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Blood serum triiodothyronine in Karagouniko and Chios rams as affected by season in a temperate environment

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Abstract The current research investigated triiodothyronine concentrations in Karagouniko and Chios rams in a temperate climate zone, considering the periods January-June and July-November, characterized by a gradual increase and decrease of daylight length, respectively. The animals raised in the farm of the Artificial Insemination Center of Karditsa ($39^0$21′18′′N, $21^0$54′19′′E), Greece, and the meteorological variables air temperature, relative humidity, and sunshine, as well as photoperiod, were considered for a more detailed analysis. T-tests were used to detect possible changes in the concentration of triiodothyronine in both sheep breeds and in the abovementioned meteorological variables. January-June coincided with significantly higher ($P < 0.05$) triiodothyronine concentrations in Karagouniko and Chios rams (0.82 ng/ml and 0.77 ng/ml, respectively) compared to July-November (0.72 ng/ml in Karagouniko and 0.67 ng/ml in Chios rams). Air temperature and sunshine followed the opposite pattern ($P < 0.05$). Karagouniko rams showed higher triiodothyronine concentrations than Chios rams, irrespective of the season. Our findings shed light on an important aspect of the thyroid gland function in Karagouniko and Chios sheep under temperate environmental conditions.

Keywords: air temperature, daylight length, sheep, sunshine, thyroid hormone

1. Introduction

Triiodothyronine concentrations in sheep significantly impact growth and development (Eswari et al 2001), and are influenced by photoperiod (PHOTO) in a quantitative manner (Dufourny et al 2016), likely contributing to the seasonal regulation of gonadotropin-releasing hormone and the transition from estrus to anestrus (Kantas et al 2008a).

Daylight length has been shown to positively affect triiodothyronine concentration in sheep (Gündoğan 2007; Kantas et al 2008a), but studies have reported lower triiodothyronine concentrations with higher air temperatures (AIRT) (Altin et al 2018; Silva et al 2019). According to Altin et al (2018) and Matsoukis (2022), the impact of high AIRT is associated with relative humidity (RH), indicating that RH may affect triiodothyronine concentration in sheep. The same may be true for sunshine (SUNS), which has been shown to have a close relationship with AIRT (Van den Besselaar et al 2015; Matuszko et al 2022).

Despite studies on various morphological and physiological parameters in popular Greek sheep breeds Karagouniko and Chios (Kantas et al 2008b; Matsoukis et al 2019; 2022ab; 2023), little is known about their triiodothyronine concentration in relation to environmental factors (Gündoğan 2007; Kantas et al 2008a), based on a thorough search of the available literature in English. Therefore, this study aimed to investigate triiodothyronine concentrations in Karagouniko and Chios rams, considering the critical environmental parameters PHOTO, AIRT, RH, and SUNS.

2. Materials and Methods

The experimental material consisted of six rams from the Karagouniko sheep breed (*Ovis aries* L.) and six rams from the Chios sheep breed, which were raised on a farm located in Karditsa Municipality, Greece, from January to November (Matsoukis et al 2023). The warm temperate climate of the surrounding area was characterized by Yassoglou et al (2017). Blood was collected from the jugular vein of each ram once a month, and the obtained serum was frozen at -20°C until the assay of the hormone triiodothyronine. The radioimmunoassay (RIA) method using the commercial T_3 [I-125] RIA kit (Izotop, Institute of Isotopes Co. Ltd. Budapest, Hungary) with an assay range from 0.0 nmol/l to 12.0 nmol/l was employed. The competition between unlabelled T_3 and a fixed quantity of 125I-labelled T_3 for a limited number of binding sites on T_3-specific antibodies formed the basis of the assay. The amount of tracer bound by the antibody was inversely proportional to the concentration of unlabelled ligand (IZOTOP 2023). The magnetic immunosorbent (IS) was added to the antigen-antibody complex, which binds to the solid particles and is then separated by magnetic precipitation. The radioactivity in the solid phase allowed for the construction of a standard curve and quantitative determination of samples. Polystyrene tubes were used to add 100 µl of standards, control serum, and samples,

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followed by 100 µl of the tracer and 500 µl of the antiserum to each tube. The tubes were incubated at room temperature for two hours, and 500 µl of IS containing paramagnetic particles buffered with 0.1% NaN_3 was added. After incubation for 15 min at room temperature, magnetic separation occurred with the aid of special magnetic bases. The IS particles were allowed to settle for 10 min, and the supernatant was removed by suction. The radioactivity of the tubes was measured in a gamma counter (1470 Wizard, PerkinElmer, Turku, Finland) for 60 sec, and T_3 concentrations were determined by reducing concentrations from the standard curve with a sensitivity of 0.06 ng/ml (Stratakos 2015).

The seasons of January-June and July-November were considered to detect a potential association between the surroundings and the triiodothyronine concentration in both breeds based on the progressive increase and decrease of daylight length in the experimental period. A more comprehensive analysis was performed for each season using the common meteorological variables AIRT, RH, SUNS, and PHOTO. The triiodothyronine data were pooled into the two seasons mentioned above. Matsoukis et al. (2019; 2022ab; 2023) provide further information about the experimental site, material, and environmental data.

Paired t-tests (Ross and Willson 2017; Rietmann et al 2023) were applied to investigate possible differences in triiodothyronine for each breed between January-June and July-November. Independent t-tests (Kim 2015; Myassar and Bani 2022) were used to detect potential differences in the studied hormone between the two sheep breeds. Statistical analysis of meteorological data followed that implemented in a recent authors' work (Matsoukis et al 2023), and MS Excel 2010 and IBM SPSS Statistics 23 were employed for executing Statistics (Kamoutsis et al 2018) with significance set at $P \leq 0.05$.

3. Results and Discussion

The mean seasonal PHOTOs were found to be substantially the same for January-June and July-November (12.47 h and 12.37 h, respectively). Mean AIRT and SUNS for January-June were observed to be lower by 6.68 °C and 1.62 h, respectively, as compared to their respective values for July-November (20.86 °C and 7.27 h). These differences were found to be significant ($P < 0.05$). However, relative humidity exhibited negligible and non-significant differences ($P > 0.05$) between the two examined subperiods.

Higher values of triiodothyronine were confirmed for Karagouniko ($P = 0.035$) and Chios sheep ($P = 0.006$) in January-June (0.82 ng/ml and 0.77 ng/ml, respectively) as compared to their respective values in July-November (0.72 ng/ml and 0.67 ng/ml, respectively; Figure 1). The increase in daylight length in January-June was found to have an impact on the higher hormone values, and vice versa, contrary to PHOTO, which showed a trivial change between the two subperiods. The inappreciable fluctuations in the body weight of the experimental rams supported this hypothesis. These results on the change of triiodothyronine concentration in relation to the examined daylight length pattern agreed with the results of Kantas et al (2008a) for Karagouniko ewes and contradicted those reported by Gündoğan (2007) for Chios rams. The experimental animals in the current study were raised in the same region (Thessaly) as those in Kantas et al (2008a).

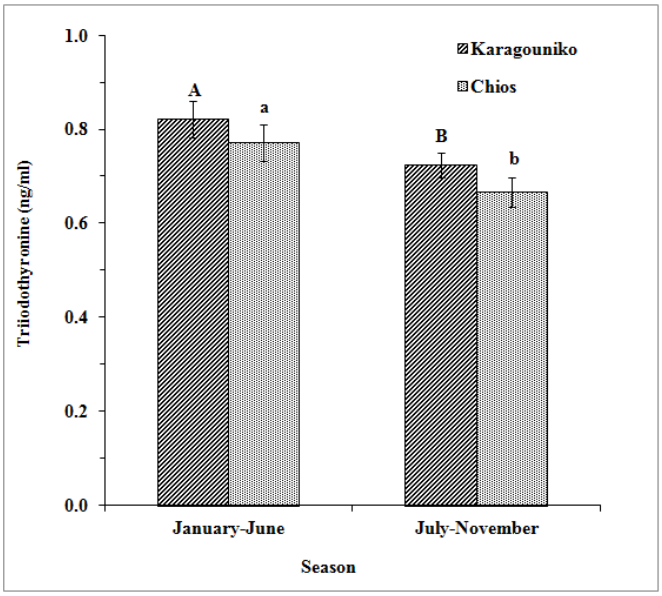

Figure 1 Triiodothyronine concentration of Karagouniko and Chios rams concerning season. In each column, the bar represents the standard error of the mean. Significant differences between the respective means, separately for Karagouniko sheep (upper case letters) and Chios sheep (lower case letters) at $P \leq 0.05$, following paired t-tests, were denoted by different letters above columns.

The influence of meteorological parameters on the concentrations of triiodothyronine in experimental rams was found to be significant, as indicated by the coincident higher values of AIRT and SUNS and lower values of triiodothyronine observed in the months of July to November, in contrast to the months of January to June. This finding is supported by previous studies on other sheep breeds by Starling et al (2005) and Todini (2007). However, the observed association cannot be applied to RH, which remained unchanged. The negative association between AIRT and triiodothyronine in this study is consistent with the results of Starling et al (2005) and Todini (2007), but in contrast to the findings of Gündoğan (2007), who reported significantly higher serum triiodothyronine in the summer and autumn in Chios rams. It should be noted that the experimental Karagouniko and Chios rams were raised in different environments compared to the Chios rams in Gündoğan's study. There is currently no literature on the relationship between triiodothyronine and RH or SUNS in sheep, but given the relationship between these meteorological parameters, further investigation is warranted. It has been reported that SUNS and triiodothyronine show a negative correlation in male llamas (*Lama glama* L.) (Gauly et al 1997).

In this study, the triiodothyronine concentrations did not significantly differ between the Karagouniko and Chios rams in January to June ($P = 0.364$), July to November ($P =$

0.172), or the whole period (P = 0.129). However, the triiodothyronine concentration in Karagouniko was numerically higher than that of Chios, possibly due to differences in genetic potential (Matsoukis et al 2023). Karagouniko ewes have been reported to have lower triiodothyronine concentrations (Kantas et al 2008a), while Chios rams have been reported to have higher concentrations (Gündoğan 2007). Gender-related differences in triiodothyronine have also been reported in various mammalian species (Waner and Nyska 1988; Celi et al 2003).

This study sheds light on the effect of the environment on the concentrations of triiodothyronine in Karagouniko and Chios rams. It is a crucial step in understanding the relationship between the physiology of these sheep breeds and their environment, which can aid in the development of better exploitation programs. The findings of this study can also serve as a basis for further investigation of the relationship between triiodothyronine and meteorological parameters in sheep.

4. Conclusions

Higher concentrations of triiodothyronine were observed in Karagouniko and Chios rams during the period of increasing daylight length (January-June) compared to the period of decreasing daylight length (July-November). It was noted that there existed an inverse relationship between triiodothyronine levels and air temperature, including sunshine. Additionally, the study showed that Karagouniko rams exhibited higher concentrations of triiodothyronine than Chios rams.

Ethical considerations

The research met all the requirements demanded by the Ethical Committee of the Artificial Insemination Center of Karditsa, Greece.

Conflict of Interest

The authors have no conflicts of interest to declare.

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