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HEMATOLOGICAL PROFILE OF THREE SPECIES OF *Hipposideros* spp. (HIPPOSIDERIDAE) AS AN ADAPTATION IN CAVE HABITAT, IN GUNUNG SEWU GEOPARK AREA, INDONESIA

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ABSTRACT

Bats that feed on insects and are members of the Hipposideridae family, category Chiroptera is widely dispersed worldwide, including *Hipposideros* spp. There are 29 species of 73 species that inhabit Indonesia. Most of these species use caves as their roosting habitat. In cave habitats, with different physicochemical conditions from surface habitats, the hematological profile can be a physiological indicator in responding to habitat conditions. This study aims to analyze the hematological profile of *Hipposideros* spp. as a parameter of physiological adaptation in the cave habitat in Gunung Sewu Karst Area, Indonesia. Sampling was carried out purposively in six caves locality of the Gunung Sewu karst area. Bats were collected with misnet (12 x 3 m), and Harpnet was installed at the entrance of the cave at 17.00 - 21.00 WIB. The Blood samples from each bat were taken intravenously, as much as 1 mL, on the Forearm and inserted into a 1.5 mL microtube that had been given EDTA anticoagulant. A total of 20 parameters were observed from erythrocyte, leukocyte, and platelet profiles. These parameters use a Hematology Analyzer for analysis. Data differences in hematological profiles between species and sex were analyzed using one-way ANOVA (sig= 95%), respectively. The relationship trend between the physicochemical parameters of the roosting area in the cave with erythrocytes and hemoglobin was then analyzed using a univariate linear model with multiple regression. All statistical analyses were performed using the Paleontological statistics program (PAST) ver. 4.09 and R. Studio v1.4.1717-3. We found three species (*Hipposideros diadema, H. larvatus,* and *H. ater*) with 70 individuals. This study concluded that there were differences in the hematological profiles between the three *Hipposideros* species based on species and gender, although some samples did not show significant differences based on statistical tests. Erythrocytes and hemoglobin can be used as parameters of physiological adaptation to cave habitats. This is indicated by an increase in erythrocytes and hemoglobin followed by a decrease in air temperature and oxygen levels, as well as increased humidity and ammonia levels in cave habitats. These study results can be supported if similar studies analyze the hematological profile of non-cave-roosting bat. It is recommended that future studies analyze the hematological profile of non-cave-roosting bats around the caves of the studies or in areas with similar conditions.

Keywords: Bat cave, hematology, *Hipposideros*, Microchiroptera, physiological adaptations.

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INTRODUCTION

Hipposideros is a genus of the Hipposideridae family that has been distributed worldwide. This bat belongs to one of the insectivorous bats. In Indonesia, there are 29 species out of 73 in the world (Maryanto *et al.*, 2019; Iucnredlist, 2021). Moreover, seven species of this genus are found on Java island. *Hipposideros* spp. Ecologically plays an important role as a predator of agricultural pests (Ramteke *et al.*, 2012; Wijayanti, *et al.* 2011). Naturally, they feed on different groups of insects, including Hemiptera, Lepidoptera, and Coleoptera. These bats hunt using foliage gleaners and narrow space bats strategies. This strategy allows them to combine their echolocation capabilities to find prey in dense forests and vegetation (Fenton, 1990; McKenzie *et al*., 1995; Prakarsa, 2013).

Hipposideros is also known as bat cave (Sedgeley, 2003; Altringham and Senior, 2005; Kofoky, *et al*., 2006). Every animal, including bats, has a tolerance limit to the carrying capacity to keep it alive. In habitat, not all of that capacity can be used by animals because it is influenced by three main factors, including access, biotic and abiotic factors (Chase and Leibold, 2003; Pocheville, 2015). Caves with dark characteristics, high humidity, relatively lower oxygen levels compared to the habitat outside the cave, and also high levels of ammonia make the limited membership of the cave community (Wijayanti *et al*., 2011). Therefore, many animals can not use the cave as their habitat (Kurniawan *et al.* 2022). *Hipposideros* spp. has become bats that can adapt to cave habitats and are categorized as habitual trogloxene (Prakarsa, *et al.* 2021).

Physiological adaptation is closely related to the physicochemical conditions of the habitat. In cave habitats with microclimatic conditions, the hematological profile can be used as a physiological indicator of animals respond to their habitat conditions. Parameters in the hematological profile are used to detect health status (including genetic disease), nutrition, stress levels, animal populations' management, and the degree of protection (Hall *et al*., 2014; McMichael *et al*., 2015; Saimina *et al*., 2019). Hematological profiles provide material on many elements of immunology and metabolic activity, for example, the ability to bind oxygen for energy needs in metabolic processes. Fitria & Sarto (2014) stated that Animals' hematological profiles could be impacted by factors including their activity and stress level of animals, gender, age, environmental conditions, and amount of anxiety, as well as bats (Ekeolu & Adebiyi, 2018).

Hematological studies of bats in Indonesia are still lacking, specifically Microchiroptera bats, including the Genus Hipposideros (Wijayanti *et al*., 2011; Rahma *et al*., 2017; Saimina *et al*., 2019; Mubarok *et al*., 2021). So this research needs to be done. This study aims to analyze the hematological profile of *Hipposideros* spp. as a parameter of physiological adaptation in the cave habitat in Gunung Sewu Karst Area, Indonesia.

MATERIALS AND METHODS

Ethical Clearance Write it in the start of the materials and methods: This research has obtained ethical clearance approval from the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada

Field Study: This research was conducted from October 2021 to March 2022. Sampling was carried out purposively in six caves in the Gunung Sewu karst area, including Cerme Landak Cave, Toto Cave, Sioyot Cave, Plelen Cave, and Sodong Cave (Figure 1). These caves are located in the Gunung Sewu Geopark, which belongs to Gunung Kidul Regency, Special Region of Yogyakarta, and Wonogiri Regency, Central Java, Indonesia. This place has an annual average temperature of around 25.1ºC, and rainfall is about 1837 mm per year.

Sampling and Identification: Bats are caught and treated following the guidelines recommended by the American Society of Mammalogists (Sikes *et al*., 2011; Sikes, 2016). Catching bats using a misnet (12 x 3 m) and a harpnet, placed across the entrance of the cave at 17.00 - 21.00 WIB. Identification was carried out by echolocation identification (batsound) and morphometric measurements, which included: body weight (W), sex (S), head and body length (HB), ear/ear length (E), forearm (Fa), tibia (TB), hindfoot (HF), and tail length (T).

Physicochemical Parameters: Physicochemical parameteric data were measured, including air temperature, humidity, oxygen levels, and air ammonia levels of the sample roosting area in the cave. The average temperature and humidity were measured using a digital Thermo Hygro Meter Digital (HABOTEST HT 618), oxygen levels with the Oxygen Meter Tester (Smart Sensor AR8100 O2 Detector Analyzer Oxygen Tester), and ammonia using the Ammonia Tester (Gas Detector Smart Sensor Amonia Tester AR-8500 NH3).

Hematological Profile: Xetamine-Xylazine (50 mg/kg body weight) was used to anesthetize the bats. The Blood samples from each bat were taken intravenously, as much as 1 mL, on the Forearm, and inserted into a 1.5 mL microtube that had been given EDTA anticoagulant. A Hematology Analyzer Sysmex XP-100 was used to measure 20 parameters from the erythrocyte, leukocyte, and platelet profiles. Hematology analyses were carried out at the LPPT Laboratory of Gadjah Mada University and the Laboratory of IDB FMIPA Universitas Negeri Yogyakarta. The twenty hematological parameters consist of Red blood cells (RBC), hemoglobin (HGB; g/dL), hematocrit (HCT; %), mean corpuscular volume (MCV; fL), mean corpuscular hemoglobin (MCH; pg), mean corpuscular hemoglobin concentration (MCHC;g/dL), red cell distribution width standard deviation (RDW-SD), red cell distribution width coefficient counts made up the measured hematological (RDW-CV), white blood cells (WBC), lymphocytes (#LYM), neutrophils (#NEUT;), lymphocyte percentage per microliter blood (% LYM), neutrophil percentage per microliter blood (% NEUT), mixed number (#MXD;), mixed percentage were the parameters on the leukocyte profile (% MXD), the number of platelets (PLT), the platelet distribution width (PDW), the mean platelet volume (MPV), the platelet large cell ratio (P-LCR), and the platelet crit (PCT).

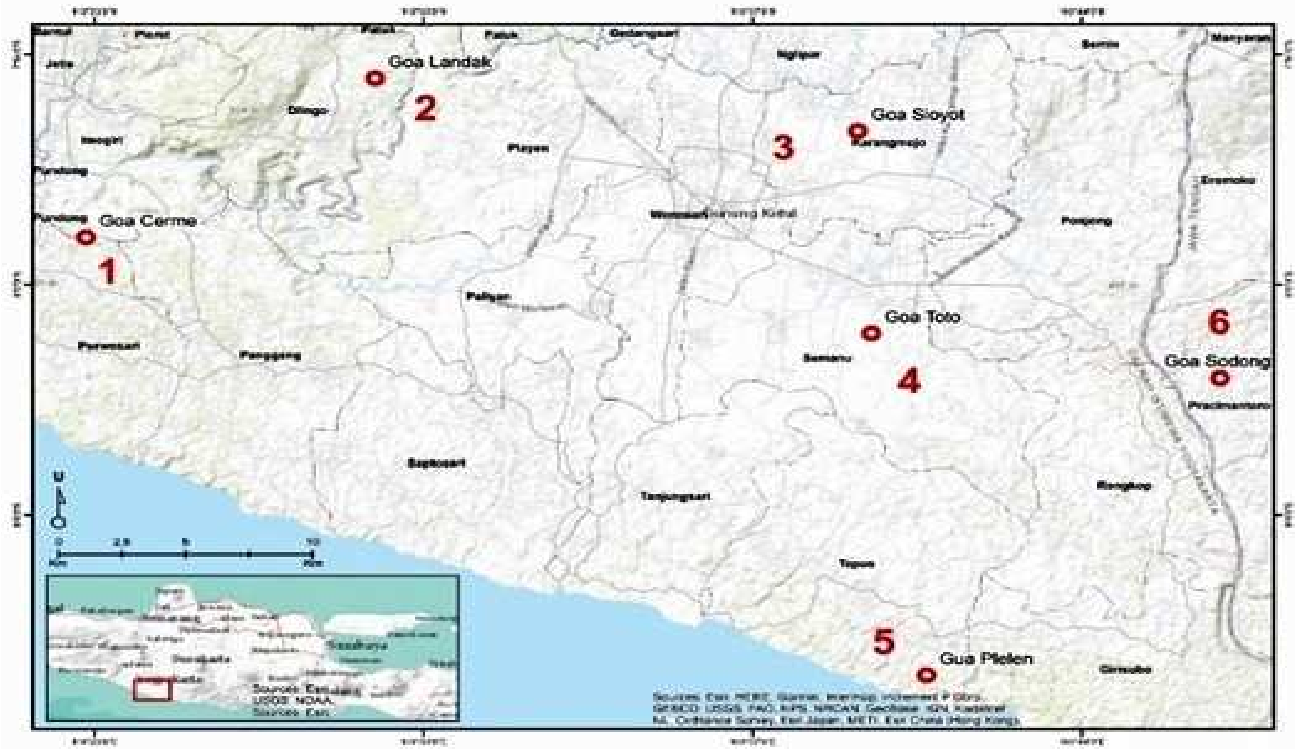

Figure 1. Gunung Sewu Karst Area, Sampling locations (1. Cerme cave; 2. Landak cave; 3. Toto Cave; 4. Sioyot cave; 5. Plelen cave; and 6. Sodong cave).

Statistical Analysis: The results of hematological measurements were tabulated, and the data were tested for normality by applying a 95% confidence level for the Shapiro-Wilk reliability test. One-way ANOVA analysis ($P < 0.05$) was performed to analyze the differences in hematological profiles by species and sex categories. The relationship trend between the physicochemical parameters of the roosting area in the cave with erythrocytes and hemoglobin were analyzed using a univariate linear model with multiple regression. All statistical analyses were performed using the Paleontological statistics program(PAST) ver. 4.09 (Hammer *et al.*, 2001) and R. Studio v1.4.1717-3.

RESULTS

We found three species of *Hipposideros*, including *Hipposideros diadema*, *Hipposideros larvatus*, and *Hipposideros*. ater. Seventy individuals of the three species collected from six caves (sampling sites) were used in this study. Detailed data on the number of individuals by sex is presented in Table 1.

Table 1. Number of individuals caught from the three *Hipposideros* species

Species	a.m	a.f	j.m	j.f	Number of each species
H. diadema	8	8	4	4	24
H. larvatus	8	8	4	2	22
H. ater	8	8	4	4	24
		Total number caught			70

a.m: adult male, a.f: adult female, j.m: juvenile male, j.f: juvenile female

Erythrocyte Profile: The average value of erythrocyte profiles in three *Hipposideros* species can be seen in Table 2. According to ANOVA analysis, males and females of *H. diadema, H. larvatus,* and *H. ater* showed significant differences in the number of erythrocytes (RBC) (P=0.021, P=0.000, and P=0.047) and red cell distribution width standard deviation (RDW-SD) (P=0.000, P=0.000, and P=0.033)..

Table 2. Erythrocyte profile of three species of *Hipposideros* from Gunung Sewu Karst Area

	H.d				***H.l***				***H.a***			
	a.m	**a.f**	**j.m**	**j.f**	**a.m**	**a.f**	**j.m**	**j.f**	**a.m**	**a.f**	**j.m**	**j.f**
RBC(106/mL)*	10.09 ±1.03	8.66 ± 1.14	8.54 ± 2.35	8.15 ± 1.56	10.01 ± 0.9	8.21 ± 1.1	10.15 ± 1.45	8.06 ± 1.55	9.93 ± 1.15	8.12 ± 1.34	9.64 ± 1.55	8.24 ± 1.61
HGB(g/dL)	19.90 ± 1.18	19.10 ± 1.57	19.15 ± 1.84	18.65 ± 1.68	18.65 ± 1.01	18.17 ± 1.24	18.20 ± 1.70	18.25 ± 2.05	17.60 ± 1.3	17.70 ± 1.36	17.25 ± 1.66	17.30 ± 1.22
HCT(%)	56.25 ± 2.11	60.60 ± 2.78	55.57 ± 1.95	60.02 ± 1.55	53.40 ± 1.45	54.10 ± 1.7	52.95 ± 1.58	53.95 ± 1.85	53.60 ± 1.63	53.90 ± 1.9	53.21 ± 1.55	53.45 ± 2.09
MCV(fL)	60.70 ± 2.41	61.15 ± 2.43	60.20 ± 2.18	60.65 ± 1.95	55.40 ± 1.75	56.30 ± 1.5	55.11 ± 1.95	56.17 ± 1.63	48.20 ± 1.55	49.25 ± 1.8	47.90 ± 1.14	49.01 ± 1.22
MCH(pg)	21.25 ± 1.01	22.10 ± 0.91	20.85 ± 1.65	21.70 ± 1.35	21.70 ± 1.04	21.00 ± 1.23	21.20 ± 1.54	20.77 ± 1.9	20.70 ± 1.07	20.95 ± 1.3	20.24 ± 1.75	20.55 ± 1.65
MCHC(g/dL)	33.50 ± 1.27	31.50 ± 1.80	33.04 ± 1.82	31.01 ± 1.52	30.00 ± 1.10	30.25 ± 1.62	29.80 ± 1.50	30.02 ± 1.85	29.25 ± 1.3	29.00 ± 1.35	28.58 ± 1.25	28.25 ± 1.55
RDW-SD(fL)*	43.40 ± 1.91	38.00 ± 1.05	42.90 ± 2.15	37.60 ± 1.89	34.50 ± 1.15	33.40 ± 1.05	34.20 ± 2.02	32.80 ± 1.25	30.50 ± 1.2	29.60 ± 1.03	30.02 ± 1.85	29.15 ± 1.74
RDW-CV(%)	16.70 ± 1.22	15.96 ± 1.14	16.15 ± 2.62	15.40 ± 1.75	16.80 ± 0.9	16.50 ± 1.03	16.25 ± 1.25	16.15 ± 1.36	15.20 ± 1	14.10 ± 1.42	14.75 ± 1.55	13.55 ± 1.26
BW(g)	42.20 ± 1.83	46.20 ± 2.65	37.51 ± 2.56	33.72 ± 2.70	15.74 ± 1.4	17.00 ± 1.45	10.53 ± 1.64	11.27 ± 1.73	6.90 ± 1.7	7.55 ± 0.86	4.63 ± 1.23	4.81 ± 1.15

H.d:*H.diadema*, H.l:*H.larvatus*, H.a:*H..diadema*. a.m: adult male, a.f: adult female, j.m: juvenile male, j.f: juvenile female.
The asterisk symbol indicates a significant parameter. The asterisk symbol on RBC indicates a significant difference in RBC between adult males and adult females in each species. The atherisk on RDW-SD showed significant differences in RDW-SD between adult males and adult females in each species.

Table 3. Leukocyte profile of three species of *Hipposideros* from Gunung Sewu Karst Area

	H.d				*H.l*				*H.a*			
	a.m	a.f	j.m	j.f	a.m	a.f	j.m	j.f	a.m	a.f	j.m	j.f
WBC(103/mL)*	6.30 ± 2.3	5.49 ± 2.56	5.4 ± 1.55	3.95 ± 1.92	5.15 ± 3.22	5.02 ± 2.94	4.25 ± 1.55	4.19 ± 1.09	4.60 ± 2.03	4.92 ± 1.54	3.15 ± 1.12	3.45 ± 1.55
#LYM(103/mL)	4.10 ± 1.46	3.70 ± 1.74	3.55 ± 1.74	2.05 ±1.25	3.30 ± 1.5	3.20 ± 1.47	2.4 ± 1.64	2.3 ± 1.17	2.38 ± 1.74	3.10 ± 1.6	1.53 ± 1.26	1.99 ± 1.21
#NEUT(103/mL)	2.20 ± 2.14	2.30 ± 2.19	1.65 ± 1.45	1.4 ± 1.75	1.85 ± 1.8	1.85 ± 1.7	0.77 ± 0.85	0.45 ± 0.34	1.25 ± 1.67	1.52 ± 1.9	0.35 ± 1.06	0.75 ± 1.02
%LYM(%)	63.49 ± 1.87	68.38 ± 2.35	66.5 ± 1.52	67.54 ±1.88	62.60 ± 1.7	54.34 ± 1.95	59.85 ± 1.91	60.17 ± 1.63	51.73 ± 1.4	63.11 ± 2.04	60.9 ± 1.45	61.13 ± 1.23
%MXD(%)	-	-	-	-	-	-	-	-	-	-	-	-
%NEUT(%)	36.51 ± 2.19	31.62 ± 2.7	33.5 ± 1.82	32.46 ± 1.45	37.40 ± 1.95	45.66 ± 2.05	40.15 ± 1.50	39.83 ± 1.85	48.27 ± 1.9	36.89 ± 1.85	39.1 ± 1.55	39.87 ± 1.57
#MXD(103/mL)	-	-	-	-	-	-	-	-	-	-	-	-
BW(g)	42.20 ± 1.83	46.20 ± 2.65	37.51 ± 2.56	33.72 ± 2.70	15.74 ± 1.4	17.00 ± 1.45	10.53 ± 1.64	11.27 ± 1.73	6.90 ± 1.7	7.55 ± 0.86	4.63 ± 1.23	4.81 ± 1.15

H.d:*H.diadema*, H.l:*H.larvatus*, H.a:*H..diadema*. a.m: adult male, a.f: adult female, j.m: juvenile male, j.f: juvenile female.
The asterisk symbol indicates a significant parameter. The asterisk symbol on WBC indicates a significant difference in WBC between adult males and adult females in each species

Table 4. Plateletprofile of three species of *Hipposideros* from Gunung Sewu Karst Area

	H.d				***H.l***				***H.a***			
	a.m	**a.f**	**j.m**	**j.f**	**a.m**	**a.f**	**j.m**	**j.f**	**a.m**	**a.f**	**j.m**	**j.f**
PLT(103/mL)	319 ± 2.74	443 ± 2.25	234 ± 1.65	414 ± 2.03	254 ± 1.75	215 ± 2.13	209 ± 1.59	229 ± 1.76	198 ± 2.32	209 ± 1.93	117 ± 1.88	201 ± 1.93
PDW(fL)	17.2 ± 1.25	10.70 ± 1.90	15.4 ± 1.55	9.95 ± 1.84	8.55 ± 1.50	9.1 ± 1.47	6.72 ± 1.65	8.93 ± 1.19	7. 1 ± 1.05	7.92 ± 0.95	5.66 ± 1.62	7.54 ± 1.95
MPV(fL)	6.60 ± 0.50	8.80 ± 0.74	5.36 ± 1.13	9.04 ± 1.14	7.51 ± 0.55	7.13 ± 0.75	5.94 ± 1.4	7.72 ± 1.52	6.34 ± 0.65	-	-	-
P-LCR(%)	-	-	-	-	-	-	-	-	-	-	-	-
PCT(%)	0.28 ± 0.04	0.63 ± 0.07	0.13 ± 0.11	0.49 ± 0.27	0.30 ± 0.03	0.52 ± 0.07	0.19 ± 0.11	0.37 ± 0.18	0.12 ± 0.09	0.24 ± 0.07	0.11 ± 0.08	0.14 ± 0.12
BW(g)	42.20 ± 1.83	46.20 ± 2.65	37.51 ± 2.56	33.72 ± 2.70	15.74 ± 1.4	17.00 ± 1.45	10.53 ± 1.64	11.27 ± 1.73	6.90 ± 1.7	7.55 ± 0.86	4.63 ± 1.23	4.81 ± 1.15

H.d:*H.diadema*, H.l:*H.larvatus*, H.a:*H..diadema*. a.m: adult male, a.f: adult female, j.m: juvenile male, j.f: juvenile female.

Platelet Profile: The average value of platelet profiles in three *Hipposideros* species can be seen in Table 4. According to ANOVA analysis, males and females of *H. diadema, H. larvatus,* and *H. ater* did not show significant differences in platelet profiles.

Leucocyte Profile: The average value of leukocyte profiles in three *Hipposideros* species can be seen in Table 3. According to ANOVA analysis, males and females of *H. diadema, H. larvatus,* and *H. ater* showed significant differences in the number of leukocytes (WBC) (P=0.000, P=0.011, and P=0.000)..

Physicochemical relationship of roosting habitat with RBC, HGB, and WBC: The results of physicochemical measurements in the roosting habitat of three species of *Hipposideros* showed that *H. diadema* could roost at the habitat with the highest temperature (26.1 ± 1.24 ºC) compared to the other species (*H. larvatus* (25.5 ± 1.35 ºC) and *H. ater* (25.1 ± 1.52 ºC)). Meanwhile, *H. ater* can adapt to roosting habitats with the highest humidity (81 ± 1.51%). The complete differences in the adaptability of the three species to physicochemical conditions in their roosting area can be seen in Table5.

Table5. Physicochemical, erythrocyte, hemoglobin, dan leukocyte of three species *Hipposideros*

Species	T (ºC)	H (%)	O_2 (%)	NH3 (ppm)
H. diadema	26.1 ± 1.24	79 ± 1.17	22.7 ± 0.88	1790 ± 74
H. larvatus	25.5 ± 1.35	75 ± 1.33	21.8 ± 1.05	1953 ± 46
H. ater	25.1 ± 1.52	81 ± 1.51	20.4 ± 1.12	2131 ± 13

T = temperature, H = humidity, O_2 = oxygen, NH3 = ammonia level, RBC = erythrocyte, HGB = hemoglobin, WBC = leucocyte

The multiple regression equation for the physicochemical relationship of habitat with erythrocytes and hemoglobin of three *Hipposideros* is presented in Table 6.

The results of multiple regressions of the effect of the physicochemical roosting habitat of each species on the levels of erythrocytes and hemoglobin showed that the physicochemical parameters (humidity, temperature, oxygen, ammonia) had significant or insignificant to the levels of erythrocytes, hemoglobin, and leukocytes (Table 7). From the results of the analysis, there is a tendency for the number/levels of erythrocytes and hemoglobin to increase along with a decrease in air temperature, an increase in humidity, a decrease in oxygen levels, and an increase in ammonia levels in the roosting habitat of three species of *Hipposideros*. However, this is not in line with leukocyte levels which are not significantly affected by the high and low physicochemical parameters.

Table 6. Multiple regression equation of habitat physicochemical relationship with erythrocytes and hemoglobin

Species	physicochemical with erythrocytes	physicochemical with hemoglobin
H. diadema	Y_e=5.345-0.537X1+0.062X2-0.741X3+0.003X4	Y_h=36.473-0.300X1+0.017X2-0.502X3+0.721X4
H. larvatus	Y_e=11.603-0.187X1+0.012X2-0.025X3+0.001X4	Y_h=16.811-0.022X1+0.110X2-0.124X3+0.061X4
H. ater	Y_e=12.598-0.204X1+0.103X2-0.216X3+0.020X4	Y_h=4.967-0.372X1+0.032X2-0.433X3+0.113X4

Y_e: erythrocyte, Y_h: hemoglobin, X1: temperature, X2: humidity, X3: oxygen, and X4: ammonia

Table7. The results of multiple regression of the relationship between cave physicochemical parameters, erythrocyte, hemoglobin, and leukocytes.

Hematological parameters	physicochemical parameters	*H. diadema*	*H. larvatus*	*H. ater*
	Constanta/Intercept	5.345*	11.603*	12.598*
erythrocyte	Temperature	-0.537**	-0.187.	-0.204.
	Humidity	0.062**	0.012*	0.103*
	O_2	-0.741**	-0.025*	-0.216*
	NH	0.003**	0.001*	0.020*
	R-square	0,989	0.596	0.755
	Constanta/Intercept	36.473**	16.811*	4.697 *
hemoglobin	Temperature	-0.300*	-0.022.	-0.372.
	Humidity	0.017*	0.110*	0.032*
	O_2	-0.502*	-0.124*	-0.433*
	NH	0.721*	0.061*	0.113*
	R-square	0,948	0.725	0.687
	Constanta/Intercept	2.812	5.024	8.441
Leukocytes	Temperature	-0.254	-0.378.	0.311
	Humidity	-0,128	0.032	-0.098
	O_2	-0.095	-0.457	-0.696
	NH	0.012	0.110	0.004·
	R-square	0,425	0.513	0.577

Signif. Code : 0 '***', 0.001 '**', 0.01 '*', 0.05 '.'

DISCUSSION

Erythrocyte Profile: The data of bat hematology is not only used as a reference collection but is also useful for understanding the physiological adaptations of various bat species health conditions and, with no exceptions for genetic disorders, nutrition, stress activity, and thus will be able to help, at least, to determine in consideration conservation status (Viljoen *et al.*, 1997, Ratnasooriya *et al.*, 2011; Hall *et al.*, 2014; Saimina *et al.*, 2019). Generally, male animals had more erythrocytes, hemoglobin, leukocytes, and platelets than female animals. Within the research, the profiles of the three species *H. diadema, H. larvatus*, and *H. ater* had low values or similar conditions along with the hematological profiles of rats, mice, and some fruit bats (Wolford *et al.*, 1986; Smith *et al.*, 1994; Rahma *et al.*, 2017). However, the location of the roosting may affect the structure of the hematological profile. Such conditions are the example of the incident in *Rousettus aegyptiacus*, where the number of hemoglobin, hematocrit, and erythrocytes of female *R. aegyptiacus* collected via garden habitats was more than that of males collected from caves (Van der Westhuyzen, 1988) or other examples from Mubarok *et al.* (2021) in *Cynopterus brachyotis* females from urban habitats had higher erythrocytes and leukocytes than females from non-urban habitats.

In this study, erythrocytes from the three species tended to be strongly influenced by environmental conditions. These environmental factors in the form of physicochemical factors in the roosting habitat influence erythrocytes and hemoglobin in the three species of *Hipposideros*. Erythrocyte levels increase when air temperature decreases, humidity increases, oxygen decreases, and ammonia levels increase. Likewise, hemoglobin levels increase along with decreasing air temperature, increasing humidity, decreasing oxygen levels, and increasing ammonia in their roosting habitat. This research supports the claims made by Ekeolu & Adebiyi (2018) and Mubarok *et al.* (2021) that external factors, including humidity, heat, and respiration rate, might affect the bat erythrocyte profile.

Furthermore, Wijayanti *et al.* (2011) also revealed that high erythrocytes and hemoglobin in cave bats were influenced by decreased air temperature, high humidity, low oxygen levels, and high ammonia levels. In general, species that live in caves have relatively higher levels of erythrocytes than those that live outside the cave (Wijayanti *et al.*, 2011). This is strongly influenced by the environmental conditions of the cave habitat having high ammonia levels and low O_2 levels (Wijayanti, *et al.*, 2011; Prakarsa *et al.* 2021). Therefore, it makes bats can adapt and survive. One of these adaptations is a physiological adaptation by increasing the number of erythrocytes and hemoglobin. These conditions allow bats to be able to meet the oxygen needs in their bodies. On the other hand, flying activity also affects the hemoglobin in bats (Saimina *et al.*, 2019).

Hemoglobin levels in bats are higher than those in other large mammals. According to Arévalo *et al.* (1991), Arad & Korine (1993), Mubarok *et al.* (2021), modifications in their vascular permeability brought on by ambient air and flight activities are responsible for this. The significant difference from other Erythrocyte parameters is the Red Cell Distribution Width Standard Deviation (RDW-SD) between adult males and females related to hormonal factors from sex but not to age (Balasubramaniam *et al.*, 2008; Padalia *et al.*, 2014). According to Putra & Bintoro (2019), the higher the RDW value, the greater the variation in cell size. A common cause of increased RDW is a nutritional deficiency (Tseliou *et al.*, 2014). This RDW is also a marker of certain diseases, such as liver, kidney, and inflammation disorders (Lippi *et al.*, 2008; Tseliou *et al.*, 2014).

In this study, the physicochemical parameters of the bat roosting habitat were also influenced by the physical condition of the cave passage. Habitats with high organic matter deposits have high ammonia levels and lower oxygen levels compared to cave passages with minimal or no organic material deposits. In another case, for example, a tunnel with an active underground river flow has higher oxygen levels and lower ammonia levels. This is in line with the research of Winkelmann *et al.* (2000) and Duran & Centano, (2002), which revealed that the physicochemical parameters in this cave habitat are influenced by cave characters, such as the physical shape of the chamber, subsurface flow, and organic material.

Leukocyte Profile: The leukocyte profile is beneficial in the field of conservation. This is because it shows the animal's stress level and can be directly related to stress hormone levels (Davis *et al.*, 2008). According to Smith *et al.* (1994), in mammals, as in mice and rats, the overall amount of leukocytes, neutrophils, lymphocytes, and platelets decreases over time. In this study, both males and females between species showed significant differences in leukocytes. In detail, the number of male *H. diadema* and *H. larvatus* leukocytes was higher than that of the female. In contrast, *H.ater* had high leukocyte counts in females(Table3). In this study, leucocytes of the three species were not affected by physicochemical parameters so that the three species could adapt to the cave habitat. According to Ekeolu & Adebiyi (2018), leukocytes can reveal details regarding antibody issues affecting the animal, like inflammation and infection. According to McLaughlin *et al.* (2007), species' immune systems can be stimulated, a normal reaction during the processing and captures procedure, which can result in high lymphocyte counts in certain animals. In this study, the Ideals of the Neutrophil-Lymphocyte Count Ratio (NLR) of *H. diadema, H. larvatus,* and *H. ater* were 0.58, 057, and 0.51 respectively. This NLR value could serve as a measure of an animal's discomfort and a diagnosis of severe infectious disorders in mammals (Davis *et al.*, 2008; Dhabhar *et al.*, 1996; Rahma *et al.*, 2017). The NLR values from this study were all species below 5. This indicated that the condition of the three species did not experience severe infection and had low stress (Hung *et al.*, 2011; Kahramanca *et al.*, 2014; Man *et al.*, 2021).

Platelet Profile: Blood clotting is strongly influenced by the condition of Platelets (Periayah, *et al.*, 2017). This study showed that *H.diadema* and *H.ater* had higher platelet counts in females than in males. In contrast, male *H.larvatus* had a higher platelet count than females, although statistically, it did not show a significant difference (>0.05). The same thing was also found in several species from the Genus *Myotis*, *Scotophilus*, and *Pipistrellus;* males had higher platelets than females, which were not significantly different (Rashid *et al.*, 2016). This study platelet values were generally normal or in the range of 150 x 103/mL – 450 x 103/mL, except for male juvenile *H. ater,* who had platelets of 117 x 103/mL (Table 5). In adults, values below this range lead to thrombocytopenia, but in juveniles, it cannot be ascertained, this is considering age, gender, genetics, the time of year, and pre-analytical conditions all have a significant impact on platelets. (Biino *et al.*, 2011; Rashid *et al.*, 2016). According to Padalia *et al.* (2014), Platelets in males will often decrease with age, but not in females. Typically, a platelet count higher than the reference interval for the species is reactive thrombocytosis and has no direct pathological importance (Eclinpath. 2021). However, according to Alonso & Cox (2015), Platelets also play a role as a defense in responding to viral and bacterial infections.

Conclusion: Hematological profiles of the three species of *Hipposideros* (*H. diadema, H. larvatus*, and *H. ater*) from the karst area in Gunung Sewu, Indonesia, showed differences between species in both males and females. However, not all of them showed significant differences based on statistical tests. Erythrocyte and hemoglobin levels can be used as parameters of physiological adaptation to cave habitats. This can be shown by the increase in erythrocytes and hemoglobin, along with the tendency of air temperature and oxygen levels to decrease and increased humidity and ammonia levels in cave habitats. These study results can be supported if similar studies analyze the hematological profile of non-cave-roosting bat. It is recommended that future studies analyze the hematological profile of non-cave-roosting bats around the caves of the studies or in areas with similar conditions.

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REFERENCES

Altringham, J. D. and P. Senior (2005). Social systems and ecology of bats. In Ruckstuhl, K.E., Neuhaus, P., (Eds.), Sexual segregation in vertebrates: Ecology of the two sexes (pp. 280–302). Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO 9780511525629.016.

Arad, Z. and C. Korine (1993). Effect of water restrictionon energy and water balance and smoregulationof the fruit bat Rousettus aegyptiacus. J. Comparative Physiology, 163(5), 401-5. https://doi.org/ 10.1007/BF00265645.

Alonso, A.L. and D. Cox (2015). Platelet Interactions with Viruses and Parasites. in S. Kerrigan, N. Moran (eds.), The Non-Thrombotic Role of Platelets in Health and Disease, Intech Open, London. 10.5772/60747. https://doi.org/ 10.5772/60747.

Arévalo, F., G. Perez-Suarez and P. Lóapez-Luna. (1991). Oxygen-binding properties of bat hemoglobins.Archives Internationales de Physiologie, deBiochimie et de Biophysique, 99(1), 77-81. https://doi.org/10.3109/ 13813459109145907.

Balasubramaniam, U., P. Pandeya, S. Sairam, D. Suhasini and L. Sekhar (2008). Hematological and Biochemical Values in Indian Population. A Research Initiative of the Apollo Hospital Educational and Research Foundation (AHERF).

Biino, G., C. Balduini, L. Casula, P. Cavallo, S. Vaccargiu, and D. Parracciani (2011). Analysis of 12,517 inhabitantsof a Sardinian geographic isolate reveals thatpredispositions to thrombocytopenia and thrombocytosisare inherited traits. Haematologica, 96, 96–101. https://doi.org/10.3324/haematol.2010.029934

Chase, J.M., and M.A. Leibold (2003). Ecological Niches: Linking Classical and Contemporary Approaches. University of Chicago Press, 7 p. ISBN 9780226101804. https://doi.org/ 10.7208/chicago/9780226101811.001.0001.

Davis, A.K., D.L. Maney and J.C. Maerz (2008). The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology. 22(5), 760–772. https://doi.org/ 10.1111/j.1365-2435.2008.01467.x.

Dhabhar, F.S., A.H. Miller, B.S. McEwen and R.L. Spencer (1996). Stressinducedchanges in blood leukocyte distribution – role of adrenal steroidhormones. J. Immunology, 157, 1638–1644.

Duran A.R. and J.A.S. Centano (2002). Temperature selection by tropical batsroosting in caves. J. Thermal Biology, 28, 465-468.

https://doi.org/10.1016/S0306-4565(03)00046-9.

Eclinpath (2021). Platelet Number. Acces on Dec 31. https://eclinpath.com/hemostasis/tests/platelet-number.

Ekeolu, O.K. and O.E. Adebiyi (2018). Hematology anderythrocyte osmotic fragility of the Franquet'sfruit bat (Epomops franqueti). J. Basicand Clinical Physiology and Pharmacology, 29(4), 391-394. https://doi.org/10.1515/jbcpp-2017-0169.

Fenton, M.B. (1990). Foraging Behavior and Ecology of Animal-Eating Bats. In. Sariano, PJ. 2000. Functional Structure of Bat Communities in Tropical Rainforest and Andean Cloud Forests. Ecotropicos, 13 (1), 1-20.

Fitria, L. and M. Sarto (2014). Profil hematologi tikus (*Rattus norvegicus* Berkenhout, 1769) galur wistar jantan dan betina umur 4, 6, dan 8 minggu. Biogenesis: Jurnal Ilmiah Biologi, 2(2), 94-100. https://doi.org/10.24252/bio.v2i2.473.

Hammer, O., D.A.T. Harper, and P.D. Ryan (2001). Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica, 4(1).

Hall, J., K. Rose, C. Smith, C. De Jong, D. Phalen, J. Austen, and H. Field (2014). Health assessmentof the Christmas Island flying fox (Pteropus melanotus natalis). J. Wildlife Diseases, 50(3), 447-458. https://doi.org/10.1515/jbcpp-2017-0169.

Hung, H.Y., J.S. Chen, C.Y. Yeh, C.R. Changchien, R. Tang, P.S. Hsieh, W.S. Tasi, J.F. You, Y.T. You, C.W. Fan, J.Y. Wang, and J.M. Chiang (2011). Effect of preoperative neutrophil-lymphocyte ratio on the surgical outcomes of stage II colon cancer patients who do not receive adjuvant chemotherapy. International J. Colorectal Disease, 26(8),1059–1065. https://doi.org/10.1007/s00384-011-1192-x.

Irfan, M. and A. Shabbir (2016). Comparative Seasonal Haematology of Two Bat Species, Scotophilus heathii and Pipistrellus pipistrellus, in a Subtropical Area of Pakistan. Pakistan J. Zoology, 48 (5), 1503 – 1510.

IUCN. (2021). The IUCN Red List of Threatened Species. Version 1.3. https://www.iucnredlist.org. (accessed: October 11, 2021).

Kahramanca, S., G. Ozgehan, D. Seker, E.I. Gökce, G. Seker, G. Tunç, T. Küçükpınar, and H. Kargıcı (2014). Neutrophil-to-lymphocyte ratio as a predictor of acute appendicitis. Turkish J. Trauma and Emergency Surgery, 20(1), 19–22. https://doi.org/10.5505/tjtes.2014.20688.

Kofoky, A., D. Andriafidison, F. Ratrimomanarivo, H.J. Razafimanahaka, D. Rakotondravony, P.A. Racey, and R.K.B. Jenkins (2006). Habitat use, roost selection and conservation of bats in Tsingy de Bemaraha National Park, Madagascar. Biodivers Conserv. https://doi.org/10.1007/978-1-4020-6320-6_14.

Kurniawan, I.D., C. Rahmadi, R.T.M. Akbar, and T.B.P. Prakarsa (2022). Stygobiotic isopod Stenasellus sp. in Sarongge Jompong cave, Tasikmalaya karst area, Indonesia. Biodiversitas 23: 1495-1504. https://doi.org/10.13057/biodiv/d230338.

Lippi, G., G. Targher, M. Montagnana, G.L. Salvagno, G. Zoppini, and G.C. Guidi (2008). Relationship between red blood cell distribution width and kidney function tests in a largecohort of unselected outpatients. Scandinavian J. Clinical and Laboratory Investigation, 68, 745-8. https://doi.org/10.1080/00365510802213550.

Man, M.A., R.M. Rajnoveanu, N.S. Motoc, C.I. Bondor, and A.F. Chis (2021). Neutrophil-to-lymphocyte ratio, platelets-to-lymphocyte ratio, and eosinophils correlation with high-resolution computer tomography severity score in COVID-19 patients. PLOS ONE, 16(6), e0252599. https://doi.org/10.1371/journal.pone.0252599.

Maryanto, I., Maharadatunkamsi, A.S. Achmadi, S. Wiantoro, E. Sulistyadi, M. Yooneda, A. Suyanto and J. Sugardjito (2019). Cecklist oh The Mammals of Indonesia. Research Center for Biology, Indonesian Institute of Science (LIPI). Bogor, Indonesia. 18 p.

McKenzie, N.L., A.C. Gunnel, M. Yani and M.R. Williams (1995). Correspondence Between Flight Morphology and Foraging Ecology in Some Paleotropical Bats. In. Kingston, T., Francis, C.M., Akbar, Z., Kunz, T.H., 2003. Species Richness in An Insectivorous Bats Assemblage from Malaysia. J. Tropical Ecology, 19, 1-12. https://doi.org/10.1017/S0266467403003080.

McLaughlin, A.B., J.H. Epstein, V. Prakash, C.S. Smith, P. Daszak, H.E. Field and A.A. Cunningham (2007). Plasma biochemistry and hematologicvalues for wild-caught flying foxes (*Pteropusgiganteus*) in India. J. Zoo and Wildlife Medicine, 38(3), 446-452. https://doi.org/10.1638/1042-7260(2007)38[446:PBAHVF]2.0.CO;2.

McMichael, L., D. Edson, A. McLaughlin, D. Mayer, S. Kopp, J. Meers and H. Field (2015). Haematology and plasma biochemistry ofwild black flying-foxes (*Pteropusalecto*) inQueensland, Australia. PLoS ONE, 10(5), 1-13. https://doi.org/10.1371/journal.pone.012574.

Mubarok, H., N.S.N. Handayani, T. Arisuryanti and I. Maryanto (2021). Haematology profile of fruit bats Cynopterus spp. from special region

Yogyakarta, Indonesia. Malaysan Applied Biology. 50(1), 1–9. https://doi.org/10.55230/mabjournal.v50i1.17.

Padalia, M.S., R.S., Trivedi, K. Karmur, Kansagara, and P. Bhanderi (2014). Effect of aging on WBC count and Platelet count. J. Research in Medical and Dental Science. 2 (4), 67–70. https://doi.org/10.5455/jrmds.20142413.

Periayah, M.H., A.S. Halim, and A.Z.M. Saad (2017). Mechanism Action of Platelets and Crucial Blood Coagulation Pathways in Hemostasis. International J. Hematology-Oncology and Stem Cell Research, 11(40), 319-327.

Pocheville, A. (2015). "The Ecological Niche: History and Recent Controversies". In Heams, Thomas; Huneman, Philippe; Lecointre, Guillaume; *et al.* (eds.). Handbook of Evolutionary Thinking in the Sciences. Dordrecht: Springer. 547–586 pp. ISBN 978-94-017-9014-7.

Prakarsa, T.B.P. (2013). Diversitas, karakteristik habitat roosting, dan analisis mangsa alami kelelawar subordo Microchiroptera Penghuni Gua Di Kawasan Karst Tuban dan Karst Menoreh. Unpublished MS Thesis, Fakultas Biologi Universitas Gadjah Mada, Yogyakarta.

Prakarsa, T.B.P., I.D. Kurniawan, and S.T.J. Putro (2021). Biospeleologi Biodiversitas Gua, Potensi, dan Permasalahannya. Bintang Pustaka Madani, Yogyakarta.

Putra, B.F.K., and U.Y. Bintoro (2019). Red cell Distribution Width sebagai Prediktor Penyakit Kardiovaskuler. Analisis CDK. 46 (11), 693–696.

Rahma, A., D. Hanadhita, D.D. Cahyadi, S. Supratikno, H. Maheshwari, A.S. Satyaningtijas, and S. Agungpriyono (2017). Haematological study offruit bat, Cynopterus tithaecheilus, in: 1stInternational Conference in One Health (ICOH2017). Atlantis Press, 164-168 pp. https://doi.org/10.2991/icoh-17.2018.32.

Ramteke, A.V., S.B. Zade, and K.G. Patil (2012). Small Rhinolophid Bat *Hipposideros* Speoris In Agroforestry System. Bionano Frontier, 5 pp.

Rashid, N., M. Irfan, M.S. Nadeem, and A. Shabbir (2016). Comparative seasonal haematology of two bat species, Scotophilus heathii and Pipistrellus pipistrellus, in a subtropical area of Pakistan. Pakistan J. Zoology, 48(5):1503-1510.

Ratnasooriya, W., P. Udagama-Randeniya, W. Yapa, P. Digana, and M. Dharmasiri (2011). Haematological parameters of three species of wild caught microchiropteran bats, Miniopterus schreibersii, Taphozous melanopogon and Hipposideros lankadiva in Sri Lanka. J. Science of the University of Kelaniya Sri Lanka, 2, 27–40. https://doi.org/10.4038/josuk.v2i0.2744.

Saimina, A.J., E. de Queljoe, and H.J. Lengkong (2019). Deskripsi hematologi kelelawar di gunung Tangkoko berdasarkan pemeriksaan darah lengkap. Pharmacon, 8(3), 243-251. https://doi.org/10.35799/pha.8.2019.29388.

Sedgeley, J.A. (2003). Roost site selection and roosting behaviour in lesser short-tailed bats (*Mystacinatuberculata*) in comparison with long-tailed bats (Chalinolobus tuberculatus) in Nothofagus forest, Fiordland. New Zealand J. Zoology, 30(3):227-241. https://doi.org/10.1080/03014223.2003.9518341.

Sikes, R.S. (2016). Animal Care and Use Committee of the American Society of Mammalogists. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J. Mammal., 97, 663–688. [CrossRef] [PubMed]. https://doi.org/10.1093/jmammal/gyw078.

Sikes, R.S. and W.L. Gannon (2011). The Animal Care and Use Committee of the American Society of Mammalogists. Guidelines of theAmerican Society of Mammalogists for the use of wild mammals in research. J. Mammal. 92, 235–253. [CrossRef] https://doi.org/10.1644/10-MAMM-F-355.1.

Smith, C.A., C.M. Andrews, J.K. Collard, D.E. Hall, and A.K. Walker (1994). Color atlas of comparative diagnostic & experimentalhematology. Wolfe Publishing, Barcelona. 9-15 pp. https://doi.org/10.1002/hon.2900120406.

Suyanto, A., (2001). Seri Panduan Lapangan: Kelelawar di Indonesia.Pusat Penelitian dan Pengembangan Biologi-LIPI, Bogor.

Tseliou, E., J.V. Terrovitis, E.E. Kaldara, A.S. Ntalianis, E. Repasos, L. Katsaros, L., *et al.* (2014). Red blood cell distribution width is a significant prognostic marker in advanced heartfailure, independent of hemoglobin levels. Hellenic J. Cardiolology.55, 457-61.

Van der Westhuyzen, J. (1988). Haematology and ironstatus of the Egyptian fruit bat, Rousettus aegyptiacus. Comparative Biochemistry and Physiology Part A: Physiology, 90(1), 117-120. https://doi.org/10.1016/0300-9629(88)91015-8.

Viljoen, M., M. Van der Merwe, G. Bower, P.F. Levay, and A.S. Grober. (1997). Peripheral blood caracteristics of gravid Screibers' long-fingered bats Miniopterusschreibersii natalensis (Microciroptera: Vespertilionidae). South African J. Science, 93, 414-418.

Wolford, S.T., R.A. Schroer, F.X. Gohs, P.P. Gallo, M. Brodeck, H.B. Falk, and R. Ruhren. (1986). Reference range data base for serum chemistryand hematology values in laboratory animals. J. Toxicology and Environmental Health, 18(2), 161-188.

https://doi.org/10.1080/15287398609530859.

Wijayanti, F., D.D. Solihin, H.S. Alikodra and I. Maryanto (2011). Eritrosit dan hemoglobin pada kelelawar gua di kawasan karst Gombong, Kebumen, Jawa Tengah. Jurnal Biologi Indonesia, 7(1), 89-98. https://doi.org/10.47349/07012011/89.

Winkelmann, J.R., F.J. Bonaccorso and T.L. Strickler (2000). Home Range of Southern Blossom Bat, Syconycteris australis in Papua New Guinea. Tropical Biology, 66, 126–132.