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Seasonal thyroid hormone alterations in Kermani sheep: An adaptive thermoregulatory mechanism for heat stress survival

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ABSTRACT

Elevated ambient temperatures adversely affect livestock production. Among domestic animals, sheep in southern Iran experience heat stress for seven months annually based on the Temperature-Humidity Index (THI), sheep in this region experience heat stress conditions for seven months of the year. Understanding the endocrine mechanisms that regulate metabolic homeostasis may provide insights into how sheep cope with such stress. Thyroid hormones—namely triiodothyronine (T3) and thyroxine (T4)—play fundamental roles in metabolic processes and thermoregulation. In this study, plasma levels of T3 and T4 were measured in Kermani sheep during winter and summer. Both hormones were significantly lower in summer than in winter ($P < 0.007$). However, the calculated T3/T4 ratio was significantly higher in summer ($P < 0.001$), indicating a 15-fold increase in the conversion of T4 to T3 compared to winter. Although overall thyroid hormone production declined during summer, although overall thyroid hormone concentrations declined during summer, the increased T3/T4 ratio suggests possible alterations in peripheral thyroid hormone metabolism and conversion, which may contribute to the adaptive response to high ambient temperatures. Further studies measuring rT3 levels and deiodinase activity are required to confirm the underlying mechanisms. In conclusion, seasonal modulation of thyroid hormone dynamics represents a key thermoregulatory strategy in Kermani sheep.

Keywords: Kermani sheep, thyroid hormones, heat stress, adaptation, thermoregulation

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Introduction

Livestock productivity is adversely affected by extreme climatic conditions. High environmental temperatures combined with forage scarcity significantly impair the well-being and performance of ewes during summer months. In sheep, rectal temperature rises when ambient temperature exceeds 32 °C, and hyperthermia develops when heat production surpasses heat loss. The efficacy of thermoregulatory mechanisms in sheep is largely dependent on breed and individual genetic makeup (Sevi and Caroprese, 2012). Under high-temperature exposure, sheep activate a series of physiological compensatory mechanisms that facilitate acclimatization to extreme environments and help maintain vital functions through substantial changes in biological functioning. Extensive rearing systems remain the most common method of sheep production in developing countries. In such systems, sheep often graze over long distances under intense solar radiation, with restricted grazing time during the hottest months. Under the influence of multiple concurrent stressors, grazing small ruminants have evolved a suite of adaptive mechanisms that promote survival under harsh environmental conditions. Sheep tolerate these stressors primarily by modifying their thermoregulatory pathways. Thyroid hormones are critical regulators of metabolic adjustments, thermogenesis, growth, organ function, wool follicle maturation, and cartilage-to-bone differentiation. Their dynamics are influenced by sex, breed, age, physiological state, dietary iodine supplementation (Nudda et al., 2013), feed composition (Katole et al., 2011), and treatment with propylthiouracil (PTU). For instance, pregnant ewes treated with PTU for 35 days exhibited significantly lower T3 and T4 concentrations compared to controls (Gifford et al., 2007). Similarly, ewes treated with PTU for 66 days postpartum had reduced thyroid hormone levels (Wells et al., 2003).

A phytoestrogen-rich diet also lowered blood T4 levels relative to control sheep (69.7 vs. 57.2 ng/mL) (Skipor et al., 2012). Interestingly, thyroid hormone levels in twin-pregnant ewes decreased more rapidly than in single-pregnant ewes during gestation (Abdollahi et al., 2013). In pregnant Merino ewes, blood T4 (62.9–64.4 ng/mL) and T3 (1.41–1.88 ng/mL) have been reported (McDowall et al., 2011). Nellore sheep showed no significant seasonal differences in T4 (48.39–53.42 ng/dL) and T3 (2.07–2.25 ng/dL) between summer and winter (Karthik et al., 2021). From early to late gestation, T3 decreased from 1.49 to 1.27 ng/mL and T4 from 38.74 to 37.92 ng/mL in pregnant ewes, whereas non-pregnant ewes had significantly higher T3 (1.81 ng/mL) and T4 (48.5 ng/mL) concentrations (Colodel et al., 2010). Reduced thyroid hormone levels, particularly T4, during hot summer months have been reported in male lambs, with cooling systems restoring blood concentrations (Koluman and Daskiran, 2011). In shaded versus unshaded sheep, blood thyroid hormone levels were higher in the unshaded group (Liu et al., 2012). The T3/T4 ratio is considered an indirect indicator of alterations in thyroid hormone metabolism because it reflects the relative balance between circulating T4 availability and its peripheral conversion to T3. This conversion is regulated by deiodinase enzymes, which play a central role in determining thyroid hormone bioavailability and tissue-specific responses.

Therefore, seasonal changes in the T3/T4 ratio may provide insights into adaptive modifications of thyroid hormone metabolism under environmental stress, although direct evaluation of deiodinase activity is required to confirm these mechanisms. To date, seasonal patterns of serum thyroid hormone concentrations in the Kermani sheep breed have not been characterized. Establishing the seasonal profile of thyroid hormones in this locally adapted breed may provide a better understanding of endocrine mechanisms associated with heat tolerance and adaptation. Such information could be valuable for developing appropriate management strategies and improving production efficiency of sheep raised in hot and semi-arid environments. Therefore, this study aimed to investigate thyroid hormone levels in Kermani sheep during summer and winter in the southern part of Kerman province (Jiroft).

Materials and Methods

Study Site and Animals

The study was conducted during summer and winter at the research station of the University of Jiroft, Iran (28°4′ N latitude, 57°4′ E longitude, 712 m altitude). Based on meteorological data, the average monthly Temperature-Humidity Index (THI) values for April, May, June, July, August, September, October, November, December, February, and March were 23.4, 26.4, 29.4, 31.6, 29.6, 28.0, 26.4, 19.7, 18.0, 14.0, and 13.8, respectively. According to Marai et al. (2007), a THI below 22.2 indicates no heat stress. These data indicate that sheep in this region experience heat stress from April through October (seven months), with no heat stress from November to March. Twenty-nine mature male lambs aged 11–13 months, weighing 29–35 kg, were selected for the study The same 29 animals were followed throughout the study and blood samples were collected from the same individuals during both summer and winter seasons. All animals received routine vaccinations (enterotoxemia, foot-and-mouth disease) and anthelmintic treatment (via drench) one month prior to the experiment. During the study period, sheep grazed on 270 hectares of land belonging to the university research station. They were housed in well-ventilated sheds with asbestos roofing (2.4 m height) and open sides, under proper hygienic conditions. Animals had ad libitum access to high-quality drinking water and salt blocks. All experimental procedures were conducted in accordance with the guidelines of the Iranian Council of Animal Care and were approved by the Animal Ethics Committee of the University of Jiroft.

Blood Sampling and Hormone Assays

Blood sampling was conducted from mid-June to mid-September (summer) and from December to March (winter). Sheep were held in a corral in the morning before grazing. Blood samples were collected in four sessions per month via jugular venipuncture using 7 mL evacuated blood collection tubes (Medical Polymer Co., Ltd., China). Samples for serum analysis were placed in an ice bath and immediately transported to the laboratory. Serum was separated by centrifugation at 3,000 × g for 15 minutes at 4°C and stored at −20°C until analysis. Thyroid hormone concentrations (total T3 and total T4) were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Monokit ELISA kits, Monobind Inc., USA) according to the manufacturer's instructions. The analytical sensitivity of the T3 assay was 0.1 ng/mL, with intra-assay and inter-assay coefficients of variation (CV) of 5.2% and 8.1%, respectively. For the T4 assay, the sensitivity was 0.2 μg/dL, with intra-assay and inter-assay CVs of 4.8% and 7.6%, respectively. All samples were analyzed in duplicate, and the mean values were used for statistical analysis.

Statistical Analysis

Data were analyzed using SAS software (Version 9.4, SAS Institute Inc., Cary, NC, USA). The normality of data distribution was assessed using the Univariate procedure. As the data were normally distributed, parametric tests (T test procedure) were employed. Differences in serum thyroid hormone concentrations (T3 and T4) and T3/T4 ratios between summer and winter seasons were compared using paired-sample t-tests, as the same animals were sampled in both seasons. Results are presented as mean ± standard error of the mean (SEM). Statistical significance was set at $P < 0.05$.

Results

Meteorological data and THI values are presented in Figure 1. According to THI thresholds, sheep were under heat stress for seven months of the year and free from heat stress for five months. Serum concentrations of both thyroid hormones were significantly lower in summer than in winter ($P < 0.001$ for T3; $P < 0.007$ for T4). Mean T3 levels were 0.62 ± 0.03 ng/mL in summer versus 2.23 ± 0.12 ng/mL in winter, representing a 3.6-fold increase in winter. Mean T4 levels were 1.5 ± 0.21 μg/dL in summer versus 9.37 ± 0.41 μg/dL in winter, a 6.2-fold increase in winter. In contrast, the calculated T3/T4 ratio was significantly higher in summer (0.3 ± 0.029) than in winter (0.02 ± 0.001, $P < 0.001$). These results are illustrated in Figure 2 and Figure 3.

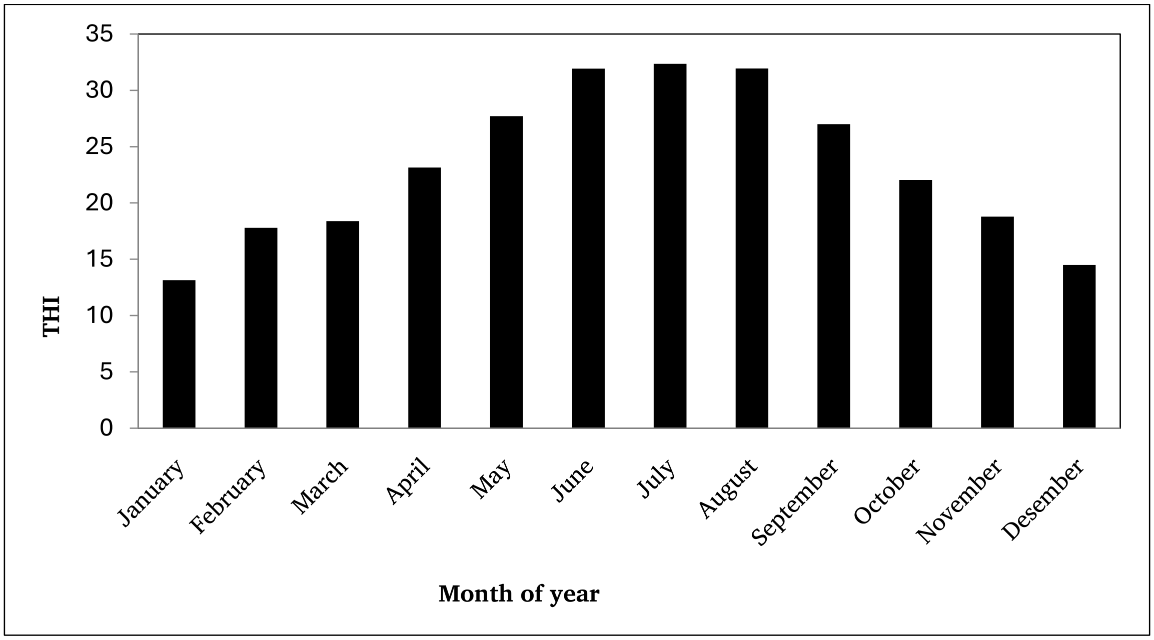

Figure 1. Monthly Temperature-Humidity Index (THI) values at the study location (Jiroft, Iran) throughout the year. The horizontal dashed line at THI = 22.2 indicates the heat stress threshold according to Marai et al. (2007), with values above this line representing heat stress conditions. Data indicate that sheep experienced heat stress from April through October (seven months) and were free from heat stress from November through March (five months). THI values were calculated using monthly temperature and relative humidity data obtained from the Jiroft Meteorological Station.

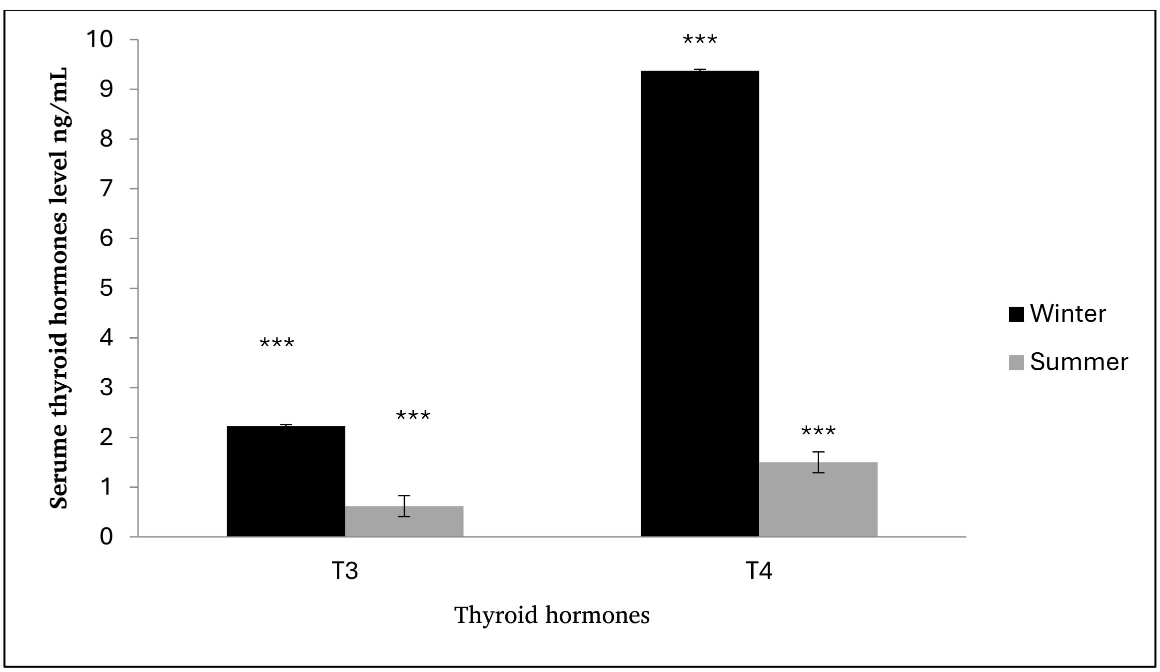

Figure 2. Serum thyroid hormone concentrations in Kermani sheep during summer and winter seasons. (A) Triiodothyronine (T3) concentration; (B) Thyroxine (T4) concentration. Data are presented as mean ± SEM (n = 29). ***$P < 0.001$ for T3; **$P < 0.01$ for T4,

comparing summer vs. winter using paired t-tests. Both T3 and T4 concentrations were significantly lower during summer compared to winter, indicating seasonal modulation of thyroid hormone levels in response to heat stress.

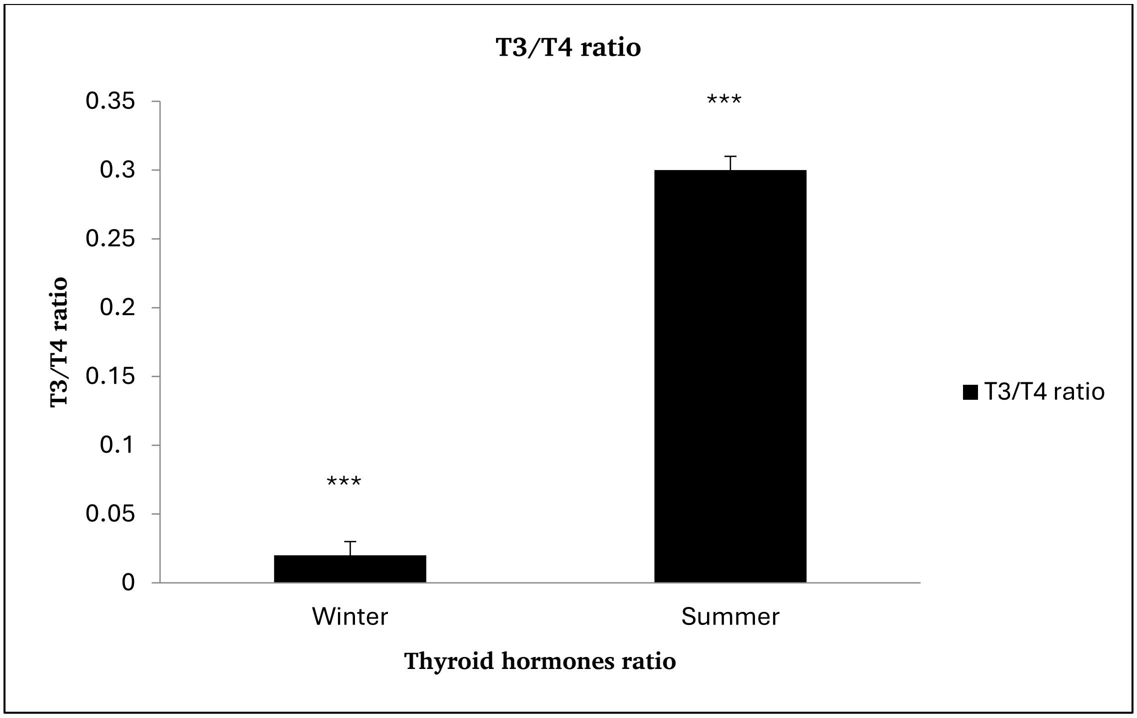

Figure 3. T3/T4 ratio in Kermani sheep during summer and winter seasons. Data are presented as mean ± SEM (n = 29). ***$P < 0.001$, comparing summer vs. winter using paired t-tests. The T3/T4 ratio was significantly higher during summer compared to winter, suggesting possible alterations in peripheral thyroid hormone metabolism and enhanced conversion of T4 to T3 under heat stress conditions.

Discussion

The present study demonstrated significant seasonal variations in circulating thyroid hormone concentrations in Kermani sheep, with markedly lower T3 and T4 levels during summer compared to winter. These findings are consistent with previous reports in various sheep breeds and other livestock species exposed to heat stress conditions (Koluman and Daskiran, 2011; Liu et al., 2012; Marai et al., 1995). The observed reduction in thyroid hormone levels during summer likely represents an adaptive thermoregulatory response aimed at decreasing basal metabolic rate and endogenous heat production, thereby facilitating heat dissipation and maintaining thermal homeostasis (Sevi and Caroprese, 2012).

Changes in thyroid hormone levels under thermal stress may involve alterations in deiodinase activity and other regulatory mechanisms. Deiodinases regulate thyroid hormone signaling by controlling the conversion of T4 to active T3 and the inactivation pathways involving T2 and reverse T3 (rT3). Although changes in these pathways may contribute to seasonal adaptation, direct measurements of rT3 concentration and deiodinase activity are required to confirm their involvement in Kermani sheep under heat stress conditions. Hypothyroidism can result from nutritional, environmental, or psychological changes. Reduced thyroid hormone levels in sheep have been reported following rapeseed consumption (Mandiki et al., 1999), attributed to glucosinolates and their derivatives, particularly goitrin, which inhibit thyroid hormone biosynthesis. Given the crucial role of thyroid hormones in thermoregulation and metabolic adjustments, even short-term temperature fluctuations can impact these endocrine pathways and their responsiveness to acute stressors.

Hot summer conditions exert a rapid and profound effect on thyroid hormone concentrations. Our findings are consistent with previous reports of declining T4 levels in male lambs during hot summers, with cooling systems reversing this decline (Koluman and Daskiran, 2011). Similarly, unshaded sheep had higher thyroid hormone levels than shaded ones (Liu et al., 2012). Changes in thyroid hormone levels under thermal and psychological stress are primarily mediated by alterations in deiodinase activity. Deiodinases modify the biological activity of thyroid hormones by converting T4 to active T3 or inactivating T3 to T2 and T4 to reverse T3 (rT3). These enzymes not only regulate circulating T3 levels but also provide dynamic control of thyroid hormone signaling. Among the three deiodinase types, type 3 deiodinase (DIO3) is responsible for converting T4 to rT3, which reduces T3 signaling and metabolic rate (Russo et al., 2021). A short period of severe hyperthermia has been shown to produce a fivefold rise in rT3 and a halving of T3 levels (Konits et al., 1984). The most notable finding of this study was the significantly higher T3/T4 ratio during summer despite lower absolute T3 and T4 concentrations. This observation suggests potential alterations in peripheral thyroid hormone metabolism, possibly involving enhanced conversion of T4 to the more biologically active T3 or increased production of reverse T3 (rT3).

However, it is important to note that the T3/T4 ratio is influenced by multiple factors, including hormone synthesis, secretion, peripheral conversion, and clearance rates. Therefore, the observed increase in this ratio should be interpreted as an indirect indication of altered thyroid hormone dynamics rather than definitive evidence of enhanced T4-to-T3 conversion. Interestingly, reverse T3 has been reported to exert protective effects against cerebral ischemia injury in both in vitro and in vivo models (Rastogi et al., 2018). It likely exerts negative feedback on the hypothalamic-pituitary-thyroid axis without mediating other T3 functions, thereby reducing circulating T4 levels. The brain expresses relatively high levels of DIO3, which enhances local T4 inactivation via rT3 production (Bianco et al., 2019).

Previous studies have reported that short-term heat stress in steers significantly decreased T4 and T3 levels while increasing the T3/T4 ratio, with rT3 levels remaining unchanged (Kahl et al., 2015). Similarly, in cattle adapted to long-term heat conditions, plasma T3 reduction was associated with decreased thyroid activity and downregulation of DIO1 and DIO2, accompanied by upregulation of DIO3 (Pereira et al., 2008). In agreement with these findings, our results showed a significantly higher T3/T4 ratio during summer despite lower absolute T3 and T4 levels, suggesting enhanced rT3 synthesis. Similar patterns have been observed in heat-stressed female goats, where T4 decreased but T3 remained unchanged (Sharma et al., 2013), and in Friesian calves, where summer thyroid hormone levels were significantly lower than in winter (Marai et al., 1995). In agreement with previous studies, our results showed a significantly higher T3/T4 ratio during summer despite lower absolute T3 and T4 concentrations. This finding may indicate alterations in peripheral thyroid hormone metabolism; however, the T3/T4 ratio is influenced by multiple factors, including hormone synthesis, conversion, and clearance. Therefore, the observed increase in this ratio should be interpreted as a possible indication of altered thyroid hormone dynamics rather than direct evidence of enhanced T4-to-T3 conversion or increased rT3 production.

Several limitations of this study should be acknowledged. First, we did not measure rT3 concentrations or deiodinase enzyme activities, which would have provided direct evidence for the proposed mechanisms. Second, we did not assess other physiological parameters such as rectal temperature, respiratory rate, or heart rate, which could have provided additional context for interpreting the endocrine responses. Third, the study was conducted on male lambs only; sex and age-related differences in thyroid hormone responses to heat stress warrant further investigation. Fourth, while we observed significant seasonal differences, the relatively small sample size ($n = 29$) may limit the generalizability of our findings. Despite these limitations, our findings contribute to the understanding of endocrine adaptations in sheep breeds indigenous to hot and semi-arid environments. The observed seasonal modulation of thyroid hormone dynamics in Kermani sheep likely represents an adaptive thermoregulatory strategy that facilitates survival under prolonged heat stress conditions. These results may have practical implications for developing management strategies to improve productivity and welfare of sheep in similar climatic regions.

Conclusion

In summary, these results demonstrate a significant effect of hot summer conditions on circulating thyroid hormone levels in Kermani sheep. The observed changes may be associated with alterations in thyroid hormone regulation and metabolism under seasonal thermal stress. Seasonal modulation of thyroid hormone profiles may contribute to the thermoregulatory adaptation of this breed; however, further studies are required to clarify the underlying mechanisms

Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this manuscript. No financial or personal relationships with other people or organizations have inappropriately influenced this work.

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