RESEARCH ARTICLE

Efficacy of Melatonin Administration Via Different Route on Reproductive Performance and Blood Mineral Profile in Chhotanagpuri Ewe

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Abstract

The present study aimed to evaluate the comparative efficacy of melatonin administration via different routes on reproductive performance and blood mineral profile of Chhotanagpuri ewe. A total of forty-two non-pregnant, non-lactating healthy ewes, not exposed to rams for 2 months, were allocated to seven equal groups each of six animals, viz., G1 (Normal day light control), G2 (Long day control, 16-18 h light; sunlight + artificial light), G3 (Long day as G2 + 3 mg melatonin daily orally), G4 (long day as G2 + 1 mg melatonin daily subcutaneous), G5 (Short day control, 8 hours sun light, rest darkness), G6 (short day as G5 + 3 mg melatonin daily orally), and G7 (short day as G5 + 1 mg melatonin daily subcutaneous). The melatonin treatment was given for one month to ewes of G3, G4, G6 and G7 and then were exposed to ram for one month, i.e., from day 61st to 90th; whereas the ewes of normal, long day and short day control (G1, G2, G5) were exposed to ram for one month from day 31st to 60th. Blood samples were collected from each animal, day before the start of experiment (day 0) and thereafter, every 30 days for five occasions. The estrus induction response was cent per cent in oral melatonin treated ewes (G3 and G6) compared to subcutaneous route (83.33%, G4 and 66.66%, G7) and controls (33.3 to 50.0%). After melatonin treatment, the overall estrus induction response and subsequent lambing rate were improved. The serum calcium level was significantly ($p<0.05$) lower in late pregnancy in G1 and G6; the serum phosphorus level was significantly ($p<0.05$) low in late pregnancy in all groups, except G5; the serum copper level was significantly lower in G6 compared to day 0 value. Furthermore, magnesium and zinc levels did not differ significantly within the treatment groups. In conclusion, the melatonin treatment can be used to improve reproductive performance of Chhotanagpuri ewe.

Keywords: Chhotanagpuri ewe, Estrus response, Fertility, Melatonin, Mineral, Photoperiod.

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Introduction

The breeding season of ewe usually begins in the summer or early fall, in response to the shortening of the day length and ends during the late winter or early spring (*Mura et al.*, 2014). The perception of light and dark alternation affects the epiphysis secretion of melatonin, with high levels secreted during night- hours and low levels during day light hours (Carcangiu *et al.,* 2013). Thus, photoperiod is a major factor influencing reproductive activity in small ruminants (Bedos *et al.,* 2014). Melatonin is a neuroendocrine signal that transduces information about the environmental light received by retina (El-Mokadem *et al.,* 2017). The use of exogenous melatonin has received much attention across the globe to control reproductive activity (Abecia *et al.,* 2011). Pharmacological use of melatonin improves reproductive efficiency in variety of sheep breeds, viz., Rasa Aragonesa, Assaf and Merino (Abecia *et al.,* 2007), Kivircil and Charollais (Cevik *et al.,* 2017), Sarda (Mura *et al.,* 2017; Mura *et al.,* 2019; Cosso *et al.,* 2021; Pulinas *et al.,* 2021) and Ossimi sheep (Abd-Allah and Daghash, 2019), and subcutaneous implants have been the most commonly utilized method of doing so (De Nicolo *et al.,* 2009; Luridiana *et al.,* 2015; Abd-Allah and Daghash, 2019). The melatonin released by the implants can mimic short days, without causing inhibition of natural melatonin secretion, thus stimulating reproductive activity in sheep (*Staples et al.*, 1992).

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Reproductive status has an important bearing on blood mineral concentration (Antunovic *et al.,* 2002). Minerals play an important role in increasing the efficiency of livestock production and reproduction as they act as catalysts in the enzymes and hormonal systems of the body (Ceylan *et al.,* 2008). 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. To date, no literature is available which depicts the role of exogenous melatonin in augmenting reproductive performance in Chhotanagpuri ewe. Thus, the present study was aimed to evaluate effect of exogenous melatonin under varied photoperiods on the fertility traits and blood mineral profile in Chhotanagpuri ewe.

MATERIALS AND METHODS

The study was conducted from February 2015 to October, 2015 following ethical approval from Institutional Animal Ethics Committee (Reference no.-139/528/RVC/IAEC); in the Department of Veterinary Physiology, College of Veterinary Science and A.H., Birsa Agricultural University, Kanke, Ranchi-6, Jharkhand. The apparently healthy, non-pregnant, non-lactating, Chhotanagpuri ewes (n=42), aged 2-3 years, weighing around 15 kg, reared under uniform management practices, were selected from Instructional Farm of the College. Animals were maintained on a standard ration (Maize 42.25%, wheat bran 37%, GNC 18.5%, Mineral mixture 2% common salt 0.25% (NRC, 1980) at the rate of 250 gm/animal/day with green fodder and water *ad-libitum*. Deworming of selected animals was done with fenbendazole @ 10 mg/kg of body weight before the start of the experiment. Selected ewes were isolated from rams at least 2 months before melatonin administration. Rams were introduced into each group after completing two month's exogenous administration of melatonin (Sigma-Aldrich). 3 mg melatonin was dissolved in 5 mL of water-ethanol (8:2 v/v) and was administered orally, for subcutaneous injection, 1-mg melatonin was dissolved in 2 mL of saline-ethanol (9:1 v/v) and was administered as s/c injection. Oral/ subcutaneous administration of melatonin was done 2 h after exposure of animals to daylight in morning.

Selected ewes were randomly divided into seven groups (G1 to G7) comprising six ewes in each group.

- G1 ewes (normal control) were exposed to normal variation in day length;
- G2 ewes (long day control), in addition to natural sunlight, were also provided artificial light for maintaining 16–18 hours light every day for one month;
- G3 ewes were managed as G2 for one month, followed by melatonin administration 3 mg/head daily orally for next 30 days;
- G4 ewes were managed as G2 for one month, followed by melatonin administration 1-mg/head, as s/c injection daily for next 30 days;
- G5 (short day control) ewes were provided only 8 hours natural daylight and were then kept in a light-proof shed for 16 hours daily for the period of one month;
- G6 ewes were managed as G5, followed by melatonin administration 3 mg/head daily orally for next 30 days, and
- G7 ewes were managed as G5, followed by melatonin administration 1-mg/head as s/c injection daily for next 30 days.

Ewes of melatonin treated group (G3, G4, G6 and G7) were exposed to ram for one month after completion of melatonin treatment, i.e., from day 61st to 90th; whereas normal control, long day control and short day control ewes (G1, G2, G5) were exposed to ram for one month, i.e., from day 30th to 60th.

Blood samples without anti-coagulant were collected from each animal day before the experiment (day 0) and thereafter every 30 days for five occasions. Blood samples were allowed to clot at room temperature in centrifuge tubes for 2-3 h and the serum was pipetted off, centrifuged at 906 x g for 10 min and stored at -20°C for further analysis. The serum calcium, phosphorus and magnesium were estimated with Tulip diagnostic kit using OCPC method, Molybdate UV method, and Calmagite method, respectively,

Table 1: Fertility parameters of Chhotanagpuri ewes in different melatonin treatment groups (n=6)

Group	Estrus response (%)	Ewe lambed	Lambing rate	Total number of lamb born	Litter Size	Average birth weight of the lamb (kg)
G1 (NC) (n)	3 (50.0)	3	50.00	3	1	1.90 ± 0.05
G2 (LDC) (n=)	3 (50.0)	3	50.00	3	1	1.90 ± 0.05
G3 (LD+MO) (n=)	6 (100.0)	6	100.00	6	1	1.98 ± 0.06
G4 (LD+MS) (n=)	5 (83.33)	5	83.33	5	1	1.96 ± 0.05
G5 (SDC) (n=)	2 (33.33)	2	33.33	2	1	1.90 ± 0.10
G6 (SD+MO) (n=)	6 (100.0)	6	100.00	6	1	1.96 ± 0.06
G7 (SD+MS) (n=)	4 (66.66)	4	66.66	4	1	1.95 ± 0.06

NC (normal control), LDC (long day control), LD+MO (long day +melatonin orally), LD+MS (long day + melatonin s/c), SDC (short day control), SD+MO (short day + melatonin orally), SD+MD (short day + melatonin s/c).

Table 2: Serum Ca, P, Mg, Cu and Zn profile of Chhotanagpuri ewes in different melatonin treatment groups at different time intervals (Mean± SE)

		Day 0	Day 30th	Day 60th	Day 90th	Day 120th	Day 150th
G1 (n=6)	Ca (mg/dL)	9.19 ± 0.25	9.16 ± 0.24	9.20 ± 0.23	8.95 ± 0.25	8.89 ± 0.24	8.83 ± 0.24
	P (mg/dL)	6.26a ± 0.21	6.23 a ± 0.19	6.22 a ± 0.21	5.92ab ± 0.15	5.70 b ± 0.13	5.59 b ± 0.13
	Mg (mg/dL)	1.73 ± 0.10	1.73 ± 0.09	1.75 ± 0.10	1.74 ± 0.10	1.73 ± 0.11	1.71 ± 0.11
	Cu (ppm)	1.29 ± 0.11	1.30 ± 0.10	1.27 ± 0.10	1.23 ± 0.09	1.17 ± 0.09	1.10 ± 0.07
	Zn (ppm)	2.16 ± 0.30	2.15 ± 0.29	2.11 ± 0.31	2.18 ± 0.35	2.26 ± 0.41	2.30 ± 0.43
G2 (n=6)	Ca (mg/dL)	9.14 ± 0.24	9.06 ± 0.24	9.15 ± 0.21	8.89 ± 0.19	8.82 ± 0.19	8.7 6 ± 0.19
	P (mg/dL)	6.29 a ± 0.20	6.02abc ± 0.19	6.16 ab ± 0.19	5.88abc ± 0.17	5.65bc ± 0.18	5.55c ± 0.19
	Mg (mg/dL)	1.73 ± 0.09	1.77 ± 0.08	1.73 ± 0.07	1.71 ± 0.08	1.70 ± 0.08	1.69 ± 0.09
	Cu (ppm)	1.30 ± 0.07	1.25 ± 0.07	1.27 ± 0.07	1.23 ± 0.06	1.18 ± 0.06	1.10 ± 0.05
	Zn (ppm)	2.26 ± 0.60	2.21 ± 0.55	2.20 ± 0.53	2.25 ± 0.55	2.35 ± 0.56	2.40 ± 0.59
G3 (n=6)	Ca (mg/dL)	9.19 ± 0.30	9.07 ± 0.26	9.20 ± 0.23	8.82 ± 0.24	8.70 ± 0.23	8.59 ± 0.23
	P (mg/dL)	6.26 a ± 0.22	5.99 ab ± 0.22	6.40 a ± 0.21	5.88 ab ± 0.21	5.58b ± 0.18	5.41 b ± 0.18
	Mg (mg/dL)	1.74 ± 0.06	1.78 ± 0.06	1.84 ± 0.06	1.79 ± 0.06	1.74 ± 0.06	1.69 ± 0.06
	Cu (ppm)	1.31 ± 0.11	1.27 ± 0.10	1.31 ± 0.10	1.23 ± 0.10	1.16 ± 0.09	1.08 ± 0.08
	Zn (ppm)	2.18 ± 0.37	2.13 ± 0.33	2.25 ± 0.33	2.35 ± 0.32	2.50 ± 0.31	2.65 ± 0.29
G4 (n=6)	Ca (mg/dL)	9.11ab ± 0.14	9.04 ab ± 0.12	9.14a ± 0.09	8.81abc ± 0.12	8.73bc ± 0.13	8.63c ± 0.15
	P (mg/dL)	6.20 ± 0.42	6.00 ± 0.40	6.25 ± 0.38	5.83 ± 0.41	5.48 ± 0.40	5.34 ± 0.42
	Mg (mg/dL)	1.77 ± 0.06	1.80 ± 0.05	1.83 ± 0.04	1.79 ± 0.04	1.75 ± 0.05	1.71 ± 0.06
	Cu (ppm)	1.30 ± 0.11	1.26 ± 0.10	1.31 ± 0.10	1.24 ± 0.10	1.18 ± 0.09	1.10 ± 0.07
	Zn (ppm)	2.23 ± 0.34	2.20 ± 0.31	2.33 ± 0.28	2.41 ± 0.29	2.53 ± 0.30	2.61 ± 0.31
G5 (n=6)	Ca (mg/dL)	9.14 ± 0.16	9.19 ± 0.15	9.20 ± 0.14	9.05 ± 0.12	9.02 ± 0.13	8.98 ± 0.14
	P (mg/dL)	6.23 ± 0.15	6.29 ± 0.14	6.33 ± 0.14	6.10 ± 0.18	5.93 ± 0.23	5.85 ± 0.25
	Mg (mg/dL)	1.74 ± 0.08	1.71 ± 0.08	1.73 ± 0.08	1.73 ± 0.09	1.74 ± 0.10	1.73 ± 0.11
	Cu (ppm)	1.30 ± 0.12	1.33 ± 0.12	1.31 ± 0.12	1.26 ± 0.11	1.23 ± 0.10	1.19 ± 0.09
	Zn (ppm)	2.20 ± 0.49	2.23 ± 0.48	2.18 ± 0.46	2.18 ± 0.48	2.20 ± 0.49	2.21 ± 0.50
G6 (n=6)	Ca (mg/dL)	9.12 a ± 0.11	9.17 a ± 0.08	9.25 a ± 0.07	8.84b ± 0.06	8.73 b ± 0.07	8.61 b ± 0.07
	P (mg/dL)	6.18 a ± 0.21	6.12 a ± 0.19	6.25 a ± 0.21	5.73ab ± 0.21	5.37b ± 0.20	5.22 b ± 0.19
	Mg (mg/dL)	1.78 ± 0.07	1.75 ± 0.06	1.80 ± 0.07	1.74 ± 0.07	1.70 ± 0.07	1.66 ± 0.06
	Cu (ppm)	1.29ab ± 0.06	1.32ab ± 0.07	1.36a ± 0.07	1.27ab ± 0.06	1.19ab ± 0.06	1.13b ± 0.06
	Zn (ppm)	2.18 ± 0.33	2.21 ± 0.29	2.30 ± 0.29	2.41 ± 0.27	2.55 ± 0.26	2.66 ± 0.25
G7 (n=6)	Ca (mg/dL)	9.15 ± 0.21	9.19 ± 0.20	9.27 ± 0.18	9.02 ± 0.16	8.95 ± 0.17	8.87 ± 0.18
	P (mg/dL)	6.27 a ± 0.17	6.23 a ± 0.17	6.31 a ± 0.18	5.94 ab ± 0.16	5.66 b ± 0.17	5.54 b ± 0.19
	Mg (mg/dL)	1.76 ± 0.05	1.73 ± 0.05	1.79 ± 0.05	1.77 ± 0.07	1.74 ± 0.08	1.70 ± 0.09
	Cu (ppm)	1.29 ± 0.06	1.31 ± 0.06	1.35 ± 0.06	1.29 ± 0.6	1.22 ± 0.06	1.16 ± 0.07
	Zn (ppm)	2.28 ± 0.32	2.30 ± 0.31	2.36 ± 0.29	2.43 ± 0.27	2.51 ± 0.24	2.58 ± 0.23

Means bearing different superscripts within the row vary significantly ($p<0.05$).

as per manufacturers' instructions. Trace minerals (copper and zinc) were estimated by AAS (ECIL 4141M, ECI, Hyderabad).

The effects of treatment protocols were assessed by using estrus response, lambing rate, prolificacy (number of lambs born per ewe lambed) and lamb weight. The data were statistically analyzed using one way analysis of variance (ANOVA) and Chi-square test (Snedecor and Cochran, 2004).

RESULTS AND DISCUSSION

The estrus induction response, lambing rate, prolificacy and average lamb birth weight observed in different groups of ewes are depicted in Table 1. The ewes subjected to 3 mg oral melatonin administration (G3 and G6) resulted in 100% estrus induction response as well as lambing rate compared to 1 mg subcutaneous melatonin (83.33%, G4 *vs* 66.66%, G7),

whereas lower estrus induction response as well as lambing rate, i.e., 50.0, 50.0 and 33.33% each were recorded in normal control (G1), long day control (G2) and short day control (G5) groups, respectively. Thus, in the present study, it seems that, apart from photoperiod, the administration of melatonin also affects the estrus induction response in ewes. Similar higher estrus response was reported in Kivircik and Charollais ewes subjected to subcutaneous melatonin implant (Cevik 2017). Conversely, El-Mokadem *et al.* (2017) recorded no significant effect of melatonin treatment on estrus response in Anglo-Nubian goats.

The present findings of higher lambing rate in melatonin treated groups of Chhotanagpuri ewes concurred well with earlier reports in Rasa Arafonesa, Assaf and Merino sheep (Abecia *et al.,* 2007), Kivircik and Charollais ewes (Cevik *et al.,* 2017), Anglo-Nubian goat (El-Mokadem *et al.,* 2017), Ossimi sheep (Abd-Allah and Daghash, 2019), Sarda sheep (Cosso *et al.,* 2021; Pulinas *et al.,* 2021) and Iberian Red Deer (Ortiz *et al.,* 2021) subjected to melatonin implant compared to untreated contemporaries. The prolificacy or litter size and average birth weight of lamb did not differ among different treatment groups in the present study. A similar non-significant effect on litter size has also been reported in melatonin-treated Sarda dairy sheep (Cosso *et al.,* 2021; Pulinas *et al.,* 2021). On the contrary , significantly higher litter size was reported in melatonin-treated Kivircik and Charollais ewes by Cevik *et al.* (2017).

The serum concentration of macro-minerals and trace minerals recorded in different groups of ewes over a period are depicted in Table 2. The significantly ($p<0.05$) lower calcium level was recorded in G4 and G6 at day 150th of sampling (around 3 months pregnancy). The values were non-significantly lower at day 150th in other groups compared to 0 day values. Like present findings, decreasing serum calcium level during pregnancy was also reported in Akkaraman sheep (Yildiz *et al.,* 2005) and Corriedale ewe (Inayat *et al.,* 2013). Conversely, ewes have a significantly higher blood calcium level during late pregnancy compared to lactating ewes by Antunovic *et al.* (2002). The decreasing calcium level with advancement of pregnancy might be due to increased calcium demand for ossification of fetal bones. Furthermore, Yatoo *et al.* (2017) also reported higher mineral requirements for pregnant Changthangi ewes compared to non-pregnant contemporaries.

The serum phosphorus level was significantly ($p<0.05$) lower at day 150th compared to day 0 in all treatment groups, except G 4 and G5, in which a non-significant decrease was noted. Similar decreasing trend of serum phosphorus level during pregnancy was also reported in Akkaraman sheep (Yildiz *et al.,* 2005) and Corriedale ewe (Inayat *et al.,* 2013). Moreover, significantly ($p<0.05$) lower phosphorus levels were observed in pregnant Changthangi ewes in summer and winter seasons compared to non-pregnant ewes by Yatoo *et al.* (2017). Singh *et al.* (2010) opined that melatonin treatment does not affect serum phosphorus level in anestrus buffalo heifers. Serum phosphorus level generally reflects intake, although they were modified by vitamin D and calcium (Kumar *et al.,* 2020).

The serum magnesium level was numerically lowest ($p>0.05$) on day 150th of sampling compared to day 0 in all treatment groups, which might be due to haemodilution, which usually occurs during late pregnancy (Elnageeb and Adelatif, 2010). Similar non-significant changes were reported in magnesium level in melatonin treated rat compared to control by Zaghloul and Gad (2014), while Baranowska- Bosiacka *et al.* (2008) found significantly higher serum magnesium level in melatonin treated rats compared to untreated contemporaries. In present study, the melatonin treatment could not produce significant effect on the serum magnesium level as blood magnesium level in body is under the control of several hormones (Rosol and Capen, 1997).

The serum copper concentration (ppm) was numerically lowest ($p>0.05$) at day 150th of sampling compared to day 0 in all groups, except G6, in which the value was significantly ($p<0.05$) lower at day 150th compared to day 60th of sampling. Unlike present trends, a significantly higher copper level (4.21 vs 2.57 ppm) was reported in Corriedale ewe in fifth month of pregnancy compared to first month (Inayat *et al.,* 2013). The periodic values of zinc level did not differ significantly within the group and a definite pattern was also lacking as pregnancy advanced. Conversely, a significantly low ($p<0.05$) zinc level (4.12 ± 0.04 vs 6.45 ± 0.10 ppm) was reported in Corriedale ewe in fifth-month pregnancy compared first month of pregnancy (Inayat *et al.,* 2013).

CONCLUSION

The present study's findings confirmed that melatonin treatment could be a useful tool to improve the reproductive efficiency of Chhotanagpuri ewe. Melatonin treatment via oral rout is more effective in estrus induction and lambing rate.

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