JOURNAL OF ANIMAL BEHAVIOUR AND BIOMETEOROLOGY

RESEARCH ARTICLE
Published Online: April 11, 2022
https://doi.org/10.31893/jabb.22020

Scrotal circumference in Karagouniko and Chios sheep in relation to the aerial environment

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Abstract Local breeds are a valuable asset in sheep farming systems in Greece and other Mediterranean countries. Among these, Karagouniko and Chios sheep hold a special position, participating in numerous genetic improvement programs, but a research gap exists on their scrotal circumference (SC) in relation to the environmental parameters photoperiod (PHOTO), air temperature, sunshine and rainfall. Therefore, four seasons were examined: April-June and July-September (Long days, increasing and decreasing photoperiod length, respectively), and October-December and January-March (Short days, decreasing and increasing photoperiod length, respectively). Repeated measures analysis of variance and linear correlation analysis were used to investigate the effect of the period on SC and the relationships of SC and each of the environmental parameters above, respectively, in both breeds. Also, independent t-tests were used to investigate SC differences between Karagouniko and Chios. A progressive increase of SC in both breeds coincided with the lowering of PHOTO from April-June to October-December. A significant correlation was confirmed between the SC of each breed and PHOTO, shedding light on the importance of this parameter on SC. Chios sheep had significantly larger ($P < 0.05$) SC (35.23 cm) than the Karagouniko one (34.59 cm). Better exploitation of these sheep breeds could be derived from the current study's findings.

Keywords: air temperature, photoperiod, ram, repeated measures

1. Introduction

One of the main characteristics of sheep originating from temperate climate regions is the seasonality of its reproduction (Gordon 1997; Rosa and Bryant 2003; Sarlós et al 2013; Carvajal-Serna et al 2019). This seasonality is detected through various behavioural, physiological and visible changes in ewes and rams (Sarlós et al 2013; Kulaksiz and Cebi Sen 2019). A visible change of seasonality in rams is reflected in the changes in their scrotal circumference (SC) to a greater or lesser extent (Oláh et al 2013; Al-Anazi et al 2017; Belkadi et al 2017). Scrotal circumference, in other words, width of the testicles at the widest point (Schoenian 2021), has been reported to be a good indicator of the ram's breeding ability (Toe et al. 2000; Moghaddam et al 2012; Milczewski et al 2015; Maksimović et al 2016). It also seems to provide a bigger scope for genetic improvement of fertility in ram lambs than does any semen characteristics (Rege et al 2000), considering its high heritability (Toe et al 2000).

In temperate climate regions, the natural photoperiod (PHOTO) is the decisive factor of the seasonal reproduction of sheep (Sarlós et al 2013; Carvajal-Serna et al 2019), thus impacting ram SC (Kafi et al 2004; Al-Anazi et al 2017; Ojeda Fermoselle et al 2021). In general, shorter PHOTO coincides with larger ram SC (Tulley and Burfening 1983; Kafi et al 2004; Budai et al 2013), closely related to estrus ewes (Abecia et al 2020). Nevertheless, other environmental parameters have been reported to potentially influence the onset and the duration of sheep anoestrous period (Rosa and Bryant 2003). Đuričić et al (2022) have reported that the common environmental parameters of air temperature (AIRT), rainfall (RNFL) and sunshine (SUNS) could impact the reproductive performance of Romanov sheep in a moderate climate. In addition, Gündoğan (2007) had reported improved reproductive performance in Daglic and Chios rams in Autumn (higher values of various sexual parameters and testosterone) when a decrease in daylight length, AIRT and RNFL took place (Afyonkarahisar province, Turkey).

Chios and Karagouniko breeds are promising sheep breeds of Mediterranean countries, especially Greece (Matsoukis et al 2022), a country that belongs to the temperate continental climate zone of the northern hemisphere (Argiriou 2022). The reproductive physiology of the breeds above is of particular interest, taking into account their participation in many genetic improvement programs (Gelasakis et al 2013; Zygoyiannis 2014; Yardibi et al 2015).

However, to the best of our knowledge, there is a gap in the literature concerning the possible seasonal variation of SC in the breeds above, considering their aerial environment in detail. Thus, our current work tests the hypothesis of the response of Karagouniko and Chios SC to seasonal changes in their aerial environment, investigating, in parallel, the possible associations of SC with PHOTO, AIRT, SUNS and RNFL.

 Received: March 3, 2022 | Accepted: April 8, 2022

2. Materials and Methods

Animal care and handling procedures followed the guidelines of the Ethics Committee of Artificial Insemination Center of Karditsa on the Use of Animals in Experiments.

The research was carried out on the farm of the Artificial Insemination Center of Karditsa Municipality, Greece, from April to March. Karditsa is characterized by a warm temperate climate (Yassoglou et al 2017) with more RNFL in the winter than in the summer and Csa climate type according to Köppen-Geiger climate classification (Anonymous 2022). The experimental material included six rams (*Ovis aries* L.) of the Karagouniko sheep breed and another six rams of the Chios sheep breed.

Starting from May, the SC of each ram was measured once a month (on its first day), except for March (the last month of the experiment), where the measurement took place twice (on its first and last days). For the measurement of SC, a Coulter Scrotal Tape (Trueman Manufacturing, Edmonton, AB, Canada) at the greatest diameter point of the scrotum was used (Dance et al 2015; Bossois Moura et al 2019) after grasping the neck of the scrotum and firmly pulling the testes down into the scrotum (Chase et al 2017).

To investigate the impact of the aerial environment on sheep SC, four seasons were taken into account based on the temporal shift in PHOTO, that is, April-June (LD1, increasing photoperiod length), July-September (LD2, decreasing photoperiod length), October-December (SD3, decreasing photoperiod length) and January-March (SD4, increasing photoperiod length). The mean seasonal values of PHOTO for LD1, LD2, SD3 and SD4 were 14.06 h, 13.51 h [long days (LD), >12 h daylight], 10.31 h and 10.89 h [short days (SD), <12 h daylight], respectively. Also, apart from PHOTO, AIRT, SUNS and RNFL were considered. Air temperature and SUNS followed a similar pattern. July-September and January-March were the warmest (26.4 ^{o}C) and coolest (7.1 ^{o}C) periods, respectively. The sunniest periods were April-June and July-September (highest SUNS duration, 8.8 h), while the wettest periods were January-March and October-December (highest total RNFL, 248.8 mm). Further details on the experimental site, experimental material, collection and processing of environmental data have been reported in Matsoukis et al (2019; 2022). Regarding SC data, averages on a monthly and seasonal basis were estimated after their pooling into LD1, LD2, SD3 and SD4 periods. To prevent undue influence of extreme values, the two (out of eighteen) extreme values of SC for each breed and period were excluded from the data before the analysis (Feldman et al 2002).

The experiment was conducted according to the one-way repeated measures design, separately for each sheep breed, considering the examined four time periods (LD1, LD2, SD3 and SD4). Paired t-tests were used for the pairwise comparison of means by using the Bonferroni correction (Verma 2016).

A linear correlation (Pearson's) analysis (Matsoukis et al 2018; Ladyka et al 2020) was conducted to detect possible relationships between the SC of each examined sheep breed and each of the studied environmental parameters using monthly averages for the whole period. Also, to investigate possible differences in SC between the two sheep breeds, independent t-tests were applied (Kim 2015). Statistics were performed using 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

Significant differences in SC of Karagouniko and Chios sheep were confirmed using the repeated measures analysis of variance (Table 1).

Scrotal circumference significantly increased in July-September, compared to April-June, for both sheep breeds. A significant increase in SC continued to exist in October-December, compared to the two preceding periods, with a decline afterwards (Figure 1). The minimum and maximum values of SC for both breeds appeared in April-June and October-December, respectively. Regarding the maximum values mentioned above, their increases for Karagouniko and Chios were 9.1% and 6.6%, respectively (compared with the respective minimum values).

Table 1 Analysis of variance for the effects of season on the scrotal circumference of Karagouniko and Chios sheep.

Scrotal circumference (cm)							
Karagouniko sheep				Chios sheep			
	df[a]	Mean square	Variance ratio		df[a]	Mean square	Variance ratio
Season	1.90[b]	44.84	40.65***	Season	1.50[c]	31.81	23.87***
Residual	28.50[b]	1.10	-	Residual	22.48[c]	1.33	-

[a]df: degrees of freedom, [b]Corrected df according to the Greenhouse-Geisser approach (estimated value of epsilon: 0.633) following the significance of Maulchy's test of sphericity (Maulchy's W: 0.307, $P = 0.006$), [c]Corrected df according to Greenhouse-Geisser approach (estimated value of epsilon: 0.500) following the significance of Maulchy's test of sphericity (Maulchy's W: 0.199, $P = 0.001$), ***: significance at $P \leq 0.001$.

When averaging SC for SD and LD, separately, a higher SC was confirmed in SD, compared to LD, for both breeds [35.63 cm (SD) and 33.56 cm (LD) for Karagouniko, 36.00 cm (SD) and 34.47 cm (LD) for Chios]. The higher SC in

Karagouniko and Chios in SD (compared to LD) or decreasing day length (compared to increasing day length) coincided with the respective higher testosterone levels of these breeds in the same environment, also noticing a similar pattern of this hormone to SC (Matsoukis et al 2022).

It has been reported that a simulation of SD results in hypothalamic-pituitary-testicular interaction, which in turn enhances increased concentration of the follicle-stimulating hormone, luteinizing hormone and testosterone. A noticeable visible effect of these hormones is an increased SC (Tulley and Burfening 1983). A similar, in general, pattern of SC to the SC of Karagouniko and Chios in our study has been reported (Tulley and Burfening 1983; Matos and Thomas 1992). Higher ram SC in SD, in comparison to LD, has also been reported by Schanbacher and Ford (1979), Tulley and Burfening (1983) and Langford et al (1987) for other sheep breeds.

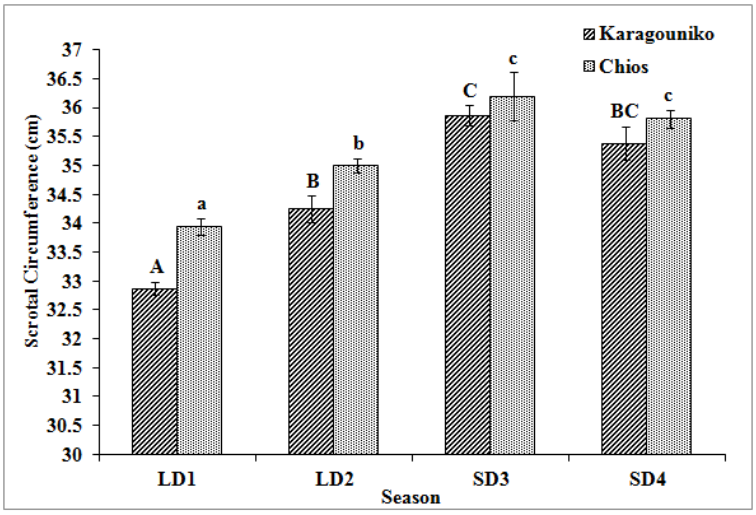

Figure 1 Effect of season on the scrotal circumference of Karagouniko and Chios sheep. In each column, the bar on it represents the standard error of the mean (n = 16). Different letters above columns indicate significant differences between the respective means, separately for Karagouniko sheep (upper case letters) and Chios sheep (lower case letters) at $P \leq 0.05$ after Bonferroni correction. LD1: April-June, LD2: July-September, SD3: October-December, SD4: January-March.

Linear correlation analysis showed the existence of a significant correlation between SC and PHOTO for both Karagouniko (r = -0.595, P = 0.041) and Chios sheep (r = -0.643, P = 0.024) while concerning the other environmental variables, no significant correlations were found between each of them and SC for both Karagouniko (r and P in the range of -0.343 to 0.304 and 0.275 to 0.417, respectively) and Chios breeds (r and P in the range of -0.375 to 0.263 and 0.230 to 0.409, respectively). The correlation between SC and PHOTO was negative, confirming in this way as well that a decreasing PHOTO length coincided with an increase of SC in our studied sheep breeds for the whole examined period.

The variation in SC of other sheep breeds within temperate climate regions, like ours, throughout the year seems to be due mainly to changes in day length (Al-Anazi et al 2017). The scrotal circumference has also been reported to vary with the level of nutrition (Azizunnesa et al 2013) and body weight (Koyuncu et al 2005). The rams used in the present study were physically mature with negligible fluctuations in their body weight. Thus, bearing also in mind the overall correlation analysis results, their SC changes could be attributed to the season and/or day length, in agreement with Kafi et al (2004), who supported a similar hypothesis for Karakul rams.

A significant correlation between day length and SC of rams of Hortobágy Black Racka sheep breed has been reported by Sarlós et al (2013), contrary to Prathibha Kaimal et al (2018), who have reported a non-significant correlation between the environmental parameter above and SC in NARI-Suwarna rams. Also, the above-mentioned authors reported a non-significant correlation between SC of the aforementioned rams and maximum AIRT, minimum AIRT, and RNFL. However, a correlation between SC of rams and the environmental temperature has been reported [Moreira et al (2001), as mentioned in Machado Júnior et al (2011); Sarlós et al (2013)]. Nevertheless, the lack of written works with the same methodology and breeds as ours limits the comparison of the current results.

When examining each period separately, significant differences in SC were confirmed between Karagouniko and Chios sheep only in LD1 (independent t-test value: -6.140 at P = 0.0001) and LD2 (independent t-test value: -2.905 at P = 0.007). The significance above could be explained by the greater SC differences between the two breeds in these periods (1.063 cm and 0.75 cm in LD1 and LD2, respectively) compared to SD3 and SD4 (0.313 cm and 0.438 cm in SD3 and SD4, respectively). After pooling, however, the SC data over the whole experimental period, for each breed, separately, significantly higher SC was pointed out for Chios sheep (35. 23 cm) than Karagouniko one (independent t-test value: -2.674 at P = 0.008). The higher SC of Chios, by 0.64 cm, compared to Karagouniko, could be attributed to their

different genetic potential, in agreement with Cárdenas-Gallegos et al (2012) for other sheep breeds. Our results open new perspectives for the more rational exploitation of Karagouniko and Chios rams, shedding light on the part of their reproductive physiology in relation to their surroundings.

4. Conclusions

In summarizing, decreasing day length led to larger scrotal circumference in both Karagouniko and Chios sheep breeds, irrespective of long days or short days. On average, the larger scrotal circumference was confirmed in short days (October-March) compared to long days (April-September), appearing a maximum in October-December, in both breeds. Chios sheep was characterized by larger scrotal circumference in comparison to Karagouniko sheep for the whole experimental period.

Conflict of Interest

The authors declare no conflict of interest.

Funding

The State Scholarships Foundation of Greece partly funded the research for this work.

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