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Study on erythrocyte osmotic fragility in apparently healthy cholistani camels reared under intensive farming system

Estudio sobre fragilidad osmótica de eritrocitos en camellos Cholistani aparentemente sanos criados en sistemas de cría intensiva

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ABSTRACT

The present study is the first of its kind conducted with an aim to assess the level of Erythrocyte Osmotic Fragility as affected by various factors (varying levels of temperature and pH, age and gender) in apparently healthy Cholistani breed of one-humped camels (n=27) being reared under intensive farming system. Blood was aseptically collected and assessed for %age hemolysis (H%) as per prescribed protocols. Results revealed that the H% decreased with increasing pH and with increasing temperatures, and values within reference range were noticed at the pH of 7.4 and 8.5, and at the temperatures of 37°C and 50°C ranging from 0.4±0.1% to 0.9±0.1%, at NaCl concentration of 0.85g/L. Regarding age, adult camels had significantly (P≤0.05) higher H% (1.0±0.2%) as compared to that in young camels (0.68±0.01%) at the NaCl concentration of 0.85g/L. Gender, however, had a non-significant (P≥0.05) effect on H% for male and female camels. This preliminary study furnishes an insight into physiological adaptations of one-humped camel/camel blood in differing environment and will enable to attain maximum productivity in times of stress through appropriate management and feeding practices. We recommend using EOF as a routine hematological attribute and also recommend further studies on H% in pathological conditions of camels.
Keywords: erythrocytic fragility, one-humped camels, hemolysis, hematology, gender.

RESUMEN

El presente estudio es el primero de su tipo, realizado con el objetivo de evaluar el nivel de fragilidad osmótica de los eritrocitos (FOE) afectada por varios factores (niveles variables de temperatura y pH, edad y género) en camellos de una joroba de la raza Cholistani aparentemente sananos (n =27) criados bajo un

sistema de producción intensivo. La sangre se obtuvo asépticamente y se evaluó para determinar el porcentaje de hemólisis (%H) según los protocolos prescritos. Los resultados revelaron que él %H disminuyó con el aumento del pH y con el aumento de la temperatura, y se notaron valores dentro del rango de referencia al pH de 7.4 y 8.5, y a las temperaturas de 37°C y 50°C que oscilaron entre 0.4 ± 0.1% y 0.9 ±0.1%, a una concentración de NaCl de 0.85g/L. Con respecto a la edad, los camellos adultos tuvieron un %H más alto (1.0±0.2%) (P≤0.05) en comparación con los camellos jóvenes (0.68±0.01%) a la concentración de NaCl de 0.85g/L. Sin embargo, el género no tuvo un efecto significativo (P≥0.05) en él %H para camellos machos y hembras. Este estudio preliminar proporciona una idea de las adaptaciones fisiológicas del camello de una joroba en diferentes entornos y permite alcanzar la máxima productividad en momentos de estrés a través de prácticas adecuadas de manejo y alimentación. Recomendamos usar FOE como un atributo hematológico de rutina y también recomendamos más estudios sobre %H en condiciones patológicas de camellos.
Palabras clave: fragilidad eritrocítica, camellos de una joroba, hemólisis, hematología, género.

INTRODUCTION

Since last decade or so, hematology and its associated aspects such as hemato-oncology and hematochemistry have gained a strong footing in veterinary clinical practice as reliable diagnostic and management tools throughout the world. The physiological, pathological, nutritional and metabolic status of an animal is well verified through these tools. Furthermore, hematology also provides the difference between normal state of an animal from stressed state arising due to stressors which may be physical, psychological, nutritional, or environmental (Farooq *et al*., 2011). Interpretation of hematological results is quite complicated in animals (especially camels) owing to extreme physiological variations with gender, age, breed, reproductive stats and various physiological conditions. Amongst hematological attributes, the erythrocytic variables *viz.* hemoglobin concentration (Hb), packed cell volume, total erythrocyte count, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration) are considered more potent in detection of various pathological conditions of an animal.

The osmotic fragility of the membrane of erythrocyte (EOF) is, in fact, a measure of degree of resistance of erythrocytic membranes to osmotic swelling/lysis in salt solutions and is a reliable hematological test which provides level of integrity and elasticity of erythrocytic membrane. It provides information of pathophysiological and pharmaco-toxicological insight of erythrocyte membrane (Igbokwe, 2018; Igbokwe *et al*., 2016). Various intrinsic (species, breed, genetics, age, sexual dimorphism, pregnancy and lactation, and membrane composition) and extrinsic factors (pH, temperature, season, type of media, method of sampling, handling and preservation) affecting EOF have been reviewed extensively (Igbokwe, 2018; Oywale *et al*., 2011).

The Cholistan desert of Pakistan, its indigenous livestock, and its prevailing livestock production systems have been reviewed earlier (Ali *et al*., 2009; Farooq *et al*., 2010). Briefly, the barren but fascinating desert is an extension of Great Indian Desert sprawling at an area of 26000 km^2. Transhumaie and nomadic pastoralism are the two main livestock production/rearing systems. The total livestock population of the desert as per Livestock Census, 2018 has been reported as 12,32,546 heads, out of which the camel population is 16037 heads. The two main breeds of camels are reared here on the basis of their utility, locality, bodily conformity and size *i.e*. Marrecha or Mahra (riding and dancing camel) and Barela (milch breed) (Farooq *et al*., 2011; Ali *et al*., 2009).

Literature review reveals that a hefty research work has been conducted on various aspects and associations of EOF in goats (Igbokwe *et al*., 2016; Bello *et al*.,2020), horses (Yaqub *et al*., 2014), chicken (Ogbuagu *et al*., 2018; Yagil *et al*., 1976), cattle (Ayres *et al*, 2014; Pati *et al*., 2017), rats (Mijares *et al*., 2010) and camels (Abdalmula *et al*., 2019; Ghoke *et al*., 2013; Lektib *et al*., 2016) from various parts of the world. There still is a paucity of similar research for one-humped camels being reared in Pakistan. The present study is hence the first of its kind which was conducted with an aim to assess the level of EOF as affected by various factors (varying levels of temperature and pH, age and gender) in apparently healthy Cholistani breed of one-humped camels (Marrecha) being reared under intensive farming system. This preliminary study will furnish an insight into physiological adaptations of one-humped camel/camel blood in differing environment and will enable to attain maximum productivity in times of stress through appropriate management and feeding practices.

MATERIALS AND METHODS

Study Site and Animals. The study was carried out in the Livestock Farm, of the university, Pakistan in May and June, 2022. The Cholistani one-humped camels (n=27) of Marrecha breed being reared under intensive farming system of the Livestock Farm of the university were incorporated in this study. All the animals were provided shaded and open area within a fenced compound. Stall feeding was being carried out for these animals with no grazing. They were well fed, clinically healthy and free from internal/external parasites. Routine treatment for parasitic infestation was carried out twice in a year and their health was regularly monitored by qualified veterinarians. Animals had free access to salt lick, 2 times water in winter and 3 times in summer.

The camels consisted of 13 males and 14 females, and 11 young (≤1 year) and 16 adults (≥1 year and up till 9 years) as per dentition and farm records. Pregnancy and lactational

state of the adult females was confirmed through the farm records allied with physical examination.

Blood Collection. Blood (5mL) was aseptically collected through jugular vein of the animals under appropriate restraining and was transferred into blood collection vacutainers (Becton Dickinson and Company®, NJ, USA) containing 3.2% buffered sodium citrate solution as coagulant. Same technique of restraining, same time of blood collection and same personnel were used in order to minimize stress to the animals. Collected blood samples were transported in ice box to the Physiology Laboratory of the university and were analyzed for EOF within 1 hour.

Osmotic Fragility. For each of the study samples, a 200 mL of NaCl solution was prepared in a concentration of 0.1 to 0.85%, and a pH of 7.4 as prescribed by Faulkner and King (Faulkner & King 1970). From these solutions, set of ten test tubes were prepared in such a manner that each test tube contained 5mL with a concentration from 0.1 to 0.85%. They were arranged serially in test tube racks. For each study sample, a separate set of these test tubes was utilized. Regarding the protocol of the test, a drop of blood was put in each test tube, mixed through gentle mixing, and were allowed to rest for 30 mins at pH of 7.4, under room temperature. Later, centrifugation of the tubes was carried out at 1500G for 20 mins. Supernatant was extracted and Hb was determined spectrophotometrically at 540nm. Following formula was used for determining level of hemolysis in percentage (Faulkner & King 1970):

$$\%\text{age Hemolysis:} = \frac{\text{Optical density of solute}}{\text{Optical density of distilled water}} \text{ x } 100$$

Statistical Analysis. Statistical Package for Social Sciences (SPSS for Windows Version 12, SPSS Inc., Chicago, IL, USA) was utilized for the purpose of data analyses. Data was presented as mean (±SE) values for EOF. Effect of different independent factors such as pH, temperature, age and gender was calculated through GLM using multivariate analysis. The LSD was implied as a post-hoc test where applicable. Significance was kept at P≤0.05.

RESULTS

The overall mean (±SE) values for H% as affected by varying levels of pH (6.5, 7.4, and 8.5) and temperatures (4°C, 37°C, and 50°C) are given in Table 1 and 2, respectively. Similar trends were revealed regarding H% both for pH and temperature. The H% decreased with increasing pH and with increasing temperatures, and values within reference range were noticed at the pH of 7.4 and 8.5, and at the temperatures of 37°C and 50°C ranging from 0.4±0.1% to 0.9±0.1%, at NaCl concentration of 0.85g/L.

The results regarding effect of age and gender are presented in Figure 1 and 2, respectively. Regarding age, adult camels had significantly (P≤0.05) higher H% (1.0±0.2%) as compared to that in young camels (0.68±0.01%) at the NaCl concentration of 0.85g/L. Gender, however, had a non-significant (P≥0.05) effect on H% for male and female camels.

Table 1. Overall mean (±SE) values for percentage hemolysis in Cholistani camels (n=27) as affected by varying levels of pH

Concentrations (NaCl g/L)	pH			Overall
	6.5	7.4	8.5	
0.05	96.5±0.3^{a}	94.3±0.4^{b}	92.2±0.5^{c}	94.3±0.3
0.1	91.9±0.5^{a}	89.5±1.0^{a}	78.6±0.3^{b}	86.7±0.9
0.2	81.4±0.4^{a}	77.6±0.5^{b}	72.8±0.4^{c}	77.2±0.6
0.3	48.2±0.7^{a}	28.2±0.4^{b}	17.1±0.3^{c}	31.2±2.0
0.4	20.5±0.7^{a}	15.9±0.5^{b}	13.0±0.2^{c}	16.5±0.5
0.5	15.1±0.6^{a}	12.5±0.5^{b}	9.7±0.3^{c}	12.4±0.4
0.6	7.2±0.2^{a}	5.8±0.2^{b}	4.0±0.1^{c}	5.7±0.2
0.7	4.4±0.2^{a}	3.1±0.2^{b}	2.3±0.1^{c}	3.3±0.1
0.8	2.7±0.1^{a}	2.1±0.2^{a}	1.4±0.1^{b}	2.1±0.1
0.85	1.2±0.1^{a}	0.9±0.1^{a}	0.4±0.1^{b}	0.8±0.1

Different superscripts within rows differ at P≤0.05.

Table 2. Overall mean (±SE) values for percentage hemolysis in Cholistani camels (n=27) as affected by varying levels of temperature

Concentrations (NaCl g/L)	Temperature			Overall
	4°C	37°C	50°C	
0.05	96.3±0.5^{a}	95.1±0.3^{b}	97.2±0.3^{a}	96.2±0.2
0.1	96.3±0.3^{a}	92.6±0.4^{b}	9.6±0.7^{b}	94.2±0.4
0.2	83.6±0.4^{a}	52.3±0.5^{b}	84.9±0.4^{a}	73.6±2.3
0.3	54.2±0.4^{a}	28.6±0.3^{b}	56.2±0.6^{c}	46.3±1.8
0.4	42.6±0.4^{a}	20.9±0.2^{b}	43.7±0.5^{a}	35.8±1.6
0.5	31.8±0.4^{a}	11.0±0.1^{b}	31.5±0.2^{a}	24.7±1.5
0.6	21.3±0.3^{a}	7.5±0.2^{b}	26.0±0.3^{c}	18.3±1.2
0.7	16.3±0.3^{a}	6.2±0.1^{b}	17.9±0.3^{c}	13.5±0.8
0.8	7.8±0.2^{a}	2.4±0.1^{b}	8.2±0.2^{a}	6.2±0.4
0.85	0.4±0.02^{a}	0.3±0.02^{b}	0.4±0.04^{a}	0.4±0.02

Different superscripts within rows differ at P≤0.05.

DISCUSSION

It has been well established that various environmental and physiological factors play a vital role in creating variability in values of EOF. In general, all the tests which involve the study of osmotic fragility in membranes are sensitive and tedious methods which present an estimate of morphological abnormalities and the level of relationship between red cell volume and cell surface area. Resultantly, the use of such tests has brought the researchers with change in isotonic shape of red cells and their flow properties which are in fact, dependent upon shape (Bowdler *et al*., 1981; Schroter *et al*., 1990). Considering the correlation of cell surface area and osmotic fragility of RBCs, an increased surface area leads to higher cell density causing an increase in EOF as well (Ballas *et al*., 1984).

The present study is apparently first of its kind being reported with an aim of presenting effect of different temperatures (4°C, 37°C and 50°C), pH (6.5, 7.4 and 8.5), age and gender on erythrocytic membrane of Cholistani camels being reared under intensive farming system through assessment of H%. In the present study, our results show that H% for one-humped camels is higher at very high temperature (50°C) and at very low temperature (4°C) when it was compared at 37°C temperature. The results correlate with earlier studies (Igbokwe, 2018).

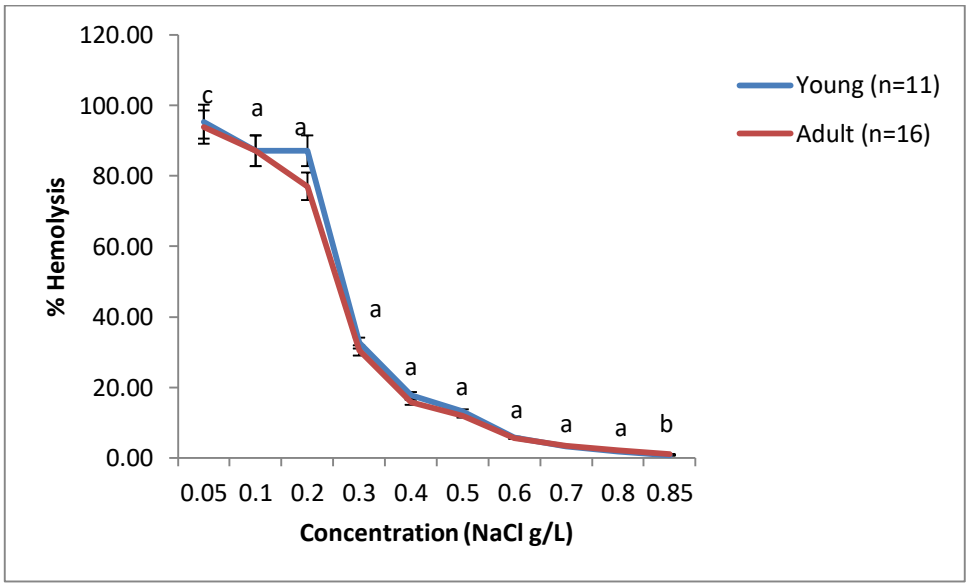

Figure 1. The % hemolysis in Cholistani camel (n=27) erythrocytes as affected by age. Different letters (a,b,c) indicate significant difference at P≤0.05

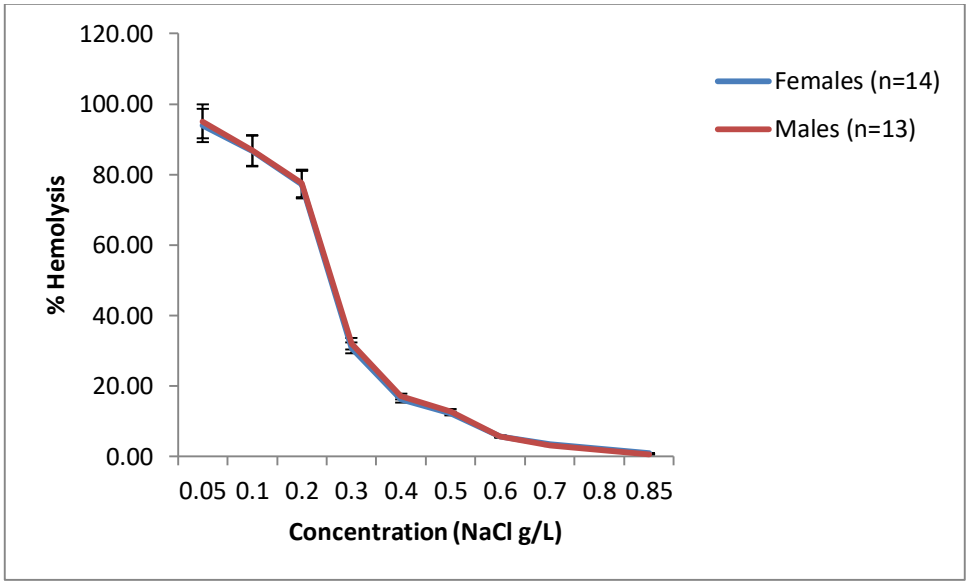

Figure 2. The % hemolysis in Cholistani camel (n=27) erythrocytes as affected by gender

Our results correlate with those by Lektib *et al*. (Lektib *et al*., 2016) in which H% in camels was very high at 50 °C and at very low temperature of 0°C. According to another study (Yagil *et al*., 1974) the resistance of erythrocytic membrane to hypotonic saline solution in a dehydrated camel is higher as compared to hydrated animals. Considering, similar studies conducted on human RBCs, similar has been concluded that high temperature affects deformability of erythrocytic membranes causing damage at 50°C (Poder *et al*., 2015). According to a previous study conducted on assessing EOF as a marker of pesticide-elicited membrane damage (Sharma *et al*., 2010) it was concluded that the vital processes of an organismal body such as growth, metabolism, reproduction, production etc are altogether altered during thermal stress owing to redistribution of body nutrients such as proteins and energy. Resultant lipid peroxidation causes enhanced fragility of erythrocytic membranes.

The results of the present study regarding the effect of pH indicated that at pH of 8.5 the hemolysis decreased whereas it tended to increase at the pH of 7.4 and 6.5. These results correlate with earlier study by Igbokwe (Igbokwe, 2018). Similar results have been reported earlier for other breeds of camels (Lektib *et al*., 2016). The RBCs are the structural and functional units of a mammalian blood which are responsible for transporting oxygen and CO_2, removing wastes, and maintaining pH through binding acids/alkalis. Enhanced metabolism in the body results in production of endogenous toxic metabolites which cause acidification of blood. This acidosis results in metalloproteins of the body removing their metals and initiation of Fenton Haber-Weiss reactions.

Resultantly, reactive oxygen species are produced which alter the fragility of RBCs inducing elevated hemolysis (Lektib *et al*., 2016; Bowdler *et al*., 1981; Schroter *et al*., 1990).

Regarding the effect of age in our study, a decreased osmotic resistance of RBCs in young camels was noticed in comparison to those for old ones. This is in contrast of certain earlier studies in which EOF in adult was greater in older than in young ones. This may be due to breed variations or seasonal variations. However, our results correlate with those of many other earlier studies (Lektib *et al*., 2016; Perk, 1966) in which EOF in young camels was reported to be higher than in older ones. According to another work on human RBCs (Walls *et al*., 1976) young RBCs were not as susceptible to thyroxine-peroxide-induced hemolysis as compared to older RBCs. Aging RBCs has a decreased activity in their G-6-PD which causes enhanced fragility of erythrocytic membranes (Seeman *et al*., 1969). A decreased glutathione concentration has plausibly been put forth for this reduction. In older RBCs, such as in the instances of blood disorders or due to physiological ageing, amorphous Hb and production of methemoglobin causes increased EOF (Keitt *et al*., 1966). Another study conducted on rat RBCs has also reported that an increase in membrane-associated dolichol causes fragility of erythrocytic membranes in older rats, which ultimately results in a 50% reduction in life of RBCs in older rats (Dini *et al*., 2001).

Regarding gender-wise study, our results show a decreased H% in female camels than in males. Our findings correlate with earlier studies (Lektib *et al*., 2016; Amin *et al*., 2012) in which EOF in males was higher in camels than in females. Similar gender-based results have earlier been reported for domestic fowl, WAD sheep, (Ferreira *et al.*, 2007) and camels (Abdamula et al., 2019) and it has been concluded that male RBCs have higher EOF and a higher susceptibility to H%. In contrast to these results of ours, other finding has reported a lower EOF in males as compared to that in females. Such findings have been reported in African giant rats (Oyewale *et al*., 1998), dogs (Yaqub *et al*., 2014) turkeys (An & Mohandas 2011) and goats (Habibu *et al.,* 2013). On the other hand, in camel, the osmotic resistance increased significantly during the green season and decreased in winter (Amin *et al*., 2007). The exceptional structural configuration of the lipoprotein and phospholipids in the camel erythrocytes has yet to be further defined and characterized. It may account for the specific particular osmotic stability of the camel erythrocytes.

CONCLUSION AND RECOMMENDATIONS

In a nutshell, the results of the present study reveal that the Cholistani camel blood show physiological levels of hemolysis at the pH of 7.4 and 8.5, and at the temperatures of 37°C

and 50°C, at NaCl concentration of 0.85g/L. Furthermore, the adult camels had higher hemolysis as compared to young ones in the present study. We recommend that EOF, though laborious, yet it may be incorporated in routine hematological tests. We also recommend level of hemolysis in camel population under various pathological conditions.

Data Availability

The data supporting these findings are available upon request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

REFERENCES

ABDALMULA A, Benashour F, Shmela M, Alnagar F, Abograra I, Buker A. 2019. Blood profile in normal one humped dromedary (*Camelus dromedarius*) camels in Libya. Part 3: Effect of sex variation on biochemical and haematological blood profile. *International Journal of Science and Basic Applied Research.* 48:9-24. ISSN: 2395-3446. https://www.gssrr.org/index.php/JournalOfBasicAndApplied/article/view/10172/4425

ALI I, Chaudhry MS, Farooq U. 2009. Camel rearing in Cholistan desert of Pakistan. *Pakistan Veterinary Journal.* 29: 85-92. ISSN:0253-8318. http://www.pvj.com.pk/pdf-files/29_2/85-92.pdf

AMIN ASA, Abdoun KA, Abdelatif A. 2012. Erythrocyte osmotic fragility curve of male and female camels (*Camelus dromedarius*). *In "Proceedings of the 3rd Conference of the International Society of Camelid Research and Development"*. Pp. 288-289. https://www.academia.edu/80370070/Effects_of_Vitamin_E_and_Vitamin_C_on_Hydrogen_Peroxide_Induced_Hemolysis_in_Moroccan_Dromedary_Camels_Camelus_Dromedarius_

AMIN A, Abdoun KA, Abdelatif AM. 2007. Seasonal variation in blood constituents of one-humped camel (*Camelus dromedarius*). *Pakistan Journal of Biological Sciences* 10: 1250-1256. https://scialert.net/qredirect.php?doi=pjbs.2007.1250.1256&linkid=pdf

AN X, Mohandas N. 2011. Erythroblastic islands, terminal erythroid differentiation and reticulocyte maturation. *International Journal of Hematology* 93: 139-143. ISSN :2578-501X. https://link.springer.com/content/pdf/10.1007/s12185-011-0779-x.pdf?pdf=button

AYRES MCC, Birgel EH, Birgel Jr E. 2014. Influence of age, sex and breed lines of Zebu nellore cattle on the erythrocytes osmotic fragility, raised in São Paulo, *Brazil. International Journal of Applied Science and Technology* 4: 70-75. ISSN 2231-3842. http://www.ijastnet.com/journals/Vol_4_No_5_October_2014/8.pdf

BALLAS SK, Clark MR, Mohandas N, Colfer H, Caswell M, Bergren M, Perkins H, Shohet S. 1984. Red cell membrane and cation deficiency in Rh null syndrome. *Blood* 63:1046-1055. ISSN 0006-4971. https://doi.org/10.1182/blood.V63.5.1046.1046

BELLO TK, Ayo JO, Oyelowo BB. 2020. Erythrocyte osmotic fragility of pubertal red sokoto goats administered ascorbic acid during the early rainy season. *Alexandria Journal for Veterinary Sciences* 66: 10-14. ISSN 2536-9520. https://www.ejmanager.com/mnstemps/31/31-1584908694.pdf?t=1693651439

BOWDLER A, Dougherty R, Bowdler NC. 1981. Age as a factor affecting erythrocyte osmotic fragility in males. *Gerontology* 27: 224-231. ISSN: 1423-0003. https://sci-hub.se/https://doi.org/10.1159/000212477

DINI B, Dolfi C, Santucci V, Cavallini G, Donati A, Gori Z, Maccheroni M, Bergamini E. 2001. Effects of ageing and increased haemolysis on the levels of dolichol in rat spleen. *Experimental Gerontology* 37: 99-105. ISSN 0531-5565. https://sci-hub.se/https://doi.org/10.1016/S0531-5565(01)00156-5

FAROOQ U, Samad H, Khurshid A, Sajjad S. 2011. Normal reference hematological values of one humped camels (Camelus dromedarius) kept in Cholistan desert. *Journal of Animal and Plant Science.* 21: 157-160. ISSN1018-7081. http://www.thejaps.org.pk/docs/21-2/11-117.pdf

FAROOQ U, Samad H, Sher F, Asim M, Khan MA. 2010. Cholistan and Cholistani breed of cattle. *Pakistan Veterinary Journal.* 30: 2074-7764. ISSN 0253-8318. http://www.pvj.com.pk/pdf-files/30_2/126-130%20_9117_.pdf

FAULKNER WR, King JW. 1970. Manual of clinical laboratory procedures. *Chemical Leather Company, UK*. https://cir.nii.ac.jp/crid/1130282269188352000

FERREIRA RF, de Mello Figueiredo Cerqueira A, Pereira AM, Guimarães CM, de Sa AG, da Silva Abreu F, Massard CL, Almosny NP. 2007. Anaplasma platys diagnosis in dogs: comparison between morphological and molecular tests. *International Journal of Applied Research in Veterinary Medicine.* 5:113. ISSN 1542-2666. https://bjvm.org.br/BJVM/article/view/478/361

GHOKE S, Jadhav K, Thorat K. 2013. Assessing the osmotic fragility of erythrocytes of rural and semiurban camels (*Camelus dromedarius*). *Camel: An international Journal of Veterinary Sciences.* 1: 75-78. ISSN: 2319-9660. https://www.indianjournals.com/ijor.aspx?target=ijor:cijvs&volume=1&issue=1&article=007

HABIBU B, Yaqub L, Ahmed I, Kawu M, Buhari H, Tauheed M, Isa H. 2013. Erythrocyte osmotic fragility and haematologic parameters of three breeds of 9-week-old broiler chickens. *International Journal of Poultry Science.* 12: 277-279. ISSN:1682-8356. https://scialert.net/fulltext/?doi=ijps.2013.277.279

IGBOKWE N. 2018. A review of the factors that influence erythrocyte osmotic fragility. *Sokoto Journal of Veterinary Sciences.* 16: 1-23. ISSN: 2315-6201. https://www.ajol.info/index.php/sokjvs/article/view/183022

IGBOKWE N, Ojo N, Igbokwe I. 2016. Effects of sex and age on the osmotic stability of Sahel goat erythrocytes. *Comparative Clinical Pathology.* 25:15-22. ISSN 1618-5641. https://link.springer.com/content/pdf/10.1007/s00580-015-2130-z.pdf

KEITT AS, Smith TW, Jandl JH. 1966. Red-cell pseudomosaicism in congenital methemoglobinemia. *New England Journal of Medicine*. 275(8): 397-405. ISSN: 0028-4793. https://www.nejm.org/doi/full/10.1056/NEJM196608252750801

LEKTIB I, Bargaâ R, Chakir Y, Belhouari A, Hammoumi A, El Khasmi M. 2016. Study of incubation conditions for erythrocytes osmotic fragility testing in dromedary camel (*Camelus dromedarius*). *International Journal of Research in Environmental Science*. 2: 22-32. ISSN 2454-9444. https://www.arcjournals.org/international-journal-of-research-in-environmental-science/volume-2-issue-2/4

LIVESTOCK & Dairy Development Department. 2018. First Real Time (Door to Door) Livestock Census. (P. Livestock & Dairy Development Department, ed.). Pp. 30. *L&DD, Punjab, Lahore, Pakistan.* https://livestock.punjab.gov.pk/system/files/ADP2019-20_0.pdf

MIJARES A, Vivas J, Abad C, Betancourt M, Piñero S, Proverbio F, Marín R, Portillo R. 2010. *Trypanosoma evansi*: Effect of experimental infection on the osmotic fragility, lipid peroxidation and calcium-ATPase activity of rat red blood cells. *Experimental Parasitology.* 124: 301-305. ISSN: 0014-4894. https://sci-hub.se/https://doi.org/10.1016/j.exppara.2009.11.002

OGBUAGU NE, Aluwong T, Ayo JO, Sumanu VO. 2018. Effect of fisetin and probiotic supplementation on erythrocyte osmotic fragility, malondialdehyde concentration and superoxide dismutase activity in broiler chickens exposed to heat stress. *Journal of Veterinary Medical Science.* 1: 477-481. ISSN: 0916-7250. https://www.jstage.jst.go.jp/article/jvms/80/12/80_18-0477/_pdf/-char/ja

OYEWALE JO, Olayerni F, Rahman SA. 1998. Blood characteristics of the Nigerian local duck (Anas platyrhynchos) I. Red blood cell characteristics. *Veterinarski Arhiv.* 68: 199-204. ISSN 0372-5480. http://www-staro.vef.unizg.hr/vetarhiv/papers/68-6/oyewale.htm

OYWALE J, Dzenda T, Yaqub L, Akanbi D, Ayo J, Oyewele O, Dare T. 2011. Alterations in the osmotic fragility of camel and donkey erythrocytes caused by temperature, pH and blood storage. *Veterinarski Arhiv.* 81(4):459-470. ISSN 0372-5480. https://hrcak.srce.hr/file/105582

PATI S, Panda SK, Behera P, Panda M. 2017. Assessment of erythrocyte osmotic fragility in cattle due to haemoprotozoan diseases. *International Journal of Science, Environment and Technology.* 6: 1560-1568. ISSN: 1735-1472. https://www.ijset.net/journal/1719.pdf

PERK K. 1966. Osmotic hemolysis of the camel's erythrocytes. I.A microcinematographic study. *Journal of Experimental Zoology.* 163(3):241-246. ISSN 2471-5646. https://onlinelibrary.wiley.com/doi/abs/10.1002/jez.1401630303?casa_token=fM8TsxFRXt4AAAAA:c9JMKcVYrXh9gBUICNF9u3C_K4hN-EBmx0AIvfC7TjWybDddPdwu4iAV5qn9pHAmZnX6yM1sxSZWzpfCoA

PODER TG, Nonkani WG, Leponkouo ÉT. 2015. Blood warming and hemolysis: a systematic review with meta-analysis. *Transfusion Medicine Reviews.* 29: 172-180. https://sci-hub.se/https://doi.org/10.1016/j.tmrv.2015.03.002

SCHROTER RC, Filali RZ, Brain APR, Jaffrey PK, Robertshaw D. 1990. Influence of dehydration and watering on camel red blood cell size: A scanningelectron microscopic study. *Respiration Physiology*. 81(3):381-390. ISSN 1569-9048. https://sci-hub.se/https://doi.org/10.1016/0034-5687(90)90118-I

SEEMAN P, Kwant W, Sauks T, Argent W. 1969. Membrane expansion of intact erythrocytes by anesthetics. *Biochimica et Biophysica Acta (BBA)-Biomembranes.* 183: 490-498. ISSN:00052736, 18792642. https://sci-hub.se/https://doi.org/10.1016/0005-2736(69)90163-1

SHARMA B, Rai DK, Rai PK, Rizvi S, Watal G. 2010. Determination of erythrocyte fragility as a marker of pesticide-induced membrane oxidative damage. *In* "Advanced Protocols in Oxidative Stress II", pp. 123-128. *Springer*. https://link.springer.com/protocol/10.1007/978-1-60761-411-1_8

WALLS R, Kumar KS, Hochstein P. 1976. Aging of human erythrocytes: Differential sensitivity of young and old erythrocytes to hemolysis induced by peroxide in the presence of thyroxine. *Archives of Biochemistry and Biophysics.* 174: 463-468. ISSN: 0003-9861. https://sci-hub.se/https://doi.org/10.1016/0003-9861(76)90374-X

YAGIL R, Luchtenstein C. Meyerstein N. 1976. Hemoconcentration and erythrocyte fragility in chickens exposed to heat and dehydration. *American Journal of Veterinary Research.* 37:103-6. ISSN: 0002-9645. https://europepmc.org/article/med/1247191

YAGIL R, Sod-Moriah UA, Meyerstien N. 1974. Dehydration and camel blood.3. Osmotic fragility, specific gravity and osmolality. *American Journal of Physiology-Legacy Content*, 226(2):305-308. ISSN 0002-9513. https://sci-hub.se/https://doi.org/10.1152/ajplegacy.1974.226.2.305

YAQUB LS, Mshelia WP, Ayo JO. 2014. Erythrocyte osmotic fragility and hematological responses of horses administered ascorbic acid and exposed to road transportation. *Journal of Equine Veterinary Science.* 34: 1324-1328. ISSN 15427412, 07370806. https://sci-hub.se/https://doi.org/10.1016/j.jevs.2014.09.015

Errata Erratum

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