REFERENCE VALUES FOR HAEMATOLOGICAL AND BIOCHEMICAL PROFILE IN ADULT INDIAN BUFFALOES

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ABSTRACT

The study was conducted on 187 graded Niliravi and Murrah buffaloes, 1 to 15 years, representing 15 districts of Punjab. Blood samples were collected two times from each buffalo. Mean values TEC, TLC, absolute lymphocyte numbers and activity of creatine kinase were higher in pregnant and non-pregnant heifers. Total serum bilirubin was higher in lactating pregnant buffaloes compared to lactating non-pregnant buffaloes. There was no difference ($P \leq 0.05$) in red cell parameters, platelet count, total leukocytes count (TLC), differential leukocyte counts (DLC), total serum protein, albumin, total bilirubin, cholesterol, plasma fibrinogen, aspartate aminotransferase (AST), alkaline phosphokinase (ALKP), gamma-glutamyl transferase (GGT), creatine kinase (CK), sodium (Na), potassium (K) and calcium (Ca) and iron metabolism analytes when compared by breed, parity and milk yield. Reference values were expressed as mean, standard deviation, range, reference intervals (5^{th} to 95^{th} percentiles) and as 95% confidence intervals of mean. The established reference values will be useful for the interpretation of haematolgical and biochemical profile for clinical diagnosis in buffaloes in the region.

Keywords: *Bubalus bubalis*, buffaloes, haematology, biochemistry, physiological status, reference values

INTRODUCTION

Haematology is the most readily available component of disease investigation. Complete blood count (CBC) invariably forming an integral part of minimum database for disease diagnosis. Apart from diagnosis it is helpful to determining the severity of illness, progression of the disease and to monitor treatment response. Next to haematology, metabolic profile is also useful in disease diagnosis. Many a times, metabolic derangements do not produce frank and characteristic clinical apparent abnormalities on physical examination. Under these circumstances, biochemical profile is helpful in detecting metabolic disturbances which helps in diagnosis. Forecasting nutritional imbalances

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and production diseases is coming up in high yielding dairy animals during transition period which is based on metabolic profile test. The metabolic profile test include many biochemical constituents *viz*. total protein, albumin, blood urea nitrogen, creatine kinase, cholesterol, for which baseline values are required in local population. According to the International Federation of Clinical Chemistry (IFCC) at least 120 animals should be used for establishing the reference values (Grasbeck *et al*., 1979). Perusal of literature on reference limits for haemato-biochemical profile shows that isolated studies are available in Indian buffaloes (Jain *et al*., 1982; Patil *et al*., 1992). However, the numbers of samples in this study do not satisfy the condition of IFCC. Haematological and biochemical values generated in a laboratory are also affected by test methodology, conditions of assays and sample handling. Therefore, the study was planned to generate the reference values in the university diagnostic laboratory by sampling more than 120 buffaloes representing all the age groups and physiological state.

MATERIALS AND METHODS

A total of 187 healthy buffaloes, aged 1 to 15 years, from 15 districts, were sampled. These animals were represented by 27 pregnant heifers, 21 non-pregnant heifers, 40 lactating pregnant buffaloes and 99 lactating non-pregnant buffaloes. Mean age of the enrolled buffaloes was 6.07±0.21 years. The criterion for the enrollment was: (a) no history of any clinical illness in last three weeks (b) body condition score between 3.0 to 3.5 (c) gestation period not more than five months. Rectal temperature and respiration rate were recorded before sample collection. Samples were collected twice for each animal during the period from September to December and January to April. Blood samples were collected and fresh blood smears were prepared immediately.

Collection of samples

Blood was collected aseptically by jugular venipuncture. Five milliliters of blood was collected in EDTA coated vials (Accuvete-PLUS, Quantum biologicals Pvt. Ltd) for complete blood count and for the estimation of fibrinogen. Thirteen milliliters of blood was collected in serum collection tubes, which was centrifuged to harvest the serum. The samples were transported on ice. Haematology was performed within 10 h of collection. Fresh blood smears were prepared immediately after blood collection. For serum biochemistry, blood was collected in 15 ml serum collection vials. Serum samples were separated within 12 h of collection. Serum was harvested (3000 rpm for 15 minutes) and stored in serum vials at -20°C for suspected analysis. The serum activity of aspartate aminotransferase (AST), alkaline phosphokinase (ALKP), gamma-glutamyl transferase (GGT) and total bilirubin were determined within 5 days of collection. The remaining biochemical constituents were analyzed within 7 days.

Analysis of samples

Haemoglobin (Hb), packed cell volume (PCV), total erythrocytes count (TEC), mean corpuscular volumes (MCV), mean corpuscular haemoglobin concentrations (MCHC), total leukocytes count (TLC) and platelet count were estimated by fully Automatic Laser Based Haematology Analyser (ADVIA 2120 Haematology system, Siemens Healthcare diagnostics Inc., USA). Differential leukocyte counts (DLC) were determined on the Leishman stained blood smears

by counting 100 cells as per Jain (1986). Stained blood smear were also examined for changes in erythrocyte and leucocyte morphology. Plasma fibrinogen was estimated by using heat precipitation method using refractometer (Schalm, 1975).

Serum was used for estimation of AST, ALKP, GGT, creatine kinase (CK), total serum proteins (TSP), total bilirubin, cholesterol, albumin, sodium (Na), potassium (K) and calcium (Ca) by fully automatic Vitros DT 350 Chemistry system (Ortho Clinical Diagnostics, Johnson & Johnson Company). Total iron (TI) and total iron binding capacity (TIBC) were determined colormetrically as described by Caraway (1963). Transferrin saturation (TS) was calculated by the formula:

$$TS\ (\%) = \frac{TI}{TIBC} \times 100$$

Statistical analysis

The statistical analysis was performed with Minitab statistical software (Minitab Inc., Version 14.2, State College, PA, USA). The numerical data on haematology and biochemistry was tested for normality. The average of the two values for each haemato-biochemical parameter was used. Descriptive statistics were calculated for each of the haemato-biochemical parameter and data were presented as mean, standard deviations (S.D.), 5th and 95th percentiles. Ninety five per cent confidence intervals (CI) were also calculated. Frequencies and proportions were calculated for categorical data. Significance of the results within the categorical variables (breed, physiological status, lactation status, milk yield, and parity) was analyzed using Student's unpaired *t*-test and Analysis of Variance (ANOVA) technique. For all tests, values of $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Haematology profile

Reference values for haematological parameters are presented in Table 1. Mean and reference intervals of Hb and PCV recorded in the present study were comparable to Jain *et al*. (1982) in Indian lactating Murrah buffaloes, Fagiolo *et al*. (2004) in Italian buffaloes and Ellah *et al*. (2014) in Egyptian buffaloes. However, lower values of mean Hb and PCV than Ciaramella *et al.* (2005) in Mediterranean buffaloes might be due to difference in nutritional status and/or climatic factors. Mean MCV values and reference intervals were lower as compared to Jain *et al.* (1982); Ellah *et al*. (2014), whereas MCHC was comparable to Jain *et al.* (1982) and higher than Fagiolo *et al.* (2004); Ellah *et al*. (2015). The reference interval for platelet count (160.0 to 304.0×10^3/µl) was similar to Ciaramella *et al.* (2005). The value of lower reference limit was higher (>2.5 times) than recorded in the Egyptian water buffaloes (Ellah *et al*., 2014; Ellah *et al*., 2015). The possible cause of difference could be clumping of buffalo's platelet as observed by Wills (2010). Reference intervals (6.48 to 12.6×10^3/µl) and mean (9.33×10^3/µl) values of TLC did not show any major variations from most of the studies (Jain *et al*., 1982; Ellah *et al*., 2014) except the mean TLC (7.96±0.80×10^3/µl) in Mediterranean buffaloes of Italy. Mean and reference intervals for absolute count of neutrophil and lymphocyte in the present study was higher than Ciaramella *et al.* (2005). On comparison with Egyptian buffaloes (Ellah *et al*., 2014; Ellah *et al*., 2015), the mean values was comparable, however the reference intervals showed wider distribution as compared to present study.

This difference was difficult to explain

because of many possible physiological and laboratory variations which may include *viz*. redistribution of leucocytes during restraint, analytical methods and climatic factors. The present study established mean and reference intervals for local buffalo's population in Punjab, which will be useful guide for interpreting haematological data during disease diagnosis. It was interesting to observe that band cells were lacking in healthy buffaloes. This was in contrast to cattle where absolute band cell numbers were present up to 0 to 120/µl (Radostitis *et al*., 2007).

Biochemical profile

Reference values for biochemical parameters are presented in Table 2. Mean and reference intervals for TSP and albumin were lower with narrower distribution compared to Tazik *et al*. (2012); Ellah *et al*. (2015). Mean total bilirubin value of 0.22 mg/dl in this study was lower (0.40 mg/dl) than reported by Ellah *et al*. (2014) and of 0.38 mg/dl by Ellah *et al.* (2015) in the Egyptian buffaloes. Reference interval for total bilirubin in this study (0.10 to 0.45 mg/dl) was narrowly distributed compared to Ellah *et al.* (2014). Mean and reference interval for the serum activity of AST was markedly higher than reported by others studies in buffaloes (Serdaru *et al*., 2011; Ellah *et al*., 2015) and lower than Bertoni *et al*. (1994); De Rosa *et al.* (2001). Mean and reference interval for the serum activity of GGT was two times higher than reported by others studies in buffaloes (Ellah *et al*., 2014; Ellah *et al*., 2015) and comparable to Bertoni *et al.* (1994). Mean and reference interval of serum ALKP activity in this study was lower than the Egyptian buffaloes (Ellah *et al*., 2014; Ellah *et al*., 2015).

Mean serum CK was 124.3 U/L, higher than values reported in pregnant buffaloes (Ali *et al*., 2011). Mean serum GGT activity was three times higher than reported by Ghanem and El-Deeb (2010) in non-pregnant buffaloes. Mean value for plasma fibrinogen from the studied animals was 420.3 mg/dl, which was higher than values reported in lactating Indian water buffaloes (Jain *et al*., 1982). Mean serum iron concentration was markedly higher than reported by Ellah *et al.* (2015) and closer to the values of Shahzadi *et al.* (2014) in buffaloes from Pakistan. Reference interval for serum iron and total iron binding capacity was higher than Ellah *et al*. (2014); Ellah *et al*. (2015). Mean and reference interval for serum cholesterol was 71.9 mg/dl and 48.4 to 98.1 mg/dl in this study. The mean values of serum sodium, potassium and calcium from were 143.6 mmol/l, 4.61 mmol/l and 10.2 mg/dl, respectively and were comparable to Egyptian buffaloes (Ellah *et al*., 2014; Ellah *et al*., 2015).

Haematological parameters in relation to physiological status

Mean TEC value of lactating non-pregnant buffaloes was lower ($P \leq 0.05$) compared to pregnant heifers, non-pregnant heifers and lactating pregnant buffaloes (Table 3). Total leucocyte count was lowest in lactating pregnant buffaloes and highest in pregnant heifers. Mean value of lymphocyte count was higher in pregnant heifers compared to lactating pregnant and lactating non-pregnant buffaloes (Table 3).

The observed changes in TEC and TLC count between heifers and adult buffaloes were similar to Ciaramella *et al.* (2005). This effect was probably due to reduced hemopoiesis due to reduced thyroid activity with aging (Jain *et al*., 1986). Higher number of lymphocytes in heifers was similar to Mammerickx *et al*. (1978) in different breeds of cattle.

Table 1. Reference value for haematology in adult healthy buffaloes (n=187).

Parameters	Mean	S.D.	Range	Reference Interval (5^{th}-95^{th} percentile)	95% CI for mean
Hb (g/dl)	11.9	1.44	8.9-15.8	9.47-14.2	11.7-12.1
TEC (10^6/µl)	7.0	0.91	5.12-9.30	5.64-9.30	6.87-7.14
PCV (%)	29.6	3.06	25.3-38.5	25.5-35.0	29.2-30.1
MCV (fl)	42.0	2.80	35.8-50.5	37.4-47.7	41.6-42.4
MCHC (g/dl)	37.4	0.80	34.8-39.2	36.0-38.5	37.3-37.5
TLC (10^3/µl)	9.33	2.04	5.80-15.5	6.48-12.6	9.04-9.62
Neutrophil count (10^3/µl)	3.52	1.05	1.36-6.45	2.01-5.39	3.37-3.67
Lymphocyte count (10^3/µl)	5.64	1.61	2.18-10.5	3.46-8.28	5.40-5.87
Eosinophil count (10^3/µl)	0.16	0.21	0-1.16	0-0.6	0.13-0.19
Monocyte count (10^3/µl)	0.002	0.01	0-0.09	0	0-0.004
Platelet count (10^3/µl)	233.2	67.3	131.5-604.0	160.0-340.9	223.4-242.9

Table 2. Reference value for biochemistry in adult healthy buffaloes (n=187).

Parameters	Mean	S.D.	Range	Reference Interval (5^{th}-95^{th} percentile)	95% CI for mean
TSP (g/dl)	6.88	0.70	5.25-9.0	5.84-8.18	6.78-6.98
Total bilirubin (mg/dl)	0.22	0.13	0.1-0.8	0.1-0.45	0.20-0.24
AST (U/L)	104.0	22.0	51.0-169.5	69.5-147.1	100.9-107.2
ALKP (U/L)	116.1	51.1	44.5-311.5	49.8-220.4	108.7-123.4
Albumin (g/dl)	2.70	0.36	2.0-4.4	2.2-3.33	2.65-2.76
CK (U/L)	124.3	39.1	47.0-287.5	73.5-205.7	118.7-130.0
Fibrinogen (mg/dl)	420.3	134.1	200.0-800.0	200.0-600.0	401.0-439.7
Cholesterol (mg/dl)	71.9	15.1	43.5-113.5	48.4-98.1	69.8-74.1
GGT (U/L)	28.4	8.04	13.0-55.0	16.0-42.8	27.2-29.6
TI (µg/dl)	159.7	33.8	81.7-242.4	100.8-215.0	154.9-164.6
TIBC (µg/dl)	344.4	82.8	185.4-571.9	218.2-490.0	332.4-356.3
TS (%)	49.7	12.6	20.3-82.2	28.7-70.8	47.8-51.5
Na (mmol/l)	143.6	11.1	122.5-174.0	125.7-164.8	142.0-145.2
K (mmol/l)	4.61	0.63	2.85-6.65	3.55-5.9	4.52-4.70
Ca (mg/dl)	10.2	1.01	8.55-13.4	8.72-12.3	10.0-10.3

Table 3. Effect of physiological status on haematological values of adult healthy buffaloes.

Parameters	Pregnant heifer (n=27)			Non-pregnant heifer (n=21)			Lactating pregnant buffalo (n=40)			Lactating non-pregnant buffalo (n=99)		
	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range
Hb (g/dl)	12.2^{a}	1.86	8.90-15.9	12.0^{a}	1.53	9.50-14.9	12.2^{a}	1.29	8.9-14.2	11.7^{a}	1.32	9.05-14.4
TEC (10^6/µl)	7.37^{a}	1.19	5.36-9.30	7.11^{ab}	0.92	5.64-8.68	7.10^{ab}	0.93	5.12-8.54	6.85^{b}	0.79	5.38-8.36
PCV (%)	30.6^{a}	3.87	25.5-38.6	29.9^{a}	3.41	25.3-36.0	29.7^{a}	2.68	25.6-34.0	29.3^{a}	2.86	25.4-37.5
MCV (fl)	41.8^{a}	2.96	37.3-47.6	41.7^{a}	2.35	38.1-47.9	41.7^{a}	2.44	35.8-46.7	42.2^{a}	3.00	37.4-50.5
MCHC (g/dl)	37.5^{a}	0.91	35.9-39.2	37.6^{a}	0.88	35.8-38.9	37.2^{a}	0.68	36.0-38.9	37.4^{a}	0.80	34.8-39.2
TLC (10^3/µl)	10.6^{a}	2.76	6.26-15.5	10.1^{ab}	1.68	6.92-14.8	8.72^{c}	1.73	5.95-12.0	9.07^{bc}	1.82	5.80-12.4
Neutrophil count (10^3/µl)	3.77^{a}	1.19	2.0-6.45	3.72^{a}	1.10	2.28-5.90	3.47^{a}	1.00	1.83-5.78	3.43^{a}	1.01	1.36-6.39
Lymphocyte count (10^3/µl)	6.63^{a}	2.18	3.25-10.5	6.18^{ab}	1.57	2.56-8.70	5.12^{b}	1.42	2.89-8.25	5.46^{b}	1.37	2.18-8.67
Eosinophil count (10^3/µl)	0.18^{a}	0.24	0-0.85	0.23^{a}	0.24	0.75	0.12^{a}	0.17	0-0.72	0.15^{a}	0.20	0-1.16
Monocyte count (10^3/µl)	0.004^{a}	0.02	0-0.06	0.008^{a}	0.02	0-0.09	0	0	0	0.001^{a}	0.01	0-0.09
Platelet count (10^3/µl)	226.6^{a}	0.37	160.0-306.0	229.3^{a}	0.55	165.5-342.0	232.0^{a}	0.74	131.5-581.0	236.2^{a}	0.74	137.0-604.0

Values having different superscript in same row differ significantly ($P \leq 0.05$).

Table 4. Effect of physiological status on biochemical values of adult healthy buffaloes.

Parameters	Pregnant heifer (n=27)			Non-pregnant heifer (n=21)			Lactating pregnant buffalo (n=40)			Lactating non-pregnant buffalo (n=99)		
	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D.	Range	Mean	S.D	Range
TSP (g/dl)	6.97[a]	0.94	5.25-9.00	7.06[a]	0.83	5.80-8.75	6.97[a]	0.68	5.55-8.55	6.77[a]	0.58	5.60-8.50
Total bilirubin (mg/dl)	0.19[ab]	0.08	0.10-0.35	0.21[ab]	0.13	0.10-0.60	0.27[a]	0.18	0.10-0.80	0.20[b]	0.11	0.10-0.60
AST (U/L)	102.7[a]	22.2	51-152.5	106.0[a]	24.8	65.5-169.5	107.2[a]	24.0	60.5-168.0	102.7[a]	20.8	56.0-161.0
ALKP (U/L)	113.8[a]	53.6	47.0-273.5	102.4[a]	44.9	45.5-235.0	131.1[a]	52.8	49.0-311.5	113.5[a]	50.4	44.5-306.5
Albumin (g/dl)	2.61[a]	0.38	2.0-3.65	2.70[a]	0.30	2.15-3.30	2.83[a]	0.36	2.20-3.65	2.68[a]	0.36	2.10-4.40
CK (U/L)	136.2[ab]	62.2	70.5-275.0	154.9[a]	57.5	81.5-287.5	115.9[b]	24.7	71.0-167.0	118.0[b]	25.6	47.0-174.0
Fibrinogen (mg/dl)	407.4[a]	156.7	200.0-700.0	476.2[a]	148.0	200.0-700.0	425.0[a]	131.6	200.0-700.0	410.1[a]	124.1	200.0-800.0
Cholesterol (mg/dl)	68.3[a]	15.5	45.0-93.0	69.2[a]	12.2	43.5-86.5	73.5[a]	13.4	45.0-100.5	72.9[a]	16.1	45.0-113.5
GGT (U/L)	28.3[a]	5.67	19.5-40.5	30.3[a]	9.86	14.0-55.0	28.5[a]	6.63	14.0-41.5	28.0[a]	8.71	13.0-51.5
TI (µg/dl)	154.1[a]	34.8	86.6-215.0	158.0[a]	29.8	81.7-213.9	162.1[a]	31.2	99.3-218.2	160.7[a]	35.6	91.0-242.4
TIBC (µg/dl)	330.1[ab]	84.9	197.6-505.6	291.6[b]	66.0	185.4-406.6	376.7[a]	81.1	185.6-518.0	346.5[a]	80.7	206.0-571.9
TS (%)	49.5[ab]	14.4	22.2-82.2	56.9[a]	11.1	40.9-77.4	46.1[b]	11.9	25.2-73.5	49.6[ab]	12.2	20.3-79.7
Na (mmol/l)	141.0[a]	10.8	122.5-159.5	140.3[a]	8.31	123.0-154.0	146.8[a]	11.8	126.0-170.5	143.7[a]	11.1	123.0-174.0
K (mmol/l)	4.76[a]	0.80	3.50-6.65	4.71[a]	0.64	3.50-6.05	4.74[a]	0.58	3.55-6.30	4.50[a]	0.59	2.85-5.95
Ca (mg/dl)	9.91[a]	0.76	8.55-11.8	10.1[a]	0.84	8.60-11.8	10.1[a]	0.93	8.55-12.8	10.2[a]	1.13	8.55-13.4

Values having different superscript in same row differ significantly ($P \leq 0.05$).

Biochemical parameters in relation to physiological status

Mean value of total bilirubin was similar in pregnant heifers, non-pregnant heifers, lactating pregnant and lactating non-pregnant buffaloes (Table 4). Mean activity of CK was highest in non-pregnant heifers followed by pregnant heifers. The result of the present study showed an inverse relationship between age and activity of CK (Table 4).

Haematological and biochemical parameters in relation to breed, parity and milk yield

It was interesting to note that values of mean as well as range of haematological and biochemical parameters were very close to each other with respect to parity. Similar to the present study, Chandra *et al.* (2008) did not record any significant difference in Hb, PCV, TEC, MCV, MCHC and TLC of graded Murrah buffaloes sampled from the age of 3 years up to 10 years and above. In contrast, Ciaramella *et al.* (2005) recorded significant fall in Hb, PCV, TEC, TLC, neutrophil and lymphocyte count in non-lactating Mediterranean buffaloes from the age of eight years and above.

There was no effect ($P \leq 0.05$) of breed and milk yield on red cell parameters, TLC, DLC and biochemical constituents. Similarly, no comparable study on haemato-biochemical parameters in relation to breed and milk yield could be traced in scientific literature. It was inferred that under the similar feeding and management conditions, no difference should be expected in haematological and biochemical parameters between Murrah and Niliravi and level of milk production.

CONCLUSION

The present study established the reference values for haematological and biochemical constituents buffaloes. Mean values for TLC, lymphocyte count and CK were higher in buffalo heifers. These values will be useful for interpreting haemato-biochemical parameters in she buffaloes in the age group of 1 to 15 years.

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