Short Communication

Hematochemical Parameters of African Lions (*Panthera leo*) Kept at Safari Zoo, Lahore, Pakistan

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ABSTRACT

Hematochemical parameters are often used by veterinarians to assess the health status of animals. The information on blood parameters of African lions (*Panthera leo*) is scarce in Pakistan, this research was conducted on captive African Lions kept at Safari Zoo, Lahore to see their blood profile. The hematochemical parameters included white blood cells (WBCs), hemoglobin (HGB), hematocrit (HCT), lymphocytes (LYM) count, RBCs count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), bilirubin, creatinine, blood urea nitrogen (BUN) were determined. Further, different biological, analytical and operational factors which impose significant effects on hematochemical factors were also considered to rationalize the results. Most of the values were in accordance with the mean values published in the literature, but values of few parameters e.g., hematocrit, platelet, AST, ALT, ALP and blood urea nitrogen were found different from the reference values. It was concluded that biological, analytical and operational factors as well as their respective partialities affect these values. The study contributes to the development of the normal values of hematochemical parameters of African lions kept at different Zoos of Pakistan and ultimately helps to improve their health status and conservation process.

The African lion (*Panthera leo*) is an important flagship species that has undergone a major decline in the population and geographic range (Bauer *et al.*, 2015). Once found in the entire continent of Africa, as well as parts of Asia and Europe, they are now only found in the sub-Saharan Africa. The International Union for Conservation of Nature (IUCN) predicts that fewer than 25,000 lions remain in Africa (Winterbach *et al.*, 2012; Riggio *et al.*, 2013). The key threat to African lion includes habitat degradation, reduction of lion prey and human–lion conflict (IUCN, 2018). In Pakistan, African lions are only available in captivity, and regular monitoring of health status, timely diagnosis of different diseases and the immediate response for treatment is mandatory for their protectionand conservation (Shrivastav, 2001).

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In the last three decades, improvement in veterinary care and other husbandry practices has resulted in increased longevity of wild animals in captivity (Krebs *et al.*, 2018). However, despite of the high prevalence of various diseases in captive animals, laboratory facilities in developing countries are insufficient for diagnosis, and there is a need to reduce difficulty in disease diagnosis in captive species. Instead of relying only on clinical symptoms, laboratory tests should be used to make final diagnosis (Budihal and Perwez, 2014). Environment and management conditions also have influence on the varied values of hematochemical parameters (Arfuso *et al.*, 2016). A detailed study of hematochemical parameters and factors affecting these values may be the great setback in species decline. Thus, knowledge about these blood parameters is important for the well-being of animals (Lacerda *et al.*, 2006). It is undoubted fact that the knowledge of normal hematochemical factors can help to distinguish the pathological conditions from the normal (Rubinoa *et al.*, 2006). Values of selected parameters can vary as laboratories differ and other factors (sex, age, animal emotions and nutrition) may also affect the blood profile. However, if all these variables are kept under

consideration, baseline standards can still be established (Plessis, 2009).

In the literature, a little information is present on the blood bio-chemistry of *Panthera leo* in Pakistan. The hematochemical parameters help in understanding the health of animals and various diseases can be diagnosed from variations in these parameters. More research-based data is required to record reference values for the hematochemical parameters of *Panthera leo,* so that the diagnosis of sub-clinical or asymptomatic infections may be done intime. The study was aimed to evaluate different hemato-chemical parameters based on which the health status of captive African lions would be determined along with the development of baseline information on these parameters for Pakistani bred, captive *Panthera leo* particularly with reference to semi natural environment.

Materials and methods

The sampling was done on African Lions (*Panthera leo*) bred and kept at Safari Zoo Lahore, Pakistan from September to December 2020. The park is a protected area covering 247 acres as a whole including more than 15 acres exclusively for the African lions. It is situated at 13 km Raiwind Road Lahore (31.3843° N, 74.2096° E) and 32 km away from main Lahore city, Pakistan.

The lions are kept in 20′×25′ indoor cages in routine and released in a large fenced 15-acres out door area (Lion safari) in rotations, i.e., only one pride is released in outdoor facility for whole day, second pride for next day and so on. All the animals ranged from 1.0-14 years in age and from 80-160 kg in average weight. The animals with recent physiological and/or concurrent abnormalities, receiving any type of medication, inadequate in body condition score, dehydrated, infested with ectoparasites and affected with any other disease were all excluded from the experiment.

A total of 20 out of 34 apparently healthy lions (6 males and 14 females) were sampled for blood (Table I). The blood samples were collected by taking animals into the squeeze cage one by one. The animals were first normalized from caging aggression and stress. The maximum 2-5 ml blood sample was drawn from dorsal or lateral coccygeal veins through 10ml disposable syringe with 18-gauge needle and shifted into a tube containing ethylenediaminetetraacetate (EDTA at 2 mg/ml of blood) as an anticoagulant (Shrivastav, 2011). The samples were then labeled and transported immediately to University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore in an icebox for further hematochemical analysis (Sajjad *et al*., 2012).

The results for all age groups and both sexes were pooled. The descriptive statistical analysis on recorded data was done by using Graphpad Prism.

Table I. Hematological and biochemical parameters of blood of *Panthera leo*.

Parameters	Mean±SD	Normal range* (Fowler, 1978)
Hematologic parameters		
WBC (10^3/µL)	13.29±3.35	8.5-18.33
LYM (10^3/µL)	3.37±1.30	1.72-6.52
RBC (10^6/µL)	10.64±1.73	7.59-13.69
HGB (g/dL)	15.12±2.32	11.8-18.5
HCT (%)	47.86±7.45	34.48-61.04
MCV (fl)	45.1±1.44	42-47
MCH (pg)	14.23±0.79	13-15.5
MCHC (g/dL)	31.61±1.47	29.6-34.1
PLT (10^3/µL)	473±22.97	189-710
Biochemical parameters		
AST (IU/L)	11.8±8.56	7.7-20.1
ALP (IU/L)	6.51±9.58	1.0-17.7
ALT (IU/L)	4.78±11.69	3.1-6.6
Bilirubin (mg/dL)	2.17±0.46	1.2-3.1
Creatinine (mg/dL)	1.66±0.55	0.98-2.61
BUN (mg/dL)	16.4±12.79	4-36

ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; HGB, hemoglobin; HCT, hematocrit; LYM, lymphocytes; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; RBC, red blood cells; WBC, white blood cells.

Results and discussion

Current study depicted that hematochemical parameters including platelets, AST, ALP, ALT and BUN were significantly different from the reference values. Values of hematocrit, platelet were apparently higher and values of AST, ALP and blood urea nitrogen were apparently lower than reference mean values (Table I). Results of the current research showed that most of the hematology parameters including WBCs (13.29 10^3/µL), LYM (3.374$10^3$/µL), RBC (10.64 10^6/µL), HGB (15.12 g/dL), MCV (45.1 fl), MCH (14.23 pg) and MCHC (31.61) were in accordance with the reference values given in Fowler (1978) and ISIS (1999) (Table I) while values of HCT, PLT were apparently higher and values of AST, ALP and BUN were apparently lower than reference mean values. The hemogram did not reveal any differences based on age and sex because of small sample size.

All the hematological values except HCT and PLT were in agreement of ISIS (1999) values. However,

Currier and Russel (1982) described that hemato-chemical values play a vital role in evaluating the health condition of individuals as well as populations. In the present study, value of HCT was recorded higher (47.86%) in comparison with reference (39.1%) and the probable reason of a high HCT value was dehydration as facilities of drinking water were not fit for experimental lions. High value of HCT (47.5 %) was reported in females at 1500 m altitude by Mortola and Wilfong (2017). Dehydration may be due to heat exhaustion as sampling was carried out in summer season. Erythrocytosis (over production of RBCs) can also be a reason for high hematocrit values.

Although clinical hematology has an important role in the care of domestic animals, the known reference values for many wildlife species are still very limited (Larsson et al., 2015). In most of the considered parameters no apparent differences were recorded in hematochemical values in comparison with reference values. Age and sex were also a matter of consideration but hemogram did not provide information about influence of these variables on these parameters. Expected reason was small sample size. Among 9 hematological and 6 biochemical parameters of blood, seven and three parameters, respectively were apparently in accordance with reference values.

According to the literature, the provided reference values may be normal for that particular animal due to variability in area, enclosure, environment and feeding habit (Thrall et al., 2012). There is an alteration in blood components in relation to physical condition of an animal's health (Togun et al., 2007). Khan and Zafar (2005) proposed that variations in these values were of valuable importance in the assessment of physiological situations of animals. Among total fifteen studied parameters, only five parameters (HCT, PLT, AST, ALP and BUN) were apparently not found matched with reference values.

In the current study, PLT count was higher 473 $10^3/\mu L$ than value given in literature 285 $10^3/\mu L$. Hawkey and Hart (1990) described the significance of establishing normal blood cell ranges and pointed out that the potential value of PLT counts is indicator of clinical anomalies in grown mammals and it should not be overlooked. Physiological thrombocytosis that may be due to stress and reactive thrombocytosis can increase PLT count in lions.

According to the literature, along with behavioral response to stress, some other responses such as physiological responses are important. Among to these responses, most significant is to rise in the production of cortisol, the stress hormone which in turn caused to increase in PLT count (Sindi et al., 2014). However, one or more reasons were applicable for the values which were deviating from reference mean values. Furthermore, most probable reasons such as health status, maintenance and ecological conditions were unknown for the lions used in the present study as reference (Erasmus, 2008). According to Afolabi et al. (2010), fluctuations in hematological parameters were frequently used to determine different states of the body and to lay down stresses caused by environmental, nutritional and/or pathological factors.

In the current study, blood samples were taken only from lions of Safari Zoo Lahore, Pakistan and according to Maas et al. (2013) lions from other localities may have different values of blood parameters due to different environmental conditions. Schalm et al. (1975) suggested that there are certain factors that may influence blood pictures of animals, such as nutrition, maintenance, animal breed, age, diseases, sex, and stress factors. In the present study, all lions were of same lineage and all were born and bred at the same place. Stress and trauma were considerable in the current study due to frequent visitors.

Values of ALP and ALT were lower than reference but it does not matter significantly. Although these indices may vary noticeably depending on method followed and instruments used (Duncan et al., 1994). BUN is an important health indicator, mainly provide information about kidney functioning as its lower values showed urea was excreting out of the body. All the probable factors discussed above can help to identify the variation in values and health status of *Panthera leo*. However, more research with large sample size can provide better reference intervals of this iconic species (*Panthera leo*) being kept in captivity.

Conclusion

In conclusion, different recorded parameters of hemato-chemical analysis have the potential to indicate health status of animals. Stress, dehydration, disease and environmental factors were concluded as major factors that have great ability to affect the studied parameters. Moreover, enclosure size and nutrition also have strong relationship with these recorded parameters. Overall animals were healthy but there is sturdy need to provide better facilities to captive animals for better health.

IRB approval

Being stationed veterinarian, Muhammad Azhar did the whole sampling and procedures related to animals at Safari Zoo Lahore by himself or accompanying the team according to the set SOP's

Ethical statement

No animal was harmed or hurt unaccountably. The sampling was done with minimum invasive protocols.

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Afolabi, K.D., Akinsoyinu, A.O., Olajide, R. and Akinleye, S.B., 2010. *Poljoprivreda,* **17**: 74-78.

Arfuso, F., Quartuccio, M., Bazzano, M. and Fazio, A., 2016. *Vet. Clin. Pathol.,* **45**: 260-270. https://doi.org/10.1111/vcp.12344

Bauer, H., Chapron, G., Nowell, K., Henschel, P., Funston, P., Hunter, L.T.B., Macdonald, D.W. and Packer, C., 2015. *Proc. natl. Acad. Sci. USA,* **112**: 14894–14899. https://doi.org/10.1073/pnas.1500664112

Budihal, S.V. and Perwez, K., 2014. *J. clin. Diagn. Res.,* **8**: 199-202.

Cheeran, J.V., 2004. *Textbook of wild and zoo animals: Care and management*. 1st edn. International Book Distributing Co, India. pp. 104-111

Currier, M.J.P. and Russel, K.R., 1982. *J. Wildl. Dis.,* **18**: 99-104. https://doi.org/10.7589/0090-3558-18.1.99

Duncan, J.R., Prasse, K.W. and Mahaffey, A.E., 1994. *Veterinary laboratory medicine*. 3rd edn. Iowa State University Press, Ames, Iowa. pp. 300.

Erasmus, H.L., 2008. *Determination of some blood parameters in the African lion* (Panthera leo). Magister Scientiae Agriculturae thesis, University of the Free State, Bloemfontein, South Africa.

Fowler, M.E., 1978. *Zoo and wild animal medicine*. 1st edn. Saunders, Philadelphia.

Hawkey, C.M., Hart, M.G., Bennett, P.M., Gascoyne, S.C., Knight, J.A. and Kirkwood, J.K., 1990. *Vet. Rec.*, **127**: 18.

International Species Information System (ISIS), 1999. *Physiological reference values*. International Species Information System. www.worldzoo.org.

IUCN SSC Cat Specialist Group, 2018. *Guidelines for the conservation of lions in Africa*. Version 1.0. Muri/Bern, Switzerland: Author. pp. 191.

Khan, T.A. and Zafar, F., 2005. *Int. J. Poult. Sci.,* **4**: 748-751.

Krebs, B.L., Marrin, D., Phelps, A., Krol, L. and Watters, J.V., 2018. *Animals*, **8**: 116. https://doi.org/10.3390/ani8070116

Lacerda, L., Campos, R., Sperb, M., Soares, E. Barbosa, P., Godinho, E., Ferreira, R., Santos, V. and González, F.D., 2006. *Arch. Vet. Sci.,* **11**: 40-44. https://doi.org/10.5380/avs.v11i2.6783

Larsson, M.H.M.A., Santo, P.L.E., Mirandola, R.M.S., Fedullo, J.D.L., Ito, F.H., Itikawa, P.H. and Pessoa1, R.B., 2015. *Acta Sci. Vet.,* **43**: 1311.

Maas, M., Keet, D.F. and Nielen, M., 2013. *Res. Vet. Sci.,* **95**: 266–268. https://doi.org/10.1016/j.rvsc.2013.01.023

Mortola, J.P. and Wilfong, D.A., 2017. *Zoology*, **125**: 10-23. https://doi.org/10.1016/j.zool.2017.07.001

Plessis, L.D., 2009. *J. Vet. Res,* **76**: 317-321.

Riggio, J., Jacobson, A., Dollar, L., Bauer, H., Becker, M., Dickman, A., Funston, P., Groom, R., Henschel, P., de Iongh, H., Lichtenfeld, L. and Pimm, S., 2013. *Biodivers. Conserv.,* **22**: 17–35. https://doi.org/10.1007/s10531-012-0381-4

Rubinoa, G., Citoa, A.M., Lacinioa, R., Bramante, G., Caroli, A., Pieragostini, E. and Petazzi, F., 2006. *J. Equine Vet. Sci.,* **26**: 475-480. https://doi.org/10.1016/j.jevs.2006.08.005

Sajjad, S., Farooq, U., Malik, H., Anwar, M. and Ahmad, I., 2012. *Turk. J. Vet. Anim. Sci.,* **36**: 346-351.

Schalm, O.W., Jain, N.C. and Carroll, E.J., 1975. *Veterinary hematology*. 3rd edn. Lea and Fabiger, Philadelphia, USA. pp. 15-218.

Shrivastav, A.B., 2001. *Intas Polivet.,* **2**: 134-136.

Sindi, S., Fiocco, A.J., Juster, R.P., Lord, C., Pruessner, J. and Lupien, S.J., 2014. *Hippocampus,* **24**: 1623-1632. https://doi.org/10.1002/hipo.22341

Thrall, M.A., Weiser, G., Allison, R.W. and Campbell, T.W., 2012. *Veterinary hematology and clinical chemistry*: 5th edn. Wiley Blackwel. New Jersey, USA.

Togun, V.A., Oseni, B.S.A., Ogundipe, J.A., Arewa, T.R., Hammed, A.A., Ajonijebu, D.C., Nwosisi I., Ogundipe, J.A., Hammed, A.A., Oyeniran, A. and Mustapha, F., 2007. *Effects of chronic lead administration on the haematological parameters of rabbits. A preliminary study*. Proceedings of the 41st Conferences of the Agricultural Society of Nigeria, Zari. p. 341

Winterbach, H.E.K., Winterbach, C.W., Somers, M.J. and Hayward, M.W., 2012. *Mammal. Rev.,* **43**: 89-110. https://doi.org/10.1111/j.1365-2907.2011.00209.x