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Seasonal Changes in Hematological and Biochemical Profile of Dairy Cows at High Altitude Cold Desert

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Abstract: Cattle are the main source of livelihood for small farmers in high altitude cold desert. They also provide draught power, social and cultural strength. Cattle, therefore, contribute to subsistence farming and enhance the sustainability of smallholder farming systems. Determination of nutritional and health status of cattle is important in modern animal agriculture. Hematological and biochemical aids have been used to identify status of cattle. Keeping this in view, the present study was undertaken to establish hematological and biochemical responses on milk production of Jersey cows at high altitude production in two different seasons (summer and winter). For each period, ambient temperature and relative humidity were recorded and the temperature-humidity index (THI) was calculated as indicator of thermal comfort for cattle. A total of 140 (70 cows in each season) clinically healthy lactating Jersey cows aged 2 – 17 years at 3327-3575-meter altitude from mean sea level in the Ladakh region of Jammu and Kashmir, India, were used to evaluate the effect of environmental conditions on Hematological parameters. The hematological parameters were estimated by using an automatic blood analyzer and biochemical test performed by the serum semi-auto analyzer. Among the hematological parameters, white blood cell (WBC), lymphocyte (LYM), granulocytes (GRAN), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT) were analyzed. The results showed a significant difference in most of the parameters due to the variation in ambient temperature, relative humidity and temperature-humidity index. A significant increase ($p<0.05$) in RBC, Hb, and HCT was recorded in winter season as compared to the summer season. MCV, MCH, MCHC and PLT levels also exhibited the similar trend whereas, the WBC, lymphocyte, monocytes, and granulocytes values found were higher in summer compared to the winter season ($p<0.05$). However, in case of biochemical profile, glucose and aspartate aminotransferase (AST) level showed a significant increase ($p<0.05$) in summer season but level of albumin went significantly higher ($p<0.05$) in the winter season. Milk production level was significantly higher ($p<0.05$) in the summer season. These results provide an insight into the hematological and biochemical responses of Jersey cows to different environmental conditions. As the hematological and biochemical profiles of dairy cows has been altered in response to the different season which ultimately affected on the milk production. This study will be helpful for the better dairy cattle management in different seasons for higher production at the cold arid high altitude region.

Key words: Hematological profile; Temperature humidity index; Erythrocytic indices; Milk production; High altitude

Introduction

Ladakh, a high altitude cold arid region; situated at 3500 m above the mean sea level in the state of Jammu and Kashmir of India, environmental conditions are very harsh. In a year, the summer season stays for approximately four months and the rest of the period as winter season. Most of the inhabitants in this region are dependent on small agriculture system and livestock farming. However, in the winter season especially the farmers are totally dependent on livestock and among them jersey cattle play an important role by providing quality milk as the major source of nutrition. Cattle provide diverse functions to farmers (1), they provide draught animal power, social and cultural functions as well as serve as security and risk reduction in rural households (2, 3). Cattle, therefore, contribute to subsistence farming and enhance the sustainability of smallholder farming systems. There are various factors that reduce cattle productivity in high altitude cold desert, the important one is severe environmental condition. Hematological measurements have been used to identify constraints on productivity in cattle (4). Blood profile as animal response indicators have been turned to serve as the basis for diagnosis, treatment, and prognosis of diseases (5, 6). Exposure of cows to hot environment stimulates

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thermoregulatory mechanisms and reduces the metabolic rates, feed intake and productivity (7). In order to maintain homeothermy, an animal must be in thermal equilibrium with its environment, which includes radiation, air temperature, air movement and humidity (8). Studies of Armstrong (1994), Kadzere *et al.,* (2002), Dikmen and Hansen (2009) suggested that the temperature-humidity index (THI) could be used as the indicator of thermal conditions and the degree of stress on cows (8-10). Variations in the Hematological profile due to season have been reported in temperate and tropical regions (11-14). However, the literature on such studies in high altitudes is scanty. Therefore, considering that the environmental conditions are major physiological stressors which affect the animal's biological system; and ultimately the animal productivity, the present study was undertaken to measure some hematological responses of Jersey cows under different environmental conditions which could be used to monitor the herd health status at high altitude region.

Materials and Methods

Ethical approval

While collecting blood samples, adequate precautionary measures were taken to minimize pain and / or discomfort to the animals and also collection of samples was carried out in accordance with the guidelines laid down by the CPCSEA laws and regulations.

Study area and experimental animals

For this study one hundred and forty healthy, lactating jersey cows; farmed in Ladakh, Jammu & Kashmir, India. All the sampling points belong within the latitude of 33.72°3.51′ ± 0.90°2.40′N, longitude 077°38.67′ ± 0°2.99′E, and 3318 ± 115.95 m above sea level. Blood samples were collected from seven different villages around the main city, Leh of Ladakh region. These coordinates of altitude, latitude and longitude of all the sampling sites/places were recorded by GPS system (Garmin GPS 72H) and presented in the table 1.

Table 1: Analyzed data of sampling sites.

S. No.	GPS Parameters	GPS Readings	
		Mean ± SD	Range
01	Altitude (Meter)	3318 ± 115.95	3327 – 3575
02	Latitude	33.72°3.51′± 0.90°2.40′	31°1.17′ - 34°6.78′
03	Longitude	077°38.67′ ± 0°2.99′	077°34.63′ - 077°44.03′

Environmental conditions

Environmental conditions during the sample collection period are presented in the graphical representation (Fig. 1).

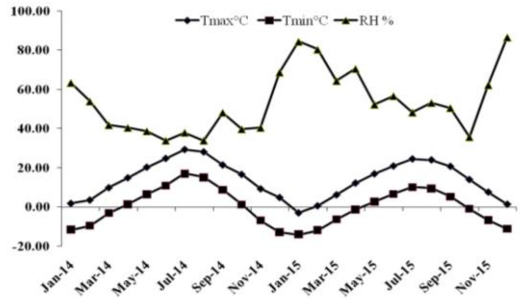

Figure 1: Environmental condition during the sampling period with maximum, minimum temperature (°C) and relative humidity (%)

All the data of environmental condition were taken from the meteorological department of Indian Air Force. For each experimental period, temperature-humidity index (THI), presented in the figure 2, used as indicator of thermal comfort for cattle, and was calculated using the U.S. Weather Bureau's Temperature Humidity Index Formula for bovine species (15):

THI [°C] = T° ambient + (0.36 * point of steam condensation) + 41.5

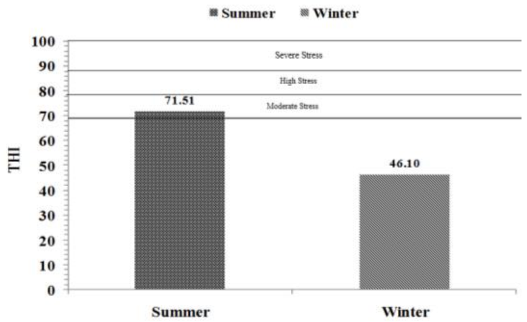

Figure 2: Temperature-Humidity index (THI) in the two different seasons

Blood sample collection and analysis

Blood was collected by venipuncture of the jugular vein during spring, between 15:00- 18:00 hrs, into vacuum tubes containing EDTA (BD Franklin Lakes, NJ, USA). Whole blood was sampled at two time points under different environmental conditions: summer and the winter. EDTA blood samples were refrigerated and analyzed for complete blood count within 6 h. from the collection. Blood analysis was performed using the PE-6800 VET fully auto Hematology Analyzer (Shenzhen Procan Electronics Inc., Guangdong, China). All samples were tested for white blood cell (WBC), lymphocyte (LYM), granulocytes (GRAN), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT). For the biochemical parameters like total protein (TP), albumin (Alb), globulin (Glb), blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase

(ALP) were analyzed by serum semi-auto analyzer (pace[vet]28 of Ocean Medical Technologies).

Statistical analysis

Data were analyzed using statistical software SPSS 22 version. Independent 't'-test was applied to determine the effects of two different seasons on Hematological profile studied. P value <0.05 was considered statistically significant.

Results

Milk production of dairy cows at this region showed significant (p<0.05) higher production level in the summer season (Table 2).

Table 2: Average milk production per day in two seasons.

Seasons	Milk production (Liter)	
	Mean ± SEM	**Range (Liter)**
Summer	4.73 ± 0.27***	1.00 - 10.00
Winter	3.54 ± 0.26	0.50 - 10.00

Values are presented as Mean ± Standard Error Mean (SEM) and different the superscript (***) indicate significant difference (p<0.05) within the same column

Table 3: Mean values ± SEM together with the relative statistical significance of Hematological parameters and WBC differential cell counts observed in experimental periods.

Parameters	Seasons			
	Summer (n=70)		Winter (n=70)	
	Mean ± SEM	Range	Mean ± SEM	Range
WBC (x10³ /µL)	38.63 ± 0.81***	3.90 - 52.50	31.62 ± 0.97	15.10 - 51.10
LYM (%)	85.18 ± 0.48***	69.20 - 92.20	80.82 ± 0.46	62.40 - 89.50
Monocytes (%)	3.85 ± 0.09	2.00 - 7.40	3.84 ± 0.10	2.00 - 6.90
GRAN (%)	15.34 ± 0.43***	8.00 - 32.20	10.98 ± 0.43	5.10 - 24.00
Lym (x10³ /µL)	31.34 ± 0.70***	3.20 - 46.30	27.11 ± 0.89	12.30 - 45.20
Monocytes (x10³ /µL)	1.51 ± 0.049***	0.10 - 2.90	1.24 ± 0.05	0.50 - 2.20
GRAN (x10³ /µL)	5.78 ± 0.17***	0.60 - 11.10	3.28 ± 0.11	1.40 - 6.00
RBC (x10⁶ /µL)	3.56 ± 0.05	2.58 - 4.51	4.91 ± 0.06***	3.44 - 6.42
Hb (g/dL)	9.39 ± 0.15	6.50 - 12.50	12.37 ± 0.16***	9.20 - 17.60
HCT (%)	21.79 ± 0.32	16.20 - 30.00	29.32 ± 034***	22.70 - 40.20
MCV (fL)	60.01 ± 0.45	50.60 - 72.70	61.52 ± 0.60*	52.20 - 82.90
MCH (pg)	25.14 ± 013	22.50 - 28.40	26.36 ± 0.27***	22.90 - 39.10
MCHC (g/dL)	42.11 ± 0.19	38.40 - 50.00	43.07 ± 0.29**	36.50 - 51.30
PLT (x10³ /µL)	298.26 ± 15.56	98.00 - 1510.00	461.18 ± 23.20***	152.00 - 1032.00

Mean ± Standard Error Mean (SEM) with superscripts (*, **, and ***) in a row differ significantly at (p< 0.05). WBC=White blood cell, LYM=Lymphocytes, GRAN=Granulocytes, RBC=Red blood cell, Hb=Hemoglobin, HCT=Hematocrit, MCV=Mean corpuscular volume, MCH= Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, PLT=Platelet

Independent 't'-test showed a statistically significant effect (P<0.05) of seasons on the following parameters: RBC, Hb, HCT, MCV, MCH, MCHC, PLT, WBC, lymphocytes, monocytes, and granulocytes (Table 3). The application of this statistics showed a statistically significant increase in RBC, Hb, HCT as MCV, MCH, MCHC and PLT levels in winter as compared to summer season, whereas, WBC, lymphocyte, monocytes, and granulocytes values were higher in as compared to winter season. In case of biochemical parameters glucose, total protein (TP), albumin (Alb), globulin (Glb), blood urea nitrogen (BUN), urea, alanine transaminase (ALT), aspertate aminotransferase (AST), and alkaline phosphatase (ALP) has been analyzed. Among all of these glucose, Alb and AST level showed the significant (P<0.05) variation in the summer season. In case of, total protein and blood urea nitrogen were slightly high in the winter season but those were not significant. There were no seasonal changes in globulin and ALT level. Moreover, uric acid and ALP level were showed insignificant (p>0.05) lower level in winter season (Table 4).

Table 4: Biochemical parameters of dairy cows in two different successive seasons.

Parameters	Seasons			
	Summer (n=70)		Winter (n=70)	
	Mean ± SEM	Range	Mean ± SEM	Range
Glucose (mg/dL)	72.23 ± 9.86**	38.78 - 776.96	53.41 ± 0.96	38.78 - 76.47
TP (g/dL)	5.91 ± 0.10	3.14 - 14.4	6.14 ± 0.16	3.71 - 14.40
Alb (g/dL)	3.66 ± 0.06	0.00 - 6.08	4.01 ± 0.10***	0.00 - 6.08
Glb (g/dL)	2.25 ± 0.11	0.03 - 10.09	2.13 ± 0.18	0.03 - 10.09
BUN (mg/dL)	18.02 ± 1.08	5.26 - 56.46	19.05 ± 1.15	5.26 - 56.20
Uric Acid (mg/dL)	1.21 ± 0.10	0.52 - 8.52	1.09 ± 0.04	0.52 - 3.44
ALT (IU/L)	29.91 ± 0.93	10.78 - 55.23	29.79 ± 0.85	10.78 – 48.00
AST (IU/L)	93.39 ± 3.01***	46.88 - 224.20	73.56 ± 1.89	46.92 - 118.07
ALP (IU/L)	97.06 ± 7.08	1.75 - 435.20	81.43 ± 6.00	1.75 - 310.23

Mean ± Standard Error Mean (SEM) with superscripts (**, and ***) in a row differ significantly at (p< 0.05) and (p<0.01), respectively. TP = Total Protein, Alb = Albumin, Glb = Globulin, BUN = Blood Urea Nitrogen, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase.

Discussion

The recorded ambient temperature and calculated THI were within the upper critical zone (Fig. 1). The ideal ambient temperature ("thermoneutral" zone) for a cow is between 5°C and 25°C (16). As ambient temperature increases, it becomes more difficult for a cow to cool herself adequately and she enters into heat stress. THI values of 70 or less are considered comfortable, 75–78 stressful, and values greater than 78 cause extreme distress with lactating cows being unable to maintain thermoregulatory mechanisms or normal body temperatures (8).

Generally, the Hematological profile is an important indicator of the physiological changes in animals (17, 18). Seasonal changes in the thermal environment influence the physiological responses of animals. Changes in Hematological parameters such as total RBC count (19), PCV (20) and RBC indices of mean corpuscular volume (MCV), mean corpuscular haemo-globin (MCH), mean corpuscular haemoglobin concentration (MCHC) of valuable in determining the adaptation of animals to the environment. Haemoglobin concentration (18) and TLC are also indicative of adaptation to adverse environmental conditions. Indeed, Hematological values are used to assess the stress and wellbeing of animals (21).

In our study, red blood cell indices showed the lower level in the summer season than the winter. As the temperature increased, body temperature of the animals also increases which is usually associated with a rise in water intake and reduced feed intake (22). Increased temperature causes the rostral cooling centre of the hypothalamus to stimulate the medial satiety centre, which inhibits the appetite centre, resulting in reduced feed intake (23). Furthermore, at high ambient temperature, peripheral vasodilatation and redistribution of cardiac output are associated with expansion of blood volume and haemodilution (24). The fluctuation during the experimental periods can be attributed to the changes in water balance. High environmental temperatures may lead to higher evaporative water loss through the skin surface, as well as the respiratory tract, thereby requiring compensatory water intake to regulate body temperature (11, 25). The RBC, Hb and HCT heat-induced depression in cows exposed to high temperatures was probably associated to haemodilution effect, because more water was transported in circulatory system for evaporative cooling (19, 20). On the other hand, as the feed intake reduces, minerals mainly iron; important for hemoglobin synthesis will not be present in the cow's body in adequate level. Meanwhile, the study area situated at the high-altitude region where hypoxic condition is very familiar (26). Vegetations are only possible at this region in the summer season. Due to vegetation, partial pressure of oxygen will be increase in the summer season than the winter season. For the low partial pressure of oxygen, certainly the erythropoietin hormone will increase which will gradually increase the erythrocytes through several mechanisms in the animal body (27). In our study, it has been found that in winter season erythrocyte profile were significantly higher (<0.05). These are the most probable reason for the low level of red blood cell indices in the cattle during summer season (24, 28-32). The significant increase of MCV, MCH, and MCHC in the winter season has been found. It might be due to the hypoxic condition in this region as discussed earlier. Due to hypoxic condition, increase of erythrocyte-stimulating factor (ESF) release will increase because relationships between the oxygen demand of tissue and the mounts of oxygen carried by blood in cattle (20, 33). However, decreased MCV in the summer season may be seen in iron deficiency and chronic blood loss. Decreasing MCH may give an early clue of impending iron deficiency, since MCH falls before MCV and decreased MCHC occurs in iron deficiency anemia (32). In the winter season most of the farmers use concentrated feed; rich in minerals and stored Lucerne. These might be the factors for higher MCV, MCH, and MCHC in the winter season. According to the previous studies (20, 34, 35), and also observed in our results, an increase in PLT values was associated with the lowering temperature. This induction can be due to low ambient temperature that is the main environmental stressing factor (35).

In the present study WBC values were higher in the summer season, confirming the results obtained in previous studies (7, 36). This could be due to the release of corticosteroids or epinephrine hormones as a result of relatively higher temperature which in turn has increased leukocyte count (37). In accordance to Narayan *et al.,* (2007), the present study revealed increased lymphocyte values in the hot period (38). These are might be due to cow's free movement in hard in the open land in the summer season. Moreover, floating of population dynamics are mostly high in the summer season as tourist loading. Infectious agents are might be growing due to the tourist loading (39). Cattle might be affected due to sub clinical parasitic infection in summer for which leucocyte profile might be higher (40, 41). However, in case of monocyte count was found to be higher in summer as compared to winter. This finding is consistent with earlier report in case of goats and cattle (42-44). The increase monocyte count recorded during summer could be associated with increased cortisol secretion. Monocytes respond to elevation of blood corticosteroid concentration (17).

In the present study, glucose and AST level were significantly higher (<0.05) in summer season but albumin level was significantly higher (<0.05) in winter season. Higher glucose level in the blood might be due to the higher fodder plants consumption in summer than the winter (45). However, total protein level was higher in winter season than the summer but that was not significant. But level of albumin significantly higher in the winter season this might be due to the dehydration in winter which may have elevated the concentration of the plasma protein (46, 47). The mean activity of indicative enzymes, AST and ALT activity related to the physiologic state, and their increase also accompanies disorder of energetic metabolism (48, 49).

The reason for describing this phenomenon that the jersey cattle in this study were mainly improved economic condition in this region. Maintaining proper management of cattles will effective for better health. Ultimately, the production level will be better. The study showed that summer season was the better for milk production than in the winter season. As discussed earlier, very harsh condition was stayed in winter season for a long-time period. Finally, Jersey cattle were more adaptive in summer season and that reflected through the milk production (32).

Conclusions

In conclusion, our results showed variations in Hematological parameters related to changes in ambient temperature, relative humidity and temperature-humidity index. This study revealed a close association between the hematological and some biochemical parameters in different seasons. The changes in hematological and biochemical profile indicated that winter season was stressful to Jersey cattle. The hematological and biochemical parameters as estimated in the present investigation could be used as measure of stress in Jersey cattle at high altitude for the winter season. Therefore, this study might be helpful for providing base line information on the hematological and biochemical profile of dairy cattle for the evaluation of nutritional status and milk production. Thereby, improved health management practices and better productivity could be possible at high altitude cold arid region.

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