The effects of melatonin application on some haematological parameters and thyroid hormones and testosterone in male goats' non-breeding season

Nurcan Dönmez^{1*}, Fikret Karaca², Ferda Belge¹, and Cafer Tayyar Ates³

¹University of Yüzüncü Yýl, Faculty of Veterinary Medicine, Department of Physiology, Van, Turkey

²University of Yüzüncü Yýl, Faculty of Veterinary Medicine, Department of Reproduction and Artificial Insemination, Van, Turkey

³University of Yüzüncü Yýl, Faculty of Veterinary Medicine, Department of Zootechnics, Van, Turkey

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In the present study, the effects of melatonin application on some haematological parameters, testosterone and thyroid hormones in male goat in out of breeding season were investigated. In this study 16 Coloured Mohair male goats were used. At the beginning of the study the animals were randomly divided into two equal groups as control and test groups. Melatonin (Regulin, Hoechst) was implanted in the test group. Thirty and 70 days after melatonin implantation, blood samples were obtained from both control and melatonin groups for determination of erythrocyte and leukocyte counts, haemoglobin concentration, haematocrit level, plasma testosterone, T_3 and T_4 concentrations. T_3 and T_4 levels were lower both 30 and 70 days after melatonin implantation in the test group compared to the values obtained from the control group. However, important decreases (P<0.05) were seen only in T_3 concentration obtained from the melatonin group on day 70 of the experiment. Testosterone values increased in the melatonin-implanted group for 30 and 70 days after implantation, but results were not significant. Accordingly, it is believed that the effects of melatonin implantation on certain haematological parameters, testosterone and thyroid hormone levels in male goats in the non-breeding season, should be taken into consideration.

Key words: male goat, thyroid hormones, testosterone

Dr. Nurcan Dönmez, Yüzüncü Yýl University, Veterinary Faculty, 65080 Van, Turkey, Phone: +90 432 225 1024/1524; Fax: +90 432 225 1127; E-mail: donmez68@hotmail.com

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^{*} Contact address:

Introduction

Melatonin released from the pineal gland is affected by the environmental photoperiod. It has been reported that organized, different physiological functions arise from seasonal changes in mammals through daily secretion (MAEDA and LINCOLN, 1990). Specifically, hypothalamus, hypophys, ovarium and testes have been reported to be affected due to changes in melatonin level. The circadian melatonin rhythm provides a critical endocrine signal to regulate secretion of the hormone involved in the onset and termination of the annual breeding season (YELLON et al., 1992; MAEDA et al., 1988). An increase in the daily period of melatonin secretion is associated with an increase in GnRH release and activation of the gonads, i.e. day breeders (GROSSE et al., 1993). This situation directed researchers to the hormones released from the affected organs (YILMAZ, 2000). The relationships between thyroid hormones and melatonin in man and in different animal species have been investigated (SOSZYNSKI et al., 1988; O'CONNOR and PERSIGER, 1996; ÖZTÜRK et al., 2000). O'CONNOR and PERSIGER (1996) have determined a relationship between melatonin and thyroid metabolism. In fact, in pineal gland-removed rats, application of one dose of melatonin was reported to affect thyroid activity at different times of the following day and through the night. Similarly, injection of melatonin in the evening to rats and mouse is reported to effect thyroid hormone synthesis during a 10-day period (SELMAOUI et al., 1997). These and other similar results show that thyroid glands activity is related to the pineal gland. In known mammals and bird species, thyroid hormones play an important role in seasonal reproduction (GERLACH and AURICH, 2000).

In this study, we aimed to investigate the effects of melatonin application in out of reproductive season on testosterone, thyroid hormones and some haematological parameters in male goats, showing seasonal changes in reproductive activity.

Materials and methods

Animals and treatments. In this study, 16 healthy Coloured Mohair male goats aged between 11 and 12 months and with similar weights were used as material. The animals were obtained from the Research Farm of the Faculty of Veterinary Science, University of Yuzuncu Yýl. The animals

were divided randomly into two equal groups as control (C, n = 8) and test groups (T, n = 8). Specific light, heat and feeding regimens were not applied to the animals during this study, and which were kept in half-open pens.

Eighteen mg of melatonin (Regulin®, Sanofi Animal Health, Hoechst UK Ltd. Milton Keynes) were implanted to the near base of ear subcutaneously by special gun to animals in the test group. Nothing was applied to animals in the control group.

Blood sampling and laboratory analyses. Blood samples were taken into heparinized tubes from both groups of animals 30 and 70 days after melatonin implantation simultaneously in order to determine thyroid, testosterone hormone levels, as well as certain haematological parameters. Erythrocyte, leukocyte counts, haemoglobin and haematocrit values were determined (KONUK, 1981). Plasma samples were stored at -20 °C until hormone analyses. Plasma T₃ and T₄ concentrations were determined non-radioactively using the DPC-IMMULITE System hormone autoanalyser (Solid-Phase, Chemiluminescent Enzyme Immunoassay) (BABSON, 1991).

The three samples taken from each goat at 9.00, 10.00 and 11.00 a.m. were pooled to reduce the effect of pulsative hormone secretion on sample values, as reported by LANGFORD et al. (1987). Plasma samples were stored at -20 °C until testosterone assay. Testosterone concentrations were measured by radioimmunoassay kit for Testosterone (Immunotech cat # 2463, with 0.1 ng/ml, France).

Student's *t*-test was used to compare differences between groups.

Results and discussion

Results obtained for T_3 , T_4 , testosterone values and certain haematological parameters for both groups are shown in Table 1.

In this study, T_3 and T_4 values of both groups were examined. T_3 and T_4 values were decreased compared to the control values obtained at the same time, 30 and 70 days after melatonin implantation. However, only T_3 values obtained 70 days after drug implantation were statistically significant (P<0.05). In a study, VRIEND et al. (1982) reported that injection of melatonin reduced plasma T_3 , T_4 and TSH concentration. On the other hand, VAUGHAN et al. (1983) reported a depression in T_4 values after melatonin injection, but

Table 1. Mean values of haematological parameters and hormone levels in the blood of control (C) and melatonin treated (T) male goats

Parameters	30 day		70 day	
	C (n = 8)	T (n = 8)	C (n = 8)	T (n = 8)
Erythrocyte (×10 ⁶ /ml)	13.20 ± 5.42	12.98 ± 1.63	16.09 ± 5.16	14.67 ± 1.00
Leukocyte (×10 ³ /ml)	10.87 ± 1.68	9.88 ± 1.81	12.07 ± 0.9	12.28 ± 0.86
Hemoglobin (gr/dl)	5.63 ± 0.63	5.35 ± 1.06	8.57 ± 0.79	7.68 ± 0.32
Haematocrit (%)	22.25 ± 0.75	25.2 ± 1.59	29.0 ± 0.91	29.5 ± 1.29
T ₃ (ng/dl)	178.96 ± 25.60	161.91 ± 25.81	177.66 ± 5.46	133.50 ±15.79*
$T_4(\mu g/dl)$	4.41 ± 0.30	3.85 ± 0.39	3.86 ± 0.12	3.63 ± 0.30
Testosterone (ng/ml)	0.69 ± 0.32	1.45 ± 0.34	1.32 ± 0.62	2.25 ± 0.66

^{*} statistical difference from control group (P<0.05)

no changes in T_3 and TSH values. Results reported in the present study support the findings of the above researchers. The differences observed in this study compared to the results of VAUGHAN et al. (1983) could be due to different animal species and the different amount of melatonin application used in this study.

Testosterone concentrations were examined in both groups. Mean testosterone concentration was higher in the melatonin group compared to control group at 30 and 70 days after implantation. However, the difference between groups was not significant. Some researches found that, depending on the dose, melatonin implantation induced a blood testosterone level lasting for days or even weeks (KENNAWAY and GILMORE, 1985; LINCOLN and EBLING, 1985; CHEMNIEAU et al., 1992; KOKALIS et al., 2000). LINCOLN and McNEILLY (1989) determined that the plasma concentrations of FSH and LH increased on short days and decreased on long days, and that there was a corresponding cycle in plasma concentrations of testosterone. KOKALIS et al. (2000) demonstrated that implantation of melatonin in rams increased the mean value, basal level and number of peaks of testosterone at the 45th day after implantation. ROSA et al. (2000) determined that melatonin implants caused an increase in plasma testosterone concentrations in rams, and that this increase was more than twice as high as the control values obtained from rams. When compared with the above studies, the increase in testosterone concentration may be speculated as being similar to the present study. Furthermore, DELGADILLO et al. (2001) reported that melatonin

implantation (on January 16) elevated plasma testosterone concentration in male coreole goats starting from February up until April. In contrast, plasma testosterone concentration in the control group remained low until mid-June.

In the present study, the number of erythrocyte, leukocyte and haematocrit values were similar in both groups, and were also similar to the values given in the literature for goats (KONUK, 1981; YILMAZ, 2000). In a study performed on albino mouse, melatonin has been reported to depress erythrocyte counts, haemoglobin and haematocrit values. Marginal increases in erythrocyte from time to time were also reported in above study. Similar increases for leukocyte were also speculated (KARIMUNGI et al., 1996). In another study carried out by KARIMUNGI and JOSHI (1996) in rats, a decrease in erythrocytes in contrast to an increase in leukocytes were reported in animals that received melatonin once or twice daily. From above the study, a modulator role can be attributed to melatonin in the haemapoesis and its fluctuation.

In conclusion, results obtained in the present study could contribute to physiological knowledge, apart from being a basis for further studies on the effects of melatonin in goats.

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SAŽETAK

Istraženi su učinci davanja melatonina na hematološke pokazatelje, testosteron i koncentraciju hormona štitnjače u jaraca izvan sezone mrkanja. Pokus je proveden na 16 Mohair jaraca podijeljenih na pokusnu i kontrolnu skupinu. životinjama pokusne skupine supkutano je dan melatonin (Regulin, Hoechst). Tridesetog i 70. dana nakon davanja melatonina svima je bila izvađena krv radi određivanja broja eritrocita i leukocita, koncentracije hemoglobina, vrijednosti hematokrita, razine testosterona u plazmi te koncentracije T₃ i T₄. Koncentracije T₃ i T₄ bile su 30. i 70. dana nakon davanja melatonina manje u pokusnoj skupini nego u kontrolnoj. Značajno povećanje (P<0,05) ustanovljeno je samo za koncentraciju T₃ u pokusnoj skupini 70. dana pokusa. Vrijednosti testosterona bile su povećane u pokusnoj skupini 30. i 70. dana nakon aplikacije, ali ne statistički značajno. Zaključuje se da valja uzeti u obzir moguće učinke davanja melatonina na neke hematološke pokazatelje te koncentraciju testosterona i hormona štitnjače u jaraca izvan sezone mrkanja.

Ključne riječi: jarac, hormoni štitnjače, testosteron