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# Variations in Thyroidal Activity during Estrous Cycle and Natural Breeding Season in Markhoz Goat Breeds

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**Abstract:** The aim of this study was to determine the profile of changes in T3, T4 and TSH levels during breeding season and estrous cycle in Markhoz (Angora) Goats. Whereas the peaks of T3 were recorded in January, concentrations of T4 and TSH were highest in October. Variations in T3 and TSH concentrations among the different months of experiments period were not significant, although T4 concentration was significantly higher during September, October and November in comparison to December and January. Weekly variations in serum T4 and TSH concentrations were directly correlated to the changes in photoperiod and temperature. Monthly variation in serum T3 and TSH did not have a significant ($p>0.05$) relationship with the changes in photoperiod and temperature, but there was a highly significant positive relationship between serum T4 and temperature. This study showed that T4 concentration was high in the early phase but decreased in the late phase of the breeding season, but T3 and TSH concentrations varied markedly from week to week. However, it appears that weekly rhythms are controlled by photoperiod and temperature because, changes in these factors resulted in different profiles of both T4 and TSH, but there was not any correlation between T3 and those factors.

**Key words:** Thyroid, photoperiod, goat, reproduction

## INTRODUCTION

Thyroid hormones are important for regulation of nutrient assimilation, metabolism, calorigenesis (Todini *et al*., 2007), growth and development (Nixon *et al*., 1988) and reproduction (Blaszczyk *et al*., 2004). Reproduction and associated events involving gonadal activity, such as estrus, pregnancy and lactation, are known to be under the control of the hypothalamo-hypophyseal-thyroid axis (Reddy *et al*., 1996; Ben Saad and Maurel, 2004; Barrett *et al*., 2007). Multiple studies indicates that thyroid hormones play an important role in reproductive seasonality among a large range of species including birds, rodents and mammals (Moenter *et al*., 1991; Dahl *et al*., 1995). It is now well known that thyroid hormones do not influence transition into the breeding season but their presence is required for its termination. In ewes, thyroid hormones must be present for the seasonal increase in hypothalamic responsiveness to estradiol negative feedback and the seasonal inhibition of pulsatile GnRH secretion that causes the transition from breeding season to anestrus (Webster *et al*., 1991; Thrun *et al*., 1997). Therefore, in the absence of these hormones the seasonal reproductive rhythm is not expressed (Moenter *et al*., 1991; Webster *et al*., 1991; Rosa and Bryant, 2003). As reviewed by Huszenicza *et al*. (2002), there is doubtless evidence concerning the involvement of thyroid hormones in negative energy balance status, in the course of certain diseases and in the process of resumption of cyclic ovarian function in ruminants.

Markhoz goat breed, known as Angora goat in other places, is the only breed raised for different colored mohairs. These goats are seasonal breeders and the breeding season commences in the September-October with the maximum estrous activity in November (Farshad *et al*., 2008). Seasonal variations in photoperiod and temperature influence reproductive activity and the secretion of a variety of hormones in mammals. T3 and T4 have been shown to vary in response to changes in photoperiod, temperature and/or reproductive state in several mammalian species (Johnson, 1986). Marked seasonal variations in thyroid activity have been reported by many researchers. These hormone variations are

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particularly important in free-ranging and grazing animals, e.g., goat (Todini *et al*., 1992) and sheep (Souza *et al*., 2002). Appropriate thyroid activity is considered crucial to sustain the productive performance in domestic animals.

In this breed of goats the role of thyroid gland in seasonal reproduction has not been studied extensively. So, the aim of the present study is to obtain better insights regarding the thyroidal hormones and TSH profiles in Markhoz does under natural photoperiod, ambient temperature and constant nutritional levels during natural breeding season. The results can be used to enhance breeding programs.

## MATERIALS AND METHODS

**Animals and management:** This experiment was performed on Markhoz does in a testing station, located in Sanandaj City, Iran (35°20′ N latitude and 47° E longitude) and lasted from mid-September (2006) to early January (2007). The experiment was carried out on 10, mature (3 years of age) does with a mean weight of (34.05±2.62 kg) at the onset of the experiment and with one kidding record. Nutritional levels were adjusted to meet maintenance requirements (NRC, 1981). Goats were fed twice a day with a diet of (530 g) alfalfa hey, (190 g) barley straw and (300 g) concentrates, whose composition was as follows: Phosphorous (0.8), Calcium (0.9), Fiber (12), Crude protein (14), TDN (65), Urea (1) percent of dry weight. They had free access to water and mineral blocks containing oligo-elements and vitamins. Each 2.5 kg block contained: magnesium 40 g, iron 10000 mg, zinc 3500 mg, manganese 3000 mg, copper 750 mg, iodine 40 mg, cobalt 25 mg, selenium 25 mg, sulfur 1500 mg, CinaQ.1 2.5 kg. In addition, animals were dewormed (using Albendazole, Sigma, USA) at the beginning of experiment. The animals were kept in open shed subjected to natural photoperiod and ambient temperature. The photoperiod varied from (12.00 h) of daylight in September to (9.00 h) daylight in January. The mean temperature in the open sheds was registered as follows: Sep. (21°C), Oct. (18°C), Nov. (10°C ), Dec. (2°C ), Jan (-4°C).

**Blood collection:** Blood samples were collected at 8 AM every 10 days, using syringes without any anticoagulant. Following the onset of breeding season and observation of standing estrus, blood samples were collected twice a week until the end of the breeding season. Samples incubated at (37°C) in water bath for 1 h and then centrifuged (15 min at 1500 g). Sera were harvested and stored at (-57°C) until assayed.

**Estrous detection:** Estrous detection was carried out twice a day for 1 h (AM and PM) using an aproned buck which was kept in a separate barn. In all does the time of occurrence of the estrous symptoms and the length of estrus duration was recorded based on visual observation. Does that exhibited a tail-flagging response and stood for mounting were considered to be in estrus (Jarrell and Dziuk, 1991).

**Hormone assays:** Serum T4 and T3 concentration were determined by commercially available RIA kit (Institute of Isotopes Ltd., Budapest). Sensitivity of assays were (0.3 nmol $L^{-1}$) for T3 and (7 nmol $L^{-1}$) for T4. The mean intra-assay Coefficient of Variation (CV) was 5.44% for T3 and 3.94% for T4. The T3 and T4 antiserum had less than 0.06% and less than 12.6% cross-reactivity for T4 and T3, respectively.

Serum TSH was assayed using a kit containing ovine TSH-antibody (Institute of Isotopes Ltd., Budapest). Sensitivity of assays was (0.011 μIU $mL^{-1}$) and the mean intra-assay Coefficient of Variation (CV) was 2.73%. All measurements of the given subjects were performed simultaneously to eliminate inter-assay variability.

**Statistical analysis:** Results are reported as the Mean±SEM. Monthly and weekly mean hormone concentrations and mean hormone concentrations during estrous cycles were also calculated and analyzed by ANOVA and Duncan's new multiple range test to compare the means (SAS, 1999). Correlation coefficients were conducted on mean weekly and monthly T3, T4 and TSH values vs. the length of photoperiod and temperature.

## RESULTS

Mean weekly concentrations of T3, T4 and TSH under natural photoperiod and temperature are shown in Table 1. The correlation between weekly concentrations of serum T4 and the length of daylight and serum T4 and temperature showed a positive significant association ($r = +0.898$; $r = +0.782$; $p<0.01$), respectively. The relationship between T3 and temperature and T3 and photoperiod were positive ($r = +0.120$) and negative ($r = -0.117$), respectively, but not significant ($p>0.05$). There were highly significant relationships between serum TSH and temperature and between serum TSH and photoperiod ($r = +0.610$; $r = +0.578$; $p<0.05$), respectively.

Monthly mean concentrations of T3, T4 and TSH under natural photoperiod and temperature are shown in Fig. 1. Highest concentration of T3 (1.18±0.14 nmol $L^{-1}$) were found in January whereas concentrations of T4 and

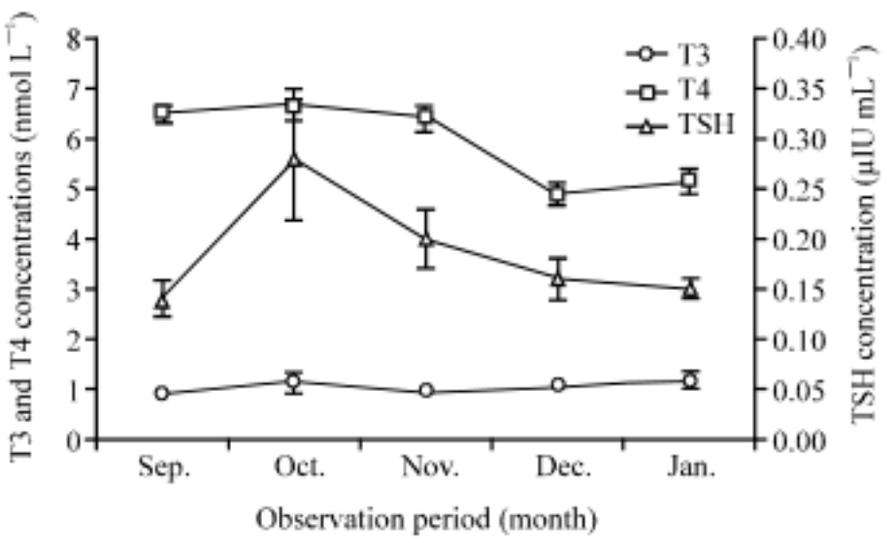


Fig. 1: Mean±SEM monthly concentrations of T3, T4 and TSH under natural photoperiod and temperature

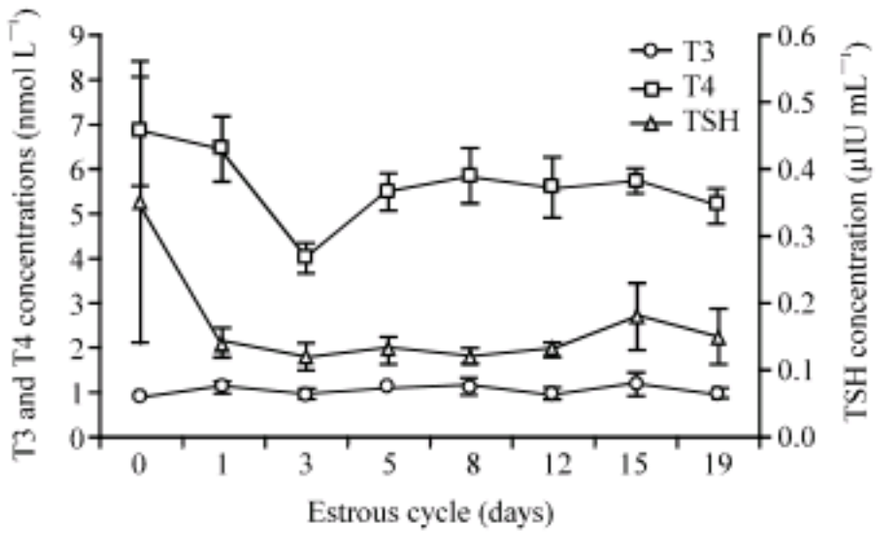


Fig. 2: Mean±SEM concentrations of T3, T4 and TSH at different days of estrous cycle

Table 1: Mean±SEM weekly concentrations of T3, T4 and TSH under natural photoperiod and temperature

| Week | Photoperiod (h) | Temperature (°C) | T3 | T4 | TSH |
|---|---|---|---|---|---|
| | | | ----------(nmol $L^{-1}$)------ | | ($\mu$IU $mL^{-1}$) |
| 1 | 11.00 | 19 | 0.90±0.03$^{ab}$ | 6.52±0.19$^{abc}$ | 0.14±0.02$^{b}$ |
| 2 | 11.00 | 22 | 1.40±0.53$^{a}$ | 6.40±0.50$^{abc}$ | 0.30±0.15$^{ab}$ |
| 3 | 11.00 | 20 | 1.10±0.30$^{ab}$ | 6.63±0.85$^{ab}$ | 0.36±0.11$^{a}$ |
| 4 | 11.00 | 18 | 1.00±0.06$^{ab}$ | 6.80±0.50$^{ab}$ | 0.25±0.08$^{ab}$ |
| 5 | 11.00 | 13 | 0.96±0.09$^{ab}$ | 7.04±0.52$^{a}$ | 0.19±0.02$^{ab}$ |
| 6 | 10.70 | 11 | 0.82±0.05$^{b}$ | 7.00±0.79$^{a}$ | 0.32±0.16$^{ab}$ |
| 7 | 10.70 | 10 | 0.85±0.04$^{b}$ | 6.90±0.50$^{ab}$ | 0.22±0.06$^{ab}$ |
| 8 | 10.01 | 9 | 0.90±0.04$^{ab}$ | 6.80±0.46$^{ab}$ | 0.16±0.03$^{ab}$ |
| 9 | 10.01 | 9 | 1.08±0.13$^{ab}$ | 6.27±0.36$^{abcd}$ | 0.19±0.03$^{ab}$ |
| 10 | 10.00 | 3 | 1.00±0.07$^{ab}$ | 5.52±0.37$^{abcde}$ | 0.13±0.01$^{b}$ |
| 11 | 9.02 | 3 | 1.06±0.12$^{ab}$ | 4.62±0.34$^{de}$ | 0.21±0.08$^{ab}$ |
| 12 | 9.02 | 2 | 0.94±0.04$^{ab}$ | 5.34±0.81$^{abcde}$ | 0.12±0.01$^{b}$ |
| 13 | 9.01 | 1 | 1.01±0.03$^{ab}$ | 4.44±0.22$^{e}$ | 0.20±0.08$^{ab}$ |
| 14 | 9.01 | 2 | 1.12±0.11$^{ab}$ | 4.82±0.24$^{cde}$ | 0.13±0.01$^{b}$ |
| 15 | 9.01 | 1 | 1.18±0.14$^{ab}$ | 5.16±0.27$^{bcde}$ | 0.15±0.01$^{ab}$ |

$^{a-e}$values with different superscripts in the same column are significantly different ($p<0.05$)

TSH were highest in October (6.7±0.31 nmol $L^{-1}$ and 0.28±0.06 µIU $mL^{-1}$), respectively. Variations in T3 and TSH concentrations were not significant ($p>0.05$) between the different months of experiment period, although T4 concentration during September, October and November was significantly higher than December and January ($p<0.05$). There was not any significant relationship between T3 and temperature or T3 and photoperiod ($r = -0.500$; $r = -0.454$; $p>0.05$), respectively. The relationships between TSH and temperature or photoperiod were not significant ($r = 0.397$; $r = 0.239$; $p>0.05$), respectively. There was a highly significant positive relationship between serum T4 and temperature ($r = +0.898$; $p<0.05$). Also a positive but insignificant correlation was observed between T4 and photoperiod ($r = +0.855$; $p>0.05$).

Mean concentrations of T3, T4 and TSH at different days of estrous cycle are shown in Fig. 2. The mean length of the estrous cycle (three cycles) in the ten does was (20.93±1.56 days). No significant changes in serum T3, T4 and TSH were detected on different days (0, 1, 3, 5, 8, 12, 15 and 19) of estrous cycle ($p>0.05$). T4 and TSH both increased around estrus but T3 decreased at this period. The maximum concentration of the T3 (1.18±0.27 nmol $L^{-1}$) was observed in 15th day of estrous cycle whereas maximum concentration of T4 and TSH were observed in estrus day (6.8±1.22 nmol $L^{-1}$ and 0.35±0.21 µIU $mL^{-1}$, respectively). T4 and TSH decreased markedly on the first and the third days of the estrous cycle; their levels were (3.97±0.29 nmol $L^{-1}$ and 0.14±0.02 µIU $mL^{-1}$), respectively. T3 exhibited gradual increase during estrous cycle.

## DISCUSSION

The highest concentrations of T4 were found during September, October and November and there appeared to be a significant relationship between T4 and temperature, but the relationship between T4 and photoperiod was insignificant. Wallace (1979) observed cyclic variations in serum T4 levels related to the ambient temperature in sheep, with the highest value occurring in September. By contrast Johnson (1986) maintains that, in mares there is no apparent relationship between temperature and T4 levels. But present analysis of weekly concentration of T4 showed a good association between T4 and the environmental conditions (photoperiod and temperature). Webster *et al.* (1991) reported that, in sheep, serum thyroxin levels are in association with daylight length. Our findings shows that T4 and TSH concentrations varied markedly from week to week, whereas serum T3 concentrations increased from week 1 to week 9, when day length was longer and temperature was higher (12 h and 12-19°C) and decreased from week 10 to week 15 when day length was shorter (9 h and 0-4°C). These results are in agreement with earlier reports that thyroxin levels in sheep and goats sera are strongly in correlation with photoperiod and ambient temperature (Souza *et al*., 2002; Todini *et al*., 2006).

Study of any correlations among the T3, T4 and TSH variations and specific procedural or physiological processes was beyond the scope of this experiment. However, the results clearly indicated that T4 and TSH could be influenced by even minor alterations in day length and temperature.

Similar to the reported results of (T3, T4 and TSH concentrations remained unchanged significantly during the estrous cycle, which is similar to the results reported by Johnson (1986). However, in other species some relationships exist between thyroid status and the estrous cycle. For example, Reimers *et al*. (1984) found that concentrations of both T4 and T3 were higher during diestrus than proestrus in bitch. Rastogi and Agarwal (1990) reported that a variation of considerable magnitude in the levels of T3 and TSH were exhibited during different phases of estrous cycle, but T4 changed insignificantly within a narrow range.

The above-mentioned results show that the concentration of T4 is high in the early phase and decreases in the late phase of the breeding season, but T3 and TSH concentrations varies markedly from week to week. However, it appears that weekly rhythms are controlled by photoperiod and temperature, because changes in these factors results in different profiles for both T4 and TSH. But there was not any correlation between T3 and the above-mentioned factors. Further studies are required to identify and characterize the physiological control of T3 and T4 secretions and to determine whether annual variations in these hormones influence reproductive seasonality or not.

M.A. Zarei and A. Farshad developed the concept and designed experiments and S. Akhondzadeh performed Field experiments and assays.

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