



Basis blood indices, serum biochemical profiles, antioxidant and oxidative profiles of endangered local buffaloes of Andaman and Nicobar Islands at different stages of reproduction

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ABSTRACT

Andaman and Nicobar Islands (ANI) have non-defined and non-descriptive breed of buffaloes. Andaman local buffaloes (ALB) are distributed in Andaman group of Islands and few numbers are available in Campbell Bay and Nicobar group of Islands. Andaman local buffaloes represent an admixture of different Indian breeds that had been brought to these islands in different phases of inhabitation and rehabilitation of migrated people. It is believed that the ALBs have the inheritance from Murrah, Nagpuri, Bhadawari and Marathwada. It is unique buffalo, well adapted to special type of tropical humid island climatic and environmental condition. The present study was designed to standardise the normal reference haematological and biochemical ranges in different stages of reproduction of Andaman local buffaloes which were maintained in the Andaman Districts of ANI, India from April 2019 to March 2020. In this study, haematological parameters, biochemical indices and antioxidant and oxidative stress profiles were estimated in healthy, normal physiological ALBs in different reproductive stages. The present study results clearly indicated that the values of haematological, biochemical and antioxidant profiles fell under the normal physiological ranges. The results of this study may serve as the reference values in which alterations due to metabolic, nutrient deficiency, physiological and health status can be compared for diagnostic and therapeutic purposes for ALBs in ANI and its neighboring countries or other parts of the country with similar environmental and climatic conditions.

Keywords: Andaman local buffaloes, Antioxidant profiles, Biochemical profiles, Haematology, Reproductive stages

ANI have non-descriptive breed of Andaman local buffaloes which are distributed in Andaman Islands and few numbers are in Campbell Bay and Nicobar groups of Islands (De *et al.* 2019) and are an admixture of different Indian buffalo breeds. Genetic root and phylogenetic history of ALB have been studied recently. It is well adapted to tropical humid island ecosystem and its population is declining rapidly due to the unavailability of proper breeding and feeding strategy. Importance of determining the biochemical and haematological indices of domestic livestock species have been well acknowledged and documented (Opara *et al.* 2006). Haematological and biochemical values can provide the strong valuable baseline information which in turn helps to assess the realistic evaluation of management practices, physiological and nutritional status of the animals and also help to diagnose and assess the health condition or status (Radostits *et al.* 2006, Jezek *et al.* 2013). Metabolic disorders, nutritional deficiencies and prevalence of the diseases can be easily detected by proper analysis as well as monitoring of blood

and other body fluids (Otto *et al.* 2000). However, this requires for establishment of normal reference values for the particular species. Pathological values are defined as those values deviating from the normal standard reference values (Radostits *et al.* 2006). Evaluation, analysis and interpretation of the obtained results mainly depend on the standard reference values for different species of the animals in different regions as well as under existing environmental or climatic conditions. Since the ALBs used in the present study did not show any significant clinical signs and/or pathological symptoms, therefore they were believed as healthy animals and the result or data observed can serve as standard reference values for these animals in future (Kaneko *et al.* 1997). The data given in the present communication can serve as the standard reference values for ALBs grown in ANI and other islands having similar climatic and nutritional conditions.

MATERIALS AND METHODS

A total of 30 ALBs maintained in Andaman districts of ANI, India were used in the present study. The study area is located at south between 6° and 14° North latitudes and 92° and 94° East longitudes. The average maximum

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temperature is 30.1°C and the minimum temperature is 23°C. Relative humidity is in the range of 82–94%. The annual rainfall is more than 3,100 mm spread over more than 7 months in a year. The samples have been collected in two seasons, viz. monsoon (April to November) and dry (December to March) as per the monsoon availability for five whole calendar years in ANI. The animals were maintained under semi-intensive condition, grazing in the forest during day time and tying them in shed during night time. The experimental animals in shed were fed daily with *ad lib.* quantity of locally available forages. Fresh water was available throughout the day.

Experimental animals were grouped into five and each group consisted of six animals. These groups were male calves, female calves, adult bulls, pregnant cows and non-pregnant cows. The young calves were selected with age of 6 months to 1 year and adult animals were selected with the age of 5 to 6 years. The pregnant status of animals was confirmed by per rectal examination. The experimental animals were clinically healthy, with good body condition score (5–6 out of 10), alert general attitude, no loss of skin elasticity, normal mucous membrane as pink, no diarrhoea, no urogenital abnormalities, no muscular abnormalities, no medication in the previous 7 days, absence of skin lesions or alopecia and absence of intestinal and blood parasites.

Blood samples were collected from jugular vein into collection tubes (20 IU of heparin/mL of blood) between 0700 and 0900 h in both seasons. Each sample was divided into two parts as one for haematological profiles analysis and another for biochemical analysis. Haematological profiles such as total red blood cells (TRBC), haemoglobin (HB), erythrocyte sedimentation rate (ESR), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular

haemoglobin concentration (MCHC) and total white blood cell (TWBC) were estimated with automatic blood analyser. The second part of blood was centrifuged at 1200 × g for 15 min at 4°C. The plasma samples were separated rapidly, labelled properly and preserved at –80°C in deep freezer for further analysis of biochemical indices and antioxidant profiles. The biochemical indices such as total protein, albumin, globulin, glucose and total cholesterol and antioxidant profiles such as total antioxidant capacity (TAC), catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) and oxidant profile such as malondialdehyde (MDA) were estimated with commercially available diagnostic kits.

The results were analysed statistically and expressed as the mean±SEM. Means were analyzed by two way analysis of variance (ANOVA), followed by the Tukey's post hoc test to determine significant differences among the different stages of reproduction and between the dry and rainy seasons in different stages of reproduction using the SPSS/PC computer program. Differences with values of $p < 0.05$ were considered to be statistically significant by using SPSS 15. Correlation between the haematological and biochemical profiles and stages of reproduction were established with correlation coefficient being done as per Pearson's method.

RESULTS AND DISCUSSION

Mean±SEM of haematological attributes (Table 1), biochemical indices (Table 2) and antioxidant profiles (Table 3) in ALBs of ANI at different stage of reproduction were analysed and revealed significant difference between the stages of reproduction. Haematological profiles were significantly ($p < 0.05$) higher in samples collected during rainy than dry season except MCV which is higher in dry

Table 1. Haematological profiles in Andaman local buffaloes of Andaman and Nicobar Islands

Parameter	Season	Buffalo male calf	Buffalo bull	Buffalo female calf	Pregnant buffalo cow	Non-pregnant buffalo cow
TRBC ($\times 10^6/\text{mm}^3$)	Rainy	7.59±0.81 ^{aAB}	9.12±0.43 ^{aC}	8.32±0.65 ^{aBC}	7.47±1.02 ^{aAB}	7.32±0.76 ^{bA}
	Dry	6.55±0.73 ^{bBC}	7.23±0.65 ^{bC}	7.14±0.78 ^{bC}	6.24±0.78 ^{bAB}	5.43±0.65 ^{aA}
Hb (g/dl)	Rainy	12.63±0.92 ^{bB}	13.57±1.21 ^{bB}	13.36±1.05 ^{bB}	11.12±0.68 ^A	10.61±0.92 ^A
	Dry	10.75±1.12 ^a	11.33±1.18 ^a	11.23±1.16 ^a	10.60±0.94	9.92±0.69
ESR (mm/h)	Rainy	6.72±0.48 ^{bB}	8.85±0.81 ^{bC}	6.82±0.68 ^{bB}	6.31±0.74 ^{bB}	4.45±0.58 ^A
	Dry	5.31±0.98 ^{aAB}	6.33±0.76 ^{aB}	5.64±0.76 ^{aAB}	4.44±0.92 ^{aA}	4.42±0.78 ^A
PCV (%)	Rainy	34.15±1.87 ^{bA}	38.22±1.64 ^{bB}	36.65±1.76 ^{bB}	33.39±1.65 ^{bA}	31.01±1.53 ^{bA}
	Dry	32.12±1.53 ^{aB}	36.48±1.32 ^{aC}	34.33±1.53 ^{aBC}	30.83±1.22 ^{aA}	28.61±1.32 ^{aA}
MCV (μm^3)	Rainy	54.10±2.23 ^{aB}	38.92±1.23 ^{aA}	49.84±2.21 ^{aB}	55.48±2.35 ^{aB}	54.87±1.73 ^{aB}
	Dry	57.83±2.23 ^{bB}	43.88±1.76 ^{bA}	52.87±1.86 ^{bB}	60.33±2.45 ^{bB}	64.65±2.64 ^{bB}
MCH (pg)	Rainy	18.54±1.23 ^{bB}	17.63±1.04 ^{bA}	17.72±1.18 ^{bA}	18.75±1.38 ^{bB}	20.76±1.32 ^{bC}
	Dry	15.86±0.79 ^{aA}	16.55±0.84 ^{aA}	15.78±0.79 ^{aA}	16.82±0.76 ^{aA}	16.84±0.33 ^{aA}
MCHC (g/dl)	Rainy	32.44±0.82 ^{bA}	43.12±1.43 ^{bB}	34.62±1.29 ^{bA}	32.26±1.72 ^{aA}	31.15±1.02 ^A
	Dry	30.21±1.31 ^{aAB}	36.56±1.24 ^{aC}	31.59±1.73 ^B	29.43 ±1.63 ^{aA}	28.45±1.27 ^{bA}
WBC ($\times 10^3/\text{mm}^3$)	Rainy	10.46±1.23 ^{bA}	12.69±1.21 ^{bB}	10.22±1.01 ^{bA}	10.14±1.11 ^{bA}	9.83±1.22 ^{bA}
	Dry	8.76±1.09 ^{aA}	10.22±1.34 ^{aB}	8.25±1.23 ^{aA}	8.08±1.07 ^{aA}	7.23±1.09 ^{aA}

Within columns means with different letters (a, b) differ significantly ($p < 0.05$) between rainy and dry seasons for different haematological profiles for different stage of reproduction. Within rows means with different letters (A, B, C, D) differ significantly ($p < 0.05$) among the stages of reproduction. n = 6 samples per group. TRBC, Total Red Blood Cell, Hb, Haemoglobin; ESR, Erythrocyte Sedimentation Rate; PCV, Packed Cell Volume; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration and TWBC, Total White Blood Cell.

Table 2. Biochemical induces in Andaman local buffaloes of Andaman and Nicobar Islands

Parameter	Season	Buffalo male calf	Buffalo bull	Buffalo female calf	Pregnant buffalo cow	Non-pregnant buffalo cow
Total Protein (g/dl)	Rainy	7.49±0.22 ^a	10.43±0.17 ^a	7.78±0.29 ^a	9.61±0.35 ^a	7.22±0.24 ^a
	Dry	5.54±0.30 ^b	7.05±0.25 ^b	5.50±0.07 ^b	7.06±0.11 ^b	5.45±0.18 ^b
Albumin (g/dl)	Rainy	3.88±0.05 ^{bAB}	4.55±0.08 ^{bB}	3.38±0.07 ^{bA}	4.03±0.16 ^{bAB}	3.24±0.24 ^{bA}
	Dry	2.89±0.03 ^{aCD}	3.25±0.05 ^{aD}	2.45±0.08 ^{aBC}	2.18±0.05 ^{aAB}	1.23±0.04 ^{aA}
Globulin (g/dl)	Rainy	4.90±0.04 ^{bBC}	5.25±0.13 ^{bC}	4.59±0.09 ^{bAB}	5.05±0.06 ^{bBC}	4.07±0.07 ^{bA}
	Dry	3.17±0.13 ^a	3.31±0.06 ^a	3.06±0.06 ^a	3.21±0.08 ^a	3.05±0.07 ^a
Glucose (mg/dl)	Rainy	59.44±0.80 ^{bB}	64.86±1.03 ^{bBC}	52.03±1.16 ^{bA}	68.46±1.19 ^{bC}	49.41±0.55 ^{bA}
	Dry	48.29±0.83 ^{aAB}	58.80±1.21 ^{aC}	45.17±0.75 ^{aA}	53.93±1.12 ^{aB}	43.92±0.70 ^{aA}
Total Cholesterol (mg/dl)	Rainy	99.65±0.77 ^{bA}	107.57±1.10 ^{bB}	100.79±1.12 ^{bA}	105.01±1.27 ^{bB}	98.10±0.40 ^{bA}
	Dry	92.04±1.14 ^{aAB}	96.32±1.23 ^{aB}	84.07±1.54 ^{aA}	97.83±1.55 ^{aB}	80.82±0.63 ^{aA}

Within columns means with different letters (a, b) differ significantly ($p<0.05$) between rainy and dry seasons for different biochemical profiles for different stages of reproduction. Within rows means with different letters (A, B, C, D) differ significantly ($p<0.05$) among the different reproductive stages. $n = 6$ samples per group.

than rainy season. Similarly, the blood profiles such as TRBC, HB, ESR, MCHC and WBC were significantly ($p<0.05$) higher in buffalo bulls, male calves followed by female calves, pregnant and non-pregnant cows whereas MCV and MCH were significantly ($p<0.05$) increased in buffalo cows, female calves followed by male calves and bulls in both rainy and dry seasons. Positive correlation was observed between reproductive stages and TRBC, HB, ESR, MCHC and WBC whereas negative correlation was observed between the reproductive stages and MCV and MCH in both the seasons.

Biochemical indices were significantly ($p<0.05$) higher in samples collected in rainy than in dry season. These biochemical indices were significantly ($p<0.05$) higher in buffalo bulls, pregnant cows followed by female and male calves and non-pregnant cows. Positive correlation was observed between the stages of reproduction and all biochemical indices. Antioxidant profiles were significantly ($p<0.05$) higher in samples collected in rainy than in dry season whereas MDA was significantly ($p<0.05$) higher in dry than in rainy season in different stages of reproduction.

Antioxidants were significantly ($p<0.05$) higher in adult bulls, pregnant cows followed by in calves and lowest was observed in non-pregnant cows in both seasons. On the contrary, MDA concentration was highest in non-pregnant cows followed by pregnant cows, adult bulls and lowest level was observed in female calves and male buffalo calves. Positive correlation was observed between reproductive stages and MDA whereas negative correlation was between stages of reproduction and antioxidant profiles in both seasons.

ALBs are well adapted to this islands humid tropical climate. Metabolic, nutritional, health and physiological status of animal can be determined by analysis and monitoring of the blood and other bio-fluids by use of different clinical, pathological and chemical procedures (Kaneko *et al.* 1997). Pathologic values are defined as the values that are deviated from the standard normal references values (Kaneko *et al.* 1997), for that, it is required to establish the normal reference values for different haematological, biochemical and antioxidant indices. In the present study, the animals used were almost healthy by

Table 3. Antioxidant and oxidant profiles in Andaman local buffaloes of Andaman and Nicobar Islands

Parameter	Season	Buffalo male calf	Buffalo bull	Buffalo female calf	Pregnant buffalo cow	Non-pregnant buffalo cow
MDA (nmol/L)	Rainy	70.38±1.67 ^{aA}	81.72±1.78 ^{aC}	74.98±2.31 ^{aB}	91.73±2.62 ^{aD}	96.37±2.54 ^{aE}
	Dry	89.33±1.19 ^{bA}	102.35±3.45 ^{bC}	94.32±1.26 ^{bB}	121.76±2.83 ^{bD}	122.75±3.12 ^{bD}
TAC (nmol/ μ L)	Rainy	38.87±1.89 ^{bB}	45.21±0.73 ^{bC}	39.74±1.83 ^{bB}	29.26±0.98 ^{bA}	28.53±1.29 ^{bA}
	Dry	24.33±1.43 ^{aA}	35.98±1.68 ^{aB}	25.73±2.47 ^{aA}	24.39±1.76 ^{aA}	23.57±1.73 ^{aA}
CAT (nmol/min/L)	Rainy	30.75±1.44 ^{bA}	37.94±1.65 ^{bC}	29.67±1.49 ^{bA}	33.32±0.89 ^{bB}	29.65±1.13 ^{bA}
	Dry	25.76±3.64 ^{aA}	32.80±1.23 ^{aB}	23.39±2.62 ^{aA}	25.44±1.32 ^{aA}	22.32±1.64 ^{aA}
GSH (nmol/L)	Rainy	34.32±1.64 ^{bA}	48.90±1.37 ^{bC}	35.98±1.03 ^{bA}	43.78±1.65 ^{bB}	34.36±1.98 ^{bA}
	Dry	24.45±1.87 ^{aA}	38.35±1.39 ^{aD}	27.64±1.12 ^{aB}	34.98±1.12 ^{aC}	23.75±0.36 ^{aA}
SOD (nmol/min/L)	Rainy	0.78±0.17 ^{bC}	0.96±0.12 ^{bE}	0.66±0.03 ^{bB}	0.87±0.13 ^{bD}	0.55±0.02 ^{bA}
	Dry	0.43±0.11 ^{aC}	0.57±0.12 ^{aD}	0.42±0.13 ^{aC}	0.36±0.15 ^{aB}	0.23±0.18 ^{aA}

Within columns means with different letters (a, b) differ significantly ($p<0.05$) between rainy and dry seasons for different biochemical profiles for different stages of reproduction. Within rows means with different letters (A, B, C, D) differ significantly ($p<0.05$) among the different stages of reproduction. $n = 6$ samples per group. MDA, Malondialdehyde; TAC, Total Antioxidant Capacity; CAT, Catalase; GSH, Glutathione; SOD, Superoxide Dismutase.

observation, palpation and percussion and did not reveal any abnormal clinical signs and/or pathological conditions, therefore, these can be considered as healthy animals and the haematological as well as the biochemical profiles of these animals can work as the standard reference values for the ALBs for future use in ANI or having similar nutritional, climatic or environmental conditions in other countries. These established standard values will be helpful to estimate the health status of these precious germplasm of ALBs in any future studies related to this bubaline species. However, the final interpretation of obtained results by laboratory analysis will depend on the standard reference values of each and every species of animal in different geographical as well as the environmental conditions.

Biochemical reports of the different physiological stages are very complex as these values are influenced by many different factors like species, breed, age, sex, nutrition, physiological status such as estrous, pregnancy, lactation and dry period, illness and also the seasonal variations (Kaneko *et al.* 1997). These reports of the biochemical investigations have shown some sort of variation indicating that these are influenced by various factors like nutrition, health status, lactation stage and season (Jezek *et al.* 2013). Study on the blood composition can address the valuable information about the general health of the animal and can be utilized to evaluate the health status of the animal. Deviation of values in certain blood and biochemical parameters from their normal ranges could be a very good guide to make diagnosis or for differential diagnosis of a particular disease or pathological condition (Radostits *et al.* 2006, Opera *et al.* 2006). Moreover, complete blood profile is an important as well as the powerful diagnostic tool in the component of a minimum database for disease diagnosis. It can also be used to monitor or watch the response to treatment or therapy, to follow up the severity of a disease or illness or for use as a starting point to formulate a list of differential diagnosis. Interpretation on the complete blood profile can be grouped into three divisions as erythrocyte, leukocyte and platelets evaluation. Each of these divisions can be interpreted separately and individually and integration of these divisions is very much important to get highest diagnostic yield or result. Haematological examination is also done as a routine screening procedure for assessment of general health (Gutienez De Lar *et al.* 1971). Blood values are also clear indicators to assess the stress and welfare of animals (Anderson *et al.* 1999).

Knowledge on the haematological values is very much useful to diagnose the different pathological as well as the metabolic disorders, which adversely or deleteriously affect the reproductive and productive performance of the buffaloes (Ahmad *et al.* 2003). Therefore, these haematological data can help to a large extent to determine the disease course and their outcome of several viral, bacterial and parasitic diseases. Factors such as breed, sex, age, seasonal variation, pregnancy, lactation, nutritional and health status of the animal alter haematological attributes

(Mirzadeh *et al.* 2010). These blood profiles in the present study were similar with those reported by Feldman *et al.* (2000) and Mohan *et al.* (2009) in Murrah buffalo. Higher TRBC was reported in adult bulls than in male calves which was due to enhancing function of androgens on erythroid progenitor cells growth in the presence of erythropoietin which in turn increased the basal metabolic rate and increased rate of erythropoiesis (Jabbar *et al.* 2012) which results into increased levels of HB and PCV in bulls as observed in the present study (Leberbauer *et al.* 2005). Moreover, significantly ($p < 0.05$) higher TRBC in female calves than in adult females was in accordance to earlier reports (Khadjeh and Papahn 2002). Similarly, results on TRBC, Hb and PCV between male and female calves showed significant alteration as reported by Beechler *et al.* (2009). The pregnant buffaloes have significantly higher TRBC, Hb and PCV than the non-pregnant buffaloes of the same experimental groups. Significantly higher concentration of TRBC was also reported in pregnant buffaloes (Jabbar *et al.* 2012) and this might be due to maternal adaptation to pregnancy to meet the requirements of the growing embryos. Further, the growing fetus during pregnancy enhances oxygen demands for its growth. This increased demand for oxygen is compensated by the endocrine system which stimulates the renal tissue to release erythropoietin (Plaschka 1997). The increased secretion of this circulating glycoprotein in turn stimulates the increased production of erythrocytes (erythropoiesis) in the bone marrow resulting into increase of TRBC during pregnancy (Lurie 1993). Higher Hb and PCV observed during pregnancy was correlated with the higher TRBC in this study. The TWBC in present study was significantly lower in young calves than in the adult buffaloes as was observed in the previous studies in different breeds of buffaloes (Mohan *et al.* 2009).

Blood glucose concentration is one of the biochemical profiles from which one may get body energy supply. Serum protein concentration suggests the balance between catabolism and anabolism of protein in the body and its concentration at any given time which in turn is a function of nutritional status, hormonal balance, water balance and other parameters affecting health status (Samanta and Das 2007). Albumin indicates a long-term protein status and plasma albumin concentrations could be changed by effect of liver function, protein and energy intake, age and protein losses during some disease condition like parasitism. Plasma albumin concentrations are indication of plasma protein levels. Physiological or pathological status or lactation stage of the cows significantly can alter the serum albumin levels (Otto *et al.* 2000). Moreover, concentration of total protein, globulin, albumin and urea-N in blood serum are the biomarkers of the adequacy or inadequacy of nitrogen in the animal diet (Sejian *et al.* 2012). In addition, serum proteins constitute a portion of the amino acid pool in the body and it is believed to be indicative of the nutritional status of the animal. These biochemical profiles in our study were within range of reference values for the adult buffaloes (Ellah 2010).

Similarly reproductive stage associated results were also reported (Gaolao cattle: Kaple *et al.* 2008, crossbred cows: Shil *et al.* 2012) with same trend as recorded in the current study. Total protein and albumin concentration were significantly ($P<0.05$) higher in monsoon than in dry seasons. High temperature humidity index (THI) has a significant negative impact on serum total protein as well as albumin concentrations (Marai *et al.* 2007). The high THI in dry summer leads to a reduction in feed intake which reduced the serum total protein concentration during dry summer (Dar *et al.* 2019). Similarly, in the present study, serum cholesterol concentration was also significantly ($P<0.05$) lower in hot dry than in the monsoon season and this finding is comparable with earlier reports (Sandhya *et al.* 2015). The lower cholesterol concentrations during dry season are due to reduced liver functions during this period (Rasooli *et al.* 2004). Moreover, the estrogen being an anabolic hormone stimulates the lipid metabolism through lipogenesis, which increases the production of total cholesterol level. The production of cholesterol is directly related with variations in the physiological status of buffaloes as lactation, pregnancy or semen production (Hafez *et al.* 2000). Decreased serum cholesterol levels may be due to reduced intake of feed containing cholesterol (Scharf *et al.* 2010).

In physiological conditions, there is a balance between the factors that promote the formation of free radicals and the levels of antioxidants. The body contains an elaborate antioxidant defense system that depends on dietary intake of antioxidant vitamins and minerals and the endogenous production of antioxidant compounds such as GSH. Reactive oxygen species are scavenged by enzymatic antioxidants (Halliwell and Gutteridge 2006) and by small molecular antioxidants. GSH appears to be essential for the activation and maintenance of cellular defences against oxidative stress, since it provides the substrate for glutathione peroxidase to detoxify peroxides. The antioxidant profiles were significantly decreased and MDA was significantly higher in summer heat stressed animal than animals in rainy season. Deficiency of antioxidants may occur due to different kinds of stress (McDowell *et al.* 2007). These free radical oxidations are activated in animals under various types of stresses and lipid peroxidation products accumulate in various organs. In our study, production of the free radical was significantly higher in buffaloes in dry than in monsoon season as was reported that heat stress/thermo humid stress stimulates excessive production of free radicals (Sivakumar *et al.* 2010). Moreover, walking or exercise in dry hot summer season is postulated to generate free radicals. These stresses can be counteracted by supplementation of antioxidants because the antioxidants are compounds that can delay autooxidation by several mechanisms (Sejian *et al.* 2012). This in turn helps to prevent cellular damage during any stressful condition.

In general, SOD concentration in buffalo serum decreases as temperature increases. SOD values were significantly ($P<0.05$) higher in monsoon than in dry season in the present study and there were significant differences

in serum activity of SOD in different stages of reproduction. These results indicated that high level SOD was in pregnant than in non-pregnant dry buffalo cows with smooth inactive ovaries. The present study shows that dry season is extra stressful to buffaloes, besides, amplification of free radicals production causes increase in the oxidative stress to the animals (Sandhya *et al.* 2015). Similar to SOD, activity of CAT was also significantly different during the different seasons. Its activity was higher in monsoon than in dry season and higher CAT activity was in pregnant than in non-pregnant buffaloes with smooth inactive ovaries indicating the relationship between the oxidative stress and ovarian function. Association was observed among the ovarian function (estradiol-17 β level), oxidative stress levels, pregnancy rates and oocyte quality. Moreover, CAT protects the genome from oxidative damage and it has role in follicular development regulation and differentiation (Park *et al.* 2016).

Significant differences were observed in mean MDA during different seasons and reproductive stages. MDA activity was higher in dry than monsoon season and in non-pregnant buffalo with smooth inactive ovaries than pregnant one. Similar results were reported by Ahmed *et al.* (2010) who has stated that MDA values are high in buffalo cows that expressed impaired fertility profiles because of inactive ovaries. Moreover, high temperature has induced higher MDA levels (Lakhania *et al.* 2018). The increased level of lipid peroxidation in hot dry season may be one of the foremost reasons for increased oxidative stress and decreased antioxidant defense and increased production of free radicals and that all the factors have increased anti-gonadotropic and anti-steroidogenic actions, summer anestrus (Williams *et al.* 2002).

Most of the analyzed haematological attributes, biochemical indices and antioxidant profiles were in normal range of different stages of reproduction, which clearly indicates that, the studied ALB populations were in healthy condition. Thus, during diagnostic procedure or measurement, it is very useful to compare the values obtained from ill or sick animals with normal reference values of healthy animal (Jezek *et al.* 2013). The values or findings of the present study may serve as the standard reference values in which deviations due to metabolic, nutrient deficiency, physiological and health status can be compared for diagnostic, prognostic and therapeutic purpose for ALBs.

REFERENCES

- Ahmad I, Gohar A, Ahmad N and Ahmed M. 2003. Haematological profile in cyclic, non cyclic and endometritic cross-bred cattle. *International Journal of Agriculture and Biology* 5: 332–34.
- Ahmed W, Bashandy M, Ibrahim A, Shalaby S I A, Abd El-Moez S, El-Moghazy F and Ibrahim S. 2010. Investigations on delayed puberty in Egyptian buffalo-heifers with emphasis on clinico-pathological changes and treatment using GnRH (Receptal®). *Global Veterinaria* 4: 78–85.
- Anderson B H, Watson D L and Colditz I G. 1999. The effect of

- Dexamethasone on some immunological parameters in cattle. *Veterinary Research Communication* **23**: 399–413.
- Beechler B R, Jolles A E and Ezenwa V O. 2009. Evaluation of hematologic values in free-ranging African buffalo (*Syncerus caffer*). *Journal of Wild life Diseases* **45**: 57–66.
- Dar A H, Kumar S, Singh D V, Sodhi M, Sharma R K, Ghosh A K, Singh B and Rahman J U. 2019. Seasonal variation in blood biochemical characteristics of Badri cattle. *Pharma Innovation Journal* **8**: 147–50.
- De A K, Perumal P, Malakar D, Muthiyar R, Kundu A and Bhattacharya D. 2019. Complete mitogenome sequencing of Andaman buffalo: an endangered germplasm of Andaman and Nicobar Islands, India. *Journal of Genetics* **98**: 97.
- Ellah M R A. 2010. Serum biochemical reference values for female buffaloes in Egypt. *Buffalo Bulletin* **29**: 141–47.
- Feldman B F, Zinkl J G and Jain N C. 2000. *Schalm's Veterinary Haematology*, 5th edn. Lippincott Williams and Wilkins, Philadelphia, USA. pp. 1085–1089.
- Gutierrez De Lar J H, Warnick A C, Cowley J J and Hentages J F. 1971. Environmental physiology in the subtropics. I. Effect of continuous environmental stress on some hematological values of beef cattle. *Journal of Animal Science* **32**: 968–73.
- Hafez E S E, Jainudeen M R and Rosnina Y. 2000. Hormones, growth factors and reproduction. *Reproduction in Farm Animals*, pp. 33–54.
- Halliwell B and Gutteridge J M C. 2006. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Archives of Biochemistry and Biophysics* **246**: 501–14.
- Jabbar L, Cheema A M, Jabbar M A and Riffat S. 2012. Effect of different dietary energy levels, season and age on hematological indices and serum electrolytes in growing buffalo heifers. *Journal of Animal and Plant Science* **22**: 279–83.
- Jezek J, Stariš J, Nemec M and Klinkon M. 2013. Deviation of biochemical variables in dairy cows with reproductive disorders: Data Analysis. *Agriculturae Conspectus Scientificus* **78**: 267–69.
- Kaneko J J, Harvey J W and Bruss M L. 1997. *Clinical Biochemistry of Domestic Animals*. Academic Press, New York.
- Kaple P M, Jagtap D G, Badukale D M and Sahatpure S K. 2008. Serum total proteins and serum total cholesterol levels in Gaolao cattle. *Veterinary World* **1**: 115–16.
- Khadjeh G H and Papahn A A. 2002. Some hematological parameters in the Iranian (Khuzestan native) buffaloes. *Indian Journal of Animal Sciences* **72**: 671–73.
- Lakhania P, Alhussienb M N, Lakhani N, Jindal R and Nayyar S. 2018. Seasonal variation in physiological responses, stress and metabolic related hormones, and oxidative status of Murrah Buffaloes. *Biological Rhythm Research* **49**: 844–52.
- Leberbauer C, Boulme F, Unfried G, Huber J, Beug H and Mullner E W. 2005. Different steroids co-regulate long term expansion versus terminal differentiation in primary human erythroid progenitors. *Blood* **105**: 85–94.
- Lurie S. 1993. Changes in age distribution of erythrocytes during pregnancy: A longitudinal study. *Gynecologic Obstetric Investigation* **36**: 141–44.
- Marai I F M, El-Darawany A A, Fadiel A and Abdel Hafez M A M. 2007. Physiological traits as affected by heat stress in sheep—a review. *Small Ruminant Research* **71**: 1–12.
- McDowell L R, Wilkinson N, Madison R and Felix T. 2007. Vitamins and minerals functioning as antioxidants with supplementation considerations. Florida Ruminant Nutrition Symposium. Best Western Gateway Grand. Gainesville, FL, 30–31 January. <http://dairy.ifas.ufl.edu/files/rns/2007/Mcdowell.pdf>. Google.
- Mirzadeh K H, Tabatabaei S, Bojarpour M and Mamoei M. 2010. Comparative study of hematological parameters according to strain, age, sex, physiological status and season in Iranian cattle. *Journal of Animal and Veterinary Advances* **9**(16): 2123–27.
- Mohan N H, Niyogi D, Waghaye J Y and Singh H N. 2009. Age-related haematological changes in Murrah buffalo (*Bubalus bubalis*) calves. *Indian Journal of Animal Sciences* **79**: 369–71.
- Opara M N, Ike K A and Okoli I C. 2006. Haematology and plasma biochemistry of the wild adult African grass cutter (*Thryonomys swinderianus*, Temminck). *Journal of American Science* **2**: 17–22.
- Otto F, Vilela F, Harun M, Taylor G, Baggasse P and Bogin E. 2000. Biochemical blood profile of Angoni cattle in Mozambique. *Israel Journal of Veterinary Medicine* **55**(3): 1–9.
- Park Y S, You S Y and Cho S. 2016. Eccentric localization of catalase to protect chromosomes from oxidative damages during meiotic maturation in mouse oocytes. *Histochemistry and Cell Biology* **146**: 281–88.
- Plaschka S G. 1997. Parametros hematologicos y bioquímicos en el parto de la yegua de raza española. *Medicina Veterinaria* **14**: 205–09.
- Radostits O M, Gay C C, Hinchcliff K W and Constable P D. 2006. *Veterinary Medicine*. 10th Edn. Elsevier Science Ltd., USA.
- Rasooli A, Nouri M, Khadjeh G H and Rasekh A. 2004. The influences of seasonal variations on thyroid activity and some biochemical parameters of cattle. *Iranian Journal of Veterinary Research* **5**(2): 55–62.
- Samanta A K and Dass R S. 2007. Effect of Vitamin E supplementation on growth, nutrient utilization, blood biochemical and enzymatic profile in male crossbred (*Bos indicus* × *Bos taurus*) calves. *International Journal of Cow Science* **3**(1&2): 34–43.
- Sandhya S C, Singh V K, Upadhyay R C, Puri G, Odedara A B and Patel P A. 2015. Evaluation of physiological and biochemical responses in different seasons in Surti buffaloes. *Veterinary World* **8**: 727–31.
- Scharf B, Carrol J A, Riley D G, Chase C, Coleman S, Keisler D, Weaver R and Spiers D. 2010. Evaluation of physical and blood serum differences in heat tolerant (*Romosinuano*) and heat susceptible (Angus) *Bos taurus* cattle during controlled heat challenge. *Journal of Animal Science* **88**: 2321–36.
- Sejian V, Singh A K, Sahoo A and Naqvi S M K. 2012. Effect of mineral mixture and antioxidant supplementation on growth, reproductive performance and adaptive capability of Malpura ewes subjected to heat stress. *Journal of Animal Physiology and Animal Nutrition* **98**: 72–83.
- Shil S K, Shaikat A H, Nath B K, Khan M F H and Khan S A. 2012. Hematobiochemical profile in lactating cows and their calves. *Journal of Bangladesh Society of Agricultural Science and Technology* **9**: 41–44.
- Sivakumar A V N, Singh G and Varshney V P. 2010. Antioxidants supplementation on acid base balance during heat stress in goats. *Asian Australasian Journal of Animal Sciences* **23**: 1462–68.
- Williams C A, Kronfeld D S, Hess T M, Saker K E, Waldron J N, Crandell K M, Hoffman R M and Harris P A. 2002. Antioxidant supplementation and subsequent oxidative stress of horses during an 80-km endurance race. *Journal of Animal Sciences* **82**: 588–94.