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Establishment of Haemato-biochemical Reference Values in Yak (*Bos grunniens*) in Cold Arid Himalayan Region of Ladakh India

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ABSTRACT

Background: Yak population is much less in comparison to total livestock population of India but still it commands importance in the life of the mountainous regions adjacent to the Himalayas. Establishing reliable reference values for hematological and biochemical parameters for apparently healthy animal populations is a quite difficult task, however it is essential to provide baseline information for assessing the population health and disease.

Methods: A total of 180 samples comprising 90 samples each of male and female yak of above one year of age were analysed for various haemato-biochemical, enzymes and trace elements. The data obtained during the study was analyzed by using Minitab statistical software Version 21.1.0.

Result: The female yaks had significantly higher haematological values of Hb, HCT and MCV values while rest of the haematological parameters showed no significant alteration between two sexes. The female yaks showed significantly lower levels of glucose, cholesterol, triglycerides and high-density lipoproteins and significantly higher levels of urea and low density-lipoproteins similarly female yaks showed higher alkaline phosphatase and lower lipase activity than male yaks. The female yaks had significantly higher trace element levels of sodium and magnesium.

Key words: Biochemical, Haematological, Kargil, Reference values, Yak.

INTRODUCTION

Living at the higher altitudes involve various environmental changes with the joint effects of cold stress and hypoxia causing severe physiological challenges and compromised cellular functions for the endothermic animals. Such a harsh environment is characterized by lower atmospheric oxygen supply, limited feed supplies and extreme cold. These regions of high altitudes are more prone and vulnerable to climate change, thus posing a great threat to biodiversity and the ecosystem. In addition to cold harshness, other factors like snow cover, late winter and early arrival of spring inevitably leads to malnutrition and weight loss among animal populations residing thereby. However, animal species over the time have developed special features through natural selection to adapt to these severe environmental conditions.

The yak (*Bos grunniens*) is the world's utmost domestic animal living and reproducing in these severe environmental conditions. The yak is the only multipurpose, large hairy bovine species that possess a great adaptability to survive in regions of higher altitudes, severe cold, lower atmospheric pressure and prolonged duration of food scarcity. Yaks are traditionally raised in high hills under a free-range system where the pasture, air and water are free from any pollution, and their products are all-natural and organic. Great insights into the adaptive evolution of yaks can be depicted under hypoxic conditions by understanding the aerobic metabolism of these animals. The Yak (*Bos grunniens*) has a distinctive genetic makeup and is a unique bovine species of economic importance in high hill and snow-covered areas (Pal and Barari, 1994).

Establishing trustworthy hematologic and biochemical reference values for a population of healthy, free-ranging animals is a difficult task, but it is crucial for giving the starting point for determining the population's health and sickness. Blood is the soul of all flesh and its significance for assessing an individual's health, a survey's chemical

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results, pathological problems, and the diagnosis and prognosis of various animal diseases cannot be overstated (Tambuwal *et al*., 2002; Alade *et al*., 2005). Additionally, it aids in separating a normal state from a stressed state, which can be physical, environmental, or related to maturation (Aderemi, 2004). Hematological measurements have several uses in establishing a systematic link and physiological adaptation, primarily involving assessing the animal health state (Kamal Shah *et al*., 2007). In dairy cattle and other animals, the alterations in these parameters have been studied (Ghergariu *et al*., 1984) sheep (Kausslish and Arora 1977). There are many factors that can affect the composition of blood in animals such as diet, management, sex, age and stress factors (Schalm *et al*., 1975). Haematological and biochemical indices are the indicator of health status and represent the impact of animal dietary care in regard to amount and feed type consumed to fulfil their physiological, biochemical and metabolic requirements (Ewuola *et al*., 2004), additionally, the presence of anti-nutritional components or elements in the feed has an impact on the hematological and biochemical values (Akinmutimi, 2004). The haematological and biochemical parameters observed between races of the same species differ greatly (Azab and Abdal-Maksoud, 1999) and it may be difficult to devise a universal metabolic profile test for the same species in this respect (Opara *et al*., 2010). These variations have further highlighted the need to establish reasonable physiological baseline values for various livestock breeds that could help in the practical evaluation of management strategy, nutrition and health status diagnosis.

Ladakh, a remote, cold and arid region located 3,500 metres above average sea level, endures challenging weather conditions all year long. About four months of the year are spent in summer, and the remaining eight are spent in winter. Environmental factors are therefore important physiological stressors that have an impact on an animal's biological system, haematological and biochemical indices and animal productivity. For the assessment of the animal's normal physiological status, the haematological and biochemical values are crucial. However, there have been very few or no studies on yak with smaller sample sizes. The discrepancy has highlighted the need for yaks to have appropriate physiological baseline values established, as this will aid in a realistic assessment of the management approaches, nutrition and diagnosis of health states.

MATERIALS AND METHODS

Animals

The study's protocols were all approved by the Institutional Animal Ethics Committee, vide No: AU/FVS/PS-57/21/5403 dated 12-08-2021. The current study was conducted in the Division of Veterinary Biochemistry, F.V.Sc. and A.H., SKUAST-Kashmir. The samples were collected from Kargil district of the Union Territory of Ladakh area in the north of Kashmir valley at an altitudes ranging from 8000 to 18000 feet above sea level and located between 32.57′ and 34.45′ latitude north and 75.35' longitude east. A total of 180 animals of > 1 year age comprising 90 each of male and female clinically health yaks were included in the study.

Sample collection

15 ml of blood was collected from the jugular vein out of which 12 ml were put in plain tube for collection of serum and 3 ml were put in a plain vacutainer tube containing EDTA and used for estimation of various haematological parameters. Samples were directly transported on ice packs to the research laboratory of the Department of Sheep Husbandry within 1 hour after collection. Samples were immediately processed in the laboratory, the samples in plain tubes were centrifuged at 3000 rpm for 15 minutes and serum was harvested. The serum was divided into two parts, the first part was stored at 4°C under refrigeration for protein and lipid analysis while the other part was stored at -20°C and used for analysis of various biochemical constituents. The samples were collected in June, July and August to obtain the baseline data.

Haematological analysis

The haematological analysis was carried out by using an automatic analyzer Erba H360 within 1-2 hours of sample collection. The blood variables measured were white blood cell count (WBCs), red blood cell count (RBCs), Haemoglobin (Hb), Haematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Red cell distribution width-coefficient of variation (RDW-CV), Red cell distribution width-standard deviation (RDW-SD), Platelets (PLT) count, Mean platelet volume (MPV), Platelet distribution width-coefficient of variation (PDW-CV), platelet distribution width-standard deviation(PDW-SD) and Platelet crit (PCT).

Biochemical analysis

Serum proteins, albumin and glucose

Serum total proteins, albumin and glucose were estimated by using an automatic analyzer Erba Chem5 through commercially available kits from ERBA diagnostics, Mannheim Germany.

Other biochemical constituents

Creatinine, total bilirubin, direct bilirubin and uric acid were estimated by using an automatic analyzer Erba Chem5 through commercially available kits from ERBA diagnostics, Mannheim Germany.

Serum lipid profile

Utilizing commercially available kits from Coral Clinical Systems-Goa, India, the serum levels of total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were measured using automatic analyzer Erba Chem5.

Serum enzyme activities

Serum enzyme activity of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH),

alkaline phosphatase (ALP), creatine kinase (CK), amylase and lipase by commercially available kits from ERBA diagnostics, Mannheim- Germany using automatic analyzer Erba Chem5.

Serum trace elements

Serum trace elements sodium (Na), potassium (K), magnesium (Mg), zinc (Zn), copper (Cu), iron (Fe), calcium (Ca) and phosphorus (P) by commercially available kits from Medsource Ozone Biomedicals Pvt. Ltd. And Accurex Biomedical PVT. Limited-Mumbai, India using automatic analyzer Erba Chem5.

Statistical analysis

The data collected for the study was under the guidelines of the approved recommendations on the theory of Reference Values from the International Federation of Clinical Chemistry (IFCC) (Solberg 1987). Minitab statistical software Version 21.1.0 was used to conduct the statistical analysis.

RESULTS AND DISCUSSION

Haematological analysis

The haematological parameters with respect to 180 male and female yaks (*Bos grunniens*) of above 1 year of age are presented in Table 1. The male yak had significantly lower HCT and MCV values of 38.24±10.4 and 73.31±19.1 with reference intervals of 17.86-58.62 and 35.88-110.74 compared to female yaks 42.99±6.9 and 86.01±28.5 with reference intervals of 29.47-65.51 and 30.15-141.87 respectively. The male yaks had lower Hb, WBC count, MCH, MCHC, RDW-CV, RDW-SD, MPV, PDW-CV, PDW-SD and PCT values and higher RBC count and PLT mean values compared to female yaks but the difference was statistically nonsignificant.

Biochemical analysis

The biochemical profile of 180 male and female yaks (*Bos grunniens*) of above 1 year of age is presented in Table 2. The male yaks had significantly higher amounts of

Table 1: Mean (±SD) of haematological parameters in yak (*Bos grunniens*) (n=180 M/F 90 each).

Parameter	Sex (M/F)	Mean (±SD)	Min	Max	Reference intervals
WBC ($\times 10^3/\mu l$)	Male	9.09±1.68	4.42	13.40	5.80-12.38
	Female	9.19±1.54	3.50	12.83	6.18-12.20
RBC ($\times 10^6/\mu l$)	Male	5.31±0.94	3.0	7.60	3.47-7.15
	Female	5.20±0.91	3.00	7.05	3.42-6.98
Hb (g/dl)	Male	10.40±1.56	4.40	14.20	7.35-13.45
	Female	11.04±1.06	8.40	13.20	8.97-13.11
HCT (%)	Male	38.24±10.40	16.20	62.80	17.86-58.62
	Female	42.99±6.9	29.20	62.80	29.47-56.51
MCV (fL)	Male	73.31±19.10	31.20	117.00	35.88-110.74
	Female	86.01±28.5	27.90	165.30	30.15-141.87
MCH (pg)	Male	19.94±3.43	12.90	32/30	13.25-26.69
	Female	21.78±3.46	13.70	29.50	15.00-28.56
MCHC (g/dl)	Male	28.44±5.89	15.70	41.40	16.90-39.98
	Female	27.19±6.64	12.20	42.50	14.18-40.20
RDW-CV (%)	Male	13.97±2.29	8.30	19.10	9.49-18.45
	Female	13.81±2.03	8.30	19.30	9.84-17.78
RDW-SD (fL)	Male	43.30±8.18	24.90	63.90	27.27-59.33
	Female	45.26±3.05	24.30	59.60	39.29 -51.23
PLT ($\times 10^3/\mu l$)	Male	254.85±57.40	110.00	371.00	142.35-367.3
	Female	253.7±43.90	150.00	366.00	167.62-339.8
MPV (fL)	Male	9.26±1.15	6.50	12.10	7.01-11.51
	Female	9.68±1.21	6.30	13.10	7.31-12.05
PDW-CV (%)	Male	15.27±1.40	10.80	19.50	12.53-18.01
	Female	15.52±1.53	12.00	19.50	12.53-18.51
PDW-SD (fL)	Male	11.50±2.91	4.80	17.50	5.80-17.20
	Female	12.52±3.05	6.00	18.60	6.55-18.49
PCT (%)	Male	0.240±0.063	0.086	0.384	0.117-0.363
	Female	0.245±0.05	0.092	0.364	0.147-0.343

Values are presented as Mean standard deviation (SD). WBC = White blood cells; RBC = Red blood cells; Hb = Hemoglobin; HCT = Hematocrit; MCV = Mean cell volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; RDW-CV = Red cell distribution width; RDW-SD = Red cell distribution width PLT= Platelet count; MPV = Mean platelet volume; PDW-CV = Platelet distribution width; PDW-SD = Platelet distribution width; PCT = Platelet crit.

glucose, cholesterol, triglycerides (TG) and high density lipoprotein (HDL) values of 91.15±6.82, 193.40±19.5, 141.80±15.3, 42.12±10.12 with reference intervals of 77.8-104.5, 155.1-231.6, 111.8-171.7, 22.29-61.95 respectively compared to female yaks 55.64± 6.7, 104.96± 24.7, 111.93± 24.6, 37.41±12.56 with reference intervals of 42.5-68.7, 56.55-153.37, 63.7-160.14, 12.8-62.00 and lower levels of urea and low density of lipoproteins (LDL) values of 40.94 ± 5.95 and 75.41±17.1 with reference intervals of 29.28-52.60 and 41.90-108.9 compared to female yaks 51.18± 10.0 and189.15±49.4 with reference intervals of 31.58-70.78 and 92.33-286.0 however, rest of the indices did not show any significant alteration between sexes.

Enzyme estimation

The enzyme profile of 180 male and female yaks (*Bos grunniens*) of above 1 year of age is shown in Table 3. The male yaks had significantly higher alanine transaminase (ALT), amylase and lipase activities of 26.12±5.20, 77.24± 16.8 and 48.84±12.9 with reference range of 15.93-36.31, 44.32-110.2 and 23.56-74.12 compared to female yaks with 22.36±2.97, 60.72±10.0 and 38.08±8.98 with reference range of 16.54-28.18, 41.12-80.32 and 20.48-55.68 activities respectively, however male yaks had significantly higher amylase activity compared to female yaks. The rest of the enzymes did not exhibit any significant difference in their activity.

Trace element estimation

The trace element levels obtained in 180 male and female yaks (*Bos grunniens*) of above I years of age is presented in Table 4. The male yaks had significantly higher levels of sodium (Na) 120.51±32.9 with a reference range of 56.03-184.99 compared to female yaks 105.84±14.4 with a reference range of 77.62-134.06. The rest of the elements did not show any significant alteration between the sexes.

The animal is healthy in the absence of any disease after going through a combination of clinical examinations and different diagnostic tests (Bailey *et al*., 1989; Klinkhoff *et al*. 1988; Pattinson and Theron 1989). The biochemical and haematological reference intervals play a key role in establishing the normal and disease state of the animal (Kumar *et al*., 2018). The reference intervals observed by European or American researchers are based on the animals enjoying better husbandry conditions and therefore may vary from the developing countries where husbandry

Table 2: Mean (±SD) of biochemical parameters in yak (*Bos grunniens*) (n=180 M/F 90 each).

Parameter	Sex (M/F)	Mean (±SD)	Min	Max	Reference intervals
Protein (g/dl)	Male	6.61±0.54	5.35	7.90	5.5-7.6
	Female	6.74±0.73	5.64	8.75	5.7-7.8
Albumin (g/dl)	Male	3.26 ± 0.30	2.51	3.96	2.70-3.90
	Female	3.13±0.33	2.10	3.99	2.5-3.8
Glucose (mg/dl)	Male	91.15±6.82	55.93	173.50	77.8-104.5
	Female	55.64±6.70	40.66	81.10	42.5-68.70
TB (mg/dl)	Male	1.60±0.32	0.20	2.52	0.99-2.20
	Female	1.12±0.35	0.29	1.90	0.44-1.80
DB (mg/dl)	Male	0.19±0.05	0.02	0.29	0.10-0.28
	Female	0.37±0.08	0.21	0.63	0.22-0.52
Urea (mg/dl)	Male	40.94±5.95	10.00	100.50	29.28-52.60
	Female	51.18±10.0	30.66	92.45	31.58-70.78
Creatinine (mg/dl)	Male	1.45±0.65	0.16	2.83	0.18-2.72
	Female	1.63±0.07	1.37	2.34	1.50-1.76
Uric acid (mg/dl)	Male	3.16±1.10	0.61	6.35	1.01-5.31
	Female	4.08±1.10	1.24	7.99	1.93-6.23
Cholesterol (mg/dl)	Male	193.40±19.5	106.20	273.10	155.1-231.6
	Female	104.96±24.7	52.74	215.20	56.55-153.37
TG (mg/dl)	Male	141.80±15.3	57.00	212.00	111.8-171.7
	Female	111.93±24.60	60.36	195.10	63.7-160.14
HDL (mg/dl)	Male	42.12±10.12	21.83	89.62	22.29-61.95
	Female	37.41±12.56	10.06	77.81	12.80-62.00
LDL (mg/dl)	Male	75.41±17.11	25.93	119.90	41.90-108.9
	Female	89.15±49.4	83.00	288.10	92.33-286.0
VLDL (mg/dl)	Male	21.96±5.02	10.90	34.80	12.13-31.71
	Female	24.63±3.85	15.90	34.90	17.09-32.17

Values are presented as Mean±standard deviation (SD). TB = Total bilirubin; DB. = Direct bilirubin; TG = Triglycerides; HDL = High density lipoprotein; LDL = Low density lipoprotein; VLDL = Very low density lipoprotein.

conditions are not up to the mark. The differences in reference intervals may be influenced by various factors such as genetic factors, feed and fodder quality, environmental conditions and good husbandry conditions, therefore relying on these reference intervals have limitations and there is need to have reference intervals to interpret the health status of the animals in this part of the world. To the best of our knowledge and literature survey the reference values for various haemato-biochemical indices in yak have not been established, therefore, the present study was planned to establish haematological and biochemical reference intervals in yaks.

In the present study, the comprehensive reference values for a wide range of biochemical, haematological, enzymatic and mineral profiles was observed in yak (*Bos grunniens*) as per the guidelines of International Federation of Clin Kical Chemists (IFCC) (Lumsden, 1998). The animals were divided into subgroups for gender to assess the variation in physiologic status. The reference intervals or reference values were observed by employing parametric and nonparametric methods. The nonparametric analysis utilizing the 90th percentile was employed for haematological parameters in the present study to avoid assumptions with respect to the distribution pattern of each parameter (Solberg, 1999). The reference range for the rest of the parameters was calculated parametrically and for the data analysis, 2.5 and 97.5 percentiles were estimated by taking the mean and ± of 1.96 standard deviations respectively (Solberg, 1996).

Table 3: Mean (±SD) of enzyme parameters in yak (*Bos grunniens*) (n=180 M/F 90 each).

Parameter	Sex (M/F)	Mean (±SD)	Min	Max	Reference intervals
AST (IU/L)	Male	27.48±6.93	11.00	46.00	13.90-41.00
	Female	30.33±6.62	15.00	46.00	17.30-43.30
ALT (IU/L)	Male	23.33±8.78	5.00	56.00	6.10-40.50
	Female	23.67±5.94	11.00	41.00	12.00-35.30
LDH (IU/L)	Male	133.45± 22.3	86.00	180.00	89.70-177.10
	Female	135.90±21.70	89.00	180.00	93.30-178.40
ALP (IU/L)	Male	28.92±7.82	11.00	47.00	13.60-44.20
	Female	48.10±14.70	18.00	99.00	19.20-77.00
CK (IU/L)	Male	18.22±3.50	10.00	27.65	11.3-25.08
	Female	16.32±1.82	9.00	22.00	12.70-19.80
Amylase (IU/L)	Male	77.00±18.5	37.00	121.00	40.2-113.20
	Female	68.20±15.90	32.00	121.00	37.00-99.30
Lipase (IU/L)	Male	42.75±12.4	16.00	72.93	18.40-67.00
	Female	42.53±11.70	19.00	73.00	19.60-65.40

Values are presented as Mean±standard deviation (SD). AST = Aspartate transaminase; ALT = Alanine transaminase; LDH = Lactate dehydrogenase; ALP = Alkaline phosphate; CK = Creatine kinase.

Table 4: Mean (±SD) of trace elements parameters in yak (*Bos grunniens*) (n=180 M/F 90 each).

Parameter	Sex (M/F)	Mean (±SD)	Min	Max	Reference intervals
Na (meq/l)	Male	134.30±9.35	112.20	158.60	115.98-152.6
	Female	155.12±17.80	101.00	197.00	116.24-186.0
K (meq/l)	Male	6.33±1.74	1.08	13.55	2.92-9.74
	Female	5.98±1.17	1.79	8.40	3.69-8.27
Mg (meq/l)	Male	2.83±0.30	2.11	3.70	2.25-3.41
	Female	10.74±1.99	3.99	20.98	6.84-14.64
Zn (μg/ml)	Male	0.92±0.14	0.60	1.22	0.65-1.19
	Female	0.88±0.21	0.37	1.76	0.08-1.28
Cu (μg/ml)	Male	0.35±0.10	0.12	0.57	0.16-0.54
	Female	0.68±0.31	0.03	1.76	0.47-1.29
Fe (μg/ml)	Male	6.03±0.49	4.97	7.80	5.07-6.99
	Female	5.18±1.57	2.00	8.90	2.11-8.25
Ca (meq/l)	Male	10.03±3.06	3.90	17.94	4.04-16.02
	Female	8.83±0.93	6.77	11.53	7.01-10.65
P (mg/dl)	Male	2.80±1.09	0.49	5.71	0.67-4.93
	Female	3.02±1.26	0.44	7.16	0.56-5.48

Values are presented as Mean standard deviation (SD). Na = Sodium; K = Potassium; Mg = Magnesium; Zn = Zinc; Cu = Copper; Fe = Iron; Ca = Calcium; P = Phosphorus.

In the present study the calculated haematological reference intervals observed for adult yaks are comparable with a single yak reported by (Schalm *et al*., 1975), (Barari *et al*., 1995), (Mondal *et al*., 1998) and 21 male and female yaks reported by Hawkey *et al*. (1983). In the present study higher WBC values of 9.09±1.68 and 9.19±1.54 with reference range of 5.80-12.38 and 6.18-12.20 in male and female yaks respectively were observed than those reported by (Hawkey *et al*., 1983) (6.6±1.2) with a reference range of (4.2-9.0). The Hb values as presented in Table 1 observed with adult male and female yaks were lower as previously reported by Hawkey *et al*. (1983) (Hb13.7±1.7 with reference range of 10.3-17.1), Schalm *et al*. (1975) (12.8 g/dl) but higher than those reported by Joshi, (1991) (9 g/dl). The increase in Hb value indicates the adoption of the animal in the environment having deficient oxygen (Winter *et al*., 1989). In the present study we observed higher MCV values in both male and female yaks than those reported by Schalm *et al*. (1975) (58.0 fl), Hawkey *et al*. (1983) (58±4.9), Barari *et al*. (1995) (49.31±1.25) and Mondal *et al*.,(1998) (54.19±2.19) and lower levels of MCHC as previously reported by Hawkey *et al*. (1983) (36.3±1.5), Barari *et al*. (1995) (40.96±0.69) and Mondal *et al*. (1998) (39.34±1.04). The MCH levels were in agreement with the earlier findings of Schalm *et al*. (1975) (20.6 pg), Hawkey *et al*. (1983) and Joshi, (1991) and lower than those reported by Mondal, (1998) (21.22± 0.77). The PLT count observed for adult male and female yaks during the present study was higher than reported by Hawkey *et al*. (1983) (251±470) with reference range of (157-345). As per the literature survey this is the first study to present the reference intervals or range with respect to HCT, RDW-CV, RDW-SD, MPV, PDW-CV, PDW-SD and PCT.

The reference range for various serum biochemical parameters, enzymes and macro and micro minerals as per the literature survey are not available for yaks, hence, this is the first study to determine the reference values for various enzymes, and macro and micro minerals in yaks. The results obtained in the present study are compared with the few studies with lesser sample sizes carried out in yak and other large bovid species. The lower protein levels as presented in Table 2 were observed in male and female yaks than those reported by Medhi *et al*. (2018) (7.22±0.14; 7.42) respectively, however similar findings have been reported by Giri *et al*. (2017) in dairy cattle in high altitude cold desert it may be due to lesser intake of water which may have resulted in the concentration of plasma proteins (Meyer and Harvey 1998). However, there was a significant reduction in albumin levels during winter, which is in contradiction to the findings of Giri *et al*. (2017).

As per the literature survey no studies have been carried out in yaks with respect to bilirubin, creatinine, uric acid, cholesterol, TG, HDL, LDL and VLDL, however these were within reference range compared to large bovid species.

CONCLUSION

In dairy animals, the impact of dietary care is indicated by the hematological and the biochemical parameters as they reflect the type and quantity of feed consumed to fulfil their physiological, biochemical, and metabolic requirements, as well as the amount of anti-nutritional elements. It may be challenging to develop a universal metabolic profile test for the same species in this regard due to the significant differences in haematological and biochemical parameters seen between races within the same species. These variations have further highlighted the need to establish reasonable physiological baseline values for various livestock breeds that could help in the practical evaluation of management strategy, nutrition and health status diagnosis.

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Disclaimers

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Conflict of interest

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