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Original Research Article

Effect of Exogenous Melatonin and Different photoperiods on Serum Glucose and Total Serum Protein levels in Chhotanagpuri Ewe.

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A B S T R A C T

Forty two (42) apparently healthy, non-pregnant, non-lactating Chhotanagpuri ewe, having body weight ranging between 14.11±0.09 to 15.38±0.06 Kg were selected and selected ewes were isolated from rams 2 months prior to melatonin administration. The selected animals were allocated randomly into seven groups viz. Group-I [Normal control], Group-II [Long day (LD) control], Group-III [LD + Melatonin administration orally (3mg/day)], Group-IV [(LD + Melatonin administration subcutaneously (1mg/day)], Group-V [Short Day (SD) control], Group-VI [SD + Melatonin administration orally (3mg/day)] and Group-VII [SD + Melatonin administration subcutaneously (1mg/day)] comprising six animals in each group. Rams were then introduced into each group after completion of exogenous administration of melatonin. Blood sample without anticoagulant in vials was collected from each animal day before the start of experiment and there after every month up to fifth month. After administration of melatonin, serum glucose was decreased in group- III, IV, VI and VII in comparison to group-I, although this decrease was not statistically significant. An increasing trend of serum glucose was observed in all groups from third to fourth month. Total protein at first month was significantly ($P<0.05$) higher in group VI in comparison to 0 day. After administration of melatonin, total serum protein concentration was significantly ($P<0.05$) decreased in group VI in comparison to first month and continued up to third month. At fifth month, total serum protein was significantly ($P<0.05$) lower in group III, IV and VI in comparison to 0 day and first month.

Keywords

Chhotanagpuri ewe, Exogenous melatonin, Photoperiod, Serum glucose, Total serum protein

Introduction

Sheep husbandry is backbone of rural economy in India. The Chhotanagpuri sheep is the only recognized breed of sheep found in Jharkhand with a few numbers in Bihar and West Bengal. This breed is maintained solely for mutton production. They produce coarse wool of poor quality. This is being utilized for carpet manufacturing. Sheep

which has a vital economy in India are mostly raised under harsh environment condition as it is seasonal breeders (Sangeetha and Rameshkumar, 2014).

Melatonin (N-acetyl-5-methoxy-tryptamine) was first identified and isolated from bovine pineal extract in 1958 as a neuro-hormone which is synthesized and secreted mainly from the pineal gland (Chakravarty and Rizvi, 2008). Since its discovery, further investigation has revealed that it is also produced by several other organs like the gastrointestinal tract, brain, eye, lungs, skin, kidney, liver, thyroid, thymus and pancreas (Fernando and Rombauts, 2014). Melatonin is an indoleamine, which is synthesized from the essential amino acid, tryptophan (Reiter *et al.*, 2013). Its production is dependent on ambient illumination, with release being suppressed by light. The supra-chiasmatic nucleus (SCN) which is the major circadian oscillator that receives light input from the retina through the retino-hypothalmic tract is the one that regulate the circadian melatonin production (Berson *et al.*, 2002).

Circadian clocks govern the timing of development, behavior, physiology, endocrinology and biochemistry, as well as photoperiodic events (Forster *et al.*, 2001). Also, circadian clocks and energy metabolism are linked because mutation in clock gene leads to metabolic syndrome in mice (Turek *et al.*, 2005). The circadian clock reportedly regulates metabolism and energy homeostasis in the liver and other peripheral tissues by mediating the expression or activity of various metabolic enzymes and transport system involved in glycogen and glucose metabolism (Green *et al.*, 2008; Lafleur, 2003; Zhang *et al.*, 2006). In addition, Challet *et al.*, (2004) observed the blood glucose increased during exposure to light and decreased during darkness in rats. A Study has demonstrated that constant darkness elevated 5’ adenosine mono phosphate (5’AMP) in the blood of mice. Hence, 5’AMP is a pivotal metabolic signal whose circulatory level determines the balance of the peripheral organ energy supply between glucose and glycogen (Zhang *et al.*, 2006). It is known that insulin secretion is not affected by photoperiod in cattle (Zinn *et al.*, 1986). In mammals, SCN activity is modulated by several neurotransmitter or neuro-hormone including melatonin (Armstrong *et al.*, 1986). However, the results of studies investigating melatonin effect on glucose metabolism in experimental animal are controversial, such as increase of blood glucose in rats (Csaba and Barath, 1971) or, on the contrary, reduction of blood glucose in rats (Lizuka, 1996).

The major site of synthesis of the serum proteins is the liver. The second major site is the immune system consisting of the monocyte-macrophage system, lymphoid and plasma cells. Structural, functional, and enzyme proteins that are synthesized in all body cells and tissues are present in plasma in minor quantities as a result of cell turnover. A seasonal rhythm was found for the serum proteins in albumin and alb/glob for sheep with acrophase between July and September for sheep (Piccione *et al.*, 2011). These changes are connected to the changes of light and temperature throughout the year (Alila-Johansson *et al.*, 2004). In fact, the hypothalamic pacemaker, directly innervated from retina, results as the principle factor involved in determining circadian rhythms (Moore-Ede, 1986). This pacemaker regulates transcriptional process also, and, in particular, makes this like an autonomous pacemaker. On the basis of the results obtained by Piccione *et al.*, (2011) it is conceivable that albumin production from liver is strictly dependent on this circadian

clock and, may be, on melatonin release (Alila-Johansson *et al.,* 2001 ; Piccione *et al.,* 2008).

There is paucity of information regarding serum glucose and total serum protein status in Chhotanagpuri sheep. Therefore, a comprehensive study was designed to observe the base line study of serum glucose and total serum protein of indigenous ewe by exogenous administration of melatonin and also the effect of different photoperiod.

Materials and Methods

The present study was conducted in Department of veterinary physiology, College of veterinary science and A.H., Birsa Agricultural University, Kanke, Ranchi-6, Jharkhand located at 23.36^{O}N latitude and 85.33^{O}E longitude with an altitude of 651 m above mean sea level. Design of experiment was approved by the Institutional Animal Ethics Committee vide letter no.-139/528/RVC/IAEC. Forty two (42) apparently healthy, non-pregnant, non-lactating Chhotanagpuri ewes, having body weight ranging between 14.11±0.09 to 15.38±0.06 Kg reared under uniform managemental husbandry practices were selected. Selected ewes were isolated from rams prior to melatonin administration. The selected animals were allocated randomly into seven groups (viz. group-I to group-VII) comprising six animals in each group. Group-I [Normal control]:- The animals in this group were exposed to normal variation in day length. Group-II [Long day (LD) control]:- In this group in addition to natural sun light, artificial light was provided to animal for maintaining 16-18 hours of light every day for the period of one month and considered as long day control. Group-III [LD + Melatonin administration orally (3mg/day)]:- In this group in addition to natural sun light, artificial light was provided for maintaining 16-18 hours of light every day for the period of one month and after that 3 mg melatonin was administered orally for one month to each animal. Group-IV [(LD + Melatonin administration subcutaneously (1mg/day)]:- In this group in addition to natural sun light, artificial light was provided for maintaining 16-18 hours of light every day for the period of one month and after that 1 mg melatonin was administered subcutaneously for one month to each animal. 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. Group-VI [SD + Melatonin administration orally (3mg/day)]:- 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 and after that 3 mg melatonin was administered orally for one month to each animal. Group-VII [SD + Melatonin administration subcutaneously (1mg/day)]:- Animals in this group were provided only 8 hours natural day light and then were kept in a light-proof shed for 16 hours exposure in dark every day for the period of one month and after that 1 mg melatonin was administered subcutaneously for one month to each animal. Rams were introduced into each group after completion of exogenous administration of melatonin. Blood samples without anticoagulant in vials were collected from each animal day before the start of experiment and there after every month up to fifth month. Without anticoagulant added blood sample was allowed to clot at room temperature in centrifuge tubes for 2-3 hours. The clots was eased away from the edge of the vessel and allowed to shrink overnight at 4^{O}C. The serum was pippeted off, centrifuged at 3000

rpm for 10 minutes and stored at -20^{O}C for further analysis of biochemical parameters. Serum glucose and Total serum protein were estimated by Spectrophotometer by using GOD/POD method as described by Trinder (1969) and Biuret Method as described by Gornall *et al.,* (1949) respectively. Data were statistically analyzed as per methods described by Snedecor & Cochran (2004).

Results and Discussion

Carbohydrate in the form of glucose is the principle source of energy for the life processes of the mammalian cells. All cells require a constant supply of this indispensable nutrient and only relatively small changes are tolerated without adverse effects on the health of the animal (Kaneko *et al.,* 1997). In present experiment, the value of serum glucose has been presented in Table-1. The mean of serum glucose was 55.81±1.96, 56.27±2.08, 56.11±2.11, 55.73±2.35, 56.49±2.72, 55.99±2.37 and 55.67±2.25 mg/dl in group I – VII, respectively on day 0.After administration of melatonin, serum glucose was decreased in group III, IV, VI and VII in comparison to group I although this decrease was not statistically significant. An increasing trend of serum glucose was observed in all groups from third to fourth month.

At fifth month, serum glucose was decreased in all groups in comparison to fourth month, but this decrease was not statistically significant. Prunet – Marcassus *et al.,* (2003) reported, blood glucose was lower when, melatonin was administered. Kassayova *et al.,* (2006) reported, prolonged melatonin administration decreases serum glucose concentration in female rats. Mahmud and Mahmud (2013) also reported melatonin (120mg/kg diet) decreases the blood glucose, which is in agreement with our findings. However, the results of studies investigating melatonin effect on glucose metabolism in experimental animal are controversial, such as increase of blood glucose in rat (Csaba and Barath, 1971) or, on the contrary, reduction of blood glucose in rats (Lizuka, 1996). Chaiyabutr *et al.,* (1982) reported during early and mid-pregnancy in goats the demand of fetus was minimal resulting little utilization while, the rate of glucose turnover increased in late pregnancy which support our finding. The values are within normal range as reported by several workers (Oshiro *et al.,* 1978 and Kaneko *et al.,* 1997). El-Sherif and Assad (2001) reported significant increased in blood glucose level in different stage of pregnancy did not agree with our findings.

Blood serum proteins serve important function in the body for maintaining colloid osmotic pressure, blood pressure, acid-base balance and transport of nutrients, hormones, enzyme etc. The major site of synthesis of the serum proteins is the liver and the second major site is the immune system consisting of the monocyte-macrophase system, lymphoid and plasma cells.

In present study, the value of total serum protein (g/dl) has been presented in Table-2. On 0 day, the mean of total protein estimated was 8.04±0.16, 8.04±0.15, 7.85±0.24, 7.92±0.38, 8.06±0.29, 8.07±0.21 and 8.00±0.28 g/dl in group I – VII, respectively. At fifth month, total serum protein was significantly ($P<0.05$) lower in groups III, IV and VI in comparison to 0 day and first month. A decreasing trend of total protein was observed in all remaining groups from second month to fifth month may be due to pregnancy, but this decrease was not statistically significant. Kassim *et al.,* (2008) observed the effect of length photoperiod on serum total protein and melatonin.

Table.1 Serum glucose concentration (mg/dl) of Chhotanagpuri ewes in different groups at different periods (Mean ± SE)

	0 Day	First Month	Second Month	Third month	Fourth Month	Fifth Month
Group – I	55.81 ±1.96	55.80 ±1.88	55.84 ±1.90	56.87 ±1.88	57.63 ±1.95	56.80 ±1.87
Group – II	56.27 ±2.08	56.57 ±1.88	56.48 ±1.87	57.57 ±1.48	58.32 ±1.24	57.53 ±1.47
Group – III	56.11 ±2.11	56.33 ±1.91	55.57 ±1.92	57.62 ±1.95	59.14 ±1.94	57.45 ±1.95
Group – IV	55.73 ±2.35	56.04 ±2.26	55.19 ±2.22	56.89 ±2.30	58.15 ±2.42	56.81 ±2.31
Group – V	56.49 ±2.72	56.16 ±2.61	56.36± 2.61	57.15 ±2.47	57.68 ±2.46	57.09 ±2.44
Group – VI	55.99 ±2.37	55.75 ±2.24	54.81 ±2.24	56.94 ±2.18	58.45 ±2.19	56.71 ±2.14
Group – VII	55.67 ±2.25	55.31 ±2.31	54.34 ±2.21	55.76 ±2.23	56.80 ±2.30	55.68 ±2.24

Means did not vary significantly between the periods and groups.

Table.2 Total serum protein concentration (g/dl) of Chhotanagpuri ewes in different groups at different periods (Mean ± SE)

	0 Day	First Month	Second Month	Third month	Fourth month	Fifth Month
Group – I	8.04^{a} ±0.16	8.05^{ABa} ±0.17	7.96^{ab} ±014	7.58^{ABab} ±0.17	7.51^{ABb} ±0.19	7.48^{ABb} ±0.20
Group – II	8.04 ±0.15	8.01^{AB} ±0.29	7.93 ±0.22	7.55^{AB} ±0.19	7.48^{AB} ±0.21	7.42^{AB} ±0.22
Group – III	7.85^{ab} ±0.24	8.10^{ABa} ±0.25	8.06^{a} ±0.16	7.44^{ABbc} ±0.16	7.34^{ABbc} ±0.16	7.22^{ABc} ±0.16
Group – IV	7.92^{a} ±0.38	7.84^{Ba} ±0.21	7.71^{ab} ±0.26	7.28^{Bab} ±0.19	7.21^{Bab} ±0.19	7.12^{Bb} ±0.17
Group – V	8.06 ±0.29	8.02^{AB} ±0.29	7.99 ±0.23	7.81^{A} ±0.20	7.76^{A} ±0.21	7.72^{A} ±0.22
Group – VI	8.07^{b} ±0.21	8.11^{Aa} ±0.23	8.04^{b} ±0.15	7.56^{ABc} ±0.13	7.45^{ABc} ±0.13	7.34^{ABc} ±0.13
Group – VII	8.00 ±0.28	8.01^{AB} ±0.20	7.98 ±0.16	$7. 65^{AB}$ ±0.17	7.56^{AB} ±0.19	7.49^{AB} ±0.20

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

Melatonin hormone concentration recorded sharp decline, when buffalo heifer was exposed to long photoperiod, but total serum protein did not show any significant difference like our finding. Oqetark *et al.*, (2004) reported melatonin treatment did not

cause a significant change in total protein levels in rat. The similar pattern was observed in our finding. Kumar (2003), Batavani *et al.,* (2006) and Verma (2012) reported decline in the protein level during pregnancy in sheep and goat, which are in agreement with our findings. The values obtained in the present experiment are within the normal range as reported by Dukes (1970), Somvanshi *et al.,* (1989) and Dutta *et al.,* (1996). Kumar (2003) reported the total serum protein concentration decrease, due to synthesis of hormones which is produced during gestation period and also due to demand of protein by growing fetus.

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