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Assessing heat tolerance through productive vs physiological indicators. Data from dairy sheep under on-farm conditions

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The search for criteria that allow the quantification of the level of thermotolerance of an animal is a major challenge in animal production. Different criteria have been proposed to date, mainly the use of routine milk recording and weather information or the collection of physiological measures related with heat stress. This study aimed at quantifying the association between indicators of heat tolerance derived from productive and physiological traits. For this purpose, two physiological traits, rectal temperature (**RT**) and respiratory rate (**RR**), and nine productive traits (milk yield, fat, protein and lactose yields and contents, casein and urea contents) were measured from June to September of 2018 in three flocks of Manchega sheep. A total of 462 lactating ewes participated in the study. Air temperature (**Ta**), relative humidity (**RH**) and associated temperature and humidity index (**THI**) were recorded inside the barn and also obtained from the closest weather station from the national meteorological network, and used to produce several measurements of heat load on animals. Based on the results of fits for quadratic and cubic regressions on the alternative heat load measures, the cubic regression on Ta and THI obtained inside the barn at time of recording yielded the best fit for physiological and productive parameters. The use of weather information taken from the official weather station closest to the farm also produced similar estimates and could be considered as a good alternative when on-farm meteorological data are not available. Two-trait random regression models that involved individual intercept and slope of response to heat load were used to obtain correlations between basal levels and heat tolerance within and across traits. Estimated correlations showed that animals with smaller vs larger basal levels of RT and RR tend to be more vs less heat tolerant (correlations up to 0.46) and that slopes of increase for RR and RT under heat stress were highly correlated (0.82). Estimated correlations between tolerance criteria from production vs physiology were up to −0.5 (between milk yield and RT), indicating that animals that show less increase in body temperature also tend to show a smaller decrease in production under heat stress. However, because of the non-unity correlation between the two types of indicators of heat tolerance, both sources of information, productive and physiological ought to be taken into account to ensure the long-term sustainability of selection programmes aiming at improving productive levels when heat stress is a concerning issue.

Implications

Improving heat tolerance is a growing concern in dairy production. This study deals with the relationship between productive (milk yield and quality) and physiological (rectal temperature and respiratory rate) indicators of heat tolerance using on-farm data from dairy sheep. The two physiological indicators were highly correlated, favouring the use of respiratory rate as a non-invasive (although labour demanding) indicator over rectal temperature. However, the correlation between productive and physiological indicators was only moderate. Current selection schemes should include not only productive but also physiological or functional indicators of heat tolerance to attain sustainably productive and heat-tolerant farming.

Introduction

Heat stress is one of the main concerns in many dairy production systems, not only in hot climates but also in more temperate regions because of the raise in temperatures associated with climate change (Arias et al., 2021). Detection of heat-tolerant animals

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can be a useful tool to improve the adaptation of farming systems to hot conditions. However, how animals can be measured to quantify their heat tolerance is not well established. Probably, the most accepted definition for a heat-tolerant animal is its capacity to regulate body temperature under high heat loads. Measurement of body temperature is normally an invasive practice, and other physiological indicators such as respiratory rate are used as proxy to measure the heat stress level of the animals. Measurement of physiological indicators is, however, expensive and difficult to carry out under field conditions. Alternatively, the use of productive data from current recording schemes together with meteorological information to estimate the individual slope of productive decay under heat stress (Misztal, 1999; Ravagnolo et al., 2000) has been broadly used to measure heat tolerance in dairy animals (Finocchiaro et al., 2005; Sánchez et al., 2009; Brügemann et al., 2011; Bernabucci et al., 2014; Carabaño et al., 2014). This approach has the advantage of being easily applicable under field conditions for populations undergoing periodical milk recording and having access to meteorological data from weather recording agencies. The use of one or another type of indicator to improve heat tolerance may have different implications. For example, improving heat tolerance through maintaining productive levels under heat stress disregarding physiological indicators could lead to the disruption of the animal's homeostasis with a loss of their adaptation abilities. On the other hand, consideration of physiological indicators of heat tolerance alone could result in a loss of productive level since lowering production is a common adaptation strategy under stressful situations (see, e.g. Berman, 2011; Hoffman, 2010).

Several authors (Spiers et al., 2004; McMillan and Van der Werf, 2007) have approached the estimation of the relationship between physiological and productive indicators of heat stress response with variable results. Part of the variability in the estimated association between productive and physiological indicators is related to the differences in the type of variable used to measure heat tolerance (level of the trait under heat stress vs change between thermoneutrality and heat stress). In addition, different degrees of association between the two types of heat tolerance indicators could be obtained from the use of different heat load measures (surrounding air temperatures vs temperature and humidity indices, ambient descriptors measured where animals are vs measures from the nearest meteorological station or daily average values vs point values).

The main goal of this study was to estimate the relationship between productive and physiological indicators of heat tolerance measured by the rate of change in the trait when heat load increases. A secondary goal was to establish the most suitable definition for the heat load variable in order to characterise the average and individual response of productive and physiological variables to heat load increases. For these goals, measures of a wide range of heat load measures and data for productive traits and the most commonly used physiological traits (rectal temperature and respiratory rate) were obtained from Manchega dairy sheep under field conditions along heat stress and thermoneutral periods. Our expectation is that the results provided can enhance the knowledge about the use of alternative indicators of heat tolerance when dealing with improving climate adaptation not only in sheep but also in other dairy ruminants.

Material and methods

Design of the study and data collection

Data were collected from 462 lactating ewes in three flocks of Manchega sheep breed, members of the Manchega sheep breed association (AGRAMA). This breed is located in the Centre-South region of Castile-La Mancha in Spain, characterised by hot and dry summers. The Manchega breed has been under a selection programme to improve milk yield for the last two decades, with noticeable results (see, e.g. Carabaño et al., 2021). The three selected flocks were chosen because of being representative of the management and productive level of the breed, because of having a large enough size to allow for sampling of a relevant number of ewes within lambing batches and because of their availability to allow the necessary visits and data collection. Latitude and altitude of these flocks ranged from 38.70 to 39.68 degrees and from 750 to 800 m, respectively. The ewes participating in the study were lambing within the same lambing batch in the flocks so that there were not large differences in lactation stage across ewes along the study. All ewes were multiparous (mean age of 50.2 ± 15.6 months) and were managed following the same reproductive cycle (three lambings each 2 years) and feeding regimen. Ewes were milked twice a day, with a similar interval between both milkings for the whole period in the three flocks (0700 in the morning and 1700 in the afternoon). The ewes were outside in the morning just after the milking for a couple of hours and then kept inside the barn for the rest of the day.

The study took place from the 18th of June to the 10th of September, 2018, to cover heat stress and thermoneutral periods. Flocks were visited at an approximate biweekly pace at time of milking to record physiological variables (respiratory rate and rectal temperature) and milk yield and to collect a milk sample to obtain other milk variables from the routine qualitative milk analysis. Before starting the experimental period, several visits to the farm were carried out as a training period to harmonise the data collection procedure. Respiratory rate was obtained by counting the number of breaths in 15 s, by flank inspection, and multiplying the results by 4. Respiratory rate was measured simultaneously by two trained persons and averaged afterwards. Rectal temperature was measured manually using a digital thermometer (SureTemp Plus 690, Welch Allyn, Chicago, IL, USA)), designed to record rectal temperature with an accuracy of ± 0.1 °C within a range from 26.7 to 43.4 °C, with interchangeable probe covers are used that helps to reduce risk of cross-contamination. Both measures and the milk sampling at each visit were done by AGRAMA personnel.

Measures of the following traits were obtained for each visit to the flocks: daily milk yield, fat yield and percentage, protein yield and percentage, lactose yield and percentage, casein percentage, milk urea content, total solid percentage, respiratory rate and rectal temperature. Milk components were measured at the Interprofessional Dairy Laboratory of Castilla-La Mancha (LILCAM, Castilla-La Mancha, Spain) by using an IR spectrophotometer (MilkoScan 4000, Foss, Hillerød; Denmark) calibrated for cow, goat and sheep milk and subjected to quality controls and interlaboratory trials with the Spanish network of official Dairy Laboratories.

Together with the physiological and productive records, hourly temperature and relative humidity were obtained from weather stations (Environmental Meter PCE-FWS 20 N, with precision of 0.1 °C for air temperature and 1% for relative humidity and range of variation 0–50 °C and 1–99% for relative humidity) installed inside the barn where the ewes are located most of the day (not at the milking parlour) and from the weather stations belonging to the national meteorological agency (AEMET) that were closest to the flocks. Distance from farms to AEMET weather stations ranged from 3 to 6 km.

Temperature and relative humidity were combined for both inside the barn and for outside AEMET weather station in a humidity index (**THI**) using the following formula (NRC, 1971),

$$THI = (1.8 \times Ta + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times Ta - 26).$$

where **Ta** was the air temperature (°C) and **RH** was the relative humidity (%) at the time of recording of the physiological traits and milk sampling.

In addition, other measures of heat load along the 24 h previous to the recording of physiological traits were considered. For that, two variables were obtained, the 24 h average of Ta or THI, called daily temperature or daily THI hereafter, and the cumulative number of degrees of Ta (**cumT**) or THI (**cumTHI**) over the thermoneutrality threshold (established from the analyses of average phenotypic response to air temperature and THI for each trait, as discussed later, at 26 °C for T and 72 for THI) over the 24 h previous to recording,

$$cumT = \sum_{i=t_0}^{i=t_{-23}} (Ta_i\text{-}26); cumTHI = \sum_{i=t_0}^{i=t_{-23}} (THI_i\text{-}72)$$

with Ta_i and THI_i being the values of Ta and THI corresponding to time *i* – the hour of recording of the physiological variables – within the previous 24 h to the measurement (from t_0 to t_{-23}).

Overall, 12 heat load variables were analysed, corresponding to the combination of: (i) weather variable (Ta or THI); (ii) origin of weather data (weather station placed on the barn or closest official weather station); and (iii) time of measurement, being either the time of physiological data collection, daily average (in a 24 h period) or sum of hourly deviations from thresholds.

Statistical analysis

Average phenotypic response

In a first set of analyses, the response of each trait to changes in heat load (**HL**) and other environmental factors was analysed by a linear mixed model with the following general equation,

$$y_{ijkmno} = Flock_i + Age_j + DIM_k + NL_m + f(HL) + ewe_n + e_{ijkmno} \quad (1)$$

where y_{ijklno} was the value of each trait (each trait was analysed separately), $Flock_i$ is the flock where animals were located (i = 1 to 3); Age_j was the class of age at lambing of the ewe (j = 1 to 4, corresponding to ≤ 2, (2, 3], (3, 4] and > 4 years of age of the ewe, respectively); DIM_k was the class of days in milk (k = 1 to 5, corresponding to ≤ 40, (40, 80], (80, 100], (100, 120] and > 120 days in milk, respectively), NL_m was the number of lambs born at the corresponding lambing (m = 1 to 3); f(HL) was the effect of the 12 alternative heat load measures, with f( ) being either a quadratic or cubic Legendre polynomial; ewe_n was the ewe that produced the record, and e_{ijklno} was the residual term. All effects were treated as fixed, except for the ewe and the residual terms, which were considered as random.

Overall, 24 models, corresponding to each of the 12 ways of defining the heat load variable and the two ways of modelling (quadratic or cubic polynomial), were solved for each trait. Software R (R Core Team, 2021) with the MCMCglmm package (Hadfield, 2010) was used for this purpose. MCMCglmm uses Bayesian inference to solve the models. Location parameters (corresponding to effects in equation [1]) are assumed to be normally distributed with a priori independence between fixed, random and residual effects. Prior values for the variance of ewe and residual effects were obtained from the raw variance of each trait, assuming a 0.20 proportion for the ewe to total variance. The software provides the values of the Markov sampling chains for the location effects and dispersion parameters, which were used to obtain the posterior mean and other statistics of the posterior distribution of the parameters of interest. Chains were composed of 10 000 samples, which were checked for convergence to the stationary distribution after discarding 2 500 samples as burn-in and retaining 1 out of 10 samples. The software also provides the deviance information criterion (**DIC**) associated with each model, which was used to establish the statistical comparison for the goodness of fit across all the models. In order to provide meaningful comparisons of goodness of fit, data had to have complete information for all the 12 heat load variables for each day of recording of the traits. After this editing, 1 841 records of 440 ewes were used to perform the statistical analyses for the phenotypic response.

Heat stress response was characterised by the slopes (derivatives) of the response curve in successive heat load points. The derivatives of the polynomial curve at selected heat load points were calculated using the Markov samples of the random regression coefficients. Final estimates of the slopes were obtained as the mean of the slope samples, once convergence to the equilibrium distribution was attained. Uncertainty of the estimated slopes was obtained from the standard deviation of the values of the slope samples.

Individual response and correlations between heat tolerance criteria

In a second set of analyses, individual responses to increases in heat load were fitted using the heat load definition that showed the best results in terms of DIC in the previous analyses, the temperature collected at the time of recording inside the barn.

In this part of the study, for the estimation of the individual responses to heat stress, we used the so-called "broken-line" random regression model (Misztal, 1999) because it provides simple and easily interpretable results. This model assumes that production remains constant up to a threshold above which the animal's ability to produce is compromised due to the high heat load and production decays. In this model, the intercept represents the basal level (under thermoneutral conditions) of the trait and the slope after the heat stress threshold (break point) could be considered as a heat tolerance indicator for each trait. As previously mentioned, the heat stress threshold was set to 26 °C for all traits.

The general statistical model was of the form,

$$y_{ijklno} = Flock_i + Age_j + DIM_k + NL_l + f(T0B) + a_{on} + a_{1n}(T0B - 26) + e_{ijklno} \quad (2)$$

where, Flock, Age, DIM, NL were fixed environmental effects, defined as in [1], f(T0B) was a cubic regression on the air temperature taken at sampling time, **T0B**, a_{on} was the random ewe effect (same as ewe_n in [1]), which can be regarded as the intercept of a random regression on the temperature for each ewe, and a_{1n} was the target parameter, the individual slope of response to temperature, represented by the linear regression of trait y on the temperature at the time of recording in the barn (T0B) above the heat stress threshold, considered as a random effect. Negative values of the covariable (T0B-26) were set to zero, since they represent thermoneutral temperatures, for which no response in the trait to changes in temperature is expected.

Multiple trait (MT) models were used to estimate correlations between the tolerance variables (slopes of individual response) among traits. Because the overall MT setting including all traits showed bad convergence, sets of analysis including two traits at a time, the two physiological traits (respiratory rate, rectal temperature) and one physiological trait with one production trait (yields and contents), plus a MT model for all production traits were run instead.

The MT models in matrix notation could be written as,

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e} \quad (3)$$

where **y** contained the values of the traits considered in each analysis, **b** was the vector of fixed effects, **X** was the incidence matrix linking effects in **b** to observations in **y**, **a** was a vector of intercepts and slopes for each trait and ewe participating in the analysis and **Z** the corresponding design matrix, including the covariables for the deviation (T0B-26), and **e** was the MT residual effect.

For the case of a bivariate model, the (co)variances for random effects in [3] were assumed to follow the following structure,

$$\mathrm{Var}(\mathbf{a}) = \mathbf{P}_{ij} \otimes \mathbf{I}_q;\ \mathrm{Var}(\mathbf{e}) = \mathbf{R}_{ij} \otimes \mathbf{I}_N,\ \text{with}\ \mathbf{P}_{ij} = \begin{bmatrix} \sigma^2_{0i} & \sigma_{0i1i} & \sigma_{0i0j} & \sigma_{0i1j} \\ \sigma_{1i0i} & \sigma^2_{1i} & \sigma_{1i0j} & \sigma_{1i1j} \\ \sigma_{0j0i} & \sigma_{0j1i} & \sigma^2_{0j} & \sigma_{0j1j} \\ \sigma_{1j0i} & \sigma_{1j1i} & \sigma_{1j0j} & \sigma^2_{1j} \end{bmatrix}, \mathbf{R}_{ij} = \begin{bmatrix} \sigma^2_{ei} & \sigma_{eij} \\ \sigma_{eji} & \sigma^2_{ej} \end{bmatrix}$$

where, $\mathbf{P}_{ij}$ and $\mathbf{R}_{ij}$ were the matrices of (co)variance components for animal and residual effects in the MT model including traits i and j, respectively. $\mathbf{P}_{ij}$ was a matrix containing the (co)variances between intercepts ($\sigma^2_{0i}, \sigma^2_{0j}, \sigma_{0i0j}$), slopes ($\sigma^2_{1i}, \sigma^2_{1j}, \sigma_{1i1j}$) and between intercepts and slopes ($\sigma_{0i1i}, \sigma_{1i0i}, \sigma_{0j1j}, \sigma_{1j0j}, \sigma_{0i1j}, \sigma_{0j1i}, \sigma_{1i0j}, \sigma_{1j0i}$). $\mathbf{R}_{ij}$ contained the residual variances and covariances for the pertinent traits. Correlations of interest were calculated from estimates of the covariances and variances in $\mathbf{P}_{ij}$. For the production traits MT model, **P** and **R** had a similar structure but a higher dimension (20×20) corresponding to intercept and slopes of the 10 production traits. Please, notice that the estimated correlations under such models represent the correlation between animal effects. It means that such correlation contains the genetic components plus other non-genetic effects related to the animal. In this sense, such a correlation is closer to the genetic one than a raw correlation because systematic effects have been removed.

The software Blupf90 (Misztal et al., 2002) was used to obtain estimates of (co)variance components. Modules Gibbs1f90, AIRemlf90 and Remlf90 were tried for different combinations of traits, being Remlf90 the algorithm of choice because of showing the best convergence behaviour. Because this module does not provide standard errors of estimated parameters, an approximation, based on Sokal and Rohlf (1995), was used to obtain 95% confidence intervals for the estimated correlations:

$$SE(\hat{r}_{ij}) = \sqrt{\frac{1 - \hat{r}_{ij}}{n - 2}}$$

where $\hat{r}_{ij}$ is the estimated correlation between traits i and j and n is the number of ewes with records participating in the estimation.

Results

Table 1 shows summary statistics for the analysed traits, including the number of records and ewes that participated in the statistical analyses. The Manchega breed is characterised by a medium level of milk yield and high levels of fat and protein contents (Ferro et al., 2017), being the productive levels of the ewes participating in this study representative of the breed milk production and milk composition. All traits showed variability, with large differences in CV across traits observed. Yields showed much larger values (around 40%) of the CV than contents (ranging from 6% for lactose percentage to 24% for fat percentage and urea content). As expected for homeothermic animals, rectal temperature showed a very small CV (1%), while the CV for respiratory rate (37%) was similar to that of the yield traits.

Table 2 shows the summary statistics depicting the distribution of values for the variables used to measure heat load in the subsequent analyses. Temperature and THI distributions were similar for barn and weather stations with correlations between them larger for temperatures (0.91 for the temperature at time of recording and the average daily temperature and 0.88 for the cumulative temperature) than for THI values (0.83 for THI0, 0.74 for daily THI and 0.47 for cumulative THI). The values of the temperature and THI ranged from thermoneutrality to moderate or high heat stress according to thresholds for heat stress onset revised by Marai et al. (2007) and Thornton et al. (2021).

Phenotypic average response

Figures showing the average phenotypic response to increasing heat load estimated in the first set of analyses for each of the 12 productive and physiological, traits, for the 12 heat load variables and for the quadratic and cubic polynomials approaches are provided as Supplementary Figs. S1 to S12. All productive traits, except lactose percentage, showed declines at higher values of the heat load, indicating the likely existence of heat stress. Regarding physiological traits, both respiratory rate and rectal temperature, showed increases as ambient temperature increased in a nearly linear trend. Temperature and THI tended to show similar patterns of response while cumulative degrees of them over the heat stress threshold showed less consistent patterns, especially for yield traits. Quadratic and cubic fits yielded similar trends, but some differences were observed. Cubic polynomials tended to show upward/downward trends at the right/left extreme of the heat load range, where information was scarcer. On the other hand, the quadratic fit showed less flexibility to allow for changes

Table 1
Number of records (N), Number of animals (Nanim), Mean, CV, Minimum (Min) and Maximum (Max) values and quantiles 25 (Q25), 50 (Median) and 75 (Q75), for the traits under analysis in Manchega sheep.

Trait[1]	N	Nanim	Mean	CV	Min	Q25	Median	Q75	Max
My (g/day)	2 385	460	1 001.4	0.47	140.0	680.0	920.0	1 280.0	2 650.0
Fy (g/day)	2 249	458	68.8	0.41	9.8	49.8	63.8	82.4	228.5
Py (g/day)	2 248	460	51.8	0.41	7.4	37.0	48.5	64.0	133.1
Lcy (g/day)	2 251	460	52.5	0.49	6.4	34.6	48.3	67.1	141.8
Fp (%)	2 314	459	7.20	0.27	2.72	5.90	6.93	8.16	13.49
Pp (%)	2 314	461	5.25	0.12	4.08	4.76	5.16	5.62	7.61
Cnp (%)	2 176	459	4.05	0.13	3.06	3.65	3.98	4.34	6.00
Lcp (%)	2 319	461	5.13	0.06	3.70	4.95	5.17	5.35	5.78
Urc (mg/l)	2 175	458	554.9	0.23	256.0	463.0	548.0	644.0	917.0
TSp (%)	2 315	459	18.37	0.13	13.50	16.72	18.02	19.55	26.54
RR (breath/min)	2 447	462	78.9	0.35	36.0	56.0	72.0	100.0	148.0
RT (°C)	2 430	462	39.1	0.01	37.8	38.9	39.1	39.4	40.3

[1] Abbreviations: M = milk; F = fat; P = protein; Lc = Lactose; Cn = Casein; Urc = Urea content; TS = total solids; RR = respiratory rate; RT = rectal temperature; y = yield; p = percentage.

Table 2
Mean, CV, Minimum (Min) and Maximum (Max) values and quantiles 25 (Q25), 50 (Median) and 75 (Q75) for temperature (T) and temperature and humidity index (THI) at time of data collection (0), daily average values (day) and accumulated degrees over the heat stress threshold[1] along the 24 h previous to collection of data (cum) inside the barn (B) or obtained from the closest official weather station(WS) in Manchega sheep.

Item	Mean	CV	Min	Q25	Median	Q75	Max
T0B (°C)	28.26	21.27	19.90	22.90	25.00	33.70	38.50
T0WS (°C)	28.40	21.20	17.10	23.80	28.60	33.30	38.40
TdayB (°C)	27.40	7.52	23.25	25.86	27.31	28.91	31.36
TdayWS (°C)	26.43	10.14	21.66	25.29	26.02	28.28	31.44
THI0B	72.97	5.76	65.23	69.28	72.36	76.88	80.34
THI0WS	72.81	6.19	61.95	69.15	72.97	76.72	81.20
THIdayB	72.61	2.22	70.44	71.16	72.71	74.11	76.34
THIdayWS	71.05	3.27	66.55	70.19	70.69	72.03	75.37
cumTB (°C)	61.03	75.18	0.00	23.10	53.30	92.10	156.20
cumTWS (°C)	45.01	113.17	0.00	7.00	24.40	78.80	154.60
cumTHIB (°C)	25.01	113.79	0.00	0.00	19.42	51.27	101.48
cumTHIWS	12.49	202.40	0.00	0.00	0.00	0.69	80.80

[1] heat stress threshold: T = 26 °C, THI = 72.

in trends along the heat load values. Overall, cubic polynomials provided better goodness of fit in terms of the DIC criterion (results not shown) and later results shown for average response correspond to the cubic polynomial.

Fig. 1 shows the resulting statistics for goodness of fit (DIC) as deviations (ΔDIC) from the reference model (where the function of the heat load was not fit), for the cubic polynomial. Models showing larger deviations from the reference are expected to provide a better fit. For all traits, the models including any of the heat load variables outperformed the control model, which indicates that heat load was a relevant factor. The daily yields and the physiological traits seemed to be best described by temperatures or THI measured inside the barn at the time of recording while the number of degrees above the heat stress threshold accumulated or the average daily values of the heat load in the 24 previous hours showed the worst results. For physiological traits, ΔDIC was much larger for heat load at time of recording than for any of the other traits. On the other hand, for content traits, smaller ΔDIC across the alternative heat load measures were found without a clear trend on the best type of measure in terms of goodness of fit. Results for the content traits were inconsistent, with cumulative degrees above the assumed thermoneutrality threshold and daily measures yielding both the best and worst performance across traits, with temperatures or THI values at the time of data collection providing intermediate results.

In order to quantify the importance of production loss and changes in physiological variables, the slopes of change under what were considered to be heat stress values for temperatures and THI are presented in Table 3. Slopes for accumulated degrees over the heat stress threshold have been omitted because of the erratic or inconsistent behaviour in response observed for those heat load definitions for some traits. The slopes presented in Table 3 for productive traits were evaluated for values of the heat load definitions that are in the descending region for each trait according to the estimated average response patterns (shown as supplementary material). Those differed for yield vs content traits. For the temperature inside the barn and measured at time of recoding, which was the heat load variable showing good or the best DIC values for all traits, daily loss (slope of the response curve) at 30 °C was 50.42/1.77/2.60/2.65 g/°C for milk/fat /protein/lactose yields. For content traits, daily loss at 34 °C were 0.15/0.04/0.04/0.18% per for fat/protein/casein/total solid percentage and 6.4 mg/l per °C for urea content. Lactose percentage rose at a rate of 0.03% per °C. For physiological traits, slopes of increase at 30 °C were 3.73 breaths/min per °C for respiration rate and 0.06 °C of rectal temperature per °C. Slopes for other heat load variables can be seen in Table 3. Values of slopes for daily THI measured in the weather station were substantially different from values of other heat load variables, probably due to the reduced variation in values observed for that variable.

Individual response and correlations between heat tolerance criteria

Summary statistics of solutions for individual intercepts and slopes of the animals for all traits are shown in Table 4. As a result of the null mean for random effects required by the method of estimation, individual estimates take positive and negative values around a value of zero. The magnitude of the range of values reflects the variability in productive and physiological traits in the sample of Manchega ewes participating in the study.

For intercepts, which represent the base values under thermoneutrality, milk yield showed a range of 1 kg of difference between the top and bottom animals. For quality traits, apart from total solid percentage, with a range of 3.8% between the best and worst ewe, fat showed the largest range of values, around 70 g for yield and 2.6% for content. Total protein and its casein fraction, together with lactose, showed smaller variability, 48 and 55 g for protein yield and lactose yield, respectively, and 2.2, 1.6 and 1.4% for protein percentage, casein percentage and lactose percentage, respectively. Urea content ranged from −172 mg/l to 173 mg/l. The physiological traits showed differences of 30 breaths per minute for respiratory rate and 0.7 °C for basal rectal temperature.

For tolerance, measured through the slopes of change by degree of temperature under heat stress, the range (i.e. difference between most tolerant and most susceptible animal) was 35 g/°C above the heat stress threshold of 26 °C for milk yield. For quality traits, the range of response varied between 3 g/°C for fat yield to 1.5 for lactose yield and from 0.04 for casein percentage to 0.3%/°C for fat percentage. For urea content, the difference in response between the two extreme animals was 12 mg/l/°C. The range of response for physiological traits was three breaths per minute/°C and 0.05 °C of rectal temperature/°C of ambient temperature above the heat stress threshold.

Table 5 shows the estimated correlations between intercepts and slopes for physiological traits. Basal values (intercepts) of both traits showed a moderate estimated correlation (around 0.40) with the tolerance variable (slope), indicating that the animals with larger basal values of respiratory rate or rectal temperature tend to be less tolerant to heat stress. The estimated correlation between basal values for the two physiological traits was low (0.24), which suggest that there is a weak relationship between respiratory rate and rectal temperature under thermoneutral conditions. However, the estimated correlation between tolerance (slopes) measured from respiratory rate and rectal temperature showed a high correlation (0.82), which implies that either trait reflects heat tolerance in a similar way.

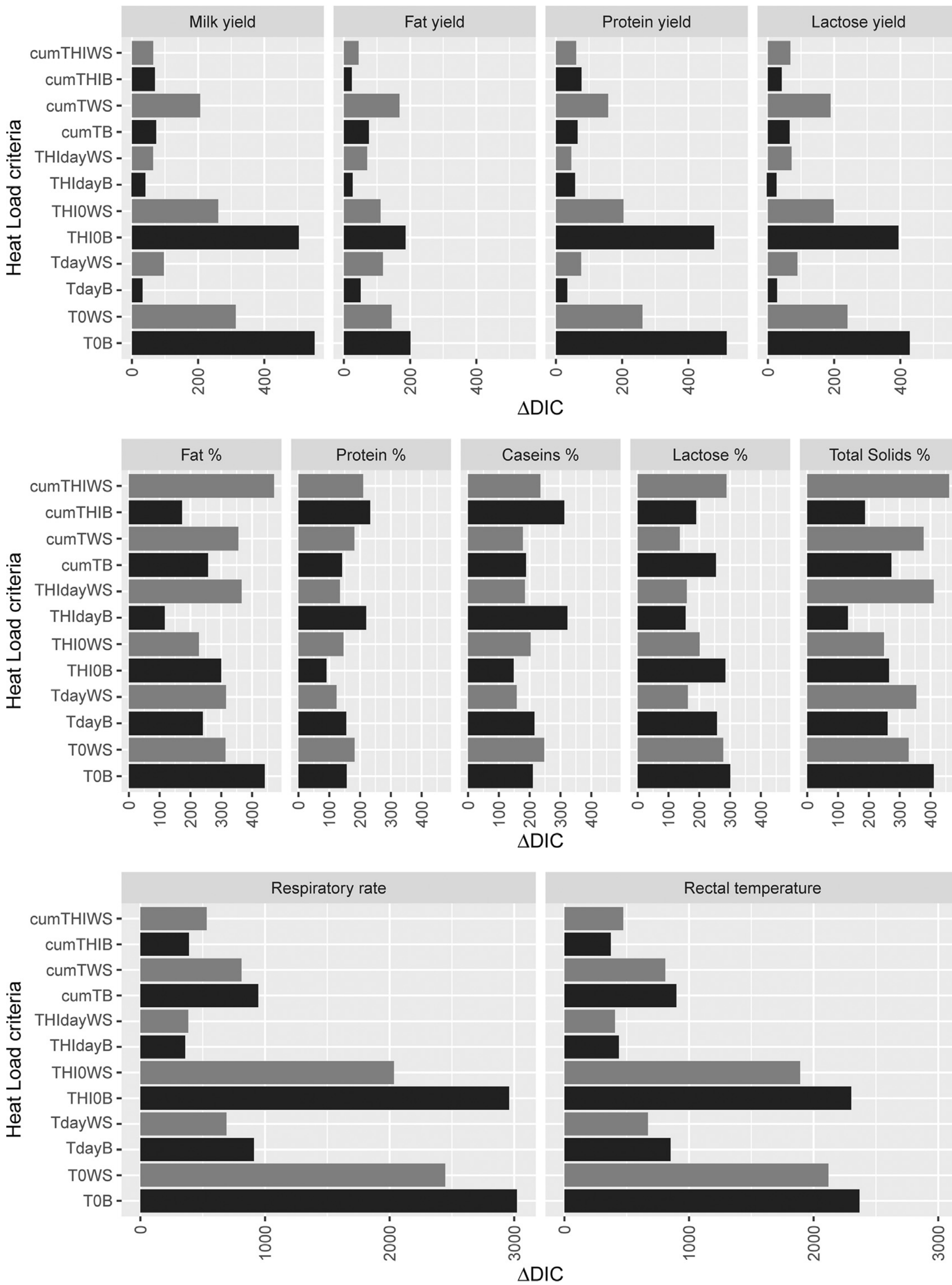

Fig. 1. Values of the difference between the deviance information criterion corresponding to the basal model that does not include the effect of the heat load and the DIC of models including heat load represented by different variables, air temperature and temperature and humidity index at time of data collection, daily values and accumulated degrees over the heat stress threshold (T_{hs} = 26 °C, THI_{hs} = 72) along the 24 hours previous to collection of data inside the barn or obtained from the closest official weather station in Manchega sheep. Abbreviations: Δ = difference with respect to the basal model; DIC = deviance information criterion; T = air temperature; THI = temperature and humidity index; 0 = time of data collection; day = daily average; Ths/THIhs = heat stress temperature/THI threshold; cum = cumulative; B = barn; WS = closest weather station.

Estimates of correlations between basal levels (intercepts) and tolerance (slopes) for production traits are shown in Table 6. Estimated correlations between intercepts were high and positive for yield traits (ranging from 0.9 for fat and lactose yields to 0.99 for milk and lactose yields). On the other hand, estimated correlations between yields and contents were smaller than expected from the widely recognised antagonism between milk production and milk quality (range from −0.10 to −0.14 between milk yield and total solid percentage and protein percentage or casein percentage, respectively). Estimated correlations between intercepts and

Table 3
Estimated slopes (±SD) of change (unit of trait/unit of heat load) under heat stress for the cubic polynomial regression on temperature (T) or temperature and humidity index (THI) measured at time of data acquisition (0) or as average of the 24 h previous to data acquisition (day) inside the barn (B) or obtained from the closest official weather station (WS) in Manchega sheep.

	Heat load variable[2]							
Trait[1]	T0B	T0WS	TdayB	TdayWS	THI0B	THI0WS	THIdayB	THIdayWS
My (g/day)	−50.4 ± 3.52	−39.3 ± 2.83	−40.3 ± 8.28	−71.4 ± 8.79	−57.4 ± 3.81	−28.5 ± 3.00	−51.2 ± 15.5	26.3 ± 6.64
Fy (g/day)	−1.77 ± 0.27	−1.67 ± 0.21	−1.65 ± 0.58	−4.78 ± 0.59	−2.62 ± 0.29	−1.71 ± 0.23	−3.19 ± 1.06	−1.07 ± 0.48
Py (g/day)	−2.6 ± 0.18	−1.89 ± 0.15	−2.03 ± 0.42	−3.17 ± 0.42	−2.91 ± 0.19	−1.39 ± 0.15	−3.15 ± 0.76	1.01 ± 0.31
Lcy (g/day)	−2.65 ± 0.20	−1.94 ± 0.17	−2.03 ± 0.47	−3.58 ± 0.48	−2.85 ± 0.21	−1.24 ± 0.17	−1.6 ± 0.83	1.68 ± 0.38
Fp (%)	−0.15 ± 0.01	−0.16 ± 0.01	−0.31 ± 0.03	−0.3 ± 0.03	−0.61 ± 0.05	−0.43 ± 0.03	−0.4 ± 0.05	−0.72 ± 0.11
Pp (%)	−0.04 ± 0.005	−0.04 ± 0.004	−0.04 ± 0.009	−0.05 ± 0.008	−0.06 ± 0.016	−0.08 ± 0.011	−0.03 ± 0.014	−0.09 ± 0.036
Cnp (%)	−0.04 ± 0.004	−0.04 ± 0.004	−0.05 ± 0.007	−0.05 ± 0.006	−0.06 ± 0.013	−0.07 ± 0.009	−0.05 ± 0.011	−0.09 ± 0.028
Lcp (%)	0.03 ± 0.003	0.03 ± 0.002	0.04 ± 0.004	0.03 ± 0.004	0.08 ± 0.008	0.05 ± 0.006	0.03 ± 0.008	0.12 ± 0.02
Urc (mg/l)	−6.4 ± 0.96	−5.82 ± 0.84	−4.21 ± 1.68	−8.47 ± 1.42	−11.26 ± 3.08	−16.79 ± 1.95	−13.07 ± 2.58	−57.8 ± 6.27
TSp (%)	−0.18 ± 0.02	−0.19 ± 0.02	−0.34 ± 0.03	−0.35 ± 0.03	−0.66 ± 0.06	−0.49 ± 0.04	−0.42 ± 0.05	−0.81 ± 0.13
RR (breath/min)	3.73 ± 0.16	4.33 ± 0.14	4.27 ± 0.52	6.79 ± 0.52	6.44 ± 0.17	5.28 ± 0.15	6.9 ± 0.70	4.31 ± 0.42
RT (°C)	0.06 ± 0.003	0.06 ± 0.002	0.08 ± 0.008	0.09 ± 0.008	0.1 ± 0.003	0.08 ± 0.003	0.1 ± 0.012	0.07 ± 0.007

[1] Abbreviations: M = milk; F = fat; P = protein; Lc = Lactose; Cn = Casein; Urc = Urea content; TS = total solids; RR = respiratory rate; RT = rectal temperature; y = yield; p = percentage.
[2] Estimated slopes measured under heat stress: for My, Fy, Py, Lcy, RR and RT, a T0/Tday of 30/26 °C and a THI0/THIday of 75/73, respectively; for Fp, Pp, Cnp, Lcp, Urc, TSp, a T0/Tday of 34/29 °C and a THI0/THIday of 79/75, respectively. Slopes for the same variables measured inside the barn or collected in the closest weather station correspond to the same T or THI value for the corresponding trait.

Table 4
Percentiles (0, 5, 95, 100) of the distribution of estimated values of individual intercepts and slopes for productive and physiological traits in Manchega sheep.

	Intercepts				Slopes			
Traits[1]	P0	P5	P95	P100	P0	P5	P95	P100
My (g/day)	−508.49	−249.87	282.68	535.58	−18.71	−10.87	9.54	16.02
Fy (g/day)	−32.86	−17.84	19.11	35.21	−1.97	−0.54	0.58	0.97
Py (g/day)	−24.02	−13.34	14.82	23.58	−1.22	−0.48	0.44	0.82
Lcy (g/day)	−29.39	−14.10	14.56	25.88	−1.03	−0.59	0.54	1.40
Fp (%)	−1.24	−0.58	0.70	1.43	−0.24	−0.07	0.07	0.16
Pp (%)	−0.71	−0.35	0.41	1.53	−0.05	−0.01	0.01	0.03
Cnp (%)	−0.58	−0.29	0.31	1.14	−0.02	−0.01	0.01	0.02
Lcp (%)	−1.06	−0.21	0.20	0.40	−0.03	−0.01	0.02	0.10
Urc (mg/l)	−172.17	−66.91	71.76	173.46	−9.00	−2.07	1.66	3.06
TSp (%)	−1.99	−0.87	0.98	1.87	−0.18	−0.07	0.08	0.18
RR (breath/min)	−12.87	−6.73	6.28	16.98	−1.42	−0.83	0.74	1.63
RT (°C)	−0.305	−0.171	0.163	0.407	−0.024	−0.015	0.015	0.030

[1] Abbreviations: M = milk; F = fat; P = protein; Lc = Lactose; Cn = Casein; Urc = Urea content; TS = total solids; RR = respiratory rate; RT = rectal temperature; y = yield; p = percentage.

Table 5
Estimated correlations (ρ) and 95% confidence interval (CI) between estimated intercepts and slopes for physiological traits in Manchega sheep.[1]

Item	Correlation	95% CI
$\rho(RR_{intercept}, RR_{slope})$	0.42	[0.31, 0.53]
$\rho(RT_{intercept}, RT_{slope})$	0.46	[0.35, 0.58]
$\rho(RR_{intercept}, RT_{intercept})$	0.24	[0.13, 0.34]
$\rho(RR_{slope}, RT_{slope})$	0.82	[0.67, 1.00]

[1] Abbreviations: RR = Respiratory Rate, RT = Rectal Temperature.

slopes of the same trait were negative for yields (ranging from −0.5 for protein yield to −0.4 for fat yield), which indicate that most productive animals will tend to be less tolerant in terms of production, although animals gathering the desired combination of productivity and tolerance can be found provided that the magnitudes of correlations are moderate. For content traits, the estimated values of those correlations varied from slightly negative (largest negative value of −0.17 for protein percentage) to slightly positive (largest positive value of 0.25 for fat percentage). An exception was LcC, which showed a large negative value estimated for the correlation between level and slope (−0.64). As a consequence of this different behaviour of yield and content traits, estimated correlations between tolerance (slopes) obtained from different productive traits were moderate to large and positive between yield traits (from 0.31 between lactose yield and fat yield to 0.97 for lactose yield and milk yield) and variable between tolerance measured from yields and content traits (from −0.54 between lactose yield and total solid percentage and −0.06 between protein yield and protein percentage to 0.14 between fat yield and urea content and between protein yield and lactose percentage). Estimated correlations between slopes for contents were also variable, ranging between 0.97 for fat percentage and total solid percentage and for protein percentage and casein percentage and −0.11 between urea content and total solid percentage. Again, lactose percentage was an exception, showing large negative estimates with most of the content traits.

Estimated correlations between intercepts and slopes of productive and physiological traits obtained from the 2 × 2 trait analyses including one productive trait and one physiological trait are presented in Table 7. Estimated correlations between intercepts of productive and physiological traits were low, especially for rectal temperature, which showed estimates under 0.2 for all productive traits. Estimated correlations between respiratory rate and productive traits were somehow larger than the correlations with rectal temperature, ranging from 0.26 for lactose yield to −0.38 for protein percentage. The interpretation of small correlations between intercepts is that basal levels of physiological traits are not related to the level of production, at least in this study.

Table 6
Estimated correlations (95% confidence interval in brackets) between intercepts (above diagonal), between slopes (below diagonal) among different traits and between intercept and slopes (diagonal) of the same trait for production traits in Manchega sheep.

Trait[1]	My	Fy	Py	Lcy	Fp	Pp	Cnp	Lcp	Urc	TSp
My	−0.46 [−0.58, −0.34]	0.91 [0.75, 1.11]	0.95 [0.78, 1.16]	0.99 [0.81, 1.2]	−0.13 [−0.23, −0.04]	−0.14 [−0.24, −0.04]	−0.14 [−0.24,-0.04]	0.22 [0.12, 0.33]	0.13 [0.03, 0.23]	−0.10 [−0.2, −0.01]
Fy	0.48 [0.36, 0.60]	−0.40 [−0.52, −0.29]	0.91 [0.75, 1.10]	0.90 [0.73, 1.09]	0.25 [0.15, 0.35]	0.01 [−0.09, 0.11]	0.03 [−0.07, 0.13]	0.15 [0.05, 0.25]	0.16 [0.06, 0.26]	0.23 [0.13, 0.34]
Py	0.96 [0.79, 1.16]	0.55 [0.43, 0.69]	−0.50 [−0.63, −0.39]	0.94 [0.77, 1.14]	−0.02 [−0.12, 0.07]	0.15 [0.05, 0.25]	0.15 [0.05, 0.25]	0.17 [0.07, 0.27]	0.21 [0.11, 0.32]	0.09 [−0.01, 0.19]
Lcy	0.97 [0.80, 1.18]	0.31 [0.21, 0.42]	0.90 [0.74, 1.09]	−0.44 [−0.56, −0.33]	−0.15 [−0.25, −0.05]	−0.16 [−0.26, −0.06]	−0.15 [−0.25, −0.05]	0.37 [0.26, 0.48]	0.10 [0.01, 0.20]	−0.09 [−0.19, 0.01]
Fp	−0.45 [−0.57, −0.34]	0.45 [0.33, 0.57]	−0.37 [−0.48, −0.26]	−0.58 [−0.72, −0.46]	0.25 [0.15, 0.35]	0.45 [0.33, 0.57]	0.50 [0.39, 0.63]	−0.20 [−0.3, −0.10]	0.22 [0.12, 0.33]	0.91 [0.75, 1.11]
Pp	−0.24 [−0.34, −0.14]	0.31 [0.21, 0.42]	−0.06 [−0.16, 0.04]	−0.37 [−0.49, −0.27]	0.56 [0.44, 0.69]	−0.17 [−0.27, −0.07]	0.98 [0.81, 1.2]	−0.23 [−0.33, −0.13]	0.33 [0.23, 0.44]	0.70 [0.56, 0.85]
Cnp	−0.29 [−0.40, −0.19]	0.24 [0.14, 0.35]	−0.14 [−0.24, −0.04]	−0.39 [−0.51, −0.28]	0.57 [0.45, 0.70]	0.97 [0.80, 1.18]	−0.07 [−0.17, 0.02]	−0.11 [−0.21, −0.01]	0.29 [0.19, 0.40]	0.76 [0.62, 0.93]
Lcp	0.28 [0.18, 0.39]	−0.49 [−0.61, −0.37]	0.14 [0.04, 0.24]	0.49 [0.37, 0.61]	−0.74 [−0.91, −0.60]	−0.65 [−0.8, −0.52]	−0.54 [−0.67, −0.42]	−0.64 [−0.78, −0.51]	−0.15 [−0.25, −0.05]	0.01 [−0.09, 0.11]
Urc	0.19 [0.09, 0.30]	0.14 [0.04, 0.24]	0.34 [0.23, 0.45]	0.17 [0.07, 0.27]	−0.16 [−0.26, −0.06]	0.10 [0.0, 0.20]	0.02 [−0.08, 0.1]	0.09 [0.0, 0.19]	−0.10 [−0.20, 0.0]	0.26 [0.16, 0.36]
TSp	−0.43 [−0.55, −0.32]	0.41 [0.30, 0.53]	−0.34 [−0.45, −0.23]	−0.54 [−0.68, −0.42]	0.97 [0.8, 1.18]	0.66 [0.52, 0.8]	0.69 [0.55, 0.84]	−0.66 [−0.81, −0.53]	−0.11 [−0.21, −0.01]	0.18 [0.08, 0.28]

[1] Abbreviations: M = milk; F = fat; P = protein; Lc = Lactose; Cn = Casein; Urc = Urea content; TS = total solids; RR = respiratory rate; RT = rectal temperature; y = yield; p = percentage.

Table 7
Estimated correlations (ρ) and 95% confidence interval (in brackets) between intercepts and slopes of physiological (Phys) traits and each productive (Prod) trait in Manchega sheep.

	$\rho(Prod_{Intercept}, Phys_{Intercept})$		$\rho(Prod_{Intercept}, Phys_{Slope})$		$\rho(Prod_{Slope}, Phys_{Intercept})$		$\rho(Prod_{Slope}, Phys_{Slope})$	
Traits[1]	RR	RT	RR	RT	RR	RT	RR	RT
My	0.22 [0.12 0.32]	0.05 [-0.04 0.15]	0.18 [0.08 0.28]	0.56 [0.44 0.69]	−0.2 [-0.31 -0.11]	−0.05 [-0.15 0.04]	−0.36 [-0.47 -0.25]	−0.45 [-0.57 -0.33]
Fy	0.04 [-0.06 0.13]	0.03 [-0.07 0.13]	0.20 [0.10 0.31]	0.30 [0.20 0.41]	−0.32 [-0.43 -0.22]	−0.33 [-0.44 -0.22]	−0.22 [-0.32 -0.12]	0.02 [-0.08 0.12]
Py	0.11 [0.01 0.21]	0.08 [-0.01 0.18]	0.24 [0.14 0.35]	0.46 [0.34 0.58]	−0.01 [-0.11 0.08]	−0.21 [-0.31 -0.11]	−0.23 [-0.33 -0.13]	−0.18 [-0.28 -0.08]
Lcy	0.26 [0.16 0.36]	0.09 [-0.01 0.19]	0.20 [0.1 0.30]	0.61 [0.49 0.76]	−0.28 [-0.39 -0.18]	−0.16 [-0.27 -0.07]	−0.37 [-0.48 -0.26]	−0.50 [-0.63 -0.39]
Fp	−0.35 [-0.46 -0.24]	−0.16 [-0.26 -0.06]	0.02 [-0.07 0.12]	−0.47 [-0.59 -0.35]	−0.54 [-0.67 -0.42]	−0.37 [-0.49 -0.27]	0.11 [0.01 0.21]	0.01 [-0.08 0.11]
Pp	−0.38 [-0.5 -0.28]	0.03 [-0.07 0.12]	0.29 [0.19 0.40]	−0.38 [-0.5 -0.28]	0.65 [0.52 0.79]	−0.16 [-0.27 -0.07]	0.63 [0.50 0.77]	0.46 [0.35 0.58]
Cnp	−0.33 [-0.44 -0.22]	0.01 [-0.09 0.11]	0.23 [0.13 0.34]	−0.41 [-0.53 -0.30]	0.54 [0.42 0.68]	−0.10 [-0.20 0.0]	0.53 [0.41 0.66]	0.43 [0.32 0.55]
Lcp	0.28 [0.18 0.39]	0.14 [0.04 0.24]	0.03 [-0.07 0.12]	0.17 [0.07 0.27]	0.15 [0.05 0.25]	0.19 [0.09 0.29]	−0.46 [-0.59 -0.35]	0.07 [-0.03 0.17]
Urc	−0.20 [-0.30 -0.10]	−0.08 [-0.17 0.02]	0.14 [0.04 0.24]	0.11 [0.01 0.21]	0.16 [0.06 0.26]	−0.03 [-0.13 0.07]	0.37 [0.27 0.49]	0.85 [0.69 1.00]
TSp	−0.37 [-0.49 -0.27]	−0.07 [-0.17 0.02]	0.13 [0.03 0.23]	−0.50 [-0.62 -0.38]	−0.53 [-0.66 -0.41]	−0.46 [-0.59 -0.35]	0.20 [0.10 0.30]	−0.11 [-0.21 -0.01]

[1] Abbreviations: M = milk; F = fat; P = protein; Lc = Lactose; Cn = Casein; Urc = Urea content; TS = total solids; RR = respiratory rate; RT = rectal temperature; y = yield; p = percentage.

Estimated correlations between intercepts of production traits and slopes of response to heat in physiological traits were weak for respiratory rate (<0.29) and moderate for rectal temperature, ranging from 0.61 for lactose yield to −0.5 for total solid percentage. Tolerance (slopes) of physiological traits showed a positive correlation with yield level (intercept), which would imply that animals with larger levels of production will tend to show larger positive slopes of response, i.e. less tolerance to heat stress.

Estimated correlations between basal levels of physiological traits and tolerance measured by slope of decrease in productive traits tended to be negative for yields (ranging from −0.33 between fat yield and rectal temperature and −0.01 between protein yield and respiratory rate) which mainly would mean that animal with a high basal rectal temperature and to a less extent respiratory rate would suffer a larger decay in yield and therefore tend to be less tolerant to heat stress. Estimated correlations for percentages were widely variable, showing either large negative values (from −0.31 to −0.54) for fat percentage and total solid percentage and positive values, 0.65 for protein percentage and 0.54 for casein percentage with respiratory rate.

Finally, estimated correlations between tolerance (slopes) from productive and physiological traits, which was one of the main targets of the study, were null or moderately negative for yields (ranging from 0.02 for fat yield and rectal temperature to −0.45 between milk yield and rectal temperature). Negative values between slopes from the two groups of traits indicate that tolerant animals for production (less negative or positive values of individual deviations for slopes) tend to be also tolerant from the perspective of the physiological variables (less positive or negative slopes). Again, for content traits, the opposite, positive estimated correlations (ranging from 0.01 between fat percentage and rectal temperature to 0.85 between urea content and rectal temperature) were observed, except for lactose percentage (-0.46).

Discussion

The main objective of this study was to determine the degree of concordance between alternative measures of heat tolerance defined as the rate of change of a trait as heat load increases (slopes of the reaction norm). In addition, estimated correlations involving basal levels of the traits (intercepts of the reaction norm) provided useful information of the expected change in production levels of traits of economic interest when improving heat tolerance.

As previously stated, the rate of increase in body temperature as heat load increases can be considered as the gold standard to measure the thermoregulatory capacity of an animal and, hence, its heat tolerance level. Results from this study showed a strong association between the rate of change in rectal temperature and respiratory rate beyond the heat stress threshold, reflecting the closeness between the activation of higher respiratory rate and increases in rectal temperature under heat stress. This result reinforces the use of respiratory rate as a non-invasive and easier to record proxy for thermal regulation. Lower levels of rectal temperature and respiratory rate have been also claimed as indicators of thermotolerance when comparing breeds adapted to high temperatures vs breeds from temperate climates in both sheep (Joy et al., 2019) and cattle (Hansen, 2004). Lower metabolic rates found in breeds originated in hot or warm climates (Hammond et al., 1996) might be associated with the lower body temperatures and respiratory rate in adapted breeds. In our study, animals with lower basal levels of those traits also tended to show smaller slopes of increase under heat stress, supporting the idea of low basal levels of respiratory rate or rectal temperature being indicators of heat tolerance. Nevertheless, sometimes respiratory rate and rectal temperature could be not related when exists conflict between thermoregulation and osmoregulation; e.g. dehydrated sheep may activate selective brain cooling in order to decrease evaporative water loss (and respiratory rate), while storing more body heat, by increasing rectal temperature. This is a very important water-saving mechanism in sheep adapted to hot and dry regions (Fuller et al., 2007; Hetem et al., 2011).

The relationship between basal levels and slopes of decay for production traits and physiological traits observed in the study provided a broad perspective of heat stress effects and identification of heat-tolerant animals. Animals that were able to maintain the body temperature (smaller slopes of increase) also tended to maintain productivity (smaller productive decays) in terms of yields under heat stress (with the largest correlation between the two types of heat tolerance indicators found for milk yield and lactose yield). Another important consideration in the identification of tolerant animals with possible implications in the long-term control of adaptation to heat is the unfavourable association found between productive levels (yields) and the ability to maintain homeothermy under heat stress (slope of increase in rectal temperature). The results found in this study confirm and quantify the generally accepted premise that a high productive level limits the ability of animals to maintain homeothermy (see, e.g. Bernabucci et al., 2010). Nevertheless, in our study, this relationship was only moderate, which might be due to the fact that productive levels are not sufficiently high in this breed as to hamper the control of body temperature or to the fact that this breed is originated in the Mediterranean area and, therefore, better adapted to high temperatures.

Although not the main goal of this study, the results relative to the response of the studied traits under thermoneutral or heat stress conditions using different ways to define the heat load on the animals are regarded also as of potential interest. Temperature or THI at the time of recording and milk sampling were the parameters that showed the best fit of the observed data for both yield traits and physiological traits, compared to daily average or cumulative values of thermal loads. This was more evident for physiological traits and in particular for rectal temperature. Increases in rectal temperature and respiratory rate with ambient temperature at the time of collection have been observed in many studies (see, e.g. Spiers et al., 2004; Atkins et al., 2018 in cattle and Slimen et al., 2019 for a meta-analysis in sheep), but never compared with the use of the accumulated or average temperatures in the previous hours or days. The results of this study suggest that response in rectal temperature or respiratory rate to increases in temperature is nearly immediate and not lagged or cumulative with respect to ambient conditions. In homeothermic animals, the increase in respiratory rate is an important heat dissipation mechanism that is activated in response to an increase in ambient temperature, and whose failure would result in an increase in body temperature. On the other hand, it is widely accepted that response in milk production is expected to lag from hours to one or two days behind the increase in temperatures (West, 2003; Spiers et al., 2004). However, in this study, the heat load measured at the time of milk sampling showed a slightly better fit than heat loads that take into account previous temperatures and relative humidity. Similar results were found in the study by Ramón et al. (2016), using test day data from the same breed, finding a slightly better fit in yields for the day of recording compared to temperatures from previous days. The fact that high temperatures are steady along the summer months in this region, as it is the case in all the Mediterranean area, may explain the lack of relevant differences when comparing lagged vs day of sample collection responses. For content traits, inconsistent results were found for the best fit for alternative types of heat load measures with a mixture of time of collection vs cumulative or daily values providing the best fit, with a trend for these last types to provide better fits.

Rates of change for traits under high temperatures were substantial for all traits, indicating that heat stress effect on both productive and physiological traits exists. In terms of percentage of change (units of the trait/one unit of the heat load variable) with respect to the mean of the value, yield traits and respiratory rate showed similar values, with rates of change of around 4% for the yield traits and respiratory rate. For rectal temperature, a smaller percentage of change was observed, 0.15% of the mean of this trait, as expected for this trait, with very small variation of values, correspondingly with the homeothermic nature of mammals. Nevertheless, rectal temperature had the highest percentage of rate of change of all traits in terms of percentage of the standard deviation of this trait, 15%. For content traits, the percentage of loss with respect to the mean of the traits ranged from 1% to 2% per degree of increase in temperature. Lactose yield showed the smallest change, 0.6% of the mean and 9% of the standard deviation of the trait per degree of increase in temperature. The estimated slopes of change for yield traits in this study match well with the estimates in the study of Finocchiaro et al. (2005) who reported slightly larger rates of loss in milk yield, around −60 g/°C in daily production, compared with the −50 g/°C slope of decrease found in this study for that trait. On the other hand, Ramón et al. (2016) in the already mentioned study carried out on the same breed reported much lower losses associated with thermal stress, around −0.2 g/°C of maximum daily temperature for fat or protein yield, while in our study, the slopes of decay were around −2.0 g/°C. The fact that a closer follow-up of production recording, compared with the monthly recording of a very large number of animals and the use of daily average temperature in the study of Ramón et al. (2016), might explain a smaller ability to capture heat stress losses in that study, as suggested in Freitas et al. (2006) in dairy cattle.

The only trait that did not show losses associated with heat stress was lactose percentage, despite the decrease in lactose yield. Baumgard et al. (2011) observed an over-reduction of lactose secretion in cows under heat stress compared with pair-fed cows under thermoneutrality, inferring that reduced milk lactose output under heat stress may be an effect of the changes in the metabolic routes deriving from an abnormal liver functioning. These authors suggest that under heat stress altered rates of hepatic gluconeogenesis would ultimately reduce glucose delivery to the mammary gland and thus, the lactose synthesis. However, no significant changes in lactose percentage in milk in the heat-stress cows was observed in that study. Given that lactose is the main regulator of the osmotic pressure in milk, and consequently of milk volume, the extra reduction of lactose secretion associated with heat stress may cause an extra reduction of milk volume, that may result in no decreasing (and possibly the small increase observed in our study) of lactose percentage.

The results from using temperature and relative humidity inside the barn vs data from the closest weather station provided insights about the feasibility of using information from weather stations located close to farms, which is the common practice in studies dealing with large milk recording systems. Our results showed high correlations between both, barn and weather station thermal variables, especially for temperatures and less for THI. Estimates of rates of change of the trait per degree or unit of the heat load variable were very similar for both measures for all traits. However, the relationship between barn and close weather stations will mostly depend on the buffering capacity of the facility, which in turn, will determine its level of thermal inertia over the day and magnitude of differences between heat load inside and outside the barn. Overall, although barn temperatures are expected to represent a closer value of the heat load and fluctuations that animals bear, the use of WS values could be a good compromise when using data from commercial farms where no barn collection of meteorological information is available. This was also the conclusion of the study of Freitas et al. (2006) in dairy cattle.

Conclusion

Measures of thermal tolerance through changes in productive or physiological indicators showed that measurement of the environmental conditions at time of recording of the physiological traits and collection of milk samples provides the best fit for both physiological and yield traits. The use of meteorological information from weather stations close to the farms provided similar estimates of the response of traits to increasing heat loads as the meteorological information collected on the farm, which validates the use of weather station data when no on-farm information is available.

Heat-tolerant animals defined as those that are able to maintain body temperature tend to be also tolerant in terms of being able to maintain or diminish losses in production associated with heat stress. However, because of the non-unity correlation between the two types of criteria and because of the difficulty of maintaining homeothermy under high levels of production, both sources of information, productive and physiological, ought to be taken into account when pursuing the improvement of heat resilience farming in the long term.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100662.

Ethics approval

Animal manipulations were performed according to the Spanish Policy for Animal Protection RD 1386/2018 and RD 53/2013, which meets the European Union Directive 86/609 about the protection of animals used in experimentation. The study involved only routine management practices in animal husbandry.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from authors upon request.

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Declaration of interest

The authors declare that no competing interests exist.

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