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Case Study

Correlation Between Biochemical and Haematological Parameters of Sheep

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ABSTRACT

Correlations among blood biochemistry and haematological parameters of sheep in Bauchi, Nigeria were assessed between February and September, 2017. Blood samples were collected from one hundred and twenty sheep comprising Yankasa, Balami, Ouda (32 per each breed) and West African Dwarf (24) at the Abubakar Tafawa Balewa University Teaching and Research Farm, Muda Lawal, Durum and Gwallaga markets. The samples were analyzed for both haematological and biochemical parameters. White blood cell (WBC) and RBC (0.210), RBC and HB (0.569), MCV and MCH (0.539) and MCH and NTP (0.346) were positive and significant. Furthermore, others such as between WBC and HB (-0.215), MCV and PLT (-0.223), MCV and MCHC (-0.610) and LYM % and NTP (-0.571) were negative and significant. Correlations among biochemical parameters were in general positive, low to moderate and non-significant. A few such as between Na^+ and Cl^- (0.379), HCO^{3-} and CRT (0.264), TP and GLC (0.267), Cl^- and TP (0.239), CRT and AST (0.182) and UR and ALT (0.405) were positive and significant. In addition, some correlation values namely; between ALB and ALT (-3.93), ALB and GLC (-264), Na^+ and ALB (-235) and ALB and AST (-0.211) were negative and significant. The correlation coefficients between haematalogical and biochemical parameters were generally positive, low to moderate and non- significant. A few such as between Na^+

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and HB (0.239), K^+ and HB (0.192), UR and LYM (0.248), TP and LYM (0.385), ALB and MCV (0.182), AST and RCD (0.219), ALT and MCHC (0.242), GLC and LYM (0.267), TC and MCHC (0.212) and WBC and LYM (0.433) were positive and significant. Furthermore, some correlation coefficients namely; between Cl^- and MCHC (-0.256), TP and NTP (-0.185), ALT and LYM (-0.199), GLC and MCV (-0.249), TP and NTP (-0.185), ALT and LYM (-0.199), GLC and MCV (-0.249), TC and MCV (-0.185), AP and HCT (-0.207) and WBC and NTP (-0.237) were negative and significant. Therefore, there were generally positive but low to moderate correlations among haematologic, biochemical; and between haematogical and biochemical parameters. The high correlations between parameters could be used as indicators between them to reduce the need to estimate both. The blood parameters needed to be monitored frequently and appropriately controlled to ensure stability and adequate health and nutritional status of sheep.

Keywords: Haematology, Blood, Production, Animals and Well-being.

INTRODUCTION

In Nigeria, sheep contribute about 50% of the total domestically produced meat (FAO, 2007). Apart from provision of food and skin, sheep production plays a crucial role in socio-economic changes such as improvement in income and quality of life (Dagnachew *et al*., 2011).

Sheep thrive in a wide variety of environments in the tropics and sub tropics and requires less capital as they can be completely maintained on pastures, browse and agricultural waste products (FAO, 2007).

Blood is the red fluid that circulates in veins and arteries. It is made up of the liquid, plasma, and various cells and, biochemicals (Abdel-Fattah *et al*., 2013, Tambuwal *et al*., 2003). The primary function of the blood is to transport oxygen from the lungs to the body cells (Koubkova *et al*., 2003; Duke, 1975), distribute nutrients and enzymes, carry away waste products and thereby maintain the homeostasis of the internal environment (Akinmutimi, 2004). Biochemical tests have been widely used for the diagnosis of disease and nutritional status of animals. Information gained from blood parameters substantiates physical examination and together with medical history provide the basis for judgment and decision making (Cambell, 2007; Schalm, *et al*., 1975). In addition, it helps to determine the extent of tissue and organ damages and response of defence mechanism (Fantu *et al*., 2012).

The blood is the life of an animal (Cambell, 2007). Blood constituents therefore determine their well-being. Differences in feeding habit including the type consumed, altitude and other environmental factors affects blood biochemistry of animals and humans (Radostits *et al*., 2000). The blood constituents of sheep in general have been fairly well studied in the developed world and also in some locations in Nigeria. There is however scant information from most parts of Nigeria; particularly the north east. In

the Bauchi zone, work on this aspect is virtually non-existent. Since blood compositions vary due to different causes, extrapolation of information from elsewhere and from other species and breeds would hardly be meaningful. Studies have to be environment specific to be useful.

The objective of this study therefore, is to determine the effects of breed, sex, age and season on the biochemical parameters.

MATERIALS AND METHODS

Area of the Study

The study was conducted in Bauchi and environs between March and September, 2017. Samples were collected from Abubakar Tafawa Balewa University Teaching and Research Farm, Durum and Muda Lawal markets Bauchi for both dry (between November to May) and wet (between June to October) seasons. Bauchi State occupies 49,119 km^2 representing about 5.3% of Nigeria's land mass and it is located between latitude 10.314159, and longitude 9.846282. Bauchi, Nigeria is located within the GPS coordinates of 10° 18' 50.9724" N and 9° 50' 46.6152" E and at an altitude of 628m above sea level (Abubakar, 1974). It is bordered by seven states, Kano and Jigawa to the north, Taraba and Plateau to the south, Gombe and Yobe to the east and Kaduna to the west.

Climate and Vegetation

The rainfalls in Bauchi state range between 1300 mm per annum in the south and only 700 mm in the extreme north (Muhammad, 2003).

The rains are usually due to the moisture laden south westerly winds. The rains therefore start earlier (April) in southern part of the state and vary to June and July in the northern areas. The average relative humidity, daily sunshine hours and temperature values range between 35 and 94 % for the months of February and August, 5.0 and 10.0 hours in August and November and 36.6 to 12.8^0 C from April to December, respectively (Muhammad, 2003).

The soils are composed of ferruginous types on crystalline rocks, and, lithosol and juvenile formation on Aeolian sand (Areola, 19789. Bauchi state spans three vegetation zones, namely, northern guinea, Sudan and Sahel savannahs (Areola, 1978; Abubakar, 1974).

Sheep Management (Field)

Sheep used in this study are mostly reared by the nomadic Fulanis and are usually grazed along with cattle. They are moved from one grazing area to another based on availability of pasture. Grazing starts at 10:00 am ends at 5:00 pm. Usually, the only form of supplementation is provision of minerals and salt licks. Watering is twice a day at streams. At about 6:00 pm animals are driven back to the kraals. This is usually

an open space, often fenced with thorny woods. Most nomadic pastoralists have developed strategies to keep their animals healthy. They include among others the application of ethno-veterinary knowledge. They sometimes combined indigenous and conventional strategies. The commonest ailments in sheep according to pastoralists are bloat and diarrhoea. They are traditionally thought to be controlled using groundnut oil and powdered baobao leaves. Other ailments such as helminths infection, poisoning and gastric-impaction are treated using *Khaya senegalensis* (madaci) bark and leaves. Ecto-parasites are controlled using tobacco leaves and paraffin.

Market Sheep Management

Sheep brought to the market that were not sold immediately are tethered in shades or open environment with feeders and drinkers. Depending on the market situation they may remained for up to 1-2 weeks with dealers. The sheep are fed mostly with hay, cereal bran, cowpea husk or mixture. Scanty health management is practised. In the events of an outbreak, sheep are slaughtered and sold at give-away prices.

Data Collection

Blood collection

Ten millilitres of blood was collected from 120 sheep (32 each of Yankasa, Balami and Ouda and, 24 WAD) for both dry and wet seasons. Sixteen (16) samples each in both sexes were collected from Yankasa, Balami and Ouda while twelve (12) samples were collected from each of female and male WAD sheep breed. Similarly, fifty eight (58) and sixty two (62) samples were collected from young and adult sheep breeds respectively comprising sixteen (16) samples from each of adult and young Yankasa, Ouda and Balami while ten (10) and fourteen (14) samples each from young and adult WAD. Sheep were classified as young and adult by dentition according to (Isidahomen *et al.,* 2011).

The blood was collected using a hypodermic syringe through the jugular vein. For each sample, 3ml was dispensed into a bottle containing ethylene diamine tetra-acetic acid (EDTA) for haematology while the remaining 7ml was delivered into dried appropriately labelled sterile test tubes with screw caps, kept slanted and allowed to clot. The blood samples were immediately transported to the Heamatology Laboratory of Abubakar Tafawa Balewa University Teaching Hospital. The samples without EDTA were left at room temperature for about 2 hours and then centrifuged at 10000 gravities for 5 minutes to yield sera. The sera were separated into clean labelled tubes and stored at -20^{o} C until analyzed for biochemical constituents.

Packed cell volume

Capillary tubes method was used for the PCV measurement. After centrifugation of the prepared capillary tubes at 10000-15000 gravity for 5 minutes, the PCV value was read using a reader (Pratt, 1985).

Red Cell Distribution

The red cell distribution (RCD) was determined using the Sysmex haematology analyser. Coefficient of variation (CV) of RDW was used to calculate for RDW as :
RDW (CV %) = Standard deviation of RBC size X 100/ MCV (Pratt, 1985).

Haemoglobin concentration determination

Haemoglobin was determined using the cyan-methaemoglobin method (Pratt, 1985). Twenty-four empty test tubes were assembled in a rack including an extra tube for blank. Five millilitres of cyanide was poured in to each test tube followed by 0.02 ml of individual blood samples except the blank. The tubes were thoroughly mixed and allowed to stand for not less than 3 minutes after which haemoglobin concentration was read using the electronic colorimeter (GSC International 4-30421). The colorimeter was first zeroed using the blank and both the fine and coarse adjusters. Subsequently the absorbances of the samples were recorded. The final results were obtained using the HB reference table (John *et al*., 2013) Red and white blood cells count and Thin-blood smears techniques for differential count have been similarly determined according to standard procedures by (John *et al*., 2013; Pratt, 1985; Wintrobe, 1967).

Red blood cell count

An automatic pipette was used to dispense 4 ml of RBCs diluting fluid into a clean and dry tube. Thereafter 0.2 µl of blood sample was added and thoroughly mixed. The mixture was allowed to stand for 5 minutes after which the Nauber chamber was charged. The charging entails first, proper cleaning of the surface of the chamber to clear dust particles which often form artifacts. A clean cover slip was properly fitted on to the chamber, the mixture was stirred and an aliquot collected and gently dispensed “charging” from one edge under the cover slip, carefully avoiding passage of bubbles. The charged chamber was then placed under the microscope and read at low magnification (x10). Only red blood cells found within the primary/secondary and tertiary squares at the centre of the Nauber chamber were counted and recorded.

White blood cell count

Blood was first drawn to fill the WBC haemocytometer pipette to the 0.5 mark. The tip of the pipette was cleaned and the WBC diluting fluid drawn to the 1.1 mark and mixed gently avoiding bubbles formation. A cover slip was placed appropriately on the counting chamber of the haemocytometer. The fluid-blood mixture was then

transferred using a fine bore pipette in to the counting chamber. It was ensured that the mixture did not overflow. After about 2 minutes when the cells had settled to the bottom, the charged chamber was placed under the microscope and viewed using the low power objective (x10). The WBCs uniformly observed in the four larger corner squares were counted. Cells present on the outermost lines were counted on one side and those present on the line opposite were avoided.

Thin blood smears techniques for differential count

A thin blood smear was made by placing a drop of blood at one end of a clean slide and with a spreader; the blood was swiftly spread down the slide. Adequate volume of methanol was used to fix the smear onto the slides for 5 minutes. A reconstituted Geimsa stain in the ratio of 1:100 ml of distilled water was used to cover the entire surface of the fixed slides. The stain was allowed for 10 minutes after which it was washed out with laboratory jets of water. The already stained slides were left to dry and then packed. Using an oil immersion microscope, at least 100 cells were counted by moving the slide in a systematic fashion as to include the central and peripheral areas of the smear. The laboratory cell counter was used to count each of the leucocytes- neutrophil, lymphocyte, monocyte, eosinophil and basophil (Sood *et al*., 2009).

Preparation of serum samples

A whole blood sample was collected aseptically in to a clean, dried and covered test tube and, allowed to clot by leaving it undisturbed at room temperature, for 15-30 minutes. The clot was removed by centrifugation at 1,000 – 2,000 x g for 10 minutes. The resulting supernatant (serum) was then carefully removed using a pasteur pipette (Henry, 1979). Total serum protein, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), urea (UR), creatinine (CRT), albumin (ALB), total cholesterol (TC), sodium (Na^+), chloride (Cl^-), hydrogen carbonate (HCO^-_3) and potassium (K^+) were determined using the Randox Test Kits according to the manufacturer's prescriptions (Sood *et al*., 2009).

Determination of blood sugar

The blood sugar was determined using a glucometer (ACCU CHEK active serial no. GN: 10023338). The instrument was first charged before use. A single glucose stripe was later inserted into position and the glucometer allowed to zero. Immediately, blood sample was dropped onto the appropriate position on the glucose stripe and the instrument allowed few seconds to record. The blood sugar was read only when the result on the glucometer remains steady for 2 seconds but not higher.

Data Analysis

Data generated were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure of SPSS Inc. (2013). All two factors interactions were tested and only the significant ones were included in the final model. Significantly different means were compared using the Duncan Multple Range Test (DMRT). Simple correlation coefficients were also estimated.

The model utilized for ANOVA was as follows:

$Y_{ijkln} = U + B_i + S_j + A_k + Sn_l + (BS)_{ij} + e_{ijklm}$

$Y_{ijkl=}$ Observation on dependent variables

$U_{=}$ Common Mean

$B_{i=}$ effect of i^{th} breed (Yankasa, Ouda, Balami and WAD)

$S_{j=}$ effect of j^{th} sex (Male and female)

$A_{k=}$ effect of k^{th} age (Young and adult)

$Sn_{l=}$ effect of l^{th} season (Dry and wet)

$(BS)_{ij=}$ interaction effect of i^{th} breed and j^{th} sex

$e_{ijklm =}$ random error term

RESULTS

Correlation Coefficients among the Haematological Biochemical Parameters

The correlation coefficients between the haematological parameters are presented in Table 1. The associations between the parameters were mostly low to moderate, both positive and negative and, non-significant. A few such as between WBC and RBC (0.210), RBC and HB (0.569), MCV and MCH (0.539) and MCH and NTP (0.346) were positive and significant. Furthermore, others such as between WBC and HB (-0.215), MCV and PLT (-0.223), MCV and MCHC (-0.610) and LYM % and NTP (-0.571) were negative and significant. The correlation coefficients among the biochemical para meters are as shown in Table 2. Correlations among biochemical parameters were in general positive, low to moderate and non-significant. A few such as between Na^+ and Cl^- (0.379), HCO^{3-} and CRT (0.264), TP and GLC (0.267), Cl^- and TP (0.239), CRT and AST (0.182) and UR and ALT (0.405) were positive and significant. In addition, some correlation values namely; between ALB and ALT (-3.93), ALB and GLC (-264), Na^+ and ALB (-235) and ALB and AST (-0.211) were negative and significant.

Table 1: Correlation between haematological parameters

Parameters	1	2	3	4	5	6	7	8	9	10	11	12
White blood cell		0.210*	-0.215*	-0.122NS	-0.137NS	0.004NS	0.140NS	0.119NS	0.433**	-0.237**	0.255**	-0.004NS
Red blood cell			0.569**	0.268**	-0.129NS	-0.178NS	-0.011NS	-0.087NS	0.054NS	0.087NS	0.038NS	0.034NS
Haemoglobin				0.353**	0.150NS	-0.036NS	-0.138NS	-0.074NS	0.088NS	-0.126NS	0.166NS	0.278**
Hematocit					0.208*	0.026NS	-0.210*	-0.006NS	0.032NS	-0.083NS	0.014NS	0.227*
Mean Capsular Volume (MCV)						0.539**	-0.610**	-0.223*	-0.133NS	0.201*	0.095NS	0.254**
Mean Capsular Haemoglobin (MCH)							0.010NS	-0.056NS	-0.199*	0.346**	0.003NS	0.138NS
Mean Capsular Haemoglobin Com (MCHC)								0.138NS	0.081NS	-0.034NS	-0.029NS	-0.132NS
Platelets (PLT)									0.013NS	-0.019NS	0.072NS	-0.113NS
Lymphocytes (%)										-0.571**	0.397**	0.122NS
Neutrophil											-0.359**	-0.001NS
Lymphocyte												0.176NS

NS = Non-significant ** = P<0.01 * = P<0.05

Table 2: Correlation between biochemical parameters

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13
Sodium		0.126^{NS}	0.379^{**}	0.73^{NS}	0.153^{NS}	0.171^{NS}	0.427^{**}	-235^{**}	0.32^{NS}	-0.042^{NS}	0.313^{*}	0.133^{NS}	0.163^{NS}
Potassium			0.010^{NS}	0.87^{NS}	0.101^{NS}	0.032^{NS}	-0.07^{NS}	-0.004^{NS}	0.47^{NS}	0.045^{NS}	-0.015^{NS}	0.047^{NS}	0.128^{NS}
Chloride				0.080^{NS}	-0.037^{NS}	-0.166^{NS}	0.239^{**}	-0.003^{NS}	-0.081^{NS}	-0.149^{NS}	-0.077^{NS}	0.057^{NS}	0.132^{NS}
Hydrogen Carbmate					0.115^{NS}	0.264^{**}	0.097^{NS}	0.078^{NS}	0.159^{NS}	0.108^{NS}	0.039^{NS}	0.162^{NS}	-0.53^{NS}
Urea						0.101^{NS}	0.083^{NS}	-0.131^{NS}	0.159^{NS}	0.259^{**}	0.141^{NS}	0.405^{**}	0.217^{*}
Creatinine							0.012^{NS}	-0.49^{NS}	0.182^{*}	0.113^{NS}	0.148^{NS}	-0.113^{NS}	-0.016^{NS}
Total Protein								-0.149^{NS}	-0.023^{NS}	-0.090^{NS}	0.267^{**}	0.214^{NS}	0.284^{**}
Albumin									-0.211^{*}	-393^{**}	-264^{**}	-129^{NS}	0.062^{NS}
Aspartate amino transferase										0.708^{**}	0.154^{NS}	0.164^{NS}	0.014^{NS}
Alanine amino transferase											-0.188^{**}	0.289^{**}	-0.051^{NS}
Glucose												0.142^{NS}	0.091^{NS}
Total cholesterol													0.088^{NS}

NS = Non-significant * = P<0.05 ** = P<0.01

Table 3: Correlations between haematological and biochem ical parameters

	HB	HCT	MCV	MCH	MCHC	PLT	LYM %	NTP	RCD
Na^+	0.239**	0.148^{NS}	0.018^{NS}	-0.088^{NS}	-0.1060^{NS}	-0.072^{NS}	0.071^{NS}	-0.167^{NS}	-0.013^{NS}
K^+	0.192*	0.057^{NS}	0.081^{NS}	-0.016^{NS}	-0.038^{NS}	-0.053^{NS}	-0.012^{NS}	-0.089^{NS}	0.102^{NS}
Cl^-	-0.039^{NS}	-0.043^{NS}	0.135^{NS}	-0.047^{NS}	-0.256**	0.017^{NS}	0.030^{NS}	0.116^{NS}	-0.017^{NS}
HCO^{3-}	-0.077^{NS}	0.024^{NS}	-0.057^{NS}	0.007^{NS}	0.151^{NS}	-0.057^{NS}	-0.137^{NS}	0.129^{NS}	-0.025^{NS}
UR	0.137^{NS}	-0.029^{NS}	-0.056^{NS}	-0.001^{NS}	0.060^{NS}	0.047^{NS}	-0.069^{NS}	-0.012^{NS}	0.178^{NS}
CRT	-0.058^{NS}	-0.079^{NS}	0.099^{NS}	0.014^{NS}	-0.004^{NS}	-0.184^{NS}	0.044^{NS}	0.105^{NS}	0.073^{NS}
TP	0.124^{NS}	-0.059^{NS}	-0.150^{NS}	-0.062^{NS}	-0.028^{NS}	-0.044^{NS}	0.385**	-0.185*	0.192*
ALB	-0.003^{NS}	0.040^{NS}	0.182*	0.095^{NS}	-0.305**	0.014^{NS}	0.078^{NS}	0.185*	-0.06^{NS}
AST	-0.016^{NS}	0.06^{NS}	-0.005^{NS}	-0.031^{NS}	0.129^{NS}	-0.064^{NS}	-0.095^{NS}	-0.022^{NS}	0.219*
ALT	-0.067^{NS}	0.039^{NS}	-0.091^{NS}	-0.132^{NS}	0.242**	-0.006^{NS}	-0.199*	-0.016^{NS}	0.197*
GLC	0.174^{NS}	0.028^{NS}	-0.249**	-0.140^{NS}	0.218*	-0.040^{NS}	0.267**	-0.255**	-0.091^{NS}
TC	0.018^{NS}	-0.066^{NS}	-0.272**	-0.082^{NS}	0.212*	0.177^{NS}	-0.098^{NS}	0.069^{NS}	0.101^{NS}
AP	-0.079^{NS}	-0.207*	-0.039^{NS}	-0.151^{NS}	-0.037^{NS}	0.033^{NS}	0.099^{NS}	-0.004^{NS}	0.046^{NS}
WBC	-0.215^{NS}	-0.122^{NS}	-0.137^{NS}	0.004^{NS}	0.140^{NS}	0.119^{NS}	0.433**	-0.237**	-0.004^{NS}
RBC	0.569**	0.268**	-0.129^{NS}	-0.178^{NS}	-0.011^{NS}	0.087^{NS}	0.054^{NS}	-0.087^{NS}	0.034^{NS}

AST = Aspartate amino transferase (Iμ/L) ALT = Alanine amino tranferase (Iμ/L) * = P<0.05

Cl^- = Chloride ion (mmol/L) GLC = Glucose (mmol/L) ** = P<0.01

HCO^-_3= Hydrogen carbonate ion (mmol/L) TC = Total cholesterol (mmol/L)

NTP = Neutrophil (%) WBCs = White Blood Cells ($x10^3/\mu$

AP = Alkaline phosphatase (mmol/L) CRT = Creatinine (mmol/L)

TP = Total Protein (g/dl) ALB = Albumin (g/dl)

MCH= Mean Corpuscular haemoglobin (Fl) LYM (%) = Lymphocyte Percent HB= Haemog

The correlation coefficients between haematalogical and biochemical parameters are depicted in Table 3. The correlation coefficients were generally positive, low to moderate and non- significant.

A few such as between Na^+ and HB (0.239), K^+ and HB (0.192), UR and LYM (0.248), TP and LYM (0.385), ALB and MCV (0.182), AST and RCD (0.219), ALT and MCHC (0.242), GLC and LYM (0.267), TC and MCHC (0.212) and WBC and LYM (0.433) were positive and significant. Furthermore, some correlation coefficients namely; between Cl^- and MCHC (-0.256), TP and NTP (-0.185), ALT and LYM (-0.199), GLC and MCV (-0.249), TP and NTP (-0.185), ALT and LYM (-0.199), GLC and MCV (-0.249), TC and MCV (-0.185), AP and HCT (-0.207) and WBC and NTP (-0.237) were negative and significant.

DISCUSSION

Correlation Coefficients among the Haematological and Biochemical Parameters

The low to moderate correlation coefficients between haematological parameters observed in the present study agree with report by of Keneko *et al*. (2008). The high and positive correlation between MCV and LYM agrees with the findings of Keser and Bilal (2008) who reported a similar value for these parameters. The generally positive but low to moderate correlation coefficients among the biochemical parameters e.g Na^+ and ALT, UR and TC and CRT and AST have been similarly reported by Keser and Bilal (2008). Positive correlations among parameters indicate that an increase in one leads to an increase in the other. Negatively correlated values mean an increase in one will lead to a decrease in the other and vice-versa. In this study for example, an increase in WBC, RBC, MCV, Na^+, and HCO^{-3} will lead to increase in RBC, HB, MCH, Cl^- and CRT respectively while a decrease in MCV, LYM, ALB, Na^+ and WBC will lead to an increase in PLT, NTP, ALT, ALB and HB respectively.

CONCLUSION AND RECOMMENDATIONS

In this present study, there were generally moderate to high correlations among haematologic, biochemical; and between haematogical and biochemical parameters. Furthermore, the high correlations between parameters could be used as indicators between them to reduce the need to estimate both.

It can be recommended that:

There should be routine checks on biochemical parameters as their values may be helpful in assessing the health status and general conditions of the animals.

More attention should be given to the animals in terms of nutrition to maintain normal biochemical parameters values all year round.

Further studies should be carried out to include the effects of more environmental factors such as ambient temperature, altitude and relative humidity on biochemical parameters

CONFLICT OF INTEREST

The article "Prediction of first lactation milk yield from part lactation yields for Murrah and Nili Ravi buffaloes" has been reviewed by me; and was found to have no conflict of interest.

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