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Effect of Melatonin Administration on Libido, Body Weight and Serum Biochemical Profile in Magra Rams During Non-breeding Season

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ABSTRACT

Background: The present study was designed to evaluate the effects of single subcutaneous melatonin administration on body weight, libido and serum biochemical profile in Magra rams during non-breeding season.

Methods: Magra (n=12) rams, (average age: 26 months, Avg. body weight: 35 kg) were randomly divided in to two groups and rams received single subcutaneous melatonin @18 mg/ram dissolved in 1 ml corn oil were considered as treatment group (n=6) and ram received corn oil (1 ml) sans melatonin were considered as control group (n=6). Post four weeks of experiment, reaction time was observed during the semen collection @ once in a week for next six consecutive weeks for quantification of libido. Initial and final body weight was recorded to evaluate the effect of melatonin on the body weight gain. Blood was collected at fortnightly intervals *i.e.*, 0, 15, 30, 45, 60 and 75th day of experiment to evaluate serum biochemical profiles.

Result: In the present study, overall mean values of reaction time were observed significantly (P<0.05) lower in treatment group as compared to control group, however, mean values during weeks didn't differ significantly between groups. Analysis of variance didn't reveal significant interaction between the mean values of reaction time and weeks in both groups. The mean values of initial body weight, final body weight, body weight gain, daily weight gain, serum aspartate aminotransferase (AST), alanine transaminase (ALT), AST/ALT ratio, A/G ratio and cholesterol didn't differ significantly between groups. The overall mean values of serum total protein, albumin and globulin were significantly (P<0.05) higher in treatment as compared to the control group. Analysis of variance didn't reveal significant interaction between serum total protein, albumin globulin and weeks in rams of both groups. The results of the study inferred that subcutaneous administration of melatonin (@18 mg/ram) ameliorated the libido, serum total protein, albumin and globulin but has no significant effect on the body weight gain, AST, ALT, AST/ALT ratio, A/G ratio and cholesterol of Magra rams during non-breeding season.

Key words: Biochemical profile, Body weight gain, Libido, Magra ram, Melatonin, Reaction time.

INTRODUCTION

Among livestock, sheep are of vital importance to the Indian agriculture based economy as they support the livelihoods of most small and medium farmers and landless workers. In India, sheep population is increased by 14.13% from 74.26 million over previous census of 2012 (DADF, BAHS, 2019). Seasonality in breeding behavior of sheep is a marked limitation to sheep production and regulated primarily by annual variations in photoperiod and several other factors (Abecia *et al*., 2012; Sarlos *et al*., 2013). Longer photoperiod is characterized by suppression of melatonin secretion resulting in reduction of libido, sperm quality and quantity leading to a period of low productivity (Cevik *et al*., 2017; Ramírez-Ramírez *et al*., 2021). Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine derived neurohormone, naturally synthesized and secreted by the pineal gland during the dark hours (Malpaux *et al*., 1997; Teng *et al*., 2017). Shorter photoperiods are associated with a longer pattern of melatonin secretion, which exerts a stimulatory effect on the reproductive axis in short day breeders like sheep (Castle-Miller *et al.,* 2017) which results in increased reproductive hormone promoting libido, semen quality and quantity (Deng *et al.,* 2018).

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Exogenous melatonin administration can overturn the effect of seasonality on reproductive performance of sheep during non-breeding season (Rosa *et al*., 2012; Cevik *et al*., 2017; Deng *et al*., 2018). Total number of matings and reaction time are generally used as main criteria for

assessing the libido levels of male animals (Estienne and Harper, 2000). In general, the longer reaction time is considered as an indication of poor-quality of semen in cross bred bulls (Pathak *et al*., 1990). Melatonin is also associated with fat deposition in fat tissues of obese animal and effects body weight (Piccinetti *et al*., 2010; Cardinali *et al*., 2011a). According to study of Gundogan *et al*. (2004) some reproductive parameters of rams showed a correlation with some serum biochemical properties and may be used to assess reproductive performance of a ram. Magra sheep is recognized as important carpet wool breed of western Rajasthan, India. Institute distributes superior quality rams for genetic improvement of field flock under different schemes. It is observed that some of rams show poor libido during non-breeding season at field level. To the best of our knowledge and literature cited, no data are available on effect of subcutaneous melatonin injection on libido parameters, body weight and serum biochemical profile of rams concerning tropical sheep breed. Therefore, the aim of the present work was to evaluate the impact of subcutaneous melatonin injection on body weight, reaction time and serum biochemical profile of Magra ram during non-breeding season.

MATERIALS AND METHODS

The present study was conducted at ICAR -Central Sheep and Wool Research Institute, Arid Region Campus, Bikaner, Rajasthan, India in collaboration with Department of Veterinary Gynaecology and Obstetrics, College of Veterinary and Animal Science, Bikaner, Rajasthan, India during the non-breeding season (June to August, 2022). During the study period, the average temperature and per cent humidity were 33.83°C and 47, respectively. All the experimental procedures involving rams were done following the standard guidelines issued by the Institute Animal Ethics Committee (IAEC).

Experimental animals

Twelve Magra rams (average age: 26 months, Avg. body weight: 35 kg) trained to donate semen in artificial vagina were included in the study. All of the rams were kept in separate groups in open-sided, asbestos-roofed sheds with well ventilation. Before commencement of the study, all animals were clinically inspected and were found free from any kind of disease. The general health management practices for deworming, vaccination etc. were followed as per health calendar of the research institute. Animals were allowed to graze for at least 7 hours per day. The animals were fed of a concentrate mixture in evening to fulfil the protein, energy and other nutrients requirements after returning from the pasture and fresh water was offered twice in a day (ad libitum). All of the rams were kept in identical dietary and management conditions.

Experimental design

Experimental rams were divided equally into treatment (group I, n=6) and control (group II, n=6) group on the basis of body weight, pre semen evaluation, testicular biometry and mounting ability. Each ram of treatment group received single subcutaneous injection of 18 mg melatonin (Sigma Aldrich, St. Louis, MO, USA) which was dissolved in 1 ml corn oil (Sangrur Agro Ltd., Sangrur, Punjab, India), whereas each ram of control received corn oil (1ml) subcutaneously sans melatonin (El-Shalofy *et al.,* 2022). Reaction time was started to record during the semen collection after 4 weeks of melatonin administration for next 6 consecutive weeks (@once in a week) for quantification of libido. Initial and final body weight were recorded to evaluate the effect on the body weight gain by using digital weigh machine. Blood samples were collected aseptically at every fortnightly interval *i.e*., day 0, 15, 30, 45, 60, 75 of treatment in sterilized 10 ml tubes from all the rams by jugular vein puncture using 20 G needle followed by separation of serum by centrifugation (Eppendorf, 5430R, Hamburg, Germany) @3000 rpm for 10 minutes and was stored at -20°C until biochemical analysis. Serum biochemical analysis of samples was done to estimate total proteins, albumin, globulin, albumin-globulin ratio, alanine aminotransferase (ALT), aspartate aminotransferase (AST), ALT-AST ratio and cholesterol using Kinetic UV test employing commercially available test kits (Accurex Biomedical Pvt Ltd, India) and the results were read on a colorimeter (Spectrostar Nano, BMG LABTECH, Germany) as per described procedures.

Statistical analysis

The experimental data were analyzed statistically by using one way and three ways analysis of variance having 2×6 factorial design using SPSS computer programme (version 20.0). The mean values were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

In the present study, the overall mean value of reaction time (seconds) differed significantly (P<0.05) between both

Table 1. Effect of melatonin administration on reaction time (seconds) of Magra rams during non-breeding season (Mean ± SE).

Groups	Reaction time (seconds) during the weeks (n=6)						
	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Overall
Treatment	3.38±0.22	3.33±0.15	3.25±0.14	3.13±0.14	3.15±0.21	3.15±0.12	3.23±0.07^{B}
Control	4.33±0.20	4.43±0.35	4.37±0.30	4.48±0.26	4.42±0.25	4.53±0.27	4.43±0.11^{A}
Overall	3.86±0.20	3.88±0.25	3.81±0.23	3.81±0.25	3.78±0.25	3.84±0.25	

Mean values having different superscripts (A, B) in the column differed significantly (p<0.05) n=Number of semen samples per ram.

the groups having lower value in the treatment as compared to control group (Table 1). However, during the weeks, the mean values of reaction time didn't differ significantly in both the groups. Analysis of variance also didn't reveal significant interaction between groups and weeks. The results of the study are in accordance with Ramadan *et al.* (2009) and Sarswat (2021) who reported a significantly (P<0.05) lower reaction time in melatonin treated Damascus and Sirohi bucks. Similarly, Zarazaga *et al*. (2019) noticed significant effect of melatonin administration on libido score in Mediterranean bucks. On contrary to present findings, Vince *et al*. (2017) and Pool *et al*. (2020) did not observe significant increase in libido intensity by administration of melatonin in French Alpine bucks and Pole Dorset and Merino breeds of ram, respectively. In the present study, the mean values of initial body weight (Kg), final body weight (Kg) and body weight gain (Kg) didn't differ significantly between treatment and control groups (Table 2), are in accordance with observation of Kleemann *et al*. (2014) in Border Leicester rams.

In the current study, melatonin treated group exhibited non-significant effect on serum total protein, albumin and globulin during the days of experiment, however, overall effect was significant (p<0.05) on serum total protein, albumin and globulin between both the groups (Table 3). There was a non-significant (p >0.05) effect of melatonin administration on serum AST, ALT, AST: ALT ratio, Albumin: Globulin ratio and cholesterol levels on the experimental rams as compared to control groups (Table 3, 4). There is a paucity of literature to compare our results with previous finding in sheep and goat. The results of the study are in accordance with Ramadan *et al*. (2019) who observed significantly higher total protein and albumin in seminal plasma after administration of melatonin implant in buffaloes during non-breeding season. Kassim *et al*. (2008) observed sharp decline in melatonin, when buffalo heifers exposed to long photoperiod, but total serum protein did not show any significant difference like our finding. Similarly, Oqetark *et al.* (2004) also reported that melatonin treatment did not cause a significant change in total protein levels in rat. 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). Melatonin implantation 18 mg, 36 mg/lamb/day significantly (P<0.05) increased total protein and decreased globulin level in serum in Awassi Lambs (Ismail Mahmud and Karadaş, 2023).

Melatonin, as being a potent antioxidant, the present data are in accordance with observations of previous researchers who also noted a significant increase in serum globulin concentration treated by non-enzymatic antioxidant

Table 2: Effect of melatonin administration on body weight (Kg) of Magra rams during non-breeding season (Mean± SE).

Groups	Initial body weight (Kg)	Final body weight (Kg)	Body weight gain (Kg)	Daily weight gain (Kg/day)
Treatment	43.83±1.05	46±1.16	2.17±0.6	0.03±0.01
Control	43.5±2.22	45.5±2.32	2.0±0.26	0.03±0.01

Mean values having different superscripts (A, B) in the column differed significantly (p<0.05).

Table 3: Effect of melatonin administration on serum total protein, albumin and globulin of magra rams during non-breeding season (Mean± SE)

Parameters	Groups	Measurement day of treatment						
		0 day	15th day	30th day	45th day	60th day	75th day	Overall
Serum total	Melatonin	7.96±0.11	8.10±0.18	8.33±0.20	8.47±0.15	8.28±0.06	8.56±0.12	8.29±0.06^{A}
protein	Control	7.25±0.28	7.08±0.31	7.17±0.35	7.52±0.35	7.13±0.21	7.21±0.09	7.22±0.11^{B}
(gm %)	Overall	7.61±0.18	7.59±0.23	7.75±0.26	7.99±0.23	7.70±0.20	7.89±0.22	
Serum albumin	Melatonin	4.05±0.34	3.68±0.09	4.07±0.20	4.22±0.13	4.06±0.12	4.14±0.154.	04±0.077^{B}
(gm %)	Control	3.62±0.10	3.47±0.18	3.58±0.21	3.65±0.29	3.69±0.06	3.80±0.05	3.63±0.07^{A}
	Overall	3.84±0.18	3.57±0.10	3.83±0.15	3.93±0.18	3.87±0.09	3.97±0.09	
Serum	Melatonin	3.91±0.31	4.42±0.18	4.26±0.29	4.25±0.20	4.22±0.13	4.42±0.21	4.25±0.09^{A}
globulin	Control	3.63±0.26	3.61±0.24	3.58±0.28	3.87±0.19	3.44±0.16	3.43±0.08	3.59±0.08^{B}
(gm %)	Overall	3.77±0.20	4.02±0.19	3.92±0.22	4.06±0.14	3.83±0.15	3.92±0.18	
Serum	Melatonin	1.12±0.22	0.84±0.04	0.99±0.1	1.01±0.07	0.97±0.06	0.96±0.09	1.03±0.03
A/G ratio	Control	1.02±0.08	0.98±0.08	1.03±0.09	0.96±0.10	1.08±0.04	1.11±0.03	0.98±0.04
	Overall	1.07±0.11	0.91±0.05	1.01±0.06	0.98±0.06	1.03±0.04	1.03±0.05	

Mean values having different superscripts (A, B) differed significantly (p<0.05).

Table 4. Effect of melatonin administration on serum aspartate amino transaminase, serum alanine transaminase, serum AST/ALT ratio and cholesterol of Magra rams during non-breeding season (Mean± SE).

Parameters	Groups	Measurement day of treatment						
		0 day	15th day	30th day	45th day	60th day	75th day	Overall
Serum aspartate aminotransferase (IU/L)	Melatonin	118.46±10.04	119.03±7.97	118.93±14.21	122.44±10.63	127.04±9.33	126.68±7.96	122.1±3.92
	Control	122.47±10.25	123.06±4.97	126.51±7.18	128.95±8.26	129.36±7.06	128.98±8.25	126.56±3.0
	Overall	120.47±6.87	121.05±4.52	122.72±7.68	125.7±6.49	128.20±5.59	127.83±5.48	
Serum alanine transaminase IU/L) level	Melatonin	38.16±4.98	45.49±4.55	52.29±4.23	44.6±8.63	45.62±6.03	46.36±8.21	45.42±2.50
	Control	41.24±6.24	58.53±6.04	53.77±5.87	50.12±7.32	49.41±8.64	49.67±6.84	50.46±2.75
	Overall	39.71±3.83	52.02±4.11	53.03±3.46	47.36±5.46	47.52±5.05	48.02±5.12	
Serum AST/ALT ratio	Melatonin	3.25±0.31	2.79±0.42	2.36±0.32	3.03±0.33	2.99±0.36	3.08±0.42	2.91±0.15
	Control	3.30±0.52	2.22±0.23	2.45±0.19	2.86±0.44	3.02±0.50	2.77±0.30	2.77±0.16
	Overall	3.27±0.29	2.5±0.25	2.4±0.18	2.94±0.26	3.0±0.30	2.92±0.25	
Serum cholesterol (mg %)	Melatonin	201.78±11.15	200.03±12.95	200.96±11.58	204.78±9.36	204.16±5.6	205.94±6.92	202.94±3.78
	Control	198.1±8.36	195.38±2.86	195.71±7.3	200.21±12.26	197.13±7.57	192.39±6.44	196.49±3.04
	Overall	199.94±6.67	197.71±6.36	198.33±6.57	202.5±7.39	200.65±4.61	199.16±4.95	

Mean values having different superscripts (A, B) differed significantly ($p<0.05$).

(ascorbic acid) in ewes and lactating dairy cows (Monzaly, 2000; Abu El-Hamad *et al*., 2007). However, melatonin treatment has non-significant effect on seminal plasma alanine transaminase level but significant effect on seminal plasma cholesterol in buffaloes during non-breeding season (Ramadan *et al.,* 2019). Oleshchuk *et al*. (2019) found that melatonin @ 10 mg/kg body weight significantly decreased activities of serum AST and ALT in Sprague-Dawley male rats. In contrary to this, Zhang *et al*. (2017) recorded that oral administration of melatonin @ 5 mg/kg body weight for 30 days in rats alleviated the increases in the plasma of the ALT and AST activity.

Melatonin treatment mimics short photoperiods and also associated with changes in sexual behaviors, particularly libido (Daramola *et al*., 2006; Abecia *et al*., 2011). The constant-release of melatonin implants in rams may be a sufficient stimulus to activate responses at the hormonal and sexual behavioral levels (Rosa *et al*., 2012) which favors that melatonin dissolved in corn oil has similar effect as implant. Melatonin is secreted from the pineal gland into the blood stream which can inhibit fat deposition in fat tissues of obese animal models (Cardinali *et al*., 2011a) and has shown reduced body weight (Piccinetti *et al*., 2010). In humans, melatonin effectively regulates body weight and effectively used in treatment of metabolic problems (Cardinali *et al*., 2011b). Melatonin can physiologically regulate brown adipose tissue and metabolic activity in mammals and humans (Tan *et al*., 2011). But in conflict, the present experiment didn't observe the effect of melatonin on body weight and needs further research trials on metabolic disordered animals at large scale. Blood serum biochemical estimates are helpful in semen evaluation as semen attributes estimation alone are not completely satisfactory for semen appraisal in the current practice of commercial artiûcial insemination (Ashworth *et al*., 1994; Perez *et al*., 1997; Barrios *et al*., 2000). A link has been examined by previous researchers among biochemical parameters and specific reproductive parameters of rams (Gundogan *et al*., 2004).

CONCLUSION

In conclusion, findings of the present study revealed that the administration of single subcutaneous melatonin (@ 18 mg/ram) has positive effect on reaction time and biochemical parameters like serum total protein, albumin and globulin whereas have no significant effect on body weight and serum biochemical parameters like AST, ALT, AST/ALT ratio, A/G ratio and cholesterol on Magra rams. However, further studies are warranted to prove its efficacy at large scale as well as in clinically poor libido rams as well.

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Conflict of interest

All authors declare that they have no conflict of interest.

REFERENCES

Abecia, J.A., Forcada, F. and Gonzalez-Bulnes, A. (2011). Pharmaceutical control of reproduction in sheep and goats. Veterinary Clinics: Food Animal Practice. 27(1): 67-79.

Abecia, J.A., Forcada, F. and Gonzalez-Bulnes, A. (2012). Hormonal control of reproduction in small ruminants. Animal Reproduction Science. 130(3-4): 173-179.

Abu El-Hamad, M.A., Shamiah, S.M., Gabr, S.A. and Mohi El-Din, A.M.A. (2007). Physiologic responses, rumen and blood parameters and milk production of Friesian cows treated with various doses of ascorbic acid during summer months in Egypt. Journal of Agricultural Research. Kafr El-Shaikh University. 33: 502-521.

Alila- Johansson, A., Eriksson, L., Soveri, T. and Laakso, M.L. (2001). Seasonal variation in endogenous serum melatonin profiles in goats: a difference between spring and fall. Journal of Biological Rhythms. 16: 254-263.

Ashworth, P.J., Harrison, R.A., Miller, N.G., Plummer, J.M. and Watson, P.F. (1994). Survival of ram spermatozoa at high dilution: protective effect of siple constituents of culture media as compared with seminal plasma. Reproduction, Fertility and Development. 6(2):173-180.

BAHS G.O.I. Basic Animal Husbandry Statistics. (2019). Department of Animal Husbandry and Dairying, Ministry of Fisheries, animal Husbandry and Dairying, New Delhi, India.

Barrios, B., Perez-Pe, R., Gallego, M., Tato, A., Osada, J., Muino-Blanco, T. and Cebrian-Perez, J.A. (2000). Seminal plasma proteins revert the cold-shock damage on ram sperm membrane. Biology of Reproduction. 63(5): 1531-1537.

Cardinali, D.P., Cano, P., Jimenez-Ortega, V. and Esquifino, A.I. (2011a). Melatonin and the metabolic syndrome: Physiopathologic and therapeutical implications. Neuroendocrinology. 93(3): 133-42.

Cardinali, D.P., Pagano, E.S., Scacchi Bernasconi, P.A., Reynoso, R. and Scacchi, P. (2011b). Disrupted chronobiology of sleep and cytoprotection in obesity: possible therapeutic value of melatonin. Neuroendocrinology Letters. 32(5): 588-606.

Castle-Miller, J., Bates, D.O. and Tortonese, D.J. (2017). Mechanisms regulating angiogenesis underlie seasonal control of pituitary function. Proceedings of the National Academy of Sciences of the United States of America. 114(12): E2514–E2523.

Cevik, M., Yilmazer, C. and Kocyigit, A. (2017). Comparison of sexual performance and testicular characteristics of melatonin treated Kivircik and Charollais rams during the non-breeding season. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 69(2): 278-284.

Daramola, J.O., Adeloye, A.A. and Soladoye, A.O. (2006). Effect of exogenous melatonin on sexual behaviors in West African Dwarf goat. Livestock Research for Rural Development. 18(9): 1-8.

Deng, S.L., Wang, Z.P., Jin, C., Kang, X.L., Batool, A., Zhang, Y., Li, X.Y., Wang, X.X., Chen S.R., Chang, C.S. and Liu, Y.X. (2018). Melatonin promotes sheep Leydig cell testosterone secretion in a co-culture with Sertoli cells. Theriogenology. 106: 170-177.

Duncan, D.B. (1955). Multiple range and multiple F' test. Biometrics. 11(1): 1-42.

El-Shalofy, A.S., Shahat, A.M. and Hedia, M.G. (2022). Effects of melatonin administration on testicular hemodynamics, echotexture, steroids productionand semen parameters during the non-breeding season in Ossimi rams. Theriogenology. 184: 34-40.

Estienne, M.J. and Harper, A.F. (2000). PGF2a facilitates the training of sexually active boars for semen collection. Theriogenology. 54(7): 1087-1092.

Gundogan, M., Yeni, D., Ucar, M. and Ozenc, E. (2004). Relationship between some reproductive parameters and biochemical properties of blood serum in rams. Archives of Andrology. 50(6): 387-390.

Ismail Mahmud, K. and Karadaş, F. (2023). Effects of the Melatonin and Vitamin E (Alpha-Tocopherol Acetate) as Antioxidants on Biochemical Blood Parameters, Lipid Profileand Muscle Vitamin E Concentration in Awassi Lambs Fed a High-Energy Diet and Normal Diet. Archives of Razi Institute. 78(1): 249-259.

Kassim, N.S.I., Afify, A.A. and Hoda, Z.H. (2008). Effect of photoperiod length on some reproductive traits and hormonal profiles in buffalo heifers. AmericanEurasian. Journal of Agriculture and Environmental Sciences. 3(4): 646-655.

Kleemann, D.O., Kelly, J.M., Arney, L.J., Farley, I.L., Tilbrook, A.J. and Walker, S.K. (2014). Positive effects of melatonin treatment on the reproductive performance of young border Leicester rams mated to merino ewes in spring: preliminary observations. Reproduction in Domestic Animals. 49(6): 894-898.

Malpaux, B., Viguie, C., Skinner, D.C., Thiery, J.C. and Chemineau, P. (1997). Control of the circannual rhythm of reproduction by melatonin in the ewe. Brain Research Bulletin. 44(4): 431-438.

Monzaly, H.M.A. (2000). Influence of flushing and administration of vitamins E, C on body weight, blood constituents and reproductive performance of Saidi ewes. M.Sc. Thesis, Faculty of Agriculture, Assiut University Egypt.

Oleshchuk, O., Ivankiv, Y., Falfushynska, H., Mudra, A. and Lisnychuk, N. (2019). Hepatoprotective Effect of Melatonin in Toxic Liver Injury in Rats. Medicina (Kaunas, Lithuania). 55(6): 304.

Oqetark, M., Kus, I., Kavaki, A., Zararsiz, I., Iihan, N. and Sarsilmaz, M. (2004). Effect of melatonin on carbontetrachloride-induced changes in rat serum. The Journal of Physiology and Biochemistry. 60(3): 205-210.

Pathak, N., Banjamin, B.R., Mohan, G. and Sahni, K.L. (1990). Libido in relation to other reproductive traits among the crossbred bulls. Indian Journal of Animal Sciences. 60(1): 52-54.

Perez, R., Lopez, A., Castrillejo, A., Bielli, A., Laborde, D., Gastel, T., Tagle, R., Queirolo, D., Franco, J., Forsberg, M. and Rodriguez-Martinez, H. (1997). Reproductive seasonality of Corriedale rams under extensive rearing conditions. Acta Veterinaria Scandinavica. 38(1): 109-117.

Piccinetti, C.C., Migliarini, B., Olivotto, I., Coletti, G., Amici, A. and Carnevali, O. (2010). Appetite regulation: The central role of melatonin in Danio rerio. Hormones and Behavior. 58(5): 780-785.

Piccione, G., Fazio, F., Caola, G. and Refinetti, R. (2008). Daily rhythmicity of glycemia in four species of domestic animals under various feeding regimes. The Journal of Physiology and Biochemistry. 58: 271-275.

Piccione, G., Messina, V., Giannetto, C., Casella, S., Assenza, A. and Fazio, F. (2011). Seasonal variations of the serum proteins in sheep and goats. Archives Animal Breeding. 54(4): 399-405.

Pool, K.R., Rickard, J.P., Pini, T. and de Graaf, S.P. (2020). Exogenous melatonin advances the ram breeding season and increases testicular function. Scientific Reports. 10(1): 9711.

Ramadan, T.A., Kumar, D., Ghuman, S.S. and Singh, I. (2019). Melatonin improved buffalo semen quality during nonbreeding season under tropical condition. Domestic Animal Endocrinology. 68: 119-125.

Ramadan, T.A., Taha, T.A., Samak, M.A. and Hassan, A. (2009). Effectiveness of exposure to long day followed by melatonin treatment on semen characteristics of Damascus male goats during breeding and non-breeding seasons. Theriogenology. 71(3): 458-468.

Ramírez-Ramírez, A.I., Delgado-Tiburcio, G.I., Cruz-Espinoza, F., Herrera-Corredor, A.C. and Gallegos-Sánchez, J. (2021). Photoperiod and its relationship to sheep reproduction. Agro Productividad. 6(10): 3-10.

Rosa, H.J.D, Silva, C.C. and Bryant, M.J. (2012). The effect of melatonin treatment in rams on seasonal variation of testicular size and semen production parameters. Small Ruminant Research. 102(2-3): 197-201.

Sarlos, P., Egerszegi, I., Balogh, O., Molnar, A., Cseh, S. and Ratky, J. (2013). Seasonal changes of scrotal circumference, blood plasma testosterone concentration and semen characteristics in Racka rams. Small Ruminant Research. 111(1-3): 90-95.

Sarswat, C., Sharma, S., Nehra, K., Sharma, P., Parashar, M. and Vaishnava, C. (2021). Effect of exogenous melatonin administration on testicular biometry, libido parameters, seminal parameters, hormone levelsand freezability of Sirohi buck semen during the non-breeding season in Southern Rajasthan. Reproduction, Fertility and Development. 33(2): 124-124.

Tan, D.X., Manchester, L.C., Fuentes Broto, L., Paredes, S.D. and Reiter, R.J. (2011). Significance and application of melatonin in the regulation of brown adipose tissue metabolism: relation to human obesity. Obesity Reviews. 12(3): 167-188.

Teng, H., Fang, T., Lin, Q., Song, H., Liu, B.and Chen, L. (2017). Red raspberry and its anthocyanins: Bioactivity beyond antioxidant capacity. Trends in Food Science and Technology. 66: 153-165.

Vince, S., Valpotic, H., Berta, V., Milinkovic-Tur, S., Samardzija, M., Grizelj, J., Spoljaric, B., Duricic, D., Nazansky, I. and ZuraZaja, I. (2017). Monitoring of libido and semen quality parameters in melatonin-treated French Alpine bucks during non-breeding season. Reproduction in Domestic Animals. 52(6): 953-961.

Zarazaga, L.A., Gatica, M.C., Hernandez, H., Chemineau, P., Delgadillo, J.A. and Guzman, J.L. (2019). Photoperiod-treated bucks are equal to melatonin-treated bucks for inducing reproductive behavior and physiological functions *via* the “male effect” in Mediterranean goats. Animal Reproduction Science. 202: 58-64.

Zhang, J.J., Meng, X., Li, Y., Zhou, Y., Xu, D.P., Li, S. and Li, H.B. (2017). Effects of melatonin on liver injuries and diseases. International Journal of Molecular Sciences. 18(4): 673.