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Hematochemical profile of Cholistani cattle being reared in the Cholistan desert of Pakistan under pastoralism

[*Perfil hemato-químico do gado Colistani criado no deserto de Cholistan, no Paquistão, sob o regime de pastoreio*]

Z. *Saeed*[1], F. *Iqbal*[2], U. *Farooq*[3], S.A. *Mahmood*[1], M.H. *Lashari*[4]*, S. *Sajjad*[5]

[1]Livestock and Dairy Development Department, Punjab, Pakistan
[2]Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan
[3] University of Bahawalpur, Faculty of Veterinary and Animal Sciences, Department of Physiology, The Islamia, Pakistan
[4] The Islamia University of Bahawalpur, Department of Zoology, Pakistan
[5]Lahore College for Women University, Department of Zoology, Lahore, Pakistan

ABSTRACT

The objective of the present study was to assess and create normal reference intervals for hematochemical profile of Cholistani cattle (n=360) being reared under pastoralism. The comparisons have been made with earlier published data on *Bos indicus* and *taurus* cattle breeds. The general health status of animals was ascertained through a thorough anamnesis from the livestock owners and clinical signs. However, theileriosis, in specific, was ruled out through blood smear examination. The animals were assigned in groups according to age: young (n=190; ≤12 months) and adult (n=170; >12 months up till 7 years); and gender: male (n=182) and female (n=178) cattle. The mean (±SE), median, range and reference intervals (25^{th} to 95^{th} percentile) for hematochemical profile were determined through descriptive statistics and differences between various groups were analyzed through Mann Whitney U test. The findings of this study may serve as reference hematochemical values for Cholistani cattle in specific and humped zebu cattle in general for assessing any physiological, pathological, or metabolic alterations.

Keywords: Cholistan, hematology, biochemistry, cattle

RESUMO

O objetivo do presente estudo foi avaliar e criar intervalos de referência normais para o perfil hemato-químico do gado colistano (n=360) criado sob pastoreio. As comparações foram feitas com dados publicados anteriormente sobre as raças bovinas Bos indicus e taurus. O estado geral de saúde dos animais foi verificado através de uma anamnese minuciosa dos proprietários de gado e de sinais clínicos. No entanto, a teileriose, em particular, foi descartada através de exame de esfregaço de sangue. Os animais foram designados em grupos de acordo com a idade: jovens (n=190; ≤12 meses) e adultos (n=170; >12 meses até 7 anos); e sexo: bovinos machos (n=182) e fêmeas (n=178). A média (±SE), mediana, faixa e intervalos de referência (percentil 25 a 95) para o perfil hemato-químico foram determinados através de estatísticas descritivas e as diferenças entre vários grupos foram analisadas através do teste Mann Whitney U. Os resultados deste estudo podem servir como valores hemato-químicos de referência para bovinos cholistani em bovinos zebuínos específicos e em geral para avaliar quaisquer alterações fisiológicas, patológicas ou metabólicas.

Palavras-chave: Cholistani, hematologia, bioquímica, gado

INTRODUCTION

Cholistani is a large sized flabby breed with small horns, long ears, well developed hump in males and large dewlap in both sexes. Owing to its speckled red, black, or brown body it is termed as 'flea-bitten' by the nomads of Cholistan. The switch of the tail is black and the elite specimen of Cholistani cows produce 15 to 20 liters of milk daily. The average body weight

*Corresponding author: mushtaq.hussain@iub.edu.pk
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recorded in a preliminary data obtained from the Government Livestock Farm, Jugait Peer, Bahawalpur varied from 500 to 600 and 300 to 400kg in male and female cattle, respectively (Farooq *et al*., 2010). This breed has been incorporated in Livestock Census 2006 of Pakistan for the first time to stay as a breed (Farooq *et al*., 2015).

Geographical location and climatic conditions of the study area (Cholistan desert, Pakistan) have been described by Farooq *et al*. (2010). Briefly, the desert is an expansion of Great Indian Desert which includes the Thar Desert in Sindh Province, Pakistan and the Rajhsatan desert, India. It starts some 30km from the main city of Bahawalpur, Punjab, Pakistan, and sprawls over an area of 26000kmm², located between the latitudes 27º42´and 29º45´North and longitudes 69º52´and 75º24´East. The climate of this area is arid subtropical and continental with low/sporadic rainfall, high temperature, low relative humidity, high rate of evaporation and strong summer winds. It is considered one of the driest and hottest areas of Pakistan with a lengthy summer season extending from May till October.

Data on its phenotypic and various productive/ reproductive traits have been published earlier from Pakistan (Farooq *et al*., 2010, 2013, 2015; Mahmood *et al*., 2013;). Furthermore, few reports on hematological profile of Cholistani breeding bulls and pregnant cows being reared at various government farms are also available (Farooq *et al*., 2017). Similarly, various molecular and hematochemical aspects of theileriosis in this breed have also been reported (Mahmood *et al*., 2013). However, to the best of our knowledge, no work has yet been reported regarding the hematological and clinical biochemistry profile of apparently healthy Cholistani cattle being reared under pastoralism. The present study has, thus, been devised with the objective to assess and create the normal reference intervals for various hematochemical attributes of Cholistani cattle being reared under pastoralism. Comparisons have been made with earlier published data on *Bos taurus* and *indicus* cattle breeds.

MATERIALS AND METHODS

The present study is, in fact, a part of a bigger study which was carried over a period of seven months with the objective to assess theileriosis in Cholistani breed of cattle in the Cholistan desert, Pakistan (Saeed *et al.,* 2016). Randomly selected "Tobas" (Man-made and Natural Reservoirs/Ponds) in the desert were visited during the study and a total of 390 Cholistani cattle were registered in the study. These 'Tobas' are a source of water both for humans and animals in the Cholistan desert. The animals of the breed were selected based on phenotype. The study was approved in full by the "Ethical Review Committee for the Use of Animals" which comes under the administrative control of the Office of Research, Innovation and Commercialization of Bahauddin Zakariya University, Multan, Pakistan. A written consent was taken on an appropriate proforma from the Cholistani pastoralists/livestock owners involved in our study. Guidelines for creating population-based reference intervals provided by the Clinical and Laboratory Standards Institute were followed (Friedrichs *et al*., 2012).

All the animals were being reared under similar management and feeding conditions of pastoralism. Split-herding is normally exercised for livestock by the pastoralists, according to which the young ones (calves in this case) are kept at their pens near the 'Tobas', while the adults are sent for grazing till night-time (Farooq *et al*., 2010). A total of 49 plant species including 29 families have been identified in Cholistan desert. Out of these, poaceae is the most dominant family with 12 species and is the main fodder of grazing cattle (Rasheed *et al.,* 2017). The study animals were selected from a total of 11 Tobas having livestock population in them, and the study was conducted from February 2015 to December 2016. The general health status of animals was ascertained through a thorough anamnesis from the livestock owners and clinical signs. The animals which were found to be lethargic, depressed, off-feed and segregated from the herd (as per the anamnesis taken from the pastoralist herders) were not included in the study. From the total registered cattle (n=390), thirty (7.6 %) were excluded from the study owing to lethargic signs (n=20, 5.1 %) and theileriosis (n=10, 2.5 %). Hence, remaining animals (n=360) were incorporated for sampling.

About 7mL of blood was collected from the jugular vein of each animal under appropriate restraining and stored as two aliquots: clotted for

harvesting serum and un-clotted (0.5M EDTA) for hematological analysis. To minimize the stress to the animal, to standardize the collection procedure, and to remove diurnal variation, all the animals were restrained with the same technique and the blood collection was carried out by the same personnel and at the same time of the day *i.e* around 05:00 pm. Each animal was sampled once, and the samples were brought to the laboratory in ice box. The un-clotted whole blood samples were processed within 08h of collection whereas serum samples were stored at -24ºC until further analyzed.

An automated Hematology Analyzer (Sysmex K21, Kobe, Japan) was used for determination of various hematological indices such as hemoglobin (Hb), packed cell volume (PCV), Red Blood Cell (RBC) Count and White Blood Cell (WBC) Count. Blood smears stained with Wright's stain were simultaneously prepared for Differential Leukocytic Count (DLC). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the prescribed formulae (Jain, 1986). The hematology analyzer was designed for human application; hence, before analysis of samples, it was validated against blood samples from 100 dogs and 100 cows as well as with manual reference methods (cynmethemoglobin photometry, hematocrit analysis, and hemocytometry) as recommended (Wassmuth *et al*., 2011; Farooq *et al*., 2017).

Regarding the serum biochemical analysis total protein (TP), creatinine, alanine transaminase (ALT), aspartate transaminase (AST) and triglycerides (TGs) were determined by using APEL PD-303S spectrophotometer (Japan) and diagnostic kits manufactured by Bio Med Diagnostics, GmbH, Germany following user's manual.

For the sake of analysis, groups were assigned as per age: young (n=190; ≤12 months) and adult (n=170; >12 months up till 7 years); and gender: male (n=182) and female (n=178) cattle. Statistical analysis was conducted using Statistical Package for Social Sciences (Windows version 12, SPSS Inc, Chicago, IL, USA) and keeping in view the guidelines provided by the American Society for Veterinary Clinical Pathology. The mean (±SE), median, range and reference intervals (25^{th} to 95^{th} percentile) for hematological and clinical biochemistry profile were determined through descriptive statistics. Difference between groups was analyzed through Mann Whitney U test.

RESULTS AND DISCUSSION

It is inevitable to deduce hematochemical profile of indigenous breeds under their local climatic conditions which will serve as a yardstick for clinicians and researchers. The present study is the first of its kind being reported on hematological and clinical biochemistry profile of indigenous Cholistani breed of cattle being reared under pastoralism in Cholistan desert of Pakistan and incorporates routine hematochemical tests. Comparisons have been made both with various *Bos indicus* and *taurus* cattle breeds.

The overall mean (±SE), median, interquartile range, minimum, maximum, 25^{th} to 95^{th} percentile of reference interval (RI) and 95% confidence interval (CI) for hematological parameters in Cholistani cattle (n=360) is given in Table 1. The overall mean (±SE) values for RBC Count, Hb, PCV, MCV, MCH and MCHC were $7.5\pm0.09\times10^{12}$/L, 110.2±0.1 g/L, 35.8±0.6 %, 48.2±0.6 fL, 15.3±0.2 pg and 310.9±0.3 g/L, respectively. These are in range reported by Jain (Jain, 1993) for zebu cattle with slight variations.

It has been ascertained through previous studies that the RBC Count of zebu cattle is higher as compared to its European counterparts which assists the animal in its thermoregulatory mechanisms (Bedenicki *et al*., 2014). A higher value of 9.6×10^{12}/L as compared to our results ($7.5\pm0.09\times10^{12}$/L) has been reported for African Sokoto cattle (Olayemi *et al*., 2007) whereas a lower value of 6.34×10^{12}/L has been given for cross bred cattle from Thailand (Boonprong *et al*., 2007). Differences in breed, geography and number of animals could be attributed to this difference.

Table 1. Overall mean (±SE), median, interquartile range, minimum, maximum, 25th to 95th percentile of reference interval (RI) and 95% confidence interval (CI) for hematological parameters in Cholistani cattle (n=360)

Attribute	Mean (±SE)	Median (IQR)	Range (Min-Max)	RI (25th to 95th Percentile)	95% CI
Red Blood Cell Count (10^{12}/µL)	7.5±0.09	7.4(2.39)	10.93(2.7-13.7)	6.3-10.0	7.3-7.7
Hemoglobin (g/L)	110.0±0.1	110.1(20.4)	130.6(50.1-180.7)	90.8-130.9	100.8-110.3
Packed Cell Volume (%)	35.8±0.6	35(10.8)	81.5(15.6-97.1)	29.1-49.5	34.6-37.0
Mean Corpuscular Volume (fL)	48.2±0.6	46(8.5)	81.5(30.9-112.4)	42.2-59.9	46.9-49.5
Mean Corpuscular Hemoglobin (pg)	15.3±0.2	15(3.17)	39.6(10.4-50.0)	13.3-20.6	14.8-15.7
Mean Corpuscular Hemoglobin Concentration (g/L)	310.9±0.3	330.1(70.1)	270(130.2-400.2)	280.5-380.5	310.4-320.5
White Blood Cell Count (10^{9}/µL)	10.4±0.4	9.2(3.2)	65.6(3.5-69.1)	7.4-19.5	9.5-11.2
Neutrophils (%)	45.9±2.7	40.1(7.2)	79.0(29.2-53.7)	42.5-55.2	8.2-11.2
Lymphocytes (%)	49.1±1.5	46(8.7)	79.1(27.1-57.2)	45.2-57.0	43.0-44.6
Eosinophils (%)	2.0±0.5	1.8(1.0)	4.2(0.0-11.0)	1.6-7.8	1.9-3.0
Monocytes (%)	1.6±0.3	1.3(0.8)	3.7(0-5)	0.8-2.4	1.0-1.1

The overall mean Hb values of present study (110.2±0.1g/L) are in accordance with the results reported for Iranian and Thai crossbred cattle (Aengwanich *et al*., 2009; Mirzadeh *et al*., 2010). These also are in line with our previous report for theileriosis in Cholistani breed (Mahmood *et al*., 2013). Seasonal factor is one of the major factors which produce evident change in Hb values being higher in summer and lower in winter (Gökçe *et al*., 2004; Kiran *et al*., 2012). Furthermore, age, physical status, geography, and method of analyses may also alter these values (Farooq *et al*., 2017).

Regarding PCV, the mean value in this study (35.8±0.6 %) is within normal reference range (24-46 %) for zebu cattle as reported by Jain (Jain, 1986). The results are also in line with previous works conducted on Nigerian Sokoto cattle (Olayemi *et al*., 2007) and Semental cattle of Iran (Mirzadeh *et al*., 2010).

Age-wise and gender-wise results for hematological parameters have been given in Table 2. The RBC Count was significantly (P≤0.05) higher whereas MCV, MCH, and MCHC were significantly (P≤0.05) lower in young Cholistani cattle as compared to their adult counterparts. A higher RBC Count for young Cholistani cattle (zebu) in the present study contrasts with results presented for *Bos taurus* breeds of cattle (Otto *et al*., 2000; Van Wyk *et al*., 2013). It has been reported that red blood cell parameters are normally lower in young animals but increase with increasing age. These changes have been plausibly attributed to a destruction of fetal RBCs and replacement of fetal Hb with adult Hb (Knowles *et al*., 2000; Onasanya *et al*., 2015). On the other hand, this replacement of fetal Hb with adult Hb in zebu cattle does not take at a swift pace and hence no substantial decrease is noticed in them (Çöl and Uslu, 2007; Onasanya *et al*., 2015). However, detailed aspects of 'Hbs witching' in zebu cattle needs further scientific navigation.

Gender-wise results showed no statistical difference between male and female Cholistani cattle (Table 2). Our results are in line with various other reports conducted on indigenous Nigerian Gudali cattle (Olayemi *et al*., 2007), African Ketuku cattle (Olayemi *et al*., 2006), Nigerian Kuri cattle (Onasanya *et al*., 2015) and Saudi indigenous Hassawi cattle (Al-Shami, 2003). On the contrary, some reports have presented higher red blood cell parameters for male cattle as compared to their female counterparts owing to a higher RBC Count and Hb level in them. A study on Iranian cattle has reported significant difference in red blood cell indices in male and female cattle being higher for males (Mirzadeh *et al*., 2010). Difference in breed, season or geography could be a plausible justification for these differences.

Table 2. Mean (±SE) values for age-wise and gender-wise hematological parameters in Cholistani cattle (n=360)

Attribute	Age-wise		Gender-wise	
	Young (n=190)	Adult (n=170)	Males (n=182)	Females (n=178)
Red Blood Cell Count (10^{12}/µL)	8.0±0.1*	6.9±0.1	7.8±0.1	7.1±0.1
Hemoglobin (g/L)	100.9±0.1	110.2±0.1	110.3±0.1	100.8±0.1
Packed Cell Volume (%)	36.6±0.9	34.9±0.6	36.6±0.7	35.1±0.8
Mean Corpuscular Volume (fL)	45.9±1.1*	50.7±0.4	47.0±0.9	49.2±0.9
Mean Corpuscular Hemoglobin (pg)	13.8±0.2*	16.9±0.3	14.8±0.3	15.7±0.3
Mean Corpuscular Hemoglobin Concentration (g/L)	310.2±0.4*	320.8±0.3	310.7±0.4	320.2±0.4
White Blood Cell Count (10^9/µL)	11.1±0.6*	9.5±0.4	10.4±0.5	10.3±0.6
Neutrophils (%)	48.5±2.4	45.8±3.0	47.5±2.1	47.8±2.0
Lymphocytes (%)	48.2±1.7	50.0±2.0	49.2±1.7	49.0±2.0
Eosinophils (%)	2.3±0.8	2.0±0.7	2.1±0.8	2.0±0.7
Monocytes (%)	1.0±0.5	2.2±0.7	1.0±0.5	2.0±0.7

*Differ significantly within a row at P≤0.05.

The overall mean (±SE), median, interquartile range, minimum, maximum, 25th to 95th percentile of reference interval (RI) and 95% confidence interval (CI) for white blood cell parameters in Cholistani cattle (n=360) are presented in Table 1. The overall mean (±SE) values for white blood cell parameters *viz*. WBC Count, Neutrophils, Lymphocytes, Eosinophils and Monocytes for the Cholistani cattle in present study were 10.4±0.4 × 10^9/L, 45.9±2.7%, 49.1±1.5%, 2.0±0.5% and 1.6±0.3%, respectively. These values and all reference intervals are in range reported by Jain (1986) for zebu cattle with slight variations.

The overall mean value (±SE) for WBC Count in present study (10.4±0.4 × 10^9/L) is within the range for cattle (4.2-12.0 × 10^9/L) reported by Jain (1986). However, higher values of 14.6±2.3 and 13.7±2.9 × 10^9/L have been reported for Hassawi cattle in Saudi Arabia (Al-Shami, 2003) and crossbred cattle of Thailand (Aengwanich *et al*., 2009), respectively. Similarly, lower values (6.2±2.9 × 10^9/L) have also been reported for indigenous Croatian cattle (Bedenicki *et al*., 2014). The differences could be due to differences in breed and physiological status of animals.

In the present study, age-wise and gender-wise results of white blood cell parameters for Cholistani cattle revealed that only WBC Count was significantly (P≤0.05) higher in young animals as compared to their adult counterparts (Table 2). Rest of all other parameters were non-significantly (P≥0.05) different between young and adult, and male and female animals (Table 2). A work conducted on Istrian cattle has reported no difference in WBC Count within various age groups (Bedenicki *et al*., 2014). Similarly, yet another study conducted on crossbred cattle of Thailand also reported no effect of age or gender on WBC Count of cattle (Aengwanich *et al*., 2009). However, while studying African short horn zebu cattle it has been reported that young animals have higher WBC Count as compared to adults which is in line with our results. The difference in various results could be because of different geography or different breed under study. A detailed future study on immunological aspects of Cholistani cattle could unearth the role and level of circulating WBCs in their blood.

The overall mean (±SE), median, interquartile range, minimum, maximum, 25th to 95th percentile of reference interval (RI) and 95% confidence interval (CI) for clinical biochemistry profile in Cholistani cattle (n=360) are presented in Table 3. The overall mean (±SE) values for serum biochemical parameters for Cholistani cattle in present study *viz*. TP, creatinine, ALT, AST, and TGs were 70.2±1.08g/L, 122.3±2.0µmol/L, 28.7±0.5U/L, 57.4±1.1U/L

and 0.29±0.004mmol/L, respectively. These values do not coincide with former reports which have shown higher or lower values than ours. A higher value for TP (80.1±0.1g/L) and lower for creatinine (88.4µmol/L) has been reported for Swiss Brown cattle (Knowles *et al*., 2000). Similarly, a higher value for AST (67.4±19.8U/L) has been reported for Turkish crossbred cattle (Gökçe *et al*., 2004). Difference in breed or physiological status could be few of the underlying factors for these differences.

Table 3. Overall mean (±SE), median, interquartile range, minimum, maximum, 25th to 95th percentile of reference interval (RI) and 95% confidence interval (CI) for clinical biochemistry profile in Cholistani cattle (n=360)

Attribute	Mean (±SE)	Median (IQR)	Range (Min-Max)	RI (25th to 95th Percentile)	95% CI
Total Protein (g/L)	70.2±1.08	70 (10.4)	90.2 (30.8-130.0)	60.4-90.5	70.0-70.4
Creatinine (µmol/L)	122.3±2.0	114.9 (35.3)	238.6 (0-238.6)	106.0-185.2	118.3-126.2
Alanine Transaminase (U/L)	28.7±0.5	29 (10.7)	51 (3.0-54.0)	23.2-45.7	27.6-29.7
Aspartate Transaminase (U/L)	57.4±1.1	55 (24.0)	127 (17.0-144)	45.0-87.0	55.2-59.6
Triglycerides (mmol/L)	0.29±0.004	0.28 (0.08)	0.68 (0.1-0.78)	0.23-0.44	0.28-0.3

Table 4. Mean (±SE) values for age-wise and gender-wise clinical biochemistry profile in Cholistani cattle (n=360)

Attribute	Age-wise		Gender-wise	
	Young (n=190)	Adult (n=170)	Males (n=182)	Females (n=178)
Total Protein (g/L)	60.8±0.1*	70.7±0.1	70.1±0.1	70.3±0.1
Creatinine (µmol/L)	106.0±0.02*	123.7±0.03	113.7±0.03	114.9±0.02
Alanine Transaminase (U/L)	28.6±0.7	28.7±0.7	28.2±0.7	29.1±0.8
Aspartate Transaminase (U/L)	56.4±1.5	58.6±1.5	55.6±1.5	59.0±1.6
Triglycerides (mmol/L)	0.29±0.6	0.26±0.5	0.29±0.7	0.27±0.5

*Differ significantly within a row at P≤0.05.

The age-wise and gender-wise results for serum chemistry revealed that TP and creatinine were significantly (P≤0.05) lower in young animals as compared to adults in this study (Table 4).

All the remaining parameters were non-significantly (P≥0.05) different between males and females, and young and adult Cholistani cattle. A similar trend has been reported for indigenous Angoni cattle of Mozambique, Africa and small ruminants of Pakistan (Otto *et al*., 2000; Kiran *et al*., 2012; Van Wyk *et al*., 2013). Factors affecting serum clinical biochemistry parameters have extensively been reviewed and it has been concluded that age, season, physiological status, and stress may alter their circulating levels (Mohri *et al*., 2007; Onasanya *et al*., 2015). Age-related increase in many clinical biochemistry parameters have also been confirmed for small ruminants (Awolaja *et al*., 1997).

CONCLUSION

The findings of this study may serve as reference hematological and clinical biochemistry values for Cholistani cattle in specific and humped zebu cattle in general. They may be utilized for comparison under various physiological, pathological, and metabolic alterations for diagnostic and prognostic approach. Future horizons include study on immunological profiling of this breed with a larger sample number and larger set of parameters.

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AUTHOR'S CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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