregnancy	06	85 – 110	95.00 ± 3.81[ad]	87.20	9.33
Pooled Total	72		113.66 ± 2.40	416.73	20.41

Table 2: One-way ANOVA of seven independent groups showing P-value corresponding to F- statistic

Source	Sum of squares SS	Degrees of freedom νν	Mean square MS	F statistic	p-value
Treatment	13,762.0000	6	2,293.6667	9.4205	1.9726e-07
Error	15,826.0000	65	243.4769		
Total	29,588.0000	71			

levels during the lactation and quiescence phases were 107.0 ± 3.05 mg/dl and 97.50 ± 3.66 mg/dl, respectively. Statistical analysis showed no significant differences between these two stages, suggesting that glucose levels remain relatively stable during lactation and quiescence.

In contrast, a marked increase in glucose levels was observed during the recrudescence, oestrous, and early pregnancy stages. The mean glucose levels during these phases were 140.0 ± 2.82 mg/dl, 126.0 ± 6.32 mg/dl, and 128.50 ± 6.90 mg/dl, respectively. Statistical comparisons indicated that there were no significant differences in glucose levels among recrudescence, oestrous, and early pregnancy. However, glucose levels during these three stages were significantly higher than those recorded during lactation, quiescence, mid-pregnancy, and advanced pregnancy.

The glucose levels declined during the later stages of pregnancy. During mid-pregnancy, the mean glucose level was 115.00 ± 4.78 mg/dl, whereas during advanced pregnancy, it decreased further to 95.00 ± 3.81 mg/dl. Statistical analysis showed that the glucose levels in mid and advanced pregnancy were not significantly different from those in lactation and quiescence. This pattern suggests that glucose metabolism in pregnant females may shift as pregnancy advances, leading to a decline in glucose levels.

The overall mean glucose level for all reproductive stages combined was calculated as 113.66 ± 2.40 mg/dl, representing the average metabolic glucose level across the reproductive cycle. These findings highlight the dynamic changes in glucose metabolism associated with reproductive activities in female *Taphozous kachhensis*. The significant rise in glucose levels during recrudescence, oestrous, and early pregnancy suggests increased metabolic demands during these phases, potentially due to

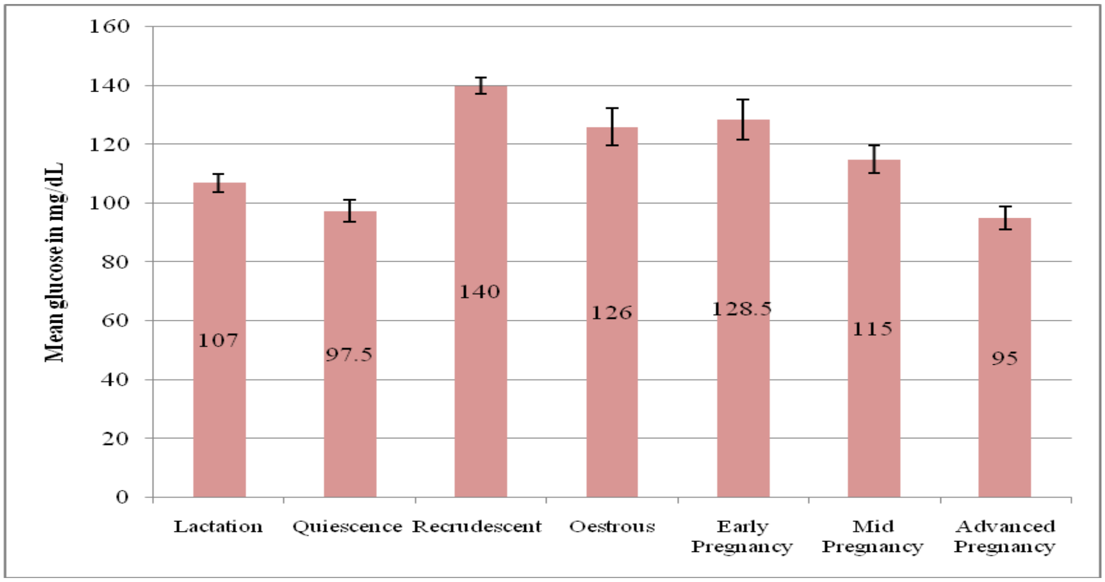

Fig.1: Mean glucose in female *Taphozous kachhensis during* reproductive cycle.

physiological preparation for conception and early fetal development. The subsequent decline in glucose levels during mid and advanced pregnancy may indicate metabolic adaptations to sustain fetal growth while maintaining maternal energy balance. Figure 1 provides a visual representation of glucose fluctuations throughout the reproductive cycle, reinforcing the statistical findings. These results emphasize the intricate relationship between glucose regulation and reproductive physiology in female *Taphozous kachhensis*, with distinct metabolic patterns observed at different reproductive stages.

Discussion

Carbohydrate digestion ultimately results in the formation of simple glucose, which is absorbed by enterocytes. When glucose levels in the bloodstream increase significantly, the condition is termed hyperglycemia. Clinically, persistent hyperglycemia accompanied by glucosuria, or glucose in urine, is a characteristic diagnostic marker of diabetes mellitus. Elevated glucose levels are generally associated with increased endocrine activity, as observed in conditions such as hyperthyroidism, hyperpituitarism, and hyper-adrenalism, or during pregnancy. Conversely, a decreased glucose level, referred to as hypoglycemia, may result from hyperinsulinism or from reduced endocrine activity, such as in hypothyroidism, hypopituitarism, hypoadrena-lism, or starvation.

Glucose homeostasis in mammals is maintained through several metabolic processes. The amino acids derived from protein digestion and glycerol obtained from fat digestion serve as precursors for gluconeogenesis, a process in which the liver synthesizes glucose. Additionally, glucose is released into the bloodstream through glycogenolysis, a process where glycogen stored in hepatocytes is hydrolyzed into glucose. The regulation of glucose levels is primarily controlled by the pancreatic hormones insulin and glucagon. Excess glucose is stored in the form of glycogen in the liver and muscles, and once these storage sites are saturated, any surplus glucose is converted into fat and stored as adipose tissue. When glucose is needed in circulation, glycogen is broken down and released into the bloodstream to maintain energy homeostasis.

Kettelhut *et al.* (1980) and Veiga *et al.* (1982) studied glucose homeostasis in cats and rats that were fed a high-protein diet. Their findings revealed that mammals consuming protein-rich diets exhibited stable glucose levels during fasting, whereas those fed carbohydrate-rich diets experienced a severe drop in fasting glucose levels.

In the present investigation, the mean pooled glucose level in female *Taphozous kachhensis* was recorded as 113.66 ± 2.40 mg/dl. The significant variations observed in glucose levels during different reproductive stages may be attributed to fluctuations in food availability and diet composition. Desai *et al.* (2012) reported that in female *Taphozous nudiventris*, the mean glucose level was 95.0 ± 4.66 mg/dl, which aligns closely with our findings, where the glucose range in female *Taphozous kachhensis* was between 77 mg/dl and 160 mg/dl.

The study also documented significant variations in mean glucose levels among females during different reproductive phases. The highest glucose level of 140.00 ± 2.82 mg/dl was recorded during the recrudescent stage in November. Elevated glucose values during November, December, and January could be attributed to the abundant availability of protein-rich food sources during these months or possibly due to physiological stress. The decline in glucose levels observed during advanced pregnancy may be linked to the increased utilization of glucose for fetal development. Similar findings have been reported by Abdel-Rachied *et al*. (2014), who observed significantly higher glucose levels ($P < 0.01$) in stressed birds and mammals compared to their non-stressed counterparts. Stress induced by capturing and handling animals could be a potential contributing factor to elevated glucose levels, as suggested by Wesson *et al*. (1979) and Widmaier and Kunz (1993).

Kelm *et al*. (2011) investigated glucose metabolism in nectar-feeding bats and found that glucose levels increased rapidly within 10 to 30 min post-feeding. The magnitude of blood glucose spikes was strongly correlated with the quality of glucose consumed. Interestingly, flight activity played a role in regulating post-feeding glucose levels, as bats that engaged in flight exhibited lower glucose spikes compared to resting bats. Thirty minutes after feeding, blood glucose levels in bats that flew were significantly lower than in resting bats, indicating that flight facilitates glucose utilization. This underscores the variability in glucose levels based on dietary intake, physical activity, environmental factors, and the time of day, as also observed by Peng *et al.* (2017).

Unlike other mammals, bats utilize glucose in a unique manner. Instead of storing excess glucose in the liver as glycogen or in adipose tissue as lipids, they consume most of the glucose directly from the bloodstream, making them highly susceptible to fasting (Kelm *et al*., 2011; Amaral *et al*., 2019). Typically, glucose mobilization from liver glycogen and adipose tissue lipid stores occurs through gluconeogenesis and glycogenolysis (Widmaier and Kunz, 1993; Pinheiro *et al*., 2006; Amaral *et al*., 2012a, 2012b). Reproductive activity in insectivorous bats demands significant energy expenditure, which is met by utilizing stored energy reserves such as glycogen, lipids, and fatty acids accumulated in the previous life cycle stages (Kunz *et al*., 1998; Srivastava and Krishna, 2008). The mobilization of these energy reserves through gluconeogenesis and glycogenolysis could explain the increased glucose levels observed during recrudescence (140.0 ± 2.82 mg/dl), oestrous (126.0 ± 6.32 mg/dl), and early pregnancy (128.50 ± 6.90 mg/dl) (Corssmit *et al*., 2001; Tappy, 2004).

A comparative analysis of glucose levels in different bat species provides valuable insights. In *Pteropus poliocephalus*, the mean glucose level in adult females was 123.78 mg/dl and in subadult females was 122.52 mg/dl, as reported by Edson *et al*. (2018). McMichael *et al.* (2015) recorded a mean glucose level of 122.52 mg/dl in wild-caught adult *Pteropus alecto*. Abdel-Rachied *et al*. (2014) reported that non-stressed *Rousettus aegyptiacus* had a mean glucose level of 76.60 ± 4.99 mg/dl, while stressed individuals had a significantly higher glucose level of 127.36 ± 17.62 mg/dl. Heard and Whittier (1997) observed glucose levels of 152.22 mg/dl in adult *Pteropus vampyrus* and 154 ± 36 mg/dl in adult *Pteropus hypomelanus*, while juvenile *Pteropus hypomelanus* had a mean glucose level of 148 ± 32 mg/dl.

Korine *et al*. (1999) reported no significant seasonal differences in glucose levels in *Rousettus aegyptiacus*. However, they observed daily fluctuations, with higher glucose levels in the morning hours. Similar observations were made by Widmaier and Kunz (1993) in captive bats. In non-hibernating species, glucose levels remain within the normal mammalian range, which is between 80 to 100 mg/dl during fasting and 120 to 140 mg/dl post-meal. In contrast, in the hibernating bat *Myotis lucifugus*, glucose levels during hibernation fall as low as 28 mg/dl, whereas after arousal, glucose levels rise to 155 mg/dl (Bauman, 1990).

Srivastava and Krishna (2008) linked increased feeding activity in *Scotophilus heathi* to elevated corticosterone levels, which induce hyperinsulinemia, leading to increased fat accumulation. Peng *et al*. (2019) observed that phytophagous bats clear blood glucose faster than insectivorous bats. In phytophagous bats, blood glucose levels return to fasting levels within 30 to 45 min, whereas insectivorous bats fail to do so even after two hours following an intraperitoneal glucose injection.

From this discussion, it can be concluded that blood glucose values are highly dynamic and not only vary across reproductive stages but also fluctuate throughout the day in mammals. Flight activity plays a crucial role in postprandial glucose regulation, and glucose levels in bats are influenced by hormones such as corticosterone, insulin, and glucagon, as well as factors like habitat, environmental conditions, food availability, reproductive status, and the time of day.

Ethical Statement

All in vivo experiments involving animals were conducted in accordance with ethical guidelines, and prior approval for the study was obtained from the Institutional Animal Ethics Committee (IAEC) of N.H. College, Bramhapuri.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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