RESEARCH ARTICLE

Effect of Exogenous Melatonin and Different Photoperiods on Differential Leucocyte Count in Chhotanagpuri Ewe

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Abstract

Forty-two healthy, non-pregnant, non-lactating Chhotanagpuri ewe, having body weight ranging from 14.11 ± 0.09 to 15.38 ± 0.06 kg, were selected and isolated from rams 2 months before melatonin administration. The selected animals were allocated randomly into seven groups each of 6 animals, *viz.*, Group-I [Normal control], Group-II [Long day (LD) control], Group-III [LD + melatonin administration 3 mg/day orally], Group-IV [(LD + melatonin administration s/c @ 1 mg/day], Group-V [Short day (SD) control], Group-VI [SD + melatonin administration 3 mg/day orally] and Group-VII [SD + melatonin administration s/c @ 1 mg/day]. Rams were then introduced into each group after completion of exogenous administration of melatonin for one month. Blood samples without anticoagulant in vials were collected from each animal at day-0 (a day before the start of experiment), 30th, 60th, 90th, 120th and 150th of the experiment. Among different periods, lymphocyte % in the third and fourth months of the experiment was significantly ($p < 0.05$) lower in all groups (group-I to VII) in comparison to day-0, first, and second months. In the fifth month, lymphocyte % was significantly ($p < 0.05$) higher in group III, IV, VI, and VII compared to the third and fourth months of the experiment. Neutrophil % in the third and fourth months of the experiment was significantly ($p < 0.05$) higher in all groups in comparison to 0 days, first, and second months. In the fifth month, neutrophil % was significantly ($p < 0.05$) lower in group III and VI than in the third and fourth months. Monocyte % was significantly ($p < 0.05$) higher in group III and IV than group VII at first month. In the third month, monocyte % was significantly ($p < 0.05$) higher in group IV and VI than in group I and group II.

Keywords: Chhotanagpuri Ewe, Differential leucocyte count, Exogenous Melatonin, Photoperiod.

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Introduction

The major functions of the white blood cells and their differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms, and produce or at least transport and distribute antibodies in immune response (Etim, 2015). Thus, animals with low white blood cells are exposed to a high risk of infection. In contrast, those with high counts are capable of generating antibodies in the process of phagocytosis and have a high degree of resistance to disease (Soetan *et al*., 2013) and enhancing adaptability to local environment and disease prevalent conditions (Isaac *et al*., 2013). According to Etim *et al*. (2014[a,b]) immune status is a function of leucocytes, lymphocytes, and neutrophils. Lymphocytes are known to play critical roles in the immune defense system of both men and animals (Etim *et al*., 2014[b]).

Melatonin signaling is necessary for photoperiodic adjustment in immune responses; it is difficult to parse the direct differential effects of melatonin on immune cells from indirect modulatory actions on other neuroendocrine axes, including the systems that control gonadal and adrenal steroids. However, the hypothesis that melatonin acts on immune function via downstream hormonal effects has not been born out, as gonadectomy and steroid replacement has minimal effect on photoperiodic plasticity in the immune system (Prendergast *et al*., 2005).

Exogenous melatonin administration increased the total leucocytic count and lymphocyte percentage in squirrels (Rai

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et al., 2009) and goats (Singh *et al*., 2014). Rai and Haldar (2003) reported that daily subcutaneous injection of melatonin at 17:30 to 18:00 hr significantly increased the lymphocyte count of blood in adult male squirrels, while pinealectomy decreased total leucocyte count and percent lymphocyte count in peripheral blood and bone marrow. The precise mechanism responsible for this melatonin-induced increase in the total leucocytic count and lymphocyte percentage is not precise. However, several mechanisms may be involved in this respect. The objective of this experiment was to determine differential leucocyte count in Chhotanagpuri sheep after exogenous administration of melatonin following different photoperiods.

Materials and Methods

The present study was conducted in the Department of Veterinary Physiology, College of Veterinary Science and AH, Ranchi-6, Jharkhand (India) located at 23.36°N latitude and 85.33°E longitude with an altitude of 651 m above mean sea level. The Institutional Animal Ethics Committee approved the design of the experiment vide letter No. 139/528/RVC/IAEC. Forty-two healthy, non-pregnant, non-lactating Chhotanagpuri ewes, having mean body weight ranging from 14.11 ± 0.09 to 15.38 ± 0.06 kg, reared under uniform managemental practices were selected. The selected ewes were isolated from rams before melatonin administration. They were allocated randomly into seven groups (*viz.*, group-I to group-VII) comprising six animals in each group. Group-I [Normal control]: The animals were exposed to normal variation in day length. Group-II [Long day (LD) control]: In this group in addition to natural sunlight, artificial light was provided to animals for maintaining 16-18 hours of light every day for one month and considered as long day control. Group-III, ewes were managed as in group II, additionally melatonin was administered @ 1mg/day, orally during 31st to 60th day (one month). In group-IV, ewes were managed as in group II, additionally melatonin was administered @ 1mg/day, subcutaneously, during 31st to 60th day (one month). Group-V [Short day (SD) control]: Animals in this group were provided only 8 hours natural day light and were then kept in a light-proof shed for 16 hours exposure in dark every day for the period of one month. This group served as short day control. In group-VI, ewes were managed as in group V, additionally melatonin was administered @ 1mg/day, orally, during 31st to 60th day (one month). In group-VII, ewes were managed as in group V, additionally melatonin was administered @ 1mg/day, subcutaneously, during 31st to 60th day (one month). Rams were introduced into each group after completion of exogenous administration of melatonin for one month. Blood samples were collected from each animal for six occasions; first at day-0 (day before the start of experiment) followed by 30 days intervals for five occasions, Blood (0.5 mL) was collected from each animal in clean EDTA coated tubes, and differential leucocytes count (%) was counted as per Schalm *et al.* (1975). Data were analyzed statistically (Snedecor and Cochran, 2004).

Results and Discussion

The average value of lymphocytes present in different treatment groups recorded at different intervals is presented in Table 1. Lymphocyte % at third and fourth month was significantly ($p<0.05$) lower in all groups (group I to VII) in comparison to 0 day, first and second month, which may be attributed to increased physiological stress during early pregnancy leading to lymphopenia (Awodu *et al*., 2002). In the fifth month, lymphocyte % was significantly ($p<0.05$) higher in groups III, IV, VI, and VII compared to the third and fourth month of the experiment due to the high pregnancy rate in these groups. An increasing trend of lymphocyte % was observed in all remaining groups in the fifth month, but this increase was statistically non-significant (Table 1). Bozdogan and Baysal (2003) observed a significant increase in lymphocyte % during pregnancy in Tuj sheep. Kandiel *et al.* (2016) reported that lymphocyte percent count in Barki sheep in mid-stage (90-100 day) was higher than early-stage (50-60 day) of pregnancy, which was similar to our findings. This may be as a result of building the immunity of the fetus. After exogenous melatonin administration, lymphocyte % increased in all melatonin treated groups compared to 0 day and first month, but this increase was statistically non-significant. Kaushlendra and Haldar (2012) reported a strong positive correlation of melatonin and lymphocyte % in female

Table 1: Lymphocyte (%) of Chhotanagpuri ewes in different groups at different periods (Mean ± SE)

Group	*0 Day*	*First Month*	*Second Month*	*Third Month*	*Fourth Month*	*Fifth month*
Group – I	$59.99^{a} \pm 1.68$	$59.73^{a} \pm 1.12$	$59.65^{a} \pm 1.14$	$54.13^{b} \pm 1.76$	$52.77^{b} \pm 1.99$	$56.14^{A\,ab} \pm 0.86$
Group – II	$60.48^{a} \pm 1.20$	$59.34^{ab} \pm 1.16$	$59.94^{a} \pm 1.20$	$54.11^{c} \pm 1.78$	$53.26^{c} \pm 1.80$	$55.93^{Abc} \pm 0.89$
Group – III	$59.80^{a} \pm 1.16$	$58.50^{a} \pm 0.87$	$60.37^{a} \pm 0.98$	$50.64^{c} \pm 0.93$	$49.13^{c} \pm 0.77$	$53.55^{Bb} \pm 0.81$
Group – IV	$59.07^{a} \pm 1.11$	$57.99^{a} \pm 1.11$	$59.84^{a} \pm 1.15$	$51.21^{c} \pm 1.15$	$50.0^{c} \pm 1.27$	$54.58^{ABb} \pm 0.62$
Group – V	$58.97^{ab} \pm 0.74$	$60.01^{a} \pm 1.21$	$59.30^{ab} \pm 1.29$	$54.23^{c} \pm 1.76$	$53.41^{c} \pm 1.72$	$55.74^{ABbc} \pm 0.83$
Group –VI	$59.44^{a} \pm 1.31$	$60.57^{a} \pm 0.85$	$61.20^{a} \pm 0.76$	$50.61^{c} \pm 1.01$	$49.12^{c} \pm 0.75$	$53.95^{ABb} \pm 0.63$
Group–VII	$59.55^{a} \pm 1.51$	$60.68^{a} \pm 1.34$	$61.43^{a} \pm 1.38$	$53.25^{c} \pm 1.63$	$51.80^{c} \pm 1.71$	$55.58^{ABb} \pm 0.76$

Means bearing different superscript vary significantly ($p < 0.05$) within the groups (a, b, c) and between the groups (A, B).

goats. Exogenous administration of melatonin increased the lymphocyte % in squirrels (Rai *et al*., 2009) and goats (Singh *et al*., 2014), which agrees with our findings.

The neutrophil percent count of a ewe in different groups at different intervals is presented in Table 2. Neutrophil percent in the third and fourth month experiment was significantly ($p<0.05$) higher in all groups than 0 day, first, and the second month. An increase in neutrophils is associated with a decrease in lymphocytes and vice-versa (Etim, 2015). In the fifth month, neutrophil % was significantly ($p<0.05$) lower in group III and VI than in the third and fourth months. A decreasing trend of neutrophil % was observed in all remaining groups in the fifth month. This may be due to an increase in lymphocyte %, but this decrease was statistically non-significant. This is likely due to impaired neutrophilic apoptosis in pregnancy. The neutrophilic cytoplasm shows toxic granulation. Neutrophilic chemotaxis and phagocytic activity are depressed, mainly due to inhibitory factors present in the serum of pregnant females (Jessica *et al*., 2007). After exogenous administration of melatonin, neutrophil % decreased in melatonin treated group compared to 0 day and first month, but this decrease was statistically non-significant (Table 2). Hodallah *et al*. (2011) reported that different doses of melatonin for 5 successive weeks produced a decrease in heterophil (neutrophil) in all treated groups versus control at the end of the first and second weeks of treatment. The overall mean of the heterophil (neutrophil) percent was lower in all treated groups versus control, confirming our findings.

The average initial monocyte percent on 0 day was 4.80 ± 0.35, 5.15 ± 0.19, 5.62 ± 0.22, 5.86 ± 0.17, 5.87 ± 0.10, 5.64 ± 0.17, and 5.71 ± 0.13% in group I to VII, respectively (Table 3). In the third month, monocyte % was significantly ($p<0.05$) higher in group IV and VI than group I and group II, which might be due to the high pregnancy rate. There is an absolute monocytosis during pregnancy, especially in the first trimester (Chandra *et al*., 2012), supporting our present finding. Monocytes help prevent fetal allograft rejection by infiltrating the decidual tissue possibly, through PGE2 mediated immunosuppression (Kline *et al*., 2005).

The value of eosinophil percent recorded (Table 4) showed that on 0 day, the mean eosinophil % was 10.26 ± 0.53, 9.50 ± 0.54, 9.76 ± 0.43, 9.93 ± 0.69, 10.10 ± 0.55, 9.86 ± 0.54, and 9.83 ± 0.87 in group I-VII, respectively. Aikhoumobhogbe and Orheruata (2006) reported a non-significant change in eosinophils during pregnancy in cows. The average of basophil percent in different treatment groups at different intervals is presented in Table-5. The initial basophil percent on day 0 was 1.01 ± 0.07, 0.80 ± 0.19, 1.06 ± 0.08, 1.14 ± 0.07, 1.05 ± 0.08, 1.07 ± 0.15, and 0.87 ± 0.08% in group I-VII, respectively. Awodu *et al*. (2002) found no variation in basophils during pregnancy. Edlestam *et al*. (2001) also reported that eosinophil and basophil counts do not change significantly during pregnancy. Our findings also revealed no significant change in eosinophil and basophil counts, which confirms the findings of the above workers.

There was significantly ($p < 0.05$) lower eosinophil % at the fifth month in group-I compared to 0 day. Eosinopenia

Table 2: Neutrophil (%) of Chhotanagpuri ewes in different groups at different periods (Mean ± SE)

Group	0 Day	First month	Second month	Third month	Fourth Month	Fifth month
Group – I	$23.92^{b} \pm 1.40$	$24.09^{b} \pm 0.96$	$24.20^{b} \pm 0.98$	$29.18^{ABa} \pm 1.58$	$30.03^{ABa} \pm 1.76$	$27.31^{Bab} \pm 0.75$
Group – II	$24.05^{c} \pm 0.89$	$24.66^{bc} \pm 0.86$	$24.44^{bc} \pm 0.84$	$29.15^{ABa} \pm 1.69$	$29.70^{ABa} \pm 1.72$	$2\ 7.64^{ABab} \pm 0.93$
Group – III	$23.74^{c} \pm 0.92$	$24.41^{c} \pm 0.63$	$23.24^{c} \pm 0.58$	$32.13^{Aa} \pm 0.50$	$33.39^{Aa} \pm 0.40$	$29.71^{Ab} \pm 0.49$
Group – IV	$23.96^{c} \pm 0.91$	$24.48^{c} \pm 0.81$	$23.13^{c} \pm 0.80$	$30.86^{ABab} \pm 1.23$	$31.66^{ABa} \pm 1.34$	$28.04^{ABb} \pm 0.80$
Group – V	$23.98^{bc} \pm 0.61$	$23.32^{c} \pm 0.85$	$23.80^{bc} \pm 0.95$	$28.02^{Ba} \pm 1.63$	$28.79^{Ba} \pm 1.70$	$27.15^{Bab} \pm 0.98$
Group –VI	$23.96^{c} \pm 0.79$	$23.28^{c} \pm 0.32$	$22.88^{c} \pm 0.34$	$32.10^{Aa} \pm 0.52$	$33.39^{Aa} \pm 0.43$	$29.72^{Ab} \pm 0.43$
Group–VII	$24.01^{bc} \pm 1.08$	$23.28^{c} \pm 0.88$	$22.69^{c} \pm 0.94$	$29.61^{ABa} \pm 1.59$	$30.62^{ABa} \pm 1.70$	$27.45^{ABab} \pm 0.97$

Means bearing different superscript vary significantly ($p < 0.05$) within the groups (a, b, c) and between the groups (A, B).

Table 3: Monocyte (%) of Chhotanagpuri ewes in different groups at different periods (Mean ± SE)

Group	0 Day	First Month	Second Month	Third month	Fourth Month	Fifth month
Group – I	$4.80C^{e} \pm 0.35$	$5.54^{BCc} \pm 0.10$	$5.61^{bc} \pm 0.11$	$5.74^{Cbc} \pm 0.15$	$6.17^{Cab} \pm 0.13$	$6.44^{ABa} \pm 0.30$
Group – II	$5.15^{BCe} \pm 0.19$	$5.39^{Cce} \pm 0.11$	$5.53^{bce} \pm 0.14$	$5.82^{BCbc} \pm 0.13$	$6.37^{BCa} \pm 0.20$	$5.95^{Bab} \pm 0.20$
Group – III	$5.62^{ABc} \pm 0.22$	$5.82^{ABbc} \pm 0.12$	$5.72^{bc} \pm 0.05$	$6.24^{ABCab} \pm 0.15$	$6.45^{ABCa} \pm 0.20$	$6.26^{ABab} \pm 0.29$
Group – IV	$5.86^{Ab} \pm 0.17$	$5.99^{Ab} \pm 0.13$	$5.65^{b} \pm 0.11$	$6.48^{Aa} \pm 0.15$	$6.91^{Aa} \pm 0.14$	$6.70^{Aa} \pm 0.18$
Group – V	$5.87^{Aabc} \pm 0.10$	$5.66^{ABCc} \pm 0.14$	$5.83^{bc} \pm 0.13$	$6.31^{ABab} \pm 0.20$	$6.39^{BCa} \pm 0.16$	$6.29^{ABab} \pm 0.27$
Group –VI	$5.64^{ABc} \pm 0.17$	$5.74^{ABCc} \pm 0.11$	$5.71^{c} \pm 0.11$	$6.44^{Aab} \pm 0.22$	$6.72^{ABa} \pm 0.17$	$6.10^{ABbc} \pm 0.22$
Group–VII	$5.71^{ABcd} \pm 0.13$	$5.46^{cd} \pm 0.13$	$5.60^{cd} \pm 0.15$	$6.08^{ABCbc} \pm 0.17$	$6.79^{ABa} \pm 0.20$	$6.27^{ABb} \pm 0.21$

Means bearing different superscript vary significantly ($p < 0.05$) within the groups (a,b,c,d,e) and between the groups (A,B,C).

Table 4: Eosinophil (%) of Chhotanagpuri ewes in different groups at different periods (Mean ± SE)

Group	*0 Day*	*First month*	*Second Month*	*Third month*	*Fourth Month*	*Fifth month*
Group – I	$10.26^{a} \pm 0.53$	$9.54^{ab} \pm 0.43$	$9.40^{ab} \pm 0.35$	$9.90^{ab} \pm 0.32$	$9.92^{ABab} \pm 0.15$	$9.06^{b} \pm 0.34$
Group – II	9.50 ± 0.54	9.62 ± 0.58	9.22 ± 0.52	9.92 ± 0.45	$9.56^{B} \pm 0.17$	9.39 ± 0.20
Group – III	9.76 ± 0.43	10.11 ± 0.35	9.60 ± 0.46	9.97 ± 0.47	$9.94^{AB} \pm 0.31$	9.43 ± 0.20
Group – IV	9.93 ± 0.69	10.45 ± 0.48	10.27 ± 0.34	10.32 ± 0.28	$10.22^{AB} \pm 0.25$	9.54 ± 0.22
Group – V	10.10 ± 0.55	9.94 ± 0.51	9.95 ± 0.51	10.29 ± 0.32	$10.31^{A} \pm 0.31$	9.79 ± 0.34
Group –VI	9.86 ± 0.54	9.39 ± 0.40	9.19 ± 0.53	9.86 ± 0.30	$9.80^{AB} \pm 0.28$	9.31 ± 0.27
Group–VII	9.83 ± 0.87	9.65 ± 0.66	9.29 ± 0.45	10.01 ± 0.39	$9.72^{AB} \pm 0.21$	9.71 ± 0.22

Means bearing different superscript vary significantly ($p < 0.05$) within the groups (a, b) and between the groups (A, B).

Table 5: Basophil (%) of Chhotanagpuri ewes in different groups at different periods (Mean ± SE)

Group	*0 Day*	*First Month*	*Second Month*	*Third month*	*Fourth Month*	*Fifth month*
Group – I	$1.01^{AB} \pm 0.07$	1.08 ± 0.05	1.12 ± 0.23	1.03 ± 0.12	$1.09^{AB} \pm 0.08$	$1.02^{AB} \pm 0.08$
Group – II	$0.80^{B} \pm 0.19$	0.97 ± 0.14	0.85 ± 0.18	0.98 ± 0.10	$1.08^{AB} \pm 0.06$	$1.08^{AB} \pm 0.06$
Group – III	$1.06^{AB} \pm 0.08$	1.14 ± 0.11	1.06 ± 0.21	1.00 ± 0.12	$1.07^{AB} \pm 0.9$	$1.04^{AB} \pm 0.02$
Group – IV	$1.14^{A} \pm 0.07$	1.06 ± 0.16	1.09 ± 0.25	1.26 ± 0.18	$1.18^{A} \pm 0.09$	$1.13^{A} \pm 0.06$
Group – V	$1.05^{AB} \pm 0.08$	1.13 ± 0.07	1.11 ± 0.25	1.13 ± 0.05	$1.05^{AB} \pm 0.04$	$1.02^{AB} \pm 0.06$
Group – I	$1.07^{AB} \pm 0.15$	1.00 ± 0.17	0.99 ± 0.25	0.97 ± 0.11	$0.91^{B} \pm 0.10$	$0.90^{B} \pm 0.11$
Group–VII	$0.87^{AB} \pm 0.08$	0.91 ± 0.08	1.02 ± 0.30	1.04 ± 0.06	$1.05^{AB} \pm 0.05$	$0.98^{AB} \pm 0.02$

Means bearing different superscript vary significantly ($p < 0.05$) between the groups (A, B).

follows stress conditions in which the hypothalamic-adenohypophyseal-adrenocortical response occurs. Adrenocorticotropic hormone stimulates the production of cortisol from the adrenal cortex, which causes eosinopenia (Reece, 2005).

The basophil % changed significantly ($p<0.05$) in melatonin-treated group-IV at the fourth and fifth months of the experiment. Marginal alteration is shown in the group which was not at par with the control might be due to the individual variation and environmental condition. Basophils have receptors for immunoglobulin E (IgE), which are produced in allergic reactions. Thus in hypersensitivity reactions, the released compounds help initiate and promote the inflammatory changes to combat the invading etiological agent (Reece, 2005).

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References

Aikhoumobhogbe, P.U., & Orheruata (2006). Haematological and biochemical indices of West African dwarf goats vaccinated against pestes des petit ruminants (PPR). *African Journal of Biotechnology*, *5*(9), 743-748.

Awodu, O.A., Enosolease, A.G.U., & Famodu, A.A. (2002). Leucocyte count in pregnant Nigerian woman with sickle cell trait. *African Journal of Reproductive Health*, *6*(3), 112-116.

Bozdogan, O. and Baysal, A.I. (2003). The effect of age, sex, housing system and pregnancies on some blood parameters of Tuj sheep. *Turkish Journal of Veterinary and Animal Sciences*, *27*, 521-524.

Chandra, S., Tripathi, A.K., Mishra, S., Amzarul, M., & Vaish, A.K. (2012). Physiological changes in haematological parameters during pregnancy. *Indian Journal of Haematology and Blood Transfusion*, *28*(3), 144-146.

Edlestam, G., Lowbeer, C., & Kral, G. (2001). New reference value for routine blood samples and human neutrophilic lipocalin during third trimester pregnancy. *Scandinavian Journal of Clinical Laboratory and Investigation*, *61,* 583-592.

Etim, N.N. (2015). Reference values for haematological parameters of sheep: a review. *The American Journal of Innovative Research and Applied Sciences*, *1*(1), 14-21.

Etim, N.N., Enyenihi, G.E., Akpabio, U., & Offiong, E.A. (2014[a]). Effects of nutrition on haematology of rabbits: A review. *European Scientific Journal*, *10*(30), 413-424.

Etim, N.N., Offiong, E.E.A., Williams, M.E., & Asuquo, L.E. (2014[b]). Influence of nutrition on haematological parameters of pigs. *American Journal of Biological and Life Science*, *2*(2), 46-52.

Hodallah, H.A., Gamed, S.E., Hamdy, A.S., & Mabrouk, A.A.D. (2011). Effect of melatonin on some hematological parameters and immune status of broiler chicks. *Journal of Agricultural Science,3*, 2.

Isaac, L.J., Abah,G., Akpan, B., & Udo, E.I. (2013). Haematological properties of different breeds and sexes of rabbits. *Proceeding of 18th Annual Conference of Animal Science Association of Nigeria,* pp. 24-27.

Jessica, M., Badger, F., Hseih, C.C., Troisi, R., Lagiou, P., & Polischman, N. (2007). Plasma volume expansion in pregnancy: implication for biomarkers in population studies. *Cancer Epidemiology Biomarkers,16*, 1720.

Kandiel, M.M.M., El-khaiat, H.M., & Mahmoud, K.G.M. (2016). Changes in some hematological and hormonal profile in Bakri sheep with various reproductive statuses. *Small Ruminant Research*, *136,* 87-95.

Kaushalendra, & Haldar, C. (2012). Correlation between peripheral melatonin and general immune status of domestic goat, *Capra hircus*: A seasonal and sex dependent variation. *Small Ruminant Research*, *107,* 147.

Klein, A.J., Williams, G.W., & Hemandeznino, J. (2005). Dimer concentration in normal pregnancy : A new diagnostic threshold are needed. *Clinical Chemistry*, *51*(5), 825-829.

Prendergast, B.J., Bilbo, S.D., & Nelson, R.J. (2005). Short day lengths enhance skin immune responses in gonadectomised Siberian hamsters. *Journal of Neuroendocrinology*, *17,* 18-21.

Rai, S., & Haldar, C. (2003). Pineal control of immune status and hematological changes in blood and bone marrow of male squirrels (*Funambulus pennant*) during their reproductive active phase. *Comparative Biochemistry and Physiology - Part C: Toxicology and Pharmacology*, *136*(4), 319-328.

Rai, S., Haldar, C., & Singh, R. (2009). Modulation of immunity in young-adult and aged squirrel, Funnambulus pennant by Melatonin and Parachlorophenylalanin. *Immunity and Aging*, *6,* 5.

Reece, W.O. (2005). *Dukes Physiology of Domestic Animals*, 12th edn. Panima Publishing Corporation, New Delhi, India.

Schalm, O.W., Jain, N.C., & Carrol, E.J. (1975). *Veterinary Hematology*. 3rd edn., Philadelphia, Lea and Febiger, USA.

Singh, A.K., Ghosh, S., Basu, P., & Haldar, C. (2014). Daily variation in melatonin level, antioxidant activity and general immune response of peripheral blood mononuclear cells and lymphoid tissues of Indian goat *Capra hircus* during summer and winter. *Indian Journal of Experimental Biology, 52,* 467- 477.

Snedecor, G.W. and Cochran, W.G. (2004). *Statistical Method*. 8th edn. The Iowa State University Press, Ames, Iowa, USA.

Soetan, K. O., Akinrade, A.S., & Ajibade, T.O. (2013). Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn. *Proceeding of 38th Annual conference of Nigerian Society of Animal Products*, pp. 49-52.