



DETERMINATION OF THE EFFECT OF BODY SIZE ON THE HEMATOLOGICAL PARAMETERS IN THE SPECIES OF *Natrix tessellata* (TESSELLATED WATER SNAKE) DISTRIBUTED IN ÇANAKKALE (TURKEY)

| Çiğdem Gül * | Murat Tosunoğlu | Hazal Bilgin | and | Bengi Baycan |

Çanakkale Onsekiz Mart University | Faculty of Arts and Sciences | Department of Biology | Çanakkale | Turkey |

| Received | 11 October 2018 |

| Accepted 21 November 2018 |

| Published 26 November 2018 |

| ID Article | Çiğdem-ManuscriptRef.1-ajira101118 |

ABSTRACT

Background: There are hematological studies on the size and number of blood cells on the *Natrix tessellata* species. However, in the *Natrix tessellata* species, detailed hematological studies, including plasma biochemistry and effect of body size on blood parameters, were not found. **Objectives:** In this study, changes in hematological parameters of *Natrix tessellata* (Tessellated water snake), which is distributed in Çanakkale, were investigated. Total body length of all samples were measured. Hemoglobin and hematocrit values, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, morphology of blood cells, glucose, calcium, cholesterol, triglyceride, creatinine, uric acid, total protein, iron, albumin, phosphorus, magnesium parameters were determined. **Methods:** Field trials were carried out between May and July around Karabiga. Total of 24 samples of *Natrix tessellata* were captured and morphologically taken from their natural environment in order to be able to perform morphological and hematological results. For evaluation of hematological parameters, approximately 1 ml of blood was taken with Caudal Ven 5 ml diameter injector, transferred in biochemistry tubes with lithium heparin, and centrifuged to separate the plasma part. Plasma biochemical parameters were determined by autoanalyzer. Erythrocyte and leukocyte counts using a hemacytometer, and hemoglobin amount Sahli method, the hematocrit value of the plasma by measuring the proportion of blood cells, the mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration calculating the mathematical hematological results. **Results:** The reference ranges of hematologic parameters were determined according to the growth of *Natrix tessellata*, and the effect of body size on hematological parameters was determined. Mean erythrocyte hemoglobin concentration and total protein values were found to be significantly positive correlations, albumin values were found to be significantly negative correlations. It was determined that the other parameters examined did not change depending on body size. **Conclusions:** A study involving the effect of body size on hematological parameters in *Natrix tessellata* has not been found, it is expected that there will be a scientific shortage of differences in some hematological parameters and it is expected to lead the hematologic studies related to body size in other snake species.

Keywords: *Natrix tessellata*, Plasma biochemistry, Hematology, Morphology

1. INTRODUCTION

Determination of blood parameters in reptile species as well as in different species; may be helpful in assessing the physiological, health and environmental conditions of populations [1,2,3,4,5,6].

There are hematological studies on the size and number of blood cells, plasma biochemistry on the *N. tessellata* species [7,8,9,10]. However, in the *N. tessellata* species, detailed hematological studies, including effect of body size on clinical hematology and plasma biochemistry, were not found. There are studies on the effect of body size on blood parameters in different reptile species [11,12,13,14,15,16]. However, such studies have not been found in snake species.

The purpose of this study is; the determination of the some hematological parameters (erythrocyte count, leukocyte count, hemoglobin and hematocrit values, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, glucose, urea, calcium, cholesterol, triglyceride, creatinine, uric acid, total protein, iron, albumin, phosphorus, magnesium) of the *N. tessellata* (Tessellated water snake) and determination of whether all the parameters differ according to the body size.

2. MATERIALS AND METHODS

2.1 Collecting Animal Samples:

The 24 *N. tessellata* samples studied were captured around the Karabiga (35T526225; UTM4469255; sea level) at midday between May and July 2018. Only healthy and mature animals were studied, and they were collected generally around the water resource with the method of hand capture. All the procedures on the samples were made with the permission of Çanakkale Onsekiz Mart University, Animal Experiments Ethics Committee with the decision numbered 2017/04-16.

After the samples were brought to the laboratory and the necessary blood samples were taken, the snakes were left behind in the habitats from which they were collected.

Total body length (TBL) were made with digital caliper. In this study, a total of 24 *N. tessellata* samples were classified as subadult between 35-59 cm and 60-103 cm adult according to total body lengths.

2.2 Hematological Analyses:

For the hematological analyses, 1 mL of blood from the dorsal coccygeal vein of all samples was centrifuged in a Cence L500 centrifuge at 4.000 rpm for 10 min and the plasma part was separated from the blood cells. From the plasma obtained, glucose, urea, calcium, cholesterol, triglyceride, creatinine, uric acid, total protein, iron, albumin, phosphorus and magnesium values were measured by means of an Elimat autoanalyzer.

The red blood cell counts (RBC) and white blood cell counts (WBC) were carried out using a Neubauer hemocytometer, where standard Hayem's solution for red blood cells and Turk's solution for white blood cells were used as a diluting solution. Hematocrit (HCT) was determined using the micro-hematocrit method [17]. The tubes were then spun in a micro-hematocrit centrifuge at 12.000 rpm for 5 min and the hematocrit (HCT) was calculated with a total blood level divided by the blood cell level. Hemoglobin concentration (Hb) was measured by the Sahli method with a Sahli's Hemoglobinometer [17]. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated mathematically by taking the above-mentioned results into consideration [17].

2.3 Statistics:

The descriptive statistics were conducted using SPSS (v10.0). The Pearson correlation test is used to determination of whether all the hematological parameters differ according to the body size.

3. RESULTS

A total of 24 *N. tessellata* samples taken from the vicinity of Karabiga were divided into 2 groups according to total body lengths (35-59 cm subadult; 60-103 cm adult) and descriptive statistics of obtained hematological parameters are given in Table 1.

Table 1: Descriptive statistics of hematologic results of subadult individuals.

Parameters	SUBADULT					ADULT				
	N	Minimum	Maximum	Mean	SD	N	Minimum	Maximum	Mean	SD
TBL (cm)	10	35.00	57.50	50.23	8.565	14	60.00	103.00	69.53	10.498
RBC (mm ³)	9	130000.00	450000.00	287777.77	84079.988	14	160000.00	440000.00	260000.00	88055.926
WBC (mm ³)	10	1000.00	3100.00	2320.00	713.052	14	1200.00	3600.00	1957.14	827.149
Hb (g/dL)	10	3.60	9.20	5.94	2.225	14	4.20	9.20	6.61	1.853
HCT (%)	10	12.00	54.00	31.30	12.649	14	11.00	35.00	24.35	7.771
MCV (μ ³)	9	821.43	1846.15	1206.11	370.660	14	523.81	1650.00	976.67	338.706
MCH (μg)	9	153.33	400.00	225.54	85.190	14	131.82	380.95	264.87	62.831
MCHC (%)	10	14.29	30.00	19.70	4.314	14	16.57	38.18	28.56	6.758
Glucose (mg/dL)	10	14.89	246.10	97.81	76.078	13	18.74	189.00	73.55	49.113
Urea (mg/dL)	6	3.00	9.00	5.70	2.797	14	3.40	20.00	9.44	5.507
Cholesterol (mg/dL)	9	33.03	554.40	229.58	169.803	14	33.03	545.28	271.74	194.345
Creatinine (mg/dL)	10	.01	8.20	1.97	2.422	14	.13	2.40	1.05	.541
Uric acid (mg/dL)	9	7.02	27.00	12.52	6.014	13	5.00	23.00	10.84	4.640
Albumin (g/dL)	10	2.06	5.96	3.16	1.126	14	.01	5.54	2.52	1.393
Calcium (mg/dL)	10	12.96	40.90	20.77	8.368	14	11.39	33.54	22.29	5.131
Triglyceride (mg/dL)	10	10.82	336.60	58.51	98.420	13	13.59	339.40	54.64	87.559
Total protein (g/dL)	10	1.45	3.90	2.34	.829	14	1.17	6.25	3.01	1.692
Phosphorus (mg/dL)	9	.01	4.56	1.86	1.463	13	.10	1.92	.97	.682
Magnesium (mg/dL)	10	.32	10.10	5.75	2.903	14	.98	9.11	4.15	2.129
Iron (mg/dL)	6	7.90	52.20	24.25	19.332	12	9.30	121.70	40.68	34.810

TBL: Total Body Length; **RBC:** Red Blood Cell Counts; **WBC:** White Blood Cell Counts; **Hb:** Hemoglobin concentration; **HCT:** Hematocrit; **MCV:** Mean Corpuscular Volume; **MCH:** Mean Corpuscular Hemoglobin; **MCHC:** Mean Corpuscular Hemoglobin Concentration; **N:** Number of specimens; **SD:** Standard Deviation.

It was determined that there was a significant correlation between MCHC value and body size ($P \leq 0.05$). There was a positive correlation between the statistical results and an increase in MCHC as body size increased ($r = 0.454$; $P = 0.026$). (Table 2; Figure 1).

Table 2: The table presents the pearson correlation test results for blood cell counts.

		Correlations							
		TBL	RBC	WBC	Hb	HCT	MCV	MCH	MCHC
TBL	Pearson Correlation	1	-0.012	0.074	0.378	-0.045	-0.220	0.235	0.454*
	Sig. (2-tailed)		0.957	0.730	0.069	0.834	0.313	0.281	0.026
	N	24	023	24	24	24	23	23	24

*: Correlation is significant at the 0.05 level (2-tailed); **: Correlation is significant at the 0.01 level (2-tailed); **TBL**: Total Body Length; **RBC**: Red Blood Cell Counts; **WBC**: White Blood Cell Counts; **Hb**: Hemoglobin concentration; **HCT**: Hematocrit; **MCV**: Mean Corpuscular Volume; **MCH**: Mean Corpuscular Hemoglobin; **MCHC**: Mean Corpuscular Hemoglobin Concentration

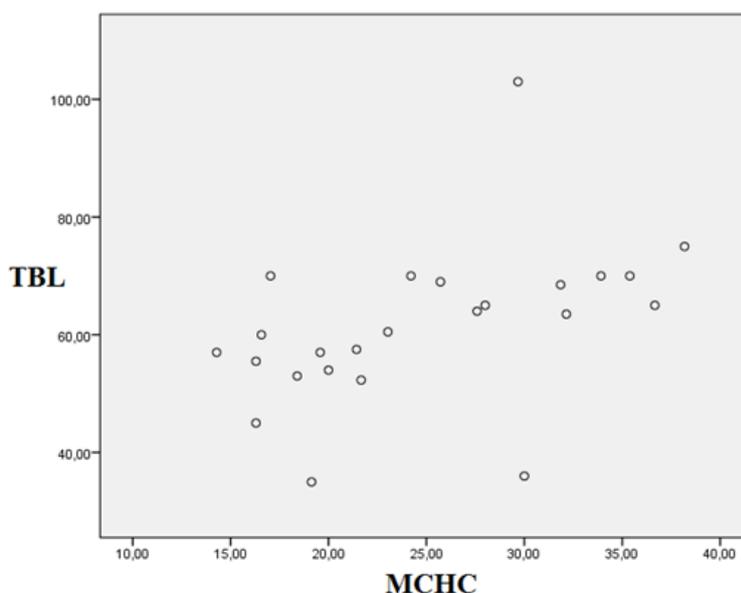


Figure 1: The graph showing the positive correlation between body size and MCHC value. (**TBL**: Total Body Length; **MCHC**: Mean Corpuscular Hemoglobin Concentration).

There was no correlation between body size and the number of erythrocytes, leukocyte count, hemoglobin, hematocrit, mean erythrocyte volume (MCV), mean erythrocyte hemoglobin (MCH) values ($P \geq 0,05$).

According to the results of plasma biochemistry; total protein and albumin values were found to be a significant correlation between body size ($P \leq 0,05$). It has been found that there is a negative correlation between albumin value and body size, albumin value decreases as body size increases ($r = -0,572$; $p = 0,003$) (Table 3; Fig 2). The total protein value was found to be statistically positive according to the results, and as the body size increased, the total protein value also increased ($r = 0,424$; $p = 0,041$) (Table 3; Fig 3).

Table 3 : The table presents the pearson correlation test results in plasma biochemistry data.

		Correlations												
		TBL	Glucose	Urea	Cholesterol	Creatinine	Uric acid	Albumin	Calcium	Triglyceride	Total protein	Phosphorus	Magnesium	Iron
TBL	Pearson Correlation	1	-0.072	0.236	0.212	-0.166	-0.269	-.572**	-0.220	-0.048	0.420*	-0.093	-0.148	-
	Sig. (2-tailed)		0.745	0.345	0.331	0.439	0.227	0.003	0.302	0.829	0.041	.682	.490	0.906
	N	24	23	18	23	24	22	24	24	23	24	22	24	16

** : Correlation is significant at the 0.01 level (2-tailed); * : Correlation is significant at the 0.05 level (2-tailed); **TBL**: Total Body Length.

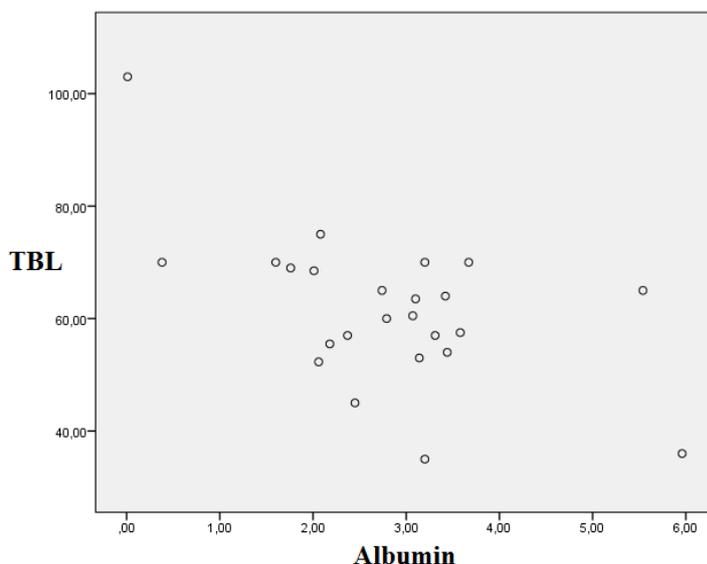


Figure 2: The graph showing negative correlation between body size and albumin value. (TBL: Total Body Length).

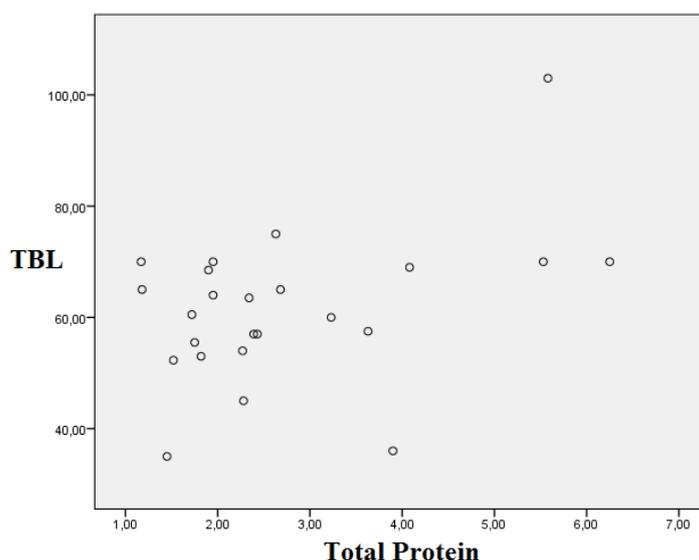


Figure 3. Graph showing the positive correlation between body size and total protein value. (TBL: Total Body Length).

There was no correlation between body size of glucose, calcium, cholesterol, triglyceride, creatinine, uric acid, iron, phosphorus and magnesium parameters ($P \geq 0,05$).

4. DISCUSSION

There are studies on the effect of body size on blood parameters in different reptile species. According to Bolten and Bjorndal (1992) and Fong et al., (2010), total protein, hematocrit and aspartate aminotransferase values were altered depending on body size in the species *Chelonia mydas* [11, 12]. Variation in aspartate aminotransferase, total protein and albumin values of *Varanus varius* species was observed by Scheeling et al., (2011). Hofmeyr et al. (2017) reported that the hemoglobin concentration in *Psammobates geometricus* was similar in female and juvenile individuals [16,13]. However, since there is no study on the effect of body size on hematological parameters in snake species, no comparison can be made with the results obtained.

5. CONCLUSION

A total of 22 hematological parameters were examined in the *N. tessellata* specimen and were determined by correlation tests in which 3 hematological parameters showed significant changes depending on the size.

As a result, it was determined that the body size of *N. tessellata* was affected on some hematological parameters. MCHC and total protein values were found to be significantly positive correlations, albumin values were found to be significantly negative correlations. It was determined that the other parameters examined did not change depending on body size. A study involving the effect of body size on hematological parameters in *N. tessellata* has not been found, it is expected that there will be a scientific shortage of differences in some hematological parameters and it is expected to lead the hematologic studies related to body size in other snake species.

Acknowledgment

This work was supported by Çanakkale Onsekiz Mart University The Scientific Research Coordination Unit, Project number: FBA-2017-1215.

6. REFERENCES

- Dickinson V.M., Jarchow J.L. ve Trueblood M.H. Hematology and Plasma Biochemistry Reference Range Values for Free-ranging Desert Tortoises in Arizona. *Journal of Wildlife Diseases*. 2002, 38: 143-153. Available: <https://www.ncbi.nlm.nih.gov/pubmed/11838205>.
- Jacopson, E. R., Gaskin, J. M., Brown, M. B., Harris, H. K., Gardiner, C. H., Lapointe, J. L., Adams, H. P., Reggiardo, C. Chronic Upper Respiratory Tract Disease of Free-Ranging Desert Tortoises (*Xerobates agasizii*). *Journal of Wildlife Diseases*. 1991, 27: 296-316. Available: <http://www.jwildlifedis.org/doi/10.7589/0090-3558-27.2.296?code=widas-site>.
- Lopez-Olvera, J. R., Montane, J., Marco, I., Martinez-Silvestre, A., Soler, J., Lavin, S. Effect of Venipuncture Site on Hematologic and Serum Biochemical Parameters in Marginated Tortoise (*Testudo marginata*). *Journal of Wildlife Diseases*. 2003, 39 (4): 830-836. Available: https://www.researchgate.net/publication/8912023_Effect_of_venipuncture_site_on_hematologic_and_serum_biochemical_parameters_in_marginated_tortoise_Testudo_marginata
- Pages, T., Peinado, V.I., Viscor, G. Seasonal Changes in Hematology and Blood Chemistry of the Freshwater Turtle *Mauremys caspica leprosa*. *Comparative Biochemistry and Physiology*. 1992, 103A (2): 275-278. Available: <https://www.sciencedirect.com/science/article/pii/030096299290579F>
- Raphael, B.L., Klemens, M.W., Moehlman, P., Dierenfeld, E., Karesh, W.B. Blood Values in Free-Ranging Pancake Tortoises (*Malacochersus tornieri*). *J. Zoo Wildl. Med.* 1994, 25: 63-67. Available: https://www.jstor.org/stable/20095335?seq=1#metadata_info_tab_contents
- Tosunoğlu M., Gül Ç., Yılmaz N. ve Topyıldız H. Hematological Reference Intervals of Some Snake Species in Turkey. *Turkish Journal of Zoology*. 2011, 35(2): 237-243. Available: <http://dergipark.gov.tr/tbtzkzoology/issue/12622/153218>
- Arıkan H. ve Çiçek K. Morphology of Peripheral Blood Cells from Various Species of Turkish Herpetofauna. *Acta Herpetologica*. 2010; 5 (2): 179-198. Available: <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.828.1943&rep=rep1&type=pdf>.
- Arıkan H., Göçmen B., Atatürk M.K., Kumluca Y. ve Çiçek K., 2009. Morphology of Peripheral Blood Cells from Various Turkish Snakes. *North-Western Journal of Zoology*. 2009, 5 (1): 61-73. Available: <http://www.biozoojournals.ro/nwjz/content/v5.1/nwjz.051108.Arıkan.pdf>.
- Çiğdem Gül, Nurihan Hacıoğlu Dođru and Murat Tosunođlu. Impact of some hematological and microbiological factors on two natrx species in the biga str eam (ÇANAKKALE, TURKEY). *American Journal of Innovative Research and Applied Sciences*. 2018; 6(4): 203-209. Available: <https://www.american-jiras.com/%C3%87%C4%9Fdem-ManuscriptRef.1-ajira160418.pdf>
- Tok C.V., Tosunođlu M., Gül Ç., Yiđini B., Türkakin M., Saruhan G. ve Kaya S. Erythrocyte Count and Size in Some Colubrids (Reptilia: Ophidia) from Turkey. *Russian Journal of Herpetology*. 2006,13 (2): 97-100. Available: <http://rjh.folium.ru/index.php/rjh/article/view/801>
- Bolten, A. B., Bjorndal, K. A. Blood Profiles for a Wild Population of Green Turtles (*Chelonia Mydas*) in the Sothern Bahamas: Size-Specific and Sex-Specific Relationships. *Wildl. Dis.* 1992, 28: 407-413. Available: http://www.widcast.org/What/Country/Bahamas/Docs/Bolten_and_Bjorndal_1992_Green_blood_profiles.pdf.
- Fong, C., Chen, H., Cheng, I., 2010. Blood profiles from wild populations of green sea turtles in Taiwan. *Journal of Veterinary Medicine and Animal Health*. 2010, Vol. 2 (2) pp. 008-010. Available: https://academicjournals.org/article/article1379426488_Fong%20et%20al.pdf.
- Hofmeyr, M.D., Henen, B.T., Walton, S. Season, sex and age variation in the haematology and body condition of geometric tortoises *Psammobates geometricus*. *African Zoology*. 2017, 52(1): 21-30. Available: <http://www.bioone.org/doi/abs/10.1080/15627020.2017.1284575>.
- Lopez, J., Ldo. Vet., M. Sc., Waters, M., B. V. Sc., M. Sc., Routh, A., B. V. Sc., Cert. Zoo. Med., Rakotonahary, T.F., D.V.M., Woolaver, L., Ph. D., Thomasson. A., Holmes, E., B. Vet. Med., M. Vet. Med., Hanspeter W. Steinmetz, Dr. med. vet., Dipl. A.C.Z.M., 2017. Hematology and Plasma Chemistry of the Ploughshare Tortoise (*Astrochelys yniphora*) in a Captive Breeding Program. *Journal of Zoo and Wildlife Medicine*. 2017, 48(1): 102-115. Available: <http://researchonline.rvc.ac.uk/10799/>
- Omonona, A.O., Olukole, S.G., Fushie, F.A. Haematology and Serum Biochemical Parameters in Free-Ranging Africa Side Neck Turtle (*Pelusios sinuatus*) in Ibadan, Nigeria. *Acta Herpetologica*. 2011, 6(2): 267-274. Available: <http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.905.7143&rep=rep1&type=pdf>
- Scheeling, T.F., Jessop, T.S. Influence of capture method, habitat quality and individual traits on blood parameters of free-ranging lace monitors (*Varanus varius*). *Aust vet j.* 2011, 89(9): 360-365. Available: <https://www.ncbi.nlm.nih.gov/pubmed/21864309>
- Tanyer G. *Hematoloji ve Laboratuvar*. 1985, Ayyıldız Matbaa A.Ş. 111-122.



Cite this article: Çiğdem Gül, Murat Tosunođlu, Hazal Bilgin and Bengi Baycan. DETERMINATION OF THE EFFECT OF BODY SIZE ON THE HEMATOLOGICAL PARAMETERS IN THE SPECIES OF *Natrix tessellata* (TESSELLATED WATER SNAKE) DISTRIBUTED IN ÇANAKKALE (TURKEY). *Am.J. innov. res. appl. sci.* 2018; 7(5): 241-245.

This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>