

Seasonal variation of thyroxine concentration in Karagouniko and Chios rams in temperate environmental conditions



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Abstract It is well known that the concentration of the thyroid hormone thyroxine increases as day length increases, and the other way around, in Karagouniko and Chios ewes but based on openly international English literature, nothing is known about this hormone on the rams of the breeds mentioned above concerning a meticulous analysis of their aerial environment. So from this perspective, the current research was conducted to investigate the thyroxine concentrations of Karagouniko and Chios rams, taking into account two periods, January-June and July-November, when the day length was increasing and decreasing, respectively. Apart from photoperiod, the meteorological variables air temperature, relative humidity, and sunshine were taken into consideration in the farm of the Artificial Insemination Center of Karditsa (39°21'18''N, 21°54'19''E), Greece (temperate climate zone), where the experimental animals raised. The possible change of thyroxine concentration in both sheep breeds and the possible changes of the abovementioned meteorological variables between the examined periods were detected using t-tests. Higher thyroxine values ($P < 0.05$) were confirmed in January-June (55.11 ng/ml in Karagouniko and 47.72 ng/ml in Chios rams), in comparison to July-November (49.03 ng/ml in Karagouniko and 44.14 ng/ml in Chios rams). Air temperature and sunshine followed a reverse course ($P < 0.05$). In both periods, Karagouniko rams were characterized by higher concentrations of thyroxine than Chios rams ($P < 0.01$). Our results add more insight into the physiology of Karagouniko and Chios sheep related to thyroxine and temperate aerial environment.

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

1. Introduction

Thyroxine, the main secretory product of the thyroid gland in sheep (Chopra et al 1975; Todini 2007), is a noticeable indicator of its metabolic and nutritional status (Todini et al 2007) and plays an integral role in its physiology and seasonality of reproduction (Whitlock et al 2012). Thyroxine has been reported to facilitate the transition into anestrus (Thrun et al 1996; 1997) and early ovulation (Menegatos et al 2008).

Day length, that is, photoperiod (PHOTO), affects the concentration of thyroxine in sheep (Zamiri and Khodaei 2005; Menegatos et al 2006; Whitlock et al 2012) with a positive association between them (Menegatos et al 2006; Kantas et al 2008b). An inverse association, however, between thyroxine and air temperature (AIRT) has been reported in this animal species (Todini 2007; Whitlock et al 2012). Also, the relative humidity (RH), which is in close relation with AIRT (Matsoukis 2022), and solar radiation seem to have an impact on the thyroxine concentration of sheep (Leite et al 2018). The same suggestion can be accordingly made for sunshine (SUNS) which has a strong positive relationship with solar radiation (Paltineanu et al 2002).

Karagouniko and Chios sheep breeds are considered two of Greece's most popular indigenous sheep breeds (Triantaphyllopoulos et al 2017), a country in the temperate continental climate zone (Hellenic National Meteorological Service 2022). Thyroxine concentrations have been studied in the ewes of the sheep mentioned above breeds concerning day length (Kantas et al 2008b) and in the rams of the Chios breed about the season (Oikonomidis et al 2019). However, based on the international literature written in the English language (openly accessible published scientific books and articles in peer-reviewed scientific journals), nothing is known about the concentrations of thyroxine in Karagouniko and Chios rams concerning a meticulous analysis of their aerial environment. This information could be valuable, considering the importance of these sheep breeds in farming systems (Matsoukis et al 2022b). Therefore, the current work investigates the thyroxine concentrations of Karagouniko and Chios rams, considering critical parameters of their aerial environment, that is, PHOTO, AIRT, RH, and SUNS.

2. Materials and Methods

The experimental procedure took place in a farm (39°21'18''N, 21°54'19''E) of Karditsa Municipality, Greece,



from January to November. A warm temperate climate characterizes this region (Yassoglou et al 2017). Karagouniko and Chios sheep breeds (*Ovis aries* L.) were the experimental material, with six rams for each breed included.

Blood samples from the jugular vein of each ram were collected in tubes once a month, except in November, when blood collection took place twice, and placed in a cooler. After the transportation of the blood to the laboratory, where it was centrifuged, the serum obtained was frozen at -20 °C until the assay of the thyroxine. The chosen method for this purpose was the radioimmunoassay (RIA) method using the commercial T4 [I-125] RIA kit (Izotop, Institute of Isotopes Co. Ltd. Budapest, Hungary) with an assay range from 0.0 nmol/l to 320.0 nmol/l and sensitivity better than 7.0 nmol/l (IZOTOP 2019).

The possible impact of the aerial environment on the thyroxine concentration in both sheep breeds, taking primarily into account the increase and decrease of day length, led us to consider two seasons; January-June and July-November when, in general, day length increased and decreased, respectively (Menegatos et al 2006). In each season, the common meteorological variables AIRT, RH, and SUNS were considered for a more detailed analysis. Apart from estimating averages of thyroxine for each breed concerning the examined period, the available data (from animals and the environment) were pooled into the two seasons mentioned above. Matsoukis et al (2019; 2022a, b) have provided further details on the experimental site, material, and environmental data.

T-tests are widely used statistical tools for significant differences concerning various variables (Jenkins et al 2006; Matsoukis et al 2018; Yunerati Beyleto et al 2022). In this regard, also taking into account the nature of our examined variables, unpaired two-sample t-tests for autocorrelated data (Wilks 2019) and paired t-tests (Ross and Willson 2017) were applied for the detection of possible differences of each meteorological variable and thyroxine concentration (separately for each breed), respectively, between the investigated two seasons. In addition, after meeting the necessary assumptions, independent t-tests were used to find possible differences of thyroxine between the two sheep breeds (Kim 2015). Statistics was executed with the aid of MS Excel 2010 and IBM SPSS Statistics 23 (Kamoutsis et al 2018), with results to be considered significant at $P \leq 0.05$.

3. Results and Discussion

The mean seasonal PHOTOs for the examined seasons January-June and July-November were very close, 12.47 h and 12.37 h, respectively. Regarding the other meteorological variables, AIRT and SUNS took significantly higher values ($P < 0.05$) in July-November (20.86 °C and 7.27 h, respectively) compared to January-June (14.18 °C and 5.65 h), while in the case of RH, where the values of the respective means were approximately equal, around 70%, no significant differences ($P > 0.05$) confirmed (Figure 1).

The increase in day length in January-June coincided with significantly higher thyroxine values ($P < 0.05$) in both

sheep breeds (55.11 ng/ml in Karagouniko rams and 47.72 ng/ml in Chios rams), in comparison to July-November (49.03 ng/ml in Karagouniko and 44.14 ng/ml in Chios rams), when daylength decreased (Figure 2). The different direction of change of day length between January-June and July-November explains the different thyroxine concentrations in both sheep breeds to some degree. This hypothesis is strengthened because PHOTO remained practically unchanged for the two experimental periods (in average terms), combined with the statistically different values of AIRT and SUNS between these periods. Also, the negligible fluctuations of the body weight of the experimental rams (Matsoukis et al 2022b) support the hypothesis mentioned above, bearing in mind that a positive relation has been reported between thyroxine concentration and body weight in sheep (Hashem 2014; Veena et al 2018; Aljubouri et al 2021). The thyroxine pattern concerning the direction of day length change in our experiment is in agreement with the results of other researchers for Karagouniko ewes (Kantas et al 2008a; Menegatos et al 1994), Chios ewes (Kantas et al 2008b) as well as Karagouniko × Mytilene ewes (Menegatos et al 2006).

The significant increases of AIRT and SUNS, combined with the significant decreases of thyroxine in the same period, July-November, in comparison to January-June, led us to the assumption that the impact of the abovementioned meteorological variables on thyroxine concentration in both Karagouniko and Chios rams is noteworthy, contrary to RH which remained practically the same. A similar inverse relationship between thyroxine in sheep and AIRT has been reported by Valtorta et al (1982), Webster et al (1991), Todini (2007), Hashem (2014), Leite et al (2018) and Pehlivan et al (2020). In addition, Leite et al (2018) reported that Morada Nova ewes showed lower concentrations of thyroxine resulting from lower RH values, while, to the best of our knowledge, there is no relevant literature on a possible relation between SUNS and thyroxine in sheep, an issue which needs further investigation. However, a negative correlation has been reported between thyroxine in male llamas (*Lama glama* L.) and SUNS (Gauly et al 1997).

In both January-June and July-November periods, the application of independent t-tests showed that the rams of Karagouniko were characterized by significantly higher concentrations of thyroxine (by 7.39 ng/ml and 4.89 ng/ml, respectively) compared to Chios rams ($P < 0.01$). The same was confirmed after pooling the thyroxine data over the whole examined period for each breed separately (52.07 ng/ml in Karagouniko and 45.93 ng/ml in Chios rams). The difference in thyroxine concentration between Karagouniko and Chios rams may be attributed to their different genetic potential (Matsoukis et al 2022b). The abovementioned thyroxine pattern aligns with the results of Kantas et al (2008b), who reported higher thyroxine concentration in Karagouniko ewes than in Chios. In addition, based on our current results, lower thyroxine concentrations were found for Karagouniko and Chios rams compared to the respective concentrations in the ewes of these breeds reported by the

researchers mentioned above. Osorio et al (2017) found higher concentrations of thyroxine in male sheep concerning female ones, contrary to Todini et al (1992), who published

lower thyroxine concentrations in male goats (*Capra hircus* L.) compared to female goats.

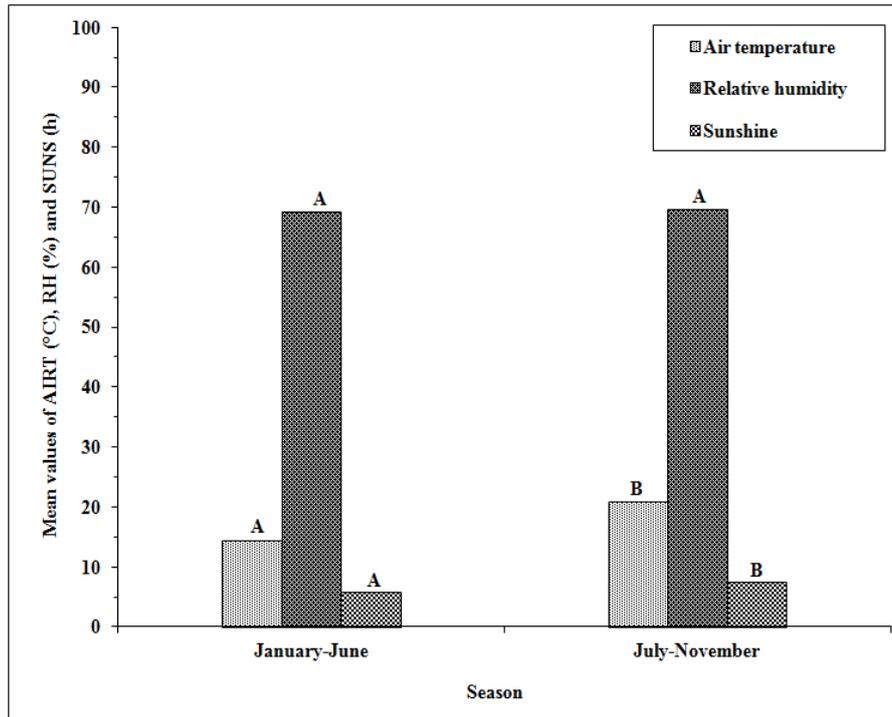


Figure 1 Mean seasonal values of air temperature (AIRT), relative humidity (RH), and sunshine (SUNS) in the experimental area. Different letters above columns indicate significant differences between the respective means, separately for AIRT, RH, and SUNS at $P \leq 0.05$ following unpaired two-sample t-tests for autocorrelated data.

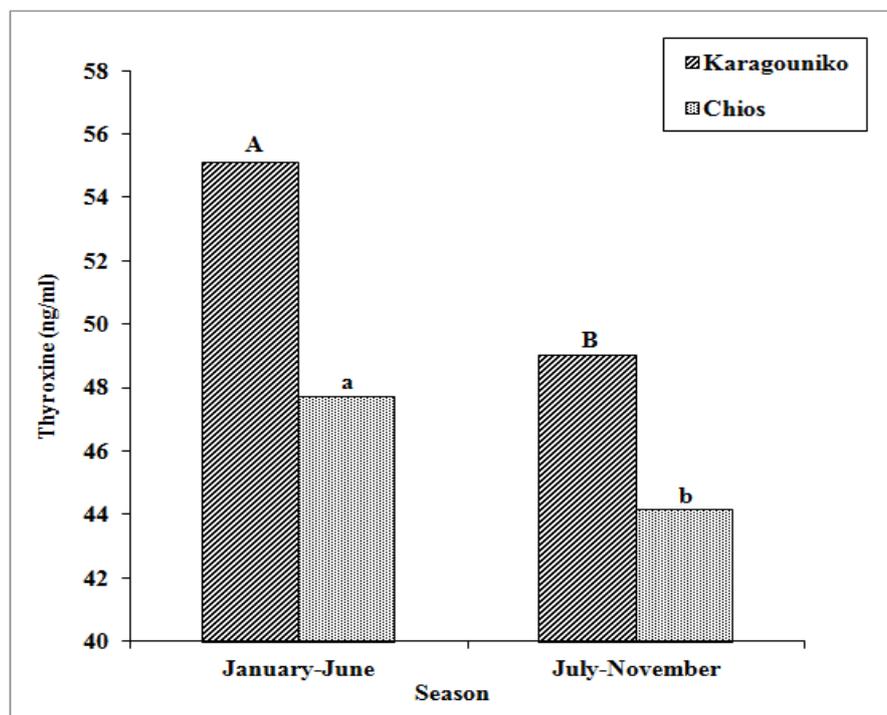


Figure 2 Effect of season on thyroxine concentration of Karagouniko and Chios rams. Different letters above columns indicate significant differences between the respective means, separately for Karagouniko sheep (upper case letters) and Chios sheep (lower case letters) at $P \leq 0.05$ following paired t-test.

In the current work, a first approach was made to elucidate the impact of the environment on the concentrations of thyroxine in male sheep of Karagouniko and Chios breeds in as much detail as possible. Our findings could be used for a more successful breeding scheme for these breeds.

4. Conclusions

Higher thyroxine concentrations in Karagouniko and Chios rams were confirmed in January-June, when day length was increasing, compared to July-November, when day length was decreasing. On the other hand, air temperature and sunshine took lower values in January-June compared to July-November, inversely impacting thyroxine. In both periods mentioned above, Karagouniko rams exhibited higher thyroxine concentrations than Chios.

Ethical considerations

The Ethical Committee of the Artificial Insemination Center of Karditsa, Greece, approved the study protocol.

Conflict of Interest

The authors declare that there is no conflict of interest.

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