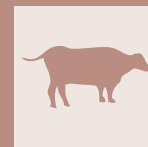


Effect of melatonin implantation on haematological parameters in anestrus lactating buffalo during summer season under tropical conditions



TAMER RAMADAN^{1*}, RAKESH SHARMA², SUSHIL PHULIA², INDERJEET SINGH²

¹ Animal Production Research Institute, Agricultural Research Center, 4 Nadi El-Said, 12311 Dokki, Giza, Egypt

² Animal Physiology and Reproduction Division, ICAR-Central Institute for Research on Buffaloes, 125001, Hisar, Haryana, India

SUMMARY

The purpose of this study was to investigate the effects of melatonin implantation in Murrah buffaloes on some haematological parameters during summer season under tropical conditions. Twelve lactating Murrah buffaloes were divided into control and treated groups of six animals each. Treated buffaloes were implanted with melatonin (18 mg melatonin / 50 kg body weight) at the base of left ear. Blood samples for measuring haematological parameters collected biweekly between 0 and 84 days were analysed by haematology analyzer. Melatonin treatment had no effect on all haematology parameters during summer season under tropical conditions. With the advancement of melatonin treatment increases ($P < 0.05$) in mean corpuscular hemoglobin at day 70 after treatment and in count of mean corpuscular hemoglobin concentration and red blood cell distribution with-coefficient of variation at day 84 were found. In addition, melatonin revealed an increase ($P < 0.05$) in mean platelet volume at days 28 and 56 after treatment. On the other hand, the advancement of melatonin treatment exhibited gradual decrease ($P < 0.05$) in neutrophil, platelets and plateletcrit count 84 days after treatment. Other haematological parameters were not affected by advancement of day after treatment. In general, melatonin implantation did not affect the haematological parameters to counteract the summer stress conditions in lactating buffaloes under tropical conditions.

KEY WORDS

Melatonin implantation, Lactating buffalo, Haematology, Summer season.

INTRODUCTION

Buffaloes are short day breeders with reproductive efficiency adversely influenced by unfavorable biometeorological factors¹. Stress, the obvious reaction of the animal, disturbs body homeostasis causing detrimental effects. Livestocks undergo various types of stresses such as physical, nutritional, chemical, psychological and thermal. Among all, heat stress is the most concerning constraint on animal production nowadays under the ever changing climatic scenario. It is the perceived discomfort and physiological strain associated with exposure to the uttermost hot temperature. It stimulates sort of complex responses which are fundamentals in the preservation of cell survival. Among many adverse effects, heat stress causes the aberration of reproductive functions, oxidative stress, enzymatic dysfunction and electrolyte imbalances. These physiological adjustments are essential to maintain normal body functions and to prevent hyperthermia².

Melatonin has been reported to play a fundamental role in the biology of body cells³ and to influence a wide range of physi-

ological processes for all organs and cells of the body⁴. However, the effect of melatonin on blood parameters is controversial and what is available in literature is contradictory. Karimungi and Joshi⁵ reported that melatonin treatment once or twice daily for two weeks induced a decrease in the red blood cells (RBCs) count and in the erythrocyte indices (MCV, MCH and MCHC) of rats. Durotoye and Rodway⁶ found that subcutaneous implants of melatonin led to reduction in RBCs count and packed cell volume (PCV) in ewes, while increased MCV. Out-of-season breeding in deep anestrus buffaloes, requests the use of melatonin^{7,8}. Melatonin is a hormone produced and stored in the pineal gland during the day and secreted during the dark, starting after sunset and ending at sunrise. It controls the reproductive rhythm in diverse livestock species, like goats and sheep (short-day species), and horses (long-day species), especially at higher latitudes⁹. Melatonin-mediated pathways regulate GnRH pulsatility and, therefore, the activity of the reproductive neuroendocrine axis. It also modulates prolactin secretion by acting on the hypophysis. Melatonin has been shown to be a highly effective antioxidant and free radical scavenger¹⁰. By virtue of its antioxidant properties melatonin, quenches the oxidants including nitric oxide, arrests lipid peroxidation, and acts synergistically with other classic antioxidants such as glutathione peroxidase (GPx), su-

Corresponding Author:

Tamer Ramadan (tamereweda1174@Yahoo.com).

peroxide dismutase (SOD), vitamin E, and selenium¹¹. The main function of melatonin is to serve as an antioxidant to protect organisms from ubiquitous oxidative stresses. It is considered to be more effective than glutathione (GSH) and mannitol in scavenging free radicals. It was found that melatonin has the ability to neutralize damaging reactive oxygen species (ROS) and reduce lipid peroxide concentrations and DNA damage, thereby, improving the viability of germ cells¹². There is a limited data available on the effect of melatonin implantation on the haematology parameters of lactating buffaloes.

The aim of the present study was to analyse the effects of melatonin implantation on some blood haematology parameters in anestrus lactating buffaloes to serve as indicators of their sustainability to the expenditure of melatonin treatment for preventing summer-induced decline in body functions.

MATERIALS AND METHODS

The present study was conducted at the animal farm of the Central Institute for Research on Buffaloes, Hisar, India (29° 10' N, 75° 41' E), using anestrus lactating buffaloes during the out-of-breeding season (from June to September). All procedures and experimental protocols were conducted in accordance with the «Guide for the Care and Use of Agricultural Animals in Research and Teaching»¹³.

Animals and management

Twelve lactating Murrah buffalo (parity: 2-4, body condition score: 4-5, milk yield 7-9 kg/day and body weight: 400-500 kg) at day 65-70 of lactation were used in the present study. The study was conducted during the hot-humid months from June to first of September when ambient temperatures and relative humidity ranged from 35 to 45°C and 35 to 80%, respectively. Daily maximum and minimum temperatures and Relative humidity (RH) were recorded at Department of Agriculture Meteorology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. The daily records of humidity and temperature were obtained from the closest meteorological station, approximately 10 km far from the farm. The temperature-humidity index (THI) was calculated from the equation¹⁴. $THI = (1.8 * AT + 32) - [(0.55 - 0.0055 * RH) * (1.8 * AT - 26)]$, where AT = average temperature (°C), RH = Relative humidity (%). The mean values of temperature and THI in different weeks are described in Figure 1. The experimental period was divided into: low (< 70); moderate (70-80) or high (> 80) THI. Lactating buffaloes were confined for the entire period of study to a barn with access to an open sheltered space. They were subjected to teasing and observed for visual signs of estrus twice per day for one hour but they failed to exhibit estrus characteristics. They were fed on roughage and concentrate supplement according to their body weight requirements¹⁵. Chaffed green fodder and wheat straw were also offered. Water was accessibly available at all times. Animals were free from diseases and were clinically normal with a healthy appearance. Lactating buffaloes were milked twice a day, morning (04:00 a.m.) and evening (03:00 p.m.).

Experimental design

Lactating buffaloes were randomly allocated to melatonin non-implanted (control) and implanted (treated) groups (n = 6 each). In melatonin-treated group, animals were administered

2 × 4 mm absorbable melatonin implants (18 mg melatonin / implant, Regulon, CEVA Animal Health Limited, Chesham, Buckinghamshire, UK) at the base of left ear using an implanter. Total implants inserted to each animal were calculated on the basis of body weight (one implant / 50 kg)¹⁶. These implants were designed to release melatonin for at least 60 days, although their functionality can extend to more than 100 days without disturbing the endogenous secretion of melatonin¹⁷.

Blood haematology parameters

Blood samples were collected via jugular venipuncture into a heparinized vial (at 06:00 a.m.). On both treated and control groups biweekly blood sampling were taken throughout the experiment between days 0 to 84. Collected blood samples were analysed for estimation of haematological parameters which included count of red blood cells (RBC), white blood cells (WBC), lymphocytes (LY), monocytes (MO), Neutrophil, Eosinophil, Basophil, percentage of lymphocytes (LY %), monocytes (MO %), Neutrophil (%), Eosinophil (%), Basophil (%), hemoglobin (Hb), hematocrit (HCT/PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width-coefficient of variation (RDW-CV), platelets (PLT), plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDWC) using hematology analyzer (HA-22-CLINDIAG).

Statistical analysis

All data records were tested for normality with the Shapiro-Wilk (W) test from the UNIVARIATE procedure¹⁸, and results indicated that all data were distributed normally ($W > 0.90$). Data for the effect of melatonin was analyzed using PROC MIXED of SAS (SAS Inst., Inc., Cary, NC) for repeated measures. Data for the effect of melatonin implantation on blood haematology were analyzed by adapting the following model:

$$Y_{ijk} = \mu + T_i + D_j + (T \times D)_{ij} + e_{ijk}$$

where Y_{ijk} is the observed value of the dependent variable determined from a sample taken from each animal, μ is the overall mean, T_i is the fixed effect of the i^{th} treatment ($i = 1, 2$), D_j is the fixed effect of the j^{th} day ($j = \text{day 0:day 84}$), $(T \times D)_{ij}$ is the first-order interaction between treatments and days and e_{ijk} is the residual error. Significant differences among means within each classification were tested using least square differences 0.05.

RESULTS

Means of ambient temperature, relative humidity and THI during the experimental period were 32.55 ± 2.56 °C, 60.81 ± 18.08 % and 83.14 ± 1.78 % respectively (Figure 1). Haematological parameters of anestrus lactating buffalo as affected by melatonin implantation are displayed in Tables 1 and 2. Melatonin implantation had no effect on all haematological parameters during summer season under tropical condition. After melatonin implantation gradual decrease ($P < 0.05$) take place in neutrophil, PLT and PCT (Figure 2). On the other hand, with the advancement of treatment an increase ($P < 0.05$) in MCH was found at day 70 after treatment. Also, increase ($P < 0.05$) in counts of MCHC and RDW-CV was observed at day 84 and in MPV at days 28 and 56 after melatonin implantation (Figure 3). Other haematological parameters were not affected by

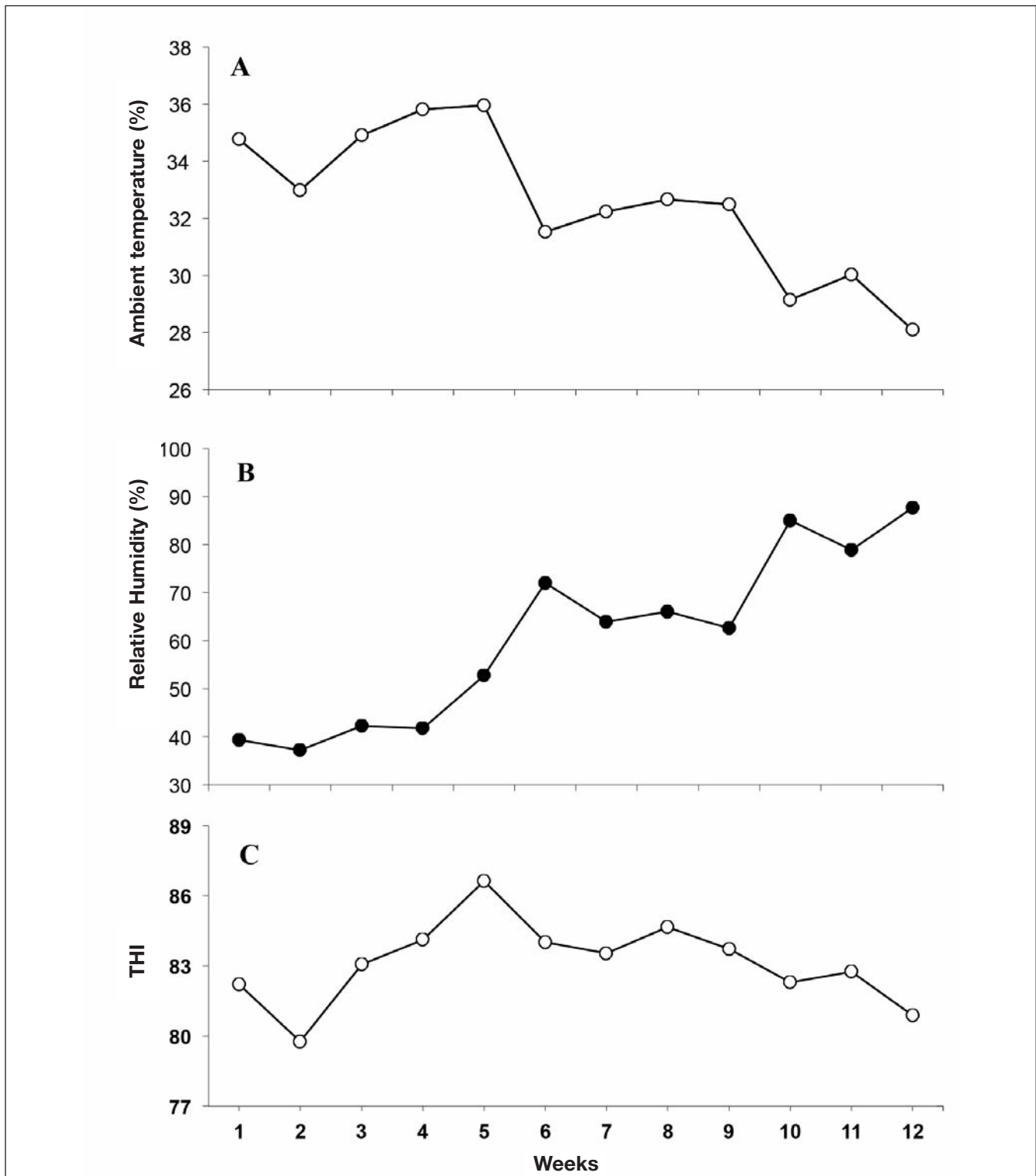


Figure 1 - The weekly average ambient temperature (A), relative humidity (B) and temperature-humidity index (THI) over the experimental period.

advancement of day after melatonin implantation of lactating buffalo during summer season under tropical conditions.

DISCUSSION

The significance of reference values on haematological indices was recognised as being useful in determining the general health status of animals¹⁹, an aid for differential diagnosis of clinical conditions and for monitoring response to therapy²⁰. Although

melatonin implantation had many beneficial effects on improving reproductive performance in heifers and lactating buffaloes^{7,8} and on semen quality of buffalo bulls²¹ it had no obvious effects on haematology of lactating buffaloes.

The blood system is sensitive to temperature changes and is an important indicator of physiological responses to stressors. Several factors such as species, breed, sex, age, nutrition, diseases, physiological stage and seasonal variations can affect the pattern of haematological values^{22,23}. Quantitative and morphological changes in blood cells are associated with heat stress. Other stud-

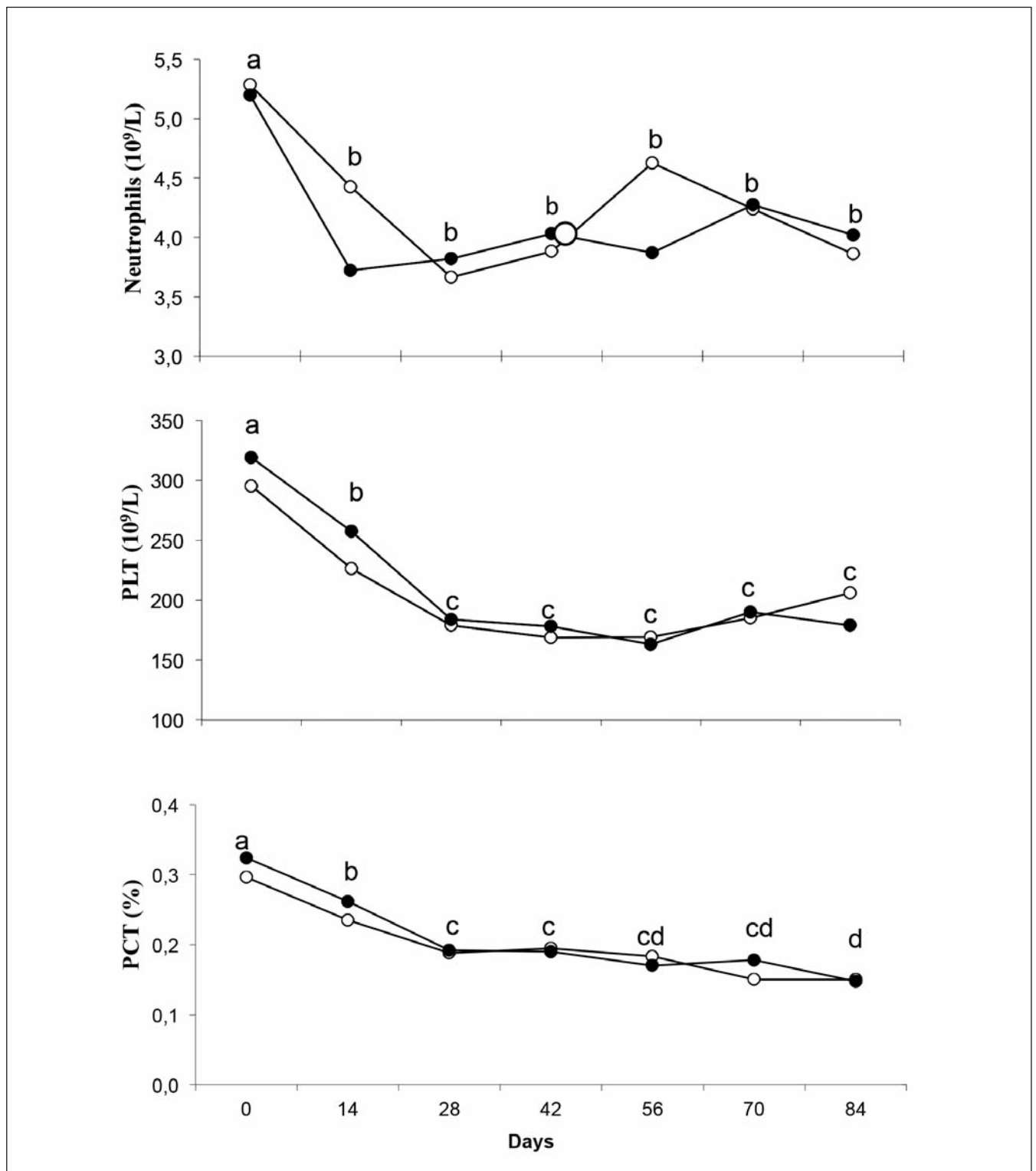


Figure 2 - Effect of day after melatonin treatment on some blood haematological parameters neutrophils ($10^9/L$), platelets ($10^9/L$) and PCT (%) of control (○) and treated (●) groups of lactating buffalo during summer season (Least square means \pm SEM).

ies show the variations in hematocrit values, mean erythrocytes count and hemoglobin^{24,25}. Poor nutrition, which occurs in animals under long-term heat stress, reduces the number of erythrocytes and hemoglobin level, resulting in a decrease of red blood cells in the bloodstream²⁶. With the rise in environmental temperature, the animal loses liquids through the respiratory tract, reducing the blood plasma volume and increasing the concentration of hematocrit. If physical exertion is prolonged, dehydration occurs, and thus, loss of fluids by the evaporative

process, results in more hematocrit increase. Thermal stress may cause hyperthermia and potentially have several physiological side effects. It is known to alter the homeostatic mechanisms of animals resulting in impaired erythropoiesis.

In the present study, mean values for all haematological parameters were situated in the normal physiological limits without statistical significant difference between implanted and unimplanted animals. However, the mean RBCs and Hb were found to be numerically lower after melatonin implantation.

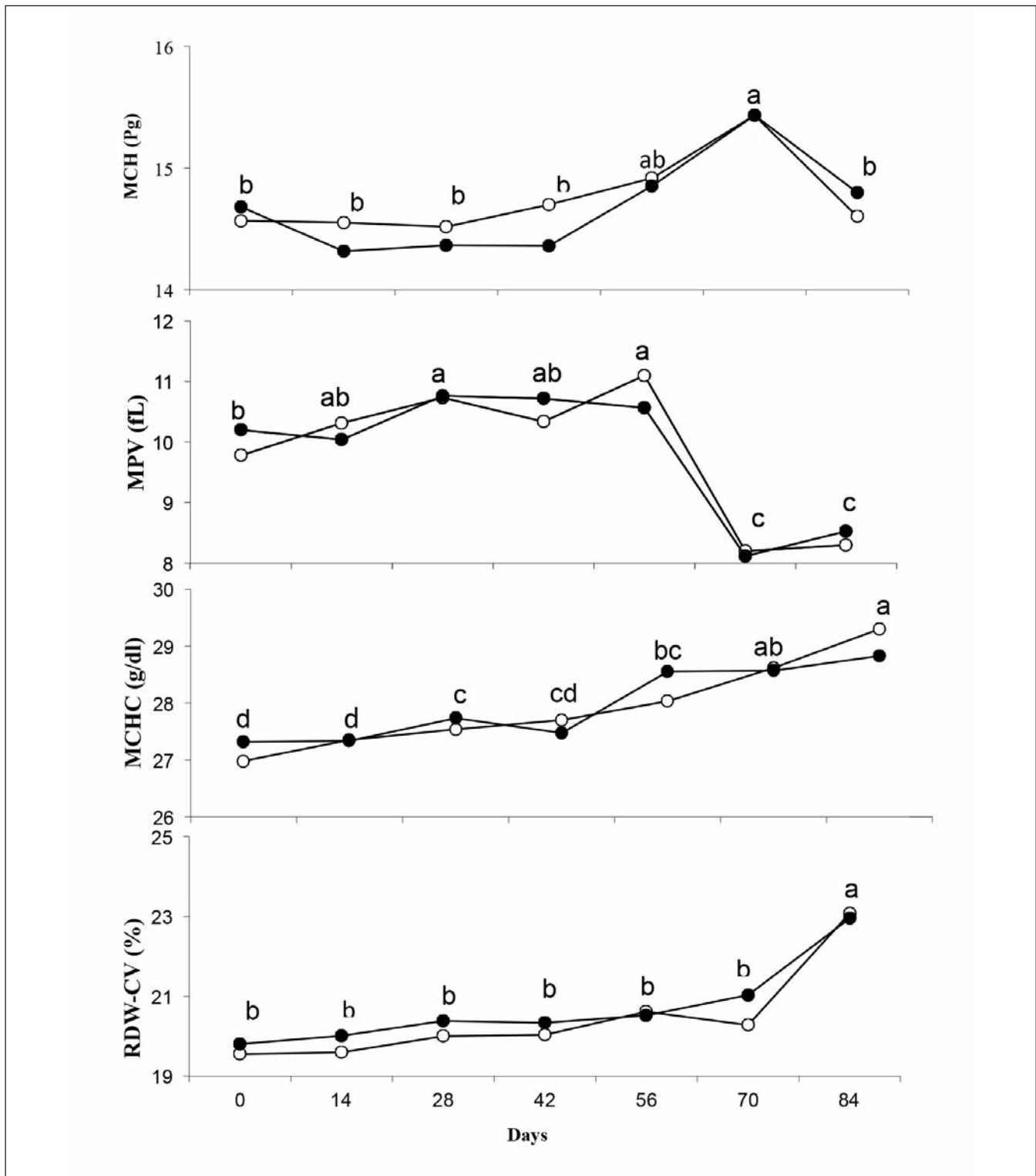


Figure 3 - Effect of day after melatonin treatment on some blood haematological parameters MCH (Pg), MPV (fL), MCHC (g/dl) and RDW-CV (%) of control (○) and treated (●) groups of lactating buffalo during summer season (Least square means \pm SEM).

The variation in RBCs may be attributed to the fact that the high environmental temperature increases the animals oxygen consumption through increased respiration rate. The high oxygen intake increases the partial pressure of oxygen in blood, decreases erythropoiesis, which in turn reduces the number of circulating RBCs and Hb values²⁷. Kumar et al.²⁸ also recorded low mean Hb in summer stressed Beetal goats as compared to pre-summer values.

No literature concerning the effect of melatonin implantation

on haematological parameters in lactating buffaloes during summer season are available. However, Anwar et al.²⁹ found that melatonin treatment in rats increased RBCs, Hb and PCV numerically. On the other hand, Durotoye and Rodway⁶ reported that implants of melatonin in ewes reduced RBCs count and PCV. The average values for Hb varied insignificantly in control (10.18 ± 0.23 g/dL) and treated groups (9.83 ± 0.23 g/dL). However, the level of hemoglobin was influenced by the season with the highest values obtained in summer season²³. Also,

Table 1 - Effect of melatonin treatment on blood haematological parameters of lactating buffalo during summer season (Least square means \pm SEM).

Parameter	Treatment (T)		SEM
	Control	Melatonin	
RBCs ($10^{12}/L$)	6.84	6.77	0.16
Hb (g/dL)	10.18	9.83	0.23
WBC ($10^9/L$)	8.98	8.92	0.30
Lymphocytes ($10^9/L$)	4.90	4.41	0.26
Monocytes ($10^9/L$)	0.27	0.26	0.10
Neutrophil ($10^9/L$)	4.28	4.13	0.16
Eosinophil ($10^9/L$)	0.09	0.08	0.01
Basophil ($10^9/L$)	0.011	0.012	0.01
HCT (%)	36.43	35.80	0.79
MCV (fL)	53.26	52.97	0.48
MCH (Pg)	14.75	14.68	0.12
MCHC (g/dl)	27.93	27.97	0.14
RDW-CV (%)	20.45	20.71	0.19
PLT ($10^9/L$)	204.25	210.07	7.09
PCT (%)	0.19	0.20	0.01
MPV (fL)	9.82	9.85	0.14
PDWC (%)	36.37	36.28	0.39

Fagiolo et al.³⁰ reported higher Hb values in lactating buffaloes, during the summer season (13.62 g/dL) compared to winter (11.37 g/dL).

A plausible explanation for the decrease in Hb levels during thermal stress could be the increased attack of reactive oxygen molecules on the erythrocyte membrane which is rich in lipid content, and ultimate lysis of RBC or inadequate nutrient avail-

ability for Hb biosynthesis due to decreased voluntary intake as the animal consumes less feed under heat stress³¹. During summer stress a significant depression in Hb levels may also be due to haemodilution effect where more water is infused into the circulatory system for evaporative cooling³². At high temperature, peripheral vasodilation and redistribution of cardiac output are associated with expansion of blood volume and result in haemodilution³³.

Moreover, the increase in Hb concentration obtained in the control group of the present study may be attributed either to its direct stimulatory effect on bone marrow²⁹ or indirectly through stimulation of some cytokines, which have a powerful stimulatory effect on bone marrow cells proliferation. Another possible explanation for the increase in Hb levels after goat exposure to sunshine and melatonin treatment during thermal stress could be the protection of bone marrow from damage by free radicals due to melatonin antioxidant effect³⁴. The average percentages of hematocrit ($36.43 \pm 0.79\%$ in control and $35.80 \pm 0.79\%$ in treated buffaloes) were between those in summer (40.75%) and those in winter period (32.63%) as reported by Fagiolo et al.³⁰ and Enculescu et al.²³.

Total WBC count in the current study were similar to those reported by Enculescu et al.²³ and Garkal et al.²². No available literature could be traced regarding the effect of melatonin on this parameter in lactating buffaloes treated with melatonin. Hasin et al.³⁵ showed that melatonin treatment was found to induce a significant increase in total leucocyte count in goats and these results are consistent with those previously reported showing that administration of melatonin increased the TLC in broiler chicks³⁶, rats²⁹ and squirrels³⁷. The precise mechanism responsible for this increase is not clear. However, several mechanisms could be involved in this respect. One possible mechanism may be the direct action of melatonin either on bone marrow³⁸ or on lymphatic tissue³⁹ to accelerate leukocytopoiesis⁴⁰. The second possible mechanism may be an indirect ac-

Table 2 - Effect of day after melatonin treatment on blood haematological parameters of lactating buffalo during summer season (Least square means \pm SEM).

Parameter	Day (D)							
	0	14	28	42	56	70	84	
RBC ($10^{12}/L$)	7.03	7.00	6.94	6.75	6.64	6.68	6.60	
Hb (g/dL)	9.70	10.09	10.05	9.97	9.85	10.27	10.12	
WBC ($10^9/L$)	9.24	9.26	8.70	8.20	8.54	9.72	8.99	
Lymphocytes ($10^9/L$)	4.31	5.50	4.85	4.11	4.21	4.69	4.92	
Monocytes ($10^9/L$)	0.30	0.19	0.21	0.23	0.28	0.31	0.31	
Neutrophil ($10^9/L$)	5.24 ^a	4.07 ^b	3.74 ^b	3.95 ^b	4.25 ^b	4.25 ^b	3.94 ^b	
Eosinophil ($10^9/L$)	0.06	0.11	0.09	0.08	0.09	0.07	0.06	
Basophil ($10^9/L$)	0.011	0.010	0.010	0.013	0.010	0.016	0.011	
HCT (%)	36.37	36.45	36.98	36.39	35.84	35.93	34.87	
MCV (fL)	54.08	52.91	52.50	53.00	52.91	53.91	52.50	
MCH (Pg)	14.62 ^b	14.43 ^b	14.44 ^b	14.53 ^b	14.88 ^{ab}	15.43 ^a	14.70 ^b	
MCHC (g/dl)	27.15 ^d	27.34 ^d	27.63 ^{cd}	27.58 ^{cd}	28.29 ^{bc}	28.59 ^{ab}	29.06 ^a	
RDW-CV (%)	19.67 ^b	19.80 ^b	20.19 ^b	20.18 ^b	20.56 ^b	20.65 ^b	23.01 ^a	
PLT ($10^9/L$)	307.10 ^a	241.75 ^b	181.50 ^c	173.42 ^c	166.17 ^c	187.71 ^c	192.47 ^c	
PCT (%)	0.31 ^a	0.24 ^b	0.19 ^c	0.19 ^c	0.17 ^{cd}	0.16 ^{cd}	0.14 ^d	
MPV (fL)	9.99 ^b	10.17 ^{ab}	10.75 ^a	10.52 ^{ab}	10.83 ^a	8.15 ^c	8.41 ^c	
PDWC (%)	35.88	36.35	37.23	36.01	35.79	36.41	36.60	

^{a,b} Within a row, means with different superscript differ ($P < 0.05$).

tion through reduction of corticosterone hormone, which is associated with the elevation of the leukocytic count²⁹. A third possible mechanism may be the protective effect of melatonin on bone marrow from damage by the free radicals due to its antioxidant effect³⁴.

The advancement of day of treatment revealed significant increase in total lymphocytes, Eosinophils and Basophils percentages in days 42, 84 and 70 in lactating buffalo. Similarly, administration of melatonin increased the total leukocytic count and lymphocyte percentage in broiler chicks³⁶, rats²⁹, immature chicks⁴¹ and squirrels³⁷. Rai and Haldar⁴² reported that daily subcutaneous injection of melatonin increased significantly the lymphocyte count in adult male squirrels, while pinealectomy decreased it along with percent lymphocyte count in peripheral blood and bone marrow. The WBC counts of birds injected subcutaneously with 40 mg melatonin/kg BW/day for 7 days were significantly higher than the WBC counts of saline-injected birds⁴¹. The increase in neutrophil counts after day 0 of the experiment commencement could be attributed to lactational stress leading to the release of endogenous corticosteroids⁴³. The monocyte counts recorded in this study were in accordance with those of Ellah et al.⁴⁴ in heifers and of Ali and Shukla⁴⁵ in normal cyclic post-partum buffaloes.

Advancement of day of treatment showed an increase in MCH and MCHC of lactating buffalo towards the end of experiment. Lee et al.⁴⁶ described a significant decrease in haematocrit value and RBC counts in dairy cows exposed to high temperatures. This decrease was probably caused by a rise in erythrocyte destruction; haemodilution effect could also participate here, because more water was transported to the circulatory system for evaporative cooling. Nadia⁴⁷ indicated that heat stress decreases MCH and MCHC, and increases MCV value in heat stressed Japanese quail. Nonetheless, the values of MCV, MCH, and MCHC did not vary significantly between groups of Murrah buffaloes in the current study. The increased RDW-CV at day 84 of the experiment was probably due to enhanced erythropoiesis. On the other hand, gradual decrease in total neutrophils, PLT and PCT with advancement of day of treatment were obtained. Current findings for PLT counts were in tune with those recorded by Das et al.⁴⁸ on lactating Mehsani buffaloes.

Little is known about the antioxidant defense mechanisms in anestrus buffalo. Oxidative stress, caused by different metabolic processes, is controlled by various antioxidant defense mechanisms including antioxidant enzymes⁴⁹. Ramadan et al.⁸ reported that melatonin implantation increased superoxide dismutase (SOD) activity in anestrus lactating buffalo during non-breeding season. Therefore, it stimulates the activities of enzymes involved in metabolizing reactive oxygen species (ROS) and preserves cell membrane fluidity. Indeed, melatonin was shown to be twice as potent as vitamin E in removing peroxyl radicals⁵⁰, and it is more effective in scavenging hydroxyl radicals than glutathione and mannitol⁵¹. Melatonin also protects and stimulates the activities of antioxidant enzymes such as SOD⁵² which acts as antioxidant that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide.

CONCLUSIONS

The values obtained for haematological parameters in melatonin treated and untreated buffalo groups were within normal physiological limits. These values could be useful for in-

terpretation of haematological parameters in lactating buffaloes. The responsiveness of lactating buffalo to melatonin implantation in summer season showed non significant differences in all blood parameters. No significant deviations in different haematological indices of lactating buffalo during summer seasons after melatonin treatment were declared.

Acknowledgements

This research was funded by a research grant awarded by CV Raman International Fellowship for African Researchers under Post-Doctoral Fellowship, New Delhi, India.

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and the unnecessary discomfort to the animals was avoided.

Conflict of interest statement

The authors declare no conflict of interest.

References

1. Phogat J.B., Pandey A.K., Singh I. 2016. Seasonality in buffaloes reproduction. *Int J Plant Anim Environ Sci*, 6: 46-54.
2. Lowe T.E., Christian C.J., Ingram J.R., Hairrs P. 2001. Impact of climate on thermal rhythm in pastoral sheep. *Physiol Behav*, 74: 659-64.
3. Reiter R.J., Robinson J.O. 1995. Melatonin: your body's natural wonder drug. In Reiter, R.J. & Robinson (Eds), Bantam Books, 1540 Broadway, New York, New York 10036.
4. Lamosova D., Zeman M., Jurani M. 1997. Influence of melatonin on chick skeletal muscle cell growth. *Comp Biochem Physiol Part C: Pharmacol Toxicol Endocrinol*, 118 C, 375-375.
5. Karimungi M.G., Joshi B.N. 1996. Diurnal sensitivity in melatonin - induced hematological changes in the male albino rat. *Biol Signals*, 5 (5): 283-290.
6. Durotoye L.A., Rodway R.G. 1996. Effect of melatonin on hematological indices in the ewe. *Veterinarski Archiv*, 66 (2): 87-95.
7. Ramadan T.A., Sharma R.K., Phulia S.K., Balhara A.K., Ghuman S.S., Singh I. 2014. Effectiveness of melatonin and controlled internal drug release device treatment on reproductive performance of buffalo heifers during out-of-breeding season under tropical conditions. *Theriogenology*, 82: 1296-302.
8. Ramadan T.A., Sharma R.K., Phulia S.K., Balhara A.K., Ghuman S.S., Singh I. 2016. Manipulation of reproductive performance of lactating buffaloes using melatonin and controlled internal drug release device treatment during out-of-breeding season under tropical conditions. *Theriogenology*, 86: 1048-1053.
9. Chemineau P., Malpau B., Delgado J.A. 1992. Control of sheep and goat reproduction: use of light and melatonin. *Anim Reprod Sci*, 30: 157-84.
10. Mauriz J.L., Collado P.S., Veneroso C., Reiter R.J., Gonzalez-Gallego J. 2013. A review of the molecular aspects of melatonin's anti-inflammatory actions: recent insights and new perspectives. *J Pineal Res*, 54: 1-14.
11. Gitto E., Tan D.X., Reiter R.J., Karbownik M., Manchester L.C., Cuzzocrea S. 2001. Individual and synergistic antioxidative actions of melatonin: studies with vitamin E, vitamin C, glutathione and desferrioxamine (desferoxamine) in rat liver homogenates. *J Pharm Pharmacol*, 53: 1393-1401.
12. Wang F., Tian X., Zhang L., Tan D.X., Reiter R.J., Liu G. 2013. Melatonin promotes the in vitro development of pronuclear embryos and increases the efficiency of blastocyst implantation in murine. *J Pineal Res*, 55: 267-274.
13. Federation of Animal Science Societies (FASS). 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. FASS, Champaign, IL.
14. Kendall P.E., Webster J.R. 2009. Season and physiological status affects the circadian body temperature rhythm of dairy cows. *Livest Sci*, 125: 155-160.
15. NRC. 2001. Nutrient Requirement of Dairy Cattle, seventh revised edition, National Academy of Science, Washington, D.C., USA.
16. Papachristoforou C., Koumas A., Photiou C. 2007. Initiation of the breed-

- ing season in ewe lambs and goat kids with melatonin implants. *Small Rumin Res*, 73: 122-126.
17. Forcada F, Zarazaga L., Abecia J.A. 1995. Effect of exogenous melatonin and plane of nutrition after weaning on estrous activity, endocrine status and ovulation rate in Salz ewes lambing in the seasonal anestrus. *The-riogenology*, 43: 1179-93.
 18. SAS. 2000. SAS user's Guide: Statistics, version 8 Edition SAS Inst., Inc., Cary, NC.
 19. Beechler B.R., Jolles A.E., Ezenwa V.O. 2009. Evaluation of hematologic values in free-ranging African buffalo (*Syncerus caffer*). *J Wildlife Diseases* 45: 57-66.
 20. Uppal G., Raina V., Sharma A., Anand M., Kumar R., Khaitan B.K., Grover J.K. 2005. Use of simple haematological, biochemical and clinical parameters to monitor response of multiple myeloma patients on high dose thalidomide therapy. *Indian J Cancer*, 42: 46-50.
 21. Ramadan T.A., Kumar D., Ghuman S.S., Singh I. 2019. Melatonin-improved buffalo semen quality during non-breeding season under tropical condition. *Dom Anim Endocrinol*, 68: 119-125.
 22. Garkal R.A., Keken P.M., Kharde P.M., Munde V.K., Narladkar B.W., Vaidya M.S. 2016. Effect of different housing systems on haematological parameters of buffaloes. *Buffalo Bulletin*, 35 (2) :191-198.
 23. Enculescu M., Nicolae I., Bota A. 2017. The Season Influence on blood parameters in lactating Buffalo females. *Anim Sci Biotechnol*, 50 (1): 10-14.
 24. Ribeiro N.L., Costa R.G., Pimenta Filho E.C., Ribeiro M.N., Crovetto A., Saraiva E.P., Bozzi R. 2016. Adaptive profile of Garfagnina goat breed assessed through physiological, haematological, biochemical and hormonal parameters. *Small Rumin Res*, 144: 236-241.
 25. Ribeiro M.N., Neila L., Bozzi R.R., Costa R.G. 2018. Physiological and biochemical blood variables of goats subjected to heat stress - a review. *J Applied Anim Res*, 46 (1): 1036-1041.
 26. Swenson M.J., Reece W.O. 2006. *Dukes - Fisiologia dos Animais Domésticos*. Rio de Janeiro: Guanabara Koogan S.A.
 27. Maurya V.P., Naqvi S.M.K., Joshi A., Mittal J.P. 2007. Effect of high temperature stress on physiological responses of Malpura sheep. *Indian J Anim Sci*, 77: 1244-1247.
 28. Kumar M., Jindal R., Nayyar S. 2011. Lipid peroxidation and antioxidant activities in summer stressed goats. *Indian Vet J*, 88 (10): 74-78.
 29. Anwar M.M., Mahfouz H.A., Sayed A.S. 1998. Potential protective effects of melatonin on bone marrow of rats exposed to cytotoxic drugs. *Comp Biochem Physiol Part A: Molecul Integr Physiol* 199 (2): 49-501.
 30. Fagiolo A., Lai O., Alfieri L., Nardon A. Cavallina R. 2004. Environmental factors and different managements that influence metabolic, endocrine and immuno responses in water buffalo during lactation, Proc. Seventh World Buffalo Congress, Manila, Philippines, 24-26.
 31. Srikandakumar A., Johnson E.H., Mahgoub O. 2003. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Rumin Res*, 49: 193-198.
 32. El-Nouty F.D., Al-haidary A.A., Salah M.S. 1990. Seasonal variations in haematological values of high and average-yielding Holstein cattle in semi-arid environment. *J King Saud Univ Sci*, 2 (2): 172- 173.
 33. Ganong W.F. 2003. Circulation. In: *Review of Medical Physiology*. Lange Medical Books/McGraw-Hill. New York. pp. 518.
 34. Ahmed H.H., Essawy G.S., Salem H.A., Abdel Daim M.A. 2005. Melatonin has a strong antioxidant activity and improves liver and kidney functions in broiler chicks. *Egyptain J Basic Appl Physiol* 4 (1): 77-92.
 35. Hasin D., Bora A., Goswami J., Barua A. 2017. Saleque A. and Sarmah B. K. effects of direct sunshine and melatonin on physiological responses and haematological profiles in beetal and assam hill goat in assam. *Vet-erinary Practitioner*, 18 (1) : 89-93.
 36. Abozakra A.A., El-sayed M., Elshazly K.A., Saad M.F. 1998. The influence of melatonin on the immune response to IBD vaccination in broilers. *J Zagazig Vet Med 4th Vet Med Zag Congress*, 600-608.
 37. Rai S., Haldar C., Singh R. 2009. Modulation of immunity in young-adult and aged squirrel, *Funambulus pennanti* by melatonin and p-chlorophenylalanine. *Immunity and Aging*, 6: 1-8.
 38. Kuci S., Becker J., Veit G., Haldar C., Handgretinger R., Attanasino A. 1987. Circadian variations in the immunomodulatory role of the pineal gland. *Neuroendocrinol Lett*, 9 (5): 287.
 39. Maestroni G.J., Conti A. 1993. Melatonin in relation to the immune system. In. *Melatonin Biosynthesis, Physiological Effect and Clinical Applications*. Yu, H.S. and Retier, R.J. (Eds). CRC Press, New York. pp. 289-309.
 40. Conti A., Gattera N.H., Maestroni G. 1992. Role of pineal melatonin and melatonin- induced immune-opioids in murine leukemogenesis. *Med Oncol Tumor Pharcother* 19 (2): 87-92.
 41. Brennan C.P., Hendricks G.L., El-Sheikh T.M., Mashaly M.M. 2002. Melatonin and enhancement of immune responses in immature male chickens. *Poultry Sci*, 18: 371-375.
 42. Rai S., Haldar C. 2003. Pineal control of immune status and hematological changes in blood and bone marrow of male squirrels *Funambulus pennanti* during their reproductive active phase. *Comp Bioch Physiology Toxicology Pharmacology*, 136 (4): 319-328.
 43. Prava M., Dixit N.K., Tolankhomba T.C. 2012. Leukocyte picture of Frieswal cattle. *Indian Vet J*, 89: 13-15.
 44. Ellah M.R.A., Hamed M.I., Ibrahim D.R., Rateb H.Z. 2014. Serum biochemical and haematological reference intervals for water buffalo *Bubalus bubalis* heifers. *J South African Vet Assoc*, 85: 962.
 45. Ali R., Shukla S.P. 2012. Haemato-biochemical changes in post-partum anoestrus buffaloes during low breeding season. *Researcher*, 4: 55-58.
 46. Lee J.A, Roussel J.D., Beatty J.F. 1976. Effect of temperature-season on adrenal cortical function, blood cell profile and milk production. *J Dairy Sci*, 59: 104-108.
 47. Nadia M.A. 2003. A study of some physiological, productive and reproductive parameters of Japanese quail under stress condition. PhD. thesis Department of poultry production. Faculty of Agricul. El- Fayoum. Cairo Univ.
 48. Das H., Lateef A., Panchasara H.H., Parsani H.R., Patel J.B., Shah N.M. 2013. Profile of lactating Mehsani Buffalo (*Bubalus bubalis*). *Technical Bulletin No. 04/2013*. Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar.
 49. Rock C.L., Jacob R.A., Bowen P.E. 1996. Update on the biological characteristics of the antioxidant micronutrients: vitamin C, vitamin E and the carotenoids. *J American Diets Assoc*, 96: 693-702.
 50. Pieri C., Marra M., Moroni F., Recchioni R., Marcheselli F. 1994. Melatonin: a peroxyl radical scavenger more effective than vitamin E. *Life Sci*, 55: 271-276.
 51. Hardeland R., Reiter R.J., Poeggeler B., Tan D.X. 1993. The significance of the metabolism of the neurohormone melatonin: antioxidative protection and formation of bioactive substances. *Neurosci Biobehav Rev*, 17: 347-57.
 52. Karbownik M., Reiter R.J. 2000. Effetti antiossidanti della melatonina nella protezione contro i danni cellulari causati dalle radiazioni ionizzanti. *Procedi Soc Experim Biol Med*, 225: 9-22.