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Effect of heat stress on milk production traits and milk coagulation properties in dairy sheep

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ABSTRACT

Heat stress in farmed animals is becoming a problem of great importance even in temperate climates, especially due to the increase in average global temperatures. Previous studies conducted on sheep have reported negative effects of heat stress on milk yield and composition and on the cheesemaking properties. The study of the effect of heat stress in sheep is complicated by the seasonality of calving and the overlap of the effects of the lactation stage with those of the climate. The aim of this work was to study the effects of heat stress in dairy sheep through a repeated measures model, separating the lactation stage and climate effects. The dataset included 2,695 repeated measurements of 555 Sardinian sheep, raised in 34 farms in Sardinia. The analyzed phenotypes were milk yield, fat, protein, lactose and somatic cell contents, milk coagulation properties (rennet clotting time, curd firming time, curd firmness), and individual laboratory cheese yield (ILCY). Temperature and humidity values of the day of the survey and of the 3 previous days were provided by the regional agency for the protection of the environment (Agenzia Regionale per la Protezione dell'Ambiente of the Sardinia Region). Climate data were then used to calculate the respective temperature and humidity indices. The effect of the temperature-humidity index (THI) was evaluated using a linear mixed model that considered stage of lactation (DIM), parity, maximum value of hourly THI calculated over 24 h (THI_{max}) class (divided into quartiles), DIM × THI interaction, and sampling date; the farm and the animal were considered random effects. The DIM × THI interaction was significant for all parameters except somatic cells and curd firmness. Milk yield showed an increase from the first to the third THI_{max} class and a reduction in the fourth class (THI > 73); this

trend was the same in the 3 lactation stages (early, mid, and late). Fat content showed an opposite trend, independent of the lactation stage. Proteins decreased linearly at the beginning and mid lactation whereas they showed no significant changes at the end of lactation. Lactose was reduced in the fourth class of THI_{max}, both in mid and late lactation. Rennet clotting time showed different patterns depending on DIM, whereas ILCY showed worse values as THI_{max} increased, especially in the early and mid lactation. The study of THI in the different phases of lactation made it possible to separate the 2 effects on the parameters studied and highlighted how in dairy sheep, heat stress leads to a reduction in production and a general worsening of the quality of the milk.

Key words: ewes, milk traits, heat stress, milk coagulation ability

INTRODUCTION

Climate changes are presenting serious challenges to the livestock industry. Animal thermoregulatory mechanisms are challenged when temperature rises above the 25°C threshold (Asseng et al., 2021), compromising the ability to preserve homeothermy (van Wettere et al., 2021). The condition of heat stress (HS) occurs when the animal is no longer able to dissipate the excess of heat to maintain the body thermal balance (Bernabucci et al., 2014). Among the different climate variables that can be used to evaluate the condition of HS, air temperature, and humidity are commonly recorded by meteorological stations. The temperature-humidity index (THI) is one of the most common indices used to measure the HS condition. By combining temperature and humidity of the air into a single value, it is able to account for the different sensitivity among species (Bohmanova et al., 2007; Yan et al., 2020).

Studies on the effect of HS on animal performances have been carried out mostly on dairy cattle (Bernabucci et al., 2014; Carabaño et al., 2014; Nzeyimana et al., 2023). Al-

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

though small ruminants are generally considered tolerant to warm conditions (Vieira et al., 2023), the effect of HS is becoming a relevant concern also for the dairy sheep industry. About the 50% of global dairy sheep production comes from countries of the Mediterranean area (Pulina et al., 2018), a hot spot considered particularly vulnerable to climate changes (Carabaño et al., 2021).

Previous studies reported negative effects of HS on sheep milk yield (Finocchiaro et al., 2005; Peana et al., 2007; González-Ronquillo et al., 2021), composition (Sevi et al., 2001; Ramón et al., 2016; Mehaba et al., 2021; González-Ronquillo et al., 2021), and coagulation properties (Sevi et al., 2011). The latter result is of particular importance for the dairy sheep industry, because almost all sheep milk is processed into cheese (Cellesi et al., 2019). Moreover, an increase in milk SCC has been reported in sheep under HS conditions, evidencing negative effects on the animal health (Sevi et al., 2001).

The investigation of the effect of HS on dairy sheep performance is hampered by the overlapping effects of climate and lactation stage. In the traditional Mediterranean dairy sheep farming system, the production cycle is seasonal, with lactations that start in winter to late spring and end at the beginning of the summer (Pulina et al., 2018). Almost all ewes are in mid-late lactation when high temperatures occur. It is therefore difficult to disentangle the depressive effect of HS from the physiological decline of milk production that occurs at the end of the lactation (Macciotta et al., 1999). The use of repeated measures for each animal (i.e., the different test-day records along the lactation) may help to address this issue. In particular, the inclusion in the statistical model of an interaction term between lactation stage and THI may allow for testing the effect of different classes of the meteorological factors in the different lactation stages.

The aim of this work was to investigate the effect of thermal stress on milk production traits of dairy sheep using repeated test-day measures per each individual to disentangle the effect of the HS from the effect of lactation stage.

MATERIALS AND METHODS

Animal and Milk Production Data

The study was carried out on 555 pluriparous Sarda breed ewes farmed in 34 flocks located in Sardegna (Italy). A total of 2,695 test-day measures were recorded in the period from January to July 2015. The number of records per animal ranged from 3 to 7 (average 4.8 ± 1.2). Measured traits were milk, fat, and protein yields; fat, protein, and lactose contents; SCS, and milk coagulation properties (MCP). Milk composition was determined by using a Milkoscan 6000 instrument (MilkoScan, Foss

Electric, Hillerød, Denmark). A Fossomatic instrument (Foss Electric) was used to determine SCC. The SCS was computed as $\log_2(\text{SCC}/100) + 3$. Milk coagulation properties were determined with a Formagraph instrument (Foss Electric). Milk samples (10 mL) were coagulated by the addition of 200 μL of rennet solution (Hansen Naturen 225, with $80\% \pm 5\%$ chymosin and $20\% \pm 5\%$ pepsin, Pacovis Amrein AG, Bern, Switzerland) diluted to 0.8% in distilled water (Zannoni and Annibaldi, 1981). This analysis continued for 30 min after rennet addition. The following parameters were recorded: rennet clotting time (RCT, min): the time from the addition of rennet to the beginning of coagulation; curd firming time (k20, min): the time from the start of coagulation until an amplitude for curd firmness of 20 mm; curd firmness (mm, a30): measured the curd consistency 30 min after the addition of rennet. Individual cheese yield was measured according to the methods proposed by Othmane et al. (2002), modified as described by Manca et al. (2016).

Weather Data

Weather data consisted of temperature and relative humidity considering hourly data recorded 24 h before the last milking of each day (test day) and also 1, 2, and 3 d before milking. Meteorological data were analyzed using the hourly data from 1700 h of the previous day to 1600 h of the day of milking during solar time and from 1600 to 1500 h during summertime. Data were provided by the Agenzia Regionale per la Protezione dell'Ambiente of the Sardinia Region (ARPAS) and were obtained from the nearest meteorological stations of the Weather Forecast Service of Sardinia (the average distance between farms and meteorological stations was 9.5 ± 6.3 km).

To assess the effects of HS conditions on milk yield and composition traits and on milk coagulation ability, the temperature-humidity index (THI) was calculated hourly by combining the air temperature (aT, in $^{\circ}\text{C}$) and average relative humidity (RH, %) with the following expression (Kliber, 1964):

$$THI = \left\{ 1.8aT - \left[\left(1 - \frac{RH}{100} \right) (aT - 14.3) \right] \right\} + 32$$

The maximum value of hourly THI calculated along the 24 h (THI_{max}) was used as biometeorological variable in the analysis because test-day milk yield is more sensitive to the extreme values (Brügemann et al., 2012; Peana et al., 2017). Four THI_{max} values were tested in the present work: values calculated for the day of the test, and 1, 2, and 3 d before test day.

Table 1. THI_{max} thresholds calculated as 25th, 50th, and 75th percentiles of its distribution THI relative to the day of the test and 1, 2, or 3 d before

THI_{max} class	Distance from test day (d)			
	-3	-2	-1	0
1	$THI < 56$	$THI < 56$	$THI < 56.9$	$THI < 57.7$
2	$56 \leq THI < 62.1$	$56 \leq THI < 62.3$	$56.9 \leq THI < 63.6$	$57.7 \leq THI < 64$
3	$62.1 \leq THI < 73$	$62.3 \leq THI < 72.8$	$63.6 \leq THI < 73.1$	$64 \leq THI < 72.8$
4	$THI \geq 73$	$THI \geq 72.8$	$THI \geq 73.1$	$THI \geq 72.8$

Statistical Analysis

The effects of THI_{max} on milk yield, composition, and coagulation properties were assessed using the following mixed linear model:

$$Y_{ijklmno} = + DIM_i + PAR_j + THI_k + (DIM \times THI)_{ik} + Test\text{-}day_l + flock_m + a_n + e_{ijklmno}, \quad [1]$$

where DIM_i is the fixed effect of the i th class of days in milk with 3 levels (1 = early lactation, ≤ 100 d; 2 = mid lactation, from 101–150 d; 3 = late lactation, ≥ 151 d); PAR_j is the fixed effect of j th parity class with 7 levels (2–8); THI_k is the fixed effect of the k th class of THI_{max} (with 4 levels based on the THI_{max} distribution); $DIM \times THI$ is the fixed effect of the interaction between the class of DIM and the class of THI; Test-day _{l} is the fixed effect of the l th date of sampling; flock _{m} is the random effect of the flock; a_n is the random effect of the animal; $e_{ijklmno}$ is the residual.

The 4 THI_{max} classes were created using the 25th, 50th, and 75th percentiles as thresholds (Table 1). Peana et al. (2007) suggested to use 8 different THI classes for Sarda dairy sheep. However, this criterion would have resulted in a small size or empty levels of the classification factor, especially as far as the interaction term ($DIM \times THI$) is concerned. The adoption of quartiles allowed for having more balanced classes and for estimating the interaction. In a preliminary step, climate variable classes were defined using THI_{max} measured the day of the test or 1, 2, or 3 d before. The THI_{max} class that showed the best F -value was then used in the final model for each variable.

Model [1] was run using the PROC MIXED of SAS software (SAS Institute Inc.). The 2 random effects were assumed to be distributed as $N(0, I\sigma_{fl}^2)$ and $N(0, I\sigma_{an}^2)$, where I is an identity matrix, and σ_{fl}^2 and σ_{an}^2 are the variance components associated with the flock and the animal effect, respectively. The contribution of the flock (r_{fl}^2) and animal (r_{an}^2) effects to the phenotypic variance of the trait were calculated as:

$$r_{fl}^2 = \frac{\sigma_{fl}^2}{\sigma_{fl}^2 + \sigma_e^2} \text{ and}$$

$$r_{an}^2 = \frac{\sigma_{an}^2}{\sigma_{an}^2 + \sigma_e^2},$$

where σ_e^2 is the variance component associated with the random residual term.

RESULTS

The distribution of records among the different combinations of THI_{max} and DIM classes is reported in Table 2. Although higher classes of THI_{max} tend to have more records in the later stages of lactation, they are represented in all DIM classes, thus allowing to estimate the effect of the meteorological variable across different lactation stages. Figure 1 shows the relationship between some of the investigated traits (milk yield, fat and protein contents, RCT) and the THI_{max} values. The trends of raw means along the different THI values were not well defined. However, milk yield showed a decreasing pattern, whereas fat and protein contents exhibited the opposite behavior. The trend observed for RCT was almost stable along the THI_{max} values (Figure 1).

The statistical significance of the THI_{max} effect (relative to d -3, -2, -1 and to the day of the test) on the different traits is reported in Table 3. Somatic cell score, k20, and a30 were not affected by THI_{max} classes. Table 3 also shows the magnitude of the effect (in terms of F -value) of THI_{max} measured in the 4 different days, which varied according to the considered trait. For lactose, the largest effect was observed for THI_{max} measured 3 d before the sampling, whereas for milk, fat, and protein yields, fat percentage, and RCT the largest effect was found 2 d before. Finally, the THI_{max} measured 1 d before the sampling had its largest effect on protein percentage and ILCY. Thus, the THI_{max} used in the subsequent statistical analyses are highlighted in bold in Table 3.

The $DIM \times THI_{max}$ class interaction was significant for all the studied variables except for fat and protein

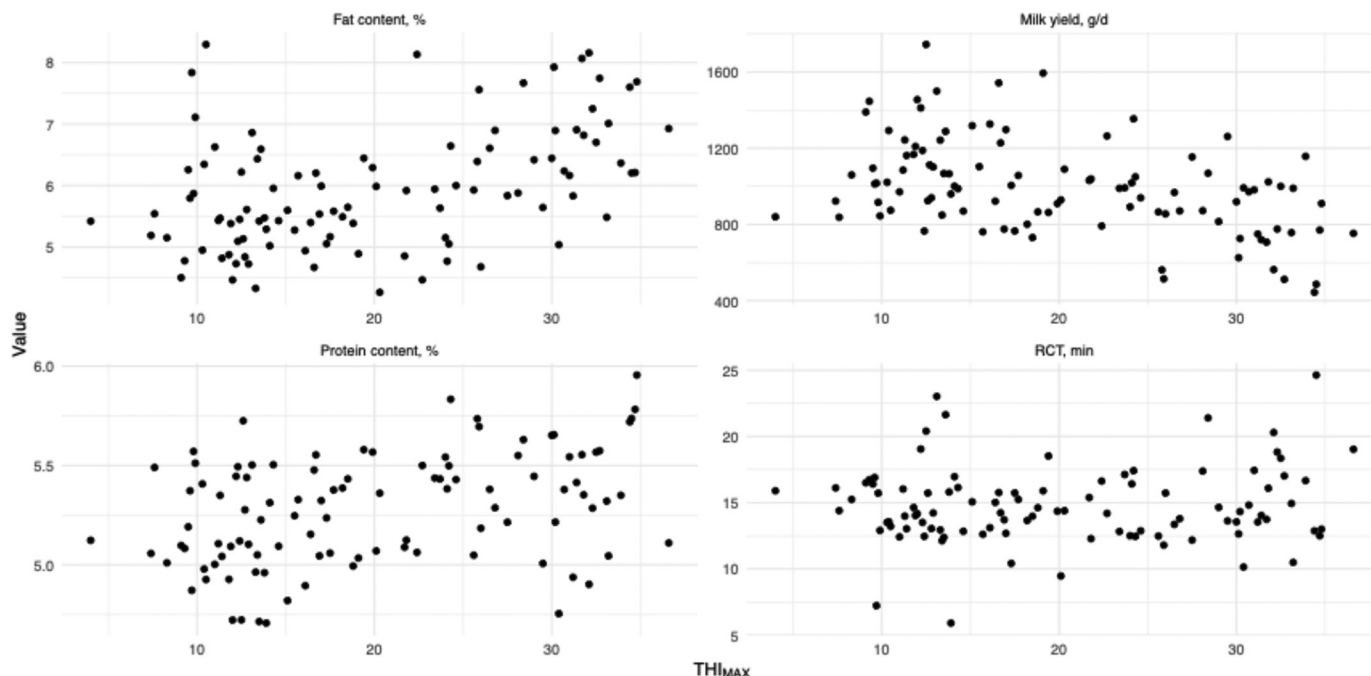


Figure 1. Raw means of milk yield, fat content, protein content, and rennet coagulation time along different THI_{max} values.

yields, SCS, and a30 (Table 4). Least squares means of the different levels of $DIM \times THI_{max}$ class interactions are reported in Figures 2, 3, 4, 5, 6, 7, 8, and 9. Milk yield in early lactation (Figure 2) increased from the first class of THI_{max} to the second and third ($P < 0.01$), with the fourth class not significantly different from the others. It exhibited a decrease in class 4 compared with class 3 or with classes 2 and 3 in mid and late lactation, respectively. Milk fat yield (Figure 3) showed a tendency to decrease in the third and fourth THI_{max} classes, even though a significant difference was observed only in late lactation. A similar pattern was observed for protein yield (Figure 4) with the fourth THI_{max} class showing lower values in mid and late lactation. Milk fat content (Figure 5) exhibited an opposite pattern, decreasing from the first class of THI_{max} to the third in early lactation, and increasing from the second to the fourth in late lactation. In middle lactation, the pattern was less definite. Milk protein content

(Figure 6) showed a linear decrease across THI_{max} classes in the early- and, with a lower magnitude, mid-lactation stages. The effect was not significant in the later stage. In early lactation, the decreases of protein content in the last THI_{max} class ($THI > 73$) were 13.6%, 11.5%, and 6.5% of the value of the first, second, and third THI class, respectively. Lactose content (Figure 7) increased from the second to the third THI class in early and mid lactation. A decrease in the fourth THI_{max} class can be observed in mid and late lactation.

The lowest values for RCT (Figure 8) were observed in the third THI_{max} class in early lactation, and in the first and the third in late lactation, respectively. No significant differences were observed in mid lactation. No significant difference was retrieved for k20 among THI_{max} classes within stage of lactation. The ILCY linearly decreased from the second to the last THI_{max} class in early and mid lactation; in late lactation, ILCY decreased from the second to the third class of THI_{max} , maintaining the same value at the last THI class (Figure 9).

The contribution of the flock effect to the total phenotypic variance (Table 5) was larger for yield traits compared with composition and MCP, whereas the animal effect contribution exhibited an opposite pattern. The contribution of flock ranged from 3% (curd firming time) to 44% (protein yield), whereas the animal contribution ranged from 8% (fat percentage and individual cheese yield), to 37% (SCC).

Table 2. Distribution of records in the different combinations of THI_{max} and DIM classes ($n = 2,695$)

THI_{max} class	DIM class		
	<100	$100 \leq DIM < 150$	≥ 150
1	482	163	9
2	332	315	36
3	96	247	335
4	17	91	573

Table 3. *F*-value (and associated *P*-value) of THI effect for milk yield, composition, and milk coagulation properties; THI relative to d -3, -2, -1 and day of the milk yield recording and milk sampling¹

Trait	THI _{max} effect, <i>F</i> -value (<i>P</i> -value)			
	-3 d	-2 d	-1 d	Test day
Milk yield, g/d	6.41 (0.0003)	9.87 (<0.0001)	9.14 (<0.0001)	5.22 (0.0014)
Fat yield, g/d	1.71 (0.1622)	2.70 (0.0443)	0.97 (0.4063)	0.31 (0.8165)
Protein yield, g/d	14.23 (<0.0001)	15.69 (<0.0001)	7.35 (<0.0001)	4.05 (0.007)
Fat content, %	6.47 (0.0002)	12.47 (<0.0001)	10.52 (<0.0001)	3.82 (0.0097)
Protein content, %	11.95 (<0.0001)	16.84 (<0.0001)	17.75 (<0.0001)	10.58 (<0.0001)
Lactose content, %	16.99 (<0.0001)	9.39 (<0.0001)	11.77 (<0.0001)	13.89 (<0.0001)
Somatic cell score	0.99 (0.3954)	0.67 (0.5699)	0.67 (0.5684)	0.96 (0.4093)
Individual cheese yield, %	19.27 (<0.0001)	18.69 (<0.0001)	21.43 (<0.0001)	6.46 (<0.0001)
Rennet coagulation time, min	2.80 (0.0385)	9.49 (<0.0001)	0.31 (0.8161)	0.58 (0.6291)
Curd firming time, min	0.96 (0.4128)	1.34 (0.2610)	0.26 (0.8519)	2.10 (0.0984)
Curd firmness, mm	1.69 (0.1676)	0.80 (0.4917)	0.86 (0.4637)	0.87 (0.4571)

¹Within each line, the value in bold represents the largest *F*-value and indicates the measure used in the subsequent statistical analysis.

DISCUSSION

In the traditional dairy sheep farming system of Mediterranean countries, the evaluation of HS effect on production traits is hampered by the seasonality of the production cycle that results in an overlapping of the effects of temperature and lactation stage. The use of repeated measures for each animal and the inclusion THI_{max} class × lactation stage interaction in the statistical model used in the present study allowed for disentangling the 2 main effects. Results obtained in the present work confirm an effect of HS on dairy sheep, and this effect was detected, in many cases, at different lactation stages.

The THI is probably the most widely used meteorological indicator to assess the condition of HS in livestock. However, different formulas for its calculation, estimates of thresholds for defining comfort and stress conditions, and the optimum time distance from the day of the milk test can be found in literature. In the present work, thresholds for THI_{max} groups were defined according to the percentiles of the biometeorological value

distribution (Table 1). This criterion was adopted to have groups of homogeneous size, avoiding empty or poorly represented factor levels in the statistical design. However, it could represent an issue for a detailed investigation of the HS effect. Class intervals based on previous studies on HS in dairy sheep would have probably led to results that were easier to interpret. For example, Peana et al. (2017) used 8 THI classes in their study on Sarda sheep. A larger number of THI classes, especially for the highest values of the biometeorological index, would allow to better estimate HS effects and to discriminate between conditions of mild and serious discomfort. Conversely, biologically defined thresholds and smaller class intervals would have resulted also in a poor representation of some groups and in a subsequent model overparameterization. An alternative approach could be the fitting of the response to thermal load by using a reaction norm function implemented in random regression models (Carabaño et al., 2021). This strategy could allow for a more detailed modeling of the genetic component of individual response, but it requires an a priori definition

Table 4. Effect of different factors (*P*-values) on the considered variables of milk yield, composition, and milk coagulation properties

Trait	Sampling date	DIM class	Parity	THI class	DIM × THI
Milk yield, g/d	<0.0001	<0.0001	<0.0001	<0.0001	0.0003
Fat yield, g/d	0.0021	<0.0001	<0.0001	0.0443	0.7610
Protein yield, g/d	0.7241	<0.0001	<0.0001	<0.0001	0.1115
Fat content, %	<0.0001	<0.0001	0.0016	<0.0001	<0.0001
Protein content, %	<0.0001	<0.0001	0.0773	<0.0001	<0.0001
Lactose content, %	<0.0001	0.0257	<0.0001	<0.0001	0.0103
SCS	0.0006	0.0264	<0.0001	0.3954	0.0970
Individual cheese yield, %	<0.0001	0.0362	0.0607	<0.0001	<0.0001
Rennet coagulation time, min	0.0703	0.5914	0.0636	<0.0001	<0.0001
Curd firming time, min	0.0929	0.7349	0.0503	0.0984	0.0247
Curd firmness, mm	0.6996	0.3838	0.0502	0.1676	0.3616

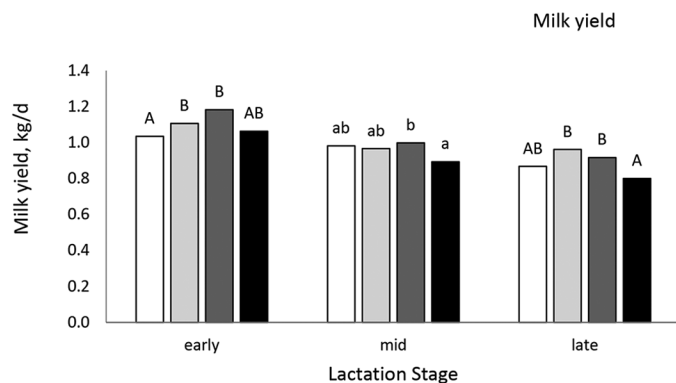


Figure 2. Effect of THI (■ = THI ≤ 56; ■ = 56 < THI ≤ 62.3; ■ = 62.3 < THI ≤ 73; ■ = THI > 73) on milk yield across lactation stages. Different letter within lactation stage indicates significant differences (lower case: $P < 0.05$; upper case: $P < 0.01$).

of a threshold of HS. Moreover, the limited number of records per animal of the present work would result in computational issues.

In any case, the first 2 THI_{max} classes of the present work (Table 1) at least partially correspond to the 2 no-discomfort classes (<60 and 60–65) identified by Peana et al. (2017) for the Sarda dairy sheep. Moreover, the third THI_{max} class (Table 1) partially covers a class of no-discomfort (60–65) and 2 classes representing possible or mild discomfort (65–68 and 68–72). Finally, the starting value of the fourth THI_{max} class represents the threshold above which discomfort, alert, danger, and emergency THI conditions occur. Values higher than 82 (emergency class) were not recorded in the present work, and very few animals (6 out of 2,695) experienced THI values in the danger class.

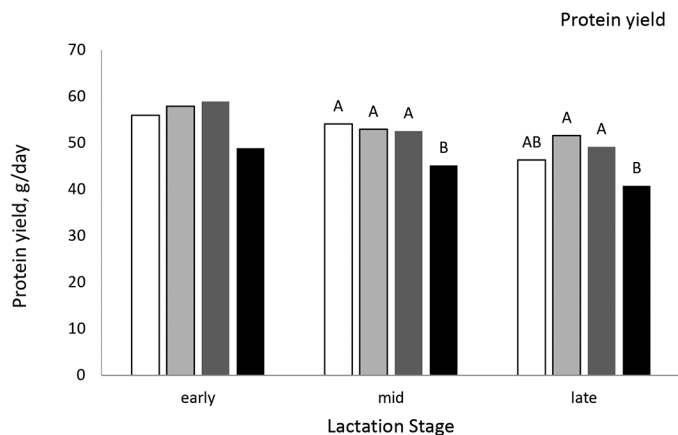


Figure 4. Effect of THI (■ = THI ≤ 56; ■ = 56 < THI ≤ 62.3; ■ = 62.3 < THI ≤ 73; ■ = THI > 73) on protein yield across lactation stages. Different letter within lactation stage indicates significant differences (lower case: $P < 0.05$; upper case: $P < 0.01$).

Regarding the optimum time distance from the day of the milk test to be considered, early investigations in dairy cattle consider the THI 3 d before the test as the more influential on the animal production response (Bohmanova et al., 2007; Aguilar et al., 2009). Thresholds for defining the HS condition in Italian Holsteins were obtained by averaging the THI values measured for 8 d before the test for milk yield and 12 d before the test for milk composition, respectively (Bernabucci et al., 2014). More recently, the average maximum daily THI value of the 5 d before test day was used (Landi et al., 2023, 2024). In the present work, the THI_{max} values measured on the day of the test, and on 1, 2, or 3 d before were compared, as previously suggested in dairy sheep (Finocchiaro et al., 2005; Peana et al., 2017). To select the most influential

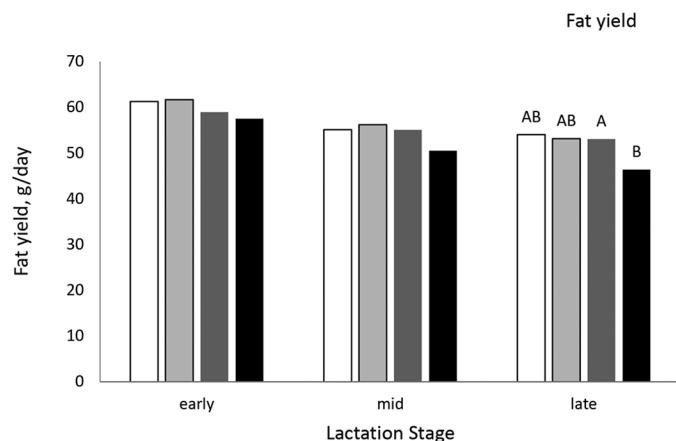


Figure 3. Effect of THI (■ = THI ≤ 56; ■ = 56 < THI ≤ 62.3; ■ = 62.3 < THI ≤ 73; ■ = THI > 73) on fat yield across lactation stages. Different letter within lactation stage indicates significant differences (lower case: $P < 0.05$; upper case: $P < 0.01$).

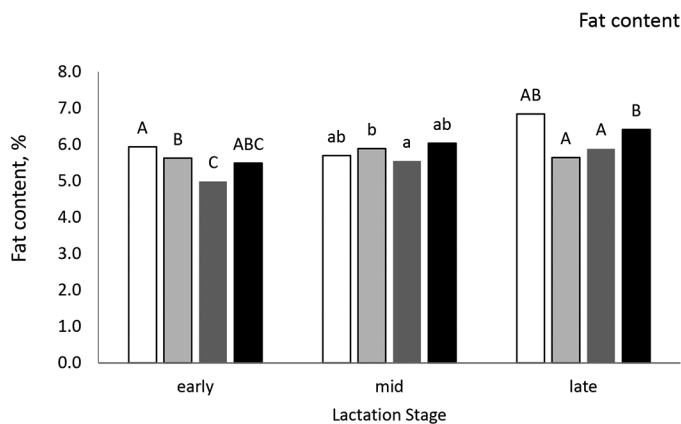


Figure 5. Effect of THI (■ = THI ≤ 56; ■ = 56 < THI ≤ 62.3; ■ = 62.3 < THI ≤ 73; ■ = THI > 73) on fat content across lactation stages. Different letter within lactation stage indicates significant differences (lower case: $P < 0.05$; upper case: $P < 0.01$).

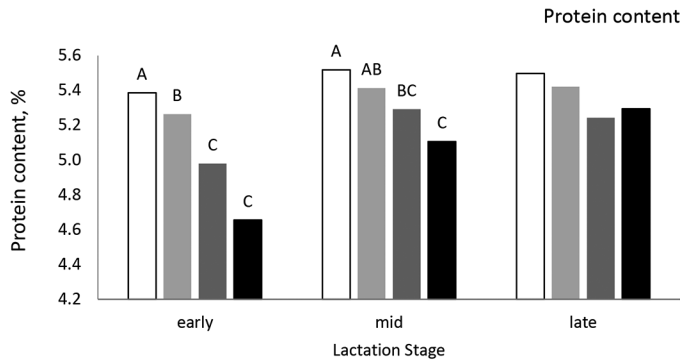


Figure 6. Effect of THI (■ = THI ≤ 56; ■ = 56 < THI ≤ 62.3; ■ = 62.3 < THI ≤ 73; ■ = THI > 73) on protein content across lactation stages. Different letter within lactation stage indicates significant differences (lower case: $P < 0.05$; upper case: $P < 0.01$).

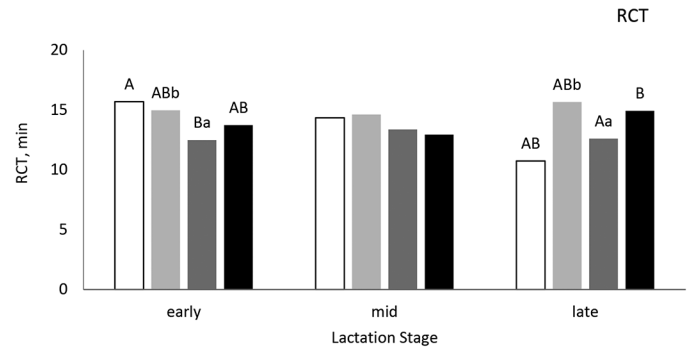


Figure 8. Effect of THI (■ = THI ≤ 56; ■ = 56 < THI ≤ 62.3; ■ = 62.3 < THI ≤ 73; ■ = THI > 73) on rennet coagulation time (RCT) across lactation stages. Different letter within lactation stage indicates significant differences (lower case: $P < 0.05$; upper case: $P < 0.01$).

value of THI_{max} , the statistical model was run 4 times, and each referred to a different day of THI_{max} recording (i.e., 3 d before, 2 d before, 1 d before, same day). The THI_{max} with the largest F -value (Table 3), which seems to be trait dependent, was included in the final model. Ramón et al. (2016) pointed out that the exposure to a 3-d period of high heat load before the test day may result in an effect not significantly different from that derived from the heat load on the day of control. In our study, the THI measured in the same day of the test never showed the largest effect. Interestingly, lactose content and SCS (even if not significant) had the largest effect of THI_{max} measured 3 d before. However, a study on Merino sheep underlined that animals usually recover within few days from the HS exposure (Alhidary et al., 2021). The strong relationship between lactose and SCS has been largely investigated both in cattle (Costa et al., 2019) and sheep (Carta et al., 2023). Looking at milk yield, the THI_{max} measured 2 d before the test had an effect almost 2 times

larger than the THI measured in the same day (Table 3). A huge difference among days was observed for both fat content and RCT. For the former, the THI measured 2 d before the test had an effect 3 times larger than the value measured the same day of the test. RCT had the largest effect of THI measured 2 d before the test; this effect was more than 3 times larger than the value measured 3 d before the test, whereas the THI measured one day before or the day of test were not significant.

The effect of THI on milk traits was studied within the different stages of lactation to avoid the overlapping effects of THI and DIM. This aspect is clearly evidenced by the comparison between Figure 1 and Figures 2, 3, 4, 5, 6, 7, 8, 9. The DIM × THI interaction was significant for all the studied variables except for SCS and A30 (Table 4). Previous works on dairy sheep reported negative (Finocchiaro et al., 2005; Peana et al., 2007; Ramón et al., 2016) or no effects (Mehaba et al., 2021) of THI on milk yield. In the present work, milk yield showed

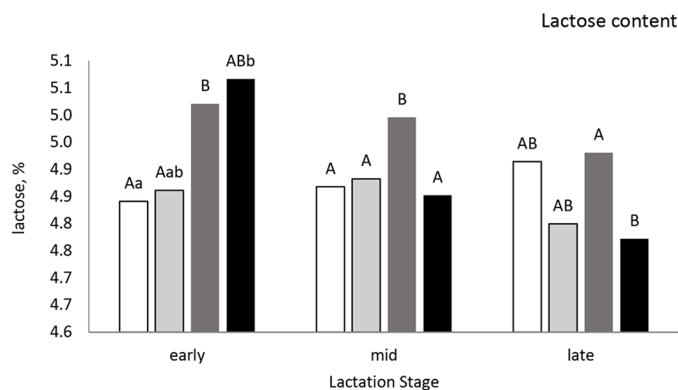


Figure 7. Effect of THI (■ = THI ≤ 56; ■ = 56 < THI ≤ 62.3; ■ = 62.3 < THI ≤ 73; ■ = THI > 73) on lactose content across lactation stages. Different letter within lactation stage indicates significant differences (lower case: $P < 0.05$; upper case: $P < 0.01$).

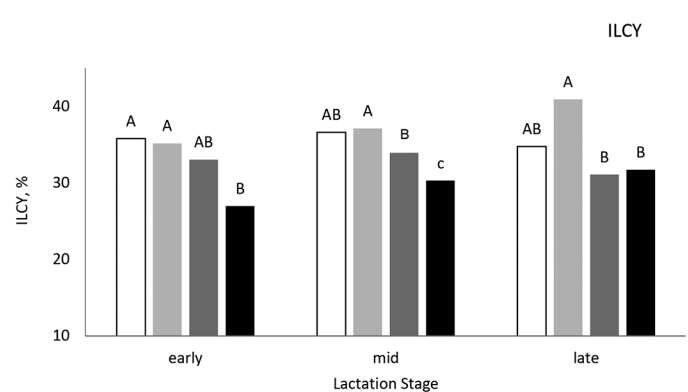


Figure 9. Effect of THI (■ = THI ≤ 56; ■ = 56 < THI ≤ 62.3; ■ = 62.3 < THI ≤ 73; ■ = THI > 73) on individual cheese yield (ILCY) across lactation stages. Different letter within lactation stage indicates significant differences (lower case: $P < 0.05$; upper case: $P < 0.01$).

Table 5. Contribution of the flock (r_n^2) and animal (r_{an}^2) random effects to the total phenotypic variance

Trait	r_n^2 , %	r_{an}^2 , %
Milk yield, g/d	41	18
Fat yield, g/d	31	15
Protein yield, g/d	44	15
Fat percentage, %	14	8
Protein percentage, %	8	34
Lactose, %	4	29
SCS	8	37
Individual cheese yield, %	15	8
Rennet coagulation time, min	8	21
Curd firming time, min	3	7
Curd firmness, mm	7	16

a slow decline approximately after THI equal to 72 across lactation stages (data not shown). A similar result was previously reported by Finocchiaro et al. (2005) in sheep, even if with a different extent of reduction. No or limited effect of HS on milk yield in lactating ewes in their second or late part of lactation was reported (Sevi et al., 2001; Mehaba et al., 2021). In the present work, milk yield at the fourth THI_{max} class (>73) showed a reduction in comparison to the third class (62.3–73) in middle (−10.5%) and late (−12.7%) lactation. Values corresponding to $THI_{max} \geq 73$ were effective in reducing the milk production of Valle del Belice sheep (Finocchiaro et al., 2005). In Sarda dairy sheep, Peana et al. (2017) found a significant decay in milk production (−16%) for THI values increasing from 65 to 68, to 72 to 75. Sevi et al. (2001) observed a milk yield reduction in Comisana sheep exposed to $THI > 80$, even if only in 2 wk of the experiment. Possible explanations for this high threshold could be related to the late stage of lactation of animals, and to the high-energy diet provided, which allowed to have similar DMI of not-exposed animals (Sevi et al., 2001). Indeed, the reduction of DMI in heat-stressed animals is the main cause of the milk yield reduction. Differences in the THI effect on milk yield according to lactation stages were reported also in dairy cattle (Bernabucci et al., 2010) and buffalo (Stefani et al., 2021).

As far as the milk composition is concerned, the most relevant depressive effect of HS was exhibited by protein content affected by the THI measured 1 d before the test (Table 3). The linear decrease of this trait with the increase of THI_{max} , observed in early and mid lactation is quite surprising (Figure 6). Peana et al. (2017) suggested a THI_{max} value of 68 as minimum threshold of the discomfort zone for Sarda sheep. Results of the present study highlight a higher susceptibility of protein content to HS in comparison with fat, in agreement with the findings of Bernabucci et al. (2010) in dairy cattle. Negative effects of HS on milk protein content have been widely reported in cattle (Bernabucci et al., 2014;

Nasr and El-Tarabany, 2017), and, to a lesser extent, in dairy sheep (Mehaba et al., 2021). The reduction of milk protein content can be ascribed to different causes. Tian et al. (2015) demonstrated that HS induces a shift in the nitrogen metabolic pathway in dairy cows, leading to the reduction of milk protein content. In contrast, HS often results in a reduction of DMI, thus lowering the available dietary CP and energy (Collier et al., 1982), directly related to the milk protein concentration (Pulina et al., 2006; Nudda et al., 2020).

The response of milk fat content to HS at each lactation stage was less definite compared with protein. In general, a decrease across the first 3 THI_{max} classes was observed, especially in early lactation. The higher fat content in the fourth THI_{max} class compared with the previous 2 observed in the late lactation was probably due to the reduction of milk yield at $THI > 72$. Negative effects of HS on milk fat content and production were reported in dairy cattle (Nasr and El-Tarabany, 2017) and sheep (Mehaba et al., 2021). Other studies found no reduction of milk fat content for high THI values, both in cows (Rhoads et al., 2009; Maggiolino et al., 2020) and sheep (Peana et al., 2007). Under HS condition, the lactating animals are in negative energy balance due to the reduction of energy intake and to the increase of maintenance costs (Bernabucci et al., 2010), similarly to early lactating animals. Increasing blood concentration of nonesterified fatty acids (NEFA) is typical in transitioning cows, with these compounds representing a significant source of energy and being precursors for milk fat synthesis. It should be then hypothesized that fat mobilization occurs during HS, providing alternative source for the milk fat than the de novo synthesis in the mammary gland. However, the level of plasma NEFA was often found unchanged in heat-stressed dairy cows and in sheep (Sano et al., 1983; Bernabucci et al., 2010; Abeyta et al., 2023), indicating a capacity to retain adipose tissues. This is probably the result of HS-induced hyperinsulinemia (Baumgard and Rhoads, 2013; Abeyta et al., 2023), as insulin is intensely antilipolytic.

The THI_{max} effect on the lactose content (Figure 7) showed different patterns across lactation stages. The largest value was obtained in the third THI_{max} class (68–72), but then it remained high in the fourth class in early lactation, whereas it decreased in middle and late lactation, respectively. Previous studies reported no significant decrease of lactose content in heat-stressed cows and sheep when milk yield remain unaffected (Sevi et al., 2001; Peana et al., 2007; Mehaba et al., 2021) and a slight reduction in lactose content when milk yield was reduced (Rhoads et al., 2009).

Sheep milk is almost all destined for cheese production. Thus, MCP are of interest for the dairy sheep industry. The effect of HS on MCP largely vary according to the

considered species (Beux et al., 2017; Costa et al., 2020). In the present study, a slight effect of HS on the MCP was observed (Table 3): THI_{max} registered 3 or 2 d before the test was significant for ILCY and RCT, respectively. The decrease of ILCY observed in the last class of THI at each stage of lactation (Figure 9) partially confirms previous observations on the negative effect of HS on sheep milk ability to be processed into cheese (Todaro et al., 2014).

Results of the present work show that the effects of thermal stress on milk traits in sheep in some cases are in agreement with reports for dairy cattle, even if with a lower magnitude. These differences can be mostly ascribed to the ability to reduce metabolic and endogenous heat production and increase heat dissipation (Bernabucci et al., 2010). These animal features depend on several morpho-physiological characteristics differences between the 2 species (e.g., the basal heat production; salivary glands size and thus, saliva secretion; the surface area of absorptive mucosa; the efficiency in the ability to recycle urea from blood to the rumen), that result in a higher metabolic rate and a lower retention mechanism of kidney of cattle compared with small ruminants (Macfarlane and Howard, 1972; Bernabucci et al., 2010). Furthermore, a possible explanation of the difference effect of HT between sheep and cattle can be found in a reduction of environmental resilience of dairy cows caused by the high selection pressure for milk production traits. (Aguilar et al., 2010; Santana et al., 2015). The lower effect of HS on dairy sheep can be then related to a higher tolerance of this species to the thermal stress, as consequence of a lower genetic selection pressure (Finocchiaro et al., 2025). The animal effect considered in the present work confirmed a large importance of both flock and individual variability on milk composition traits. Recently, a study on the same sheep breed (Carta et al., 2023) reported heritabilities for milk yield and SCC: Estimates were 0.19 ± 0.05 and 0.27 ± 0.05 for milk and somatic cells, respectively. Larger heritability (0.38) for milk yield was reported in a composite sheep breed (Marshall et al., 2024). In the same study, the heritability estimated for SCS (0.20) was lower than the animal contribution computed in this study (0.37) for the same trait.

CONCLUSIONS

In the present work, the effect of HS of dairy sheep, farmed under the typical Mediterranean system were investigated. The interaction between THI_{max} classes and lactation stages allowed to disentangle, at least partially, the effect of the climate variable from that of the lactation curve shape. Heat stress affected milk traits in different ways depending on the time lag from the test day and on the considered trait. Heat-stressed sheep decreased their

milk production and lactose and protein concentrations, whereas milk fat content increased, probably due to a concentration effect. A negative effect was observed also on the MCP in terms of an increase of RCT and decrease of cheese yield. The worsening of animal performance and milk quality confirms the importance to find strategies to reduce the thermal stress also in small ruminants.

NOTES

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Nonstandard abbreviations used: a30 = XXX; HS = heat stress; ILCY = individual laboratory cheese yield; k20 = XXXX; MCP = milk coagulation properties; NEFA = nonesterified fatty acids; RCT = rennet clotting time; THI = temperature-humidity index.

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