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LINEAR ANALYSIS OF RUMEN THERMOLOGGER DATA TO COMPARE TOLERANCE TO HEAT CHALLENGE IN EIGHT SHEEP BREEDS

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SUMMARY

The aim of this study was to analyse rumen temperature (RT) in eight sheep breeds recorded for a four-month period during a hot and arid summer in Mediterranean South Africa. Challenges in linear mixed model analysis of the automatically recorded rumen thermologger data included the effect of drinking, a high level of autocorrelation between frequent observations, and a visible lag period between the hourly recorded temperature humidity index (THI) and RT. Marked differences in RT existed between breeds at high THI, with lowest to highest RT predicted for the Namakwa Afrikaner, Dorper, Meatmaster, Dormer, Ile de France, Merino, South African Meat Merino, and Dohne Merino. The low ranking of the composite and purely indigenous breeds supported the hypothesis that these breeds have a higher capacity to tolerate heat stress.

INTRODUCTION

In South Africa (SA), a large proportion of sheep producers favour exotic Merino-type breeds for intensive and extensive meat and wool production. These breeds produce large carcasses and, at varying levels, an additional income through wool. However, they are expected to be less heat-tolerant, based on respiration rate (Cloete *et al.* 2021). The indigenous sheep breeds of South Africa are known for their distinctly short, hairy coats, slender frames, and fat tails – attributes often assumed related to adaption to high heat load (Molotsi *et al.* 2019). However, standardised testing that includes indigenous South African breeds is rare, and when it has been done, was conducted over short exposure times. Moreover, the review by McManus *et al.* (2020) concluded that variance in heat tolerance is not well explained by the hair-type vs. wool-type criteria. Considerable variation could also exist within these groups, depending on the history of each breed.

A comparison among breeds requires a measure of animal strain in response to different levels of heat stress. Recently, the use of automated rumen thermologgers was proven to provide a reliable proxy for core temperature in sheep (Vesterdorf *et al.* 2022) after already having shown potential for use in cattle (Liang *et al.* 2013). This method delivers automated repeated measurements at a very high density (~ every 10 mins) over extended periods (~ months or years). However, the high-density form of the data differs from that usually evaluated by linear mixed models by, most notably, not qualifying as independent observations.

Here we report on a preliminary analysis and results after using this method to compare eight well-known sheep breeds over a hot and dry summer season in Mediterranean SA. Although other approaches to interpret these data exist (Vesterdorf *et al.* 2022), the use of the linear mixed model framework was a deliberate decision and some of the challenges encountered in this process are discussed from the point of view that similar data could in future substantiate a genetic analysis. From this data, across breed prediction curves including exotic, composite, and indigenous breeds are presented to contribute to the current gap of similar datasets.

MATERIALS AND METHODS

Study site and period. The experiment was conducted at the Langgewens research farm near Moorreesburg in the Western Cape Province of SA. The farm is situated in the winter-grain cropping-pasture area, which is known for hot, dry summers with maximum temperatures commonly exceeding 30°C. Data recording commenced in early summer for roughly four months from 21 November 2023 to 4 March 2024.

Genetic resources. The eight sheep breeds included the Merino, Dohne Merino, South Africa Meat Merino (SAMM), Ile de France (IDF), Dormer, Dorper, Meatmaster, and the Namakwa Afrikaner (NA). For each breed, eight entire ram lambs were randomly screened from within the Langgewens multibreed sheep flock (Cloete *et al*. 2021). This flock is managed as a single group throughout the year, thus ensuring a standardised background for individuals from different breeds. The lambs were born during April – July of 2023, and the average (± SD) age of rams at the start of the experiment was 168 ± 18 days.

Data. Climate data recorded at a weather station installed close to the paddocks were used to derive an hourly measure of heat stress using the temperature humidity index (THI) as defined by LPHSI (1990). Once each month, the live weight (LW; Kg), fat depth (FATD; in mm), and coat depth (COATD; in mm) were measured on each animal.

Rumen temperature (RT) was recorded using custom made thermologgers (Bryn O Morgan Industries, Perth), which were built into rumen capsules. The loggers were programmed to record RT every 5 minutes and calibrated against a certified platinum sensor thermometer (CENTER 376) before and after deployment in the animals. Each logger recorded 34,327 measurements over the experimental period. Following Vesterdorf *et al.* (2022), any measurement that showed an absolute change > 0.40°C from the previous measurement was flagged along with the next 11 measurements (in total, one hour) as being associated with drinking. In contrast to Vesterdorf *et al.* (2022), these values were set to missing instead of imputing an average. The remaining datapoints were used to calculate an average RT value for every hour, to reduce the level of pseudo-replication and because climatic data were only available at hourly intervals. Lastly, the total dataset spanning across four months was subset to four periods within each month spanning seven days chosen manually to: (1) include both low and high average THI values; (2) be separated by at least 20 days from another replicate period; and (3) not including the dates of animal handling. Finally, the data consisted of 64 animals recorded for four replicates (Month) each with 168 (7 days x 24) hourly RT measurements.

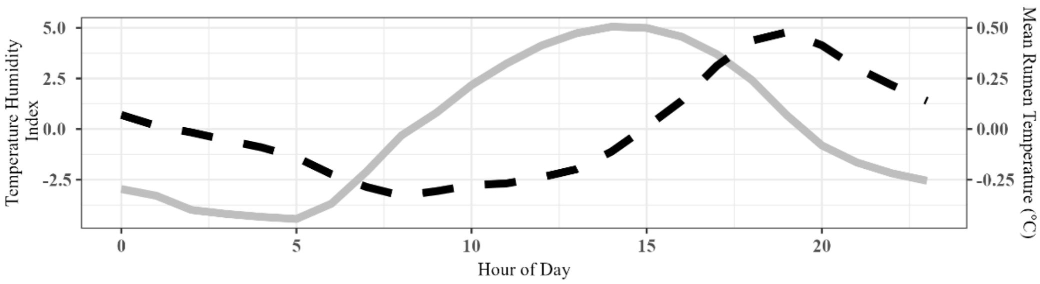

Figure 1. Hourly mean of the temperature humidity index (solid, gray line) and rumen temperature (dashed, black line) as recorded across the four months of the experiment. Values were scaled to zero by the overall mean(s)

The average raw means for a typical daily 24-hour cycle are shown in Figure 1. Several issues arise when analysing these data. Firstly, the co-dependence on a daily cycle causes an issue when both THI and an hour-of-day term are included in the model. Ignoring this term might not be ideal, since there could be factors other than THI, such as rumination (Liang *et al.* 2013), that can affect the diurnal rhythm of RT. Secondly, a marked lag effect is present on almost all days with THI reaching its peak at approximately 2pm, when the average RT is still increasing to peak later at around 5pm. This complicates any regression of $RT_i \sim THI_i$ where *i* is hour of the day. To overcome this issue, the row-axis linking records was redefined to allow for different starting points for each daily 24-point cycle. The nominated starting point was chosen based on the respective lowest values according to the raw means in Figure 1, being 5am and 8am, respectively. Thus, a regression $RT_i \sim THI_i$ was defined as, for example, the effect of THI at 2pm on the RT at 5pm.

Statistical analysis. Followed by building a linear mixed model using ASREML V4.2 software (Gilmour *et al.* 2021). Fixed effects were tested using conditional Wald F-statistics and included Breed (8 levels), the environmental variable THI (15:32) and the animal measurements LW (29:69 kg), FATD (0.09:0.53 mm) and COATD (8.3:55.6 mm) as linear covariates. The addition of random terms followed, using changes in Log-Likelihood values compared to the nested model. Random effects included animal ID (64 levels) as well as animal within month compounded (ID.Month: 248 levels) as identity terms. The model design also included testing of a first order autoregressive structure (AR1) accounting for the temporal autocorrelation between samples $t+1$ and t where $t = 1$ to $t = 168$ within a Month. Finally, prediction of RT according to variation in THI was allowed to follow a curvilinear trend modelled by a cubic smoothing spline with eight knot points nested within Breed.

RESULTS AND DISCUSSION

Model definition. The fixed effects in the model included a significant interaction between THI x Breed ($P < 0.01$), while the covariates LW, FATD, and COATD did not affect RT ($P > 0.05$). Random terms that were retained included ID ($P < 0.01$) and ID.Month ($P < 0.05$). As expected, measurements taken at adjacent timepoints were strongly correlated ($P < 0.01$) with the AR1 autocorrelation parameter p = 0.91. However, it is not yet certain whether this is the best possible approach to incorporate high density data into a linear mixed model framework. The study of Liang *et al.* (2013) used daily means of THI and RT, where some information was lost but with reduced dependence between observations. A study of genetic analysis of comparable datasets (vaginal thermometers in sows) using a repeatability model exists (Freitas *et al.* 2023), but it is not clear how dependence between adjacent measurements was accounted for.

From preliminary analyses, it is notable that using splines to model a daily RT cycle (i.e. a prediction of the trend in Figure 1) also produced informative results as long as THI was not modelled. A viable alternative might be to categorise days according to mean temperature (cool, mild, hot, etc.) and to obtain these spline solutions within each category for each breed. Alternatively, excluding THI and estimating AR1 for each breed could be a good indicator of resilience to repeated changes in heat load (Ghaderi Zefreh *et al.* 2024). Using this approach, groups with a greater fluctuation from the general mean of RT will have higher measures of temporal autocorrelation over the observed period.

Breed response curves to THI. The predicted change in RT for each of the eight sheep breeds is shown in Figure 2. A slow initial increase in RT with increased heat load accords with the trends shown by Liang *et al.* (2013). At THI between 25 – 27, there was a curvilinear increase in RT in all breeds, but high variability for predicted RT when THI exceeded 30. At the highest THI, the RT (in ˚C) for Dohne Merino (40.5 ± 0.1), SAMM (40.4 ± 0.1), and Merino (40.4 ± 0.1) were the highest, while the IDF (40.3 ± 0.1), Dormer (40.2 ± 0.1) and Meatmaster (40.2 ± 0.1) trended intermediately, while the Dorper (40.0 ± 0.1) and Namakwa Afrikaner (39.9 ± 0.1) had the lowest RT values. These differences could be judged almost negligible at the absolute level, but it is important to consider the extremely narrow distribution of the RT data (CV = 1.3% in the pruned dataset). Based on eye temperature and respiration rate, Cloete *et al.* (2021) similarly showed that the Namakwa Afrikaner (indigenous) and composites with indigenous parent breeds (Meatmaster and Dorper) were better able to manage heat stress than were purely exotic Merino type breeds. It is of interesting to observe a similar result based on RT data with the contention that this capacity for heat tolerance is not explained by any of the measurable differences in LW, FATD, or COATD. However, some response curves, such as that of the IDF, is difficult to explain, and more information is needed to comprehend re-ranking of breeds across different THI levels.

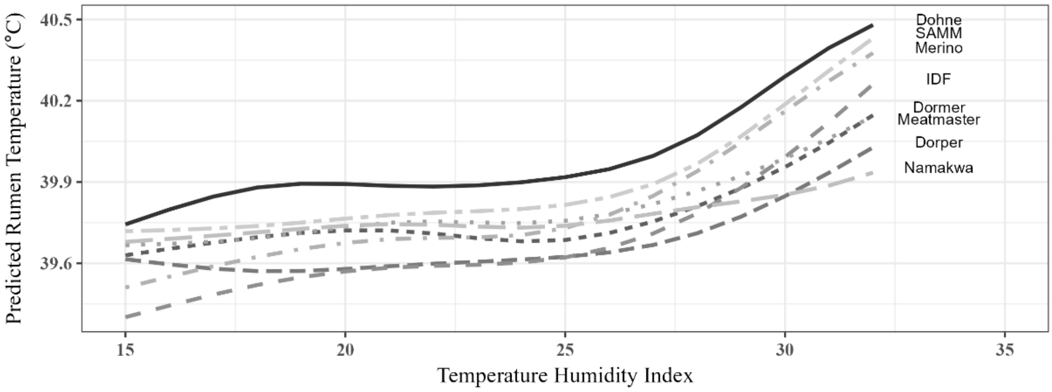

Figure 2. Predicted rumen temperature for eight sheep breeds across the observed temperature humidity index

CONCLUSION

Incorporating high density thermologger data into the standard linear mixed model framework creates challenges, but a large-scale genetic analysis remains feasible in future. On the breed level, indigenous types appear to have a quantifiable advantage under high heat load that is not explained by weight or coat depth. This points to an adaptive developmental history of breed genetics, but a larger dataset would be helpful in confirming or refuting these results.

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