

Studies on the response of lambs and ewes to changes in the ambient climate owing to climate change

By

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The crest of Stellenbosch University is centered behind the text. It features a shield with a red and white checkered pattern, a blue chief with a white cross, and a red and white banner at the bottom with the motto 'Pacta sunt quibus recti'. The crest is surrounded by a red and white wreath.

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Declaration

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Summary

This study investigated the impact of climate instability on neonatal lamb behaviour as a proxy for lamb survival in a winter lambing season in the Western Cape, as well as the impact of increased temperatures on heat stress indicator traits in mature ewes in the Western and Eastern Cape. Historic data were used to establish the relationship of neonatal lamb behaviour with cold stress and to derive genetic parameters for early lamb behaviour. Merino lambs of the Elsenburg flock were phenotyped for behaviour latencies from birth to first standing (LTBS) and from standing to first suckling (LTSS). These data were analysed in relation to a cold stress gradient (CSG) derived from a combination of wind, rain, and temperature. The flock consisted of a line selected for number of lambs weaned per ewe mated (NLW; the High or H-Line) and a line selected against NLW (Low or L-Line). H-line lambs progressed faster from standing to first suckling than L-line lambs. Increased CSG-levels resulted in 18% faster progress in LTBS when mild conditions at $800\text{kJm}^{-2}\text{h}^{-1}$ were compared to stressful conditions at $1200\text{kJm}^{-2}\text{h}^{-1}$. LTSS was compromised by 76.0% as CSG-values increased from $800\text{kJm}^{-2}\text{h}^{-1}$ to $1200\text{kJm}^{-2}\text{h}^{-1}$. Heritability estimates were 0.22 for LTBS and 0.06 for LTSS. The maternal heritability for LTBS was 0.06 and the dam permanent environment variance ratio 0.09 for LTSS. The traits were not genetically correlated. Further research is required to understand the responses of lambs to cold stress. Average temperatures are likely to increase, resulting in hotter and dryer conditions in South Africa. The impact of these changes on animal production and welfare is not well-defined. Three trials were conducted on 10 to 20 mature, dry ewes at 2 locations to determine the homeothermic response of nine sheep breeds between 2016 and 2022. These breeds were the Dohne, Dorper, Dorper, Ile de France, Meatmaster, Merino, Namaqua Afrikaner, South African Mutton Merino (SAMM) and White Dorper. They were studied in different combinations across trials. On days forecast to be hot at noon, animals were assessed under cool conditions in the morning and hot conditions at noon/afternoon by monitoring individual rectal temperature, eye temperature (thermal imaging), spot temperature, and respiration rate. The increased heat in the afternoon sessions markedly increased all traits across trials. In general, hair breeds (Dorper, White Dorper, Meatmaster, and particularly Namaqua Afrikaner) were able to maintain lower basal respiration rates compared to the wool breeds originating from temperate regions (Dohne, Dorper, Ile de France, Merino, and SAMM). These results suggest that hair sheep and hardy indigenous breeds such as the Namaqua Afrikaner may cope better with the anticipated higher heat load in the future when compared to the breeds originating from temperate regions. Rectal temperature was low-moderately repeatable across trails, while outcomes for respiration rate and spot temperature were more variable and commonly affected by the reranking of ewes across sessions as well. Eye temperature was not repeatable. There is still marked scope for research on ovine adaptation to heat stress conditions in South Africa.

Opsomming

Die impak van 'n onstabiele klimaat is ondersoek deur neonatale lamgedrag as aanduiding van lamoorlewing in die Wes-Kaap en hittestresindikatoreienskappe se reaksie op 'n verhoogde vlak van hittestres in die Wes- en Oos-Kaap te ondersoek. Historiese data is gebruik om die verwantskap van lamgedrag met kouestres te ondersoek en terselfdertyd genetiese parameters vir vroeë lamgedrag te beraam. Merinolammers op Elsenburg is aangeteken vir die gedragstye van geboorte tot staan (LTBS) en van staan tot suip (LTSS). Hierdie data is in verhouding tot 'n kouestresgradiënt (CSG), gebaseer op wind, reën en temperatuur, ontleed. Die kudde het uit lyne wat vir aantal lammers gespeen (NLW; die Hoë of H-Lyn) en teen NLW (Lae of L-Lyn). H-lyn lammers het vinniger van staan tot suip gevorder as L-lyn lammers. 'n Toenemende CSG het gelei tot 'n 18% vinniger vordering in LTBS gelei, as gematigde toestande van $800\text{kJm}^{-2}\text{uur}^{-1}$ met stresvolle toestande van $1200\text{kJm}^{-2}\text{uur}^{-1}$ vergelyk is. LTSS is met 76.0% benadeel soos CSG-waardes toegeneem het van $800\text{kJm}^{-2}\text{uur}^{-1}$ na $1200\text{kJm}^{-2}\text{uur}^{-1}$. Oorerflikhede was 0.22 vir LTBS en 0.06 vir LTSS. Die maternale oorerflikhede was 0.06 vir LTBS, terwyl die maternale permanente omgewing 0.09 was vir LTSS. Die eienskape was nie geneties gekorreleerd nie. Verdere navorsing word benodig om die reaksie van lammers op kouestres te verstaan. Gemiddelde temperature word verwag om toe te neem, wat daartoe lei dat omstandighede in Suid-Afrika warmer en droeër gaan word. Die invloed van hierdie veranderinge op diereproduksie en -welsyn is nog nie goed gekwantifiseer nie. Drie proewe is met 10 tot 20 volwasse, droeë ooie is op twee lokaliteite uitgevoer om die homeotermiese reaksie van nege skaaprasse op hoër temperature tussen 2016 en 2022 te bestudeer. Hierdie rasse was die Dohne, Dorper, Dorper, Ile de France, Meatmaster, Merino, Namakwa Afrikaner, Suid-Afrikaanse Vleismerino (SAMM) en Wit Dorper. Die rasse is in verskillende kombinasies oor proewe bestudeer. Die diere is onder koel toestande in die môre en warm toestande oor die middag bestudeer deur die monitoring van individuele rektale temperatuur, oogtemperatuur (met termiese beelde), koltemperatuur, en respirasietempo. Die warmer toestande oor middae het gelei daartoe dat alle eienskappe verhoog het. Haarrasse (Dorper, Wit Dorper, Meatmaster, en veral Namakwa Afrikaner) was beter in staat om 'n laer basale respirasietempo te handhaaf as wolrasse en ander rasse van gematigde streke (Dohne, Dorper, Ile de France, Merino, en SAMM). Hierdie resultate dui daarop dat haarskape en geharde, inheemse rasse soos die Namakwa Afrikaner beter op die verwagte hoër hittelading sal reageer as rasse uit die gematigde streke. Rektale temperature was laag-matig herhaalbaar oor proewe, terwyl uitkomstes vir respirasietempo en koltemperatuur meer variërend was en ook deur die interaksie tussen ooie en warm versus koeler omstandighede beïnvloed is. Oogtemperatuur was nie herhaalbaar nie. Daar is steeds ruimte vir verdere navorsing op die aanpassing van skape by hittestres in Suid-Afrika.

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Preface

This thesis is presented as a compilation of 8 chapters. Each chapter is introduced separately and is written according to the style of the South African Journal of Animal Sciences.

Chapter 1 **General Introduction and Project Aims**

Chapter 2 **Literature Review**

Chapter 3 **Materials and Methods**

Chapter 4 **Research Chapter**

Genetic and environmental parameters for birth weight and behaviour of neonatal Merino lambs in relation to cold stress

Chapter 5 **Research Chapter**

Responses to heat in ewes from indigenous and commercial South African sheep breeds: Preliminary Results

Chapter 6 **Research Chapter**

Quantifying *ovine* responses to hot conditions, as well as between and within animal components of variation in common heat stress indicators in a Western Cape flock

Chapter 7 **Research Chapter**

Quantifying *ovine* responses to hot conditions, as well as between and within animal components of variation in common heat stress indicators in a Northern Cape flock

Chapter 8 **General Discussion and Conclusions**

Outputs

The following chapter has been published in an accredited peer reviewed conference proceedings:

Chapter 5: Cloete, S.W.P., van der Merwe, D.A., Steyn, S., Muller, A., Melgar, J., Cornwallis, C.K. & Brand, T.S., 2021. Responses to heat in ewes from indigenous and commercial South African sheep breeds: preliminary results. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* 24, 59-62. (ISSN number – 1328-3227)

The following work from this study has been presented as oral or poster presentations or published as a conference paper at national and international symposia:

- S.W.P. Cloete, D.A. van der Merwe, S. Steyn, A. Muller, J. Melgar, C.K. Cornwallis, T.S. Brand, 2021. Responses to heat in ewes from indigenous and commercial South African sheep breeds: Preliminary Results. 24th Association for the Advancement of Animal Breeding and Genetics Conference. 2-4 November 2021. On-line with local hubs in Australia (Brisbane Qld, Armidale NSW, Melbourne Vic, Adelaide, SA, and Perth WA) and New Zealand (Dunedin). (Conference paper).
- S.W.P. Cloete, C.L. Nel, S. Steyn, T.S. Brand, 2021. Future climatic instability: Assessment of environmental stressors in sheep. 56th Grassland Society of Southern Africa Conference. 26-30 July 2021. Surval Boutique Olive Estate, Oudtshoorn, Western Cape. (Invited oral presentation).
- S. Steyn, S.W.P. Cloete, T.S. Brand, 2022. Responses of Merino, White Dorper and Meatmaster ewes to an increased heat load. 73rd Annual Meeting of European Federation of Animal Sciences Conference. 4-9 September 2022. Alfândega Congress Centre, Porto, Portugal. (Oral presentation).
- S. Steyn, S.W.P. Cloete, C.L. Nel, A.J. Scholtz, J.J.E. Jasper & T.S. Brand, 2022. Genetic and environmental parameters for birth weight and behaviour of neonatal Merino lambs in relation to cold stress. 53rd Annual South African Society for Animal Sciences Congress, 26-28 September 2022, Pietermaritzburg, KZN. (Oral presentation).

List of abbreviations

ANOVA	- Analysis of Variance
bpm	- breaths per minute
BW	- Birth weight
CSG	- Cold stress gradient
CV	- Coefficient of variation
GLM	- General linear model
H	- High
IDF	- Ile de France
L	- Low
LTBS	- Latency from birth to standing
LTSS	- Latency from standing to apparent first suckling
N/A	- Not applicable
NLW	- Number of lambs weaned per ewe mated
SAMM	- South African Mutton Merino
SD	- Standard Deviation
SE	- Standard Error
SME	- Starvation-mismothering-exposure
THI	- Temperature-humidity index
W	- Body weight

Table of Contents

Declaration	ii
Summary	iii
Opsomming	iv
Acknowledgments	v
Preface	vi
Outputs	vii
List of abbreviations	viii
Table of Contents	x
Chapter 1. General Introduction and Project Aims	1
References	4
Chapter 2. Literature Review	5
2.1 Introduction	5
2.2 Climate Change	6
2.3 Heat Stress Impact	8
2.3.1 Behavioural and physiological patterns	8
2.3.2 Ewe fertility	10
2.4 Ewe and Lamb Interactions	10
2.4.1 Physiological aspects	11
2.4.2 Behavioural aspects	11
2.5 Inclement Weather and Neonatal Survival	12
References	15
Chapter 3. Materials and Methods	19
3.1 Interventions facilitating the recording of those data needed for this study	19
3.1.1 Identification of individuals	19
3.1.2 Tinytag Plus 2 Data Logger and Weather Screen	19
3.1.3 Lamb Behavioural Traits	20
3.1.4 Body Weight Measurement	21
3.1.5 Fat Depth Measurement	21
3.1.6 Fleece Depth Measurement	22
3.1.7 Respiration Rate Recording	23
3.1.8 Core Body Temperature Measurements	23
3.1.8.1 Standard Clinical Thermometer – Rectal Temperature	23
3.1.8.2 Laser-based Thermometer – Spot Temperature	24
3.1.8.3 FLIR E75 Thermal Camera – Eye Temperature	24
3.1.9 Accelerometers – activity monitoring	25
References	27

Chapter 4. Genetic and environmental parameters for birth weight and behaviour of neonatal Merino lambs in relation to cold stress **29**

4.1 Abstract	29
4.2 Introduction	30
4.3 Materials and Methods	31
4.3.1 Animals, Selection Procedures, and Location	31
4.3.2 Observations and Recordings	32
4.3.3 Cold stress gradient	33
4.3.4 Statistical Analysis	34
4.4 Results	35
4.4.1 Descriptive statistics	35
4.4.2 Fixed effects	36
4.4.3 Cold stress gradient	37
4.4.4 Log Likelihood values for random effects	37
4.4.5 Genetic parameters	38
4.5 Discussion	39
4.5.1 Descriptive statistics	39
4.5.2 Fixed effects	39
4.5.3 The effect of the cold stress gradient (CSG) on neonatal progress	41
4.5.4 Random effects	43
4.5.5 Genetic correlations	44
4.6 Conclusion	45
References	46

Chapter 5. Responses to heat in ewes from indigenous and commercial South African sheep breeds: Preliminary Results **48**

5.1 Abstract	48
5.2 Introduction	48
5.3 Materials and Methods	49
5.4 Results and Discussion	50
5.5 Conclusion	52
References	53

Chapter 6. Quantifying ovine responses to hot conditions, as well as between and within animal components of variation in common heat stress indicators in a Western Cape flock **54**

6.1 Abstract	54
6.2 Introduction	54
6.3 Materials and Methods	56
6.3.1 Animals, Location and Recordings	56
6.3.2 Temperature-Humidity Index (THI)	57
6.3.3 Statistical Analysis	58
6.4 Results	59
6.4.1 Descriptive statistics	59
6.4.2 Temperature-Humidity Index (THI)	60
6.4.3 Breed effects on live weight, fat depth and fleece depth	61
6.4.4 Fixed effects	62
6.4.5 Log Likelihood values and random effects	70

6.5 Discussion	71
6.5.1 Descriptive statistics	71
6.5.2 THI	71
6.5.3 Breed effects	72
6.5.4 Heat stress indicator traits	72
6.5.4.1 Rectal Temperature	72
6.5.4.2 Respiration Rate	73
6.5.4.3 Spot Temperature	75
6.5.5 Between ewe variation	75
6.6 Conclusion	76
References	77

Chapter 7. Quantifying *ovine* responses to hot conditions, as well as between and within animal components of variation in common heat stress indicators in a Northern Cape flock **80**

7.1 Abstract	80
7.2 Introduction	80
7.3 Materials and Methods	83
7.3.1 Animals, Location and Recordings	83
7.3.2 Temperature-Humidity Index (THI)	84
7.3.3 Statistical Analysis	84
7.4 Results	85
7.4.1 Descriptive statistics	85
7.4.2 Temperature-Humidity Index (THI)	86
7.4.3 Breed effects on live weight, fat depth and fleece depth	87
7.4.4 Fixed effects	88
7.4.5 Log Likelihood values and random effects	95
7.5 Discussion	96
7.5.1 Descriptive statistics	97
7.5.2 THI	97
7.5.3 Breed effects on live weight, fat depth and fleece depth	98
7.5.4 Heat stress indicator traits	98
7.5.4.1 Rectal Temperature and Respiration Rate	98
7.5.4.2 Spot Temperature	103
7.5.4.3 Eye Temperature	104
7.5.5 Between ewe variation and repeatability	105
7.6 Conclusion	105
References	107

Chapter 8. General Discussion and Conclusions **111**

8.1 Conclusions and Recommendations	111
8.1.1 The impact of cold stress on neonatal lambs	111
8.1.2 The reaction of mature ewes to elevated levels of heat and an increased THI	112
References	115

Chapter 1: General Introduction and Project Aims

It is projected that the climate of the Western Cape will become more unstable, including severe heat during the summer as well as more severe cold weather (cold snaps) during winter. It is a reality that climate change is occurring and that there is limited local knowledge on the responses of livestock to elevated temperatures as well as the effectiveness of interventions to curb the effects of extreme temperatures (Cloete *et al.*, 2000; Cloete, 2012; Meissner *et al.*, 2013; Rust & Rust, 2013). Therefore, it is of paramount importance to come up with interventions that will assist the farming community to cope with these events. Animal-based records to guide breeding and management decisions for alleviating climate stress have not yet been quantified under local conditions. It is thus important to assess the available breeds that form part of the available South African *ovine* genetic resource for their ability to withstand increased temperatures and inclement conditions expected in the future. It is also important to be able to quantify potential differences between breeds, as well as between individuals within breeds to understand the mechanisms underlying the ability of sheep to adapt to heat stress conditions. Additionally, research on the genetics of early lamb behaviour is also significant as a proxy for lamb mortality in relation to climate.

The small stock industry has provided local farmers with a stable, reliable income over many years, despite being farmed in a relatively arid climate, on poor soils and under marginal conditions (Cloete & Olivier, 2010). In fact, rural stability and the sustainable usage of vast tracts of agricultural land depend on sheep farming for viable rural communities. This study will add to the existing knowledge regarding viable and sustainable sheep farming on the provincial and national levels to the advantage of both commercial and communal/smallholder farmers. It will contribute to stable rural communities, job opportunities as well as agro-industries with specific reference to the red meat and wool commodities.

This study focusses on *ovine* genetic resources under institutional control to fully utilise the comprehensive data that are commonly recorded in the respective resource flocks. Sheep found on two research farms, managed by the Western Cape Department of Agriculture, namely Langgewens and Elsenburg were used. Langgewens is the Western Cape research farm with the highest means for maximum, minimum, and average temperature as well as the only research farm that boasts maximum mean monthly temperatures in excess of 30°C for two months, namely January and February. Temperatures at Tygerhoek and Elsenburg are quite similar. Therefore, Langgewens Research Farm was considered the logical location for conducting research involving heat stress in ewes belonging to widely divergent breeds in the Western Cape. In contrast, Elsenburg is the research farm with the greatest likelihood of inclement weather during a winter lambing season. This locality was thus used as the location of a study on the effect of cold stress on the behaviour of neonatal lambs by adding climate data to a historic data set.

Sheep are also maintained on research farms in the Northern Cape, namely Upington and Carnarvon. In this province, Upington is considered in a league of its own as far as temperatures are

concerned with maxima exceeding 35°C from December to February and should be the preferred Northern Cape farm, while Carnarvon appears to be fairly like the hotter Western Cape research farms. However, neither of these farms in the Northern Cape were utilised for the project due to unforeseen circumstances. The project leader resolved this issue by proceeding with the project on Rooikop Farm located near Sutherland in the Northern Cape.

Due to climate change, the commencement of Project ACU-2021-21966 (AP/BR/S/TB102) was postponed until end January and February 2022. The reason for this is that the ambient climate in the Northern Cape and Western Cape was not ideal for the specific traits to be measured during December 2021 and start of January 2022 (e.g., temperature did not exceed 30°C for more than three consecutive days). In fact, an unexpected mild summer season was experienced up to the end of January in both provinces. Therefore, the project proceeded from 24 January 2022 to 27 January 2022 in the Western Cape on Langgewens Research Farm. The study was therefore continued at the Rooikop Farm (give coordinates) in the Sutherland district in the Northern Cape from 21 February 2022 to 24 February 2022.

One major problem the research team came across during the heat stress project at Langgewens Research Farm involved the Thermal Camera needed to measure the eye temperature of the sheep on Tuesday 25 January 2022. Due to this technical issue, the research team was only able to capture the eye temperature of each individual ewe once during the period Project ACU-2021-21966 (AP/BR/S/TB102) took place at Langgewens Research Farm. The technical issue could only be solved after the Thermal Camera (see section 3.1.8.3 in Chapter 3 for description) was updated and reformatted on 4 February 2022. Reasons for this malfunction could have been due to exposure to the ambient heat and direct sunlight that occurred when images were taken of the sheep at Langgewens. This problem was alleviated by using an umbrella to provide shade over the camera (black in colour) to prevent direct thermal radiation. As a fringe benefit, the umbrella caught the attention of the sheep, causing them to look straight at it (and thus also the camera), making it easier to capture images of individual sheep. Accordingly, this solution did not scare the animals, it simply made them look up as it was an unfamiliar object. This is accepted as normal, since sheep are considered as a quite curious species (Lighthouse Farm Sanctuary, 2020; Schoenian, 2021a).

The animals used in this study were exposed to environmental conditions typically experienced by sheep in the region throughout a normal summer (i.e., 2 to 4 days during which daytime temperatures reached 30°C and above). As sheep are commonly maintained under exposed conditions with minimal resources in terms of shade and other shelter in both provinces, the experimental animals were maintained in pens without any form of shade. The experimental animals were maintained in the same flock and were able to socialize in a natural way as well as to express the full range of behavioural repertoires particular to the species. Recordings of these animals were taken on days forecast to be hot during the cooler mornings as well as the hot afternoons to provide a range of conditions. The morning recording sessions started between 06h00 and 07h00 at both Langgewens Research Farm and Rooikop Farm. Whereas the afternoon recording sessions at

Langgewens Research Farm started roughly around 13h40, while recordings at Rooikop Farm started between 14h00 and 15h00. The reason behind the afternoon recording sessions starting at different times on the two farms, was simply due to the difference in maximum temperature occurring at a certain time for that specific locality as well as the distance between the paddocks. Repeated records were needed to derive between-animal variance components to estimate repeatability, as well as the re-ranking of breeds or individuals under different sets of conditions.

The aim of the studies presented in this dissertation is to guide husbandry decisions in the commercial and smallholder sectors through providing information regarding tolerance for heat and cold in South African sheep. This project will complement and contribute to research partially funded by the industry and dealing with *ovine* robustness, fitness, and adaptation to variable and often limiting environmental conditions. As such, the project will contribute to ethical animal production practices, while also adding to rural stability.

This study highlights the importance of adapting sheep to the changing climate scenario reflecting hotter summer temperatures. The effect of heat stress was emphasized as the most crucial factor that negatively influences livestock production. Physiological responses to heat stress were considered the immediate response/adaptive mechanisms by which the animals survive the stress impact. These included respiration rate, rectal temperature, and spot temperature which promote welfare and facilitate adaptation of animals to a specific environment. These traits were assessed as potential heat stress indicator traits that may help to quantify the response in heat stressed sheep. It is assumed that heat stressed sheep rely heavily on the physiological response mechanisms to cope with the adverse environmental conditions. Heat stress indicator traits which emerge in this pathway may serve as useful indicators of animal welfare in the changing climatic situation. These discussions vividly indicate the importance of studying in detail the physiological adaptive mechanisms in different sheep breeds. It is contended that these efforts can aid the farming community to identify the suitable agroclimatic zone suitable for specific sheep breeds. It is unlikely that the present study will provide all the answers to this problem. Therefore it is likely that the outcomes of this study needs to be refined in further research in the Northern and Western Cape areas of South Africa.

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Chapter 2: Literature Review

2.1 Introduction

Areas in the North-Western parts of the country have become hotter and drier because of climate change (Blignaut *et al.*, 2009), while there is consensus among analysts that the Western Cape is also likely to become hotter and drier (Meissner *et al.*, 2013). It is also projected that the climate of the Western Cape and southern interior of the Northern Cape will become more severe in terms of extreme cold weather (cold snaps) during winter (TerraClim, 2021). These anticipated developments in the Western Cape prompted the establishment of the SmartAgri project (see <http://www.greenagri.org.za>). This study seeks to provide findings and guidelines with reference to expected future environmental changes to the local farming community to assist efforts to adapt the local sheep industry to the changing environment.

In times of scarcity, sheep are able to utilise low-quality biomass and convert it into milk, meat and wool products (Cloete & Olivier, 2010; Indu & Pareek, 2015; Pérez *et al.*, 2020). As the sheep industry becomes more popular, it is essential to implement strategies that favour inexpensive and straightforward techniques to facilitate sheep production (Indu & Pareek, 2015). The dominant breeds in the local ovine genetic resource include the wool breeds Merino and Dohne Merino, the dual-purpose SA Mutton Merino (SAMM), the Dormer and Ile de France terminal sire breeds, as well as meat breeds like the Dorper and Meatmaster (Cloete *et al.*, 2014). Unimproved, fat-tailed genotypes are also available, albeit in smaller numbers (Cloete & Olivier, 2010). The mainstream breeds as well as indigenous breeds, such as the Namaqua Afrikaner, have been evaluated in terms of production, reproduction, and product quality (see Schoeman *et al.*, 2010 for a review of the small stock genetic resources in South Africa). However, comparative studies on the heat tolerance of those sheep breeds constituting the South African ovine genetic resource were not found in the scientific literature.

A study on ovine heat tolerance was recently conducted in Brazil, indicating substantial differences between the 11 sheep breeds that were tested (McManus *et al.*, 2016). The authors reported that breeds originating from temperate areas struggled to cope with high heat loads, as indicated by those heat tolerance indicators studied. In contrast, hair sheep like the South African Dorper were more capable of withstanding heat, but the best heat-coping capacity was observed in Santa Ines ewes indigenous to Brazil. The study by McManus *et al.* (2016) mapped out areas of Brazil where animals belonging to specific breeds would be capable of producing at acceptable levels under certain farming conditions. However, information as far as variation between individuals within breeds is concerned, could not be found in this paper.

In anticipation of more intense winter storms in future, cold stress is defined by the chill index which is derived from daily rainfall, wind speed and ambient temperatures (Nixon-Smith, 1972; Donnelly, 1984). A study evaluating lamb vitality and mortality of positively selected High (H) line

relative to negatively selected Low (L) line sheep in response to cold stress, revealed that H-line lambs performed better under cold stress conditions during a winter lambing season (Nel *et al.*, 2021a). The improved performance included lower mortality rates as well as higher neonatal rectal temperatures in H-line lambs, particularly under increased cold stress. These results suggested that H-line lambs were able to maintain their core body temperature more effectively than their L-line contemporaries. Additionally, the lower mortality rates suggest that the H-line lambs had an improved ability to cope with the stressful environment.

Knowledge of within and between breed variation in indicators of heat tolerance of local breeds would be of paramount importance in breed choice for sustainable production under the more intense heat conditions expected in the future, as well as for the knowledge of genetic variation available for breeders to adapt their animals to such conditions (Silva *et al.*, 2022). It is also extremely important to have knowledge regarding the genetic selection of improved reproduction in sheep that supports lamb robustness under cold stress conditions (Nel *et al.*, 2021a). It is crucial lambs have the ability to self-sustain and thermoregulate to maintain core body temperature during the neonatal period (Plush *et al.*, 2016). This would be particularly important in breed choice for sustainable production under the more intense cold, wet, and windy conditions expected with the greater likelihood for more intense winter storms in the future (Walsh *et al.*, 2014; Francis *et al.*, 2017; NOAA, 2017).

Information pertaining to breed and animal effects on heat stress indicators as well as to lamb vitality and mortality on cold stress indicators, are vital components of an integrated climate management programme to allow farmers to make informed choices for sustainable production systems.

2.2 Climate Change

Climate change is humanity's greatest concern ever to be encountered and poses a risk to both global ecological balances and economic security (Rashamol *et al.*, 2018). According to Rashamol *et al.* (2018) the primary consequence of climate change is the constant rise in global earth surface temperature which in turn has a marked influence on global food security. As an important agricultural enterprise, livestock is the principal contributor to the global food security and is critically threatened by climate change (Rosegrant *et al.*, 2009; Nardone *et al.*, 2010). According to the Food and Agriculture Organization (FAO) of the United Nations, the global demand for livestock products are expected to double by 2050 due to the improved standard of living and the growing human population (McGuire, 2015). Climate change induced heat stress has been established as one of the crucial factors affecting livestock production (Steinfeld *et al.*, 2006; Rashamol *et al.*, 2018). Sejian *et al.* (2010) and Indu & Pareek (2015) stated that heat stress may result in the reduction of meat production, milk production and quality, reproductive efficiency, as well as general animal health.

Sheep form an integral component of most livestock production systems as they are capable of adapting to a wide variety of environmental conditions (Cottle, 2010; Indu & Pareek, 2015). Cattle

(2010) argues that the global adaptability and success of sheep is confirmed by their status as the world's most diverse mammalian livestock species, contributing some 25% to the global number of farm animal breeds. Accordingly, sheep breeds are listed by region, confirming that the *ovine* species is indeed represented in farming landscapes throughout the world, ranging from arid to high rainfall areas and from sea level to the highest mountains (Cottle, 2010). Environmental temperatures are expected to increase globally, where sheep are likely to be exposed to higher temperatures more frequently for prolonged periods (Rashamol *et al.*, 2018; Van Wettere *et al.*, 2021). Under increased stress resulting from external drivers such as increased temperatures owing to climate change, sheep and goats are more resilient than alternative livestock species, particularly dairy cattle (Henry *et al.*, 2012; Rust & Rust, 2013; Indu & Pareek, 2015). Animals generally differ in their susceptibility and tolerance to thermal stress across breeds (Singh *et al.*, 2017). As a result, it is anticipated that heat stress will heavily impact the wool and meat industries (Indu & Pareek, 2015; Van Wettere *et al.*, 2021).

In India, there are 40 registered sheep breeds according to the nodal agency for the registration of livestock breeds, NBAGR. The adaptability of these breeds to local climatic conditions in general and particularly to heat stress, are relatively diverse (Singh *et al.*, 2016). Physiological adaptations sheep exhibit during increased environmental temperatures above the upper critical temperature ($\geq 31^{\circ}\text{C}$) includes reduced metabolic heat production as well as increased heat dissipation, while behavioural adaptations sheep favour during high heat loads include the increased use of shade and a reduction in activity (Van Wettere *et al.*, 2021). As heat is a major source of stress in livestock, it has a significant impact on their reproduction and production abilities (Singh *et al.*, 2016; Singh *et al.*, 2017; Rashamol *et al.*, 2018). When raised under extreme climatic conditions the least variation in traits indicative of heat or cold tolerance are expected in animals that are adapted to respectively hot or cold climatic conditions (Singh *et al.*, 2017). In effect, heat stress resulted in a reduced average daily gain, body weight (W), growth rate as well as body total solids, which was also reflected by impaired reproduction, causing severe economic losses to the sheep and goat industries (Indu & Pareek, 2015; Singh *et al.*, 2016).

Heat stress is considered a limiting factor in sheep production as it alters follicular growth, steroid secretion, and gene expression. Differences in heat tolerance and susceptibility have been observed in sheep where this variation in susceptibility/tolerance can be examined at DNA level (Singh *et al.*, 2017) to identify superior germplasm underlying adaptability, which can then be utilised for the selection of animals tolerant to heat stress (Singh *et al.*, 2016; Singh *et al.*, 2017). Two indicators of heat stress, considered under physiological parameters, include changes in respiratory rate and pulse rate. These rates were found to be significantly higher under heat stress conditions compared to normal conditions in the study conducted by Singh *et al.* (2016) during the months of February and June at the Arid Region Campus of CSWRI, Bikaner, India.

Heat stress impact

2.3.1 Behavioural and physiological patterns

Heat stress is defined as the sum of forces external to a homeothermic animal that acts to disrupt body temperature from the resting state (Yousef, 1985). A combination of environmental factors such as temperature, relative humidity (RH), air movement, solar radiation, and precipitation could result in the occurrence of heat stress (Bohmanova *et al.*, 2007; Indu & Pareek, 2015; Wills, 2016). Heat stress occurs when the physiological systems of the body fail to regulate body temperature within the normal range of around 37°C (Zaheer, 2019). Heat stress progresses to hyperthermia at elevated body temperatures (>40°C), resulting in extreme discomfort as well as physiological strain when heat loads from the environment (external) and internal production exceeds the capacity of animals to dissipate heat (Wills, 2016; Li *et al.*, 2018). Common indicator traits reflecting heat stress includes respiration rate, rectal temperature, spot temperatures on the skin, pulse rate, and sweating rate (McManus *et al.*, 2009; Joy *et al.*, 2020).

Li *et al.* (2018) furthermore reported that the hypothalamus controls homeostatic mechanisms through various neuroendocrine pathways, leading to divergent behavioural and endogenous responses that can be measured. An animal's response to heat stress can therefore be measured by heart rate, activity level, hematological, as well as other physiological traits in addition to respiration rate, rectal temperature, spot temperature, pulse rate, and sweating rate listed above (Indu & Pareek, 2015; Li *et al.*, 2018; Joy *et al.*, 2020). Accordingly, respiration rate, rectal temperature, spot temperature measurements have been identified as indicators to quantify the impact of heat stress on livestock (Shilja *et al.*, 2016).

Homeostatic functions of sheep under heat stress conditions are modified due to the heat increment that exceeds heat loss (Singh *et al.*, 2016). Singh *et al.* (2016) mentions that "heat stress elicits an integrative physiological and endocrinal modulation altering overall metabolism and helping the animal sustain during the stressful period". Heat-shock proteins are crucial for cellular homeostasis and are produced when cells are exposed to heat and other stresses such as exercise, infection, inflammation, oxygen deprivation, water stress, as well as exposure to toxins, heavy metals, and pesticides (Singh *et al.*, 2017). Heat-shock proteins are molecular chaperones (Ponomarenko *et al.*, 2013; Singh *et al.*, 2017) that prevent non-specific protein aggregates to form and assist cellular proteins to acquire their native structure (Singh *et al.*, 2017), and/or restore denatured proteins, the assembly of their complexes, transportation through membranes as well as their participation in signal transduction (Ponomarenko *et al.*, 2013).

Protein synthesis in the cell is suppressed when there is a nonlethal increase in temperature above the physiological norm, activating the heat-shock factors and enhancing the transcription of heat-shock genes (Ponomarenko *et al.*, 2013). Heat-shock factors are defined as the transcriptional activators of genes (heat-shock genes) regulated by thermal stress, that encode for heat-shock proteins (Garbuz, 2017; Gomez-Pastor *et al.*, 2018). Like heat-shock proteins, heat-shock genes are

also activated by numerous stress factors, including excessive heat, oxidative stress, hypoxia and virus infection (Garbuz, 2017). In contrast, when exposed to lethal temperatures, apoptosis (also known as programmed cell death) is initiated (Ponomarenko *et al.*, 2013). Heat-shock proteins inhibit apoptosis, providing cells with thermal stability if stress had to reoccur or is prolonged (Ponomarenko *et al.*, 2013).

The thermo-neutral zone of sheep in general ranges between 12 °C and 27°C (Marai *et al.*, 2007; Sejian *et al.*, 2017). However, adult hair breeds are considered to have an upper critical temperature of up to 34 °C (Do Prado Paim *et al.*, 2013), highlighting their natural tolerance for higher temperatures. The activation of compensatory and adaptive mechanisms in sheep allow them to efficiently tolerate temperatures above their upper critical temperature of their thermo-neutral zone, without markedly compromising their productivity (Pérez *et al.*, 2020). The degree of heat stress can be analysed using the temperature-humidity index (THI) (López *et al.*, 2015; Li *et al.*, 2018). The THI is defined as a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress. Sheep in general start to experience heat stress above THI of 22.2 (Marai *et al.*, 2001; López *et al.*, 2015; McManus *et al.*, 2016; Li *et al.*, 2018). According to Marai *et al.* (2007) and López *et al.* (2015), certain sheep breeds (i.e. hair breeds) only start to show signs of heat stress between a THI of 23.3 and 24. Thus, more research is required to determine the precise inflection points when heat stress symptoms in sheep become apparent. Hair sheep breeds' greater tolerance to heat stress conditions are related to genetic and phenotypic adaptations, as well as their activation of physiological, metabolic and endocrinological mechanisms (Macías-Cruz *et al.*, 2013, 2016). These mechanisms assist in maintaining an adequate body water balance and normothermic conditions at a low energy cost.

Respiration is defined as the intake of oxygen and elimination of carbon dioxide in a thermo-neutral state (Da Silva *et al.*, 2017). This leads to the evaporation and dissipation of moisture from the respiratory tract that in turn maintains thermal balance (Fuquay, 1981; Da Silva *et al.*, 2017; Rashamol *et al.*, 2018). In response to high heat loads, sheep start to pant to increase respiratory evaporation (Indu & Pareek, 2015). These mechanisms to balance heat gain and heat loss are crucial for preventing heat stress in animals (Fuquay, 1981; Da Silva *et al.*, 2017), where respiration rate acts as an initial warning sign of the heat stress condition in livestock (Nienaber & Hahn, 2007). An increase of respiration rate can visually be observed through the noticeable increase in respiratory side movements from thermo-neutral conditions (McManus *et al.*, 2009; Indu & Pareek, 2015; Dalcin *et al.*, 2016). This is an indication that the sheep are trying to maintain homeostasis through dissipating heat from their body (Nienaber & Hahn, 2007). Respiration rate tends to increase in livestock with higher ambient temperatures and relative humidities (RH) (Upadhyay *et al.*, 2009; Rashamol *et al.*, 2018). If the increased respiration rate fails to maintain core body temperature, the rectal temperature of the animal will increase in response to this excessive heat load within the body. In a review by Kadzere *et al.* (2002), it was evident that the performance in livestock was already compromised in response to as little as $\leq 1^{\circ}\text{C}$ rise in rectal temperature. An increased rectal

temperature was also related to increased THI in Sahiwal and Karan-Fries cattle as well as Murrah buffalo breeds (Bhan *et al.*, 2013; Sailo *et al.*, 2017).

Significant changes in body weight (W), body condition score, growth and the physiology (respiration rate, pulse rate, and rectal temperature) were evident in ewes that experienced heat stress (Indu & Pareek, 2015). Physiological adaptability in response to heat stress conditions varied between indigenous, crossbred and purebred animals (Rashamol *et al.*, 2018). Accordingly, indigenous livestock breeds displayed less variation in heat stress indicators when compared to their contemporary temperate breeds.

2.3.2 Ewe fertility

Fertility is defined as the number of ewes lambing per 100 ewes mated. During the mating period, heat stress negatively impacted upon fertilization, embryo survival, as well as pregnancy rates. This effect was observed up to three weeks after mating (Van Wettere *et al.*, 2021). According to Van Wettere *et al.* (2021), fertilization rates decreased when ewes were exposed to high temperatures of 32°C either during early oestrus or shortly before the onset of oestrus. Heat stress did not impair fertilization when experienced a day or more after oestrus but rather reduced lambing rate (~80%) due to embryo loss (Van Wettere *et al.*, 2021).

Pregnancy rates are greatly reduced when ewes experience heat stress five days prior to the onset of oestrus, the day of onset of oestrus or one day after the onset of oestrus, as well as when heat stress episodes started three or five days post-mating (Van Wettere *et al.*, 2021). Extreme environmental heat caused extensive embryonic deaths in *ovine* species (Griffiths *et al.*, 1970). In general, a meta-analysis has concluded that ewes experiencing heat stress are 2.4 times less likely to get pregnant than ewes under thermo-neutral conditions (Van Wettere *et al.*, 2021). To reduce the negative impact of heat stress on pregnancy rates during the peri-oestrus period, ewes can be shorn and exposed to lower temperatures overnight. These interventions alleviated the effects of HS, resulting in normal fertilization rates (Van Wettere *et al.*, 2021).

2.3 Ewe and Lamb Interactions

The early neonatal behaviour of lambs subjected to varying levels of cold exposure is studied in Chapter 4. Some background as to the behaviour of ewes and lambs during this period is needed as background information to digest the information reported in this chapter.

2.4.2 Physiological aspects

The rapid change in ovarian steroids is the first significant physiological event expressed in the parturient ewe, where a decrease in the plasma concentration of progesterone is integrated with an increase in oestradiol (Nowak *et al.*, 2000). A few hours following parturition, oestradiol returns to basal concentrations. Nowak *et al.* (2000) also observed a parallel increase in prolactin, while high concentrations are maintained postpartum due to the suckling stimulation of the young. The mechanical stimulation of the genital tract is the second most important event and is driven by the expulsion of the fetus (Nowak *et al.*, 2000). This is associated with a short, but significant release of an important neurotransmitter referred to as oxytocin. At parturition, oxytocin significantly increases in the cerebrospinal fluid. Equivalent to prolactin, oxytocin is also maintained postpartum through suckling activity (Nowak *et al.*, 2000).

Ovarian steroids, such as progesterone and oestradiol, are thought to be indirectly responsible for the onset of maternal care in ovine and caprine species (Nowak *et al.*, 2000). Therefore, high doses of these ovarian steroids could induce maternal responsiveness in nonpregnant ewes, but only a few maternal behaviour characteristics would be expressed. Progesterone and oestradiol thus seems to have a priming role, where exposure to these ovarian steroids influences the maternal behaviour in response to the expulsion of the fetus (Nowak *et al.*, 2000). Léavy *et al.* (1996) found that 80% of ewes developed complete maternal behaviour in response to the presentation of a newborn lamb after they were primed with ovarian steroids and experienced five minutes of vaginocervical stimulation. Conversely, in the absence of such mechanical stimulation, only 20% of ewes developed complete maternal behaviour in response to the presentation of a newborn lamb.

Nowak *et al.* (2000) has shown that maternal responses of oestrogen-treated ewes can be induced with intracerebroventricular injections of oxytocin. However, it was found that oxytocin is ineffective for vaginocervical stimulation when given without oestrogen priming. Opiates and corticotrophin-releasing factors are neurotransmitters that can modulate the effect of central oxytocin (Nowak *et al.*, 2000). During parturition, the paraventricular nucleus and the medial preoptic area are two of the oxytocin releasing sites that seem to trigger maternal behaviour in the ewe (Nowak *et al.*, 2000). Nowak *et al.* (2000) indicated that complete maternal responsiveness could be induced with infusions of oxytocin in the paraventricular nucleus, while the infusion of oxytocin in the medial preoptic area only reduces the rejection behaviour of ewes towards their newborn lambs. It was concluded that oxytocin could act at different sites to exert its effects on different components of maternal behaviour conducive to offspring care.

2.4.3 Behavioural aspects

Prior to parturition it can generally be observed that ewes, cows and does show a strong tendency to isolate themselves from the flock (Alexander *et al.*, 1979; Allan *et al.*, 1991; Lidfors *et al.*, 1994; Nowak, 1996). This is a significant preliminary step in the development of the mother-young bond,

since it facilitates early interactions as well as protects the dyad from disturbances by relatives, predators, and other parturient ewes that may be attracted to the amniotic fluid of the ewe giving birth (Nowak *et al.*, 2000). The immediate function of the mother-young relationship is primarily to provide offspring with a secure source of nutrition, protection against predators, as well as guidance (Nowak *et al.*, 2000). However, this isolation from the main flock could lead to increased lamb mortality during severe weather conditions (Broster *et al.*, 2010). Neonatal lamb mortality can therefore be reduced through using shelterbelts to prevent these ewes from moving too far away from shelter. Additionally, the shelterbelts need to be installed in such a way that still allows for parturient ewes to separate themselves from the rest of the flock prior to parturition (Alexander *et al.*, 1983). In doing so, ewe-lamb separation caused by disturbances from other ewes and lambs can be avoided.

Primiparous ewes (i.e. ewes that have only been pregnant and given birth for the first time) tend to reject their young more often than their experienced older flock mates, due to lack of experience which could potentially lead to lamb mortality (Nowak *et al.*, 2000). Abnormal maternal behaviour, particularly in primiparous ewes, include temporary delays in the expression of maternal care (e.g., grooming, licking, nursing) or preventing lambs to suckle in terms of behavioural disturbances (i.e., backing away from the lamb(s), circling to prevent access to the udder, and/or showing aggression towards the lamb(s) by butting). This delay in access to the udder reduces the chance of neonate survival, especially under harsh climatic conditions (Nowak *et al.*, 2000; Alexander, 1988; Cloete and Scholtz, 1998). Another component influencing the expression of maternal behaviour is nutrition, which is considered an environmental factor (Nowak *et al.*, 2000). Consequently, if ewes lack adequate nourishment during gestation, it could depress maternal behaviour at lambing and in turn increase neonatal mortality. This could be avoided through providing ewes with supplementary feeding, which is considered as an adaptive intervention (Nowak *et al.*, 2000). Desirable maternal behavioural traits that aid in the neonates' first suckling attempts, involve standing still, implementing a slight hunched posture to enable access to udder, as well as nudging the lamb in a position that facilitates suckling (Cloete & Scholtz, 1998). The absence of the aberrant behaviours (backing, circling, and butting) mentioned above also facilitates early suckling under paddock conditions.

Research on maternal behaviour of ungulates have shown that ewes bearing more than one lamb have limitations in their care for multiples, even though they show higher nursing and licking activities than single-bearing ewes (Nowak *et al.*, 2000). Since a twin-bearing ewe needs to divide her care between her two lambs, both would individually receive less stimulation than administered to singletons, causing a delay in mother-young bonding. For multiple-bearing ewes, a field situation leads to a higher risk of abandonment and uneven maternal bonding due to accidental separation, especially within the first four hours of birth (Nowak *et al.*, 2000). This usually occurs when: multiples are born several meters apart, other dams interfere as well as ewes who move away from the birth site to graze. In effect, these abandoned lambs have little chance of survival. It is considered that the time ewes spent on the birth site mostly influences the strength of bonding or litter size awareness

rather than that of dam age (Nowak *et al.*, 2000). According to Broster *et al.* (2010), both litter size and shelter type influence the mother-young interaction. For the same shelter type, it was observed that singles had greater ewe-lamb contact than twins (Broster *et al.*, 2010). Under different shelter designs, ewe-lamb contact in twins were higher for those with shrub belts than those with Hessian rows.

2.4 Inclement Weather and Neonatal Survival

With good management and planning 4-6 weeks prior to lambing, death from cold and starvation in the neonate can be avoided by shearing ewes (in turn causing ewes to seek shelter under severe conditions), providing these ewes with proper nutrition (Stott & Slee, 1985), as well as reducing windspeed at lamb height through establishing shelterbelts (Broster *et al.*, 2010). Events associated with the starvation-mismothering-exposure complex (SME; explains lamb deaths relating to a variety of causes) and dystocia are considered as the two main drivers of neonatal lamb mortality (Broster *et al.*, 2010). It is thought that hypothermia is sometimes caused by failure to feed as a result of the SME complex (Haughey, 1991). Starvation-mismothering-exposure complex generally occurs in smaller lambs as well as those born as multiples (Broster *et al.*, 2010). Plush *et al.* (2016) stated that thermogenesis is a crucial component of the SME complex concerning neonatal lamb mortality and is characterized by depleted fat reserves and inadequate milk ingesta.

Thermogenesis is described as the dissipation of energy through the production of heat and occurs in specialized tissues, including brown adipose tissue and skeletal muscle (Stott & Slee, 1985). There are two high risk periods of neonatal hypothermia, i.e. birth to 5 hours of age and 12 to 36 hours of age (Eales *et al.*, 1982). The major cause of hypothermia in the first period could be ascribed to excessive heat loss, while hypothermia in the second period mainly resulted from depressed heat production in association with the depletion of energy reserves (Stott & Slee, 1985). Therefore, a viable lamb must strongly be homeothermic at birth and possess sufficient energy reserves which is also replenished by suckling an attentive dam with an adequate colostrum and milk supply.

The study of Stott & Slee (1985) indicated that lambs born to cold-treated ewes (i.e. ewes shorn to a fleece depth of ± 5 mm and housed in a climate chamber maintained at 6°C) were able to regulate their rectal temperature during a cold test. The peak metabolic response following a nor-epinephrine (also known as nor-adrenaline) injection was about 1-5 times greater in lambs of cold-treated ewes than in lambs born to control ewes (i.e., full fleece ewes housed in individual pens at $\pm 6^{\circ}\text{C}$) as well as in lambs born to warm-treated ewes (i.e., full fleece ewes housed in a climate chamber maintained at 26°C). The mean elevation of the peak metabolic response over thermo-neutral metabolic rate was respectively: 2-8, 1-8 and 1-7 times in the lambs born to cold-treated ewes, control ewes and warm-treated ewes, respectively. This elevation was sustained for longer in the lambs of cold-treated ewes (Stott & Slee, 1985). Thus, the total metabolic response to nor-epinephrine was significantly greater in lambs of cold-treated ewes. During late pregnancy, Stott & Slee (1985) concluded that

exposing ewes to cold favoured foetal brown adipose tissue deposition, which increased neonatal capacity for non-shivering thermogenesis. Non-shivering thermogenesis accounts for at least 50% of the heat-generating mechanisms in a lamb at birth (Plush *et al.*, 2016).

Essentially non-shivering thermogenesis is achieved through the metabolic activity of the brown adipose tissue, which in turn is regulated by endocrine and neural networks (Duyne *et al.*, 1960; Himms-Hagen, 1984, 1985; Girard *et al.*, 1992; Cannon & Nedergaard, 2011). According to Plush *et al.* (2016) differences in neonatal cold resistance could potentially exist due to differences in the activity or level of the brown adipose tissue reserves. Sequentially, the maturation of the controlling neural and endocrine networks at birth may also be involved. Although the maturity of lambs has been shown to be capable of improvement (Bloomfield *et al.*, 2003; Dwyer & Morgan, 2006; Symonds, 2013; Plush *et al.*, 2016), none of the research have specifically tested cold resistance in these individuals. Further investigation of thermogenesis and its physiological control at birth, using controlled and repeatable tests, are required to clarify the mechanisms underlying differences in resistance to cold exposure in neonatal lambs (Plush *et al.*, 2016).

Against this background, a series of experiments were conducted to provide information regarding tolerance for heat in South African sheep. The following aims were set:

- to study the genetics of early lamb behaviour as a proxy for lamb mortality in relation to climate,
- to record non-invasive indicators of heat tolerance in resource flocks in the Western Cape and Northern Cape Departments of Agriculture,
- to obtain breed differences and repeatability estimates (to act as an indication of putative genetic variation).

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Chapter 3: Materials and Methods

3.1 Interventions facilitating the recording of those data needed for this study

This study hinged on the ability of observers/handlers to identify animals from a distance without undue disturbance. It also included records that are not commonly recorded in local sheep breeding operations. A section on the data recording practices were included against this background.

3.1.1 Identification of individuals

Paint branding, spray markers, marking crayons and neck-tags are available sources of temporary identification used in a sheep flock (Schoenian, 2021). Stock-marker spray offers a clean and easy way to mark livestock (especially sheep and goats) with reliable ozone friendly colour aerosol (Leader Products, 2022). It ensures a clear marking of the fleece with long-lasting colours. Generally, the use of stock-marker spray is avoided in wool breeds, as it could reduce the quality of the wool. Ordinary paint should never be used on sheep, especially wool breeds. Only fluids which have specifically been made for sheep, such as Etro-Mark®, should be applied on sheep as residual paint leaving a permanent stain make it difficult to scour the wool (Schoenian, 2021). Therefore, neck-tags were the preferred source of identification for animals from a distance in Chapters 6 and 7 of the current study. Stock-marker spray was used in Chapter 4, where it was important to identify ewes and their lambs over a longer period. Accordingly, the lambs were side-branded after being groomed by their dams for at least 15 minutes with a unique number identical to that applied to the sides of their dams (Cloete & Scholtz, 1998). Multiple lambs were also identified according to their order of birth.

Neck-tags are the least used form of sheep identification as they are difficult to see when the animals are in a group (Schoenian, 2021) as they are also more easily obscured by other sheep. However, neck-tags could be used as temporary identification as they are easily removed and recycled. For Chapters 6 and 7 of the current study, the number of ewes placed per paddock were just a few and allowed for easy identification from a distance. Thus, the use of neck-tags was efficient as the individuals could not form large groups and were only observed for a short period of 3 to 4 days. Neck-tags were positioned around each individual ewe's neck, sufficiently tight not to fall off, but loose enough to allow for easy breathing and movement (Schoenian, 2021).

3.1.2 Tinytag Plus 2 Data Logger and Weather Screen

A Tinytag Plus 2 Data Logger (TGP-4500) was used together with a 'Stevenson' type weather screen to measure the environmental relative humidity (RH) and temperature at 10-minute intervals. This allowed for linkage between the ambient conditions and the time of recording the ewe traits for the duration of the trial at Rooikop Farm (Chapter 7). The Tinytag Plus 2 Data Logger has an internal temperature (-25°C to +85°C) and relative humidity (0-100% RH) probe. It was specifically designed

for measuring temperature and humidity, with an additional dew point measurement, in a variety of harsh outdoor and industrial applications (Tinytag, 2019, 2022). Housed in a robust and waterproof case (IP68), this TGP-4500 datalogger allows for outdoor monitoring of environmental or climatic changes and has a built in sensor (9903-1550) and temperature/RH probe (9903-1555) with user programmable alarms, a reading capacity of 32 000 bytes, high reading resolution and fast data offload (Tinytag, 2019, 2022; Preservation Equipment Ltd, 2022). The coated RH probe has good resistance to moisture and condensation that ensures measurement reliability (Preservation Equipment Ltd, 2022).

The weather screen protected the Tinytag Plus 2 Data Logger (TGP-4500) from direct sunlight and precipitation. This 'Stevenson' type louvre (or weather) screen allows for air to circulate freely around the enclosed TGP-4500 data logger and has internal dimensions of 110mm diameter x 90mm high (Preservation Equipment Ltd, 2022). At Rooikop Farm (Chapter 7), this unit was deployed in the center of the paddocks at sheep head-height level to obtain representative temperature and RH data readings.

The hourly climate (temperature and RH) data used for Chapter 6 was acquired from the registered Western Cape Department of Agriculture's Weather Station Portal at Langgewens Research Farm (<https://gis.elsenburg.com/apps/wsp/#>). The weather data used for Chapter 4 was also acquired from the registered weather station at Elsenburg Farm.

3.1.3 Lamb Behavioural Traits

Lambs usually attempt to stand shortly after birth, and usually succeed doing so within 30 minutes of birth. Subsequently, lambs will succeed in suckling within 30 to 45 minutes after standing which amounts to about 60 to 75 minutes after birth (Arnold & Morgan, 1975). Lamb behaviour traits considered in the current study included the latency from birth to standing (LTBS) and latency from standing to apparent first suckling (LTSS). These traits are commonly affected by breed and selection line (Stott & Slee, 1985; Cloete & Scholtz, 1998; Dwyer & Lawrence, 2000; Matheson *et al.*, 2012).

Lambs in Chapter 4 originated from two selection lines of Merino sheep that were based on maternal ranking values for lambs reared per joining, namely the High (H) and Low (L) lines (Cloete & Scholtz, 1998). H-line lambs descended from ewes that reared more than one lamb per joining (i.e., reared twins at least once), while L-line lambs descended from ewes that reared less than one lamb per joining (i.e., ewes were barren or lost all lambs born at least once) and were used as replacements (Cloete & Scholtz, 1998). Accordingly, progeny of ewes that reared one lamb per joining were accepted in both lines on occasion, depending on the average reproduction of the selection lines and the number of replacements needed. Selection decisions were mainly based from three or more maternal joinings, particularly in the case of rams (Cloete & Scholtz, 1998). Besides death or severe teeth and udder malfunction, ewes remained in the breeding flock for five joinings when selected. For the duration of the observations in Chapter 4, these selection lines were

maintained at Elsenburg Experimental Farm, located near Stellenbosch in the south-western region of South Africa (Cloete & Scholtz, 1998).

At Elsenburg Experimental Farm (Chapter 4), 10 lambing paddocks (0.5 Ha kikuyu) were used for the research covered in Chapter 4, where 5 paddocks were reserved for usage during the day, and another 5 were floodlit for usage at night. To help ewes to adapt to the presence of observers for the trial discussed in Chapter 4, one to two of the six trained observers continuously monitored the lambing flocks for three weeks during peak lambing (Cloete & Scholtz, 1998). Accordingly, the observers were able to approach the ewes closely (<10m) without unnecessary disturbances. Since the lamb behaviour traits were well-defined, it was easy for the trained observers to assess these traits through direct observation when patrolling the area. As the first behavioural observation in lambs, LTBS was defined as the latency from expulsion to standing for > 10 seconds. Subsequently, the LTSS was defined as the latency from standing to apparent first suckling (Cloete & Scholtz, 1998).

3.1.4 Body Weight Measurement

A Gallagher W210 scale was used to obtain the body weights of individual ewes before and after the trial in Chapters 6 and 7. The scale was calibrated prior to the sheep being weighed by using subjects of a known weight. The platform of the Gallagher W210 scale was placed on a stable, flat and level surface (bolted to the load bars) that was clear from touching any rails and gates. For accurate weight (W) results, it was ensured that the ewes were completely on the platform and that there were no obstructions, such as stones or dung etc., underneath the platform (Gallagher Group Limited, 2014). Also, the neck tags were put on after or removed prior to weighing the ewes. The W210 weigh scale was set on the manual mode as the facility did not necessarily allow for a smooth flow of animals across the platform and some of the ewes tended to be restless (Gallagher Group Limited, 2014). The units of weight were set to kilograms (kg). The 'Zero' button was pressed prior to weighing the ewes (i.e., if the zero icon was not displayed on-screen) to zero-out any weight that could have been on the platform. Once the 'Weigh' button was pressed, the scale weighed the ewe and locked on to the measurement that was displayed on-screen for data accumulation.

For Chapter 4, individual lamb birth weights (BW) were recorded to the nearest 0.1 kg within 24 hours of birth (Cloete & Scholtz, 1998).

3.1.5 Fat Depth Measurement

Ultrasound images of fat depth were captured at the mid-rib position, perpendicular to the spine of each individual ewe, using a Mindray DP 30V ultrasound scanner with a 7.5 MHz linear transducer. At the scanning site, the wool or hair was gently parted to allow for placement of the transducer probe, with minimal pressure being applied, to obtain and capture a clear image of the fat depth. Ultrasound gel was utilized as a coupling agent to improve conductivity between the transducer

probe and the skin. Once the best image was obtained, it was frozen and on-screen measurements of the fat depth of each individual ewe was taken (Mindray DP 30 Digital Ultrasonic Diagnostic System Imaging System 1.0).

The recording of fat depth measurements was considered necessary for this project to identify whether differences in fat depth between individual ewes could be linked to the occurrence of heat stress. Therefore, fat depth measurements were taken during the afternoon of the day before the intended recording sessions in Chapters 5 and 7.

3.1.6 Fleece Depth Measurement

A Digital Caliper, usually referred to as a Digital 'Vernier' Caliper, is a precision instrument that is used for measuring internal and external distances accurately (Ryan, 2004; Synotronics, 2021; RS Components, 2022). Synotronics (2021) stated that a caliper is a device with a measuring scale and a "jaw" that is used to measure an object's dimensions, where a specific type of caliper, known as a Vernier Caliper, has two scales. These scales are referred to as the main scale and a vernier scale. The vernier scale is an auxiliary scale that appears alongside the instrument's main scale and provides the option of higher resolution, allowing for measurement resolutions as precise as 0.01mm (Synotronics, 2021). A Digital Caliper takes linear measurements like that of the Vernier Caliper but has an additional LSD screen that displays the measurements automatically. This digital caliper has a built-in ABS (absolute) scale that enables the caliper to be used immediately once power is turned on without origin resetting, similar to using a manually operated Vernier Caliper (Bengatouch (PTY) LTD, 2020; RS Components, 2022). Digital calipers are less complex to use than the Vernier Caliper, as the measurements are read from a LCD display and through pressing the inch/mm button, the distance can be read as metric (mm) or imperial (inches or fractions of inches) units (Ryan, 2004). Thus, this instrument can be used to measure fleece depth in sheep.

For this project, wool depth of Merino, Dohne Merino, South African Mutton Merino (SAMM), Ile de France (IDF), and Dorper ewes as well as hair length of Namakwa Afrikaner, Meatmaster, Dorper and White Dorper ewes were measured in millimeters (mm) using a Mitutoyo Digital Precision Caliper of the Absolute Digimatic Caliper Series (Chapter 6 and 7). To initiate the measuring of fleece depth, the wool or hair of the ewe was gently parted to allow for a clear visual display of the beginning and end edges of the fleece for accurate placement of the caliper's external jaws. The external jaws were then moved apart, where the locking screw is tightened to ensure the external jaws are in a fixed position. At this stage, the measurement in mm could be read from the digital display (Ryan, 2004).

3.1.7 Respiration Rate Recording

Respiration rate is considered the amount of work an animal must do to maintain homeostasis (Indu & Pareek, 2015; McManus *et al.*, 2016). It is the first trait that serves as an indicator of breed and

individual differences in heat stress in sheep. The respiration rate of each individual ewe was measured non-invasively from a distance by a team consisting of a recorder and a scribe (Chapters 6 and 7) (Gaughan *et al.*, 2000a; Marai *et al.*, 2001; Matheson *et al.*, 2012; Hamzaoui *et al.*, 2013; Macías-Cruz *et al.*, 2016; McManus *et al.*, 2016; Shilja *et al.*, 2016). The recorder was responsible for counting the number of respiratory side movements for 30 seconds. The resultant count was doubled to obtain respiration rate as bpm. The scribe used a digital stopwatch for timekeeping and communicated with the recorder of when to start and when to stop counting the respiratory side movements. Respiration rates were recorded twice daily, once in the morning and then during the afternoon (Sejian *et al.*, 2010; Matheson *et al.*, 2012b; Bhan *et al.*, 2013; McManus *et al.*, 2016; Shilja *et al.*, 2016; Seixas *et al.*, 2017). The same recorder was maintained for the duration of the project; however, a different scribe was utilized on the two farms where data were recorded. Counting the respiratory side movements was not as easy as it seemed, therefore a few of the individuals' respiration rates were counted more than once, since all ewes did not stand still for the whole duration of 30 seconds and the recorder occasionally lost sight of the respiratory side movements. The time of each recording was documented for linkage back to hourly ambient temperature and humidity data readings (Macías-Cruz *et al.*, 2016; McManus *et al.*, 2016; Seixas *et al.*, 2017).

3.1.8 Core Body Temperature Measurements

At both Langgewens Research Farm in the Western Cape (Chapter 6) and Rooikop Farm in the Northern Cape (Chapter 7), the core body temperature of individual ewes was measured twice daily, once in the morning and then during the afternoon. The time of each recording was documented for linkage back to hourly ambient temperature and humidity data readings. For Project ACU-2021-21966 (AP/BR/S/TB102), the following equipment and methods were used to measure core temperature of individual ewes:

3.1.8.1 Standard Clinical Thermometer – Rectal Temperature

Rectal temperature is a direct measure of core body temperature in livestock and associates strongly with numerous physiological traits relating to heat stress (Indu & Pareek, 2015; Da Silva *et al.*, 2017). A rise in rectal temperature in response to high environmental temperatures and humidity denotes the failure of thermo-regulatory mechanisms in the body of the animal in the face of high levels of heat stress (Kadzere *et al.*, 2002; Marai & Haeeb, 2010; Rashamol *et al.*, 2018).

The rectal temperature was recorded using a standard clinical thermometer by inserting the thermometer directly in the rectum of individual sheep, as required for the recording of data necessary to determine potential breed differences for heat tolerance in sheep. Rectal temperature measurements using clinical thermometers occasionally tend to be inaccurate due to inadequate insertion depth (Naylor *et al.*, 2012). Provided that the same method is used to record rectal

temperature throughout the study, representative figures can however be obtained. This procedure is also necessary to obtain an objective assessment of core temperature to relate to the other measurements discussed under sections 3.1.8.2 and 3.1.8.3. For recording rectal temperature, individual ewes were non-invasively restrained for approximately 90 seconds by two to three farmhands. Simultaneously, a research assistant measured the individual rectal temperatures, that was documented by a scribe with the corresponding time of measurement.

3.1.8.2 Laser-based Thermometer – Spot Temperature

Genetic differentiation between sheep breeds is dependent on coat traits, including height (relating to fleece depth), colour, hair length and density, as well as on skin traits including thickness and sweat gland density (Do Prado Paim *et al.*, 2013; Joy *et al.*, 2020; Silva *et al.*, 2022). It was evident that the three leading phenotypic traits (fleece depth, density and length) affected thermography temperature measurements and are considered significant for heat tolerance characterization in sheep (Do Prado Paim *et al.*, 2013). Therefore, when analyzing whether breed differences existed in response to certain environment conditions, these traits need to be evaluated.

For the occurrence of heat exchange between the body of an animal and its surrounding environment, the animal's skin becomes important (Rashamol *et al.*, 2018) with regards to spot measurements of the surface temperature. Spot measurements of skin temperatures is a non-invasive indirect measure of core body temperature (McManus *et al.*, 2016) that is directly associated with increased blood flow to the skin surface and vasodilation of the skin capillary bed (Indu & Pareek, 2015; Shilja *et al.*, 2016; Katiyatiya *et al.*, 2017). Spot temperatures vary between individuals based on the exposure of the cheek to direct sunlight. During each recording session on Langgewens Research Farm (Chapter 6) and Rooikop Farm (Chapter 7), spot temperatures on the cheek of individual ewes were measured using a laser-based thermometer simultaneous to the measuring of rectal temperature by another research assistant. Increased spot temperature in response to high surrounding environmental temperatures can partially be attributed to the fact that the exposure of sheep to heat stress alters their blood flow, leading to the redistribution and increase of blood flow to their skin surfaces (Indu & Pareek, 2015; Rashamol *et al.*, 2018).

3.1.8.3 FLIR E75 Thermal Camera – Eye Temperature

A FLIR E75 Thermal Camera was used to record the core body temperature of ewes through thermal imaging. Thermal imaging is a non-invasive tool that gives a good indication of the core body temperature of the animal and is considered a suitable recording method (Do Prado Paim *et al.*, 2013; McManus *et al.*, 2016) for heat stress indicator traits in sheep. Accordingly, thermal imaging was obtained using an emittance coefficient of 0.95 to measure the eye temperature of all ewes studied in the project on Rooikop Farm (Chapter 7). The research team was unable to record the eye temperature of individual ewes at Langgewens Research Farm due to technical issues with the

FLIR E75 Thermal Camera. Thermal measurements of eye temperature were taken in the morning at low temperatures and again in the afternoon during the hottest hours. Records were obtained without handling the ewes to avoid stress unrelated to heat. The camera operator took individual images of the eyes from the side of the ewes' head, ensuring to stand outside their flight zone (± 2 to 3 m) to prevent them from becoming restless. Additionally, to prevent the thermal camera from overheating due to exposure to the ambient heat and direct sunlight when images were taken of the sheep, an umbrella was used to provide shade over the camera (black in colour) to prevent direct thermal radiation. As a fringe benefit, the umbrella caught the attention of the sheep, causing them to look straight at it (and thus also the camera), making it easier to capture images of individual sheep. As sheep are generally a quite curious species (Lighthouse Farm Sanctuary, 2020; Schoenian, 2021a), this action did not scare them, it simply made them look up as it was an unfamiliar object. Thermal images were captured on the camera once the operator determined it to be in focus and downloaded to a laptop computer. This was necessary to determine whether there was a significant change in ewe response mechanisms to different ambient temperatures (Do Prado Paim *et al.*, 2013). FLIR® Tools software was used to retrieve the individual ewe eye temperature from the thermal images generated in this way (FLIR systems, 2017).

3.1.9 Accelerometers – activity monitoring

Although not used in this study, accelerometers are another recording method that may be convenient and of great value for future research purposes that was not used during the current study. Accelerometers, also referred to as activity sensors, can be utilized for tracking the position of an animal and record their movement and activity or inactivity patterns, as it contains several sensors that record location and transmits velocity and acceleration data in a single or all three dimensions (Brito *et al.*, 2020), including static forces such as lying down, and movements such as walking. This device can be attached to different parts of the body of the animals such as the neck, ear, back, feet, and legs, where it then classifies a variety of activities, for instance feeding, panting, standing, walking, ruminating, gait, lameness, as well as grazing behaviour (Brito *et al.*, 2020). Therefore, this device can be useful in outdoor production systems and commercial livestock operations. As accelerometers has a great potential to generate large-scale datasets, it can be utilized in the field of research for studies pertaining to breeding schemes (Brito *et al.*, 2020). Additionally, these devices are small, affordable and capable of generating and transferring real-time data to data center stations (i.e., databases) through wireless sensor networks when embedded into wireless sensors (Brito *et al.*, 2020). In terms of detecting and recording indicators of heat stress in sheep and other livestock species, such as increased respiratory movements (i.e., panting) as well as an increased water intake, a reduced feed intake, and reduced locomotion, accelerometers can be attached around the neck of the animal and utilized to determine whether that animal is subjected

(26)

to heat stress during certain times of the day.

Individual research chapters, each constituting a complete paper that are ready to submission to an accredited scientific journal, follows on from these introductory chapters. Chapter 5 was published in the peer-reviewed and accredited Proceedings of the Association for the Advancement of Animal Breeding and Genetics (ISSN number – 1328-3227).

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Chapter 4: Genetic and environmental parameters for birth weight and behaviour of neonatal Merino lambs in relation to cold stress

4.1 Abstract

Lamb mortality remains a significant welfare and production issue, constraining ethical and sustainable sheep production. Lamb survival is benefitted by early suckling and colostrum intake, while it is impaired by inclement weather involving a combination of rain, wind, and a low temperature. It is important to understand the effect of cold exposure on neonatal behaviour and progress to suckling in lambs to curb lamb mortality. The dual aims of this study were to use historic behavioural data to establish the relationship of neonatal lamb behaviour with cold stress and to derive genetic parameters for early lamb behaviour. It was hypothesized that cold stress conditions would impair early suckling activity and that lamb behaviour was heritable. Historic data of Merino lambs from 1993 to 2002 of the Elsenburg Merino flock for birth weight (BW in kg) and behaviour latencies (time intervals in minutes), namely: from birth to first standing for >10 sec (LTBS) as well as from standing to first suckling for >10 sec (LTSS), were analysed in relation to a cold stress gradient (CSG). The CSG was derived from a combination of wind, rain, and temperature. The flock consisted of a line selected for number of lambs weaned per ewe mated (NLW; the High or H-Line) and a line selected against NLW (Low or L-Line). ASReml was used to obtain regressions of fixed linear and random cubic spline components of LTBS and LTSS on the CSG and to derive genetic parameters for all traits. Overall, H-line lambs were somewhat heavier at birth and progressed faster from standing to first suckling than L-line lambs ($p > 0.05$). Regressions of neonatal lamb progress on random spline components as well as interactions of the regression variables with selection line were not significant ($p > 0.05$) and the analyses reduced to modelling the fixed linear component. Increased levels of cold stress resulted in faster progress in LTBS ($p < 0.05$). Expressed relative to mild conditions at a CSG of $800 \text{ kJm}^{-2}\text{h}^{-1}$, LTBS was reduced by 17.6% on the observed scale at a higher CSG of $1200 \text{ kJm}^{-2}\text{h}^{-1}$ ($p < 0.05$). In contrast, LTSS was compromised at higher CSG values ($p < 0.05$), increasing markedly by 76.0% from $800 \text{ kJm}^{-2}\text{h}^{-1}$ to $1200 \text{ kJm}^{-2}\text{h}^{-1}$. Direct single-trait heritability estimates were 0.16 ± 0.05 for BW, 0.22 ± 0.07 for LTBS, and 0.06 ± 0.05 for LTSS. The inclusion of the maternal genetic variance ratio resulted in an improvement in the log-likelihood ratio for BW and LTBS, yielding single-trait estimates of 0.37 ± 0.03 and 0.06 ± 0.03 , respectively. Single-trait dam permanent environment variance ratio was 0.09 ± 0.03 for LTSS. Three-trait genetic parameters were close to the corresponding single-trait values. Genetic correlations among traits were not significant. Further research is required to understand the different responses to cold stress between LTBS and LTSS.

4.2 Introduction

Since lamb mortality remains a significant welfare and production issue, it is an ongoing constraint for efficient sheep production (Cloete & Scholtz, 1998). The majority of lambs die during or shortly after birth, while the rate of lamb mortality is double, if not more, for twin lambs compared to lambs born as singles (Brien *et al.*, 2014). Nowak *et al.* (2000) mentions that early suckling behaviours and the intake of colostrum are facilitated by the bond between the ewe and her lamb(s), which is crucial for the survival of lambs. The following abnormal ewe behaviours towards their lambs are considered to have significant effects on lamb mortality: abandonment; aggression towards the lamb or refusing access to suckle through backing or circling; as well as prolonged parturition (Alexander, 1988; Cloete & Scholtz, 1998). However, recognizing and grooming the lamb, co-operating with suckling attempts, maintaining close contact with the lamb, absence of aggression towards the lamb, and not deserting the lamb, are considered maternal behavioural traits that contribute to lamb survival (Cloete & Scholtz, 1998; Dwyer & Lawrence, 2005).

According to Mukasa-Mugerwa *et al.* (2000), lamb losses tend to be high during the perinatal period due to cold stress (i.e., hypothermia due to inclement weather conditions causing a rapid decline in ambient temperature), starvation (as a result of inadequate colostrum intake owing to delayed suckling or competition with siblings), mismothering, failure to adapt outside the uterine environment, as well as dystocia. Particularly in young animals, inadequate nutrition affects the severity of several infections, which in turn leads to lamb mortality (Mukasa-Mugerwa *et al.*, 2000). This agrees with findings from Nowak *et al.* (2000) and Brien *et al.* (2014), stating that neonates face major risks aside from the birth process itself. Abhorrent or lethargic ewe and lamb behaviour may result in the lamb succumbing due to inadequate milk ingestion and depleted fat reserves. This leads to a series of events presenting with diagnosis suggestive of a complex termed as starvation-mismothering-exposure (SME) (Cloete & Scholtz, 1998). Cloete & Scholtz (1998) also indicate that SME is a consequence of exposure to cold, wet, and windy conditions that interact to result in slow lamb progress in the neonatal stage, occasionally linked to inadequate maternal care. Therefore, both the ewe and lamb contribute to the survival of the lamb (Cloete & Scholtz, 1998; Mukasa-Mugerwa *et al.*, 2000; Nowak *et al.*, 2000; Dwyer & Lawrence, 2005; Brien *et al.*, 2014). In effect, the ewe must give birth to a live lamb without complications, provide a reliable source of colostrum and milk, and a stable mothering environment. Moreover, the lamb needs to adapt outside the uterine environment through being able to stand and suckle in a reasonably short period after birth to sustain its energy levels (Brien *et al.*, 2014) while also regulating their own core temperature (Plush *et al.*, 2016).

Owing to a plausible drop in core temperature from approximately 39°C to 35°C within one hour of birth under adverse conditions, lambs must be capable of thermoregulation to maintain homeothermy during the neonatal period (Plush *et al.*, 2016). Thermoregulation around birth is often tough for lambs, as inclement weather conditions may prevail during the lambing. In accordance with

results reported by Cloete & Scholtz (1998), Plush *et al.* (2016) postulated that thermogenesis is a crucial component to counter the impact of SME concerning neonatal lamb mortality. Thermogenesis is characterized by the dissipation of energy through the production of heat and occurs in specialized tissues, including brown adipose tissue and skeletal muscle (Stott & Slee, 1985). In lambs, at least 50% of the heat-generating mechanisms are accounted for by non-shivering thermogenesis (Plush *et al.*, 2016). This is primarily achieved through the metabolic activity of brown adipose tissue. Endocrine and neural networks sequentially regulate this metabolic activity. It is evident that differences in the level and activity of the brown adipose tissue could partially reflect the differences in neonatal cold resistance (Plush *et al.*, 2016).

This study sought to model the neonatal responses of the lambs against the cold stress gradient (CSG) defined by Nel *et al.* (2021). The latter study as well as that of Slee *et al.* (1991), indicated a clear relationship of inclement weather with neonatal survival. The objectives of this study were to test whether neonatal lamb progress was affected by ambient conditions through the analysis of historic data and also to partition animal effects into its direct genetic, maternal genetic and maternal permanent environment components in neonatal lambs. The effect of considering the CSG on the derived variance ratios were studied simultaneously.

4.3 Materials and Methods

4.3.1 Animals, Selection Procedures, and Location

Using maternal ranking values for lambs reared per mating, two lines (namely High (H) line and Low (L) line) of Merino sheep were divergently selected from the same base population from 1986 to 2002 (Cloete & Scholtz, 1998; Cloete *et al.*, 2009). The maternal ranking value was based on number of lambs weaned per ewe mated (NLW) using a ranking table based on the formula published by Turner (1978). These divergent lines were selected from available ewes on the Tygerhoek research farm of the Western Cape Department of Agriculture after being allocated at random from a Merino line that was selected for an increased ratio of secondary:primary wool follicles (Heydenrych *et al.*, 1984). Once ewes were selected, they remained in the breeding flock for at least five matings, aside from cases of death and teeth or udder malfunction (Cloete & Scholtz, 1998). As a result, no selection on reproduction was directed at the current flock. Approximately 120 breeding ewes represented each selection line at the onset of the study, with the H-line gradually increasing to 130-150 breeding ewes. However, L-line ewe numbers dropped to about 40-80 breeding ewes resulting from a relatively poor reproduction (Cloete *et al.*, 2009). Initially, five rams represented each selection line where they were used for only one breeding season until 1992. From 1992, one to two rams in each selection line was carried over to the next year to provide sire links across years (Cloete *et al.*, 2009).

The resource flock was maintained at the Tygerhoek Experimental Farm from 1986 to 1992, before being transferred to the Elsenburg Experimental Farm near Stellenbosch during 1993 for

intensive studies on ewe and lamb behaviour in the period from 1993 to 2002 (see Cloete & Scholtz, 1998; Cloete *et al.*, 2021), the period covered in this study. The two lines studied were maintained as a single flock except during mating for a 10-year period (1993 to 2002) period. Mating was performed in single-sire groups to 4-5 rams (Cloete & Scholtz, 1998). During mating in the summer (January – February) and lambing in the winter (June – July), ten irrigated 0.3-0.5 ha kikuyu (*Pennisetium clandestinum*) paddocks were utilised (Cloete & Scholtz, 1998; Cloete *et al.*, 2009; Cloete *et al.*, 2021). Flock management after the lambing observations during 1993 to 2002 was described by Cloete & Scholtz (1998) and Cloete *et al.* (2021). In short, ewes and their lambs were drifted off the kikuyu lambing paddocks and joined in groups of 30-40 on lucerne (*Medicago sativa*) paddocks. From there, groups were joined after tail docking and moved to larger dryland paddocks where they grazed dryland lucerne and medic (*M. truncatula*) pastures. Oat (*Avena sativa*) fodder crops were also regularly utilised during winter. During spring and early summer, oat crop residues and standing hay were available from time to time to supplement dryland lucerne and medic pastures. Supplementary feeding was rarely needed as nutrition was mostly adequate throughout the year (Cloete & Scholtz, 1998). Ewes were annually shorn within 3 - 4 weeks prior to the commencement of lambing.

4.3.2 Observations and Recordings

For identification from a distance and without undue disturbance, ewes were side-branded using stock-marker spray (Cloete & Scholtz, 1998; Cloete *et al.*, 2021). The lambing flocks were continuously observed by one to two of six trained observers for approximately three weeks during peak lambing (Cloete & Scholtz, 1998; Cloete *et al.*, 2021). Soon after lambing commenced, observations begun and continued until the number of ewes lambing per day did not warrant continued supervision. To prevent the possibility of biases in favour of specific groups, the observers were not informed of the selection line, dam age, birth type (i.e., number of lambs carried), nor the sex of lambs. Furthermore, the lamb behavioural traits recorded were well-defined making them easier to assess by direct observation by trained observers continuously patrolling the area (Cloete *et al.*, 2021).

Cloete & Scholtz (1998) found ewes to adapt quickly to the presence of the observers as they wandered around and could be approached closely (< 10 m) without unnecessary disturbance. Five lambing paddocks were utilised throughout the day, while another five 'floodlit' paddocks were utilised at night (Cloete & Scholtz, 1998). According to set criteria, assistance at birth was provided once it was realized that ewes were struggling to deliver a lamb within a certain period of time (Cloete & Scholtz, 1998). Thus, assistance was implemented after a time lapse of: (i) 3.5 to 4.0 hours from the first definite sign of parturition; (ii) 2 hours after the lamb was presented at the vulva; (iii) 2 hours from the birth of a preceding litter mate; or (iv) 30 minutes from the appearance of a head or a limb of the presenting lamb.

Individual lamb records included selection line, birth year, birth type, sex, and dam age (Cloete & Scholtz, 1998; Cloete *et al.*, 2021). Triplets were pooled with twins and denoted as multiples. For identification, after lambs were groomed by their dams for at least 15 minutes, individual lambs were side-branded using stock-marker spray with a unique number equivalent to that applied to the sides of their dams (Cloete & Scholtz, 1998; Cloete *et al.*, 2002a). Lambs born as multiples were also identified according to the order of birth. The following two lamb behavioural traits were recorded, namely the latency from birth to standing for at least 10 seconds (LTBS) and the latency from standing to apparent first suckling (LTSS) (Cloete & Scholtz, 1998; Cloete *et al.*, 2002a). Cloete *et al.* (2021) used the same data set, but only considered the latency from birth to apparently suckling. The two defined latency traits denoting early lamb progress were also related to cold stress in this study. It was deemed important to separate the traits, as it was reasoned that the effect of cold stress might not be similar for LTBS and LTSS. Birth weight (BW) was recorded within 24 hours of birth to the nearest 0.1 kg (Cloete & Scholtz, 1998).

The following ewe behavioural traits were considered to prolong LTSS, namely: (i) backing away from the lamb(s); (ii) circling to prevent access to the udder; (iii) and/or acting with aggression towards the lamb(s) by butting (Alexander, 1988; Cloete & Scholtz, 1998; Cloete *et al.*, 2021). Additionally, the following three behavioural traits were thought to aid in the lambs' first suckling attempts, namely: (i) standing still, (ii) adopting a slight hunched posture to enable access to the udder, and (iii) nudging the lamb in a position that will facilitate suckling (Cloete & Scholtz, 1998; Cloete *et al.*, 2021). An overall maternal co-operation score was given to the ewe through totalling these recordings on a 6-point scale (three behaviours where the absence of a trait counted in favour of the ewe and three traits where the presence of a trait was favourable) (Cloete & Scholtz, 1998). These scores were primarily a trait of the ewe in the earlier study of Cloete *et al.* (2021), and were used as a covariate in analyses on LTSS to account for desirable ewe behaviour assisting these early suckling attempts.

4.3.3 Cold stress gradient

Climate data were obtained from the Elsenburg weather station (situated at 33°51'S 18°50'E, alt: 171 m) which was about 1000 m from the lambing paddocks (Nel *et al.*, 2021a). Weather data were used to derive an environmental stress parameter, here denoted as the cold stress gradient (CSG; Nixon-Smith, 1972; Donnelly, 1984). Heat loss, as based on the combined effects of daily wind, rainfall, and temperature was estimated as:

$$\text{CSG} = [11.7 + 3.1V^{(0.5)}][40 - T] + 481 + 418(1 - e^{(-0.04R)})$$

In this equation, the CSG was the derived heat loss ($\text{kJ m}^{-2} \text{h}^{-1}$), V was the average wind speed (m s^{-1}), T was the mean temperature ($^{\circ}\text{C}$), and R was the total rainfall (mm) for a 24-hour period.

The database containing lamb data were updated with the CSG, as experienced on the day of

birth of the specific lamb. Nel *et al.* (2021a) used a CSG based on the first 3 days of life and showed that neonatal mortality to 3 days of age increased with an increased CSG so defined. In contrast, the CSG was related to neonatal latency traits in this study. The climate data for the day of birth were thus used to study the effects of cold stress on neonatal progress. Since the daily tagging of all lambs commenced at 08h00, the previous day was taken as the birth date. In the absence of hourly weather data, it was assumed that the CSG so derived would be fairly representative of the conditions during the neonatal phase of individual lambs.

4.3.4 Statistical Analysis

The latency traits, LTBS as well as LTSS, were not normally distributed (Table 4.1), therefore not conforming to the assumptions for analysis of variance. To normalize these traits and to account for some very short time intervals, latency traits were transformed to natural logarithms after 3 was added to individual values. For the initial single-trait analyses, ASReml4 was used to assess the fixed effects affecting birth weight as well as the neonatal lamb behaviour traits (Gilmour *et al.*, 2015). Fixed effects considered were birth year (1993 – 2002, excluding 2000 and 2001 where no climate data were available), selection line (H-line and L-line), birth type (single or multiple), sex (ram or ewe), and age of dam (2 - 6+ years) as well as significant two-factor interactions. To account for differences in ewe behaviour, dam cooperation scores for individual lambs were added as a linear covariate to analyses on LTSS. The effect of the CSG was then added to the fixed effects models fitted, by making use of cubic splines (Verbyla *et al.*, 1999) in ASReml4. These analyses typically fitted a fixed linear component, random deviations from linearity conforming to a smooth trend and random deviations from linearity not subscribing to a smooth trend. A log-likelihood ratio test was used to test for the significant differences in models. Once the operational model was specified, other random effects were added. Additionally, the log-likelihood ratio test is a chi-square distributed random variable, with degrees of freedom equal to the difference in the number of random effects. A difference of 1.92 was considered as a significant ($p < 0.05$) improvement in the log-likelihood when the addition of one random effect at a time was compared to the reduced model. The direct-maternal genetic correlation was not modelled, as the previous study by (Cloete *et al.*, 2021) based on the full data set including lamb data for 2000 and 2001, found that this effect was not significant for the latency of birth to first suckling. Therefore, the following genetic models for analyzing lamb traits were implemented (in matrix notation):

$$y = Xb + Z_1a + e \quad (1)$$

$$y = Xb + Z_1a + Z_3m + e \quad (2)$$

$$[\text{Correlation (a,m)} = 0]$$

$$y = Xb + Z_1a + Z_2c + e \quad (3)$$

$$y = Xb + Z_1a + Z_3m + Z_2c + e \quad (4)$$

$$[\text{Correlation (a,m)} = 0]$$

In these analyses, y was a vector of observations for lamb birth weight or behavioural traits; b , a , m , and c were vectors of fixed effects, direct genetic variances, maternal genetic variances as well as maternal permanent environmental variances respectively; X , Z_1 , Z_2 , and Z_3 were the corresponding incidence matrices relating the respective effects to y ; e was the vector of residuals. It was assumed that the variance structure for the model was:

$$V(a) = A\sigma^2_a; V(m) = A\sigma^2_m; V(c) = I\sigma^2_c; V(e) = I\sigma^2_e,$$

with A being the numerator relationship matrix and I being an identity matrix; σ^2_a , σ^2_m , σ^2_c , and σ^2_e were respectively the direct genetic variance (also referred to as direct additive variance), maternal genetic variance (as a trait of the lamb), maternal permanent environmental variance, and environmental (residual) variance. Estimates of heritability (h^2), maternal heritability (m^2) and maternal permanent environmental effects (c^2) were calculated as ratios of estimates of σ^2_a , σ^2_m , and σ^2_c , respectively, to the phenotypic variance (σ^2_p).

A complete pedigree file based on 10355 individuals born from 1986 to 2020 as the progeny of 282 sires and 2141 dams were used in all analyses. The log-likelihood ratios obtained from all four analyses were used to identify the random effects models best fitting the data for each trait (Snyman *et al.*, 1995).

4.4 Results

4.4.1 Descriptive statistics

The descriptive statistics for BW, LTBS and LTSS are given in Table 4.1. Coefficients of variation ranged from 20.9% for BW to 126.4% for untransformed LTSS (Table 4.1). Untransformed means did not conform to normality as far as skewness and kurtosis were considered. The log transformation normalized both distributions and rendered more reasonable coefficients of variation of around 25%. Coefficients of variation of the traits analysed exceeded 20%, suggesting that all traits were variable.

Table 4.1 Descriptive statistics for birth weight, transformed and untransformed latencies for behavioural traits (LTBS and LTSS) recorded in the study.

Trait	Observations (n)	Mean \pm SD	CV (%)	Range
Birth Weight (kg)	2167	3.98 \pm 0.83	20.9	1 - 7
<u>Raw means:</u>				
LTBS (min)	1326	35.5 \pm 40.6	114.4	1 - 393
LTSS (min)	1265	42.8 \pm 54.1	126.4	1 - 661
<u>Transformed means:</u>				
LTBS (min)	1326	3.28 \pm 0.81	24.7	1.39 – 5.98
LTSS (min)	1265	3.40 \pm 0.89	26.2	1.39 – 6.38

SD – Standard Deviation; CV – Coefficient of Variance; LTBS – Latency from birth to standing; LTSS – Latency from standing to apparent first suckling.

4.4.2 Fixed effects

The predicted means with back-transformed values for BW, LTBS and LTSS are given in Table 4.2. Selection line, birth type, sex, and dam age all significantly affected the BW of lambs ($p < 0.05$) born in the study. H-line lambs were slightly heavier than L-line lambs; lambs born as singles were markedly heavier than lambs born as multiples, ram lambs were heavier than ewe lambs; and the progeny of mature dams were heavier than lambs born to 2-year-old dams (all $p < 0.05$; Table 4.2).

LTBS was only affected by sex (Table 4.2), indicating that ewe lambs stood up faster once they were born compared to their ram contemporaries ($p < 0.05$). LTBS was unaffected by ($p > 0.05$) selection line, birth type, and dam age.

Table 4.2 Predicted means for fixed effects affecting the lamb traits considered (\pm SE). Back-transformed means of traits that were derived after transformation to natural logarithms are given in parentheses.

Fixed Effects	BW (kg)	LTBS (min)	LTSS (min)
<u>Selection Line</u>	**	0.29	**
H-line	4.15 \pm 0.02	3.29 \pm 0.02 (23.9)	3.31 \pm 0.03 (24.3)
L-line	4.01 \pm 0.03	3.26 \pm 0.05 (23.0)	3.47 \pm 0.05 (29.0)
<u>Birth Type</u>	**	0.87	**
Single	4.56 \pm 0.03	3.27 \pm 0.03 (23.4)	3.23 \pm 0.04 (22.2)
Multiple	3.60 \pm 0.02	3.28 \pm 0.03 (23.5)	3.55 \pm 0.04 (31.8)
<u>Sex</u>	**	**	0.94
Ram	4.21 \pm 0.02	3.34 \pm 0.03 (25.1)	3.39 \pm 0.04 (26.6)
Ewe	3.95 \pm 0.02	3.21 \pm 0.03 (21.9)	3.39 \pm 0.04 (26.7)
<u>Age of Dam (Years)</u>	**	0.72	0.31
2	3.82 \pm 0.04	3.28 \pm 0.05 (23.5)	3.50 \pm 0.06 (30.2)
3	4.11 \pm 0.03	3.30 \pm 0.04 (24.2)	3.35 \pm 0.05 (25.5)
4	4.15 \pm 0.03	3.28 \pm 0.04 (23.6)	3.34 \pm 0.06 (25.4)
5	4.18 \pm 0.04	3.29 \pm 0.04 (23.8)	3.39 \pm 0.06 (26.6)
6+	4.14 \pm 0.03	3.22 \pm 0.05 (22.1)	3.36 \pm 0.06 (25.7)
Dam cooperation score (n)	N/A	N/A	** -0.237 \pm 0.025

**Significant ($p < 0.05$); Actual F-value for $p > 0.05$. BW – Birth weight; LTBS – Latency from birth to standing; LTSS – Latency from standing to apparent first suckling; H – High; L – Low; N/A – Not applicable.

LTSS was influenced by selection line as well as birth type (Table 4.2). H-line lambs progressed faster to suckling than their L-line contemporaries ($p < 0.05$) with a 19.3% (expressed relative to the H-Line) difference in the geometric means for this time interval between the two selection lines. Lambs born as singles were the quicker to progress to suckling once they could stand, where multiples had a 43.2% (expressed relative to singles) longer LTSS than singles ($p < 0.05$). Sex and dam age had no significant influence on the LTSS of lambs ($p > 0.05$; Table 4.2). An increase of one unit in dam cooperation score was associated with an approximately 7% reduction in LTSS when expressed relative to the overall log transformed mean ($p < 0.05$; Table 4.2).

4.4.3 Cold stress gradient

The addition of the CSG to the traits indicative of neonatal progress was considered next. LTBS was only affected ($p < 0.05$) by the fixed linear component of the CSG. Neither the spline component nor interactions of either the linear or spline components with selection line was significant. The linear regression of LTBS on the CSG amounted to -0.00061 ± 0.00020 min on the log scale or -0.02% of the overall log transformed mean ($p < 0.05$). In essence, this meant that LTBS declined from 3.35 ± 0.04 (25.5 min) at $800 \text{ kJm}^{-2}\text{h}^{-1}$ to 3.18 ± 0.05 (21.0 min) at $1200 \text{ kJm}^{-2}\text{h}^{-1}$. The significance of other fixed effects and predicted fixed effect means were largely similar compared to the model excluding the CSG.

As for LTBS, LTSS was independent of random spline effects and of interactions of selection line with the CSG ($p > 0.05$). The linear regression of LTSS on the CSG amounted to 0.00127 ± 0.00022 min on the log scale or 0.04% of the overall log transformed mean. It was evident that LTSS was markedly compromised at high levels of cold stress, with means of 3.17 ± 0.05 (20.8 min) at $800 \text{ kJm}^{-2}\text{h}^{-1}$ and 3.68 ± 0.06 (36.6 min) at $1200 \text{ kJm}^{-2}\text{h}^{-1}$. Results indicated that H-Line lambs and singles progressed quicker from standing to suckling, as also deduced from the model excluding the CSG. Predicted means for other fixed effects were also like in the initial analysis in Table 4.2. The lack of a significant interaction effect of selection line with the CSG suggests that H-Line lambs would be able to progress quicker from standing to suckling than L-Line contemporaries, irrespective of the level of cold stress experienced.

4.4.4 Log-Likelihood values for random effects

Log-likelihood ratios and the models that best fitted the data for BW, LTBS and LTSS are given in Table 4.3. The best model fitted for BW included direct additive as well as maternal additive effects. Likewise, the inclusion of direct additive and maternal additive effects fitted the data best for LTBS. However, the best model for LTSS included direct additive as well as dam permanent environmental effects.

Table 4.3 Log-likelihood ratios for birth weight and lamb behavioural traits (LTBS and LTSS) in Merino lambs of the H- and L-lines under five different models of analysis. The “best” models are denoted in bold and italic font.

Model Fitted	BW (kg)	LTBS (min)	LTSS (min)
Fixed effects only	-308.376	-180.602	-458.921
+ h^2	-167.967	-156.858	-453.835
+ $h^2 + c^2$	-94.1193	-155.099	-448.856
+ $h^2 + m^2$	-68.9166	-154.837	-452.697
+ $h^2 + m^2 + c^2$	-68.7666	-154.631	-448.856

Variance ratios: h^2 – direct heritability; m^2 – maternal heritability; c^2 – dam permanent environment; BW – Birth weight; LTBS – Latency from birth to standing; LTSS – Latency from standing to apparent first suckling; H – High; L - Low.

4.4.5 Genetic parameters

Single-trait variance component and ratio estimates for BW, LTBS and LTSS are given in Table 4.4. Direct heritability (h^2) estimates amounted to 0.16 for BW, 0.22 for LTBS and 0.06 for LTSS. The estimates for BW and LTBS were more than twice the corresponding standard error (SE), while the estimate for LTSS was in the same range as the corresponding SE (Table 4.4). However, the direct additive effect for LTSS was retained by default. Maternal additive variance ratios (m^2) for BW and LTBS were respectively 0.37 and 0.06. The dam permanent environmental variance ratio (c^2) amounted to 0.09 for LTSS.

Table 4.4 Single-trait variance components and ratio estimates (\pm SE) for birth weight and respective behavioural traits (LTBS and LTSS) in Merino lambs of the H- and L-lines.

Variance components and ratios	Trait		
	BW (kg)	LTBS (min)	LTSS (min)
<u>Components</u>			
Direct additive (σ^2_a)	0.0792	0.1010	0.0397
Maternal genetic (σ^2_m)	0.1830	0.0301	N/A
Maternal PE (σ^2_c)	N/A	N/A	0.0632
Residual (σ^2_e)	0.2354	0.3321	0.6082
Total phenotype (σ^2_p)	0.4975	0.4632	0.7111
<u>Variance ratios</u>			
h^2	0.16 \pm 0.05	0.22 \pm 0.07	0.06 \pm 0.05
m^2	0.37 \pm 0.03	0.06 \pm 0.03	N/A
c^2	N/A	N/A	0.09 \pm 0.03

Variance ratios: h^2 – direct heritability; m^2 – maternal heritability; c^2 – dam permanent environment; BW – Birth weight; LTBS – Latency from birth to standing; LTSS – Latency from standing to apparent first suckling; H – High; L – Low; N/A – Not applicable.

Estimates for h^2 stemming from the three-trait analysis (Table 4.5) were similar for BW and within 0.03 for LTBS and LTSS compared to the corresponding single-trait estimates. The h^2 estimates for BW and LTBS were more than double the corresponding SE, whereas the estimate for LTSS was below double the corresponding SE. There was some evidence that some genetic variation in LTSS repartitioned to h^2 in the three-trait analysis, although the standard errors in the respective analyses were too high to consider it as a significant repartitioning. The m^2 estimates for BW and LTBS as well as the c^2 for LTSS were at least double the corresponding SE (Table 4.5).

None of the genetic correlations among traits were significant ($p > 0.05$). The direction of the genetic correlations, however, suggested that lambs heavier at birth would putatively take longer to progress from birth to standing and that lambs taking longer to progress from birth to standing would also progress slower from standing to first suckling, and those lambs heavier at birth will progress faster from standing to first suckling (Table 4.5). In most cases phenotypic correlations were smaller in magnitude than genetic correlations. Apart from the phenotypic correlation between LTBS and LTSS, the phenotypic correlations resembled the corresponding genetic correlations in sign. The maternal genetic correlation between BW and LTBS was below 10% and not significant.

Table 4.5 Three-trait (co)variance component and ratio estimates (\pm SE) for birth weight and respective behavioural traits (LTBS and LTSS) in Merino lambs of the H- and L-lines.

Variance components and ratios	Trait		
	BW (kg)	LTBS (min)	LTSS (min)
<u>Components</u>			
Direct additive (σ^2_a)	0.0775	0.1145	0.0610
Maternal genetic (σ^2_m)	0.1817	0.0294	N/A
Maternal PE (σ^2_c)	N/A	N/A	0.0542
Residual (σ^2_e)	0.2366	0.3196	0.5846
Total phenotype (σ^2_p)	0.4959	0.4645	0.6750
h^2 in bold on the diagonal, r_G below and r_P above the diagonal*			
BW	0.16 \pm 0.05	0.03 \pm 0.03	-0.11 \pm 0.03
LTBS	0.31 \pm 0.22	0.25 \pm 0.07	-0.07 \pm 0.03
LTSS	-0.16 \pm 0.30	0.35 \pm 0.28	0.09 \pm 0.05
m^2 in bold on the diagonal, r_M below the diagonal*			
BW	0.37 \pm 0.03		
LTBS	-0.04 \pm 0.19	0.06 \pm 0.03	
c^2 in bold	N/A	N/A	0.08 \pm 0.03

* r_G – genetic correlation; r_M – maternal genetic correlation; r_P – phenotypic correlation; PE – permanent environment; BW – Birth weight; LTBS – Latency from birth to standing; LTSS – Latency from standing to apparent first suckling; H – High; L – Low; N/A – Not applicable.

4.5 Discussion

4.5.1 Descriptive statistics

Coefficients of variation% (CV%s) for BW was 22.6% (Cloete *et al.*, 2009) and 20.8% (Burger, 2019) for Merino lambs, these values coincide with the estimated 20.9% found in this study (Table 4.1). In Afrino lambs, the CV for BW amounted to 16.4% (Snyman *et al.*, 1995). Similar CV for BW were obtained for wool (14.2%), dual-purpose (16.5%) and meat (19.2%) breeds in a study by Safari *et al.* (2005). CVs for LTSS and LTBS in this study were both higher than the 16.3% CV derived when these traits were combined in a single figure as the latency of birth to suckling (Cloete *et al.*, 2021).

4.5.2 Fixed effects

The BW of the lambs was significantly influenced by selection line, birth type, sex, and dam age (Table 4.2). The effects of birth type, sex, and dam age are consistent with those reported by Gardner *et al.* (2007), Juengel *et al.* (2018) and Sveinbjörnsson *et al.* (2021).

H-line lambs were 3.7% heavier ($p < 0.05$) than L-line lambs at birth (Cloete & Scholtz, 1998), a figure according with the 3.5% BW difference (expressed relative to the L-line) between the two selection lines in the current study (Table 4.2). This result is thought to be related to the distinct reproduction performance between the two selection lines, where H-lines are subjected to improved reproduction performance and L-lines to poor reproduction performance (Cloete *et al.*, 2009). For that reason, it is considered that L-line ewes' ability to bear lambs for a certain period of time is inferior to that of H-line ewes. On the phenotypic level, it was found that there was no significant difference in BW between the H-line and L-line lambs from 1986 to 1992 and from 1998 to 2002 (Cloete *et al.*, 2003). However, from 1993 to 1997, the phenotypic means for BW of H-line lambs tended to be greater than that of L-line lambs. Subsequently, no consistent genetic differences in

favour of either line for BW were found up to 2002 (Cloete *et al.*, 2003). The phenotypic and genetic effects of selection lines on BW were not consistent even though H-line lambs were somewhat heavier than L-line contemporaries on the phenotypic level.

Lambs born as singles had approximately 21% (expressed relative to the singles) higher BWs than lambs born as multiples ($p < 0.05$), which is in accordance with results found in literature (Cloete, 1993; Gardner *et al.*, 2007; Sveinbjörnsson *et al.*, 2021). Accordingly, due to the limited capacity of maternal uterine space, individual BWs decrease as the litter size increases (Gardner *et al.*, 2007). Expressed relative to the multiples, singles were 26.7% heavier than the multiples in the current study, which agrees with the difference of 27.8% reported by Cloete *et al.* (2003).

In this study, ram lambs were slightly heavier than ewe lambs at birth, which is consistent with results found in literature (Juengel *et al.*, 2018; Sveinbjörnsson *et al.*, 2021), where the BW of ewe lambs was 93.8% of the BW of rams. According to Cloete *et al.* (2003), ram lambs were 6.7% heavier than ewes and agrees with the 6.6% (expressed relative to the ewes) obtained from Table 4.2.

Progeny from mature ewes were significantly ($p < 0.05$) heavier than lambs born to 2-year-old ewes, where body weight increased as dam age increased from 2 to 5 years of age and decreased at the age of 6 years to values similar to those observed in 4-year-old dams (Table 4.2), as was found in other studies (Cloete *et al.*, 2003; Juengel *et al.*, 2018). The BW of lambs born to 2-year-old ewes were on average 92.2% of the BW of lambs born to older ewes (3-year-old to 6+-year-old ewes), which is consistent with other studies (Sveinbjörnsson *et al.*, 2021). The reason behind this difference in lamb BWs between immature and mature ewes could be due to fetal growth being 'maternally constraint' and is related to both the limited uterine space of the ewe as well as the ewe's ability to supply sufficient nutrients with regards to the intrauterine environment (Gardner *et al.*, 2007; Cloete *et al.*, 2009). Additionally, some nutrients are partitioned to somatic growth in immature (2-year-old) ewes as they have not yet reached their mature size, leading to competition for resources with intrauterine lamb growth. Maternal body condition score prior to conception as well as late gestational energy intake significantly affected lamb BW (Gardner *et al.*, 2007).

LTBS was only significantly influenced by the sex of Merino lambs (Table 4.2). Ewe lambs roughly had a 12.7% (expressed relative to the ram lambs) shorter LTBS compared to their ram contemporaries, where ram lambs took approximately 3.2 minutes longer to stand once they were born. Similarly, Dwyer (2003) reported that Suffolk ram lambs were more vulnerable than ewe contemporaries and therefore took longer to progress to standing. East Friesian ewe lambs were also faster to attempt to stand, stand, and stand firmly compared to the ram lambs (Simonetti *et al.*, 2018). The results in the current study are in correspondence with that of Cloete & Scholtz (1998), whom reported that ram lambs took 4 minutes longer to stand (28 vs. 24 minutes). It needs to be stated that the latter study overlapped with this study from 1993 to 1998. Given that data for 2000 and 2001 had to be dropped from this study because of incomplete climate records, the studies had a lot of data in common. In contrast, Cloete *et al.* (2002a) found that sex did not affect ($p > 0.05$) LTBS of South African Mutton Merino (SAMM) and Dormer lambs, whereas Cloete (1993) reported

that Dorner and SAMM lambs born to mature ewes progressed faster ($p < 0.05$) from birth to standing compared to the lambs born to maidens. Other research, such as studies by Matheson *et al.* (2012) and Cloete *et al.* (2021), studied the effects of sex solely on the latency from birth to suckling, whereas this interval was divided into LTBS and LTSS in this study. However, Matheson *et al.* (2012) indicated that ram lambs were usually slower to go through behavioural progression, which agrees with the basic outcome of the current study.

LTSS was independent of sex and dam age, but was significantly influenced by selection line, birth type and dam cooperation score (Table 4.2). The effects of sex on the progress of lambs to suckle seem to vary between studies, with some studies reporting sex differences (Dwyer, 2003; Dwyer & Lawrence, 2005) and other not (Wassmuth *et al.*, 2001; Cloete & Scholtz, 1998; Cloete *et al.*, 2002a; 2021). Simonetti *et al.* (2018) reported that East Friesian ewe lambs only reached the udder faster than ram lambs, and that there was no significant difference between the LTSS of ewe and ram lambs. Opposed to the nonsignificant ($p > 0.05$) effect of dam age on LTSS in this study, Cloete (1993), Cloete & Scholtz (1998) and Cloete *et al.* (2002) indicated that lambs born to mature ewes progressed faster ($p < 0.05$) for LTSS than lambs born to younger ewes. This agrees with results reported by Dwyer (2003) and Dwyer *et al.* (2005). On the contrary, Matheson *et al.* (2012) found that lambs born to mature ewes progressed slower to suck compared to lambs born to younger (i.e., first parity) ewes. The effects of birth type and dam cooperation score correspond to results found in literature (Cloete, 1993; Cloete & Scholtz, 1998; Cloete *et al.*, 2002a). On the other hand, Simonetti *et al.* (2018) stated that only when BW was fitted to the model as a covariate and twin lambs' BW was equivalent to singletons, then twin lambs tended to have a shorter LTSS than singletons. H-line lambs were approximately 19.3% (expressed relative to the L-line) faster for LTSS compared to L-line lambs. This result is in correspondence with that of Cloete & Scholtz (1998), but with a 26.3% faster progression for LTSS in H-line lambs. Cloete *et al.* (2021) mentioned that the reason behind H-line lambs progressing quicker to suckling than L-line lambs could possibly relate to the fact that these H-line lambs were born to dams with superior maternal cooperation scores. In their study, the significant ($p < 0.05$) difference in favour of the H-line in the latency from birth to suckling (not analysed in this study) was removed when dam cooperation score was included in the model. According to Cloete *et al.* (2021), it appears that the effect of selection line on LTSS could partially be reflected by 'better' behaviour in H-line ewes, therefore not only solely depending on the instinctive ability of lambs themselves. The inclusion of maternal cooperation in the study of Cloete & Scholtz (1998) failed to remove the line difference in LTSS, as was also found in this study. Lambs born as multiples took on average 9.6 min longer for LTSS compared to their contemporary singles, according to similar results found in other literature (Cloete & Scholtz, 1998; Cloete *et al.*, 1998).

4.5.3 The effect of the cold stress gradient (CSG) on neonatal progress

It must be conceded that, although exact birth time was available for individual lambs, it was impossible to link it directly to hourly climate, which was not recorded at that stage. Individual lambs

could thus have been exposed to the ambient conditions for varying periods of time. When modelling the CSG, LTBS and LTSS were relatively independent of most of the fixed effects in this study as was also reported by Slee *et al.* (1991) as well as the analysis without the CSG (Table 4.2). It was nevertheless clear that increased levels of cold stress resulted in faster progress in LTBS ($p < 0.05$). This could potentially be due to neonatal lambs experiencing discomfort immediately after birth at high CSG conditions, resulting in them standing to try and make up for the loss of energy caused by shivering to maintain body temperature. In order for lambs to increase heat production to maintain body temperature in inclement weather conditions, neonatal lambs need to metabolise brown fat and increase muscular activity by shivering (Alexander & Williams, 1968). In correspondence to this study, Held (2021) stated that lambs are well-equipped to handle cold environments due to their brown fat reserves that could supply energy directly. However, once this fat supply is depleted within a few hours of birth, colostrum intake is supposed to act as the primary source of energy. Research has also shown that when lambs are exposed to cold conditions, their parasympathetic nervous system is stimulated, which in turn triggers the release of nor-adrenaline from the sympathetic nerves in brown fat (Himms-Hagen, 1985). Nor-adrenaline together with cold exposure act by stimulating the metabolism of brown adipose tissue that enables heat production. Adrenaline appears to have a similar effect in stimulating non-shivering thermogenesis. Additionally, the metabolism of brown adipose tissue by the stress hormones, adrenaline, nor-adrenaline, and glucocorticoids, is thought to be a key mechanism of thermogenesis in young lambs (Plush *et al.*, 2016). This is essential since newborn lambs are not always immediately able to suckle to allow diet induced thermogenesis. A study by Alexander and Williams (1966) revealed that neonatal behaviour and the establishment of the ewe-lamb bond is significantly affected by cold exposure. Additionally, teat-seeking behaviour was inhibited by cold, wet and windy conditions, especially when the lamb's rectal temperature was below 38°C (Alexander & Williams, 1966).

Expressed relative to mild conditions at a CSG of 800 kJm⁻²h⁻¹, LTBS was reduced by 17.6% on the observed scale at a higher CSG of 1200 kJm⁻²h⁻¹ ($p < 0.05$). It could be hypothesized that, under mild conditions, lambs might feel more comfortable while being groomed by an attendant dam without an urgent need to suckle to make up for the lost energy. On the contrary, when a lamb's core body temperature steadily drops due to a lack of energy caused by more extreme cold conditions, they run the risk of becoming weak and unthrifty (Held, 2021). Lambs so affected may lose the ability and desire to stand. It stands to reason that lambs confronted with moderate to severe cold stress conditions may thus stand sooner after birth because they seek sustenance to supplement their depleting brown fat reserves.

In contrast, LTSS was compromised at higher CSG values ($p < 0.05$), increasing markedly by 76.0% from 800 kJm⁻²h⁻¹ to 1200 kJm⁻²h⁻¹. It could be argued that standing under cold, windy conditions is energetically expensive as wind chill may result in excessive levels of heat loss over a larger exposed body surface, leading to a greater lethargy and an increased LTSS (Bird *et al.*, 1984; Haughey, 1991; Erickson, 2018; Whitworth & OSU Extension, 2021). From results obtained in the

study by Nel *et al.* (2021b), the significant interaction between rectal temperature and the CSG during the first two days from birth suggested that differences in rectal temperature between the H- and L-lines were dependent on ambient conditions. When born under more extreme conditions (i.e., an increased CSG), rectal temperature of L-line lambs declined faster than in their H-line contemporaries, suggesting that L-line lambs are more susceptible to elevated levels of cold stress. On the other hand, H-line lambs could maintain homeothermy fairly well across the full range of the CSG (Nel *et al.*, 2021b). Accordingly, the resistance to heat loss for H-line lambs was higher than that of L-line lambs, as reflected by the non-similar trends for the two selection lines. Overall, this study as well as that of Cloete & Scholtz (1998) indicated that H-line lambs were able to obtain nourishing colostrum sooner than L-line contemporaries across the CSG. This adaptation was probably also reflected in the trends in rectal temperature reported by Nel *et al.* (2021b).

4.5.4 Random effects

In the current study, h^2 for BW was 0.16, with a corresponding m^2 estimate of 0.37 (Tables 4.4 and 4.5). In wool breeds, the estimates of h^2 for BW ranged from 0.15 to 0.20, while estimates for m^2 ranged from 0.09 to 0.15 (Cloete *et al.*, 2001; Kushwaha *et al.*, 2009). Similar values for h^2 (0.22) and m^2 (0.09) of BW were estimated for Afrino lambs (Snyman *et al.*, 1995). Additionally, the latter author also found a c^2 estimate of 0.12 for a total maternal effect of 0.21. In SAMM and Dormer lambs, h^2 estimates for BW amounted to 0.11 and 0.21, respectively, while m^2 was estimated at 0.16 in Dormer lambs, and the c^2 estimates amounted to 0.15 in SAMM and 0.18 in Dormer lambs (Cloete *et al.*, 2002a). Brien *et al.* (2014) reported h^2 estimates of 0.05-0.35 for BW in Merino lambs, and 0.05-0.39 in lambs of Australian meat breeds, while m^2 was estimated at 0.14-0.29 in Merino lambs and 0.22-0.35 in lambs of Australian meat breeds. The h^2 estimates for BW reported above also coincide with estimates obtained from meat breeds (0.15), dual-purpose breeds (0.19), and wool breeds (0.21), as reported in an extensive review of genetic parameters for wool, growth, meat and reproduction traits in sheep by Safari *et al.* (2005).

Estimates of h^2 and m^2 for LTBS amounted to 0.22-0.25 and to 0.06, respectively (Tables 4.4 and 4.5). In SAMM and Dormer lambs, estimates of h^2 for LTBS amounted to 0.10 and 0.22, respectively (Cloete *et al.*, 2002a). Similarly, Brien *et al.* (2010) reported a h^2 estimate of 0.10 for time lambs took to stand. Matheson *et al.* (2012) reported a h^2 estimate of 0.40 for lamb vigour, scored on a 5-point scale from 1 (very vigorous) to 5 (very weak) at 5 minutes of birth. This trait can also be seen as a trait reflecting lamb vitality, as LTBS and LTSS in this study. According to Cloete *et al.* (1998), h^2 (0.10) and c^2 (0.09) effects equally influenced LTBS of lambs in dual-purpose breeds, where c^2 might have been related to maternal behaviour patterns in the neonatal phase that are not transferred to their ewe progeny. Although there is some variation in these figures, indications are that LTBS are consistently affected by both direct and maternal effects.

For the LTSS, h^2 was estimated at 0.06-0.09, with a corresponding c^2 estimate of 0.08-0.09 (Tables 4.4 and 4.5). An h^2 estimate of 0.15 was reported by Brien *et al.* (2010) for time lambs took

to contact the udder. For Suffolk lambs, Matheson *et al.* (2012) found that suckling assistance, scored on a 5-point scale from 1 (suckling within 1 hour without assistance) to 5 (still requiring suckling assistance at 3 days of age) had a moderate h^2 of 0.32, suggesting that genetic solutions to improve lamb vigour traits could lead to substantial progress. Estimates of h^2 for the LTSS was 0.08 in SAMM lambs and 0.12 in Dormer lambs (Cloete *et al.*, 2002a). In SAMM lambs, c^2 for LTSS was estimated at 0.17 (Cloete *et al.*, 2002a). Cloete (1993) reported that the h^2 of LTSS was smaller than twice its standard error for Dormer and SAMM lambs, as in the present study. For LTSS in dual-purpose breeds, h^2 was estimated at 0.07, while c^2 amounted to 0.19 (Cloete *et al.*, 1998). Thus, the progress of lambs in terms of LTSS during the neonatal phase consistently had relatively small h^2 estimates. In this case, there was clear evidence that the maternal environment provided by the ewe could benefit or compromise LTSS in neonatal lambs. This is possibly partially accounted for by including maternal cooperation score in analyses on LTSS (Cloete & Scholtz, 1998; Cloete *et al.*, 2021). Significant genetic variation was detected when maternal cooperation score were analysed as a dam trait (Cloete *et al.*, 2021). Moreover, genetic trends for the lines were divergent, suggesting that maternal cooperation improved with time in the H-line, while it deteriorated in the L-line.

4.5.5 Genetic correlations

Although not significant, the magnitude of the genetic correlation of BW with LTBS was moderate at 0.31 (Table 4.5). There was no significant relationship between BW and LTBS at the maternal genetic level (-0.04) (Table 4.5). On the phenotypic level, BW was also not significantly related to LTBS at 0.03 (Table 4.5). Matheson *et al.* (2012) reported a high and positive genetic correlation of 0.68 with a moderate and positive phenotypic correlation of 0.39 of birth assistance scored on a 5-point scale from 1 (unassisted, easy delivery within 30 min) to 5 (veterinary intervention required) with lamb vigour (defined previously) Suffolk lambs. These results suggest that lambs requiring the least assistance at birth were more active and vigorous shortly after birth, thus unassisted lambs were able to stand up faster. Cloete *et al.* (2002a) reported negative phenotypic correlations of BW with neonatal lamb progress, ranging from -0.10 to -0.17 in both the SAMM and Dormer breeds. However, a significant maternal permanent environmental correlation was estimated at -0.75 between BW and LTBS (Cloete *et al.*, 2002a). This is an indication that heavier lambs at birth would progress faster under relatively mild conditions in an autumn lambing in South Africa. It is thus impossible to make firm conclusions with reference to the direction and/or magnitude of correlations of BW with LTBS based on the present knowledge, suggesting that further studies are needed.

At respective values of 0.31 and -0.26, BW was not significantly genetically related to either LTBS or LTSS ($p > 0.05$; Table 4.5). This corresponds to the low genetic correlation of 0.13 between BW and latency of birth to suckling (Cloete *et al.*, 2021). However, Dwyer & Lawrence (2005) indicated that lamb BW was only significantly genetically related to the time it took lambs to reach the udder. A low and negative phenotypic correlation (-0.11) was estimated between BW and LTSS (Table 4.5). On the phenotypic level, latency of birth to suckling was also not related to BW at -0.06

(Cloete *et al.*, 2021). For Suffolk lambs, a high and positive genetic correlation of birth assistance with suckling assistance (0.54) was reported by Matheson *et al.* (2012), while the phenotypic correlation between these two traits was low and positive (0.29). This suggests that lambs needing assistance at birth were likely to need suckling assistance too. The maternal genetic correlation of BW with latency of birth to suckling was low and negative at -0.02 (Cloete *et al.*, 2021), thus supporting the lack of a significant relation between BW and LTSS.

Although the genetic correlation of 0.35 between LTBS and LTSS was moderate in magnitude, it was not significant and should be interpreted with caution. Nevertheless, it suggested that lambs taking longer to progress from birth to standing, would likely also take longer to progress from standing to apparent first suckling and vice versa (Table 4.5). At -0.07, LTBS was not phenotypically related to LTSS (Table 4.5). Suffolk lambs had high and positive genetic (0.80) as well as phenotypic (0.60) correlations of lamb vigour and suckling assistance (Matheson *et al.*, 2012). Lambs that were very vigorous at birth (i.e., with low scores; see earlier definition) were also less likely to require suckling assistance. For East Friesen lambs, Simonetti *et al.* (2018) reported that LTBS was significantly positively related (Pearson's $r=0.56$; $p < 0.001$) to the time it took lambs to reach the udder. Yet, there was no significant correlation (0.100; $p=0.591$) between LTBS and LTSS for these lambs (Simonetti *et al.*, 2018). Information from literature on behavioural correlations is scarce. However, a significant positive and moderate correlation (Pearson's $r=0.48$) was found for Romanov lambs between LTBS and LTSS, but not for Finnsheep or Suffolk lambs (Fahmy *et al.*, 1997).

4.6 Conclusion

Cold stress compromised LTSS in neonatal lambs, explaining the higher levels of lamb mortality under cold stress conditions. Selection in the H-Line improved the ability of lambs to progress from standing to apparent first suckling across the range modelled for the CSG. Furthermore, there was evidence of genetic variation (in terms of direct and maternal effects) in neonatal lamb behaviour, yet these results still vary between studies. Other studies found that the lamb's failure to reach the udder was associated with hypothermia and lower rectal temperatures in lambs. This suggests that inclement weather conditions influence lamb behaviour through a combination of discomfort, a depletion of energy reserves and hypothermia. Further research is required to understand the response to cold stress that differed between LTBS and LTSS.

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Chapter 5: Responses to heat in ewes from indigenous and commercial South African sheep breeds: Preliminary Results

5.1 Abstract

Average temperatures are likely to increase, resulting in hotter and dryer conditions in South Africa. The impact of these changes on animal production and welfare is not well-defined. Two trials were conducted to determine the homeothermic response of eight sheep breeds. In 2016, the study included seven breeds, namely Dohne, Dormer, Dorper, Meatmaster, Merino, South African Mutton Merino (SAMM) and White Dorper. The White Dorper was replaced by an unimproved indigenous breed, the Namaqua Afrikaner, in 2017. Ranges of ewes per breed were 10-14 in 2016 and 12-15 in 2017. On days forecast to be hot at noon, these animals were assessed under cool conditions (19-24°C) in the morning and hot conditions at noon (30-33°C) by monitoring individual average eye temperature using thermal imaging (only in 2016) as well as counting flank movements to derive respiration rate (both years). The increased heat load in the afternoon markedly increased both traits. Breed interacted with the time of the day. During 2016, there were suggestions that the hair breeds (Dorper, White Dorper and Meatmaster) were able to maintain lower basal respiration rates in the morning compared to the other breeds. Respiration rate in the generally cooler 2017 study increased by more than threefold from the morning to the afternoon in Merino, Dohne, SAMM and Dormer ewes, more than twofold in the Dorper and Meatmaster and by only 84% in the Namaqua Afrikaner breed. These results suggest that hair sheep and hardy indigenous breeds may cope better with the anticipated higher heat load in the future. There is still marked scope for further research on *ovine* adaptation to heat stress conditions in South Africa.

5.2 Introduction

Sheep form an integral component of most livestock production systems throughout the world, the species being able to adapt to a wide variety of environments. The adaptability and success of sheep is confirmed thereby that they are the world's most diverse mammalian livestock species (Cloete, 2012). A list of sheep breeds by region confirmed that the ovine species is indeed globally successful and represented in widely divergent farming landscapes throughout the world.

It is generally accepted that the western parts of Southern Africa will become hotter and drier under the impact of climate change (Meissner *et al.*, 2013). Considerable areas of South Africa are already marginal owing to constraints of climate and soil (Cloete & Olivier, 2010). Given the ability of sheep to adapt to marginal conditions, the species plays an important role in both the commercial and smallholder animal agricultural sectors. Under the increasing challenge posed by external drivers, such as temperature change, sheep and goats were reported to be more resilient than other

livestock species (Rust & Rust, 2013). The South African ovine genetic resource encompasses specialist wool and meat breeds, terminal sire breeds, dual-purpose breeds as well as unimproved, indigenous fat-tailed types (Cloete & Olivier, 2010). By the number of weaning weight records, the most important South African breeds are the Merino, Dohne, SAMM, Dorper, Dormer and Meatmaster (Cloete *et al.*, 2014). Although it is only found in conservation flocks at present (Qwabe, 2011), the unimproved indigenous Namaqua Afrikaner breed performed well in fitness traits when compared to commercial breeds (Cloete *et al.*, 2016).

Against this background, it is important to assess these breeds for their ability to withstand high temperatures. It is also important to quantify potential differences between breeds, as well as between individuals within breeds to understand the mechanisms underlying the ability of sheep to maintain homeothermy under heat stress conditions.

5.3 Materials and Methods

Two studies were conducted on the Langgewens research farm of the Western Cape Department of Agriculture in the Swartland district, where it is common for the diurnal maximum temperature to exceed 30°C during the summer. The homeothermic response of seven sheep breeds, namely the Dohne, Dormer, Dorper, Meatmaster, Merino, SAMM and White Dorper were assessed, by monitoring individual eye temperature using thermal imaging as well as respiration rate over four sessions. The Merino, Dohne, SAMM and Dormer originated from breeds developed in temperate regions, whereas the Dorper, White Dorper and Meatmaster were composite hair breeds with temperate and heat-adapted breeds as parents. Respiration rate was determined by counting flank movements over a 30 second interval and then express it as breaths per minute (bpm). Sheep were monitored for two sessions during the cooler mornings and for two sessions during hotter afternoons over a three-day period from 31 October to 2 November 2016.

Experienced stockmen released ewes in groups of three to four from a crush into an outside yard where they could be approached to approximately 3 to 4 meters. Individual ewes had numbered tags tied around their necks to allow identification from a distance. Average eye temperature was recorded by an operator equipped with a thermal camera while a second operator counted the flank movements of individual sheep. A scribe recorded the respiration rate of individual ewes, while also acting as a time-keeper. When all sheep in a group were processed, the group was moved to a separate holding yard before the next group was assessed. This routine was followed until all ewes were processed. Several temperature forecast services were used to identify days for breath counting and eye temperature recording with a likely spread of temperatures well in the thermo-neutral zone (19-24°C ambient temperature according to the weather station) in the morning, to increase to a range where some individuals/breeds may experience heat stress (>30°C ambient temperature according to the weather station; see Marai *et al.*, 2007) in the afternoon.

The second study involved the same breeds with the exception of the White Dorper, which was

replaced by the Namakwa Afrikaner. Apart from this change, the same basic procedure was followed during 7 and 8 November 2017. The thermal camera was not available at this stage and the recordings were restricted to respiration rate. The mean (\pm SD) sizes of the breed groups were 12.4 ± 1.3 (ranging from 10-14 per group) during 2016 and 13.0 ± 1.1 (ranging from 12-15 per group) during 2017. All ewes were purchased from reputable breeders within each breed, but possible family relationships were unknown. The ewes were already on the farm for at least 7 months (including the Mediterranean winter) when assessed.

Mixed model methods were used to analyse the data with ASReml4 (Gilmour *et al.*, 2015) within years (2016 and 2017). The model fitted was the following:

$$y_{ijkl} = \mu + b_i + t_j + b_i t_j + ewe_{ijk} + e_{ijkl}$$

with y_{ijkl} = the i^{th} eye temperature or respiration rate observation on the ijk^{th} ewe; μ = the overall mean; b_i = the i^{th} breed (as described within years); t_j = the j^{th} time of day (morning or afternoon); $b_i t_j$ = the breed x time of day interaction; ewe_{ijk} = the random effect of the ijk^{th} ewe and e_{ijkl} = the random error term. The between-ewe variance component so derived was used to estimate the repeatability of the trait under consideration. Random ewe effects were then interacted with the time of the day to assess the variance associated with the re-ranking of ewes under hotter conditions in the afternoon.

5.4 Results and Discussion

Two studies mean (\pm SD) temperatures derived from weather station data indicated morning temperatures during recording of $23.6 \pm 1.6^\circ\text{C}$ during 2016 and $18.9 \pm 2.6^\circ\text{C}$ during 2017. Corresponding means for the afternoon recording were respectively $32.6 \pm 1.4^\circ\text{C}$ and $30.2 \pm 1.2^\circ\text{C}$. The 2017 recording were thus done under somewhat cooler conditions, especially in the mornings. Ewe breed, time of day and the interaction between these fixed effects were significant in 2016 (Table 5.1). The average eye temperature increased from $35.5 \pm 0.1^\circ\text{C}$ in the morning to $36.7 \pm 0.1^\circ\text{C}$ at noon ($p < 0.01$). However, these responses were not similar for all breeds (Table 5.1). Eye temperature increased by around 2% for White Dorper, Dormer, Dorper and Merino ewes, but by much more (3.7 to 8.7%) in the case of SAMM, Dohne and Meatmaster ewes. Respiration rate similarly increased from 75 ± 2 bpm in the morning to 122 ± 2 bpm at noon ($p < 0.01$). In this case there was evidence of differentiation according to origin, as the breeds from temperate regions (Dormer, SAMM, Merino and Dohne) generally exhibited smaller increases of 43 to 58% from morning to noon, compared to 83 to 100% observed in hair sheep (White Dorper, Dorper and Meatmaster). These results stem from the fact that the heat-adapted hair sheep generally had lower basal respiration rates of 54 to 60 bpm in the mornings, compared to 77 to 96 bpm for the breeds originating from temperate regions. The between-ewe variance component went to the boundary of parameters space (zero) for average eye temperature while the repeatability of respiration rate

amounted to 0.26 ± 0.06 . Interacting ewe with the time of the day resulted in estimates of 0.22 ± 0.07 for the repeatability and 0.17 ± 0.08 for the re-ranking term. The regression of respiration rate on eye temperature yielded a coefficient of 5.0 ± 1.1 breaths per minute for one °C increase in eye temperature ($r = 24$; $p < 0.01$).

Table 5.1 Estimated means (\pm SE) for respiration rate and average eye temperature of the respective breeds during cool (morning) and hot (noon) periods during 2016.

Traits and Time	Breed						
	WD	Dorper	SAMM	Dorper	Merino	Dohne	MM
Average eye temperature (°C)							
Morning	35.6 \pm 0.32	35.9 \pm 0.29	34.4 \pm 0.29	36.1 \pm 0.31	36.2 \pm 0.34	35.0 \pm 0.29	35.1 \pm 0.29
Noon	36.3 \pm 0.31	36.6 \pm 0.30	37.4 \pm 0.31	36.9 \pm 0.31	36.8 \pm 0.34	36.6 \pm 0.29	36.4 \pm 0.28
Increase	1.97	1.94	8.72	2.22	1.66	4.27	3.70
Respiration rate (bpm)							
Morning	58 \pm 4.9	96 \pm 4.7	77 \pm 4.7	60 \pm 4.8	94 \pm 5.3	89 \pm 4.7	54 \pm 4.5
Noon	108 \pm 4.8	137 \pm 4.7	118 \pm 4.7	120 \pm 4.8	133 \pm 5.3	132 \pm 4.7	104 \pm 4.5
Increase	83.1	42.7	53.2	100.0	58.3	48.3	92.6

The increase from morning to noon is expressed relative to the mean for the morning. WD – White Dorper; SAMM – South African Mutton Merino; MM – Meatmaster; bpm – breaths per minute.

During 2017, overall respiration rate increased by approximately three-fold from morning to noon as temperatures increased (from 33 ± 1 bpm in the morning to 95 ± 1 bpm at noon ($p < 0.01$)). The interaction of breed with time of day was again highly significant ($p < 0.01$). The respiration rate of ewes was quite similar in the cooler mornings, ranging from 31 bpm (Dorpers and Dohnes) to 38 bpm in Merinos (Table 5.2). The smaller differences between breeds could be related to the lower morning temperatures during 2017. Responses to the higher heat loads at noon were again highly breed-specific. The respiration rate of the unimproved fat-tailed Namaqua Afrikaner increased by 84% from the morning session to the noon session. The respiration rate of the other hair sheep (Dorper and Meatmaster) increased by more than 2-fold, while the respiration rate of the breeds from temperate origin increased by more than 3-fold. The repeatability of respiration rate amounted to 0.18 ± 0.06 . When the ewe x time of the day interaction was added, most of the variance repartitioned toward the interaction (re-ranking) term, yielding respective estimates of 0.08 ± 0.07 and 0.28 ± 0.09 .

The ability of adapted, indigenous genotypes to better cope with heat stress across species was reviewed by (Cloete, 2012). It was evident that indigenous sheep breeds were better able to cope with heat stress in Egypt and India. The Namaqua Afrikaner, in particular, was described in the literature as a slender breed with long legs to assist in the dissipation of excess heat (Qwabe, 2011; Snyman *et al.*, 2013). The ability of this breed to cope with heat conditions as well as its resistance to external parasites (Cloete *et al.*, 2016) indicate that it may play an important role under challenging and poorly resourced conditions (Molotsi *et al.*, 2020). Although other hair sheep (White Dorper, Dorper and Meatmaster) also performed better than the temperate breeds for respiration rate, they were not quite as well adapted as the Namaqua Afrikaner.

Table 5.2 Estimated means (\pm SE) for respiration rate of the respective breeds during cool (morning) and hot (noon) periods during 2017.

Traits and Time	Breed						
	NA	Dorper	SAMM	Dorper	Merino	Dohne	MM
Respiration rate (bpm)							
Morning	32 \pm 3.7	32 \pm 3.9	34 \pm 3.7	31 \pm 3.8	38 \pm 3.7	31 \pm 3.7	32 \pm 3.4
Noon	58 \pm 3.9	121 \pm 3.8	112 \pm 3.7	87 \pm 3.8	122 \pm 3.8	94 \pm 3.7	74 \pm 3.4
Increase	84.4	378.1	329.4	280.6	321.1	303.2	231.3

The increase from morning to noon is expressed relative to the mean for mornings. NA – Namaqua Afrikaner; SAMM – South African Mutton Merino; MM – Meatmaster; bpm – breaths per minute.

5.5 Conclusion

Adapted livestock such as particularly the Namakwa Afrikaner, but also the Meatmaster and Dorper, may cope better under challenging climate change scenarios than breeds from temperate regions such as the Merino, Dohne, SAMM and Dorper. An easily recorded indicator trait such as respiration rate could be considered as a tool to improve within-breed heat tolerance by selection under low-input systems. The provision is that future studies should allow a better understanding of the interaction of random ewe effects with the ambient conditions, represented in this study by cooler mornings and hotter afternoons.

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Chapter 6: Quantifying *ovine* responses to hot conditions, as well as between and within animal components of variation in common heat stress indicators in a Western Cape flock

6.1 Abstract

Data from multi-breed resource flocks at the Langgewens Research Farm were accumulated from 24 to 27 January 2022 and used to quantify responses of ewes to an increased heat load. In total, 80 ewes were used, 10 each of 3 wool breeds (Merino, Dohne and SA Mutton Merino or SAMM), two terminal sire breeds (Dorper and Ile de France), 2 composite breeds with indigenous content (Dorper and Meatmaster) and an unselected indigenous fat-tailed breed, the Namaqua Afrikaner. These breeds were recorded for rectal temperature, spot temperature and respiration as indicators of heat stress over three days, involving 3 morning and 3 afternoon recording sessions. The mornings were appreciably cooler than the afternoons, resulting in means for all traits increasing from the morning to the afternoon session. A clear breed difference for spot temperature suggested that higher cheek temperatures were obtained in the dark-headed breeds, namely the Dorper and Namaqua Afrikaner. The means for respiration rate of Namaqua Afrikaner and Meatmaster sheep were lower than that of the Dorper. Respiration rates of the other breeds (Dohne, Dorper, Ile de France, Merino and SAMM) accordingly increased above that of the Dorper. Rectal temperature (0.16) and spot temperature (0.17) were low-moderately repeatable, but all between-ewe variance partitioned to a term accounting for the reranking of ewes from the morning to the afternoon sessions. This effect was applicable to spot temperature (0.19) and respiration rate (0.20). Based on the variation in respiration rate, it was contended that the breeds with indigenous content would adapt better to the anticipated hotter conditions expected in future.

6.2 Introduction

It is generally accepted that the western parts of Southern Africa will become hotter and drier under the impact of climate change (Meissner *et al.*, 2013). It is important for an animal to remain healthy and maintain its productivity and longevity through maintaining body temperature within physiological limits (Marai *et al.*, 2007). Cloete & Olivier (2010) stated that considerable areas of South Africa are already marginal owing to constraints of climate and soil. Animal production is likely to be adversely influenced by increased levels of heat stress in livestock species in association with climate change (Indu & Pareek, 2015; Rashamol *et al.*, 2018).

Nationally, sheep are by far the dominant small ruminant species in numbers (Cloete & Olivier, 2010). Although goats are considered the most adaptable species in severe climatic conditions (Aziz, 2010; Silanikove & Koluman, 2015), sheep from breeds native to arid and semi-arid regions are also

better adapted to heat stress and are able to survive in precarious extensive conditions (Shinde & Sejian, 2013; Al-Dawood, 2017). It is thus important to assess the available breeds that form part of the available South African ovine genetic resource for their ability to withstand increased temperatures (Aziz, 2010; Silanikove & Koluman, 2015; Sejian *et al.*, 2017). It is also important to be able to quantify potential differences between breeds, as well as between individuals within breeds to understand the mechanisms underlying the ability of sheep to adapt to heat stress conditions. Sheep experiencing heat stress initiate compensatory and adaptive mechanisms (i.e., behavioural and physiological changes) to re-establish homeothermy and homeostasis in attempt to maintain their body temperature (Chapter 3; Henry *et al.*, 2012; Indu & Pareek, 2015). This in turn will promote welfare and favour survival in sheep residing in environments with extreme temperatures.

It is a reality that climate change is occurring and that there is limited local knowledge on the responses of livestock to elevated temperatures. The effectiveness of interventions to curb the effects of increased temperatures is also understudied (Cloete *et al.*, 2000; Cloete, 2012; Meissner *et al.*, 2013; Rust & Rust, 2013). This background warrants a study on the ability of different sheep breeds to cope with elevated temperatures characteristic of most of pastoral South Africa. It is foreseen that these climatic extremes will become more common in future, adding to the priority of this research.

There are several measurements, generally referred to as physiological responses, that serve as indicators of breed and individual differences in heat stress in sheep (McManus *et al.*, 2016; Rashamol *et al.*, 2018). Cardinal physiological responses to heat stress that help maintain the heat balance and homeostasis of heat stressed animals include respiration rate, pulse rate, rectal temperature, sweating rate, and skin temperature (Chapter 2; Indu & Pareek, 2015; Rashamol *et al.*, 2018). These physiological responses were established as ideal indicators or biomarkers for quantifying and thus assessing the severity of heat stress in farm animals (Indu & Pareek, 2015; McManus *et al.*, 2016; Shilja *et al.*, 2016; Rashamol *et al.*, 2018).

Marai *et al.* (2007), Dalcin *et al.* (2016), Singh *et al.* (2016), El-Tarabany *et al.* (2017), and Rashamol *et al.* (2018) found that when livestock are exposed to heat stress, their respiration rate is the first to increase to compensate for the excessive internal heat load. If exposure to heat stress continues, the rectal temperature rises, indicating that the animal is unable to maintain homeostasis (i.e., fails to maintain core body temperature). Spot temperature is an additional heat stress indicator trait, which is directly associated with vasodilation of the skin capillary bed and increased blood flow to the skin surface that promotes heat dissipation in attempt to maintain thermal stress internally (McManus *et al.*, 2009; Indu & Pareek, 2015; Shilja *et al.*, 2016; Katiyatiya *et al.*, 2017).

Rectal temperature is considered a direct indicator of the core body temperature in animals (McManus *et al.*, 2016). Non-invasive indirect measures that could act as proxies for core body temperature include spot-measurements of skin temperature using a laser-based thermometer and the measurement of eye temperature using thermal imaging. Records derived from respiration rate,

spot temperatures, rectal temperature and thermal imaging were obtained to further the objectives of Project ACU-2021-21966 (Stellenbosch University) and AP/BR/S/TB102 (Eisenburg - DECRA). Respiration rate and thermal images were measured from a distance under paddock and sheep-yard conditions with minimal inputs, except for manpower. There is, however, a need to calibrate respiration rate and eye temperature against rectal temperature as a true measure of core body temperature. As wool cover forms a barrier over which heat exchange needs to take place, fleece depth and cover type (wooly or hairy – see do Prado Paim *et al.*, 2013) was recorded.

This study sought to test whether ewes from a divergent genetic background differ in their heat-tolerance, and whether between-animal variances (repeatability) could serve as a proxy for genetic variation across breeds and be used to identify genotypes and individuals less subject to heat stress. Therefore, non-invasive indicators of heat tolerance in the Western Cape were recorded to obtain breed differences and repeatability estimates for traits putatively related to heat tolerance in South African sheep. Rectal temperature was also recorded as a standard to relate the other proxy traits to.

6.3 Materials and Methods

6.3.1 Animals, Location and Recordings

The multi-breed resource flock maintained at Langgewens Research Farm in the Western Cape near Moorreesburg, supplied the experimental animals for this project. A total of 80 ewes recorded for this study was drafted from the mixed-age flock already present on Langgewens Research Farm and consisted of 10 Merino, 10 Dohne Merino, 10 South African Mutton Merino (SAMM), 10 Dorper, 10 Ile de France (IDF), 10 Dorper, 10 Namaqua Afrikaner and 10 Meatmaster ewes.

Prior to the start of the experiment, several temperature forecast services (www.meteoblue.com; www.yr.no; www.accuweather.com; www.weathersa.co.za) were consulted to identify days that are likely to have a spread of temperatures well in the thermo-neutral zone (20-28°C ambient temperature according to the weather station) in the morning, to increase to a range where some individuals or breeds may experience heat stress (30-35°C ambient temperature according to the weather station) towards noon. Specific care was taken to also record on days when most animals are likely to experience heat stress (35°C and higher) at noon and during the afternoon. Recording involved six sessions over three days to ensure that a wide range of ambient conditions were covered. The Western Cape Department of Agriculture's Weather Station Portal (<https://gis.elsenburg.com/apps/wsp/#>) was used to obtain hourly temperature and humidity data readings over the three trial days from 25 to 27 January 2022.

The animals were brought into the sheepyard at Langgewens Research Farm during the afternoon of the day before the recording sessions commenced (i.e., 24 January 2022). At this stage, the ewes were put through a crush for the measurement and recording of physiological traits, namely body weight, fat depth and wool depth. To enable identification from a distance during recording

sessions, each individual ewe received a uniquely numbered neck-tag that was gently fitted around the neck of a specific animal according to their unique neck circumferences. Subsequently, the group of ewes was divided into 10 smaller paddocks with 8 ewes (one ewe per breed) per paddock to facilitate the recording of individual heat stress indicator traits. Ewes were fed medic hay overnight. One to two hours prior to the start of each recording session, ewes within the same paddock were split into two groups of four ewes to facilitate an easier flow of measurements. After each recording session, the ewes were released to roam freely within their initial larger paddock. On the three days of recording heat stress indicator traits (25-27 January 2022), respiration rates were the first measurements, followed by rectal temperatures and spot temperatures that were recorded simultaneously. As the final measurement, the recording of eye temperatures through thermal imaging was only implemented during the first session on the first day of the trial at Langgewens Research Farm due to technical failure relating to the E75 Thermal Camera. These recordings were duplicated in the cooler mornings as well as during the hotter afternoons, resulting in two recording sessions per day. On the final day, following the afternoon recording session, the animals were given an opportunity to cool down through provision of drinking water. Following the cool-down session, each individual ewe was put through the crush once again to be weighed and for the removal of their neck-tags. Lastly the animals were grouped together, returned to their flocks of origin, and managed according to the existing Heat Stress SOP for this study.

The handling of animals may have resulted in an increased core temperature for the records obtained when animals were restrained. The same treatment applied to all animals, so it is considered unlikely that it would affect the ranking of animals for the spot and rectal temperatures. A team consisting of a recorder and a scribe counted the number of respiratory side movements for all animals in a group for 30 seconds until all groups were counted. A standard clinical thermometer was used to measure rectal temperatures, while a laser-based thermometer was used to measure spot temperatures on the cheek. For wool depth and hair length measurements, a Digital Caliper was used. Fat depth measurements were taken using a Mindray DP 30V ultrasound scanner with a 7.5 MHz linear transducer. The time of the recording was noted to allow linkage back to the hourly ambient conditions recorded by the Weather Station Data Portal of the Western Cape Department of Agriculture. Refer to section 2.8 of Chapter 2: Literature Review for a detailed description on the instruments and devices used to perform the required measurements of the individual heat stress indicator traits and other physiological traits, as well as the recording of ambient climate data.

6.3.2 Temperature-Humidity Index (THI)

THI for Langgewens Research Farm was computed from the available climate data retrieved from the Weather Station Portal of the Western Cape Department of Agriculture (<https://gis.elsenburg.com/apps/wsp/#>), reporting environmental temperature and relative humidity (RH) values that were recorded hourly. The equation by Marai *et al.* (2001) was used to compute

the THI for the current study. All ambient temperature (TC) values are in degrees Celsius (°C), and RH is a percentage (%).

$$\text{THI} = \text{TC} - ((0.31 - 0.31 \times \text{RH}/100) \times (\text{TC} - 14.4))$$

6.3.3 Statistical analysis

The trait means for the change in body weight (W, kg) was compared between the ewe breed groups by analysis of variance (ANOVA) using the general linear models (GLM) procedure of SAS Enterprise Guide. Since the data acquired for the fat depth (cm) trait did not conform to normality, the trait means were derived through performing a non-parametric test (i.e., the Kruskal-Wallis test), using the NPAR1WAY procedure of SAS Enterprise Guide, which is equivalent to the one-way ANOVA but not assuming normality (SAS Version 7.1, 2021). Since the fleece depth (mm) trait violated both the assumptions of normality and homoscedasticity, it was necessary to transform this data. This was done through non-linear transformation with the intention to modify the shape of the fleece depth distribution in order to bring the values closer to meeting the underlying assumptions (Meyers *et al.*, 2009). Since the raw data of fleece depth was found positively skewed, Meyers *et al.* (2009) suggested that either square root transformation, log transformation or reflected inverse transformation should be performed to reduce this positive skewness. The transformed data of fleece depth from log base 10 and natural log both resulted in the same outcome and reduced the skewness down to approximately -0.11. However, the natural logarithm transformation attained the best univariate normal distribution. Through applying natural logarithm transformation, the fleece depth data was normalized, where the trait means were derived by Welch's Variance-Weighted ANOVA using the ANOVA procedure of SAS Enterprise Guide. The influence of the main effect of breed (Merino, Dohne Merino, SAMM, Dormer, IDF, Dorper, Namakwa Afrikaner or Meatmaster) on trait means of the various physiological ewe traits were evaluated. Differences among the breed groups were regarded as significant at $p \leq 0.01$. The breed effects were expressed as arithmetic means with their respective standard errors (SE).

The heat stress indicator traits (rectal temperature, spot temperature, eye temperature and respiration rate) that were recorded across three days with two sessions (morning and afternoon) were subjected to mixed model analyses, using ASReml 4.2 (Gilmour *et al.*, 2015). This software allows for the prediction of means of selected fixed effects and regressions in unbalanced designs using linear mixed models. Ewe breed and day of recording were fitted as fixed effects. As response variables, the heat stress indicator traits were regressed on the ambient climatic conditions as reflected by the THI. The THI was based on the actual temperature and humidity at the time of measurement. The regressions in ASReml depended on a fixed linear component and random cubic spline components, reflecting random deviations from linearity conforming to a smooth trend (Verbyla *et al.*, 1999) where appropriate. Two-factor interactions were fitted between ewe breed and recording day, as well as between breed and relevant regression variables as appropriate. The

random effect of ewes was added to an operational model that included the appropriate fixed and regression components for a specific heat stress indicator trait. Additionally, random ewe effects were interacted with session (morning and afternoon) to account for the reranking of ewes between cooler morning and hotter afternoon conditions. Since the session was confounded with regressions on the ambient climatic conditions experienced, it was not considered as a fixed effect in the operational model. Log-likelihood ratios were used to compare random effects models when an additional random effect was fitted, as described by Snyman *et al.* (1995).

The fixed effects of breed, day (both as defined before) and session (morning or afternoon), as well as two-factor interactions among them were considered next. Significant main and interaction effects were presented in Tables and Figures.

6.4 Results

6.4.1 Descriptive statistics

The descriptive statistics for body weight (*W*) before and after the trial, fat depth and fleece depth as well as heat stress indicator traits are given in Table 6.1. Coefficients of variation (CV) ranged from 10.3% for natural log transformed fleece depth to 37.9% for untransformed fleece depth (Table 6.1). Only *W* traits and heat stress indicator traits conformed to normality as far as skewness and kurtosis were considered. CV of the fat and untransformed fleece depth traits analyzed exceeded 20%, suggesting that these traits were highly variable. When the population variances were considered prior to analyzing the respective traits, the raw and transformed means of fleece depth did not conform to homoscedasticity. This is due to the Dorper and Namakwa Afrikaner breeds having much greater variation compared to their contemporaries and thus contributed to the unequal population variances. The animal-based temperature records had means between 35.9 and 38.6, while the mean for respiration rate amounted to almost 125 bpm (Table 6.1). The CV for rectal temperature was very low at 1.03%, while it was also below 10% for spot temperature. Untransformed respiration rate was highly variable, with a CV of almost 46%.

Table 6.1 Descriptive statistics for body weight before and after the trial, fat depth and fleece depth traits as well as heat stress indicator traits recorded for the eight divergent sheep breeds considered (n = 480) in the study on Langgewens Research Farm.

Trait	Observations (n)	Mean \pm SD	CV (%)	Range
<u>Phenotypic</u>				
Body Weight Before (kg)	80	79.1 \pm 11.9	15.1	55.0 – 99.8
Body Weight After (kg)	80	76.7 \pm 13.0	17.0	50.6 – 100.8
Fat Depth (cm)	80	0.207 \pm 0.07	32.7	0.09 – 0.44
<u>Fleece Depth (mm)</u>				
Untransformed	80	37.4 \pm 14.2	37.9	10.4 – 79.2
Log transformed	80	3.56 \pm 0.37	10.3	2.34 – 4.37
<u>Heat stress indicators</u>				
Rectal temperature ($^{\circ}$ C)	480	38.9 \pm 0.4	1.03	37.1 – 40.3
Spot temperature ($^{\circ}$ C)	480	35.9 \pm 1.6	4.46	32.0 – 42.9
<u>Respiration rate (bpm)</u>				
Untransformed	480	124.3 \pm 56.3	45.3	18 - 226
Log transformed	480	124.3 \pm 56.3		18 - 226

SD – Standard Deviation; CV – Coefficient of Variance; bpm – breaths per minute

6.4.2 Temperature-humidity index (THI)

Environmental temperature (T in $^{\circ}$ C) and RH (%) were significant factors that affected the THI for the duration of the study and are illustrated in Figure 6.1, where THI was computed according to Marai *et al.* (2001). It was observed that the THI-values differed significantly on average between the morning (THI = 19.8 \pm 0.89) and afternoon (THI = 29.1 \pm 0.28) sessions for the three-day trial period (Figure 6.1). Accordingly, the THI-values for the afternoon recording sessions were similar ($p > 0.05$) across the three trial days with a maximum and minimum THI of 30.5 and 27.6, respectively. This is an indication that the ewes most likely experienced extreme heat stress between 14h00 and 16h00 on each day. THI-values for the morning recording sessions were also similar ($p > 0.05$) for each day and reflected a maximum THI of 23.3 and minimum THI of 16.4, suggesting that heat stress was absent to moderate in ewes at 06h00 to around 09h30, respectively. Overall, THI was the highest on Day 2 and lowest on Day 3 for the afternoon recording sessions, with THI being the highest on Day 1 and lowest on Day 2 for the morning recording sessions (Figure 6.1).

(61)

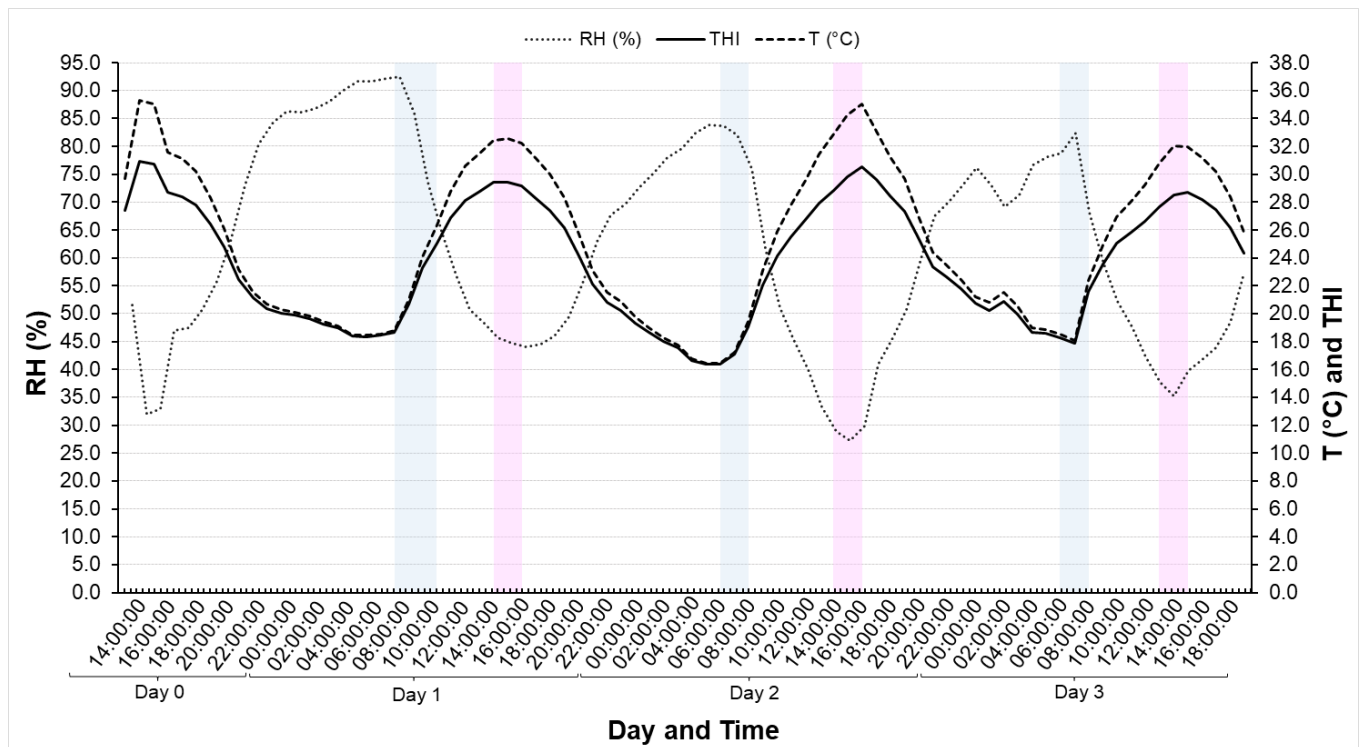


Figure 6.1 The environmental temperature (T), relative humidity (RH), and temperature-humidity index (THI, where T was expressed relative to °C) for the duration of the trial (24 – 27 January 2022) at Langgewens Research Farm. Blue shading represents the morning recording sessions; Pink shading represents the noon/afternoon recording sessions.

6.4.3 Breed effects on live weight, fat depth and fleece depth

The change in W differed significantly between the ewe breeds, with the highest means for Namakwa Afrikaners and Meatmasters (Table 6.2). Merinos, Dorpers, IDF, and Dohne Merinos were intermediate and not different from the other breeds. SAMM and Dormers had the lowest means for the change in W, which was significantly lower than the means for the other breeds. Fat depth was independent of breed ($p > 0.05$), with an average trait mean of 0.207 cm (Table 6.2). Significant differences were found among the breeds for the fleece depth data after transformation to natural logarithms, with the highest fleece depth value presented in the Namakwa Afrikaner ewes. The Dohne Merinos, Merinos and Dorpers were intermediate and not different ($p > 0.05$) from the other breeds. The Dormer, IDF, Meatmaster and SAMM ewes presented the lowest means and was different from the other breeds ($p < 0.05$; Table 6.2).

Table 6.2 Arithmetic means (\pm SE) for W change, fat depth, and Log transformed fleece depth traits in ewes of different breeds on Langgewens Research Farm.

Effect	Traits		
	Change in W (kg)	Fat depth (cm)	Fleece depth (mm)
Overall mean	-2.38 \pm 0.31	0.207 \pm 0.01	3.56 \pm 0.04
<u>Breed</u>	**	13.5	**
Dohne Merino	-2.24 \pm 0.87 ^{a, b}	0.267 \pm 0.02	3.64 \pm 0.03 ^{a, b}
Dorper	-0.60 \pm 1.02 ^b	0.199 \pm 0.01	3.40 \pm 0.07 ^b
Dorper	-2.44 \pm 0.83 ^{a, b}	0.201 \pm 0.02	3.57 \pm 0.22 ^{a, b}
IDF	-2.44 \pm 0.65 ^{a, b}	0.199 \pm 0.02	3.30 \pm 0.04 ^b
Meatmaster	-3.76 \pm 0.77 ^a	0.165 \pm 0.01	3.42 \pm 0.11 ^b
Merino	-2.62 \pm 1.25 ^{a, b}	0.224 \pm 0.03	3.63 \pm 0.05 ^{a, b}
Namakwa Afrikaner	-4.12 \pm 0.34 ^a	0.182 \pm 0.01	4.02 \pm 0.10 ^a
SAMM	-0.84 \pm 0.63 ^b	0.220 \pm 0.03	3.48 \pm 0.04 ^b

**Significant ($p < 0.05$); Actual F-value for $p > 0.05$; ^{a, b} Denote significant differences ($p < 0.05$) in rows within traits. W – Body Weight; IDF – Ile de France; SAMM – South African Mutton Merino.

6.4.4 Fixed effects

The significance of fixed and interaction effects for each heat stress indicator trait are provided in Table 6.3. All traits except respiration rate were affected ($p < 0.01$) by breed and day, where respiration rate was only affected by breed. The fixed linear regressions of all heat stress indicator traits on the relevant THI variable were significant for all traits and interacted with breed for spot temperature and respiration rate (all $p < 0.01$). The heat stress indicator traits, rectal temperature and respiration rate, both required the inclusion of a cubic spline (as well as respiration rate's interaction with breed) to model deviations from linearity conforming to a smooth trend (Table 6.3).

Table 6.3 The levels of significance for fixed effects of breed and day of recording as well as the regression variables on the temperature-humidity index (THI) and interactions between these main effects.

Effect	Trait		
	Rectal Temperature ($^{\circ}$ C)	Spot Temperature ($^{\circ}$ C)	Respiration Rate (bpm)
Breed	**	**	**
Days	**	**	0.068
Breed x Days	0.713	0.959	0.713
THI Linear	**	**	**
Breed x THI Linear	0.113	**	**
Breed x THI Spline	**	N/A	**

**Significant ($p < 0.01$); Actual P-value are provided for $p > 0.05$; N/A – Not Applicable; bpm – breaths per minute; THI – temperature-humidity index

Overall, rectal temperature was higher in Dohne Merino and Ile de France ewes than in Merino, Namaqua Afrikaner, Meatmaster and Dorper ewes, which in turn had higher means than SAMM and Dorper ewes (Table 6.4). In contrast, spot temperatures trended higher ($p < 0.05$) in Dorper and Namaqua Afrikaner ewes compared to the other breeds. The respiration rate of Namaqua Afrikaner and Meatmaster ewes were the lowest, then Dorper ewes, compared to the other breeds (All $p < 0.05$). Rectal temperature trended higher on Day 1 while spot temperature trended higher on Day 2

compared to the other two days. Furthermore, respiration rate roughly remained the same across all three days ($p > 0.05$).

Table 6.4 Predicted means (\pm SE) for the fixed effects of breed and day of recording on the heat stress indicator traits that were analysed.

Effect	Trait		
	Rectal Temperature ($^{\circ}$ C)	Spot Temperature ($^{\circ}$ C)	Respiration Rate (bpm)
Breed			
Dohne Merino	39.0 \pm 0.04 ^c	35.8 \pm 0.17 ^a	4.76 \pm 0.06 (117) ^c
Dorper	38.8 \pm 0.04 ^a	36.7 \pm 0.17 ^b	4.59 \pm 0.06 (99) ^b
IDF	39.0 \pm 0.04 ^c	35.5 \pm 0.17 ^a	4.83 \pm 0.06 (125) ^c
SAMM	38.8 \pm 0.04 ^a	35.8 \pm 0.17 ^a	4.76 \pm 0.06 (116) ^c
Merino	38.9 \pm 0.04 ^b	35.8 \pm 0.17 ^a	4.84 \pm 0.06 (126) ^c
Namaqua Afrikaner	38.9 \pm 0.04 ^b	36.4 \pm 0.17 ^b	4.43 \pm 0.06 (84) ^a
Meatmaster	38.9 \pm 0.04 ^b	35.5 \pm 0.17 ^a	4.42 \pm 0.06 (83) ^a
Dorner	38.9 \pm 0.04 ^b	35.5 \pm 0.17 ^a	4.87 \pm 0.06 (131) ^c
Day of Recording			
1	38.9 \pm 0.05 ^b	35.8 \pm 0.10 ^a	4.68 \pm 0.03 (107)
2	38.7 \pm 0.06 ^a	36.2 \pm 0.10 ^b	4.73 \pm 0.03 (113)
3	38.7 \pm 0.05 ^a	35.6 \pm 0.10 ^a	4.65 \pm 0.03 (105)

^{a, b, c} Denote significant differences ($p < 0.05$) in columns within effects. bpm – breaths per minute; IDF – Ile de France; SAMM – South African Mutton Merino

The interaction of breed with the linear and spline components is provided in Figure 6.2. This graph was very crowded with all breeds present and were thus split in three separate graphs. The wool breeds (Merino, Dohne and SAMM) are presented in Figure 6.2 (a), the terminal sire breeds (Dorner and Ile de France in Figure 6.2 (b) and the breeds with indigenous content (Dorper, Meatmaster and Namaqua Afrikaner) in Figure 6.2 (c). The graphs were standardised for the range on the y-axis to facilitate comparisons among graphs. Regression lines for Dohnes and Merinos were quite similar, but the line for SAMM appeared to be more linear in nature. Trends for the terminal sire breeds were quite similar, but the trendline for the Namaqua Afrikaner breed was quite different than those of all other breeds. It appeared if the associated change with increased THI levels were shallower in the breeds with indigenous content in Figure 6.2 (c). It is notable that a widespread, indicative of high levels of individual variation, were observable within and across breeds at any given THI-level.

(64)

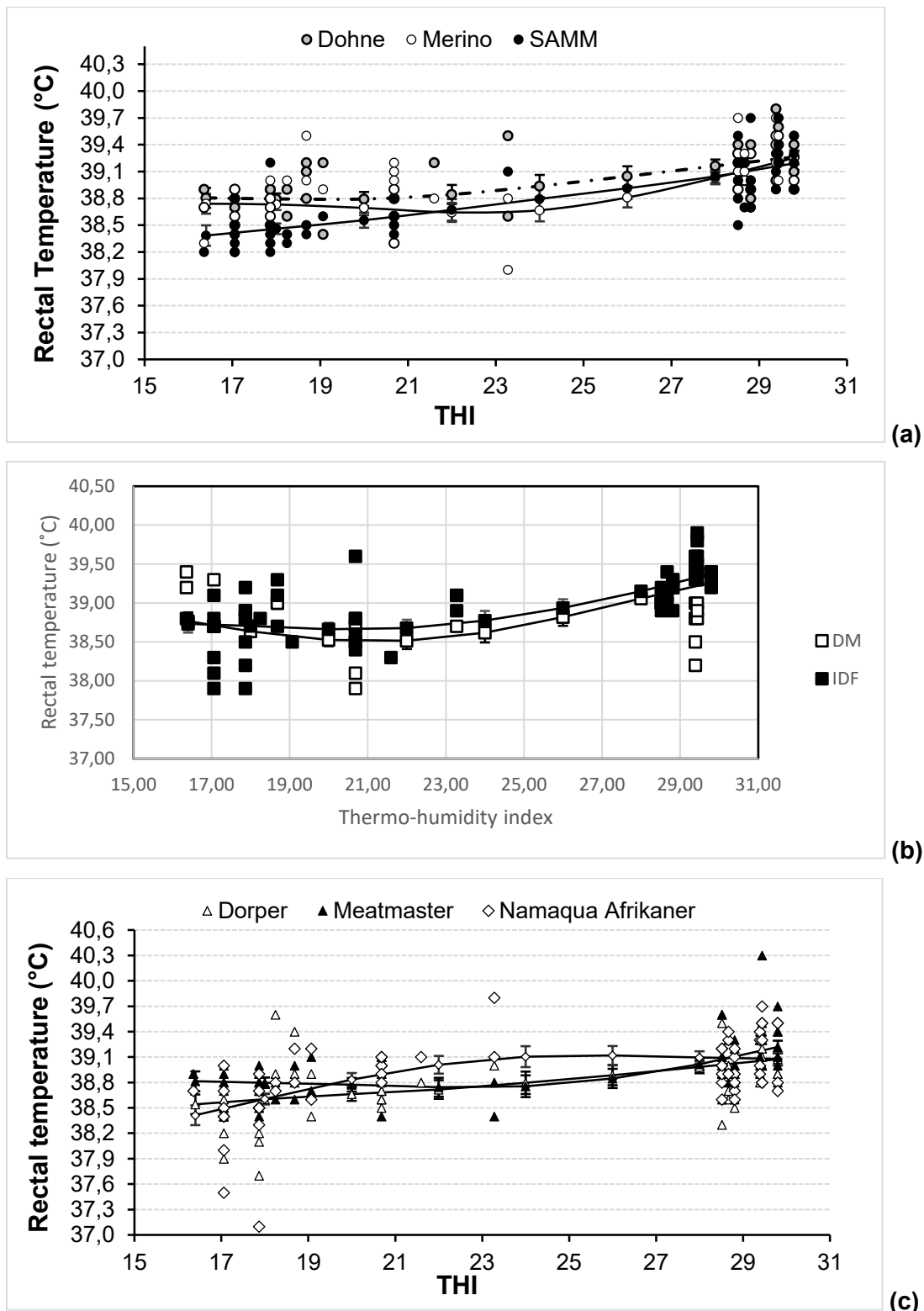


Figure 6.2 Scatterplot depicting the relationship of recorded rectal temperatures of ewes from wool breeds (Dohne, Merino and South African Mutton Merino - SMM) (a), Terminal sire breeds (Dorper and Ile de France - IDF) (b) and indigenous breeds (Dorper, Meatmaster and Namaqua Afrikaner (c) with the temperature-humidity index (THI). Standard errors on the respective breed regression lines could be used to compare breed differences at different THI-levels.

Spot temperatures were also subject to an interaction between breed and the linear component of the regression on the THI. This interaction was mostly driven by different trajectories for the breeds with dark heads (Dorper and Namaqua Afrikaner) and the wool breeds. As demonstration, Figure 6.3 depicts a scatterplot for the dark-headed breeds and the fine wool breeds (Merino and Dohne)

with applicable regression lines to allow the assessment of the interaction. It is clear that the trendlines for Dorpers and Namaqua Afrikaners had a steeper slope than the lines for the wool breeds. It was also evident that the spread of values around the graphs were markedly increased during the afternoon recording sessions.

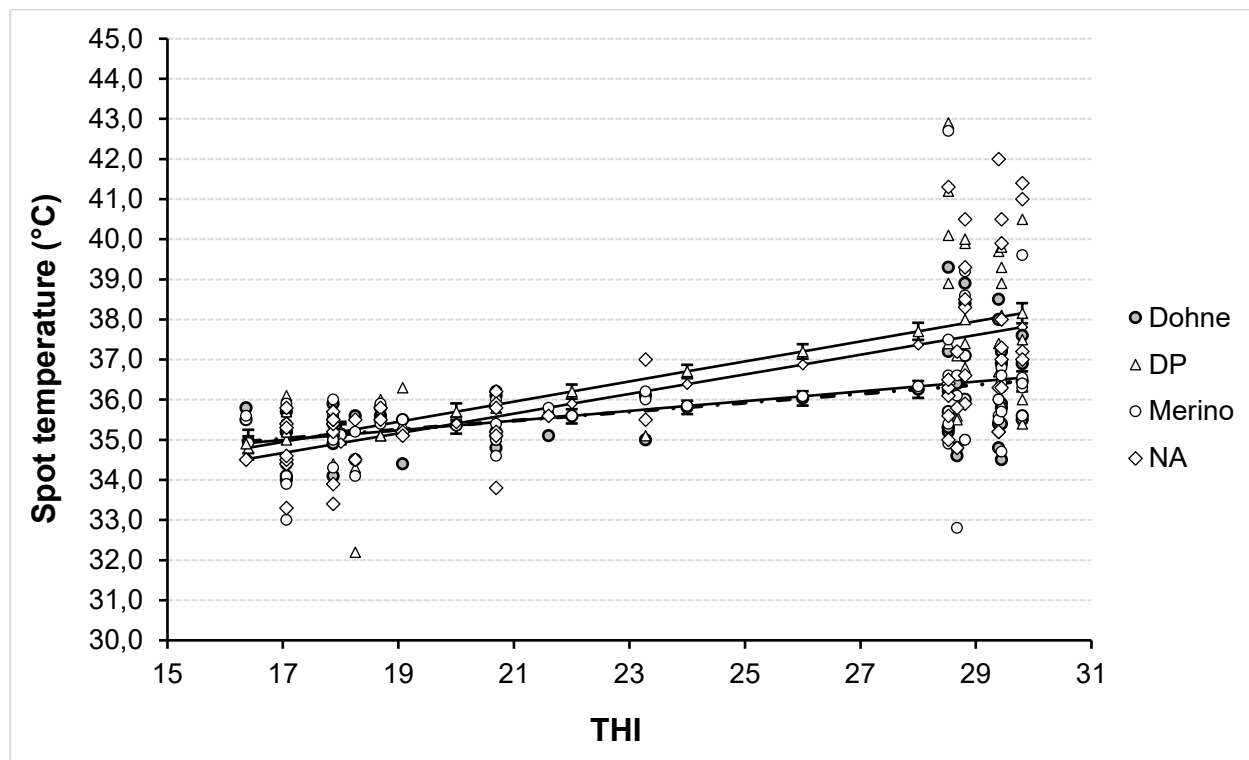


Figure 6.3 Scatterplot depicting the relationship of recorded spot temperatures of ewes from wool breeds (Dohne and Merino) with dark-headed indigenous breeds (Dorper - DP and Namaqua Afrikaner - NA (c) with the temperature-humidity index (THI). Standard errors on the respective breed regression lines could be used to compare breed differences at different THI-levels.

The interaction of breed with the spline components of the THI for respiration rate is depicted in Figure 6.4 using observed values and regressions based on predictions of geometric means. It is believed that plotting the graph on the observed scale will ease interpretation by the reader. As the graphs were very crowded when all the breeds were considered, it was decided to only plot values for a fine wool breed (Merino), a terminal sire breed (IDF), a meat breed with indigenous content (Dorper) and an unimproved indigenous breed (the Namaqua Afrikaner). It was clear that, although the regression for the IDF started at lower levels compared to Merinos, it inclined faster and were above the line for Merinos at high THI-values. The Dorper and Namaqua Afrikaner breeds were at similar levels and below the breeds originating from temperate environments at low THI-levels. The curve for Dorpers inclined faster than that of Namaqua Afrikaners. The latter breed was thus substantially below all the other breeds at moderate and high THI-levels.

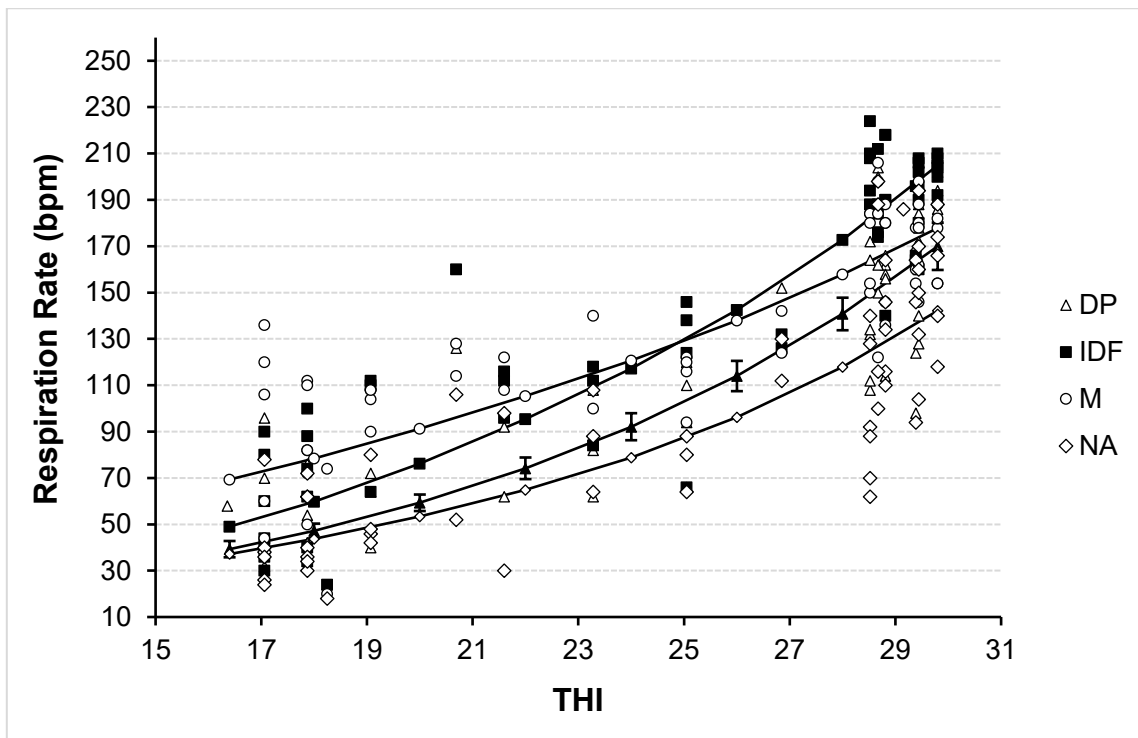


Figure 6.4 Scatterplot depicting the relationship of recorded respiration rate (breaths per minute - bpm) on the observed scale of ewes from a wool breed (Merino – M), a terminal sire breed (Ile de France – IDF), a breed with indigenous content (Dorper – DP) and an unimproved indigenous breed (Namaqua Afrikaner – NA) with the temperature-humidity index (THI). Standard errors on the respective breed regression lines could be used to compare breed differences at different THI-levels.

Like respiration rate, the graphs for rectal temperature were very crowded when all the breeds were considered. Thus, the interaction of breed with the spline components of the THI for rectal temperature is depicted in Figure 6.5 using observed values and regressions based on predictions of means in Merino, IDF, Dorper, and Namaqua Afrikaner ewes. There were no clear discrepancies for the regressions between the Dorper, IDF and Merino breeds. Although the regression for the Namaqua Afrikaner started at similar levels compared to the other breeds, it inclined faster and was above the line for Dorpers, IDF and Merinos at moderate to high THI-values, to which the Namaqua Afrikaner's regression stabilised at a THI of 26, and slowly declined from there to similar levels compared to the other three breeds.

(67)

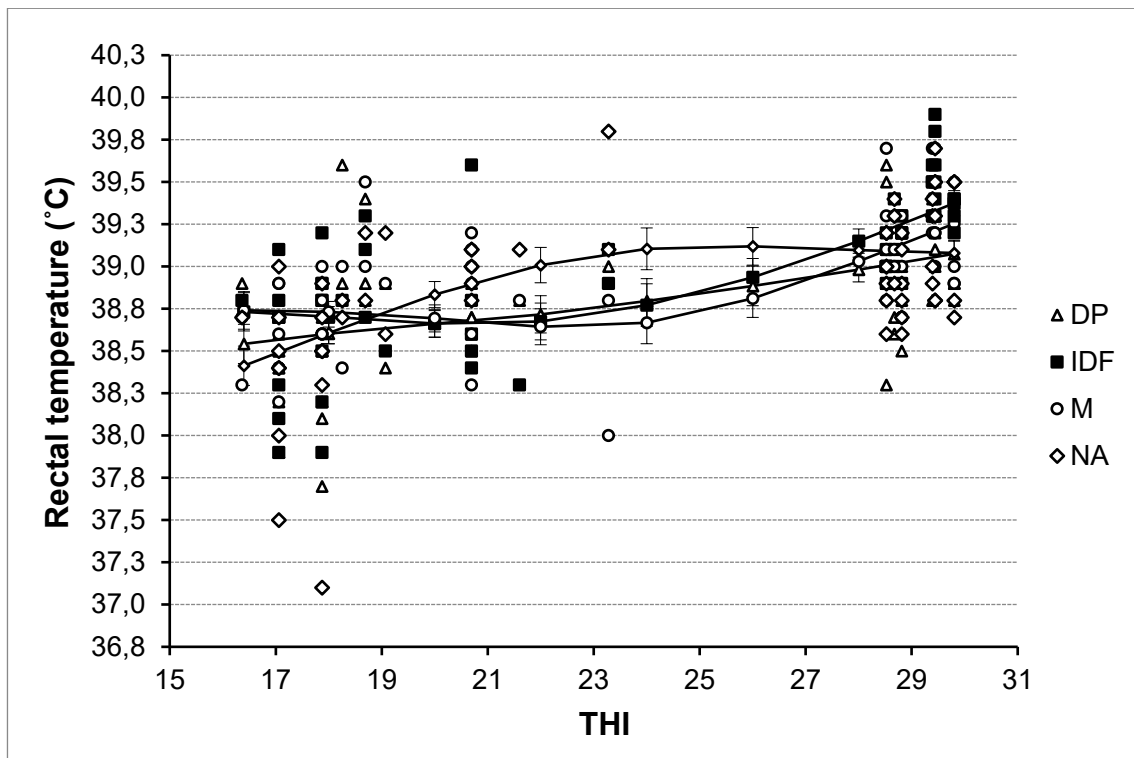


Figure 6.5 Scatterplot depicting the relationship of recorded rectal temperature on the observed scale of ewes from a wool breed (Merino – M), a terminal sire breed (Ile de France – IDF), a breed with indigenous content (Dorper – DP) and an unimproved indigenous breed (Namaqua Afrikaner – NA) with the temperature-humidity index (THI). Standard errors on the respective breed regression lines could be used to compare breed differences at different THI-levels.

Additional analyses were conducted by only considering the three fixed effects considered, namely breed, day and session as well as all two-factor interactions. The statistical information of this analysis is provided in Table 6.5. Main effects were significant for all traits. All traits were independent of the breed x day interaction, but the other interactions were significant ($p < 0.05$) for spot temperature and respiration rate. The only interaction that approached significance ($p = 0.07$) for rectal temperature was the breed x session interaction.

Table 6.5 The levels of significance for fixed effects of breed and day of recording as well as the regression variables on the temperature-humidity index (THI) and interactions between these main effects.

Effect	Trait		
	Rectal Temperature (°C)	Spot Temperature (°C)	Respiration Rate (bpm)
Breed	**	**	**
Days	**	**	**
Breed x Days	0.629	0.931	0.209
Session	**	**	**
Breed x Session	0.070	**	**
Day x Session	0.768	**	**

**Significant ($p < 0.01$); Actual P-value are provided for $p > 0.05$; N/A – Not Applicable; bpm – breaths per minute

Overall, all three traits increased ($p < 0.01$) from the morning to the noon/afternoon session. This increase amounted to 1.3 % for rectal temperature (38.7 ± 0.02 vs. 39.2 ± 0.02); 4.9 % for spot temperature (35.0 ± 0.1 vs. 36.7 ± 0.1); and 19.7 % for respiration rate transformed to natural

logarithms (4.26 ± 0.020 vs. 5.10 ± 0.020 , respective geometric means 70.8 and 164.8). The geometric means on the observed scale suggested a 2.3-fold increase in respiration rate. Means depicting the interaction of breed with session are provided in Table 6.6. When differences between morning and afternoon session were compared within breeds, the % increases ($p < 0.05$) in rectal temperature expressed relative to morning values ranged from 1.0% (Dohne, Dorper, Meatmaster, and Namaqua Afrikaner) to 1.6% (Dorner, Ile de France, and SA Mutton Merino). The Merino was intermediate at 1.3%. The increase ($p < 0.05$) in spot temperature as a % of morning values ranged from 3.4% (Dohne, Ile de France, Merino, and SA Mutton Merino) to more than 7% (Dorper and Namaqua Afrikaner). The Dorner and Meatmaster breeds were intermediate, with increases just below 5%. It is of note that the largest increases were in the two breeds with coloured heads, namely the Dorper and Namaqua Afrikaner, while the smallest increases were for the wool breeds.

Table 6.6 Predicted means (\pm SE) depicting the two-factor interaction between breed and recording session (morning or afternoon) for the heat stress indicator traits, namely rectal temperature, spot temperature and respiration rate.

Traits and Effects	Breed							
	Dohne	Dorner	Dorper	IDF	MM	Merino	NA	SAMM
<u>Rectal temperature (°C)</u>								
Morning	38.8	38.6	38.6	38.7	38.8	38.7	38.7	38.5
Afternoon	39.2	39.2	39.0	39.3	39.1	39.2	39.1	39.1
% Difference*	1.0	1.6	1.0	1.6	1.0	1.3	1.0	1.6
<u>Spot Temperature (°C)</u>								
Morning	35.2	34.6	35.3	34.9	34.7	35.2	35.1	35.2
Afternoon	36.4	36.3	38.1	36.1	36.3	36.4	37.6	36.4
% Difference*	3.4	4.9	7.9	3.4	4.6	3.4	7.1	3.4
<u>Respiration Rate (bpm) with geometric means on the observed scale in brackets</u>								
Morning	4.48 (88)	4.38 (80)	4.09 (60)	4.35 (78)	3.93 (51)	4.51 (91)	3.99 (54)	4.34 (77)
Afternoon	5.22 (167)	5.26 (192)	5.06 (157)	5.26 (192)	4.90 (135)	5.14 (171)	4.89 (132)	5.21 (183)
% Difference*	16.5	20.1	23.7	20.9	24.7	14.0	22.6	20.0

Standard errors (SE) per trait were 0.06 for rectal temperature, 0.2 for spot temperature and 0.06 for respiration rate; the % differences are expressed relative to morning values within traits. IDF – Ile de France; MM – Meatmaster; NA – Namaqua Afrikaner; SAMM – South African Mutton Merino; bpm – breaths per minute.

When respiration rate was considered, increases of below 17% were recorded in the specialist wool breeds (Dohne and Merino) where afternoon values were less than two-fold that of morning values on the observed scale. It is notable that these increases are relative to higher base values of around 90 bpm on the observed scale. Geometric means for morning respiration rates of the breeds from temperate regions (Dohne, Dorner, Ile de France, Merino, and SA Mutton Merino) ranged from 77 to 91 bpm and exceeded those means ranging from 51 to 60 for breeds with indigenous content (Dorper and Meatmaster) as well as the unimproved, indigenous Namaqua Afrikaner. The same pattern emerged for the higher afternoon respiration rates, geometric means for breeds from temperate regions ranging from 167 for Dohnes to 192 for the Dorner and Ile de France breeds. These means were higher ($p < 0.05$) than those for the breeds with indigenous content and the

Namaqua Afrikaner, ranging from 132 (Namaqua Afrikaner) to 157 (Dorper). Geometric means of respectively 132 and 135 for Namaqua Afrikaners and Meatmasters were accordingly lower than the mean for Dorpers.

The interaction between day of recording and session is presented in Figure 6.6 for spot temperature and in Figure 6.7 for the natural logarithm of respiration rate. The interactions were, among others, driven by a higher ($p < 0.05$) spot temperature and respiration rate during the morning session on Day 1. In the case of spot temperature, Day 2 was also characterised by a higher ($p < 0.05$) mean than the other two days, contributing to the observed interaction.

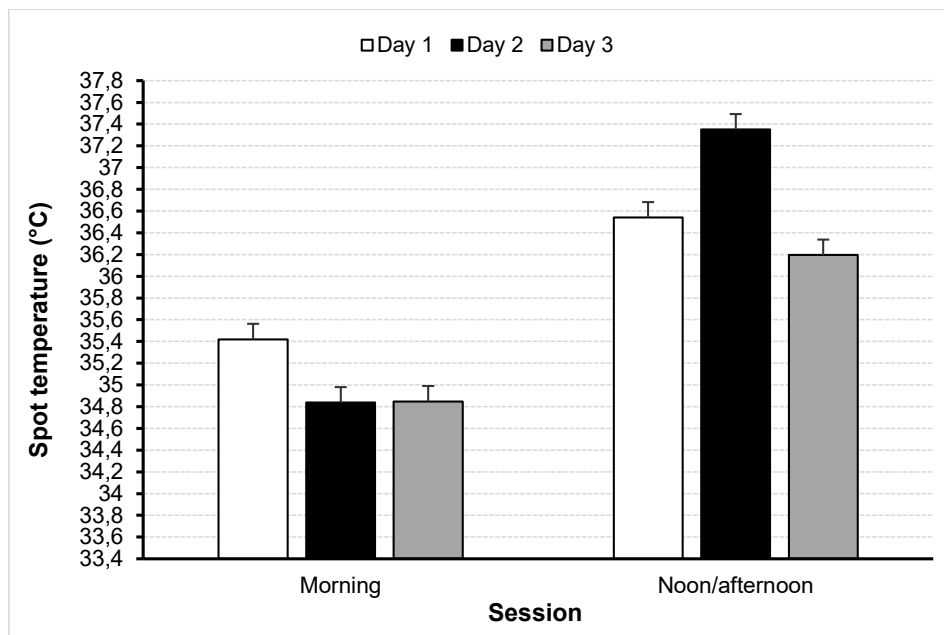


Figure 6.6 Predicted means for the interaction between days and session for spot temperature. Vertical bars above the means represent standard errors.

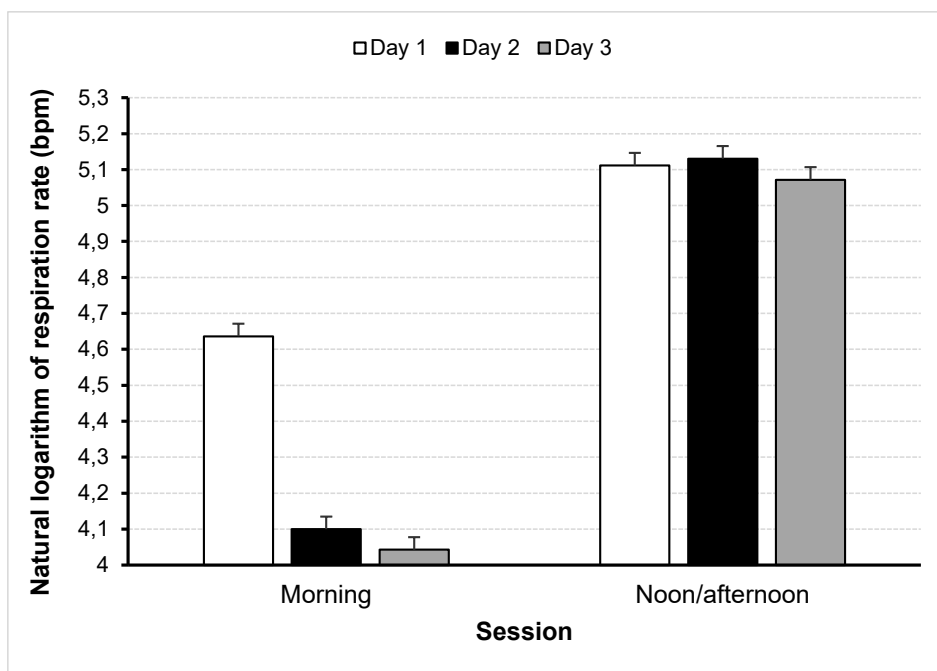


Figure 6.7 Predicted means for the interaction between days and session for log transformed respiration rate. Vertical bars above the means represent standard errors.

6.4.5 Log Likelihood values for random effects

The inclusion of random ewe effects improved the log-likelihood values above those of the operational model for rectal temperature, spot temperature and respiration rate (Table 6.7). The inclusion of effect ewe x session as a single random effect also resulted in improved log-likelihood values for spot temperature and respiration rate, when compared to the operational model. When both random sources of variation (ewe and ewe x session) were added to the operational model a further improvement in the log-likelihood was seen for spot temperature but not for respiration rate. In fact, all the random variation for ewes seemed to partition to ewe x session, as the random ewe variance component went to the boundary of parameter space (zero). Subsequent results will therefore focus on the variance ratios derived for rectal temperature, spot temperature and respiration rate.

Table 6.7 Log-likelihood values for the operational model and for models with added random effects for the heat stress indicator traits. The “best” models are denoted in bold font.

Model Fitted	Trait		
	Rectal Temperature (°C)	Spot Temperature (°C)	Respiration Rate (bpm)
Operational model	254.072	246.891	-405.204
+ Ewe	264.454	276.822	-398.613
+ Ewe x Session	260.924	292.360	-397.206
+ Ewe + Ewe x Session	264.729	294.804	-396.290

Between-ewe repeatability coefficients amounted to 0.16 for rectal temperature and 0.17 for spot temperature (Table 6.8). The corresponding ewe x session variance estimates amounted to 0.31 ± 0.08 for spot temperature and 0.20 ± 0.05 for respiration rate. When the reranking of ewes from the cooler mornings to the hotter afternoons were considered, the between ewe variance was repartitioned to ratios of almost double the magnitude for ewe and ewe x session effects in the case of spot temperature (Table 6.8). The reranking among ewes appeared to be the dominant source of variation from animal origin that contributed to variation in respiration rate as described above.

Table 6.8 Variance components and ratios for random ewe effects as well as random ewe x session effects to model the reranking of ewes from cooler mornings to hotter afternoons for rectal temperature and respiration rate for rectal temperature and respiration rate.

Variance components and ratios	Trait		
	Rectal Temperature (°C)	Spot Temperature (°C)	Respiration Rate (bpm)
<u>Components</u>			
σ^2_{ewe}	0.0143	0.0151	N/A
$\sigma^2_{ewe \times session}$	N/A	0.0280	0.3360
$\sigma^2_{residual}$	0.0742	0.0465	1.3509
$\sigma^2_{phenotype}$	0.0884	0.0896	1.6869
<u>Ratios</u>			
ewe ²	0.16 ± 0.05	0.17 ± 0.07	N/A
ewe x session ²	N/A	0.31 ± 0.08	0.20 ± 0.05

σ^2_{ewe} – random ewe effects; $\sigma^2_{ewe \times session}$ – random ewe x session effects; $\sigma^2_{residual}$ – random residual effects; $\sigma^2_{phenotype}$ – total phenotypic variance; N/A – Variance component or ratio not significant ($p > 0.05$).

6.5 Discussion

The primary objective of this study was to explore the respective adaptive mechanisms relating to the effect of THI on physiological responses of a wide variety of ewe sheep breeds under hotter summer conditions in the Western Cape. Numerous factors impact heat tolerance in sheep, including the physiological status and genetic composition of the animal, as well as the feeding regime (Marai *et al.*, 2007; McManus *et al.*, 2009). For this reason, the sheep used in this study were not late pregnant or lactating, since these physiological states may have affected outcomes. THI is considered a sensitive indicator for stressful climatic conditions (Silanikove, 2000; El-Tarabany *et al.*, 2017), where respiration rate, rectal temperature, and spot temperature are considered as significant indicators of heat stress in sheep (Marai *et al.*, 2007; Dalcin *et al.*, 2016; McManus *et al.*, 2016; Singh *et al.*, 2016; El-Tarabany *et al.*, 2017; Rashamol *et al.*, 2018).

6.5.1 Descriptive statistics

From Table 6.1 it was observed that the overall CV for ewe body weight (15.1-17%) was greater than those reported in adult wool (12.4%), dual-purpose (10.6%) and meat (6.0%) ewe breeds (Safari *et al.*, 2005). Ruiz-Ortega *et al.* (2022) reported a similar CV range (14.9-16.1%) for body weight in Barbados Blackbelly adult ewes compared to the present study, while Sabi ewes had a lower CV of 11.8% for body weight (Matika *et al.*, 2003). The CV for fat depth reported in a review by Safari *et al.* (2005) was more than 10% smaller than that illustrated in Table 6.1. The untransformed fleece depth obtained in the present study had a similar CV compared to the 38.96% found in a study by McManus *et al.* (2011). For rectal temperature, Santa Inês and crossbred ewes had a CV of 0.33 (Furtado *et al.*, 2017), which is much lower than the overall CV reported for the 8 sheep breeds in the present study. However, the CV for rectal temperature in Barbados Blackbelly ewes was four times the magnitude (Ruiz-Ortega *et al.*, 2022) compared to the 1.03% seen in Table 6.1. Ruiz-Ortega *et al.* (2022) reported a similar CV for respiration rate in Santa Inês and crossbred ewes compared to the untransformed means reported in present study.

6.5.2 THI

Diurnal variations in ambient temperature, RH and THI were observed during the trial (Figure 6.1). Daily ambient temperatures were on average 19.9°C, with maxima and minima of 26.4°C and 16.5°C, respectively during the morning sessions. During the afternoon sessions, daily ambient temperature amounted to 32.7°C on average, with maxima and minima of 34.3°C and 32.0°C, respectively. Across the three trial days, RH ranged from 62.6% to 89.2% during the morning sessions and from 27.3% to 45.6% during the afternoon sessions (Figure 6.1). THI during the morning sessions reflected none to moderate levels of heat stress, with THI-values of 19.4 on average, maxima 25.1 and minima 16.4, while the afternoon sessions reached THI-values classified

as dangerous (THI > 27) according to Seixas *et al.* (2017), with an average THI of 29.1 across the three-day trial period. These results coincide with the average THI-levels of 15.7 and 29.2 that was obtained during the morning and afternoon, respectively (Seixas *et al.*, 2017).

Rectal temperature, spot temperature and respiration rate were all dependent on the THI (Table 6.3). This coincides with the study by Seixas *et al.* (2017), reporting a direct relationship between the heat stress indicator traits (rectal temperature and respiration rate), where lower rectal temperatures and respiration rates imply that animals are more adapted to their tough surrounding environmental conditions. These relationships were further complicated by interactions of breed with the regressions for all three traits (Figures 6.2 to 6.5; Table 6.3).

Yousef (1985), Maurya *et al.* (2007), Indu & Pareek (2015) all reported that respiration rate was markedly lower at 08h00 than at 12h00 and 16h00 and is in accordance with the present study (Figure 6.7). Similarly, Sejian *et al.* (2012) and Da Silva *et al.* (2017) found higher respiration rates in the afternoon, presumably under hotter conditions than in the morning, presumably under cooler conditions. During the morning recording sessions (thermoneutral conditions), diurnal variations for spot temperatures and respiration rates are directly related to the environmental temperatures (Figures 6.1, 6.6 and 6.7; Table 6.5). On the contrary, during the afternoon (under heat stress conditions), circadian fluctuations in spot temperatures depend on the environmental temperature, while changes in respiration rates are subjected to the presence of minimal differences in the thermal gradient between the core temperature of the sheep and the environmental temperatures, the latter also reflected by the THI (Figures 6.1, 6.3, 6.4, 6.5). This corresponds to the results reported by Macías-Cruz *et al.* (2016), with the exception that their thermoneutral conditions refer to the spring season, while heat stress conditions refer to their summer season results.

6.5.3 Breed effects

Overall, the ewes in the present study lost weight (Table 6.2), which is an expected outcome for animals that have been exposed to heat stress conditions and agrees with literature (Hamzaoui *et al.*, 2013; Kandemir *et al.*, 2013; Mehaba *et al.*, 2021). Additionally, the Namaqua Afrikaner and Meatmaster breeds had the greatest weight loss, which significantly differed from the SAMM and Dormer breeds with the least weight loss. The latter breeds had an intermediate change in body weight and were not different from the rest. This is in correspondence with results from Shinde & Sejian (2013), also reporting that sheep exposed to thermal stress experienced a reduction body weight which could be due to high energy usage caused by increased respiration rates.

6.5.4 Heat stress indicator traits

6.5.4.1 Rectal Temperature

Overall rectal temperatures between breeds were quite similar, except for the lowest values recorded in the SAMM and Dorper ewes and the highest values in the Dohne and Ile de France ewes (Figures 6.2 and 6.5; Tables 6.4 and 6.6). The average rectal temperatures obtained during the morning

recording sessions, presumably under thermoneutral conditions (Table 6.6), coincide with the rectal temperature range of 38.1-38.5°C reported in sheep under similar conditions (Furtado *et al.*, 2017; Machado *et al.*, 2020). On the contrary, higher rectal temperatures (>39.0°C) were reported in Naimey, Merino, Omani, Santa Inês x Dorper cross sheep under cool environmental conditions (Srikandakumar *et al.*, 2003; Al-Haidary, 2004; Dos Santos *et al.*, 2021). The 1.3% increase from the morning to afternoon recording sessions is markedly lower than 3.76% in Osmanabadi goats (Shilja *et al.*, 2016), but is similar to the 0.98% in Hu ewes and 1.76% (Li *et al.*, 2018) in Omani sheep (Srikandakumar *et al.*, 2003). The average rectal temperature reported in Table 6.6 for the afternoon sessions are similar to average standard of 39.1°C (Seixas *et al.*, 2017), but below the normal range 38.3-39.9°C (Marai *et al.*, 2007; Okourwa, 2015), suggesting the ewes in the present study maintained their rectal temperatures within the normal range and did not experience heat stress. The rectal temperatures reported during the afternoon sessions (i.e., under heat stress conditions) (Figures 6.2, 6.5; Table 6.6.) were markedly lower than the average 39.8°C obtained for Santa Inês and crossbred sheep exposed to extreme heat stress (THI = 33.77) (Gesualdi Júnior *et al.*, 2014). Belhadj Slimen *et al.* (2019) and Mehaba *et al.* (2021) also reported higher rectal temperatures than the present study.

It is important to note that the number of animals used per breed was fairly low, which could account for the little variation observed among the breeds as well as between the morning and afternoon sessions. Thus, for future experiments more individuals per breed should be considered to obtain results of greater value.

6.5.4.2 Respiration Rate

From Figures 6.4 and 6.7 as well as Table 6.6, it can be perceived that there was diurnal variation in respiration rate and is in correspondence with Ashutosh *et al.* (2000) as well as Macías-Cruz *et al.* (2016). An average respiration rate of 27 bpm is considered normal for sheep (Seixas *et al.*, 2017; Sailo *et al.*, 2017), which was clearly not the case for the ewes recorded in this study under thermoneutral conditions (morning sessions) (Table 6.6). Silanikove (2000) states that respiration rates above 80 bpm indicate that sheep start to experience heat stress, while a respiration rate of 120 and above is considered as severe heat stress, and those reaching 200 bpm and above indicate extreme levels of heat stress.

Lower respiration rates were observed during the morning compared to noon/afternoon and circadian variations can be seen in Table 6.6 and Figure 6.7, which is in agreement with Macías-Cruz *et al.* (2016) who found that the maximum change in respiration rate occurred at 18h00, with the hotter environmental conditions resulting in an average value of 172 bpm, while cooler environmental conditions had a much lower average respiration rate of 75 bpm. These values were appreciably higher than those reported for Dorper, Meatmaster and Namaqua Afrikaner ewes, but lower than the respiration rates reported for Dormer, IDF, Merino and SAMM ewes. Physiological adaptability generally determines variation in circadian rhythms for respiration rate that is

implemented by sheep to maintain homeothermy and to avoid dehydration under hot climatic conditions (Macías-Cruz *et al.*, 2016). In accordance with Figures 6.4 and 6.7, Osmanabadi goats had significantly lower respiration rates in the morning compared to the afternoon (Shilja *et al.*, 2016). In response to heat stress conditions, respiration rates increased with 14.0-24.7% from the morning to the afternoon recording sessions (Figure 6.4; Table 6.6).

Overall, Namaqua Afrikaner and Meatmaster ewes seemed to be less subjected heat stress, with the Dorpers slightly more subjected to heat stress levels under comparable ambient conditions. The temperate breeds (Dohne, IDF, SAMM, Merino and Dormer) exhibited higher overall respiration rates (Tables 6.4 and 6.6). It needs to be stated that respiration rates were similar over days for the afternoon sessions (Figure 6.7; Table 6.4), but varied for the morning sessions, with Day 1 having the highest respiration rates (Figure 6.7). This could be due to the longer duration of the particular morning session on Day 1, which resulted in the ewes being exposed to higher levels of heat stress (average maximum THI = 26.9 in the morning).

Under thermoneutral conditions (average morning THI = 20.17), the Meatmaster, Namaqua Afrikaner and Dorper ewes had the lowest respiration rates of < 61 bpm, with SAMM, IDF and Dormers obtaining on average values between 77 bpm and 88 bpm, while Dohnes and Merinos had the highest respiration rates of 88 bpm and 91 bpm, respectively (Table 6.6). Similar to the results reported in the present study, respiration rates of Chokla sheep were 79 bpm and Avivastra sheep 57 bpm under moderate to severe climatic conditions (THI = \pm 23.3) reported by Ashutosh *et al.* (2000). Accordingly, the ewes' respiration rates were significantly higher between 14h00 and 16h00 than during the morning sessions between 06h00 and 09h00, as seen in Table 6.6 and Figure 6.7. Romo-Barron *et al.* (2019) also reported lower respiration rates and a similar range to the present study (i.e., 41-101 bpm) in ewes under thermoneutral conditions.

When sheep are subjected to heat exposure, they increase their respiratory evaporation (i.e., evaporative cooling) to maintain thermal balance. Accordingly, increased respiration rates are generally a primary indication of heat stress in livestock, which promotes evaporative cooling through dissipating heat from the body (Al-Haidary, 2004; Nienaber & Hahn, 2007; Romero *et al.*, 2013; Macías-Cruz *et al.*, 2016; Ruiz-Ortega *et al.*, 2022). Additionally, in response to changing environmental conditions, respiration rate is used as an adaptive mechanism in attempt to maintain their thermal balance through evaporating and dissipating moisture from the respiratory tract (Da Silva *et al.*, 2017; Rashamol *et al.*, 2018).

Under extreme heat stress conditions, reaching levels considered as dangerous (THI > 27) by Seixas *et al.* (2017), an average THI-value of 29.1 was observed in the afternoon recording sessions for the duration of the trial (Figure 6.1). The maximum average respiration rates obtained in this study (Table 6.6) are markedly higher than the 160 bpm reported in hair ewe breeds by (Meza-Herrera *et al.*, 2015), but similar to the 180 bpm reported for Barbados Blackbelly ewes by Ruiz-Ortega *et al.* (2022). Likewise, Romo-Barron *et al.* (2019) reported similar respiration rates in ewes exposed to heat stress conditions (i.e., 109-203 bpm). All ewes experienced severe levels of heat

stress during the afternoon recording sessions as their respiration rates ranged between 120 to 200 bpm, where some individuals across all breeds reached extreme and dangerous levels of heat stress (i.e., respiration rates ranged above 200 bpm).

6.5.4.3 Spot Temperature

Overall, spot temperatures were the highest in the Namaqua Afrikaners and Dorpers, which did not differ ($p < 0.05$) from one another, while the other breeds had lower and similar ($p > 0.05$) spot temperatures (Table 6.4). Additionally, the average spot temperatures of the ewes trended higher in breeds with darker coat and skin colour on their cheek (site of measurement). Since the spot temperatures were taken from the cheek of animals, it was evident that those with dark brown or black coated heads, such as the Dorper and Namaqua Afrikaner breeds, tended to have higher temperatures when measured, particularly during the afternoon (Table 6.6; Figure 6.3). This corresponds to literature stating that dark coat colours are known to absorb solar radiation and light coat colours reflect it (Henry *et al.*, 2012; Do Prado Paim *et al.*, 2013; Pérez *et al.*, 2020; Ruiz-Ortega *et al.*, 2022). This was observed especially when sheep were exposed to direct sunlight (i.e., not hiding their head beneath another ewe's belly). Hence, the surface temperature (i.e., spot measurements) is related to the association between coat or skin colour and its reflectance (i.e., lighter coats reflect solar radiation, while darker coats absorb it) as well as the capacity of peripheral vasodilatation (McManus *et al.*, 2009; Do Prado Paim *et al.*, 2013; Indu & Pareek, 2015; Shilja *et al.*, 2016; Katiyatiya *et al.*, 2017).

Accordingly, diurnal variation in spot temperatures can be observed from Figures 6.3 and 6.6 as well as Table 6.6. This corresponds to the lower spot temperatures recorded at 08h00 than at 12h00-16h00 in the summer (Rashamol *et al.*, 2018; Ruiz-ortega *et al.*, 2022). Spot temperatures were significantly higher on Day 2 compared to Day 1 and 3, which were similar ($p > 0.05$; Table 6.4). Day 2 also recorded the highest spot temperatures during the afternoon sessions, while Day 1 recorded the highest spot temperatures during the morning sessions (Figure 6.6). Spot temperatures increased with 3.4-7.9% from the morning to afternoon recording sessions (Table 6.6). This increase was markedly lower compared to the 20.96% increase in spot temperature of Osmanabadi goats from the morning to afternoon recording sessions.

6.5.5 Between ewe variation

The only traits that were low-moderately repeatable throughout in this study, was spot temperature and rectal temperature (Table 6.8). In contrast, the between ewe variance when to zero when ewe x session was also modelled. Ewe x session variance ratios amounted to 0.20 for respiration rate and 0.31 for spot temperature. It may be contended that the trends observed for dark-headed Namaqua Afrikaner and Dorper ewes might have contributed to this result. No comparable values were found for sheep, but Brien *et al.* (2010) accordingly reported that rectal temperatures of lambs

were lowly heritable. It is widely accepted that repeatability represents the upper boundary of heritability.

6.6 Conclusion

This study indicated clear between-breed variation, particularly for spot temperature and respiration rate. Overall means for rectal temperature, although there was significant between-breed variation, ranged narrowly between 38.8 and 39.0 °C. It was postulated that the black and brown face cover of Dorper and Namaqua Afrikaner ewes probably contributed to the significant breed variation as well as the repeatability of spot temperature in this study. Results for respiration rate clearly indicated that the lowest respiration rate at high temperatures were in Namaqua Afrikaner and Meatmaster ewes, with Dorpers being intermediate and the ewe breeds from temperate environments having the highest respiration rates. Rectal temperature was low-moderately repeatable on the short term (within a three-day cycle). The role of this trait in current flock selection hinges on repeatable ewe differences being sustained over the longer term.

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Chapter 7: Quantifying *ovine* responses to hot conditions, as well as between and within animal components of variation in common heat stress indicators in a Northern Cape flock

7.1 Abstract

The responses to cooler conditions in the morning, as well as higher heat loads in the afternoon were assessed from 21 to 24 February 2022 for Merino, White Dorper and Meatmaster (n=20 for each breed) ewes at the Rookop farm in the Northern Cape. Data of breed effects on live weight gain, fat depth and fleece depth were analyzed using the GLM ANOVA procedure in SAS®. Mixed-model analyses in ASReml 4.2 were used to assess the fixed effects of ewes in response to adverse environmental conditions, as well as the repeatability of responses to heat. The heat stress indicator traits, respiration rate, rectal temperature, and spot temperature varied markedly ($p < 0.01$) in response to an increase in the THI from cooler mornings to hotter afternoons. However, the magnitude of this variance was defined by the breed of the animal, with White Dorper and Meatmaster ewes apparently better equipped to cope with higher THI-levels as based on rectal temperature and respiration rate records. Eye temperature was not affected by breed or its interactions with other fixed effects and was considered as unlikely to be of value in assessing breed-specific responses to and increased THI, Spot temperature measurements were highest in the Meatmaster ewes, followed by the Merino and then the White Dorper ewes. Differences in spot temperature might have been due to the skin or coat colour as well as coat type of ewes. In the absence of the ewe x session interaction, the repeatability for rectal temperature was moderate at 0.24. The estimate for respiration rate was markedly lower at 0.08. Accordingly, the ewes x session (morning or afternoon) interaction contributed to the between-ewe variation in rectal temperature and respiration rate, resulting in respective variance ratios of 0.19 and 0.20. The repeatability of rectal temperature remained significant at 0.16, but the repeatability of respiration rate went to the boundary of parameter space (zero). It was concluded that higher THI values significantly disturbs rectal temperature, spot temperature, respiration rate as well as eye temperature in ewes. Rectal temperature may be amenable for adaptation to allow current-flock selection if the observed repeatability coefficients could be sustained in longer-term studies.

7.2 Introduction

Sheep form an integral component of most livestock production systems throughout the world due to the species being able adapt to a wide variety of environments (Cottle, 2010). Given the ability of sheep to adapt to marginal conditions, the species already plays an important role in both commercial and smallholder animal agriculture (Cloete & Olivier, 2010; Rashamol *et al.*, 2018).

Sheep development and productivity are adversely affected by high temperatures, since it reduces feed intake and increases energy demands due to the activation of thermoregulatory mechanisms (Pérez *et al.*, 2020). Energy exchange between the environment and the animal is influenced by three factors, namely environmental and animal factors as well as thermoregulation (Srikandakumar *et al.*, 2003; Henry *et al.*, 2012). Environmental factors comprise of ambient temperature, relative humidity (RH), solar radiation and wind speed, while animal factors include breed, coat colour, stage of lactation and health status (Henry *et al.*, 2012). Thermoregulation which relates to thermoregulatory mechanisms include circulatory adjustments, sweating and panting. When livestock are exposed to prolonged heat stress, it stimulates increased dissipation of excessive body heat to the environment to counteract the heat load in their body (Rashamol *et al.*, 2018). This is brought about by the physiological responses respiration rate, rectal temperature, pulse rate, spot temperature, and sweating rate. Additionally, the environmental variables drastically change throughout the day, which then leads to the physiological response to also be affected by the time of day (Sharma *et al.*, 2013; Da Silva *et al.*, 2017).

It has been demonstrated that animals may compromise their productive performance through harnessing their adaptive mechanisms to survive in harsh environmental conditions (Kassahn *et al.*, 2009; Rashamol *et al.*, 2018), thereby affecting body weight (W), growth and their physiology (Indu & Pareek, 2015). The physiological adaptation of animals is the principal response mechanism used to maintain homeostasis when experiencing heat stress (Kassahn *et al.*, 2009). In their review, Pérez *et al.* (2020) stated that high temperatures have a negative impact on carcass characteristics and meat quality in sheep. The degree to which heat stress influences sheep productivity depends on the adaptive proficiency of a given breed, where hair breeds are regarded as generally less susceptible to heat stress (McManus *et al.*, 2011; Correa *et al.*, 2013). The severity of heat stress can be measured using a combination of ambient temperature and relative humidity (RH), termed as the temperature-humidity index (THI) (Marai *et al.*, 2001).

All animals possess a thermo-neutral zone or comfort zone, in which livestock animals are able to maintain their body temperature within a $\pm 0.5^{\circ}\text{C}$ range of the thermoneutral zone (Henry *et al.*, 2012; Indu & Pareek, 2015). This comfort zone identifies a range of environmental temperatures where heat production and heat loss from the body are equal (Srikandakumar *et al.*, 2003). The physiological functions of an animal are in balance when maintained in this thermoneutral zone (Indu & Pareek, 2015). According to do Prado Paim *et al.* (2013), the upper critical temperature for adult hair sheep is equivalent to 34°C and for adult European sheep (i.e., relating to the woolled breeds originating from temperate regions) is 32°C . Srikandakumar *et al.* (2003) also stated that the thermoneutral zone for an adult sheep with full fleece ranges between 12°C and 32°C . Marai *et al.*, (2001) stated that THI values below 22.2 correspond to an animals thermoneutral zone, where heat stress is considered to be absent (Srikandakumar *et al.*, 2003; McManus *et al.*, 2016; Li *et al.*, 2018). Accordingly, animals experience moderate heat stress when the THI ranges from 22.2 to 23.3, while severe heat stress corresponds to THI values between 23.3 and 25.6. THI values above 25.6

signifies that the animals are subjected to extreme heat stress, where THI values ranging above 27 is considered to be dangerous (Srikandakumar *et al.*, 2003; McManus *et al.*, 2016; Li *et al.*, 2018). At this stage, respiration rate and body temperature measurements are good indicators of the degree of heat stress in sheep (Indu & Pareek, 2015). Additionally, respiration rate is considered the first measurement that serves as a reliable indicator of breed and individual differences in heat stress in sheep (Gaughan *et al.*, 2000b; Eigenberg *et al.*, 2005; McManus *et al.*, 2016). Henry *et al.* (2012) stated that for a single source of stress, like heat stress, an animal will develop a phenotypic response termed acclimation. Acclimation leads to increased water intake, reduced feed intake, altered physiological functions (i.e., production and reproduction), as well as changes in respiration rate and pulse rate in animals (Henry *et al.*, 2012) in attempt to facilitate the response to heat stress (Kassahn *et al.*, 2009). However, hair sheep breeds can tolerate higher temperatures than wooled sheep, which suggests that wooled sheep start to experience heat stress at lower THI levels (Pérez *et al.*, 2020). Thus, more research is required to determine the precise inflection point when sheep start to manifest heat stress symptoms.

A paucity of data on the effect of high temperatures in South Africa on sheep exists. It is unlikely that local temperatures will at any time reach the level where a resilient livestock species, such as sheep (Rust & Rust, 2013), may suffer irreparable damage because of the failure of their coping mechanisms. This project is likely to shed more light on questions such as these. It was notable that a Google search for hyperthermia and sheep yielded mostly results on hypothermia, once again reiterating the paucity of data on sheep production under hot conditions. It was, however, established that normal ranges for ovine rectal temperatures are 38.5–40.0°C for adult sheep (see ranges at: <http://www.husbandrypunjab.org/pages/rtemp1.htm>). Previous results reported by Singh *et al.* (1980) reported that Rambouillet ewes with an average (not maximum) respiration rate of 280 breaths per minute (bpm) and rectal temperature of 41.4°C at an ambient temperature of 40°C recovered without ill-effects after the heat-stress conditions they were subjected to were lifted. Kovács *et al.* (2019) also reported that it is possible for animals to recover from heat stress conditions during cooler nights, thus alleviating some of the heat stress that accumulated during daytime.

This study sought to test whether ewes from a widely divergent genetic background differ in terms of heat-tolerance, and whether between-animal (i.e., repeatability as a proxy for heritability) and genetic variation across breeds could be used to identify individuals less subject to heat stress. Therefore, non-invasive indicators of heat tolerance in Northern Cape were recorded to obtain breed differences and repeatability estimates for traits putatively related to heat tolerance in South African sheep.

7.3 Materials and Methods

7.3.1 Animals, Location and Recordings

Animals from a commercial flock maintained at the Rooikop Farm in the Northern Cape near Sutherland were used for this project. A total of 60 mature breeding ewes recorded for this study was drafted from the mixed-age flock already present on Rooikop Farm and consisted of 20 Merino, 20 Meatmaster, and 20 White Dorper ewes.

Prior to the start of the experiment, several temperature forecast services (www.meteoblue.com; www.yr.no; www.accuweather.com; www.weathersa.co.za) were consulted to identify days that are likely to have a spread of temperatures well in the thermoneutral zone (20-28°C ambient temperature) in the morning, to increase to a range where some individuals/breeds may experience heat stress (30-35°C ambient temperature) towards noon. Recording involved six sessions over three days to ensure that a wide range of ambient conditions were covered. A Tinytag Plus 2 Data Logger together with its weather screen was set up in the center of the paddocks that were used at sheep head-height level upon arrival (see Chapter 2 for details). This Data Logger was set to record environmental conditions, including relative humidity (RH) and ambient temperature, throughout the entire sampling period at ten-minute intervals.

The experimental animals were brought into the sheeppark during the afternoon of the day before the recording sessions commenced (i.e., 21 February 2022). At this stage, the ewes were put through a crush for the measurement and recording of body weight (W), fat depth and wool depth. To enable identification from a distance during recording sessions, each individual ewe received a uniquely numbered neck-tag that was gently fitted around the neck of a specific animal according to their unique neck circumferences (see Chapter 2). Subsequently, the group of ewes was divided into 7 smaller paddocks with 7-9 ewe per paddock to facilitate the recording of individual heat stress indicator traits. These traits are considered as direct or indirect indicators of the core body temperature in animals (McManus et al., 2016). Ewes were fed lucerne hay overnight and between sessions. On the three days of recording heat stress indicator traits (22-24 February 2022), respiration rates were the first measurements, followed by rectal temperatures and spot temperatures that were recorded simultaneously. Eye temperatures through thermal imaging was the final measurement. These recordings were duplicated in the cooler mornings as well as during the hotter afternoons, resulting in two recording sessions per day. On the final day, following the afternoon recording session, the animals were given an opportunity to cool down through provision of drinking water. Following the cool-down session, each individual ewe was put through the crush once again to be weighed and for the removal of their neck-tags. Lastly the animals were grouped together, returned to their flocks of origin, and managed according to the existing Heat Stress SOP for this study.

The handling of animals may have resulted in an increased core temperature for the records obtained when animals were restrained for the measurement of rectal temperature and spot

temperature. The same treatment applied to all animals, so it is considered unlikely that it would affect the ranking of animals for these traits. A team consisting of a recorder and a scribe counted the number of respiratory side movements for all animals in a group for 30 seconds until all groups are counted. Following this recording session as well as the recording of rectal temperature and spot temperature where the animals were restrained, the recorder used a thermal camera to record eye temperature of the same animals. The time of each recording was noted to allow linkage back to the “ten-minute interval” ambient conditions recorded by the temperature and humidity sensitive probe in the Tinytag Plus 2 Data Logger.

A standard clinical thermometer was used to measure rectal temperature, while a laser-based thermometer was used to measure spot temperature on the cheek. For wool depth and hair length measurements, a Digital Caliper was used. Fat depth measurements were taken using a Mindray DP 30V ultrasound scanner with a 7.5 MHz linear transducer. An E75 Thermal Camera was utilized to capture eye temperature as an indication of core body temperatures. Refer to section 2.8 of Chapter 2: Literature Review for a detailed description on the instruments and devices used to perform the required measurements of the individual heat stress indicator traits and physiological traits, as well as the recording of ambient climate data.

7.3.2 Temperature-Humidity Index (THI)

THI was computed from data retrieved from the Tinytag Plus 2 Data Logger for environmental temperature and relative humidity (RH) values that was recorded at 10-minute intervals. The following equation by Marai *et al.* (2001) was used to compute the THI for the current study. All ambient temperature (TC) values are in degrees Celsius (°C), and RH is a percentage (%).

$$\text{THI} = \text{TC} - ((0.31 - 0.31 \times \text{RH}/100) \times (\text{TC} - 14.4))$$

7.3.3 Statistical analysis

The trait means for fat depth (cm) and the change in body weight (W, kg) were compared between the ewe breed groups by one-way analysis of variance (ANOVA) using the general linear models (GLM) procedure of SAS Enterprise Guide. Fleece depth (mm) was analyzed by Welch’s Variance-Weighted ANOVA using the ANOVA procedure of SAS Enterprise Guide to elucidate breed effects due to unequal population variances (SAS Version 7.1, 2021). The influence of the main effect of breed (Merino, Meatmaster or White Dorper) on trait means of the various physiological ewe traits were evaluated. Differences among the breed groups were regarded as significant at $p \leq 0.05$. The breed effects were expressed as arithmetic means with their respective standard errors (SE).

The heat stress indicator traits (rectal temperature, spot temperature, eye temperature and respiration rate) that were recorded across three days with two sessions (morning and afternoon) were subjected to mixed model analyses, using ASReml 4.2 (Gilmour *et al.*, 2015). Respiration rate was highly variable (Table 7.1) and the variances for morning and afternoon records were

heterogeneous. Transformation of respiration rate data to natural logarithms normalized the data sufficiently for the analyses described below. ASReml software allows for the prediction of means of selected fixed effects and regression coefficients in unbalanced designs using linear mixed models. Ewe breed and day of recording were fitted as fixed effects. As response variables, the heat stress indicator traits were regressed on the ambient climatic conditions as reflected by the THI, as based on the actual temperature and humidity at the time of measurement. The regressions in ASReml depended on a fixed linear component and a random cubic spline component, reflecting random deviations from linearity conforming to a smooth trend (Verbyla *et al.*, 1999) where appropriate. Two-factor interactions were fitted between ewe breed and recording day, as well as between breed and relevant regression variables as appropriate. The random effect of ewes was added to an operational model that included the appropriate fixed and regression components for a specific heat stress indicator trait. Additionally, random ewe effects were interacted with session (morning and afternoon) to account for the reranking of ewes between cooler morning and hotter afternoon conditions. Since the session was confounded with regressions on the ambient climatic conditions experienced, it was initially not considered as a fixed effect in the operational model. Log-likelihood ratios were used to compare random effects models when an additional random effect was fitted, as described by Snyman *et al.* (1995). A difference of 1.92 was considered as a significant ($p < 0.05$) improvement in the log-likelihood when the addition of one random effect at a time was compared to the reduced model.

Based on the outcomes of these analyses, further analyses involving the fixed effects of breed, day (as defined previously) and session (morning or afternoon) were conducted. All relevant two-factor interactions among fixed effects were also considered. For reasons outline below, these analyses were restricted to rectal temperature and respiration rate. Chi-square procedures (Van Ark, 1990) were used to test proportional differences between breeds, as based on respiration rate thresholds, for significance. Three proportions needed to be compared, necessitating the use of the Bonferoni correction for multiple comparisons (Van Ark, 1990).

7.4 Results

7.4.1 Descriptive statistics

The descriptive statistics for body weight (W) before and after the trial, fat depth and fleece depth as well as heat stress indicator traits are given in Table 7.1. Coefficients of variation (CV) for the weight traits ranged from 14.9% for body weight before recordings commenced to 34.8% for fleece depth (Table 7.1). All traits conformed to normality as far as skewness and kurtosis were considered. The CV of fat depth approached 20%, suggesting that this trait was variable. When the population variances were considered prior to analyzing the respective traits, fleece depth did not conform to homoscedasticity. This is due to the Merino breed having less variation, thereby contributing to the unequal population variances. The animal-based temperature records had means between 31.2 and

38.6, while the mean for respiration rate amounted to almost 88 bpm (Table 7.1). The CV for rectal temperature was very low at 1.3%, while it was also below 10% for eye temperature. Untransformed respiration rate was highly variable, with a CV of almost 70%. Transforming respiration rate data to natural logarithms, however, reduced this value markedly to below 20%.

Table 7.1 Descriptive statistics for body weight before and after the trial, fat depth and fleece depth traits as well as heat stress indicator traits recorded for the three divergent sheep breeds considered in the study on Rooikop Farm.

Trait	Observations (n)	Mean \pm SD	CV (%)	Range
<u>Body and Fleece</u>				
Body Weight Before (kg)	60	58.2 \pm 8.66	14.9	38.4 – 78.0
Body Weight After (kg)	60	58.7 \pm 9.44	16.1	39.8 – 80.5
Fat Depth (cm)	60	0.637 \pm 0.12	19.5	0.35 – 0.93
Fleece Depth (mm)	60	33.1 \pm 11.5	34.8	13.9 – 64.0
<u>Heat stress indicators</u>				
Rectal temperature ($^{\circ}$ C)	360	38.6 \pm 0.5	1.3	36.7 – 39.9
Spot temperature ($^{\circ}$ C)	360	31.2 \pm 4.3	13.4	18.8 – 43.6
Eye temperature ($^{\circ}$ C)	358	36.4 \pm 3.0	8.2	26.6 – 41.0
Respiration rate (bpm)				
Untransformed	360	87.5 \pm 60.7	69.3	16 – 218
Log transformed	360	4.16 \pm 0.83	19.9	2.77 – 5.38

SD – Standard Deviation; CV – Coefficient of Variance

7.4.2 THI

Environmental temperature (T in $^{\circ}$ C) and RH (%) derived from the Tinytag Plus 2 Data Logger were significant factors that affected the THI for the duration of the study and are illustrated in Figure 7.1, where THI was computed according to Marai *et al.* (2001). Overall, THI-values differed significantly between the morning (THI = 17.9 \pm 2.46) and afternoon (THI = 28.4 \pm 0.65) sessions for the three-day trial period (Figure 7.1). Accordingly, the THI-values for the afternoon recording sessions varied ($p < 0.01$) across the three days with maxima within the respective recording sessions of 28.4 (Day 1), 28.9 (Day 2) 29.6 (Day 3) and minima of 27.2 (Day 1), 27.9 (Day 2) and 28.5 (Day 3). On the contrary, THI values for the morning recording sessions were similar ($p > 0.01$) between days and reflected a maximum of 21.9 and minimum of 13.1. Overall, THI was the highest on Day 3 and lowest on Day 1 throughout the trial (Figure 7.1). The overall environmental temperatures differed significantly between the morning (T = 18.6 \pm 3.01 $^{\circ}$ C) and afternoon (T = 33.5 \pm 0.89 $^{\circ}$ C) sessions for the three-day trial period (Figure 7.1). Accordingly, environmental temperatures for the afternoon recording sessions on Day 2 and 3 differed ($p < 0.01$) from Day 1, with maxima within the respective recording sessions of 33.4 $^{\circ}$ C (Day 1), 34.4 $^{\circ}$ C (Day 2) 35.0 $^{\circ}$ C (Day 3) and minima of 31.7 $^{\circ}$ C (Day 1), 33.1 $^{\circ}$ C (Day 2) and 33.5 $^{\circ}$ C (Day 3). On the contrary, the environmental temperatures were similar ($p > 0.05$) for the morning recording sessions between days, and reflected a maximum of 23.7 $^{\circ}$ C and minimum of 13.0 $^{\circ}$ C. The overall RH differed significantly between the morning (RH = 48.1 \pm 8.60%) and afternoon (RH = 14.2 \pm 1.98%) sessions for the three-day trial period (Figure 7.1). Accordingly, RH for the afternoon recording sessions varied ($p < 0.01$) across the three days with maxima within the respective recording sessions of 16.2% (Day 1), 12.5% (Day 2) 16.2% (Day 3)

(87)

and minima of 13.6% (Day 1), 10.2% (Day 2) and 15.4% (Day 3). Likewise, the RH for the morning recording sessions on Day 2 and 3 differed ($p < 0.01$) from the RH on Day 1, with Day 1 pertaining the highest mean RH of $52.9 \pm 9.69\%$. The maxima RH during the morning recording sessions were respectively 66.1% (Day 1), 53.2% (Day 2), and 46.8% (Day 3), while the minima RH were 36.7% (Day 1), 34.3% (Day 2), and 37.0% (Day 3), respectively (Figure 7.1). For each unit increase in the THI, overall linear increases amounted to $0.0319 \pm 0.0041^\circ\text{C}$ for rectal temperature, $0.468 \pm 0.024^\circ\text{C}$ for spot temperature, $0.513 \pm 0.033^\circ\text{C}$ for eye temperature, and by 0.144 ± 0.034 bpm for log transformed respiration rate.

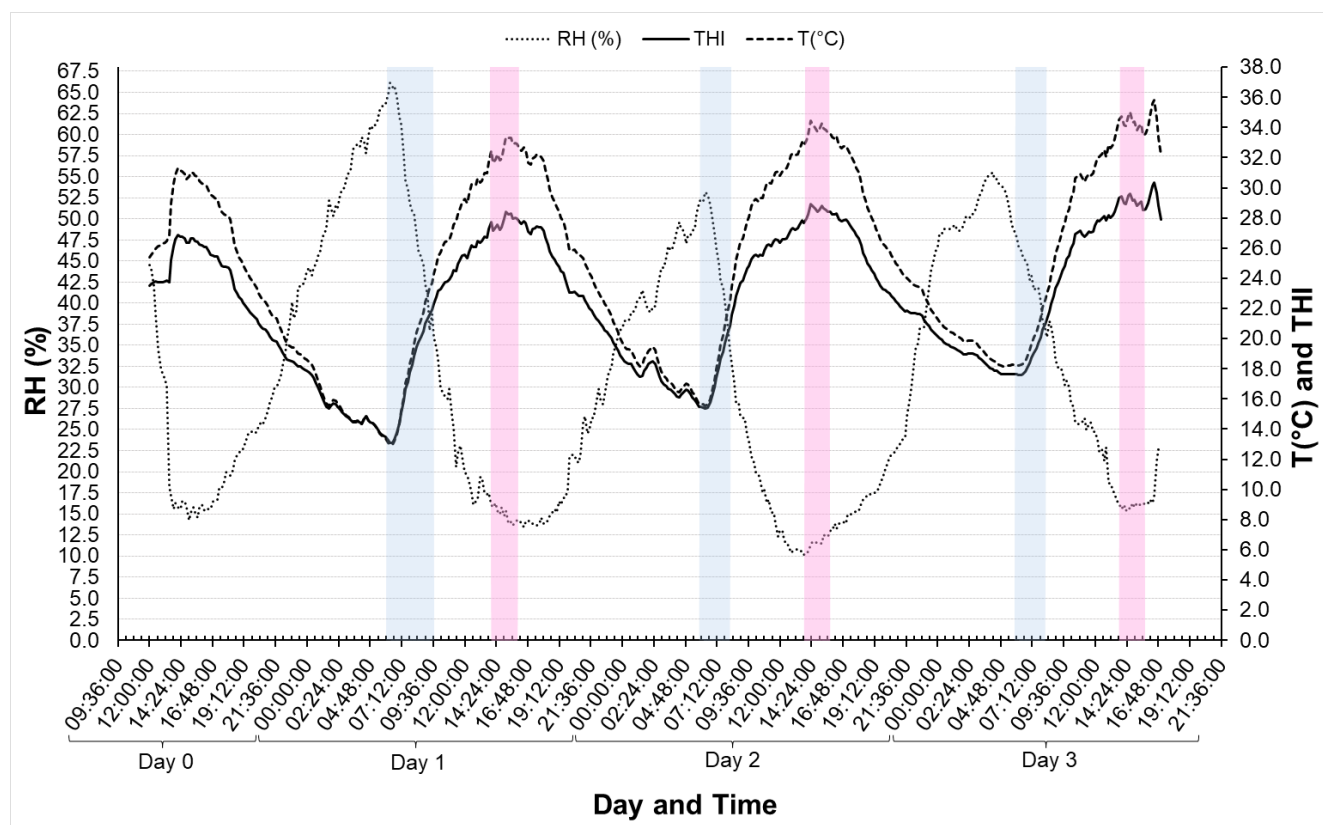


Figure 7.1 The environmental temperature (T), relative humidity (RH), and temperature-humidity index (THI, where T was expressed relative to $^\circ\text{C}$) for the duration of the trial (21 – 24 February 2022) at Rooikop Farm in the Northern Cape. Blue shading represents the morning recording sessions; Pink shading represents the noon/afternoon recording sessions.

7.4.3 Breed effects on live weight, fat depth and fleece depth

The net weight loss in Meatmasters was higher than the net weight gain in White Dorpers ($p < 0.05$; Table 7.2). The mean for Merinos was intermediate and not different from either of the other breeds. No differences ($p > 0.05$) were found among the breeds for fat depth, with an average trait mean of 0.637 cm. White Dorper ewes presented the highest fleece depth value, which was significantly higher than the means for both the Meatmaster and Merino ewes, which did not differ ($p < 0.05$; Table 7.2).

Table 7.2 Arithmetic means (\pm SE) for change in body weight (W), fat depth and fleece depth traits in ewes of different breeds on Rooikop Farm.

Effect	Traits		
	Change in W (kg)	Fat depth (cm)	Fleece depth (mm)
Overall mean	0.542 \pm 0.35	0.637 \pm 0.02	33.1 \pm 1.49
<u>Breed</u>	**	0.25	**
Merino	0.435 \pm 0.67 ^{a, b}	0.638 \pm 0.03	27.6 \pm 1.20 ^b
Meatmaster	-0.635 \pm 0.52 ^a	0.650 \pm 0.03	28.8 \pm 2.39 ^b
White Dorper	1.825 \pm 0.51 ^b	0.622 \pm 0.02	42.9 \pm 2.42 ^a

**Significant ($p < 0.05$); Actual F-value for $p > 0.05$; ^{a, b} Denote significant differences ($p < 0.05$) in rows within traits.

7.4.4 Fixed effects

The significance of fixed and interaction effects for each heat stress indicator trait are provided in Table 7.3. All traits except eye temperature were affected ($p < 0.01$) by breed and day. Additionally, the breed x day interaction was significant for spot temperature and respiration rate. The fixed linear regressions of all heat stress indicator traits on the relevant THI variable were significant for all traits and interacted with breed for rectal temperature and respiration rate (all $p < 0.01$). Respiration rate was the only heat stress indicator trait requiring the inclusion of a cubic spline (as well as its interaction with breed) to model deviations from linearity conforming to a smooth trend (Table 7.3).

Table 7.3 The levels of significance for fixed effects of breed and day of recording as well as the regression variables on the temperature-humidity index (THI) and interactions between these main effects.

Effect	Trait			
	Rectal Temperature ($^{\circ}$ C)	Spot Temperature ($^{\circ}$ C)	Eye Temperature ($^{\circ}$ C)	Respiration Rate (bpm)
Breed	**	**	0.414	**
Days	**	**	0.104	**
Breed x Days	0.39	**	0.892	**
THI Linear	**	**	**	**
Breed x THI Linear	**	0.099	0.670	*
Breed x THI Spline	N/A	N/A	N/A	**

**Significant ($p < 0.01$) *Significant ($p < 0.05$); Actual P-value for $p > 0.05$; N/A – Not Applicable

Overall, rectal temperature was somewhat higher in Merino ewes than in the other breeds (Table 7.4). In contrast, the spot temperatures trended higher in Meatmaster ewes compared to Merinos, with White Dorper ewes having the lowest means. The respiration rate of Merino ewes was also higher than in White Dorper ewes (All $p < 0.05$). Rectal temperature and spot temperature both declined from Day 1 to Day 2 and then to Day 3 (all $p < 0.05$). Respiration rate trended higher on Day 1 compared to the other two days ($p < 0.05$).

Table 7.4 Predicted means (\pm SE) for the fixed effects of breed and day of recording on the heat stress indicator traits that were analysed.

Effect	Trait			
	Rectal Temperature ($^{\circ}$ C)	Spot Temperature ($^{\circ}$ C)	Eye Temperature ($^{\circ}$ C)	Respiration Rate (bpm)
<u>Breed</u>				
Merino	38.8 \pm 0.03 ^b	32.1 \pm 0.2 ^b	36.5 \pm 0.2	4.16 \pm 0.06 (63.9) ^b
Meatmaster	38.5 \pm 0.03 ^a	32.7 \pm 0.2 ^c	36.5 \pm 0.2	3.95 \pm 0.06 (51.8) ^a
White Dorper	38.5 \pm 0.03 ^a	31.3 \pm 0.2 ^a	36.1 \pm 0.2	3.89 \pm 0.06 (49.0) ^a
<u>Day of Recording</u>				
1	38.7 \pm 0.03 ^c	33.1 \pm 0.2 ^c	36.4 \pm 0.2	4.24 \pm 0.06 (69.2) ^b
2	38.6 \pm 0.03 ^b	31.8 \pm 0.2 ^b	36.3 \pm 0.2	3.89 \pm 0.07 (48.9) ^{a, b}
3	38.5 \pm 0.03 ^a	31.0 \pm 0.2 ^a	36.5 \pm 0.2	3.87 \pm 0.07 (48.1) ^a

a, b, c Denote significant differences ($p < 0.05$) in columns within effects.

The main effects of spot temperature and respiration rate were complicated by two-factor interactions between breed and day (Table 7.5). The spot temperature of Meatmasters was distinctly higher than those of the other two breeds on Day 1 ($p < 0.05$), which did not differ. On both Days 2 and 3, Merinos and Meatmasters had a similar spot temperature ($p > 0.05$), which was higher than the comparable means for White Dorpers ($p < 0.05$). When respiration rate was considered, Merinos recorded the highest values on Day 1, followed by Meatmasters and then White Dorpers (all $p < 0.05$). The same general pattern emerged on Day 2, but the difference between Meatmasters and White Dorpers was not significant ($p > 0.05$). In contrast, no distinct breed differences were found on Day 3 ($p > 0.05$). Respiration rate was consistently higher on Day 1 compared to the other two days, irrespective of breed (all $p < 0.05$).

Table 7.5 Predicted means (\pm SE) depicting the two-factor interaction between breed and day of recording for the heat stress indicator traits - spot temperature and respiration rate.

Traits and Effects	Breed		
	Merino	Meatmaster	White Dorper
<u>Respiration Rate (bpm)</u>			
Day 1	4.48 \pm 0.06 (88.4) ^{b 3}	4.19 \pm 0.06 (66.2) ^{b 2}	4.03 \pm 0.06 (56.5) ^{b 1}
Day 2	4.03 \pm 0.08 (56.3) ^{a 2}	3.82 \pm 0.08 (45.5) ^{a 1}	3.81 \pm 0.08 (45.5) ^{a 1}
Day 3	3.96 \pm 0.08 (52.6) ^{a 1}	3.83 \pm 0.08 (46.0) ^{a 1}	3.82 \pm 0.08 (45.8) ^{a 1}
<u>Spot Temperature ($^{\circ}$C)</u>			
Day 1	32.5 \pm 0.3 ^{b 1}	34.5 \pm 0.3 ^{c 2}	32.4 \pm 0.3 ^{b 1}
Day 2	32.5 \pm 0.3 ^{b 2}	32.0 \pm 0.3 ^{b 2}	31.0 \pm 0.3 ^{a 1}
Day 3	31.3 \pm 0.3 ^{a 2}	31.3 \pm 0.3 ^{a 2}	30.5 \pm 0.3 ^{a 1}

a, b, c Denote significant differences ($p < 0.05$) in columns within traits; ^{1, 2, 3} Denote significant differences ($p < 0.05$) in rows within traits.

Graphs were presented for those traits where linear or spline components interacted ($p < 0.05$) with breed, suggesting different responses for the respective breeds to increased temperatures. It was evident that the linear regression of rectal temperature of Merinos on the THI was shallower

than in the other two breeds (Figure 7.2). In general, it seemed that the rectal temperatures of Merinos were higher than in the two other breeds during the cooler mornings, with less evidence of a distinct breed difference during the hotter part of the day.

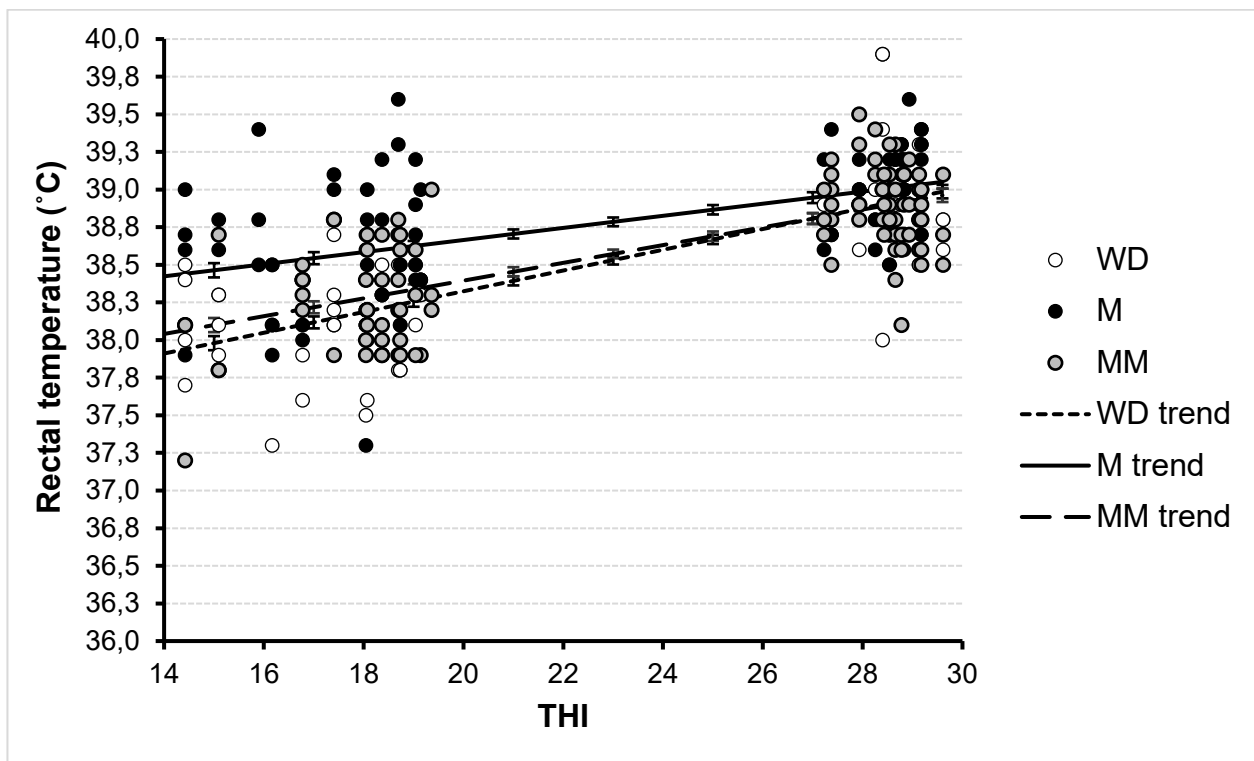


Figure 7.2 Scatterplot depicting the relationship of recorded rectal temperatures of White Dorper (WD), Merino (M) and Meatmaster (MM) ewes with the temperature-humidity index (THI). Standard errors on the respective breed regression lines could be used to compare breed differences at different THI-levels.

Figure 7.3 illustrates the relationship between natural log transformed respiration rate of individuals ewes and the THI of the environment for the duration of the trial. Respiration rate inclined curvilinearly with an increase in the THI to reach higher levels at high levels of the THI in all breeds (Figure 7.3). Trendlines for the three breeds during cooler mornings suggested higher respiration rate means for Merinos throughout when the standard errors associated with the trendlines were considered ($p < 0.05$). There were no clear differences between trendlines for Meatmasters and White Dorpers as indicated by overlapping standard errors.

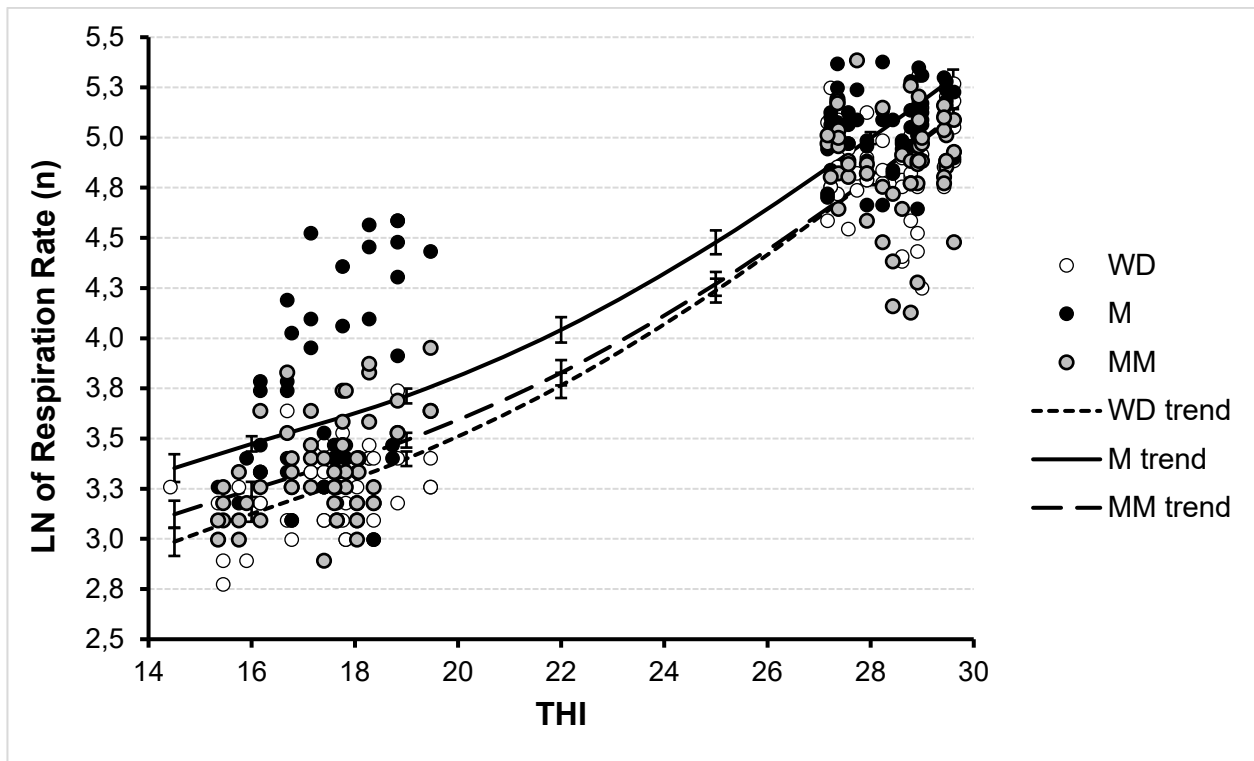


Figure 7.3 Scatterplot depicting the relationship of recorded respiration rate of White Dorper (WD), Merino (M) and Meatmaster (MM) ewes with the temperature-humidity index (THI). Standard errors on the respective breed regression lines could be used to compare breed differences at different THI-levels.

These results, reasonable as they are, prompted us to also apply a different approach to analysing the data. Because only rectal temperature and respiration rate interacted with the regression components involving the THI, these additional analyses were restricted to these traits. The levels of significance for the fixed effects of breed, day of recording (as defined before) and recording session (morning or afternoon) as well as two-factor interactions among these main effects are provided in Table 7.6. Respiration rate data were transformed to natural logarithms, as before.

Table 7.6 The levels of significance for fixed effects of breed, day of recording and session as well as two-factor interactions between these main effects.

Effect	Trait	
	Rectal temperature (°C)	Respiration rate (bpm)
Breed	**	**
Days	0.288	**
Breed x Days	0.434	**
Session	**	**
Breed x Session	**	*
Days x Session	**	**

**Significant ($p < 0.01$) *Significant ($p < 0.05$); Actual P-value for $p > 0.05$

The significance of common effects in Tables 7.3 and 7.6 (i.e., breed, day, and breed x day) were similar with the sole exception of the effect of days for rectal temperature. The effects of session and its interactions with breed and days were significant throughout (Table 7.6). The outcomes for

the effects of breed, days and the breed x day interaction were like the results reported in Tables 7.4 and 7.5 and are thus not considered. Both rectal temperature and respiration rate were higher ($p < 0.01$) in the afternoon than in the morning. Expressed relative to means for the morning, rectal temperature increased by 1.5% (respective means of $38.3 \pm 0.03^{\circ}\text{C}$ vs. $38.9 \pm 0.03^{\circ}\text{C}$) and respiration rate by 45% (3.39 ± 0.02 vs. 4.92 ± 0.02 ; respective geometric means of 29.7 and 137.7 bpm, i.e., a 4.6-fold increase). The predicted means for the breed x session interaction are given in Figure 7.4 for rectal temperature and Figure 7.5 for the natural logarithm of respiration rate. Figure 7.4 clearly indicates that Merinos had a higher ($p < 0.05$) rectal temperature in the mornings compared to White Dorpers and Meatmasters (38.5 ± 0.05 vs. 38.2 ± 0.05 and 38.1 ± 0.05 °C respectively), while no obvious breed effect ($p > 0.05$) was observed during the afternoons (range of 38.9 to 39.0 °C). This result is consistent with the outcomes of Figure 7.2.

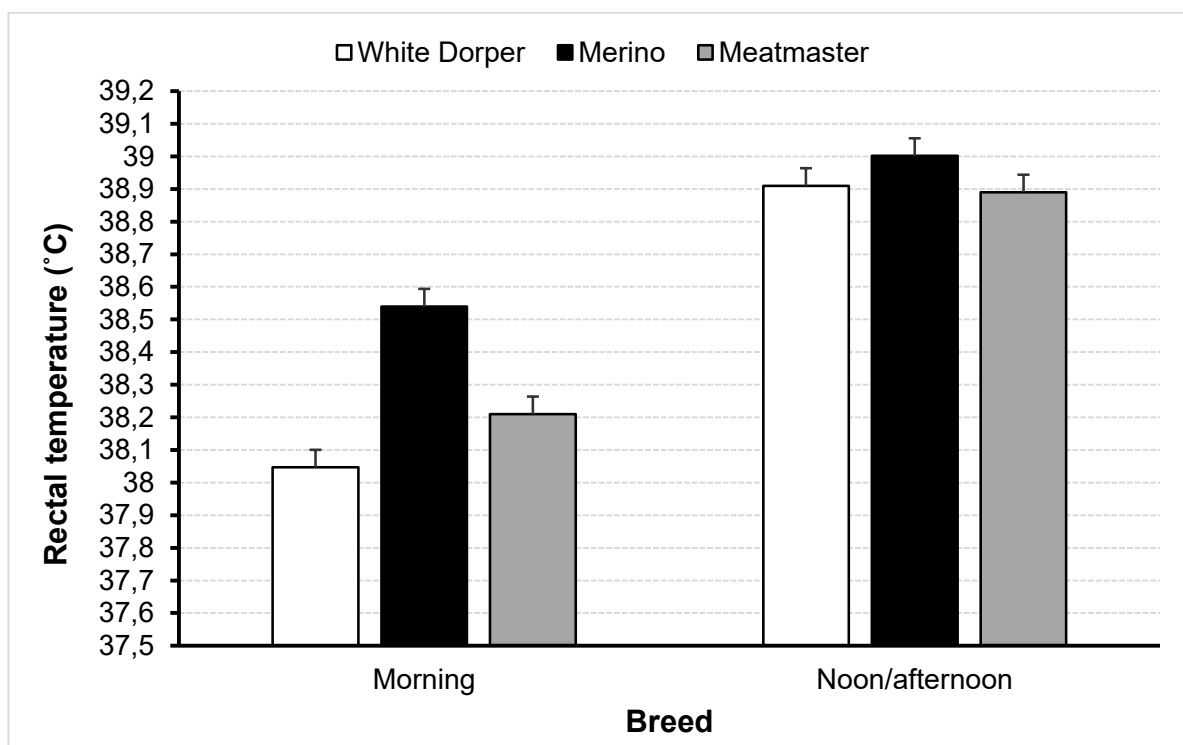


Figure 7.4 Predicted means for the interaction between breed and session for rectal temperature. Vertical bars above the means represent standard errors.

The corresponding interaction graph for the natural logarithm of respiration rate are provided in Figure 7.5. This interaction was only significant at $p < 0.05$, and Merino ewes had higher respiration rates compared to the other breeds when considered during both sessions ($p < 0.05$), without substantial differences between the other breeds. This outcome is also consistent with the results provided in Figure 7.3.

(93)

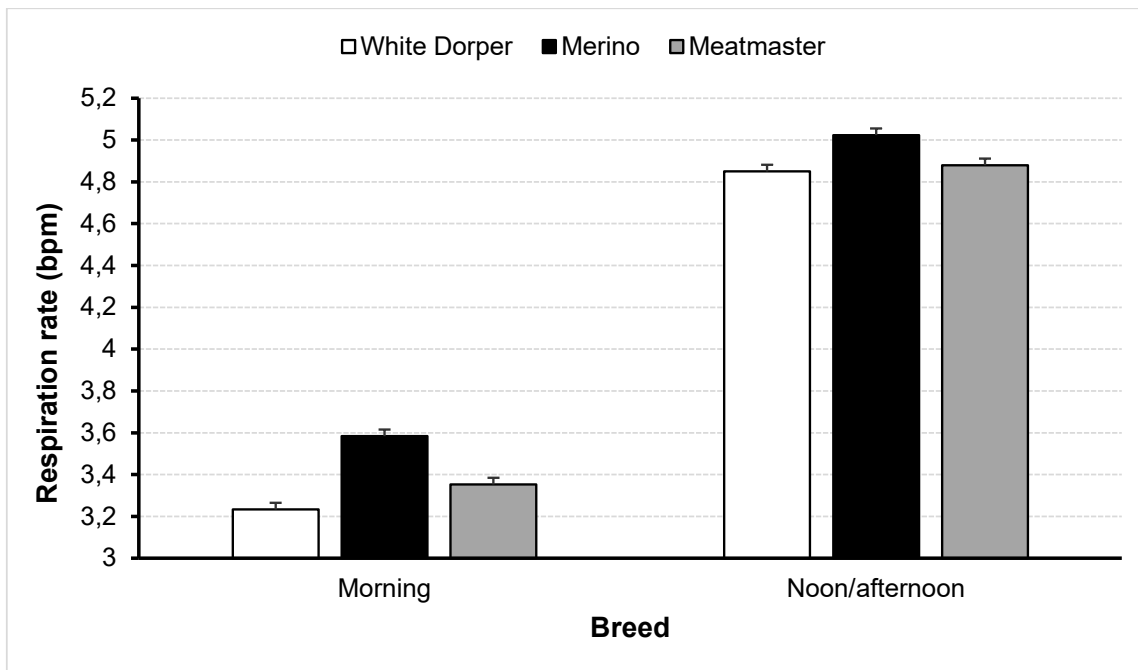


Figure 7.5 Predicted means for the interaction between breed and session for the natural logarithm of respiration rate. Vertical bars above the means represent standard errors. bpm – breaths per minute

The interaction between days and session is presented in Figure 7.6 for rectal temperature. According to this figure, morning rectal temperature was lower ($p < 0.05$) on Day 1 compared to Days 2 and 3 (38.1 ± 0.04 vs. 38.4 ± 0.04 and 38.3 ± 0.04 °C respectively). The increase in rectal temperature from the morning to the noon amounted to 2.2% on day 1, compared to 1.5 to 1.6% on days 2 and 3 (all $p < 0.05$), with no significant differences among days when assessed in the afternoon ($p > 0.05$; Range 38.9 to 39.0 °C).

(94)

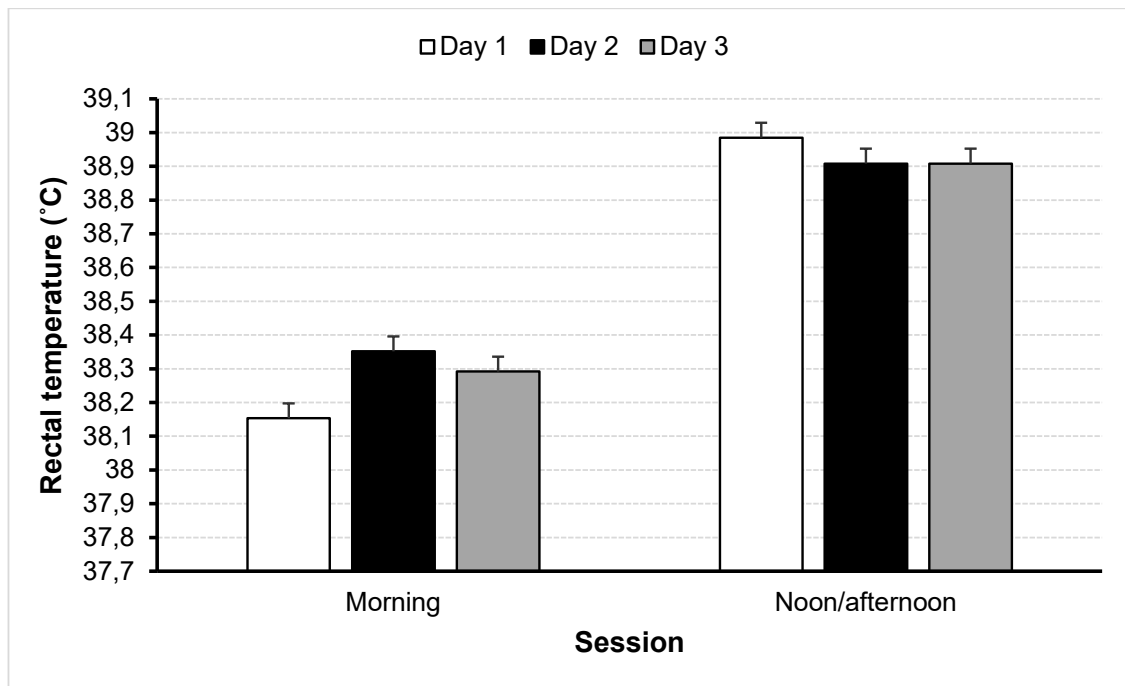


Figure 7.6 Predicted means for the interaction between days and session for rectal temperature. Vertical bars above the means represent standard errors.

Means for the natural logarithm of respiration rate, as affected by the day x session interaction are reported in Figure 7.7. During the morning session, the mean for the natural logarithm of respiration rate on Day 1 exceeded ($p < 0.05$) the means for the other two days (3.68 ± 0.03 vs. 3.23 ± 0.03 and 3.25 ± 0.03 °C respectively). Corresponding means during Days 1 and 3 exceeded $p < 0.05$ the mean for day 2 during the afternoon sessions (4.97 ± 0.03 and 5.03 ± 0.03 vs. 4.75 ± 0.03 bpm respectively).

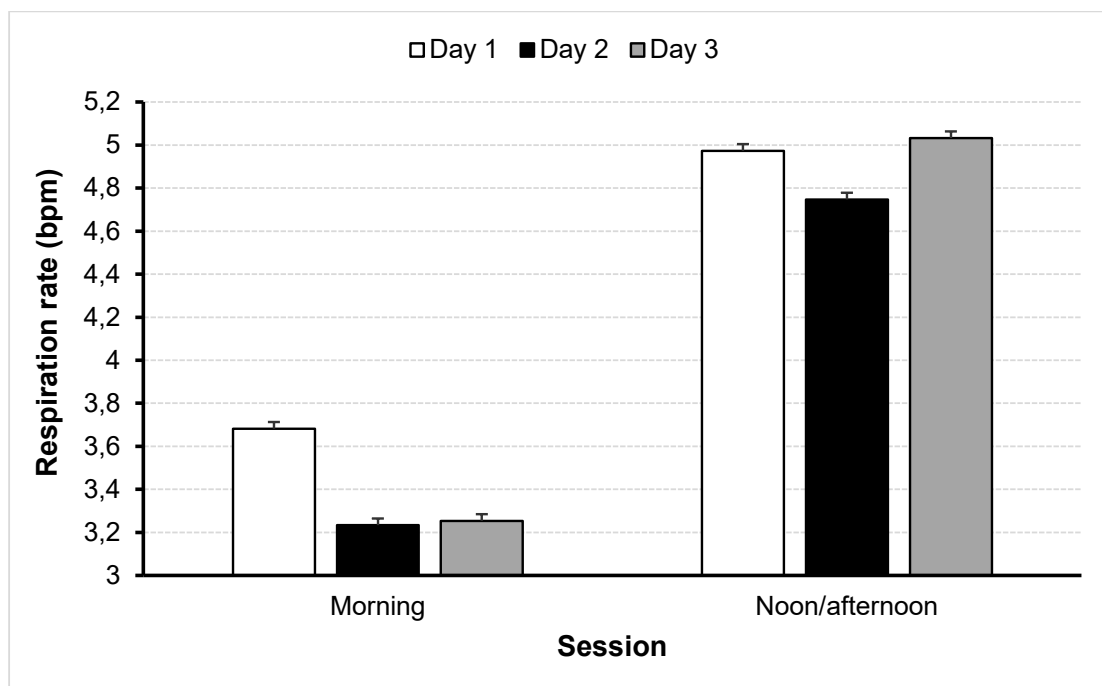


Figure 7.7 Predicted means for the interaction between days and session for the natural logarithm of respiration rate. Vertical bars above the means represent standard errors.

During the morning sessions, respiration rates were lower on days 2 and 3 (Figure 7.7) and the frequencies of ewes with respiration rates exceeding 40 bpm were very low at 0 for White Dorpers, 3 for Merinos and 0 for Meatmasters on Day 2. Corresponding figures for Day 3 were respectively 0, 0 and 1. Chi-square analyses were not attempted, since more than 20% of cells in the 3 x 2 table had a frequency below 5. On day 1, though, the proportion of Merino ewes with a respiration rate of 40+ bpm ($17/20 = 0.85$) exceeded figures for the White Dorpers ($1/20 = 0.05$) and Meatmasters ($6/20 = 0.30$) (Chi-square = 27.92; degrees of freedom = 2; $p < 0.05$). After the Bonforoni correction for multiple comparisons was applied, it was evident that the proportion of Merino ewes with a respiration rate above 40 bpm was higher ($p < 0.05$) than in the other two breeds. When the figures for the noon session were considered and related to a critical Chi-square-value of 5.99 for a 3 x 2 table, no breed differences were found between the proportions of ewes with either a respiration rate below 120 or 120+ bpm on Day 1 (Chi-square = 3.840) and Day 2 (Chi-square = 3.851). The respiration rates of almost all ewes were 120 bpm or higher during the noon session of Day 3, namely 19 for White Dorpers, 20 for Merinos and 18 for Meatmasters out of the 20 ewes recorded per breed. No statistical analysis was presented as more than 20% of the cells had frequencies below 5. It needs to be added that the proportions of ewes with respiration rates of 120 bpm and above were consistently high at 0.833 on day 1, 0.633 on Day 2 and 0.95 on Day 3. However, the corresponding proportions of ewes with a respiration rate exceeding 200 bpm was low at 0.033 on Day 1, 0.017 on Day 2 and 0.050 on Day 3.

7.4.5 Log Likelihood values and random effects

Results pertaining to the random effects models were similar for the two sets of analyses conducted. For this reason, only results for the first analyses are reported in Tables 7.2 to 7.4. The inclusion of random ewe effects only improved the log-likelihood values above those of the operational model for rectal temperature and respiration rate (Table 7.7). The inclusion of the effect ewe x session as a single random effect also resulted in improved log-likelihood values for both the latter traits, when compared to the operational model. When both random sources of variation (ewe and ewe x session) were added to the operational model, a further improvement in the log-likelihood for rectal temperature was found, but not for respiration rate. In fact, all the random variation for ewes seemed to partition to ewe x session, as the random ewe variance component went to the boundary of parameter space (zero). Subsequent results will therefore focus on the variance ratios derived for rectal temperature and respiration rate.

Table 7.7 Log-likelihood values for the operational model and for models with added random effects for the heat stress indicator traits. The “best” models are denoted in bold font.

Model Fitted	Trait			
	Rectal Temperature (°C)	Spot Temperature (°C)	Eye Temperature (°C)	Respiration Rate (bpm)
Operational model	195.474	-443.401	-397.437	336.790
+ Ewe	212.030	-443.401	-396.701	338.874
+ Ewe x Session	214.683	-442.085	-396.647	342.684
+ Ewe + Ewe x Session	217.105	-442.085	-396.647	342.684

The repeatability of rectal temperature in the absence of the ewe x session interaction was moderate at 0.24 ± 0.06 , while the corresponding estimate for respiration rate was appreciably lower at 0.08 ± 0.04 (Results were not tabulated). The corresponding ewe x session variance estimates amounted to 0.35 ± 0.06 for rectal temperature and 0.20 ± 0.06 for respiration rate. When the reranking of ewes from the cooler mornings to the hotter afternoons were considered, the between ewe variance was repartitioned to ratios of roughly equal magnitude for ewe and ewe x session effects in the case of rectal temperature (Table 7.8). The reranking among ewes appeared to be the dominant source of variation from animal origin that contributed to variation in respiration rate as described above.

Table 7.8 Variance components and ratios for random ewe effects as well as random ewe x session effects to model the reranking of ewes from cooler mornings to hotter afternoons for rectal temperature and respiration rate for rectal temperature and respiration rate.

Variance components and ratios	Trait	
	Rectal temperature (°C)	Respiration rate (bpm)
<u>Components</u>		
σ^2_{ewe}	0.0165	N/A
$\sigma^2_{ewe \times session}$	0.0193	0.0084
$\sigma^2_{residual}$	0.0660	0.0338
$\sigma^2_{phenotype}$	0.1018	0.0422
<u>Ratios</u>		
ewe^2	0.16 ± 0.07	N/A
$ewe \times session^2$	0.19 ± 0.08	0.20 ± 0.06

σ^2_{ewe} – random ewe effects; $\sigma^2_{ewe \times session}$ – random ewe x session effects; $\sigma^2_{residual}$ – random residual effects; $\sigma^2_{phenotype}$ – total phenotypic variance; N/A – Variance component went to zero when fitted with ewe x session.

7.5 Discussion

The primary objective of this study was to explore the respective adaptive mechanisms relating to the effect of THI on physiological responses of a variety of ewe sheep breeds under hotter summer conditions in the Northern Cape. Heat tolerance in sheep is influenced by numerous factors, including the physiological status and genetic composition of the animal, as well as the feeding regime (Marai *et al.*, 2007; McManus *et al.*, 2009). For this reason, the sheep used in this study were not late pregnant or lactating, since these physiological states may have affected outcomes. THI is considered a sensitive indicator for stressful climatic conditions (Silanikove, 2000; El-Tarabany *et al.*, 2017), where indicator traits such as respiration rate, rectal temperature, spot temperature and

eye temperature are expected to incline with increased levels of heat stress in sheep (Marai *et al.*, 2007; Dalcin *et al.*, 2016; McManus *et al.*, 2016; Singh *et al.*, 2016; El-Tarabany *et al.*, 2017; Rashamol *et al.*, 2018).

7.5.1 Descriptive statistics

The CV for body weights of ewes in this study ranged from 14.9% to 16.1% and were comparable to values reported for Barbados Blackbelly adult ewes (Ruiz-Ortega *et al.*, 2022). However, these CV values are higher in magnitude compared to the CV range of 6.0-12.9% for adult wool, dual-purpose and meat sheep breeds (Matika *et al.*, 2003; Safari *et al.*, 2005). The CV for rectal temperature was double the magnitude of that in Santa Inês and crossbred ewes (Furtado *et al.*, 2017), but only half the magnitude of the CV for rectal temperatures reported in Barbados Blackbelly ewes (Ruiz-Ortega *et al.*, 2022). The untransformed respiration rate's CV reported in this study was similar to that reported in the study by Ruiz-Ortega *et al.* (2022). The CV for fat depth in this study corresponds to the value of 20.4% reported in a review by Safari *et al.* (2005). Additionally, an average fat depth of 0.680 cm was obtained for ewes of different breeds in South Africa (Van Der Merwe *et al.*, 2022), which agrees with the mean obtained in this study for similar ewe breeds (Table 7.1). The CV for hair length in sheep was 38.96% (McManus *et al.*, 2011) and corresponds to the value of 34.8% for fleece depth found in this study (Table 7.1).

7.5.2 THI

High daily ambient temperature variation was observed during the trial, with a maximum of 35.8°C in the afternoon and minimum of 13.0°C in the morning (Figure 7.1). The RH also varied during the day, with the lowest RH of 10.2% in the afternoon, and maximum RH-values of 66.1% in the morning. High THI-values were observed in the afternoon (average of 28.5, and maximum of 29.6), reaching values classified as dangerous, i.e. in excess of 27 (Seixas *et al.*, 2017), while the animals were in thermal comfort during the mornings (THI < 22.2), with an average and maximum THI of 18.0 and 21.9, respectively. These results are in agreement but with lower maxima than climate data obtained from the study reported by Seixas *et al.* (2017), where average THI-levels of 15.7 and 29.2 was obtained during the morning and afternoon, respectively.

All heat stress indicator traits were dependent on the THI (Table 7.3). This corresponds to the direct relationship found between the THI and the heat stress indicator traits, respiration rate and rectal temperature (Seixas *et al.*, 2017). Additionally, lower respiration rates and rectal temperatures indicate that animals are more adapted to their surrounding environment, reflecting harsher climatic conditions. These relationships were further complicated by interactions of breed with the regressions for rectal temperature and respiration rate (Table 7.3). In accordance with the present study, Youself (1985), Maurya *et al.* (2007), Indu & Pareek (2015), and Ruiz-Ortega *et al.* (2022) also observed that respiration rate was markedly lower at 08h00 than at 12h00 and 16h00. Sejian *et al.* (2012) similarly found higher respiration rates in the afternoon than in the morning of Malpura

sheep from a semi-arid region. Accordingly, it was reported by da Silva *et al.* (2017) that the physiological responses of animals increased from the morning (around 07h00 to 10h00, presumably under cooler conditions) up to noon (around 11h00 to 14h00, presumably under hotter conditions), after which it started to decrease from the evening till night time where it plateaued for the duration thereof (around 23h00 to 06h00).

Diurnal variation for spot temperatures and respiration rates in thermoneutral conditions (morning recording sessions), are directly related to the environmental temperatures (Figure 7.1; Figure 7.5; Table 7.6). However, under heat stress conditions (afternoon recording sessions), circadian fluctuations in spot temperatures are influenced by the environmental temperature, while changes in respiration rates are dependent on the presence of minimal differences in the thermal gradient between the core temperature of the sheep and the environmental temperatures, the latter also reflected by the THI (Figure 7.1; Figure 7.3; Figure 7.5). This corresponds to the results reported by Macías-Cruz *et al.* (2016), except their thermoneutral conditions refer to the spring season, while heat stress conditions refer to their summer season results.

7.5.3 Breed effects on live weight, fat depth and fleece depth

The Meatmaster ewes gained weight during this study while no significant weight change was observed for the White Dorper and Merino ewes (Table 7.2). However, this is unexpected and inconsistent with external research (Hamzaoui *et al.*, 2013; Kandemir *et al.*, 2013; Mehaba *et al.*, 2021). Additionally, another study on ewes' response to heat stress over a 21-day trial period resulted in an overall weight loss, with an average change in body weight of -0.38 kg (Mehaba *et al.*, 2021). Shinde & Sejian (2013) also reported that sheep exposed to thermal stress experienced a reduction in lower body weight caused by high energy losses. A net weight gain in the present study in comparison with weight loss in other studies possibly reflects the short duration of the present trial, as well as the ability of ewes to compensate for accumulated daytime heat stress during the cooler nights (see Figure 7.1).

7.5.4 Heat stress indicator traits

7.5.4.1 Rectal Temperature and Respiration Rate

Since the effects of increased heat loads on rectal temperature and respiration rate were closely interlinked, these traits are combined in this discussion. At first, attention will be paid to the traits separately, which will be followed by a combined discussion on both traits. The average rectal temperature in the absence of heat stress in the morning amounted to 38.0°C in White Dorper, 38.5°C in Merino, and 38.2°C in Meatmaster ewes (Figure 7.2 and 7.4; Table 7.6), and coincides with rectal temperatures ranging from 38.1°C to 38.5°C for sheep in the morning (Furtado *et al.*, 2017; Machado *et al.*, 2020). However, these rectal temperatures were lower compared to the average rectal temperature of 39.3°C in Naimey sheep (Al-Haidary, 2004) as well as those obtained for Merino (39.5°C) and Omani (39.0°C) sheep (Srikandakumar *et al.*, 2003) under cool

environmental conditions. Similarly, Santa Inês x Dorper ewes obtained an average rectal temperature of 39.0°C in the morning (Dos Santos *et al.*, 2021). Accordingly, there was a 1.72% increase ($p < 0.05$) in rectal temperature from the morning to the noon/afternoon recording sessions (Figure 7.2 and 7.4; Table 7.6). This increase in rectal temperature was less than half of that found in Osmanabadi goats (3.76%) (Shilja *et al.*, 2016). In contrast, Li *et al.* (2018) reported a smaller increase (0.98%) in rectal temperature of Hu ewes in response to heat stress conditions. Similar to the current study, rectal temperatures increased with 1.76% in Omani sheep under hot environmental conditions (Srikandakumar *et al.*, 2003). This rise in rectal temperature from the morning to the noon/afternoon sessions (Figure 7.2; Figure 7.4) are therefore consistent with values reported in literature (Tabarez *et al.*, 2009; Romero *et al.*, 2013; Pinto-Santini *et al.*, 2014; Macías-Cruz *et al.*, 2016; Machado *et al.*, 2020).

An average rectal temperature of 39.1°C is considered normal for sheep according to Seixas *et al.* (2017), while Marai *et al.* (2007) and Okourwa (2015) reported the norm to range between 38.3°C and 39.9°C. With a maximum rectal temperature of 39.9°C for an individual White Dorper ewe (Figure 7.2), it is thought that the majority, if not all, of the ewes in this study maintained their rectal temperatures within the normal range. The higher magnitude of increase in rectal temperature in White Dorper and Meatmaster ewes when exposed to heat stress (Figure 7.4), suggests that these animals are capable of storing body heat during this phase (Srikandakumar *et al.*, 2003). Such an adaptation can reduce water loss as well as reduce the need of energy associated with respiration rate (Li *et al.*, 2018), meaning that White Dorper and Meatmaster ewes do not need to increase their respirations rates as much as the Merino ewes are required to do in response to heat stress conditions. Subsequently the White Dorper and Meatmasters use less energy for increases in respiration rates compared to the Merinos. The rectal temperatures reported in this study (Figures 7.2, 7.4, and 7.6) were lower than those reported for different sheep breeds in response to heat stress (Srikandakumar *et al.*, 2003; Al-Haidary, 2004; Li *et al.*, 2018). Under extreme heat stress conditions (THI = 33.77), Santa Inês and cross-bred (F1-Dorper x Santa Inês) sheep also recorded higher rectal temperatures amounting to 39.8°C on average (Gesualdi Júnior *et al.*, 2014), which only corresponded to the maximum individual rectal temperatures recorded in this study (Figure 7.2). Other researchers also found average rectal temperatures above 39.5°C in ewes under heat stress conditions (Belhadj Slimen *et al.*, 2019; Mehaba *et al.*, 2021).

At an ambient temperature of 32°C, the rectal temperature of sheep start to rise above normal levels (Srikandakumar *et al.*, 2003). If the RH is below 65%, open mouth panting commences at a rectal temperature of 40°C. However, it is suggested that sheep may die if their rectal temperature exceeds 41.1°C, since animal cells start to degenerate (Marai *et al.*, 2007). Srikandakumar *et al.* (2003) also stated that sheep can withstand external temperatures as high as 43°C for hours, during which both sweating and panting become essential heat regulating mechanisms.

In accordance with Figure 7.3 and Table 7.6, Shilja *et al.* (2016) reported that Osmanabadi goats had significantly lower respirations rates in the morning compared to the afternoon. In response to

heat stress conditions, the respiration rates increased to 159 bpm in Merino, and to 135 bpm in both Meatmaster and White Dorper ewes (Figure 7.5). As mentioned previously, the respiration rate of White Dorper and Meatmaster ewes tended to stabilize at higher THI levels (Figure 7.3). This could be an indication that the White Dorsers' and Meatmasters' adaptive mechanisms (i.e., respiration as a cooling mechanism relating to respiration rate) were slightly more effective in cooling down their core body temperature with increases in THI, compared to their contemporary Merino ewes. This corresponds to the statement in the study by McManus *et al.* (2016) and Macías-Cruz *et al.* (2016) that hair breeds tended to have faster responses to increased THI and ambient temperatures, implying their adaptive cooling mechanisms were slightly more effective compared to the wool breeds. Lower respiration rates were observed during the morning compared to noon/afternoon and the variation in circadian rhythms for respiration rate can be seen in Figure 7.5. These results are in agreement with that found in Correa *et al.* (2013) and Ruiz-Ortega *et al.* (2022). Macías-Cruz *et al.* (2016) who found that the maximum change in respiration rate occurred at 18h00, with the hotter environmental conditions resulting in an average value of 172 bpm, while cooler environmental conditions had a much lower average respiration rate of 75 bpm. Variation in circadian rhythms for respiration rate are generally related to physiological adaptability, which is implemented by sheep to maintain homeothermy and to avoid dehydration under hot climatic conditions (Macías-Cruz *et al.*, 2016).

An average respiration rate of 27 bpm is considered normal for sheep (Seixas *et al.*, 2017). In response to heat exposure, sheep increase respiratory evaporation (i.e., evaporative cooling) to maintain thermal balance. An increased respiration rate is an early warning signal of heat stress in livestock, which promotes evaporative cooling through dissipating heat from the body (Al-Haidary, 2004; Nienaber & Hahn, 2007; Romero *et al.*, 2013; Macías-Cruz *et al.*, 2016; Ruiz-Ortega *et al.*, 2022). Additionally, as an adaptive mechanism in response to changing environmental conditions, respiration rate is the visual representation of what occurs within the animal's body in attempt to maintain their thermal balance through evaporating and dissipating moisture from the respiratory tract. These mechanisms are defined as respiration, which is the intake of O₂ and elimination of CO₂ is (Da Silva *et al.*, 2017; Rashamol *et al.*, 2018).

It was observed that Merino ewes had the highest mean natural log, with the geometric mean in brackets, respiration rate of 3.59 (40.97 bpm) \pm 0.06 among the three breeds (Figures 7.3 and 7.5) during the cooler morning sessions. At this stage heat stress was absent (THI < 22.2) which reflected a thermoneutral environment with a corresponding THI of 17.4 \pm 0.14 (Figure 7.1). However, on Day 1 of the trial the respiration rate records were taken after rectal temperatures and spot temperatures were measured, presumably leading to the interaction depicted in Figure 7.7. Thus, on Day 2 and Day 3 of the trial, respiration rates were recorded prior to measuring individual rectal temperature and spot temperature, which contributed to the results in Figure 7.7. Taking this into consideration, Merino ewes had a much lower overall average respiration rate of 3.33 (28.6 bpm) \pm 0.06 for Day 2 and Day 3 of the trial. Individual Merino ewes varied markedly pertaining to

respiration rates, with a range of 45 to 90 bpm during low THI conditions (15.4-19.5) during the morning (Figures 7.3 and 7.5), respiration rates were similar to that of adult goats ranging on average between 23.85-25.12 bpm (El-Tarabany *et al.*, 2017). This also corresponds with fluctuating respiration rates between 15 and 30 bpm in a study by Pugh & Baird (2012). It is accepted that the basal respiration rate of sheep and goats amount to 25-30 bpm (Sailo *et al.*, 2017).

Under extreme heat stress conditions (THI > 25.6), an average THI value of 28.4 ± 0.11 was observed in the afternoon recording sessions for the duration of the trial (Figure 7.1). Accordingly, the ewes' respiration rates were significantly higher between 14h00 and 16h00 than during the morning sessions between 06h00 and 09h00, as seen in Figure 7.3. The average respiration rates of Meatmasters (4.88 (135 bpm) ± 0.03) and White Dorpers (4.88 (135 bpm) ± 0.03) ewes were significantly lower than those of Merinos (5.05 (159 bpm) ± 0.02) ewes (Figures 7.3 and 7.5). The maximum average respiration rates obtained in this study coincide with the 160 bpm reported in hair ewe breeds by (Meza-Herrera *et al.*, 2015) and 180 bpm reported for Barbados Blackbelly ewes by Ruiz-Ortega *et al.* (2022). In a comparative study, Santa Inês and crossbred (F1-Dorper x Santa Inês) sheep had average respiration rates of 113 bpm and 135 bpm, respectively, with a corresponding THI value of 33.77 (Gesualdi Júnior *et al.*, 2014). It needs mentioning that these respiration rates were recorded in the shade and determined using a flexible stethoscope placed at the laryngotracheal region, which clarifies why the respiration rates were markedly lower than what would be expected for a THI of 33.77. Respiration rates of Chokla (79 bpm) and Avivastra (57 bpm) sheep reported by Ashutosh *et al.* (2000) were much lower under moderate to severe climatic conditions (THI = ± 23.3) compared to the sheep breeds used in this study.

Overall, Merino ewes seemed to be subject to higher levels of heat stress under comparable ambient conditions than their White Dorper and Meatmaster contemporaries as based by overall higher rectal temperatures and respiration rates (Tables 7.3 and 7.4). It needs to be stated that these effects were not consistent over sessions for rectal temperature (Figures 7.2 and 7.4) and over days for respiration rate (Table 7.5) as well as over sessions (Figure 7.3). The proportion of Merino ewes with an elevated respiration rate under mild, morning conditions were also higher than in the other breeds on Day 1, when the general respiration rates of the ewes were higher compared to the other morning sessions (Figures 7.5 and 7.7). Merinos were still able to maintain homeothermy equally well compared to the other breeds under the hotter noon conditions, as reflected by rectal temperature. However, they arguably had to work harder to achieve this, as reflected by an increased respiration rate. If heat stress accrues over a longer period than in the present study, this burden may result in a decreased appetite and a lower feed intake, leading to weight loss. Although feed intake figures for the respective breeds were unavailable in this study, the lack of change in live weight in Merino ewes suggested that their intake was probably not suppressed by the level of heat stress they were subjected to. Silanikove (2000) states that respiration rates above 80 bpm indicate that sheep start to experience heat stress. Overall, if a respiration rate of 120 and above is considered as severe heat stress it was clear that between 63 and 95% of ewes were subject to this

level of heat stress according to the three recording days. The percentages of ewes with respiration rates of 200 and above (indicating extreme heat stress) were low, at between 1.7 and 5.0%. Romo-Barron *et al.* (2019) also reported that ewes under thermoneutral conditions obtained lower respiration rates than those exposed to heat stress conditions (i.e., 41-101 bpm vs. 109-203 bpm). Similar results were reported for ewes located in Southern Mexico (Meza-Herrera *et al.*, 2015; Ruiz-Ortega *et al.*, 2022).

The result that Meatmasters and White Dorpers (hair breeds) recorded lower rectal temperatures when compared to Merinos (a temperate wool breed) is consistent with those of Romero *et al.* (2013), finding higher rectal temperatures in the wool breed (i.e., Suffolk) compared to the hair breed (i.e., Pelibuey). These results suggested that hair breeds and their crosses are highly adaptable to heat stress conditions and that their thermoregulatory mechanisms maintain homeothermy very efficiently (Macías-Cruz *et al.*, 2016). Respiration rates were also lower in the White Dorper and Meatmaster breeds compared to the Merinos (Table 7.4 and Figure 7.3) during cool environmental (thermoneutral) conditions. This result could be related to the coat type in terms of its depth and density as stated by Do Prado Paim *et al.* (2013) and Titto *et al.* (2016). Wool breeds, like the Merino, have thicker and more dense coats that maintain body heat through preventing air flow (McManus *et al.*, 2011; Correa *et al.*, 2013; Do Prado Paim *et al.*, 2013). On the contrary, the White Dorper and Meatmaster hair breeds have thinner hair, which facilitates air flow across their skin, allowing easier heat exchange with the environment. Additionally, the hair breeds are known to shed their coat, which would also be to their advantage (Schoenian, 2009, 2021c). Evidence of shedding was also observed in the study animals, but not recorded.

The fixed effect of day also had an impact on overall rectal temperatures, spot temperatures and respiration rates (Table 7.3), but was affected by interaction with breed and session in some instances. Rectal temperature and spot temperature both declined from Day 1 through to Day 3 (Table 7.4), while respiration rate was highest on Day 1 and lowest on Day 3, with Day 2 being intermediate.

From Figure 7.2 to 7.5, as well as Table 7.6, it can be perceived that there was diurnal variation for rectal temperature and respiration rate. This corresponds to the results of a study on hair sheep (Macías-Cruz *et al.*, 2016), as well as in native and crossbred sheep in India (Ashutosh *et al.*, 2000). Additionally, for different seasons, diurnal patterns in rectal temperature and respiration rate coincide with diurnal changes in environmental temperatures (Mohamed, 2012). However, no other research in hair sheep reported diurnal variation in rectal temperature. Macías-Cruz *et al.* (2016) found that season interacted with the hour of day, meaning that rectal temperatures and respiration rates were both higher during the summer at given sampling hours when compared to spring. Rectal temperatures were 0.25°C and respiration rates 56 bpm higher on average during the summer season when compared to spring.

We preferred a regression approach to model the impact of the THI on the heat stress indicator traits studied, in the hope to gain information on breed-specific change-points in the reaction of

animals to heat (see McManus *et al.*, 2016; Cloete *et al.*, 2021). This was not realized, though, since there was a lack of an overlap between the cooler mornings and the hotter afternoons. Although the regressions indicated breed-specific trends for rectal temperature and respiration rate, the nature of these did not allow for the detection of specific change-points. This is in contrast to a study by McManus *et al.* (2016), where numerous change or inflection points (i.e., the moment or turning point where a significant change occurs, relating to an animal's change in response to changing environmental conditions) were obtained for different sheep breeds, including the Dorper, IDF, Merino, Santa Inês, Corriedale, Crioulo, Hampshire, Ideal, Romney, Suffolk and Texel. This information is of cardinal importance to understand how well an animal can adapt when exposed to certain environmental conditions. The inflection point for respiration rate is considered as an indication of the point at which the animal changes its respiration to compensate for environmental changes (McManus *et al.*, 2016), such as a rise in the THI, ambient temperature, or RH. Transformed respiration rate was curve-linearly related to the THI in this study, but it was impossible to obtain breed-specific change-points with the data at our disposal. A relatively poor adaptation was observed for some wool and semi-wool sheep breeds, namely Crioulo, Hampshire, Romney, and Suffolk as they generally had lower inflections points compared to hair breeds also studied (Dorper and Santa Inês) (McManus *et al.*, 2016). However, a moderate adaptation was observed for other wool and semi-wool breeds, namely Merino, IDF, Corriedale, Ideal, and Texel. This study also found that the curve denoting the curve-linear regression of respiration rate on THI was consistently higher in Merinos than in the other breeds (Figure 7.3). This result suggested that they had to resort to faster breathing to maintain homeostasis compared to the hair breeds across the THI-trajectory.

7.5.4.2 Spot Temperature

Accordingly, overall spot temperatures were the highest in the Meatmaster, then the Merino and lowest in the White Dorper (Table 7.4). However, on Day 1 of the trial, the spot temperature of the White Dorper and Merino breeds did not differ ($p > 0.05$) from one another (Table 7.5). On Day 2, there was no difference ($p > 0.05$) between the Merino and Meatmaster breeds. These results relate to the ewe's skin colour as well as coat type and colour, where dark colours are known to absorb solar radiation and light colours reflect it (Henry *et al.*, 2012; Do Prado Paim *et al.*, 2013; Pérez *et al.*, 2020; Ruiz-Ortega *et al.*, 2022). Additionally, Merinos had a near white to cream colour coat and White Dorsers had a white coat colour across their whole body, while Meatmasters had different shades of light to dark brown, with at least one individual being uniformly black. Merino ewes' coat density in terms of their 'wooled' coat type could also have resulted in the higher means for spot temperature on two days compared to their White Dorper contemporaries.

Although some breed variation was picked up by spot temperature, it was subject to daily variation (Table 7.5). Breed means were not conclusive and seemed to be affected by the greater likelihood that Meatmaster ewes had darker heads, an effect particularly evident on Day 1. The White Dorper, with a face cover consisting of chalky white medullated fibres, had a lower spot temperature

than the other breeds, except when compared to Merinos on Day 1 (Table 7.5). It is likely that this effect could be related to a greater reflectance of solar radiation by White Dorpers as suggested above. The value of spot temperature as a heat stress indicator could thus also be questioned based on these results and backing arguments.

During the day, the spot temperature was on average 20.7% lower ($p < 0.05$) for the morning recording sessions (between 06h00 and 09h00) compared to the afternoon recording sessions (between 14h00 and 16h00). This agrees with the 20.96% increase in spot temperature of Osmanabadi goats from the morning to afternoon recording sessions. Similarly, Rashamol *et al.* (2018) and Ruiz-ortega *et al.* (2022) stated that spot temperatures were lower at 08h00 than at 12h00-16h00 in the summer. In a study on Saanen goats, the spot temperature of the animals increased with 0.22°C per degree of ambient temperature exposure (Paulo & Lopes, 2014).

7.5.4.3 Eye Temperature

Opposed to rectal temperature and respiration rate, the present study did not suggest any differentiation on breed, or breed-specific trends for eye temperature. Despite arguments for the usage of this trait in the assessment of heat stress (McManus *et al.*, 2016), it seemed to be of limited value in discerning breed-specific trends in various heat stress scenarios under the conditions of the present study. Additionally, the study by McManus *et al.* (2016) was conducted on ewes housed in a shed, which can explain the contradicting significance of eye temperature as a heat stress indicator trait for the present study. The value of this trait in assessing heat stress in local sheep therefore needs to be questioned. In contrast, McManus *et al.* (2016) reported different inflection points for both respiration rate and eye temperature, as well as for breed (Dorper, IDF, Santa Inês, Merino, Corriedale, Crioulo, Hampshire, Ideal, Romney, Suffolk and Texel). It was suggested that the inflection point of eye temperature is the value where an animal's physiological mechanisms can no longer maintain its core body temperature.

Eye temperature did however differ ($p < 0.05$) between the two recording, with an average eye temperature of 34.2°C during the morning and 38.7°C during the afternoon. This physiological trait is considered an acceptable and accurate measure of the core body temperature in livestock animals (Do Prado Paim *et al.*, 2013; George *et al.*, 2014; McManus *et al.*, 2016). The results in this study indicates that the eye temperature of ewes increased as the THI of the surrounding environment increased, possibly mirroring the change in ambient conditions. Overall, eye temperature increased with 11.5% from the cooler morning sessions to the warmer afternoon sessions ($p < 0.05$). A study on hair sheep and cattle by George *et al.* (2014) revealed a high and positive correlation between the rectal temperature and eye temperature in ewes of hair breeds. The average eye temperatures of Santa Inês and Morada sheep amounted to 36.3°C in the morning (Seixas *et al.*, 2017), which was higher than the average eye temperature of 34.2°C recorded in this study. For the afternoon sessions, Seixas *et al.* (2017) reported an average eye temperature of 38.4°C in Santa Inês and Morada sheep, which is similar to the values reported in the current study for the Merino,

Meatmaster, and White Dorper breeds. Further research on eye temperature in sheep (or livestock animals in general) exposed to a variety of climatic conditions are required for analysis comparisons.

7.5.5 Between ewe variation and repeatability

The only trait that was moderately repeatable throughout, was rectal temperature (Table 7.8). Although the repeatability estimate was reduced to below 0.20 when the ewe x session term was added to the model, it was still significant. Current-flock gains may thus follow selection based on the ability of ewes to maintain their rectal temperature under heat stress conditions. It needs to be cautioned that repeatability of rectal temperature over a longer period than that used in the current study needs to be determined to objectively assess the potential of rectal temperature for selection in the current flock. If rectal temperature is repeatable over the longer term, part of the between-ewe variation may indeed be transferable to future generations. No comparable results were found in the literature on sheep. However, this argument is supported by a significant heritability estimate for rectal temperature in the report of Brien *et al.* (2010).

The repeatability of respiration rate was low and all the between-ewe variation was repartitioned to the ewe x session term (Table 7.8). It is not clear how short-term ewe effects, as well as reranking effects may be used for selection in the current flock. This topic warrants further research. We were also unable to find any comparable values in the literature at our disposal.

7.6 Conclusion

This study identified rectal temperature and possibly respiration rate as meaningful heat stress indicator traits that helped quantifying the response of sheep to elevated temperatures. The role of spot temperature and eye temperature is less obvious, unless refined by further studies. We were unable to determine the inflection points of these traits for the respective sheep breeds in this study owing to the discrete nature of morning and noon/afternoon recordings and the formation of clusters according to session. Thus, further research is mandatory to determine the precise inflection point when the ewes start to manifest heat stress symptoms. This can be achieved by accumulating more data on rectal temperature and respiration rate over a continuous range of THI-values. To achieve this, records of days with a lower maximum temperature across longer timespans are needed.

Significant breed effects pointed towards the hair breeds (Dorper and Meatmaster) being better equipped to deal with elevated THI-values in comparison to Merinos. Further research could target variation among sheep breeds in their physiological adaptive mechanisms in response to heat stress. Breeds specific trends, as well as the repeatability of the best proxy trait for core temperature, i.e., rectal temperature, may underly specific adaptive physiological mechanisms to respond to adverse environmental conditions. A better understanding of this and other heat stress indicator traits which emerge in this pathway may serve as useful indicators of animal welfare in the changing climatic situation. These arguments vividly indicate the importance of studying in detail the physiological

adaptive mechanisms to elevated THI-levels in different sheep breeds. It is seen as essential to extend the repeatability studies over longer trial periods to make it applicable to a current-flock selection scenario. Efforts such as this can aid the farming community to identify specific sheep breeds which can adapt to specific agroclimatic zones, thereby helping to ensure climate resilient sheep production. Refinement of the research presented here are needed to ensure sustainable sheep production in the Northern Cape and Western Cape areas of South Africa.

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Chapter 8: General Discussion and Conclusion

8.1 Conclusions and Recommendations

This thesis reported on two divergent aspects of the impact of climate variability on the wellbeing of sheep. In both instances, aspects of sheep behaviour were used to assess the effects of either cold and inclement weather conditions on lambs separated according to selection for number of lambs weaned per ewe mated (NLW; Chapter 4), or the impact of increased heat loads on common heat stress indicators in ewes originating from widely variable local sheep breeds (Chapters 5 to 7). These issues are dealt with separately below:

8.1.1 The impact of cold stress on neonatal lambs

This study indicated that cold stress, as expressed by a cold stress gradient (CSG) depending on temperature, windspeed and rainfall, had a profound effect on the standing and suckling behaviour of lambs (see Chapter 4). Contrary to the expectation, lambs born under cold stress conditions were somewhat faster (an 18% increase from a CSG-value of 800 $\text{kJm}^{-2}\text{h}^{-1}$ a value of 1200 $\text{kJm}^{-2}\text{h}^{-1}$) to progress from birth to standing for > 10 seconds than those born under milder conditions. In contrast, the latency of standing to first suckling for > 10 seconds was markedly impaired by 76% under cold stress conditions (1200 $\text{kJm}^{-2}\text{h}^{-1}$) compared to lambs born under mild conditions (800 $\text{kJm}^{-2}\text{h}^{-1}$). It was reasoned that cold stress could have prompted lambs to stand early to obtain sustenance to replenish dwindling resources leading to a reduced latency from birth to first standing. However, being able to do this, higher levels of cold stress were possibly associated with an element of lethargy because of a loss of energy reserves, leading to an inability to suckle soon after standing.

Genetic differences were also noted between the line selected for an increased NLW (H-Line) and one selected for a reduced NLW (L-Line). The most important of these was that H-Line lambs showed a shorted latency from standing to suckling. The absence of an interaction of selection line with a linear regression on the CSG implies that this line difference persisted even at higher levels of cold stress. Moreover, this advantage of H-Line lambs persisted when the better behaviour of H-Line ewes to facilitate their lambs' first suckling attempts was accounted for by analysis of covariance. Genetic parameter estimation also indicated that all aspects of neonatal lamb behaviour were under some genetic control. This was more evident in the latency from birth to suckling, whereas between lamb variation was mostly partitioned to the permanent environment provided by the ewe for the latency from standing to suckling. This is understandable if it is considered that maternal cooperation with the first suckling attempts of lambs were shown to be under genetic control when assessed as a trait of the ewe (see Cloete et al., 2021).

It is clear that further research is required to better understand the impact of severe weather events on the wellbeing of lambs. In this case, the latency from standing to suckling was used as a proxy trait for lamb survival. The line difference in latency from standing to suckling is supported by

divergent genetic trends in the same resource flock for lamb survival (Nel et al., 2021a) as well as a better ability to control rectal temperature in H-Line lambs (Nel et al., 2021b). There are many databases containing lamb survival as a trait in Western Cape ovine resource flocks. The linkage of these databases to readily available weather station data for the farms housing the lambs will provide a valuable resource to gain a better understanding of the impact of an increased CSG on the survival of lambs. Such studies can be conducted at minimal direct costs by the merging of resources that are already available and should be prioritised.

Further, since the influence of increased CSG-values on the latency from birth to standing was unexpected, there is scope for refining analyses to better understand this phenomenon. In this case, the neonatal behaviour data of this study can be merged with that of Cloete et al. (2002) reporting the behaviour of Dormer and SA Mutton Merino lambs, with added climate data in terms of the CSG and the Thermo-humidity Index (THI). This will provide a combined database where the separate and/or combined effects of cold stress and heat stress on neonatal lamb behaviour could be studied in more detail.

8.1.2 The reaction of mature ewes to elevated levels of heat and an increased THI

Exposure of ewes to higher levels of heat (thus an increase in THI-values) were assessed by recording data for common heat stress indicator traits on sheep flocks with a different breed composition in Chapters 5 to 7. Differences in the THI-levels animals were exposed to were obtained by recording data early in the morning under cooler conditions and during the noon/afternoon under hotter conditions. Recording took place on days with an increased forecast temperature exceeding 30°C according to available weather forecast services to ensure a reasonable spread of ambient conditions. Recording generally took place over 2 to 3 days on two locations.

All the indicator traits, namely: ¹rectal temperature (Chapters 6 and 7); ²respiration rate (Chapters 5 to 7); ³eye temperature from thermographic images (Chapters 5 and 7) and ⁴spot temperature (Chapters 6 and 7) adequately reflected the increased THI-levels during the afternoon. All traits were thus accurate descriptors of the ambient conditions on recording. Of the traits, only rectal temperature and respiration as well as spot temperature and eye temperature to a lesser extent were able to consistently differentiate among sheep breeds. Overall, ewes from breeds with indigenous content were able to withstand heat better than ewes originating from temperate breeds, as reflected by respiration rate (Chapters 5 to 7) and rectal temperature (Chapters 6 and 7). Eye temperature and spot temperature appeared to be less effective in differentiating between breeds and may be subject to bias stemming from face colour and cover as the spot temperature was determined on the face of ewes (Chapters 6 and 7). These results were supported with an improved ability to partition the effect of individual ewes from the environmental variance to obtain repeatability estimates for rectal temperature (Chapters 6 and 7) and respiration rate (Chapters 5 to 7). Although spot temperature was also subject to repeatable variation in Chapter 6, this may stem from extrinsic

factors such as face colour within breeds. Eye temperatures were inadequate to differentiate between individual ewes in Chapters 5 and 7.

The observed repeatability estimates were variable across sessions, ranging from 0.16 to 0.24 for rectal temperature, 0.16 for spot temperature and 0.08 to 0.26 for respiration rate. Corresponding ewe x session variance ratios were 0.19, 0.19 to 0.28 and 0.17 to 0.28, 0.19. Eye temperature was consistently not affected by the between-ewe variance component. The repeatability estimates of spot temperature (Chapter 6) and respiration rate (Chapters 5 to 7) as well rectal temperature to a lesser extent (Chapter 7), were complicated by an interaction of ewe x recording session (morning or noon/afternoon). Between-ewe variance components commonly went to the boundary of parameter space when this effect was fitted for spot temperature in Chapter 6 and respiration rate in Chapters 6 and 7. The only trait that was consistently repeatable at around 0.20 was rectal temperature (Chapters 6 and 7). When repeatability is seen as a maximum for heritability (in the absence of animal permanent environmental effects), it follows that rectal temperature is the only trait amenable to selection for within breed current-flock gains. Having said this, there is also a need for some serious effort in understanding the reranking of ewes within breeds between the cooler mornings and the hotter noon/afternoon recordings. Alternative methods to record traits that could serve as non-invasive heat stress indicators should also receive attention. One such trait could be respiration movements or heart rate as derived from a pedometer applied to the chest of the subject to be tested (Brito *et al.*, 2020; Silva *et al.*, 2022). As methods of recording grazing, ruminating and resting behaviour of sheep evolve, several traits not yet considered may also play a role in future. Research to develop such traits, or to adapt those already applied elsewhere to local conditions, should also have a high priority.

It is important to note that all the studies involving the response of ewes were conducted over the short term (2 to 3 days) and mostly resulted in discrete clusters of data points according to session (morning or noon/afternoon). Therefore, it is also recommended that future studies should consider repeatability of rectal temperature and respiration rate over a longer term of weeks, months or even years. If these traits are repeatable across a longer term, it would lead substance to their usage for achieving current-flock gains. Also, further studies should record data across milder days, to ensure that future data sets will allow the estimation of breed-specific inflection points at specific THI-levels. Such data will play an important role in determining the suitability of specific breeds to specific geographical regions when linking to readily available Geographical Information System (GIS) data (see McManus *et al.*, 2016). Such a study will be of great value to inform the farming community of the suitability of specific sheep breeds to the region they are farmed in. Once established, it will also be easy to update GIS maps to reflect the impact of different climate change scenarios.

It needs to be conceded that this study only reported short-term repeatability coefficients. Ideally, research should strive to obtain data suitable for the estimation of genetic (co)variances among heat stress indicator traits as well as genetic correlations with other traits of economic importance. This

can be done either on ewes with the intention of achieving current-flock gains or in lambs during studies that refine the present knowledge of the impact of cold stress or heat stress in lambs. In this respect, it is appropriate to note that Brien et al. (2010) reported that rectal temperature of lambs was lowly heritable. It may also be possible to apply a reaction norm approach to determine whether the heritability of a specific heat stress indicator stay similar across either a CSG or a THI trajectory (Brito *et al.*, 2020).

Finally, this study only scratched the surface of quantifying responses of ewes and lambs to respectively heat stress and cold stress. The recommendations provided should lay the foundation for further research. These envisaged studies could firstly use existing data merged with climate information, whereas there is also scope for secondly designing new studies by following suggestions provided.

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