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Exogenous Melatonin Modulates Scrotal and Testicular Profiles, Libido and Endocrinological Profiles in Ganjam Goat of Odisha

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Abstract

Ganjam goat is a dual purpose breed for meat and milk in Odisha and parts of Andhra Pradesh, India. Present study was conducted to assess the effect of slow release subcutaneous exogenous melatonin (MT) on sexual behaviour, scrotal circumference (SC) and testicular parameters, endocrinological and heamatological profiles in buck during winter and pre-monsoon seasons. Experimental bucks (2-3 years of age) were selected and divided randomly into two groups, Gr I: Control (n=24) and Gr II: Treatment (n=24; melatonin implant @ 18mg/50 kg B. Wt). SC and testicular parameters [testicular volume & weight and testis index], testosterone, sex behaviour profiles [reaction time, Flehmen's response and libido score] and heamatological profiles [red blood cells (TRBC), white blood cells (TWBC), haemoglobin (Hb) and packed cell volume (PCV)] were estimated. Result revealed that these experimental profiles differed between treatment and control within the seasons and among the seasons within the groups. SC, testicular volume & weight, reaction time, Hb and TWBC were significantly ($p<0.05$) higher in MT treated than in control in pre-monsoon whereas testosterone and testicular index were significantly ($p<0.05$) higher in MT treated than in control in both seasons. SC, testicular index, testicular volume & weight and Hb were significantly ($p<0.05$) higher in pre-monsoon than in winter in both groups. Testosterone, Flehmen's response, TRBC, PCV and TWBC were significantly ($p<0.05$) higher in pre-monsoon than in winter in treatment group. Study concluded that exogenous slow-release MT and pre-monsoon had significant beneficial effects in improvement of testosterone with cascading beneficial effects on SC, testicular parameters and sex behavioural profiles, which will improve the semen production, higher sperm cryo-survivability and fertility rate in Ganjam goat.

Key words: *Heamatological profiles, Endocrinological profiles, melatonin, Ganjam goat, scrotal circumference, testicular parameters, sexual behaviour*

Introduction

Ganjam goat (INDIA_GOAT_1500_GANJAM_06008) is one important breed found in southern Odisha and bordering Andhra Pradesh, India. Ganjam goat is a dual purpose breed for meat and milk. Natural mating is preferred breeding practices, however, it is associated with many drawbacks including disease transmission, poor body confirmation of kids born and inbreeding depression and therefore, productive and reproductive performance losses in Ganjam goat under field condition. SC and testicular parameters of a breeding male are not only the critical components to evaluate the breeding soundness, but also help indirectly to measure the endocrinological profiles (McGowan *et al*., 2002), spermatogenic capabilities (Perumal, 2014), semen production capacity and birth weight of progeny (Evans *et al*., 1995).

Testes and scrotum are sensitive in changes of temperatures as either high or low temperature triggers testicular degenerative changes; thereby, reduction of testicular size and weight and alteration in its consistency (McEntee, 1990). Day length and temperature humidity index (THI) are the two factors in assessing the seasonality as well as the secretory pattern of reproductive and metabolic hormones (Sekoni *et al*., 1987). Longer photo period and higher THI in summer than in winter cause considerable reduction of MT secretion (short day breeder); reduced MT stimulates increased prolactin secretion; thereby, inhibits secretion of gonadotropins (Ohashi *et al*., 1995). Irregular production of these hormones induces adverse effects on gonadal hormone production, sex behavioural profiles and sperm production in breeding males (Ohashi *et al*., 1995).

MT is secreted in brain by the pineal gland, which acts as a neurotransmitter for regulation of circadian rhythm (Reiter, 1993) as well as seasonal reproduction in mammals (Revel *et al*., 2009). In mammals, MT significantly modulates the fertility profiles in male as infertility is caused by its low concentration (Klein and Moore, 1991). Animal studies revealed clearly that MT could control or regulate the hypothalamic GnRH pulse firing frequency, thereby, changes in the gonadotropins releasing pattern, which in turn could induce testosterone production (Bittman *et al*., 1985) and subsequent improvement in gonads and reproductive system development.

MT acts as antioxidants, anti-apoptotic agent and direct scavengers of free radicals (Tomás-Zapico and Coto-Montes, 2005). MT induces the enzymes which responsible for metabolising the free radicals, thereby; it helps in protection of the fluidity and integrity of the cell membranes (Tan *et al*., 1993). MT has greater potency than Vit-E (Pieri *et al*., 1994) and glutathione/mannitol (Hardeland *et al*., 1993) in scavenging the peroxyl radicals. Though, previous studies indicated that slow release exogenous MT has significantly improved the hormone profiles, scrotal and testicular biometrics and sex behavioural profiles in different species (sheep: Buffoni *et al*., 2015; El-Battawy and Sckalcki, 2015; cattle: Aggarwal *et al*., 2005; goat: Ghosh *et al*., 2014; Jiménez *et al*., 2014; buffalo: Ramadan *et al*., 2019; Kumar *et al*., 2015; mithun: Perumal *et al*., 2018); perusal of available literature on similar line of studies revealed no study reports available in Ganjam goat. Therefore, the present investigation was designed to evaluate the effect of slow release exogenous MT implantation on scrotal and testicular attributes and its associated hormone profile, sex behavioural scores and heamatological profiles in Ganjam goat.

Materials and Methods

Experimental design

Present study was conducted at ICAR-AICRP on Goat Improvement organised goat farm, Rambha, Ganjam district, Odisha, India which is located at 19.52°N 85.1°E with elevation of 87 m MSL in two seasons, winter (January-February; THI: 73.06, sunshine hour: 9.14 and rain fall: 16.13 mm) and pre-monsoon (March-April; THI: 80.81, sunshine hours: 8.61 and rain fall: 24.63 mm). Forty eight apparently healthy (body condition score: 5-6 out of 10, classified as good) Ganjam buck of 2-3 years of age (average: 22.80 months) were selected randomly from the flock for the present study. Bucks were maintained under uniform feeding, lighting, housing and other standard managemental practices as per the farm schedule. Experimental animals were randomly divided into two groups, Gr I: Control (n = 24) and Gr II: Treatment (n = 24; melatonin implant @ 18 mg/50 kg B Wt). Due to unavailability of MT implants in India, crystalline MT powder (HiMedia Laboratories Pvt Ltd, Mumbai, India) was dissolved in refined corn oil at room temperature in quantities sufficient to make a final concentration of 18 mg/mL. Once dissolved, the suspension was used immediately on the same day (Kumar *et al.,* 2015; Perumal *et al.,* 2018). Control animals were administered with corn oil as placebo. MT supplementation was done once in a month and blood samples were collected and analysed heamatological and endocrinological profiles.

Collection of blood and estimation of experimental profiles

Blood samples were collected from the experimental bucks by venipuncture of jugular vein into heparin tubes (20 IU of heparin per mL of blood) on day 37-40 of MT implant initiation (6th week). Part of these blood samples were used for haematology profiles analysis. Remaining blood samples were centrifuged at 1200 × g for 15 min at 4°C. The plasma samples were separated rapidly, labelled and preserved at -20°C for further analysis of testosterone profile.

Blood analysis was carried out using Veterinary fully automatic hematological analyzer (Prokan, PE-6800). Heamatological profiles such as total red blood cells (TRBC), haemoglobin (Hb), packed cell volume (PCV) and total white blood cell (TWBC) were estimated. Testosterone (analytical sensitivity: 0.02 ng/mL; intra- and inter-assay coefficients of variation: 5.46 and

9.65%, respectively) were estimated using commercially available ELISA kits (Alpha Diagnostic International, Life Technologies (India) Pvt. Ltd, New Delhi, India) in 96-well clear polypropylene microplate using a MRC Microplate Reader (UT-2100C, Israel).

Measurement of the SC and testicular parameters

Testicular parameters and SC were measured by using a caliper (Mitutoya Digimatic Caliper, Japan) and a measuring tape following proper restraining of the buck (Chenoweth *et al*., 1993). Testicular volume was estimated by using the following formula for volume of an ellipsoid as described by Love *et al*. (1991) ($4/3\pi abc$, a = thickness/2; b = width/2 and c = length/2). Weight of the testes was calculated by multiplying 1.038 with volume as 1.038 is the approximate density of testicular tissue. Weight of the testes was calculated by multiplying 1.038 with volume as 1.038 is the approximate density of testicular tissue (Amann, 1990). Testis index was calculated from testis volume and body weight of the bucks as testis index (cm^3/kg): testis volume (cm^3)/body weight (kg). SC and testicular parameters were measured four times by the same operator at winter and pre-monsoon seasons for control and treatment groups.

Sex behavioural profiles

Reaction time was calculated as the time between the appearance of buck to the estrous female and the first mounting or mounting attempt. The mount may or may not be successful. The reaction time was noted by stop watch in seconds. Flehmen's response is a typical male behavior observed when a buck is brought in contact with another herd mate. After sniffing the genitals of another herd mate, the buck raises its neck, extends its chin and inhales with slightly opened mouth, tongue held in flat position and upper lip curled to the nostrils which becomes partially closed. The passive partner does not object to this procedure. Frequency of flehmen response, following 10 exposures was recorded. Libido score scored with the help of libido score card developed by Zubieta (1990) for bucks.

Statistical analysis

The statistical analysis of the data was performed as per standard procedures (Statistical Analysis System for Windows, SAS Version 9.3; SAS Institute, Inc., Cary, NC, 2001). Figures present the non-transformed data. Means were analysed by student-t test to determine the significant differences between the seasons and between the treatment groups using the SAS software/PC computer program. The mean values with a significance of $p < 0.05$ were considered to be statistically significant.

Results

Body weight of the bucks was non-significantly higher in MT treated than in control in pre-monsoon and winter seasons. It also revealed that pre-monsoon had significantly (p<0.05) higher B Wt than in winter in control (2.32%) and treatment (3.85%) groups. SC was significantly (p<0.05) and non-significantly higher in MT treated than in control in pre-monsoon (5.85%) and winter (1.59%) seasons, respectively. It also revealed that pre-monsoon had significantly (p<0.05) higher SC than in winter in control (2.96%) and treatment (7.31%) groups. Testicular index was significantly (p<0.05) higher in MT treated than in control bucks in pre-monsoon (18.23%) and winter (3.76%) seasons. It also revealed that pre-monsoon had significantly (p<0.05) higher TI than in winter in treatment (23.31%) and control (9.05%) groups (Fig. 1).

Total testicular volume was significantly (p<0.05) and non-significantly higher in MT treated than in control bucks in pre-monsoon (18.50%) and winter (2.84%) seasons, respectively. It also revealed that pre-monsoon had significantly (p<0.05) higher TV than in winter season in control (11.43%) and treatment (26.69%) groups. Total testicular weight was significantly (p<0.05) and non-significantly higher in MT treated than in control bucks in pre-monsoon (19.23%) and winter (3.12%) seasons, respectively. It also revealed that pre-monsoon had significantly (p<0.05) higher TW than in winter season in control (12.18%) and treatment (27.54%) groups (Fig. 2).

Testosterone concentration was significantly (p<0.05) higher in MT treated than in control bucks in pre-monsoon (36.91%) and winter (15.66%) seasons. It also revealed that pre-monsoon had significantly (p<0.05) and non-significantly higher TW than in winter season in treatment (26.62%) and control (4.32%) groups. Reaction time was significantly ($p<0.05$) and non-significantly lower in MT treated than in control bucks in pre-monsoon (5.99%) and winter (4.05%) seasons, respectively. It also revealed that pre-monsoon had non-significantly higher RT than in winter season in treatment and control groups. Libido score was non-significantly higher in MT treated than in control bucks in pre-monsoon and winter seasons. It also revealed that pre-monsoon had non-significantly higher LS than in winter season in treatment and control groups. Flehmen's response was non-significantly higher in MT treated than in control bucks in pre-monsoon and winter seasons. It also revealed that pre-monsoon had significantly ($p<0.05$) and non-significantly higher FR than in winter season in treatment (21.22%) and control (3.01%) groups (Fig. 3).

MT treated bucks had significantly ($p<0.05$) higher Hb (winter: 4.75% and pre-monsoon: 10.08%), TWBC (pre-monsoon: 6.64%) and non-significantly higher TRBC and PCV than in control bucks in winter and pre-monsoon seasons. Similarly pre-monsoon season had significantly ($p<0.05$) higher TRBC (treatment: 5.57%), Hb (control: 3.38% and treatment: 8.71%), PCV (treatment: 3.62%) and TWBC (treatment: 8.19%) than in winter season in the experimental groups (Fig. 4).

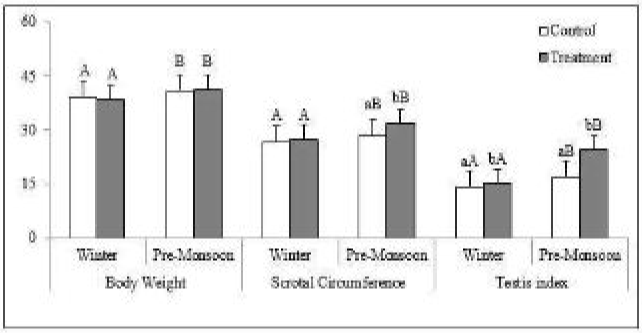

Fig 1: Effect of exogenous melatonin on body weight (kg), scrotal circumference (cm) and testis index of buck in pre-monsoon and winter seasons. Winter: January-February and pre-monsoon: March-April. Vertical bar on each point represents standard error of mean. Vertical bar with capital letters (A, B) indicates significant (p<0.05) difference between seasons within the group and small letter (a. b) indicates significant (p<0.05) difference between control and treatment groups within the season. Average Age: 22.80 months.

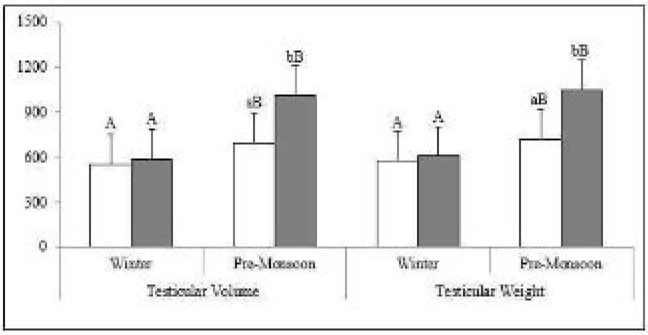

Fig 2: Effect of exogenous melatonin on testicular volume (cm^3) and testicular weight (gm) of buck in pre-monsoon and winter seasons. Winter: January-February and pre-monsoon: March-April. Vertical bar on each point represents standard error of mean. Vertical bar with capital letters (A, B) indicates significant (p<0.05) difference between seasons within the group and small letter (a. b) indicates significant (p<0.05) difference between control and treatment groups within the season. Average Age: 22.80 months.

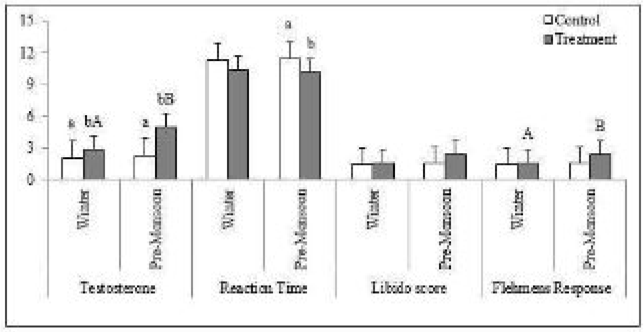

Fig 3: Effect of exogenous melatonin on testosterone (ng/mL), reaction time (sec), libido score and Flehmen's response of buck in pre-monsoon and winter seasons. Winter: January-February and pre-monsoon: March-April. Vertical bar on each point represents standard error of mean. Vertical bar with capital letters (A, B) indicates significant (p<0.05) difference between seasons within the group and small letter (a. b) indicates significant (p<0.05) difference between control and treatment groups within the season. Average Age: 22.80 months.

Discussion

Beneficial effects of exogenous MT on endocrinological attributes, biometry of testis & scrotum and sex behavioural profiles were studied in different livestock species (sheep: Buffoni *et al*., 2015; El-Battawy and Sckalcki, 2015; cattle: Aggarwal *et al*., 2005; goat: Ghosh *et al*., 2014; Jiménez *et al*., 2014; buffalo: Ramadan *et al*., 2019; Kumar *et al*., 2015; mithun: Perumal *et al.,* 2018), similar line of investigations were lacking in Ganjam buck in tropical humid coastal ecosystem of Odisha, India. However, MT is used in semen extender as an additive (Perumal *et al*., 2016) and supplement as subcutaneous injectable (Perumal *et al.,* 2018) or oral (Fatoba *et al*., 2013) form in different livestock species; which was proved as it is a potential chemical compound or antioxidants or anti-apoptotic agent to improve these experimental parameters along with semen production and *in-vitro* & *in-vivo* fertility in animal species.

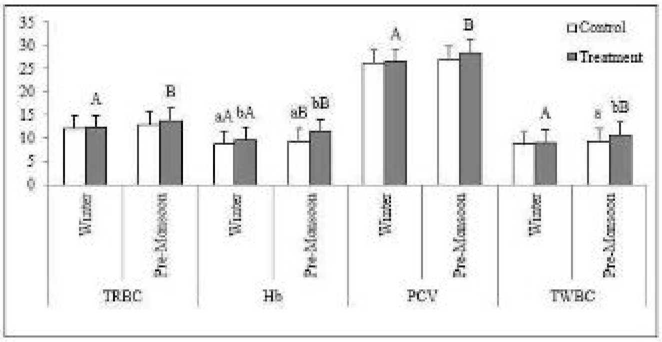

Fig 4: Effect of exogenous melatonin on haematological profiles of buck in pre-monsoon and winter seasons. TRBC: Total red blood cell ($x10^6/mm^3$), Hb: Haemoglobin (g/dl), PCV: Packed Cell Volume (%) and TWBC: total white blood cell ($x10^3/mm^3$). Winter: January-February and pre-monsoon: March-April. Vertical bar on each point represents standard error of mean. Vertical bar with capital letters (A, B) indicates significant ($p<0.05$) difference between seasons within the group and small letter (a. b) indicates significant ($p<0.05$) difference between control and treatment groups within the season. Average Age: 22.80 months.

These blood profiles estimated in our study were higher in MT treated than in untreated buck. Total WBC significantly increased in MT treated animals due to MT has pain attenuation effect, immune development effect and cytotoxic effects (Gilad *et al.,* 1998). Anwar *et al.* (1998) found that MT treatment in rats numerically increased RBC, Hb and PCV. MT protects Hb against nitrite induced oxidation which delays the formation of meth-Hb in concentration dependent manner (Hussain *et al.,* 2009). Dalia *et al.* (2013) reported that MT improved PCV value. However, Durotoye and Rodway (1996) reported a reduction in PCV in ewe following subcutaneous injection of MT. Melatonin has anti-inflammatory effects as it reduced the TNF-α and IL-1β concentrations (Bahrami *et al.,* 2018). Besides, MT modulates the inflammatory genes expression in immune cells and regulates the immune/inflammatory responses. As a potential antioxidant, MT minimises the generation of free radicals leads to reduction of pro-inflammatory cytokine production through inhibition of the activation and nuclear translocation of NF-κB (Mehrzadi *et al.,* 2018). Similar findings were reported that MT has a modulatory role in haematopoiesis and its rhythms (Zaghloul and Gad, 2014) and they opined that stimulatory effect of MT on WBC supports its immuno-potentiating action.

Reproduction in mammals is regulated by complex interactions among the physical factors viz., food, rainfall, sunshine hours, humidity and temperature (Bronson and Heideman, 1994). Photoperiod is well documented to activate this seasonal cycle by working on the neuro-reproductive-endocrine system. In our study, SC and testicular attributes were recorded higher during pre-monsoon and lowest was during winter which is in accordance with Ahmad *et al*. (2005) and is varied with seasons (Strumpt *et al*., 1993). SC and testicular diameter were higher in pre-monsoon season because during this season, pre-monsoon rain was expressed and goat was getting sufficient fodder and feeling mild environment. Whereas during winter, the goat was not getting sufficient fodder and the weather was also more towards cold as result, testes were reduced in its consistency and size. SC has significant ($p<0.05$) and positive correlation with testicular volume & weight in bovine species (Palasz *et al*., 1994) and testosterone concentration in the seminal plasma in bubaline bulls (Javed *et al*., 2000). Similar observation was reported in our study. Further, MT secretion showed significant variation among the seasons throughout the year in blood of male that may partly be explained the variation in SC and hormonal profiles observed between the different seasons in this caprine species (Perumal *et al*., 2020).

Role of MT has not been studied in reproductive physiology of Ganjam goat. Subcutaneous implant release MT continuously and does not affect the endogenous MT secretion and its diurnal rhythm. Moreover, result of MT in the implanted blood plasma suggested that subcutaneous implants were able to maintain this hormone with supra physiological plasma level at several times more than the endogenous secretion which suggested that exogenous prolonged constant supply of MT from the implant did not affect the endogenous MT secretion (Forsberg *et al.*, 1990). Testosterone was significantly ($p<0.05$) higher in treatment than in control in our study. Increased blood MT inturn stimulated the hypothalamic-pituitary-testicular axis, resulted into increased gonadotropins concentration which further inturn induced to produce higher testosterone and increased libido in Ganjam buck (Misztal *et al.*, 2002). MT directly acts on the hypothalamic GnRH neurons (Roy *et al.* 2001) which causes increased pulse secretion of GnRH with immediate higher pulsatile secretion of LH (Viguie *et al.*, 1995), which resulted into higher testosterone (Rosa and Bryant, 2003). Further, MT also has considerable effects on accessory sex glands and gonads as MT receptors are prevalent male reproductive system (Gilad *et al.*, 1998). Therefore, MT has significantly increased testosterone production, followed by development of the gonads and reproductive system. Similar report was observed in our study.

SC and testicular attributes are parameters in evaluation of breeding soundness and higher SC and testicular weight are correlated with higher sex behavioural score and also better semen production. SC and testicular attributes shown significant differences between the seasons indicated that they were significantly affected by seasons. Similarly in rams, MT improved the scrotal and testicular diameters and also significantly increased the performances of reproduction (Palacin *et al.*, 2008). The reason is that MT has capability to regularise the endocrine secretion; thereby higher spermatogenesis (Misztal *et al.*, 2002) which inturn causes increased SC and testicular mass. Moreover, MT minimised the testicular histopathological changes caused by DEHP induced toxicity (Bahrami *et al.*, 2018), which indicated that MT protects the testes and scrotum from free radicals or toxicities. Higher SC and testicular weight were due to beneficial morphological changes caused by MT as an endocrine regulator, antioxidant and anti-apoptotic agent. As reported, MT increased the level and activities of antioxidants causes minimised the lipid peroxidation and thereby decreased the fluidity of membrane of testes and scrotum (Bahrami *et al.*, 2018). Histopathological reports indicated that MT attenuated the oxidative stress stimulated injuries in sheep seminiferous tubules (Gholami *et al.*, 2015). MT has also anti-inflammatory properties as it reduced the level of TNF-α and IL-1β concentrations which was induced by DEHP in testicular tissue of mice (Bahrami *et al.*, 2018). Thus, MT protected the histo-physiology of the testis and scrotum. Again, MT has significantly enhanced the number of Sertoli cells and spermatogonia, height of germinal epithelium and diameter of luminal & tubular structures of seminiferous tubules. Thus, in our study, MT has increased the SC, testicular attributes in the Ganjam buck. Similar results were reported in other goat breeds (Chemineau *et al.*, 1992; Daramola *et al.*, 2006; Shekher *et al.* 2020).

Sexual behaviour in the form of libido score, reaction time and Flehmen's response in Ganjam buck was estimated. Sexual behaviour profiles were significantly affected by season. Similarly, these profiles were significantly higher in MT treated than in untreated buck. In our study, MT enhanced the hormone profiles especially testosterone, which inturn stimulated the libido score (D'Occhio and Brooks, 1982). This was confirmed by a positive correlation existed between sex behaviour profiles and testosterone in MT treated Ganjam bucks. Moreover, MT enhanced the health of the testicular structures and scrotum which resulted into increased SC and testicular attributes, which empowered the favourable micro-environment for Sertoli cell and Leydig cells which inturn higher testosterone production led to increased sex behavioural profiles in our study in Ganjam buck. Similar result observed in ovine species that MT implants increased the growth of testis with a higher level of testicular size, libido scores and enhanced semen production and its functional characteristics (Egerszegi *et al.*, 2014). The reaction time was shorter in MT treated than in control Ganjam buck. This finding is agreed with observations by Abecia *et al.* (2017) in rams, Ramadan *et* *al*. (2009) in Damascus male goats and Daramola *et al.* (2006) in West African dwarf bucks following treatment with MT. Similarly libido score was increased in MT supplemented than in untreated control bucks. Similar reports were reported in other species (goat: Delgadillo *et al.,* 2002; mithun: Perumal *et al*., 2020) in mithun bulls) following MT administration. However, a significantly higher frequency of Flehmen's reaction was registered in MT treated males compared to the control, which corroborates the earlier findings (Delgadillo *et al.,* 2002; Chemineau *et al*., 1992; Daramola *et al*., 2006; Abecia *et al.,* 2018).

Conclusions

Exogenous slow release MT and pre-monsoon season had significantly higher testosterone secretion led to increased scrotal & testicular biometrics and increased sex behavioural profiles in Ganjam buck. However, further study is needed to analyse the possible associations between hormone production, sex behavioural profiles, semen production and fertility profiles in Ganjam buck during different seasons to confirm the present findings.

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