Turk. J. Vet. Anim. Sci.
2008; 32(2): 137-140

Short Communication

Circadian Variations in Thyroid Hormone Levels of Nonpregnant Uniparous Fat-Tailed Iranian Ewes in Summer

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Received: 23.01.2007

Abstract: Circadian variations in serum triiodothyronine (T_3), thyroxine (T_4), free triiodothyronine (fT_3), and free thyroxine (fT_4) levels were investigated in Iranian ewes. For that purpose, blood samples were collected from the jugular veins of 15 adult clinically healthy Iranian nonpregnant uniparous ewes every 6 h (0600, 1200, 1800, and 0000) during 6 days in summer with a mean ambient temperature of 40 °C. Serum T_3, T_4, fT_3, and fT_4 were measured by radioimmunoassay. There were statistically significant differences in thyroid hormone concentrations (T_3, T_4, fT_3, and fT_4) at different hours of the day. The results revealed that serum T_3, T_4, fT_3, and fT_4 levels were highest at 1800 ($P < 0.05$). Significant 24 h periodicity in the serum levels of T_3, T_4, fT_4 and fT_3 was observed in nonpregnant uniparous Iranian ewes. It is concluded that circadian rhythm was observed in serum thyroid hormones.

Key Words: Circadian rhythm, triiodothyronine, thyroxine, nonpregnant uniparous ewes

Thyroid hormones (THs) are known to be important modulators of developmental processes and general metabolism in mammals (1). Changes in the concentration of serum thyroxine (T_4) and triiodothyronine (T_3) of sheep in seasonal heat and cold stress have already been investigated (2-4). Diurnal variations in THs have also shown changes in foals, mares, rams, rats, cats, rooster chicks (*Gallus domesticus*), and men (5-12). Iranian fat-tailed sheep are reared under the climatic conditions of Iran. The physiological importance of the fat-tailed sheep is that it provides energy during seasonal droughts and conditions of feeding deprivation, which are not uncommon under the climatic conditions of Iran (13). Since there is no available information about the circadian changes in serum THs in these sheep, the present study aimed to examine variations in the serum T_3, T_4, free triiodothyronine (fT_3), and free thyroxine (fT_4) of Iranian nonpregnant uniparous ewes at various times of the day during a 6-day period in summer, to provide data about the circadian variation in TH concentration.

This study was performed using blood samples from 15 nonpregnant uniparous fat-tailed Iranian ewes at the Animal Husbandry Unit, College of Agriculture, Shiraz University, located 20 km north of Shiraz, a tropical area in the south of Iran. Shiraz has relatively rainy mild winters and hot dry summers. Shiraz is 1540 m above sea level. The animals were fed hay (mainly alfalfa and grass) from the pasture near the husbandry unit. All animals were clinically healthy and free from internal and external parasites. All were treated with fenbendazole (Damloran Co. Borujerd, Iran) 10 mg/kg 30 days prior to the study. The prevention of internal and external parasites is routine practice in this unit. Each ewe had a separate file including all necessary records so that its characteristics, including age, sex etc., could be determined. Blood samples were taken from the jugular vein of 15 adult clinically healthy Iranian ewes with the

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mean 48 kg body weight, every 6 h (0600, 1200, 1800, and 0000) during 6 days in summer with a mean ambient temperature of 40 °C. The serum samples were separated by centrifugation at 750 × g for 15 min and stored at -20 °C until used. Serum T_4, T_3, fT_3, and fT_4 were measured by radioimmunoassay (kits available from Immunotech Company, IMMUNOTECH, Radiova, Prague, Czech Republic) in Namazi Research Center, Shiraz, Iran. The areas of validation for T_3, T_4, fT_3, and fT_4 assays included limits of detection, and precision in standard curve following sample dilution, and inter- and intra-assay coefficients of variation results were considered. Intra- and interassays for T_4 and T_3 were below 6.2%, 8.6%, 3.3%, and 8.6% respectively. For fT_4 and fT_3 the values were below 6.5%, 7.2%, 3.5%, and 7.0%, respectively. The data were expressed in SI units and analyzed by repeated measurement ANOVA and Bonferroni multiple comparisons test using SPSS/PC software (14). All values were expressed as mean and standard error (SE), and $P < 0.05$ was regarded as statistically significant.

Circadian variations in serum T_3, T_4, fT_3, and fT_4 in the Iranian nonpregnant uniparous ewes at various times of the day during a 6-day period in summer are presented in Figures 1-4, respectively. There were significant differences in TH concentrations at different times. The results revealed an increase in serum T_3, T_4, fT_3, and fT_4 levels at 1800 hours ($P < 0.05$) in the mentioned season. Circadian rhythm was observed in serum TH with the peak value in the evening. These results agreed with those reported by Weeke and Gundersen (15), who obtained a low peak in the daytime and higher levels at night for free T_3 and serum TSH in men and there was a tendency for a similar rhythm in free T_4. They also showed that a pulsatile release of hormones from the thyroid gland is governed by a pulsatile TSH secretion. Our results also agreed with those reported by Souza et al. (11), who obtained means of T_3 and T_4 that varied in peaks throughout the 24-h period with the highest concentrations occurring in the afternoon (1630 and 1430 hours, respectively). They also showed that throughout the year the highest levels were during months of long day length (October, December, and February). There were some findings such as those reported by Oki and Atkinson (12) that showed in the harbor seal that neither total nor fT_4 and T_3 displayed a

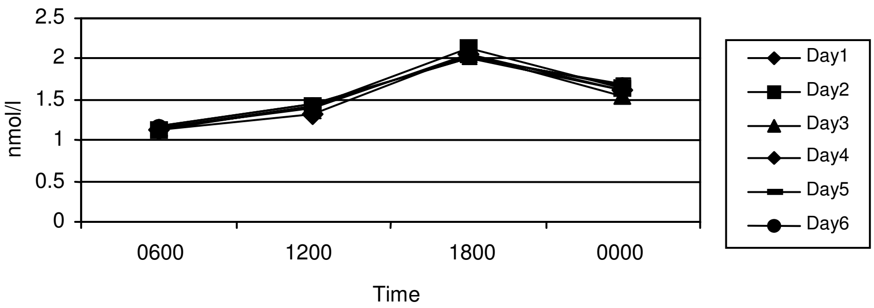

Figure 1. Variation in serum T_3 concentration (mean ± SE) in 15 nonpregnant uniparous Iranian ewes over 24 h during 6 days in summer.

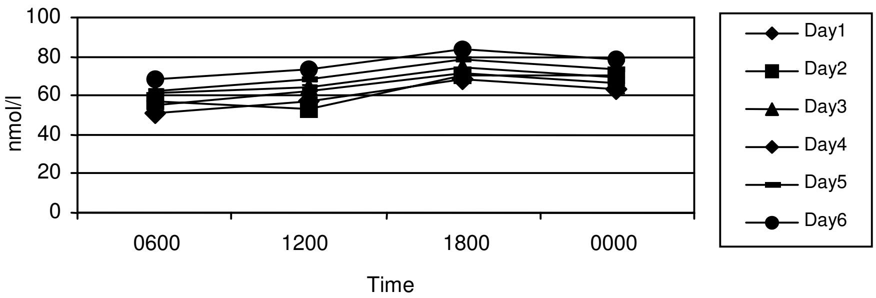

Figure 2. Variation of serum T_4 concentration (mean ± SE) in 15 nonpregnant uniparous Iranian ewes over 24 h during 6 days in summer.

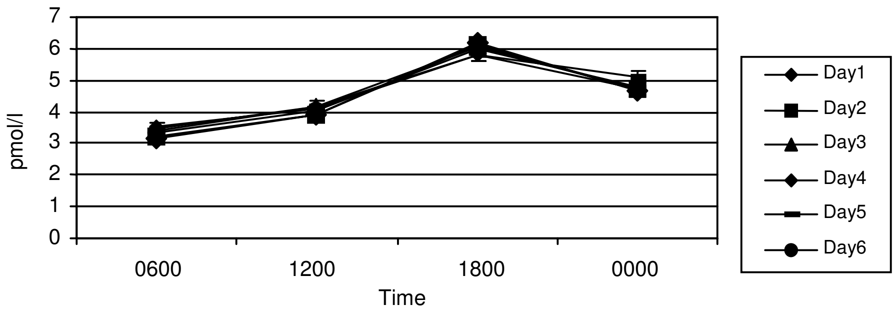

Figure 3. Variation in serum fT_3 concentration (mean ± SE) in 15 nonpregnant uniparous Iranian ewes over 24 h during 6 days in summer.

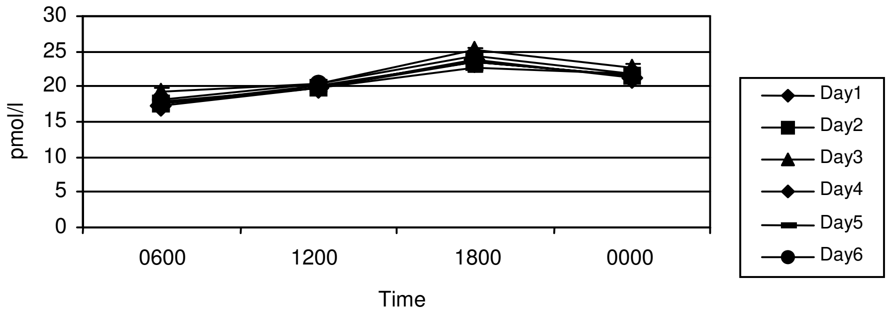

Figure 4. Variation in serum fT_4 concentration (mean ± SE) in 15 nonpregnant uniparous Iranian ewes over 24 h during 6 days in summer.

* In all cases, there are significant differences between the thyroid hormone levels at 1800 hours compared with others ($P < 0.05$).

diurnal rhythm in either season. However, T_4, T_3, and fT_3 levels were significantly higher in the winter than in the summer. There was no seasonal effect on fT_4 levels. In summer, the activity of the thyroid gland is minimal and generally the function of this gland is connected with systemic adaptation to low temperatures (12). Cold environment may be a stimulus to increase the output of thyrotrophic hormone, thereby resulting in a higher concentration of thyroid hormones in serum (3,16). Nazifi et al. (17) reported that in Iranian fat-tailed sheep the concentrations of T_3 and T_4 in cold stress conditions were higher than in heat stress conditions. During the summer, T_4 levels fell gradually in dehydrated dromedary camels and increased after rehydration, whereas in the winter T_4 levels increased in dehydrated camels (18). In contrast to the above reports, Assane and Sere (2) reported that, in Sahel Peulh ewes, plasma T_3 and T_4 levels did not show significant variations from the beginning of the cool season until the end of the dry warm season. Moreover, followed by the rainy season, maximal serum T_3 concentration was found in the summer (4).

Komosa et al. (9) reported that in mature mares diurnal rhythm was observed in concentration of T_3 only in summer months. Krebietke (5,6) found diurnal rhythms of THs in roaster chicken (*Gallus domesticus*). In agreement with our finding, Krebietke (5,6) reported that the peak value of thyroid hormones occurred at 0600 and 2100 hours, respectively. Freake et al. (19) stated that a diurnal variation was maintained in all thyroid states, with the peak value in the middle of the dark period being 3-fold higher than the nadir. However, Sturgess et al. (20) found that TSHs followed a diurnal rhythm with a peak level at 2330 hours and a trough level at 1430 hours. This study shows significant time-related variability in TSHs and thyroid hormone levels in treated hypothyroid patients. Wong et al. (8) reported that there was no influence of age (40-, 60-, and 90-day-old Sprague-Dawley rats) but there was a significant influence of strain (Sprague-Dawley vs. BH/Ztm rats) and season (summer vs. winter) on the diurnal pattern of serum TSH and T_3 levels. Flisińska-Bojanowska et al. (10) reported that a diurnal rhythm in T_3 level was found throughout pregnancy, with acrophase always at 1400 hours in mares. No diurnal rhythm in the T_4 level was observed. In mares, seasonal cyclicity in T_3 and T_4 levels was found. To summarize, in the present study circadian rhythm was observed in serum thyroid hormones of clinically healthy Iranian nonpregnant uniparous ewes, with the peak value in the evening.

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