

https://dspace.org/

# Comparison of febrile responses, thermoregulation and skin morphology in the local Kolbroek, Windsnyer and exotic Large White breeds of pigs in South Africa

**Moyo, Davison**

**2017**

http://hdl.handle.net/10539/23088

Moyo, Davison (2017) Comparison of febrile responses, thermoregulation and skin morphology in the local kolbroek, windsnyer and exotic large white breeds of pigs in South Africa, University of the Witwatersrand, Johannesburg, <http://hdl.handle.net/10539/23088>

Downloaded from a DSpace repository. https://dspace.org/

# COMPARISON OF FEBRILE RESPONSES, THERMOREGULATION AND SKIN MORPHOLOGY IN THE LOCAL KOLBROEK, WINDSNYER AND EXOTIC LARGE WHITE BREEDS OF PIGS IN SOUTH AFRICA

Davison Moyo

A Thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Doctor of Philosophy

Johannesburg, 2017

**DECLARATION**

I, **Davison Moyo**, declare that this Thesis is my own original work except where acknowledged in the text. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

……..

Davison Moyo

Signed on the ……..……. day of ……..……. 2017

## DEDICATION

In memory of my father

Ndarubva George Nhema

1938 - 2000

# PRESENTATIONS

1. **Moyo, D.**, Erlwanger, K. H., Harden, L. M. and Hetem R. S. (2014) Febrile and behavioral responses in Kolbroek, Windsnyer and Large White experimentally infected with lipopolysaccharide, polyinosinic: polycytidylic and *Staphylococcus aureus* cell walls. Poster Presentation at the 6th Cross Faculty-Graduate Symposium, University of the Witwatersrand, Johannesburg on the 29th – 30th October 2014, Johannesburg, South Africa

2. **Moyo, D.**, Erlwanger, K. H. and Hetem R. S. (2015) Comparison of the physiological responses of local Windsnyer and Kolbroek pigs and the exotic Large White pigs to cold and hot temperatures. Poster Presentation at the 43rd Physiological Society of Southern Africa Conference, Jointly hoisted by the University of the Witwatersrand and the University of Johannesburg, on the 6th – 9th September 2015, Parys, Free State, South Africa

# PUBLICATIONS

## Manuscript submitted

1. **Moyo, D.**, Gomes, M. and Erlwanger, K. H. (2016). Comparison of the skin histological characteristics of the Windsnyer, Kolbroek and Large White pigs. *Submitted to the journal: Animal* (**Manuscript Reference Number**: **ANIMAL-S-16-00888**)

## Manuscripts in preparation

*1.* **Moyo, D.**, Erlwanger, K. H., Hetem, R. S. (2017 Large White pigs display more severe sickness behaviours than local pig breeds (Kolbroek and Windsnyer), despite similar febrile responses, to viral and bacterial mimetics*. Target journal: Physiology and Behavior*

*2.* **Moyo, D.**, Erlwanger, K. H., Hetem, R. S. (2017) Effects of exposure to cold and high ambient temperatures, high relative humidity and water deprivation on the thermoregulatory and physiological responses of the local Kolbroek and Windsnyer and the exotic Large White pigs. *Target journal: Journal of Thermal Biology*

## ABSTRACT

Smallholder agriculture may be particularly vulnerable to the increased temperatures, reduced water availability and increased risk of disease anticipated under future climate change scenarios. Pigs may be particularly sensitive to the increased heat stress as they do not sweat. Local pigs are said to be better adapted than the exotic breeds and therefore may be better able to withstand some of the negative impacts of climate change however, there is not much scientific support for that claim. I therefore compared the febrile and thermoregulatory responses and skin characteristics of local (Kolbroek, Windsnyer) and exotic (Large White) pigs.

Pigs were implanted with intra-abdominal tags for measuring core body temperature and activity. Terminally, skin samples were collected from the interscapular, lateral thoraco-abdominal and ventral abdominal regions. Six week old boars of the (Kolbroek (5.4 ± 1.4 kg; n = 8), Windsnyer (8.1 ± 1.6 kg; n = 8) and Large White (6.0 ± 1.5 kg; n = 8) were used to determine the febrile responses and sickness behaviours. The pigs were injected intravenously with polyinosinic acid: polycytidylic acid (poly I:C) (0.5 mg/kg); lipopolysaccharide (LPS) (2 µg/kg) and *Staphylococcus aureus* (*S. aureus*) (1.7 x 1010 cell walls/kg) or saline (control). The exotic Large White pigs had a significantly greater ($F_{2,20}$ = 13.70; $P$ = 0.0003) Thermal Response Index (TRI) after receiving poly I:C but a lower ($F_{2,21}$ = 6.22; $P$ = 0.009) TRI in response to LPS than the local pigs. All pigs displayed anorexia and lethargy in response to poly I:C, but only the Large White and Windsnyer displayed anorexia and lethargy to LPS. Febrile temperature responses were similar between the breeds of pigs after injecting *S. aureu*s. The Large White and Kolbroek were more sensitive to *S. aureus* and had severe clinical signs when compared to the Windsnyer pigs. Following LPS and *S. aureus* administration, the Large White and Kolbroek pigs showed no body mass reduction 22 h after pyrogen administration unlike the Windsnyer which lost body mass. There were slight differences in febrile responses between the breeds; however the Large White pigs had more severe clinical signs than the local breeds of pigs after injection of the bacterial mimetics.

Four month old boars of Kolbroek (n=6; 40 ± 1.3 kg); Windsnyer (n=7; 46 ± 7.7 kg) and Large White (n=7; 60 ± 1.3 kg) pigs (Sus scrofa domesticus) were used to determine thermoregulatory responses. The pigs were exposed to 5°C (92% RH), thermoneutral (20°C) with 40% RH, 30°C with drinking water with 40% RH, 30°C with high relative humidity (60%) and 30°C with 48 h water deprivation except for the cold and thermoneutral treatments where pigs were kept for 48 h. The pigs showed remarkably similar patterns in core body temperature under all the treatments. At 5°C, local pigs employed primitive behaviours to maintain core body temperature while the exotic pigs increased activity. At 30°C compared to TNZ all pigs reduced physical activity, however, the Large White and Kolbroek had higher change in respiratory rates ($F_{4,68} = 14.96$; $P < 0.0001$) than the Windsnyer which maintained constant respiratory rates when compared to TNZ. On exposure to 30°C with 48 h water deprivation, the local breeds conserved their plasma volume unlike the Large White. The lower respiratory rates in the Windsnyer pigs may reflect their being less dependent on panting than the other breeds. Their skin histology provides support for that hypothesis as they had large ($F_{2,13} = 52.48$; $P < 0.0001$) and more superficial ($F_{2,13} = 125.60$; $P < 0.0001$) sweat glands, thin total skin layer, thinner hypodermis than the Large White pigs and Kolbroek pigs. The skin of the Windsnyer also had more melanin visible than the Kolbroek whilst the Large White had none.

Although the differences between the breeds were subtle, the Windsnyer pigs had some physiological, behavioural and morphological traits that might make them more adaptable to the changing environmental conditions than the Kolbroek and Large White pigs.

## ACKNOWLEDGEMENTS

First and foremost, I would like to thank my supervisors Associate Professor Kennedy H. Erlwanger and Dr. Robyn S. Hetem for providing the direction for my thesis. Without your support, I never would have completed let alone embarked on this study. Associate Professor K. H. Erlwanger, your personal and professional advice, patience and humour also made my post-graduate experience more rewarding. Not forgetting the Brain Function Research Group that gave me an opportunity to be part of this group of world class researchers. Dr. Lois Harden, I appreciate your willingness to become part of my committee at a later stage and the advice you provided in regards to the fever experiment. I would like to give a huge thank you to all Central Animal Services staff members who looked after my animals and were always willing to help me with any aspect of my trial and whose patience was greatly appreciated. Mr. Zipho Zwane your help with feeding and weighing the animals during the trials is greatly appreciated. To the experienced laboratory technician, Ms. Margaret Badenhorst thank you for the many hours of your time you put in preparing my pyrogens, that worked so well and gave the best responses. I would also like to thank and Dr. Denise Lowrie, of the National Health Laboratory Service (NHLS) Flow Cytometry Laboratory at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) for assisting with counting of the *Staphylococcus aureus* cell walls. Prof Paul Manger's role in assisting with the use of Image J software and Mrs. Hassan Ali's technical assistance with the processing of slides and photographing is also greatly appreciated. To Dr. Eliton Chivandi, I will never forget all the laughs and memories we have shared. I could not have survived post-graduate life without your friendship and advice. To all of my other post-graduate student friends, thank you for making these last four years at the Medical School the best of my life. Dr. Ian Murray your comments on an earlier version of this manuscript are greatly appreciated. The financial support of the Faculty Research Committee, University of the Witwatersrand, The National Research Foundation and, the Oppenheimer Memorial Trust during my second and third years of my study is acknowledged. Finally, thank you to my family and children for your unwavering support during these challenging and exciting post-graduate years. I know you don't completely understand the physiology and science behind my research but the fact that you stood behind my goals and accomplishments no matter what means the world to me.

## TABLE OF CONTENTS

**DECLARATION** ..... **i**

**DEDICATION** ..... **ii**

**PRESENTATIONS** ..... **iii**

**PUBLICATIONS** ..... **iv**

**ABSTRACT** ..... **v**

**ACKNOWLEDGEMENTS** ..... **vii**

**TABLE OF CONTENTS** ..... **viii**

**LIST OF FIGURES** ..... **xiii**

**LIST OF TABLES** ..... **xv**

**NOMENCLATURE** ..... **xvi**

**CHAPTER 1: INTRODUCTION AND JUSTIFICATION** ..... **1**

1.1 Introduction ..... 1

1.2 Hypotheses ..... 2

1.3 Thesis outline ..... 3

**CHAPTER 2: LITERATURE REVIEW** ..... **6**

2.1 Livestock industry ..... 6

2.2 Pork production – global picture ..... 7

2.3 Pork production – South Africa ..... 8

2.4 Types of breeds ..... 9

2.4.1 Large White ..... 9

2.4.2 Windsnyer ..... 11

2.4.3 Kolbroek ..... 12

2.5 Threats to local pigs ..... 13
2.5.1 Lack of political support & political instability ..... 14
2.5.2 Climate change ..... 15
2.5.3 Contribution of livestock to climate change ..... 17
2.6 Impact of heat stress on livestock ..... 19
2.6.1 Production ..... 19
2.6.2 Thermoregulation ..... 20
2.7 Skin ..... 23
2.8 Disease ..... 26
2.8.1 Fever and sickness behaviours ..... 27
2.8.2 Pyrogenic mimetics ..... 29
2.9 Aims of the research ..... 32
2.10 Study Objectives ..... 33
**CHAPTER 3: OVERVIEW OF THE METHODS ..... 35**
3.1 Fever experiment ..... 35
3.2 Thermoregulation experiment ..... 36
3.3 Skin experiment ..... 37
**CHAPTER 4: CHARACTERISATION OF THE FEBRILE AND SICKNESS BEHAVIOURAL RESPONSES INDUCED BY BACTERIAL AND VIRAL MIMETICS IN THE LOCAL KOLBROEK, WINDSNYER AND EXOTIC LARGE WHITE PIGS ..... 39**
4.1 Introduction ..... 39
4.2 Aims ..... 40
4.3 Hypothesis ..... 41
*4.4* Materials and Methods ..... 41
4.4.1 Animals and housing ..... 41
4.4.2 Surgery ..... 42

4.4.3 Core body temperature ..... 43
4.4.4 Activity ..... 44
4.4.5 Body mass and voluntary feed intake ..... 45
4.4.6 Pyrogens ..... 45
4.4.7 Experimental procedure ..... 46
4.5 Data Analysis ..... 47
4.5.1 Febrile responses ..... 47
4.5.2 Sickness behaviours ..... 48
4.6 Results ..... 50
4.6.1 Febrile response ..... 50
4.6.2 Sickness behaviours ..... 55
4.7 Discussion ..... 64
4.8 Conclusion ..... 71

**CHAPTER 5: EVALUATION OF THERMOREGULATORY AND BEHAVIOURAL RESPONSES OF THE LOCAL KOLBROEK, WINDSNYER AND EXOTIC LARGE WHITE PIGS DURING EXPOSURE TO COLD AND HOT AMBIENT TEMPERATURES, HIGH RELATIVE HUMIDITY AND WATER DEPRIVATION ..... 73**

5.1 Introduction ..... 73
5.2 Aims ..... 74
5.3 Hypothesis ..... 75
5.4 Materials and Methods ..... 75
5.4.1 Animals and housing ..... 75
5.4.2 Surgery ..... 76
5.4.3 Core body temperature ..... 76
5.4.4 Activity ..... 76
5.5 Experimental procedures ..... 76

5.5.1 Measurement of haematocrit, plasma osmolality and water intake post high temperature and water deprivation ..... 78
5.5.2 Respiratory rate and behavioural observations ..... 79
5.5.3 Data Analysis ..... 79
5.6 Results ..... 82
5.6.1 Core body temperature rhythm ..... 82
5.6.2 Activity ..... 88
5.6.3 Respiratory rates ..... 90
5.6.4 Plasma osmolality, body mass and water intake ..... 91
5.6.5 Behavioural responses ..... 94
5.7 Discussion ..... 94
5.8 Conclusion ..... 101
**CHAPTER 6: COMPARISON OF THE HISTOLOGICAL CHARACTERISTICS OF THE SKIN OF THE WINDSNYER, KOLBROEK AND LARGE WHITE PIGS ..... 103**
6.1 Introduction ..... 103
6.2 Material and methods ..... 105
6.2.1 Animals ..... 106
6.2.2 Skin sample collection and processing for histology ..... 107
6.2.3 Data analyses ..... 109
6.3 Results ..... 110
6.3.1 Epidermis ..... 110
6.3.2 Dermis ..... 112
6.3.3 Hypodermis ..... 113
6.3.4 Sweat glands ..... 119
6.3.5 Melanin ..... 120
6.4 Discussion ..... 121

6.4.1 Epidermis ..... 121
6.4.2 Dermis and hypodermis ..... 122
6.4.3 Sweat glands ..... 123
6.4.4 Melanin ..... 124
6.5 Conclusion ..... 126
**CHAPTER 7: CONCLUSIONS AND FUTURE DIRECTION ..... 127**
7.1 Fever ..... 127
7.2 Thermoregulation ..... 129
7.3 Future Research ..... 131
7.3.1 Spread of diseases ..... 131
7.3.2 Exposure to high temperatures ..... 132
**CHAPTER 8: LITERATURE CITED ..... 134**
**CHAPTER 9: APPENDICES ..... 168**
9.1 Ethical Clearance certificate ..... 169
9.2 Modifications and Extensions ..... 170
9.3 DATA USED FOR DRAWING GRAPHS THAT ARE APPEARING IN CHAPTERS 4 AND 5. ..... 172

## LIST OF FIGURES

**Figure 2-1**: A mature Large White boar .....10

**Figure 2-2**: A mature Windsnyer boar.....11

**Figure 2-3**: A mature Kolbroek boar .....12

**Figure 2-4**: Breakdown of livestock sector greenhouse gases emissions by source. ....18

**Figure 4-1**: Profile of core body temperature of three breeds of pigs following the intravenous injection of polyinosinic acid: polycytidylic acid (poly I:C; 0.5 mg/kg); lipopolysaccharide (LPS; 2 µg/kg) and *Staphylococcus aureus* (*S. aureus;* 1.7 ± 0.1 x $10^{10}$ cell walls/kg) and saline... .....53

**Figure 4-2:** Changes in activity in the three breeds in pigs after injecting them with lipopolysaccharide, polycytidylic acid: polyinosinic acid, *Staphylococcus aureus* and saline.....57

**Figure 4-3**: Change in voluntary feed intake in the three breeds in pigs after injecting them with lipopolysaccharide, polycytidylic acid: polyinosinic acid, *Staphylococcus aureus* and saline. .....60

**Figure 4-4**: Change in body mass in the three breeds in pigs after injecting them with lipopolysaccharide, polycytidylic acid: polyinosinic acid, *Staphylococcus aureus* and saline.....63

**Figure 5-1:** Core body temperature rhythm of the three breeds of pigs (left panels) under the five different experimental treatments (right panels.....86

**Figure 5-2**: The 8 h mean (panel A), maximum (panel B), minimum (panel C), and amplitude (panel D) of core temperature of three breeds of pigs during exposure to the five experimental treatments on day 4.....88

**Figure 5-3**: Change in activity (panel A) and respiratory rates (panel B) of the three breeds of pigs following exposure to the five experimental treatments. .....90

**Figure 5-4**: Comparison of the percentage change in body mass (%) (panel A) and water intake (as a % of body mass) (panel B) following 48h of water deprivation and exposure to high temperatures (30ºC) for three breeds of pigs. .....94

**Figure 6-1:** Representative vertical skin sections from the dorsal interscapular region, lateral thoraco-abdominal region and ventral abdominal region of Windsnyer, Kolbroek and Large White pigs. The three sections from the different body regions of the three breeds of pigs show the epidermis (Ep), dermis (Der) and stratum corneum (SC) and rete pegs (RP). .....115

**Figure 6-2:** Representative vertical skin sections from the dorsal interscapular region, lateral thoraco-abdominal region and ventral abdominal region of Windsnyer, Kolbroek and Large White pigs showing the relative sizes of the sweat glands in the three body regions. .....117

**Figure 6-3**: Representative vertical skin sections from the dorsal interscapular region, lateral thoraco-abdominal region and ventral abdominal region of Windsnyer, Kolbroek and Large White pigs. The figure shows a large amount of melanin pigments in the basal layer of the epidermis in the Windsnyer pigs, very little in the interscapular region in the Kolbroek pigs and no melanin pigments in Large White pigs .....118

# LIST OF TABLES

**Table 2-1**: Tonnage and percentage contribution of each source of meat consumed in the world in 2006 and 2015 .....7

**Table 2-2**: Productive efficiency of animal products.....19

**Table 4-1:** Indices of the febrile responses in three different breeds of pigs injected intravenously with polyinosinic acid: polycytidylic acid (poly I:C; 0.5 mg/kg); lipopolysaccharide (LPS; 2 µg/kg) and *Staphylococcus aureus* (*S. aureus;* 1.7 x $10^{10}$ cell walls/kg). .....54

**Table 4-2**: Behavioural scores of Windsnyer, Kolbroek and Large White pigs during an 8 h period after intravenous injection with polyinosinic acid: polycytidylic acid (poly I:C) (0.5 mg/kg); lipopolysaccharide (LPS) (2 µg/kg) and *Staphylococcus aureus* (*S. aureus*) (1.7 x $10^{10}$ cell walls/kg).....64

**Table 5-1:** Mean ± SD of plasma osmolality, osmolality ratio, haematocrit and plasma volume ratio in the three breeds of pigs before and after exposure to the 48 h water deprivation at 30ºC.....92

**Table 6-1**: Effect of the breed on the thickness of the epidermis, dermis and hypodermis of the local Kolbroek and Windsnyer pigs and the exotic Large White pigs. .....114

**Table 6-2**: A comparison of the sweat gland characteristics of the local Kolbroek and Windsnyer pigs and the exotic Large White pigs.. .....120

**Table 6-3**: A comparison of the presence of melanin in the basal layers of the skins of the local Kolbroek and Windsnyer pigs and the exotic Large White pigs. .....121

## NOMENCLATURE

| | | |
|---|---|---|
| AESC | – | Animal Ethics Screening Committee |
| ANOVA | – | Analysis of variance |
| AUSD | – | Australian Dollar |
| CAS | – | Central Animal Services |
| $CO_2$-eq | – | Carbon dioxide equivalent |
| CSFV | – | Classical Swine Fever Virus |
| DAFF | – | Department of Agriculture, Forestry and Fisheries |
| ECT | – | Evaporative Critical Temperature |
| FAO | – | Food and Agriculture Organization of the United Nations |
| FAS | – | Foreign Agricultural Services |
| FCR | – | Food Conversion Ratio |
| G | – | Gauge |
| GCIS | – | Government Communication and Information Systems |
| GDP | – | Gross Domestic Product |
| GHGs | – | Greenhouse gases |
| GPA | – | Global Plan of Action |
| Gt | – | Gigatonnes |
| IL | – | Interleukin |
| i.m. | – | Intramuscular |
| IPCC | – | Intergovernmental Panel on Climate Change |
| IUPS | – | International Union of Physiological Sciences |
| i.v. | – | Intravenous |
| LCT | – | Lower Critical temperature |
| LPS | – | Lipopolysaccharide |
| M&Es | – | Modifications and Extensions |
| MDP | – | Muramyl dipeptide |
| mmt | – | Million metric tonnes |
| NIPCC | – | Non-Governmental International Panel on Climate Change |
| NRC | – | National Research Council |
| Poly I:C | – | Polycytidylic acid: polyinosinic acid |

| | | |
|---|---|---|
| RH | – | Relative Humidity |
| SADC | – | Southern African Development Community |
| *S. aureus* | – | *Staphylococcus aureus* |
| SD | – | Standard Deviation |
| TEWL | – | Transepidermal Water Loss |
| TRI | – | Thermal response index |
| TNF-α | – | Tumour Necrosis Factor - α |
| TNZ | – | Thermoneutral zone |
| $T_b$ | – | Core body temperature |
| Tg | – | Teragrams |
| UCT | – | Upper Critical temperature |
| µg | – | Micrograms ($10^{-6}$ g) |
| UK | – | United Kingdom |
| USA | – | United States of America |
| USD | – | United States Dollar |
| USDA | – | United States Department of Agriculture |
| WHO | – | World Health Organisation |
| WMO | – | World Meteorological Organisation |

# CHAPTER 1: INTRODUCTION AND JUSTIFICATION

## 1.1 Introduction

Pigs are an important resource in the livelihood of smallholder farmers. However pigs are particularly vulnerable to high temperatures and water shortages due to their inability to sweat (Ingram, 1967) and generally thick layer of subcutaneous adipose tissue (Sokolov, 1982). In subsistence-oriented production systems farmers tend to use local pig breeds which they claim are hardy. The local breeds are generally reported to be heat tolerant, resistant to endemic diseases, survive under strained water availability and reproduce under low quality nutrition (Food and Agriculture Organisation (FAO), 1998) better than the commercially available exotic breeds.

In South Africa the main pig breeds used in subsistence farming are the Kolbroek and Windsnyer. There are unsubstantiated claims that Windsnyer and Kolbroek pigs are hardier and better adapted to their hot and dry environment than the exotic pig breeds used in intensive production piggeries in the same region. However, a literature search has indicated very little scientific data to support the anecdotal accounts that the physiological adaptability of these local pigs enables them to survive under harsher environmental conditions than the Large White breed. The research conducted to date on the local pigs has focused on traits of economic importance such as growth and carcass composition (Chimonyo et al., 2010, Kanengoni et al., 2004), birth mass, litter size and litter mass (Chimonyo et al., 2006); nutrition (Mushandu et al., 2005); herd dynamics (Chiduwa et al., 2008), internal parasites (Zanga et al., 2003) and ability to use high fibre diets (Kanengoni et al., 2002). Only one paper (Madzimure et al., 2012), has investigated anatomical adaptations and how these local pigs might cope with hot and dry conditions predicted under future climate change. The same study also measured rectal and skin temperature of Windsnyer pigs. Many researchers argue that for a complete understanding of the effects of climate change on organisms, investigations should focus on how physiology, behaviour and morphology could be used to buffer the impact of climate change (Bale et al., 2002, Helmuth et al., 2005, Portner and Knust, 2007).

Climate change, driven by an increase in heat-trapping gases emitted from anthropogenic activities, is envisaged to result in high environmental temperatures, increased frequency and duration of heat waves, and altered rainfall patterns [Intergovernmental Panel on Climate Change (IPCC, 2013)]. These changes are likely to cause heat stress, water shortages and the emergence of new and the re-emergence of old viral and bacterial diseases which may put additional pressures on livestock farming practices. In addition to climate change, local pigs are also under threat from political conflicts, disease outbreaks, the changing agricultural production systems and the lack of policies that favour the production of local pig breeds. Globally, local breeds have been lost and many more are on the verge of extinction thereby threatening pig genetic diversity (Piling and Hoffman, 2011).

Maintaining these breeds as an integral part of the communities will guarantee food security, sustainable and viable agricultural production in the future as climates and environments change. Understanding the physiological, behavioural and morphological traits is therefore important in order to describe and characterise the responses of local pigs to environmental and pathogenic challenges and help in the conservation, promotion and sustainable utilisation of a vital genetic resource.

The purpose of this thesis was to investigate whether two local breeds of pig have physiological, behavioural and morphological (skin) traits that differ to those in an exotic breed of pig that may allow the former to tolerate the effects of increased environmental temperatures, water shortages and disease burdens predicted to occur under climate change.

## 1.2 Hypotheses

This PhD thesis has three major hypotheses which are as follows:

***First Hypothesis:*** The local Kolbroek and Windsnyer pigs will show a dampened febrile response and sickness behaviours (decreased physical activity, decreased voluntary

feed intake and reduction in body mass) compared to the exotic Large White pigs when injected with bacterial and viral mimetics.

***Second Hypothesis:*** The local Kolbroek and Windsnyer pigs possess physiological and behavioural responses that will allow them to buffer the effects of high and low temperatures and deprivation of drinking water better than the exotic Large White pigs

***Third Hypothesis:*** The local Kolbroek and Windsnyer pigs have skin traits that will allow them to buffer the high temperatures better than the exotic Large White pigs.

## 1.3 Thesis outline

This thesis has a total of eight chapters including an introduction, literature review, overview of the methodology, three complimentary components, conclusions and recommendations and references. The three complementary components attempt to answer the research questions/hypotheses stated earlier. The complimentary chapters each have an introduction, materials and methods, results, discussion and conclusion sections. The complimentary chapters will be developed into manuscripts that will be submitted to peer reviewed journals. A brief description of each chapter of the thesis is provided below:

**Chapter 1**: This chapter provides a general introduction and motivation for conducting the study. It also poses major questions that the research intends to address.

**Chapter 2**: This chapter provides an overview of the livestock industry with an emphasis on pig production. It also provides background information and a review of pertinent literature on the threats and risks facing the local pig genetics, causes and effects of climate change, effects of heat stress on the productivity of livestock and the direct and indirect effects of diseases on livestock production with an emphasis on pigs.

**Chapter 3**: This chapter provides an overview of the methodology used in the experiments in each chapter.

**Chapter 4**: In this chapter I investigated the febrile and behavioural responses of pigs following injection of bacterial and viral mimetics. This study quantified and compared the febrile responses and sickness behaviours exhibited by three breeds of pigs upon being challenged with bacterial and viral mimetics. I recorded slight differences in febrile responses which were unlikely to be biologically significant. Although all pigs were generally similar in their responses to bacterial and viral mimetics, the Large White breed of pigs displayed more severe clinical signs than the local pigs after receiving the bacterial mimetics.

**Chapter 5**: In this chapter I investigated the effects of low and high temperatures, as well as high temperatures with 48 h water deprivation or high relative humidity using simulated conditions in a climate chamber. The key findings of this study were that at low temperatures, local pigs displayed primitive thermoregulatory behaviours to conserve body heat. Under the high temperatures the Windsnyer maintained constant respiratory rates while the Kolbroek and Large White pigs increased their respiratory rate. Also the local pigs showed remarkable ability to conserve body water when exposed to high temperatures with water deprivation while the Large White pig breed failed to protect their body water resulting in 13% increase in plasma osmolality. The Windsnyer pigs did not increase their respiratory rate and might have relied less on panting compared to the other breeds and could have lost heat through the skin when exposed to high temperatures. This interesting finding led to the study on the skin morphological traits which form the basis of Chapter 6.

**Chapter 6:** This chapter analyses the skin morphological traits to try and explain the differences in the responses to high temperatures between the Windsnyer, Kolbroek and the Large White pigs in Chapter 5. I analysed the differences in the thickness of the skin layers, size and depth of the sweat glands and the presence of the melanin. The major finding was that the Windsnyer breed of pig had the thinnest skin layers, larger sweat glands that were closer to the surface and more visible melanin than the Large White and Kolbroek pigs.

**Chapter 7**: This chapter gives an overall interpretation and integrates the findings in the three preceding chapters (Chapters 4-6). It also highlights the relationship of the key findings under each objective. Remarks are made on the overall significance and contribution of the research to the current knowledge on the local pigs. Recommendations on possible future research are also highlighted.

**Chapter 8**: This section contains all the literature cited in the thesis.

**Chapter 9**: This section is the appendix and it contains the ethics clearance certificate, Modifications and Extensions (M&Es).

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Livestock industry

Worldwide the livestock sector contributes to national income, human nutrition, plays a significant role in rural livelihoods (e.g. manure, draught power), employment creation and poverty alleviation (Upton, 2004). Livestock production contributes about 30% to overall agricultural production in developing countries, compared to the 53% contribution it makes to the agricultural sector in industrialised countries (Boto and La Peccerella, 2012). Livestock production is an integral part of the livelihoods of 1.3 billion farmers worldwide (Thornton et al., 2007) of which it is estimated that 800 million are subsistence farmers. Three hundred million of the subsistence farmers are in Africa [African Union InterAfrican Bureau for Animal Resources (AU-IBAR, 2013)].

The world population of major livestock species in 2014 was estimated at 1.5 billion cattle (beef and dairy), 1.2 billion sheep, 1.0 billion goats, 986 million pigs, and 23.2 billion chickens (broilers and layers) [Food and Agriculture Organization Statistics Division (FAOSTAT, 2015)]. The livestock industry is growing. The growth is being driven by the rapidly increasing per capita consumption of livestock products due to human population growth, urbanisation and income growth in the developing countries (Trostle and Seeley, 2013). The growth of the livestock industry worldwide is propelled by the growth particularly of the poultry and pig sectors. Although cattle are the most numerous (based on world population mentioned previously), many of the cattle are used for milk production and a relatively small proportion of the cattle contribute to meat consumption. Similarly, sheep and goats outnumber pigs, yet they too have a lower contribution to the meat industry as they are kept for long periods to maximize fibre production, such as wool and mohair. Thus the pork and poultry industries are growing faster than the ruminant-based industries probably due to taste preferences thereby making pork and poultry the most widely eaten meats in the world. These two meats account for 73.1% of all meat consumed followed by beef (Table 2-1). In addition to the factors identified earlier driving the increase in livestock production in general, pork production is set to continue growing due to the reduced incidence of diseases affecting

the pig industries in Asian countries (which are in the main pork producing region in the world) and the growing favourable policy support from different governments (Best, 2012). The next sections provide a brief overview of the current state of pork production.

**Table 2-1**: Tonnage and percentage contribution of each source of meat consumed in the world in 2006 and 2015

| Meat | Meat consumed (million metric tonnes) | | Increase (%) in pork consumption between 2006 and 2015 | Percentage of meat (%) |
|---|---|---|---|---|
| | **2006** | **2015** | | **2015** |
| Pork | 107.0 | 118.8 | 11.0 | 37.6 |
| Poultry | 81.0 | 112.1 | 38.4 | 35.5 |
| Beef | 65.9 | 68.3 | 3.6 | 22.3 |
| Lamb | 13.3 | 14.0 | 5.3 | 4.6 |
| **Total** | **267.2** | **313.2** | | **100.0** |

Source: (FAO Food Outlook, 2015)

## 2.2 Pork production – global picture

The increase in the demand for and production of pork is attributed to several factors. Pork is regarded as a good dietary source of high-quality protein, vitamins, and minerals (Choe et al., 2015). The increased pork production may also be a reflection of the shorter production cycle than the ruminants (USDA, 2015). The shorter production cycle results in a higher yield and thereby pork production becomes more cost effective than ruminant production. Other factors that have led to the growth of the pork industry are the increased slaughter masses, reduced production costs and improved efficiency of the production process systems (Oliveira et al., 2015). China is the largest pork producer (51%) (FAS/USDA, 2015)] and the biggest consumer of pork (52%) in the world (FAOSTAT, 2015). This is attributed to the fact that in China, pork is the meat of cultural preference and accounts for at least 70% of all meat consumed (McOrist et al., 2011).

## 2.3 Pork production – South Africa

Compared to China, the South African pork industry is small and contributes about 0.18% to the total world pork output (South African Pork Producers Organisation, 2014). Although its contribution to the global industry is fairly small, the industry increased by an average of 4.5% per annum between 2006 and 2015 (Grimbeek et al., 2016) an increase which is higher than the annual world average of 1.1% that was recorded during the same period (FAOSTAT, 2015). Despite the increased production, the amount of pork being produced is not meeting demand (Davids et al., 2014). According to the FAS/USDA (2016), between 2006 and 2015, pork production in South Africa was lower than pork consumption making the country deficient in pork production. Unfortunately literature showing actual statistics on the shortfall in pork production was only available up to 2012. The shortfall in pork production in the period 2003 and 2012 ranged between 18 000 and 33 000 tonnes and that had to be supplemented by imports to meet demand [Department of Agriculture, Forestry and Fisheries, (DAFF, 2013)]. In 2012 alone South Africa imported 27 000 tonnes of pork to the value of ZAR601 million (equivalent to USD73.36 million[1]) (DAFF, 2013). The government has pledged support to increase pork production locally to cover the shortfall and reduce import costs.

Data (profit margins, pig numbers and pork tonnage) are readily available for commercial piggeries, but not for the subsistence farming yet 28% of the pigs in South Africa are farmed by smallholder famers (DAFF, 2013). According to the 2011 Household Agricultural Census, there were 100 589 subsistence farmers in South Africa who kept between 1 and 10 pigs per household (Statistics South Africa, 2013) mainly for domestic consumption.

South Africa is unique because of its relatively large reliance on traditional farming systems by smallholder and subsistence farmers [Southern Africa Development Community, (SADC, 2016)]. Because the subsistence farmers rear local and locally adapted breeds, the existence of a large proportion of smallholder livestock farmers is

[1] ZAR1 = USD0.122063 (Exchange rate (midrate) for 2012)

an important component of food security and a source of animal genetic diversity in the region. It is thus important to define the different types of breeds.

### 2.4 Types of breeds

Local breeds are defined as “breeds that occur only in one country” whereas the locally adapted breeds are defined as” breeds which have been in the country for a sufficient time to be genetically adapted to one or more of the traditional production systems or environments in the country” (FAO, 2012). These breeds are an important genetic resource in South Africa especially for the subsistence farmers where the fittest survive and pass on their adaptive or survival traits (Madzimure et al., 2013). In contrast, the exotic breeds have been selected for high yields (i.e. productive traits) and require high inputs for exploitation of their full genetic potential (Huyen et al., 2005). The exotic breeds are defined as “breeds which are maintained in a different area from the one they were developed and including breeds that are not locally adapted” (FAO, 2012). In South Africa these breeds are found in the commercial sector.

For the purpose of this thesis I will refer to the Kolbroek and Windsnyer pigs as the ‘local’ breeds whereas the Large White pigs will be referred to as an ‘exotic’ breed to South Africa.

As this thesis was a comparative study on the local (Kolbroek and Windsnyer) pigs and an exotic (Large White) breed, the following sections will provide some descriptions of the pigs.

#### 2.4.1 Large White

The Large White breed (Figure 2-1, below) of pig was developed in Yorkshire County, England in the late 1700s (Taylor et al., 2005). The breed was developed when a small, fleshy type of pig from Canton in China was cross bred with white pigs from Yorkshire and adjacent counties to produce the Small White, Middle White and Large White breeds (Taylor et al., 2005). The Large White breed of pig reared in South Africa was

imported from the United States of America however the precise time was not ascertainable [Agricultural Research Council, (ARC, 1993)].

The Large White breed of pig is distinguished by its erect ears, slightly dished face and long deep sides. It has white hair and a pink skin. In South Africa, the Large White is the most popular commercial breed (ARC, 1993) due to its superior fertility and growth rates compared to the Landrace and Duroc breeds (Ncube et al., 2003). It has a high feed conversion efficiency of 2.5 kg feed per kg of body mass (Steyn et al., 2012).



**Figure 2-1**: A mature Large White boar (Source: Photograph taken by the author, May 2015)

The males have a mature body mass of 300-450 kg while the adult females weigh about 250-350 kg (FAO, 2009). The exotic Large White breed of pig attains reproductive maturity between five and eight months of age at which time it weighs between 81 and 104 kg (McGlone and Pond, 2003). It is well known for its excellent mothering and rearing ability of the sow as well as its docility. Large White pigs farrow an average of 10 piglets per litter (Umesiobi, 2010) and possess high percentage (90%) of piglet survival at 21 days of age (Browne, 1994), which is the age at which piglets will be less dependent on milk and will be starting to eat hard feed.

### 2.4.2 Windsnyer

The Windsnyer breed of pig (Figure 2-2) is believed to have been introduced into South Africa by the European and Chinese traders 300 to 400 years ago (Holness, 1995). This breed is also present in Zimbabwe and parts of Mozambique and Zambia where it is referred to as the 'Mukota' pig (Holness, 1973).

The Windsnyer breed of pig has a large colour variation and can either be black, reddish-brown, brown, black and white or spotted (Mhlanga et al., 1999). Some of the young even have longitudinal stripes which are typical of the young bush pig (Ncube et al., 2003). The name Windsnyer (wind-cutter) is derived from its shape as it is narrow-bodied and long-nosed (Chimonyo et al., 2005).



**Figure 2-2**: A mature Windsnyer boar (Source: Photograph taken by the author, September 2016)

The adult males have a mature body mass that ranges between 140 and 180 kg (Kanengoni et al., 2014). The mature body mass of females ranges from 40 to 120 kg (Mhlanga et al., 1999). The Windsnyer pigs attain sexual maturity at an early age with gilts showing first signs of oestrus as early as three months of age (Mhlanga et al., 1999) at which stage they weigh between 20 and 35 kg. The average litter size is seven

(Chimonyo et al., 2005). The sows have excellent mothering abilities which results in very few piglet deaths (Wickedfood Earth, 2016).

The Windsnyer pigs have a lower feed conversion efficiency than the in Large White pigs (3.5:1 vs. 2.5:1,(Steyn et al., 2012), which results in a lower daily growth rate of 299 g/day (Hoffman et al., 2005) compared to 950 g/day in Large White pigs (Visser, 2004).

### 2.4.3 Kolbroek

There are two postulations on the origin of the Kolbroek breed of pig (Figure 2-3). One suggests that they were named after the sailing ship called Colebrook that wrecked at the Cape Hangklip in 1778 (Ramsay et al., 1998). The pigs on board were then collected by farmers who settled in that area. The second school of thought is that this breed was introduced into South Africa by the European and Chinese traders 300 to 400 years ago (Holness, 1995).



**Figure 2-3**: A mature Kolbroek boar (Source: Photograph taken by the author, May 2015)

The Kolbroek breed of pig has black and white patches and is often striped at birth. It is a short and fat pig, with a short snout, pricked ears and a squashed face. This breed has a tendency to accumulate fat subcutaneously (Hoffman et al., 2005) hence its synonym “lard pig”. The Kolbroek breed of pig resembles the “Lard breed of pig” found in China.

The average live body mass of a mature male and female Kolbroek pig is 112 kg. The Kolbroek pigs like the Windsnyer reach sexual maturity early and females may show first signs of oestrus as early as three months of age (Mhlanga et al., 1999) and will weigh between 15 and 30 kg at that time. They have a good mothering ability (Chimonyo et al., 2008) and have an average litter size of between seven and eight piglets (Bailekae, 2012).

The Kolbroek has lower feed conversion efficiency (4.0:1) (Hoffman et al., 2005) than the Windsnyer and Large White pigs (Steyn et al., 2012). In addition, growth studies in South Africa have demonstrated that Kolbroek pigs grew at 440 g/day, which was faster than the Windsnyer pigs that grew at 299 g/day (Hoffman et al., 2005) but slower than the Large White pigs which had a growth rate of 950 g/day (Visser, 2004). The local breeds of pigs worldwide and in South Africa in particular face several threats which are both natural and man-made.

### 2.5 Threats to local pigs

The recent increase in the demand for animal products has prioritised breeds with high yields (exotics) thus leading to cross-breeding (Mathias and Mundy, 2005) and the dilution of genetic diversity of the local breeds (Rischkowsky and Pilling, 2007). These genetic changes have led to the loss of the breeds ability to adapt to the local environmental conditions resulting in extinction of some local breeds such as the “hairless” creole (Scarpa et al., 2003). In addition to the marginalisation of local breeds as a result of intensification of livestock production and cross breeding, local pigs are also under threat from lack of political support and political instability, climate change and disease. Such threats are likely exacerbated by low stocking densities and

inbreeding thus making a number of local pigs vulnerable to natural disasters (Halimani et al., 2010). I will now consider the threats to pig production in a bit more detail.

### 2.5.1 Lack of political support & political instability

Policy makers have generally paid little attention to low producing local breeds of pigs (Ayalew et al., 2011) resulting in the lack of policies safeguarding their genetic diversity which hinders conservation of livestock genetic diversity in southern Africa (Halimani et al., 2010). For example, farmers of local breeds are disadvantaged by the current grading systems which do not accommodate for the high fat content, shorter body length and low body mass at slaughter, and carcass of local breeds often are unable to conform to the minimum mass requirements of pork cuts (Chimonyo et al., 2010). If policies do not support local breeds, they can quickly become marginalised. For example in Vietnam, the agricultural incentives that were adopted worked against the conservation of local genetic pig resources. The promotion of exotic breeds in Vietnam resulted in a loss of 25% of the 23 million pigs which were made up mainly of the local breeds (Drucker et al., 2006), there by compromising food security.

Globally, local breeds of livestock are being extirpated. Over a 15-year period, from 1988 to 2003, between one and two local breeds became extinct every week (Cardellino, 2003). It is estimated that more than 8% of known breeds became extinct in the recent decades and another 21% are at risk of extinction (time period not indicated) (Piling and Hoffman, 2011). On realising the potential impacts on food security and loss of adaptive capacity, the United Nations, through the Food and Agriculture Organisation, started raising awareness and prompted the world community to agree on the Global Plan Action for Animal Genetic Resources (Rischkowsky and Pilling, 2007). The aim of the Global Action Plan is to encourage the characterisation, conservation, sustainable use and policy formulation for the management of local animal genetic resources at national, regional and global level (FAO, 2007a). However, for these endeavours to be successful political support is required.

Political instability and conflict can contribute to losses of genetic diversity through their impact on the territorial distribution and protection of rare and endangered breeds. For example, the 1992-95 war in Bosnia-Herzegovina reduced the national pig herd (both local and exotic breeds of pigs) by 90% and caused the loss of Bosnian mountain breed of horses and the nucleus herd of the pure-breed local Busa cattle (Rischkowsky and Pilling, 2007). The post-war rehabilitation programmes in Bosnia-Herzegovina introduced exotic breeds from across European countries. The introduction of these imported breeds quickly increased the livestock numbers but they had poor adaptability to local conditions. The introduced exotic breeds had a higher nutrient requirements and lower disease resistance which increased their production costs for the already poverty stricken farmers (FAO, 2007b). For future restocking programme to be successful, the potential impacts of such efforts on genetic diversity should be assessed and should ensure that the breeds used are appropriate to the local production environment. However, many of these local production environmental conditions are likely to change as a result of future climate change.

### 2.5.2 Climate change

The IPCC predicts an increase in global-average surface temperature of between about 2.5°C and 7.8°C by 2100, relative to the pre-industrial levels, depending on economic activity and the rate at which we switch from fossil-fuels to cleaner energy sources (IPCC, 2014). These models provide a ‘best case’ and ‘worst case’, or ‘business as usual’ predictions for the magnitude of climate change. The three climate scenarios are based on projected mean daily ambient temperatures associated with a baseline, doubling, and tripling of atmospheric carbon dioxide levels (Mader et al., 2009). It is predicted that for South Africa, the temperatures will increase by at least twice the observed global average of 0.8°C per 100 years, the rainfall will decrease (less certainty) and extreme weather events are likely to increase [Department of Environmental Affairs, (DEA, 2013)].

The increased temperature and aridity predicted for much of southern Africa is likely to adversely impact pig production. Because pigs are dependent on water for heat

dissipation, pig production is likely to decline where water availability becomes limited through reduced rainfall (Hoffmann, 2010). Currently in South Africa, the Limpopo Province is the leading producer of pork (23% of the national output) (DAFF, 2013) and it is experiencing temperatures as high as 45°C and is the driest province (FAO, 2004). Should temperatures and aridity in Limpopo increase further, pig production farms might have to relocate to other regions as these industries follow the sources of inexpensive cereals, the production of which also requires water (Gregory, 2010). Moving local breeds to new location may dilute genetic diversity, which will contribute to reduced breed viability and may lead to extinction. The smallholder farmers may be particularly vulnerable because of their low adaptive capacity. The smallholder farmers have limited skills and equipment for disaster management, inadequate financial resources, weak institutional support and are heavily dependent on rain-fed agriculture (Rockstrom, 2000). The consequences of disrupted livestock production include increased poverty and decreased food security (Thornton et al., 2013). Yet, local pigs may be more tolerant to heat and food shortages than exotic breeds, which make the conservation of genetic diversity crucial for future productivity and food security.

It is important to note that there are opposing views on the magnitude of change. The Non-Governmental IPCC refutes the huge increase in temperatures and they say that the change may be smaller than predicted by the IPCC (Idso and Idso, 2013). They (NIPCC) argue that these models use faulty parameterisations and as such may not accurately predict the future climate conditions (Idso and Idso, 2013). All models have uncertainties and limitations, particularly when modelling complex systems like global climates, yet climate change models are continuously improving with time and increasing real-world data. Indeed, many recent observed climatic changes have been accurately predicted by the climate models. Although the exact magnitude of projected climate change, particularly in Africa, may be controversial, there is little doubt that global temperatures are increasing and that the projected temperature increases in the future are likely to adversely impact on biota, including the livestock industry.

We have already observed increased temperatures, decreased rainfall reliability, and increased frequency and severity of extreme climate events in different parts of the

world as predicted by the models. Since 1880, the hottest years occurred between 1998 and 2015, with 2015 being the warmest year on record [National Oceanic and Atmospheric Administration, (NOAA, 2015)]. During the 18-year period, the temperature increases ranged between 0.54°C and 1.00°C above the pre-industrial era (NOAA, 2015). The years 2011-2015 were the warmest five-year period on record, with an increase in many extreme weather events such as heat waves [World Meteorological Organisation, (WMO, 2016)]. The frequency of droughts has increased in some areas and in the other areas flooding has been observed to have increased too. Increasing ambient temperatures are expected to exacerbate the impact of summer weather extremes on the ability of vulnerable farm animals to thermoregulate (Hahn, 1995). The observed responses to increased heat stress in all classes of livestock are reduced voluntary feed intake, animal performance, fertility levels, activity and increased livestock mortality (Das et al., 2016). Not only are livestock likely to be adversely effected by climate change but they are also contributing to greenhouse gas emissions.

### 2.5.3 Contribution of livestock to climate change

The current climatic changes have been attributed to an increase in the concentration of greenhouse gases (GHGs), including carbon dioxide ($CO_2$), methane ($CH_4$) and nitrous oxide ($N_2O$) over the last 50 years (IPCC, 2007). Livestock production itself contributes to greenhouse gas emissions through $N_2O$ and $CH_4$ production; two gases which are more potent (that is, have a higher global warming potential) than $CO_2$. $N_2O$ is 310 times more potent and $CH_4$ has 21 times more global warming potential in the atmosphere than one molecule of $CO_2$ (IPCC, 2007). All of these gases have long resident times in the atmosphere. Methane ($CH_4$) has a resident time of 12 years and nitrous oxide ($N_2O$) has a resident time of 114 years while carbon dioxide ($CO_2$) has a resident time that ranges between 5-200 years (Houghton et al., 1995). These three gases are produced mainly from enteric fermentation, manure and feed production and account for 86% of the greenhouse gases produced by the livestock sector (Figure 2-4, below). Livestock also produce water vapour, a gas with no adverse consequences. For comparative purposes, all greenhouse gases are converted to a single common unit, the carbon dioxide equivalent.

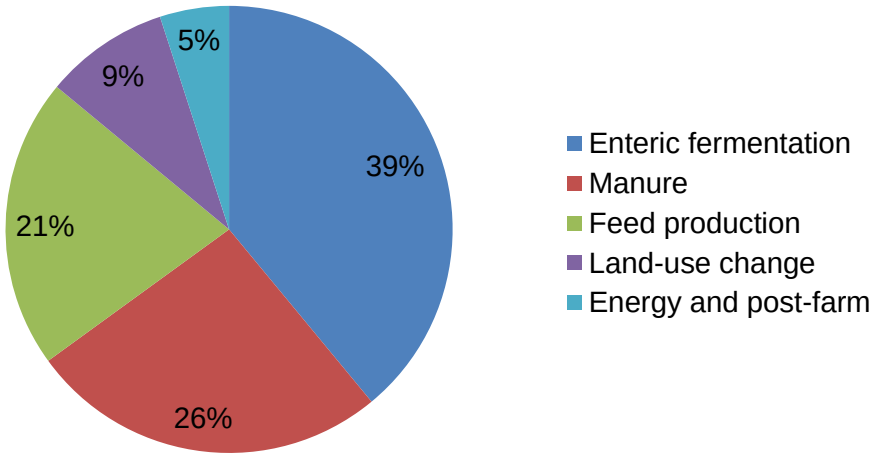



**Figure 2-4**: Breakdown of livestock sector greenhouse gases emissions by source. (Source: Chatham House analysis based on(Opio et al., 2013))

Carbon dioxide equivalent ($CO_2$-eq) is defined as "the universal unit of measurement used to standardise and compare emissions from different greenhouse gases based on their global warming potential, converted to an equivalent amount of carbon dioxide" (Worley Parsons Annual Report, 2015). For example if 1 kg of methane is emitted, this can be expressed as 21 kg of $CO_2$–eq.

Between 1995 and 2005, the global contribution to GHG by the livestock sector was estimated at 7.1 Gigatonnes (Gt) $CO_2$-eq per year, (18% of the annual global anthropogenic GHGs emissions, (Steinfeld et al., 2006) the majority of which (5.7 Gt) was produced by the ruminant supply chain (Opio et al., 2013). Monogastric livestock species contribute substantially less to GHG emissions, pigs (0.7 Gt $CO_2$-eq per year) and poultry (0.7 Gt $CO_2$-eq per year) (MacLeod et al., 2013) mainly because of differences in their digestive physiology; whereas the ruminants rely on ruminal and intestinal fermentation, the monogastrics use intestinal fermentation only.

Compared to ruminants, pig production requires lower feed per kilogram of body mass and produces lower carbon dioxide emissions contributing to GHGs per kg of meat

produced (Shields and Orme-Evans, 2015). Table 2-2 shows the differences in the production attributes of pigs and ruminants. The production efficiency of the monogastrics has been attributed to higher feed conversion efficiency, lower enteric methane emissions, and faster rates of reproduction than the ruminants (Shields and Orme-Evans, 2015).

**Table 2-2**: Productive efficiency of animal products

| Animal products | Land requirement ($m^2$ per kg body mass) | Conversion of grain (kg feed per kg body mass) | Emissions (kg $CO_2$-eq per kg body mass) | Average emissions (kg $CO_2$-eq per kg product) |
|---|---|---|---|---|
| Beef | 20 – 23 | 7 – 10 | 16 – 40 | 46.2[3] |
| Pork | 7.4 – 8.9 | 3.4 – 6 | 4.5 – 7.5[1] | 6.1[2] |
| Chicken | 6.4 – 7.3 | 2 – 4 | 1.5 – 7.3 | 5.4[2] |
| Lamb/mutton | 14 – 30 | 4 – 5[4] | 10.1 – 17.0 | 23.8[3] |
| Milk | 1.2 – 12.0 | 1 | 3.1 – 7.3 | 2.8[3] |
| Eggs | 3.5 – 67 | 2 | 1.6 – 2.9[1] | 3.7[2] |

Sources: (Stephenson, 2010); [1](Weiss and Leip, 2012); [2](MacLeod et al., 2013); [3](Opio et al., 2013); [4](Brand et al., 1991)

## 2.6 Impact of heat stress on livestock

### 2.6.1 *Production*

The increased ambient temperature with climate change is likely to directly affect livestock production as a result of increased heat stress. As ambient temperatures increase voluntary feed intake (Collin et al., 2001) and feed conversion efficiency decline (Rowlinson, 2008, Christon, 1988) (i.e. decrease productivity), animal performance declines and heat related deaths can result (Baumgard and Rhoads, 2013, Ross et al., 2015, Upah et al., 2011). In addition, heat stress can negatively impact the reproductive output of sows and boars (Edwards et al., 1968) further decreasing productivity. In sows and gilts, heat stress results in abnormal oestrus cycle, an increased proportion of abnormal ova, decreased fertility and increased embryonic and foetal mortality (Stott

and Wiersma, 1973). There is also an increase in stillbirths (Einarsson et al., 2008). Even in instances where artificial insemination is used, high ambient temperature at the time of insemination or during the first few weeks after breeding can reduce pregnancy rate in pigs (D'Allaire et al., 1996).

In boars, heat stress reduces sperm output and quality (Ross et al., 2015) and lowers libido. The lowered sperm quality presents as an increased percentage of dead spermatozoa in the ejaculate, reduced sperm motility and increased abnormal sperm all of which decrease conception rates (McGlone, 1999, Suriyasomboon et al., 2004, Wettemann et al., 1976).

### 2.6.2 *Thermoregulation*

Livestock may attempt to compensate for increased heat stress by increasing their heat loss or reducing heat gain. Thermoregulation is the means by which an animal maintains a constant core body temperature by balancing heat gain and heat loss (IUPS, 2001). Endotherms, through control by the central nervous system, employ behavioural and autonomic processes to regulate body temperature (Maruyama et al., 2003). These processes are integrated with other physiological systems such as the respiratory, digestive, cardiovascular and the motor systems (Piccione and Refinetti, 2003).

The autonomic responses employed in thermoregulation include shivering, non-shivering thermogenesis, skin vasomotor responses, and evaporative heat loss (IUPS, 2001). Autonomic responses cannot be sustained indefinitely, especially in a severely hot or cold environments and as such long term thermoregulation depends mainly on behavioural mechanisms (Cabanac, 1996). Behavioural thermoregulation entails finding or establishing an appropriate thermal environment. Baldwin and Ingram (1967) Baldwin and Ingram (1968) reported that when pigs were exposed to cold and or heat, under laboratory conditions with access to a switch controlling the heat source, they learnt how to switch on and off an infrared heat source in response to the ambient temperature. Natural behavioural responses exhibited by pigs include huddling when cold, seeking shade, wallowing in water and changing from diurnal to nocturnal feeding times when

heat stressed during the day (Ingram and Legge, 1970b). In addition pigs may respond to increasing temperatures by modifying their lying position, increased excretion and wallowing in their excreta (Huynh et al., 2005b). Breed and seasonal differences have been noted in behavioural responses. During the hot and wet summer in South Africa, the frequency and duration of behavioural heat loss activities such as wallowing, and sprawling in slurry increased in the light coloured Large White pigs compared to the local Windsnyer pigs (Madzimure et al., 2012).

Thermoregulatory behaviours help to reduce the costs of thermoregulation as they help an animal to select environmental conditions within its thermoneutral zone (TNZ). The thermoneutral condition for the pigs (including in the tropics) is dependent on the age and weight of the pigs and the TNZ for the growing-finishing pigs is between 10 and 21°C (Whitney, 2014). The TNZ is the range of ambient temperature at which temperature regulation is achieved only by control of sensible heat loss (IUPS, 2001) and without activating evaporative heat loss or metabolic heat production. Within the TNZ the metabolic rate is minimal, constant and independent of ambient temperature (Mount (1979). In addition, within TNZ homeothermy is maintained by mechanisms that require little effort such as change in posture and an increase in blood flow to the skin in order to increase conductive, convective and radiative heat loss (Renaudeau, 2005). The lower critical temperature (LCT) is the ambient temperature below which the rate of metabolic heat production of a resting thermoregulating animal must be increased by shivering and/or nonshivering thermogenesis in order to maintain thermal balance (IUPS, 2001). The temperature at which the respiratory rate begins to increase is termed evaporative critical temperature (ECT). When environmental temperature increases above ECT animals gain heat from the environment and have to actively dissipate that heat via evaporative cooling. The thermal zone of comfort is the range between the LCT and the ECT and varies depending on the environmental condition and animal related factors, such as the age and body mass of the pig (Banhazi et al., 2007).

For a 60 kg Large White pig, respiratory rate increased between 21.3°C and 23.4°C and voluntary feed intake and heat production decreased above 23°C, marking the evaporative critical temperature (Huynh et al., 2005a). When environmental

temperatures exceed the evaporative critical temperature and pigs are denied access to water for wallowing, the evaporative heat loss from the respiratory tract is inadequate to compensate for heat gain and body temperatures increase, which can lead to hyperthermia (Mount, 1979). The core body temperature of pigs is about 38.5°C (Muys and Westenbrink, 2004) and might increase to above 40°C. Under TNZ (20±2.5°C) the respiratory rates of pigs is about 19 breaths per minutes and it increases to about 95 breaths per minute when exposed to high temperatures (25-33.5 °C) (Umboh and Tulung, 2001). Because, in a dry environment, the ability of pigs to compensate for high ambient temperatures depends on evaporative heat loss from the respiratory tract, relative humidity (RH) becomes an important factor to be considered under conditions of heat stress (Renaudeau, 2005). During heat stress, an increase in RH reduces the efficiency of evaporative cooling since the vapour pressure gradient between the animal and the environment declines (Silanikove, 2000), thereby accentuated the effects of high temperature on the pigs (Huynh et al., 2005b). Evaporative cooling by panting uses water, a resource most likely to be scarce with climate change. Should water be restricted, thereby preventing animals from dissipating excess heat by evaporative means, animals may display dehydration-induced hyperthermia, a phenomenon observed in ruminants (Topps, 1975) but not studied in pigs. The decreased water availability and precipitation predicted to occur with climate change may result in a decrease in relative humidity. A lower humidity would allow more effective heat loss by evaporation. Yet some studies suggest that it is less important than ambient temperature in contributing to heat stress of pigs (Huynh et al., 2005a).

A pig's ability to acclimate to heat stress is influenced by the ambient temperature during rearing and by the breed of pig. For example, the local Creole pigs, from the Caribbean, are low producers and were better acclimated to heat stress than the exotic Large White breed (Renaudeau, 2005). Heat tolerance of low producing lines is attributed to their lower heat production as a consequence of their low productivity and maintenance requirements suggesting that low production is itself an adaptive attribute to heat stress (Renaudeau, 2005). Ingram (1977) noted that growing pigs reared at 25°C increased their respiratory rate when exposed to high temperatures (35°C), while an increase in ambient temperature had no effect on those pigs reared at 35°C. Warm-reared pigs may

show acclimation to the hotter environments predicted to occur with climate change. The local pigs are reared in hot areas where ambient temperatures often exceed 40°C in summer. Conversely, the exotic pigs are normally bred indoors with potential for climate control; therefore the local pigs may be better acclimated to high environmental temperatures likely to become prevalent with climate change.

Pigs are more sensitive to high environmental temperatures compared to ruminants because of a limited capacity to lose heat by water evaporation from the skin (Ingram, 1965). Pigs have sweat glands but not under active regulation and they do not increase sweat rate with higher ambient temperatures (Ingram, 1965). Pigs do not sweat and rely primarily on panting for evaporative heat loss. In addition the pigs have been noted to use transepidermal water as another avenue for evaporative heat loss. Transepidermal water loss is defined as the passive diffusion of water across the epidermis excluding water loss from the sweat glands (van der Valk et al., 2004). Studies conducted in exotic pigs have indicated that transepidermal water loss is a tightly controlled process when water intake is limited (Cunningham, 1968, Haggarty et al., 1994). In order to compensate for a limited capacity to lose heat via evaporative heat loss, commercial piggeries employ water drips, sprinkling and snout coolers to cool pigs (McGlone, 1999).

**2.7 Skin**

The skin provides an important barrier through which heat transfer occurs. The skin has three layers namely the epidermis, the dermis, and the hypodermis (Kanitakis, 2002). These layers influence the way animals lose or gain heat and also regulate water loss. Although the skin has many functions, for the purpose of this thesis I will only discuss the main functions that relate to temperature regulation and water loss through the skin.

Several studies in cattle have revealed that morphology of the (skin thickness, colour and density of sweat glands) as well as the structure of the coat can influence their thermoregulation (Thatcher et al., 2010, Nay and Hayman, 1956). The better adaptation to heat by *Bos indicus* cattle is primarily related to the greater sweating rates due to higher density and larger perimeter of sweat glands, quick transfer of metabolic heat to

the skin due to a lower tissue resistance and fewer hairs, thereby reducing coat resistance to heat loss (Behl et al., 2010). In pigs, the heat tolerant Creole pigs had a higher density of sweat glands compared to the Large White pigs (Renaudeau et al., 2006), however they did not do any comparative tests on the functionality of the two breeds sweat glands.

In cattle, those animals that had a thinner dermis had better thermoregulatory abilities than those that had thicker skin and tolerated heat better (Dowling, 1955, Finch et al., 1984). Breed differences in skin thickness have also been found within pigs. Madzimure et al. (2012) demonstrated that these pigs had longer hair, greater hair density and thicker fat layer than the Large White pigs.

Colour mediates the impact of solar radiation and influences the magnitude of the heat load on animals (Hamilton, 1973). The surface colouration that an animal presents to its environment affects its fitness both by determining its conspicuousness and by modifying thermal balance (Walsberg and Wolf, 1995). Amongst polymorphic mammalian species that display multiple colour forms, those with melanic pelage are prone to overheating in warm climates, as their fur absorbs solar energy at a higher rate (Fratto and Davis, 2011, Hetem et al., 2009). However previous studies have shown that not to be the case. In goats, dark coloured individuals (pigmented) had significantly lower rectal temperatures, pulse rate and respiration rate than the light coloured ones (unpigmented) around midday when exposed to radiant heat such as sunlight (Darcan et al., 2009). A similar result was obtained in the South African local Windsnyer pigs. Despite being dark coloured, the Windsnyer pigs had superior heat tolerance attributed to the lower heart rate, skin surface temperature and lower panting compared to the Large White pigs (Madzimure et al., 2012). It is notable that in the study by Madzimure et al. (2012), rectal temperature, skin surface temperature, heart rate and breathing rate measurements were taken at the same time at 08:00, 12:00 and 16:00 after every other day for the 105 day study period. Rectal temperature was measured manually by inserting a clinical digital thermometer into the rectum for 60 seconds and that could have introduced stress hyperthermia thereby giving an inaccurate reading. The skin

surface temperature was also manually measured possibly also introducing stress hyperthermia.

If radiation absorption occurs largely near the outer coat surface, then a major fraction of the resultant heat will be lost to the environment and will not contribute to the thermal load on the skin (Cena and Monteith, 1975, Kovarik, 1964, Walsberg et al., 1978). On a hot day coat insulation retards heat loss to the environment if radiation penetrates deeply into the coat before being absorbed, and a large fraction of the heat generated by solar radiation contributes to the thermal load on the skin (Walsberg and Wolf, 1995). The thermal properties of pelts have been examined in cattle (Hutchinson and Brown, 1969) and sheep (Bennet, 1964) but the pelts of local breeds of pigs have not been investigated. It is not clear how different skin colourations affect heat transfer and a pig's ability to adapt to higher temperatures predicted under climate change. It is hypothesised that the colour and coat type that an animal has may influence its ability to thermoregulate in thermally challenging conditions. However color is not the only factor which determines the amount of heat absorbed through the pelt. The dense plumage of pigeons (Wolf and Walsberg, 2000) and emus (Maloney and Dawson, 1995) prevented radiant heat from penetrating the feathers so heat load was independent of color in these species. Heat transfer depends on fur depth, hair length, density, and diameter and, on fur absorptivity and reflectivity and on the thermal environment (Armitage, 2009).

The skin also contributes to the control of body water content through water loss via the sweat glands and also through the formation of a permeability barrier that limits excessive transepidermal water loss (Lillywhite, 2006). The lipid barrier in the epidermis plays an important function in the water relations of mammals dwelling in arid environments (Hadley, 1991, Lillywhite, 2006). When drinking water is limiting, the lipid barrier in the stratum corneum forms a water tight layer that reduces water loss through the skin, thereby preventing dehydration (Haugen et al., 2003). The formation of the water tight lipid barrier in the *stratum corneum* is reliant on the transformation of cerebrosides to ceramides by the enzyme β-glucocerebrosidase (Cox et al., 2008). Animals from dry environments tend to have a greater quantity of ceramides in the

epidermis, which aids in the control of the permeability of the skin. Studies have shown that the animals in arid environments had lower transepidermal water loss than animals from wet environments (Raith and Neubert, 2000). Beside natural selection, phenotypic plasticity, reversible phenotypic flexibility and developmental plasticity has also been observed to affect the rate of transepidermal water loss depending on environmental conditions (Lillywhite et al., 2009).

Understanding the pigs physiological responses to water scarcity when challenged with heat stress will help farmers improve animal management practices and maximise productivity. The local breeds of pigs have had a long history in South Africa dating as far back as 1778 (Hoffman et al., 2005). They have been exposed to extreme conditions over many generations and therefore might be better acclimated to the high temperatures predicted to occur in southern Africa as a result of climate change. In the current study the abilities of local and exotic breeds of pigs to withstand and respond to high temperatures and water scarcity were compared in controlled laboratory trials.

### 2.8 Disease

In addition to the direct effects of climate change on livestock (i.e. increased heat stress), climate change is likely to increase the risk of disease, which was previously listed as the third major threat to local breeds. Changes in rainfall patterns and intensity, combined with shifts in temperature ranges, will change local environments allowing relocation of arthropod vectors and enabling them to survive in areas that previously were uninhabitable (Cumming and van Vuuren, 2006, Olwoch et al., 2003, Rogers and Randolph, 2000, Slenning, 2010, Elbers et al., 2015). The environmental changes will also affect the biology, transmission and epidemiology of the pathogens and vectors (Gale et al., 2009, Baylis and Githeko, 2006, Dantas-Torres, 2015).

The recent warmer winters in Europe enabled vector-borne pathogens, such as the Bluetongue virus (Purse et al., 2005), to spread across Europe as a result of expanding vector ranges. Warmer winters may also accelerate the development of pathogens or parasites that spend some of their life cycle outside their animal host (Harvell et al.,

2002) allowing disease vectors such as mosquitoes and ticks to rapidly reach critically high densities. The spread of such vectors and the pathogens they carry resulted in significant animal loses at a huge cost to the governments which had to compensate farmers and eradicate potentially infected individuals. Lyme disease (bacterial), toxic *Escherichia coli*, a new hantavirus, and cholera (water-borne diarrhoeal disease) are predicted to spread due to the envisaged global climate change (Epstein, 2001). *Vibrio cholerae*, the causative agent for cholera has been detected in feaces of livestock including pigs in the rural areas of Limpopo Province (Keshav et al., 2010) due to animals drinking contaminated water. It becomes pertinent therefore to elicit and evaluate the response of indigenous pigs to evaluate the immune responses of the indigenous pigs to a likely stressor.

### 2.8.1 Fever and sickness behaviours

As a result of climate change and the spread of pathogens, livestock hosts are likely to have to contend with novel pathogens, which are likely to initiate immune responses and result in fever. Fever is defined “as a physiological state of elevated set-point of core body temperature which is part of the defensive responses of the host to the invasion by live (microorganisms) or inanimate matter (non-microbial) recognised as pathogenic by the host” (IUPS, 2001).

The immune response plays a vital role in protecting against infectious agents. Bacterial infections result in the production of toll-like receptors while the viruses induce the Type I interferons (Machado et al., 2004). Organisms resist infections by establishing barriers and activating different classes of innate resistance and adaptive immunity. Due to the evolutionary host-pathogen arms race, some pathogens have evolved evasion mechanisms that overcome the host immune responses, resulting in acute or chronic infection accompanied with sickness behaviours. Sickness behaviour is a behavioural complex that develops during infection and is accompanied by fever, anorexia, reduction in physical activity and body mass loss (Maes et al., 2012, Lopes, 2014). Sickness behaviours are adaptive responses that enhance recovery by conserving energy to combat acute inflammation (Maes et al., 2012).

In artificial fever experiments, numerous pyrogenic mimetics such as lipopolysaccharide (LPS), muramyl dipeptide (MDP) and polyinosinic: polycytidylic (poly I:C) are used to induce fever (Sehic and Blatteis, 1996). Mimetics are small molecules, which are either synthetic or natural organic products, which share structural similarity with a native ligand, such as a toxin (Zabriskie and Visvanathan, 2007). These small molecules are recognised by the toll-like receptors found on the sentinal cells of the innate immune response (such as macrophages and dendritic cells) and can induce a strong immune response without replicating within the organism as in the case with the use of live pyrogens like viruses and bacteria (Johnson and von Borell, 1994) and have the ability to stimulate macrophages to synthesise and secrete proinflammatory cytokines (Johnson and von Borell, 1994). The pyrogenic mimetics induce symptoms of acute infection, including anorexia, lethargy, and fever (Liu et al., 2003, Hart, 1988, Maes et al., 2012). These febrile responses and associated sickness behaviours are usually of smaller and shorter magnitude compared to those of live pathogenic challenges (Sandberg et al., 2007).

The advantages of using mimetics over live pathogens include safety, convenience and more importantly reproducibility and control over doses and time of administration of the immunological challenge (Fortier et al., 2004). However it should be noted that the experimental model of injecting a non-replicating agonist does not completely mimic the physiological changes that would occur during a *bona fide* infectious process on the farms (Balaji et al., 2000). Nevertheless it allows one to better understand the physiology of infection and inflammation due to an immunological challenge (Liu et al., 2003). Live pathogens give the actual responses of the animals to real infection and this model gives the natural progression of the infection as would be expected in an animal production setting. Infection can be caused by either virulent or non-virulent pathogens. Virulent pathogens are capable of causing overt signs of disease through activating and overwhelming the immune system of the host (Surico, 2013). The use of live pathogens in experiments introduces an added cost of treating animals once injected with the pathogens. In addition the handling of pathogenic microorganisms requires precautions that guarantee the safety of humans and the environment, including laboratory personnel, and other persons who could be exposed to these microorganisms (Surico,

2013) unlike with the use of pyrogenic mimetics. For my study I will focus on the following three mimetics: polyriboinosinic-polyribocytidylic acid (poly I:C; a viral mimetic); lipopolysaccharide (LPS, a gram-negative bacteria mimetic), and *Staphylococcus aureus* (*S. aureus*, a gram-positive bacteria mimetic). The mimetic models will be discussed below.

### 2.8.2 Pyrogenic mimetics

#### 2.8.2.1 Lipopolysaccharide

Lipopolysaccharide (LPS) is a glycolipid present in the outer membrane of the gram-negative bacterial cell wall (Mani et al., 2012). *E. coli* derived LPS is used to mimic acute gram-negative bacterial infections (Galic et al., 2009) and is the most commonly used pyrogen in fever studies (Soszynski et al., 1991). Lipopolysaccharide consists of a hydrophobic domain, lipid A, through which it is inserted into the bacterial cell wall, a core oligosaccharide, and a distal oligosaccharide (Raetz and Whitfield, 2002, Elin and Wolff, 1976). The hydrophobic lipid A domain is the most biologically active portion of the LPS molecule and it is synonymously known as endotoxin because of its toxic nature (Erridge et al., 2002, Mueller et al., 2005). It acts as an endotoxin and causes strong immune responses in animals. The bacterial mimic, LPS, stimulates β lymphocytes which in turn activate macrophages to release cytokines such as interleukin-6 (IL-6), interleukin-1 (IL-1) and tumor necrotic factor-alpha (TNF-α) (Dinarello, 1984, Jessen, 2000), which subsequently increase prostaglandin synthesis (Crestani et al., 1991, Hashimoto et al., 1988, Uehara et al., 1989).

Intraperitoneally injection of LPS in pigs has been shown to cause an increase in plasma concentrations of cortisol and TNF-α, induced a febrile response, reduced voluntary feed intake, physical activity and body mass (Warren et al., 1997). The 5 and 50 µg/kg doses of LPS increased plasma concentrations of cortisol and TNF-α, while inducing anorexia, hypersomnia, and fever while the 0.5 µg/kg dose of LPS induced acute anorexia, hypersomnia and fever but did not increase the TNF-α and had small increases in cortisol. In a variety of species, injection of synthetic pyrogens induces monophasic

fever at low doses and biphasic fever at high doses (Romanovsky and Blatteis, 1995). In pigs, LPS injected intraperitoneally (i.p.) at doses 0.5-50 µg/kg resulted in a dose-dependent increase in body temperature of 0.3-2.3°C, which peaked 4 h post-injection (Johnson and von Borell, 1994). Similarly, pigs exhibited a dose-dependent response when LPS was injected intravenously (i.v.) at doses of 0.5-30 µg/kg, which resulted in an increase in rectal temperature of 1-2°C (Sakumoto et al., 2003). Although responses differed slightly between studies, with 0.8 µg/kg of LPS i.v. resulting in an increase in body temperatures between 1.5°C (Parrot et al., 1997) and 2°C (Parrot et al., 1995), i.v. administration consistently resulted in longer lasting fever responses with higher febrile temperatures. Yet, the mechanism of action of LPS appeared to be independent of the route of administration with both i.v. (Sakumoto et al., 2003) and i.p. (Warren et al., 1997, Webel et al., 1997) administration resulting in elevated tumor necrosis factor-α (TNF- α), cortisol and plasma interleukin-6 (IL-6). LPS has also been shown to induce sickness behaviours in pigs, such as lethargy (Johnson and von Borell, 1994), hypersomnia and anorexia (Warren et al., 1997), as indicated by reduced growth and feed intake (Johnson and von Borell, 1994, Sakumoto et al., 2003). Pigs therefore seem to employ many of the systemic acute-phase responses that are used by other mammals and birds to maintain homeostasis during infection (Johnson and von Borell, 1994).

#### 2.8.2.2 Staphylococcus aureus

*Staphylococcus aureus* (*S. aureus*) is a Gram positive bacterium. Cell walls, which are derived from the heat killed bacteria, are used in experiments as pyrogenic mimetics of gram-positive bacterial infections. The Gram-positive bacterial cell walls are composed of multiple peptidoglycan layers, wall teichoic acids linked to the peptidoglycan and lipoteichoic acid linked to the cytoplasmic membrane (Fournier and Philpott, 2005). Gram-positive bacteria cause inflammation by either secreting a toxin or using components of the cell wall such as the peptidoglycans. The toll-like receptor-2 functions as the transmembrane component that detects the *staphylococcal* lipoteichoic acid and phenol-soluble modulin and is involved in the synthesis of inflammatory cytokines by monocytes/macrophages in response to the cell wall components (Fournier and Philpott,

2005). Gram-positive infections such as those with *S. aureus* produce systemic cytokine responses with the peak cytokine response occurring between 50 and 75 h after the challenge whereas it occurs 1 to 5 h after in gram-negative infections (Opal and Cohen, 1999). In previous studies, *S. aureus* induced fever and sickness behaviours in goats (Mphahlele et al., 2004) however I could not find literature to suggest that it has been used in pigs.

#### 2.8.2.3 Polyriboinosinic-polyribocytidylic acid

Polyriboinosinic-polyribocytidylic acid (poly I:C) is a synthetic double-stranded RNA that is used as a viral mimetic (Galic et al., 2009). The double-stranded RNA represents a molecular pattern that is associated with viral infection, since it is generated by viruses during their life cycle (Weber et al., 2006). Viral infections are recognised by the toll-like receptors of the innate immune system. Poly I:C binds to and activates the toll-like receptor-3, a receptor that recognises the double-stranded RNA produced by most viruses during their replication (Takeda and Akira, 2005, Fortier et al., 2004). Recognition of infections by toll-like receptors results in the activation of 'nuclear factor kappa-light-chain-enhancer of activated beta cells' (NF-κB) transcription factors, resulting in the subsequent production of inflammatory cytokines (Alexopoulou, 2001, Takeuchi and Akira, 2007). Viral infections induce a cascade of cytokine synthesis and release, similar to bacterial-infection, but the primary cytokines involved in viral infections are the interferons (Majde, 2000). The interferons mediate immune reactions and cause central nervous system-mediated effects, such as fever, by activating prostaglandins (Won and Lin, 1988).

Compared to simulated bacterial infections, very little is known about the immune response of pigs to simulated viral infections. However, in rodents, poly I:C induced an acute-phase response, including fever and lethargy but not anorexia (Fortier et al., 2004), and increased plasma IL-6, TNF-α and IL-10 but did not affect interferon-gamma (IFN-γ) (Gandhi et al., 2007). Like rodents, rabbits are sensitive to poly I:C and doses as low as 2.5-50 μg/kg can induce fevers of ~1°C in magnitude (Soszynski et al., 1991). Conversely, piglets show a low sensitivity to poly I:C and fever was induced by doses

between 720 and 3600 µg/kg (Loewen and Derbyshire, 1986), with a peak in interferon titers between 4 and 8 h after injection of 3600 µg/kg. In terms of their sensitivity to poly I:C, weaned or mature pigs are thought to be intermediate, between the rodents and unweaned piglets, as interferon induction has been shown after i.v. injection of 100-500 µg/kg poly I:C (Gainer and Guarnieri, 1985, Vengris and Mare, 1972). Administration of 5 mg/kg poly I:C intraperitoneally in Large White piglets (Dilger and Johnson, 2010) has been shown to induce acute sickness behaviours (reduced voluntary feed intake, physical activity and reduced body mass) and febrile responses which last between 24 h and 48 h.

Although some studies have looked at aspects of the bacterial and immune response of exotic pigs, no data exists for local pigs in South Africa. Using a virulent Classical Swine Fever Virus (CSFV), Blacksell et al. (2006) showed that the local Moo Laat pigs of the Lao People's Democratic Republic were more tolerant to CSFV, than the exotic Landrace pigs. The Landrace pigs succumbed to CSFV on the 8$^{th}$ day while the first death in the Moo Laat pigs was recorded on the 17$^{th}$ day post-injection. The longer tolerant period in the Moo Laat pigs gives farmers an opportunity to intervene therapeutically. If a similar response was to be observed the South African local pigs, farmers may have a longer window of opportunity to save their animals. In addition, the pigs which display a greater sensitivity to pathogens may die while the pigs more resistant to pathogens may be more likely to survive. A literature search has revealed that no study has investigated febrile responses and sickness behaviours, such as lethargy and anorexia, of South African local pigs in response to both viral and bacterial pathogens.

### 2.9 Aims of the research

While some research on the effects of climate change on the performance of exotic pigs has been conducted (Mader et al., 2009, Nardone et al., 2010, St-Pierre et al., 2003) as well as in local Windsnyer pigs (Madzimure et al., 2012), there are still gaps in our understanding of the physiological responses of the local pigs in South Africa to cope

with high temperatures accompanied by water deprivation as well as diseases that are expected to become more prevalent with climate change.

There are unsubstantiated claims that local pigs are hardy and better adapted to their environment than the exotic Large White breed of pig. The literature search has indicated very little scientific data to support the anecdotal accounts that the physiological adaptability of the local pigs enables them to survive under harsh environmental conditions. The physiological information generated under the various stressors will contribute to the knowledge base that would help predict the consequences for pigs under the scenarios envisaged for climate change.

### 2.10 Study Objectives

The broad objective of this thesis is to characterise the differences between three breeds of pigs in terms of febrile responses to bacterial and viral derived pyrogens, thermoregulation (dehydrated/euhydrated states) and the skin morphological differences. The specific objectives were to:

i. investigate the differences in duration and magnitude of viral-induced fever and sickness behaviours of the two local breeds (the Windsnyer and Kolbroek pigs) and an exotic Large White breed of pig.

ii. measure the difference in duration and magnitude of bacterial-induced fever and sickness behaviours between two local breeds (the Windsnyer and Kolbroek pigs) and an exotic Large White pig.

iii. investigate the effects of water deprivation and high temperatures on the thermoregulatory abilities of two local breeds (namely Windsnyer and Kolbroek pigs) and an exotic Large White breed of pig.

iv. investigate the effects of three temperature levels (below, within and above the TNZ, RH 40%) and the combination of high temperature and high relative

humidity (60%) on thermoregulation of two local breeds (namely the Windsnyer and Kolbroek pigs) and an exotic Large White breed of pig.

v. investigate the differences in the skin characteristics between the two local breeds and an exotic breed of pig.

The following chapter gives a brief overview of the methods that were used in investigating the physiological responses and skin morphological differences between the two local breeds the Windsnyer and Kolbroek pigs and an exotic Large White breed of pig.

# CHAPTER 3: OVERVIEW OF THE METHODS

In this chapter I give a brief overview of the methods used in the three main experiments of this study. For this research intact boars were used to minimise the cyclic effects of female hormones on core body temperature once the pigs attained puberty. For example, the core body temperature of females rises by between 0.5-0.7°C when they are in oestrus (Scolari et al., 2011). In studies on thermoregulation and fever, any increase in core body temperature not related to the interventions, distorts the results and could lead to incorrect conclusions. Detailed methods of each experiment are presented under each respective chapter.

## 3.1 Fever experiment

Four-week old, uncastrated male piglets from two local breeds, the Kolbroek (n=8) and Windsnyer (n=7), and an exotic breed, the Large White (n=8) in this study (Chapter 4) were used. The piglets were abdominally implanted with temperature and activity-sensitive tags and data loggers. They then had a 10-day recovery period post-surgery. The pigs with similar body masses were matched and housed as pairs by breed in pens. They were fed a commercial ration under controlled environmental conditions in the Central Animal Services, University of the Witwatersrand. Drinking water was available *ad libitum*. The biologging technique was chosen for its remote real-time functionality (Strauss et al., 2015). Remote monitoring of core body temperature and sickness behaviour (activity of the animals after injecting them with bacterial and viral mimetics) in undisturbed animals is important as it eliminates the impact of handling stress that may distort the readings.

I characterised the febrile and sickness responses of pigs to lipopolysaccharide (gram-negative bacteria mimic), polyinosinic: polycytidylic (viral mimic) and cell walls of *Staphylococcus aureus* (gram-positive bacteria mimic). A crossover design was used where the pyrogens viz lipopolysaccharide (2 µg/kg), polycytidylic acid: polyinosinic acid (5 mg/kg) and, *Staphylococcus aureus* (1.7 x $10^{10}$ cell walls/kg) were administered intravenously in a completely randomised manner. Saline was used as a control. The

pigs had a 14-day washout period between each injection. Core body temperature and physical activity were recorded continuously every 5 minutes and monitored before and post-injection of mimetics. Voluntary feed intake and body mass were also measured before and post-injection of mimetics. A one-way ANOVA followed by the Tukey's Multiple Comparison *post-hoc* Test to assess if there were differences in peak core body temperature, time to peak core body temperature and TRIs between the three breeds of pigs in response to the different pyrogens were used. Data was tested for normality and homoscedasticity before being analysed. Febrile response data were presented graphically over a 10 h period post injection of mimetics. Sickness behavioural data were presented to indicate the extent of suppression in the first 24 h and duration of the sickness behaviours up to 72 h post-injection of mimetic.

## 3.2 Thermoregulation experiment

The pigs used in the first experiment were given a 21-day washout period before entering the second experiment (Chapter 5). The four months old uncastrated male Kolbroek (n=6) and Windsnyer (n=7) pigs and exotic Large White (n=7) pigs were used to investigate thermoregulatory and physiological traits in response to simulated conditions in a climate chamber using a method by Beatty et al. (2006) and Brown-Brandl et al. (2003). The pigs were divided into two groups of three or four pigs per breed and exposed to experimental conditions in a randomised controlled cross over design. Five experimental treatments comprised of cold (5°C), thermoneutral (20°C), hot (30°C) with *ad libitum* access to drinking water, hot (30°C) with high relative humidity (60%) and hot (30°C) with 48 h water deprivation. The pigs were subjected to these simulated conditions for 8 h a day for two days except for the cold and thermoneutral treatments in which the pigs were continuously exposed for 48 h. The pigs had a 9-day recovery period between the treatments. Core body temperature and physical activity were recorded continuously every 5 minutes using the temperature- and activity-sensitive tags which had been implanted abdominally for the fever study. Respiratory rate (breaths per minute) was also recorded during exposure to the thermal challenges. Blood was collected before and after exposure to high temperature and water deprivation treatment. The haematocrit and plasma osmolality were then measured to

determine the hydration status of pigs. Data on core body temperature and physical activity were analysed using the repeated measures two-way ANOVA. Data was tested for normality and homoscedasticity before being analysed. Data on haematocrit, plasma osmolality and the ratios were analysed using the paired student *t*-test. Comparative graphs indicating the body temperature indices (i.e. core body temperature data) of the pigs were drawn against the simulated environmental conditions tested. Graphs indicating the changes in activity and respiratory rates during exposure were plotted. A table with comparative data on the plasma osmolality and haematocrit was also constructed.

### 3.3 Skin experiment

In this study, uncastrated male pigs (Large White (n=7), Windsnyer (n=5) and Kolbroek (n=4)) aged between six and eight months old were used to investigate the skin characteristics. Full-thickness skin samples were collected from the dorsal interscapular region, lateral thoraco-abdominal region and ventral abdominal region of the bodies of the pigs. The samples were preserved in 10% formalin for at least 7 days. The samples were then processed through an automated tissue processor for 21 h and embedded in molten paraffin wax. Sections measuring 6 µm in thickness were cut using a manual microtome. Thereafter the sections from the different regions of the body were all either stained using the One-Step Mallory-Heidenhain stain, for the evaluation of general morphology or, they were stained with Fontana stain to show the presence of melanin in the skin. After staining the sections, photomicrographs were taken using a digital colour camera TV-Lens coupled to a microscope and computer with a board for image capture. The thickness of the epidermis, dermis and hypodermis; and the perimeter and depth of the sweat glands were then measured using the morphometric software, Image J. All the data were analysed using the repeated measures two-way analysis of variance ANOVA and then followed by a Tukey's Multiple Comparison *post-hoc Test* if significant differences were detected. Data was tested for normality and homoscedasticity before being analysed. A table with comparative data on the thickness of skin layers and presence of melanin was drawn up. Three punnet tables with comparative

photomicrographs showing the skin layers, and highlighting the size and depth of sweat glands as well as the presence of melanin were constructed for the three breeds.

# CHAPTER 4: CHARACTERISATION OF THE FEBRILE AND SICKNESS BEHAVIOURAL RESPONSES INDUCED BY BACTERIAL AND VIRAL MIMETICS IN THE LOCAL KOLBROEK, WINDSNYER AND EXOTIC LARGE WHITE PIGS

## 4.1 Introduction

Worldwide, infectious diseases of pigs caused by bacteria or viruses are a major problem and cost piggeries billions of dollars each year. The annual economics loss caused by infectious diseases in the pig industry in China alone is estimated at CNY40 billion (about USD6.12 billion)[2] (Zhao et al., 2012), primarily as a result of decreased productivity, morbidity, mortality as well as treatment and vaccinations costs (Holtkamp et al., 2013). In addition, infectious diseases of livestock result in disruption of international trade and rural economies, decreased market values, and increase food insecurity (Dehove et al., 2012). Subsistence farmers are particularly vulnerable to animal losses as a result of disease because they cannot afford veterinary costs, yet because of prior exposure to an array of pathogens, local breeds are proposed to be better able to withstand disease.

Following the infection of the host animal, pathogenic microorganisms activate a characteristic set of immune, physiological, metabolic and behavioural responses, known collectively as the acute phase response (Owen-Ashley et al., 2006). The acute phase response begins when macrophages produce and release a number of cytokines such as interleukin-1, interleukin-6 and tumor necrosis factor-α (Bochsler and Slauson, 2002). These cytokines induce a cascade of events that lead to the display of characteristic clinical changes such as fever and sickness behaviours. The sickness behaviours include reduction in voluntary feed intake (anorexia) and water intake (adipsia), decreased physical activity (lethargy), increased sleep, and reduction in social activities and grooming behaviour (Gabay and Kushner, 1999, Hart, 1988). Reduced feed and water intake result in the loss of body mass. Of the acute phase responses, fever and reduction in voluntary feed intake, loss of body mass and lethargy are the

[2] CNY1 = USD0.15 (Exchange rate on the 3 March 2012)

most commonly recognised and well-established responses to infection (Owen-Ashley et al., 2006).

Understanding these sickness behaviours helps improve the detection of unhealthy animals and allow the isolation of sick animals to minimise the transmission of pathogens in the herd and initiate treatment. Subsequently, the isolation and treatment will reduce economic losses due to the decline in animal performance such as reduced growth and increased mortality. If the local breeds of pigs are better adapted to withstand disease it is likely they will exhibit a muted acute phase response when challenged with a pathogenic micro-organism, compared to exotic breeds as has been shown in the Mexican hairless pigs. Experimental infection of the Mexican Hairless pigs with *Salmonella*, *Escherichia coli* and *Pasteurella* demonstrated that they had a higher concentration of interleukin-1β (IL-1β) and interleukin-4 (IL-4), (which are used as disease resistance indicators) and reflected a higher disease resistance to infection than in the commercial Yorkshire x Landrace crosses (Mejia-Martinez et al., 2008). Also a study in Vietnam showed that the local Mong Cai breed of pig had a higher concentration of leucocytes in response to a disease challenge than the Large White and Landrace pigs indicating a better disease resistance (Nguyen et al., 1996).

The South African livestock sector is unique in that there is a particularly high proportion of subsistence farmers involved with livestock farming. The subsistence farmers rely mostly on the two common local breeds the Windsnyer and Kolbroek pigs. Anecdotal evidence, based on the subjective assessment by the farmers, indicates that the local pigs are resistant to diseases. However, there is no available scientific evidence to support these claims. Hence I set out to investigate the differences in the physiological and behavioural responses to viral and bacterial mimetics of the local and exotic breeds.

## 4.2 Aims

The aim of the experiment described below was to investigate, characterise and describe the differences between febrile responses and sickness behaviours of the two

local breeds of pig and an exotic breed of pig after injecting them with bacterial and viral mimetics.

## 4.3 Hypothesis

I hypothesised that the local pigs would have a muted febrile response compared to the exotic pigs following administration of three mimetics. To test this hypothesis I measured the physiological and behavioural responses which included core body temperature, physical activity, voluntary feed intake and changes in body mass. Furthermore I recorded the clinical signs displayed by the pigs following injection of bacterial and viral mimetics.

## *4.4* Materials and Methods

All experimental procedures used in this study were approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, Johannesburg, South Africa (AESC Clearance Certificate Number 2010/58/04 – see Appendix 9.1).

### 4.4.1 Animals and housing

A total of 24 male pigs (28 days old) from three *Sus scrofa domesticus* breeds, namely Kolbroek (n=8; 5.4 ± 1.4 kg), Windsnyer (n=8; 8.1 ± 1.6 kg) and Large White (n=8; 6.0 ± 1.5 kg), were purchased from the piggery unit at the Agricultural Research Council (Irene Station, Irene, Pretoria, South Africa). On arrival at the Central Animal Services facilities the pigs were placed in temperature-controlled rooms in groups of four to reduce weaning stress. The breeds of pigs were kept separate and were provided with radiant heat (BR125 IR, 150W, Royal Philips Holland, Yongsan-Ku, South Korea) for 24 h a day for one week during the adaptation period. The pigs were injected intramuscularly with an endectocide (Dectomax, doramectin**,** 0.3 mg/kg, Intervet, Johannesburg, South Africa). Dry wheat straw was provided for bedding in the pens and it was changed daily. The pigs were fed twice a day, at 08:00 and 16:00, with a 15% crude protein maize/soybean meal-based pig grower diet enriched with vitamins and

minerals (Epol, Johannesburg, South Africa). The proximate composition of the of the commercial feed used in the study was: Crude Protein 15%, Moisture 12%, Fat 0.25%, Fibre 0.8%, Calcium 0.08 – 0.1%, Phosphorus 0.06% and Total Lysine 0.075%. Fresh feed was offered at 5% of their daily morning body mass, moistened in the ratio of 2 feed: 1 water and all individuals had equal access to the feed trough. Feed offered at 5% body mass is the recommended feeding rate for grower-finisher pigs to meet their energy requirements and allow for growth (Bay et al., 1995). Drinking water was available *ad libitum*.

To monitor the ambient conditions in the pens, air temperature and relative humidity data were collected every five minutes with a portable weather station (Hobo U12-013 Temp/RH/2 External Data Logger, Onset Computer Corporation, Pocasset, MA, USA) installed approximately 1 m above the ground. The rooms were maintained at 23 ± 2°C throughout the experimental period. Additionally, a 12:12 h light-dark cycle was maintained for the duration of the study, with lights on at 06:00. After a 14-day acclimation period, the pigs underwent surgery to implant temperature-sensitive data loggers and activity and temperature-sensitive tags.

### 4.4.2 Surgery

The pigs were deprived of food and water for 16 h before surgery and then sedated by a deep intramuscular (i.m.) injection of 11 mg/kg ketamine (Bayer Animal Health Division, Isando, Johannesburg, South Africa) and 0.3 mg/kg midazolam (Roche Products, Isando, Johannesburg, South Africa). Surgery on all the pigs was conducted in a theatre in the Central Animal Services at the University of the Witwatersrand, Johannesburg by a veterinary surgeon. Anesthesia was maintained with 2 - 6% isoflurane (Safe Line Pharmaceuticals, Florida, Johannesburg, South Africa) in 100% oxygen via an endotracheal tube. The level of anesthesia was adjusted based on the assessment of heart rate, blood pressure, blood oxygen saturation, rectal temperature (Cardell Veterinary Monitor, Model 9403 BP/$SpO_2$/ECG, CAS Medical Systems, Inc., USA), reflex responses and respiratory rate, which were monitored continuously throughout the anesthesia. An intravenous (i.v.) catheter (24G Jelco I.V. Catheter Radiopaque, Smith

Medical International Ltd, Rossendale, Lancashire, United Kingdom) was inserted in the ear vein for infusion of sterile 0.9% saline (Sabax, Johannesburg, South Africa) to maintain hydration during surgery. The ventral or lateral abdominal areas were then shaved, cleaned and sterilised (2% w/v chlorhexidine acetate, Kyron Laboratories (Pty) Ltd, Benrose, Johannesburg, South Africa). An incision was made through the skin and deeper tissues into the abdominal cavity on the ventral midline of the abdominal wall (Large White and Kolbroek pigs) or on the lateral paralumbar area (Windsnyer pigs). The temperature- and activity-sensitive tags were tethered to the inner abdominal wall to ensure the activity loggers detected whole body movement rather than visceral motility. A freely floating temperature-sensitive data logger was also placed into the abdominal cavity. The incision wounds were closed with 2/0 nylon and 2/0 vicryl sutures (Gabler Medical (Pty) Ltd, Cape Town, South Africa) and sprayed with a topical antiseptic spray (Necrospray, Centaur Labs, Johannesburg, South Africa). Each pig was then injected with an analgesic (Bupranorphine; Temgesic; 0.01 mg/kg i.m., Schering-Plough (Pty) Ltd, Woodmead, South Africa), a non-steroidal anti-inflammatory drug (NSAID) (Flunixine, Finadyne; 2.2 mg/kg i.m., Centaur Labs, Johannesburg, South Africa) and an antibiotic (Penicillin, Peni LA, 15 mg/kg i.m., Virbac Animal Health Division, Centurion, Pretoria, South Africa). Surgery was carried out under sterile conditions and lasted for approximately 30 minutes per pig.

After surgery, pigs with similar body masses were paired within breeds and then placed in pens measuring 2.1 m x 2.7 m in temperature-controlled rooms. During the first five-days of recovery pigs were provided with extra heat (infrared light) for 24 h a day to aid post-surgical recovery. After five days the heat source was withdrawn to allow animals to acclimate to the climatic conditions in the rooms. They were given at least a 10-day recovery period before the first injections were administered.

### 4.4.3 Core body temperature

The core body temperature of each pig was measured with a temperature-sensitive tag (Africa Wildlife Tracking, Pretoria, South Africa) and a temperature-sensitive data logger (StowAway XTI, Onset Computer Corporation, Pocasset, Massachusetts, USA) which

were implanted intra-abdominally. I implanted two devices into each pig in order to provide a backup of the data should one of the devices fail. The tags had the advantage of allowing us to monitor the animal's responses in real-time and also monitor the welfare of the pigs throughout the interventions. All temperature-sensitive tags and data loggers were calibrated against a high accuracy thermometer (Quat 100, Heraeus, Hanau, Germany), in an insulated water-bath, over the range of core body temperatures expected (35-43°C). Following calibration, all of the temperature-sensitive data loggers and tags were accurate to within 0.1°C. The temperature-sensitive tag allowed for the real-time measurement of core body temperature throughout the experiment at a resolution of 0.06°C. The tags transmitted core body temperature data every five minutes to an ultra-high speed downloader modem (Africa Wildlife Tracking, Pretoria, South Africa) placed in the room where the pigs were housed. Data were then relayed via the global system for mobile communication network as a short message service (SMS) and stored on a secure database, which could then be downloaded from the internet hourly using HAWK software (Africa Wildlife Tracking, Pretoria, South Africa). Each temperature-sensitive data logger had a storage capacity of 32kB, which allowed core body temperature to be recorded every 10 minutes for 7 months. The data loggers could record temperature over a range of +34°C to +46°C with a resolution of 0.04°C.

### 4.4.4 Activity

The same tag that measured core body temperature also had a chip that measured activity. Activity was measured using an omni-directional mercury activity switch (TAC433 sensor, Africa Wildlife Tracking, Pretoria, South Africa). The tag transmitted data every five minutes to the modem and data was captured as described for core body temperature above. I validated the activity measurements by behavioural observations. When pigs were sleeping (inactive), activity counts were minimal and represented less than 5% of the maximum activity recorded within a five minute period.

### 4.4.5 Body mass and voluntary feed intake

Pigs were weighed in the morning between 07:30 and 08:00, before giving them feed, using a digital platform scale (Snowrex, Adam Equipment Johannesburg, South Africa) with accuracy of ~ 20 g. The pigs were offered a pre-weighed amount of feed (5% of the combined body masses of the pair of pigs in a pen) between 08:00 and 10:00 before they received injections. Both pigs had equal access to the feed trough. Feed remaining after 2 h of feeding was measured at 10:00 prior to injection of pyrogens/saline. After the pigs had received the pyrogen/saline injection, remaining feed was returned to the pens. Voluntary feed intake was measured per pen rather than per pig (two pigs per pen). The feed remaining from the morning feed portion was weighed at 16:00 and the remnants from the afternoon portion were weighed at 18:00. Voluntary feed intake for the 8 h post-injection period was the sum of the voluntary feed intake between 10:00 and 16:00 and the voluntary feed intake between 16:00 and 18:00. I focused on this time period because, as a diurnal species, approximately 70% of the total 24 h voluntary feed intake occurred between 10:00 and 18:00.

### 4.4.6 Pyrogens

The volumes of the injection solution were calculated based on the body mass recorded on the morning of the injection. Polyinosinic acid: polycytidylic acid (poly I:C, Lot Numbers. 010M4097; 012M4032V; 080M4082, Sigma-Aldrich, Schnelldorf, Germany) was injected at a dose of 0.5 mg/kg in a volume of 0.1 ml/kg. Lipopolysaccharide (LPS, phenol extract of *Escherichia coli* 0111:B4; Lot No. 97F4089, Sigma, St. Louis, Missouri, USA) powder was dissolved in saline (sterile, pyrogen-free 0.9% saline, Sabax, Johannesburg, South Africa) and given at a dose of 2 µg/kg and administered in a volume of 0.1 ml/kg. The killed organisms (cell walls) of *S. aureus* (Lot No. 109H8602, Sigma-Aldrich Logistik GmbH, Schnelldorf, Germany) suspension were injected at a dose of approximately $1.7 \times 10^{10}$ cell walls/kg in a volume of 0.2 ml/kg. The cell walls of *S. aureus*, heat killed and fixed in formalin, were supplied as a suspension in phosphate-buffered saline and were injected without dilution. The doses of each pyrogen were based on findings from previous studies that caused febrile responses in pigs (de Groot

et al., 2007, Gainer and Guarnieri, 1985) and goats (Mphahlele et al., 2004). Sterile saline (0.9%) was injected at a volume of 0.1 ml/kg body mass.

### 4.4.7 Experimental procedure

The pyrogens and saline (control) were administered between 10:00 and 11:00. The pigs were 89 ± 13 days old at the time the first injection was administered. Each of the pig pairs received the pyrogens/saline injections in a controlled randomised crossover experimental design (i.e. repeated measurements that allowed each pig to act as its own control). I allowed a 14-day washout period between injections because tolerance window has been reported to be 14 days in pigs after injecting them with *Salmonella typhimurium* and *Escherichia coli* (Islam et al., 2015). Injections were given into the lateral ear vein, which required mild restraint of the pigs in a sling. The pigs were habituated to handling of the ear whilst in the sling for approximately five minutes per day for five days prior to the injections to reduce stress-induced hyperthermia. The ear was cleaned and disinfected (2% w/v chlorhexidine acetate in alcohol, Kyron Laboratories (Pty) Ltd, Benrose, Johannesburg, South Africa). Before injecting the pigs, the pyrogens and saline were warmed slightly by hand. Injections were administered aseptically using 14G catheters (Jelco I.V. Catheter Radiopaque, Smith Medical International Ltd, Rossendale, Lancashire, United Kingdom). After the injections, the pigs were returned to their pens so that I could monitor changes in core body temperature, body mass, activity and voluntary feed intake, and to assess clinical signs.

I monitored the pairs of pigs every half hour post-injection up to 18:00 for the changes in sleepiness and the following five clinical signs: seizures, cyanosis, vomiting, diarrhoea and hyperventilation. I used the focal-animal method that combined event- and time based approach to collect the data on the animals as described by Altmann (1974). The severity and duration of each clinical signs was assigned either a negative sign (for the absence of clinical signs) or a positive sign (for the presence of clinical signs). As indicated below Table 4-2 (page 59) the number of positive signs relates to the duration over which the clinical sign was observed, i.e. the greater the number of positive signs the longer the period over which the clinical sign was observed.

## 4.5 Data Analysis

### 4.5.1 Febrile responses

One of the Kolbroek pigs developed a hernia and was euthanised before receiving the *S. aureus* injection, which reduced my sample size to seven Kolbroek pigs for the *S. aureus* intervention. One Windsnyer pig became hypothermic (37.2°C) after receiving a poly I:C injection and never developed a fever. The data from this pig were therefore excluded from the analyses. Because the temperature-sensitive tags occasionally missed readings, I analysed data recorded from the data loggers except for one Windsnyer pig whose data logger failed. For this individual pig core body temperatures recorded by the temperature-sensitive tag was used. Prior to the failure of the data logger, core body temperatures from the temperature-sensitive tag and data logger correlated well.

The core body temperature responses of pigs after receiving saline were not significantly different between the three breeds of pigs ($F_{2,21}$ = 2.70, $P$ = 0.090) and therefore I averaged core body temperature for all pigs for the 8 h period (10:00-18:00) following saline injection (40.0 ± 0.4°C). To investigate the time of initiation and termination of fever by pyrogen per breed of pig, repeated measures ANOVA to compare each 10 min recording of core body temperature following injection of each pyrogen to that of the mean core body temperature following saline injection (40.0°C) was used. Time of initiation of fever was the first 10 minute time-point within the post-injection period at which core body temperature was significantly higher than the average core body temperature of pigs after receiving saline. The time of termination of the fever was time within the post-injection period when the core body temperature of pigs was no longer significantly greater than the average of the core body temperature of the pigs after receiving saline. The duration of fever was calculated as the difference between time of initiation and termination of the fever. The peak core body temperature during the fever was calculated as the highest body temperature recorded within 8 h after receiving pyrogens. The time to peak core body temperature was defined as the time between receiving pyrogens and the time at which the highest core body temperature was

recorded. The thermal response index (TRI) as described by (Murakami and Ono, 1987) and Lipton and Ticknor (1979) was used as a measure of the severity of each fever as it incorporates both the duration of the fever and the magnitude of the fever. The TRIs (°C.h) were calculated as the sum of the differences between each 10 min recording of core body temperature of each pig during the 8 h (10:00-18:00) post injection period of each pyrogen and the mean core body temperature of all pigs at 10-minutes intervals over the same time period after receiving saline. A one-way ANOVA followed by the Tukey's Multiple Comparison *post-hoc* Test to assess if there were differences in peak core body temperature, time to peak core body temperature and TRIs between the three breeds of pigs in response to the different pyrogens were used. Data was tested for normality and homoscedasticity before being analysed.

### 4.5.2 Sickness behaviours

#### 4.5.2.1 Activity

Change in activity (Maes et al., 2012, Harden et al., 2008, Hetem et al., 2012) was calculated as the difference between the sum of activity during an 8 h period (10:00-18:00) after each injection and the average sum of activity during an equivalent time period in the five days prior to the injection and expressed as a percentage of the average sum of activity during the equivalent period in the five days prior to the injection. There were no significant differences in the percentage change in activity between the breeds of pigs ($F_{2,21}$ = 1.91; $P$ = 0.17) following saline injection, hence the 8 h percentage change in activity values post saline injection to get an average value for all the three breeds of pigs were combined. I focused on this time period because, as a diurnal species, approximately 60% of the total 24 h physical activity occurred between 10:00 and 18:00.

#### 4.5.2.2 Voluntary feed intake

Percentage change in voluntary feed intake (Maes et al., 2012, Harden et al., 2008) per pen was calculated as the difference between voluntary feed intake between 10:00 and

18:00 on the day of the injection and voluntary feed intake for the same time period on the day prior to the injection, expressed as a percent of the feed intake on the day prior to the injection. There were no significant differences in the percentage change in voluntary feed intake between the breeds of pigs ($F_{2,9}$ = 0.33; $P$ = 0.73) following saline injection hence the voluntary feed intake values when pigs were injected with saline to get an average value for all three breeds were combined.

#### 4.5.2.3 Body mass changes

Percentage change in body mass (Maes et al., 2012, Harden et al., 2008) was calculated as the difference between the body masses measured 22 h after the pigs received the injection and the body masses measured 2 h before the injection, expressed as a percent of the body masses measured 2 h before the injection. There were no significant differences in the percentage change in body mass between the breeds of pigs ($F_{2,21}$ = 2.14; $P$ = 0.14) following saline injection hence the body mass values when pigs were injected with saline to get an average value for all three breeds were combined.

We used a one-way ANOVA to compare the sickness behaviour responses of each breed following administration of each pyrogen and the control (i.e. when pigs were injected with saline). Tukey's Multiple Comparison *post-hoc* Test was used to compare if there were differences in sickness behaviours between the breeds. Data was tested for normality and homoscedasticity before being analysed. The data was analyzed according to the linear model for repeated measures below:

$$y_{ijkl} = \mu + \alpha_i + b_j + c_k + (\alpha c)_{ik} + e_{ijk}$$

where: $y_{ijk}$ is the measured response (core body temperature and activity counts) on the $j^{th}$ breed of pig when injected with the $i^{th}$ mimetic (poly I:C, LPS and *S. aureus*),

$\mu$ is the overall mean effect,

$\alpha_i$ is the $i^{th}$ fixed breed of pig (Windsnyer, Kolbroek and Large White) effect;

$b_j$ is the random effect of the $j^{th}$ breed of pig within the $i^{th}$ treatment,

$c_k$ is a fixed effect of mimetic $k$,

$(\alpha b)_{ij}$ is the fixed interaction effect between breed of pig and treatment

$e_{ijk}$ is the random error associated with the $j^{th}$ breed of pig assigned to the $i^{th}$ treatment at time $t$

All statistical analyses were performed using GraphPad Prism 5 (San Diego CA, USA). All data are reported as means ± SD. Significance was accepted at $P < 0.05$.

## 4.6 Results

### 4.6.1 Febrile response

Figure 4-1 shows the core body temperature responses over approximately 12 h for the three breeds of pigs when they received intravenous injections of poly I:C, LPS, *S. aureus* or saline. The febrile responses to all three pyrogens were monophasic and resolved within 8 h in all breeds (Figure 4-1). Table 4-1 summarises the febrile responses of each breed in terms of the time of initiation of the fever, duration of the febrile response, peak body temperature, time to peak body temperature and 8 h thermal response index.

The time to initiate a fever after receiving poly I:C was similar between the three breeds of pigs, but the duration of the febrile response lasted approximately 150 minutes longer, and peak core body temperature was reached significantly later ($F_{2,20}$ = 6.40, $P$ = 0.0068) in the Large White pigs compared to the Windsnyer ($P$ = 0.014) and Kolbroek ($P$ = 0.015) pigs. The longer fever duration resulted in a significantly higher thermal response index (TRI) ($F_{2,20}$ = 13.70, $P$ = 0.0003) for Large White pigs compared to Windsnyer ($P$ = 0.04) and Kolbroek ($P$ = 0.003) pigs, even though there was no difference in the peak core body temperature reached ($F_{2,20}$ = 1.80, $P$ = 0.19).

The Large White pigs took nearly twice as long as the local breeds to initiate a fever in response to LPS administration (Table 4-1). The longer time to initiate the fever was also combined with earlier termination of the fever relative to the local breeds which resulted

in a shorter (1-3 h) fever duration for the Large White pigs compared to both the Kolbroek pigs and Windsnyer pigs (Table 4-1). The short fever duration in Large White pigs in response to LPS, resulted in a smaller TRI for the Large White pigs ($F_{2,21}$ = 6.22, $P$ = 0.009) compared to that of Windsnyer ($P$ = 0.01) and Kolbroek ($P$ = 0.04) pigs, even though there was no difference in peak body temperature reached ($F_{2,21}$ = 3.37, $P$ = 0.054) or time to peak body temperature ($F_{2,21}$= 4.40, $P$ = 0.056).

Both local breeds of pigs displayed a shorter time to initiate a fever (40 min earlier) after *S. aureus* injection than did the Large White pigs (Table 4-1). Despite the earlier time to initiate the fever in the local pigs there were no significant differences in the duration of fever, peak body temperature ($F_{2,20}$ = 0.48; $P$ = 0.62), time to peak body temperature ($F_{2,20}$ = 1.73; $P$ = 0.20) or TRIs ($F_{2,20}$ = 1.93; $P$ = 0.17) between the local pig breeds and the Large White pigs.

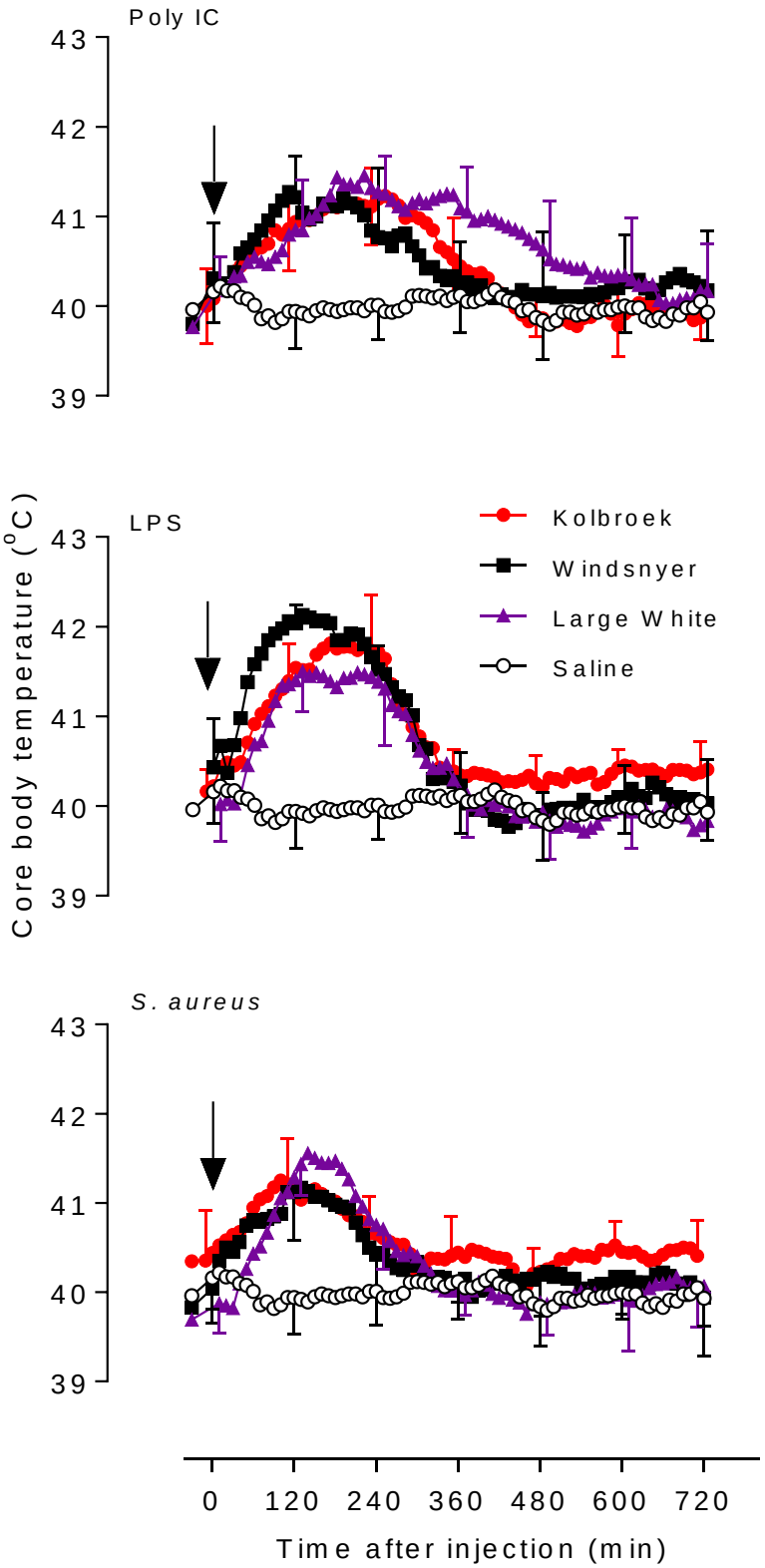

Poly IC
LPS
S. aureus
Kolbroek
Windsnyer
Large White
Saline
Core body temperature (°C)
43
42
41
40
39
0
120
240
360
480
600
720
Time after injection (min)

**Figure 4-1**: Profile of core body temperature of three breeds of pigs following the intravenous injection of polyinosinic acid: polycytidylic acid (poly I:C; 0.5 mg/kg); lipopolysaccharide (LPS; 2 µg/kg) and *Staphylococcus aureus* (*S. aureus;* 1.7 ± 0.1 x $10^{10}$ cell walls/kg) and saline. The arrows depict the time of injection. Data are mean ± SD.

**TABLE 4-1:** Indices of the febrile responses in three different breeds of pigs injected intravenously with polyinosinic acid: polycytidylic acid (poly I:C; 0.5 mg/kg); lipopolysaccharide (LPS; 2 µg/kg) and *Staphylococcus aureus* (*S. aureus;* 1.7 x $10^{10}$ cell walls/kg).

| Pyrogen | Fever Indices | Windsnyer | Kolbroek | Large White |
|---|---|---|---|---|
| Poly I:C | | (n=7) | (n=8) | (n=8) |
| | Initiation (min) | 30 | 40 | 30 |
| | Duration (min) | 380 | 390 | 540 |
| | Peak core $T_b$ (°C) | 41.5 ± 0.3[a] | 41.6 ± 0.4[a] | 41.8 ± 0.4[a] |
| | Time to peak core $T_b$ (min) | 198 ± 61[a] | 175 ± 50[a] | 262 ± 66[b] |
| | TRI - 8 h (°C.h) | 4.1 ± 1.1[a] | 5.4 ± 2.4[a] | 9.2 ± 2.0[b] |
| LPS | | (n=8) | (n=8) | (n=8) |
| | Initiation (min) | 30 | 30 | 50 |
| | Duration (min) | 490 | 360 | 300 |
| | Peak core $T_b$ (°C) | 42.0 ± 0.4[a] | 42.3 ± 0.4[a] | 41.9 ± 0.3[a] |
| | Time to peak core $T_b$ (min) | 178 ± 45[a] | 143 ± 43[a] | 178 ± 40[a] |
| | TRI - 8 h (°C.h) | 7.6 ± 1.3[a] | 7.8 ± 1.2[a] | 5.5 ± 1.6[b] |
| *S. aureus* | | (n=7) | (n=8) | (n=8) |
| | Initiation (min) | 20 | 20 | 60 |
| | Duration (min) | 290 | 240 | 260 |
| | Peak core $T_b$ (°C) | 41.5 ± 0.4[a] | 41.4 ± 0.7[a] | 41.7 ± 0.3[a] |
| | Time to peak core $T_b$ (min) | 118 ± 41[a] | 121 ± 43[a] | 149 ± 18[a] |
| | TRI - 8 h (°C.h) | 3.4 ± 1.7[a] | 4.3 ± 1.1[a] | 4.8 ± 1.4[a] |

Note: Within a row, means without a common superscript differ; $P < 0.05$ was statistical significant and one-way repeated measures ANOVA was used; The values of peak core $T_b$ and time to peak $T_b$ were calculated from individual pig values and are not directly related to 12 h average $T_b$ shown in Figure 4-1; $T_b$ – core body temperature; TRI – Thermal Response Index; Data are shown as mean ± SD.

### 4.6.2 Sickness behaviours

#### 4.6.2.1 Activity changes

Figure 4-2 shows the percentage change in activity in the 8 h post injection period (between 10:00 and 18:00) for the three breeds of pigs when they received intravenous injections of poly I:C, LPS, *S. aureus* or saline. All three breeds of pigs showed a significant reduction in activity following poly I:C injection when compared to saline ($F_{3,27} = 15.46$; $P < 0.0001$; Figure 4-2 upper panel). There was no significant difference ($P > 0.05$) in the reduction of activity between the three breeds of pigs.

The injection of LPS also resulted in a significant reduction in activity in the Large White pigs ($P = 0.0047$) and Windsnyer pigs ($P = 0.014$) ($F_{3,28} = 28.62$; $P < 0.0001$; Figure 4-2 middle panel) compared to when the pigs received saline. The Kolbroek pigs did not show a significant reduction in activity following LPS injection ($P = 0.78$). The magnitude of activity reduction was significantly greater in the Windsnyer ($P = 0.0012$) and Large White pigs ($P = 0.0054$) compared to the Kolbroek pigs.

The injection of *S. aureus* resulted in a significant reduction in activity in the Large White pigs ($P = 0.0064$) and Windsnyer pigs ($P = 0.0047$) but activity in the Kolbroek pigs did not change ($P = 0.56$) when compared to when the pigs received saline ($F_{3,27} = 13.75$; $P < 0.0001$; Figure 4-2 lower panel). There was also a greater reduction in activity in the Windsnyer ($P = 0.0026$) and Large White pigs ($P = 0.036$) compared to the Kolbroek pigs.

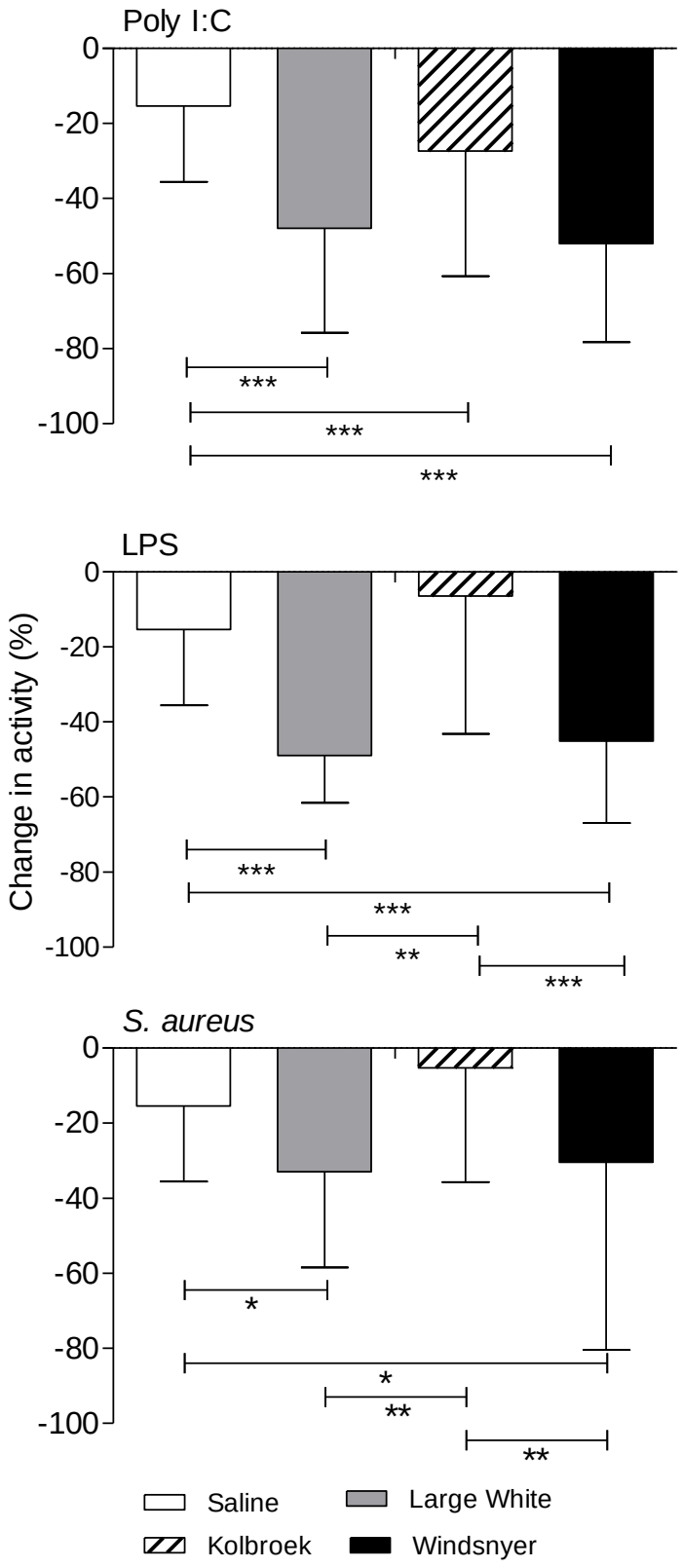

Poly I:C
0
-20
-40
-60
-80
-100
***
***
***
LPS
Change in activity (%)
0
-20
-40
-60
-80
-100
***
***
**
***
S. aureus
0
-20
-40
-60
-80
-100
*
*
**
**
Saline
Large White
Kolbroek
Windsnyer

**Figure 4-2:** Changes in activity in the three breeds in pigs after injecting them with lipopolysaccharide, polycytidylic acid: polyinosinic acid, *Staphylococcus aureus* and saline.

Change in activity was calculated as the difference between the sum of activity during an 8 h period (10:00-18:00) after each injection and the average sum of activity during an equivalent time period in the five days prior to the injection and expressed as a percentage of the average sum of activity during the equivalent period in the five days prior to the intravenous injections of poly I:C (0.5 mg/kg), LPS (2 µg/kg), *S. aureus* (1.7 ± 0.1 x 1010 cell walls/kg) and saline. Asterisks represent statistical differences (one-way repeated measures ANOVA; ** $P$<0.01; *** $P$<0.0001). Negative values represent a reduction below pre-injection levels. Data are mean ± SD.

#### 4.6.2.2 Voluntary feed intake

Figure 4-4 shows the voluntary feed intake over ~8 h for the three breeds of pigs when they received intravenous injections of poly I:C, LPS, *S. aureus* or saline. Following the injection of poly I:C, all three breeds of pigs exhibited a significant reduction ($F_{3,12}$ = 105.20; $P$ < 0.0001) in voluntary feed intake when compared to when pigs received saline (Figure 4-4 upper panel). The poly I:C injection resulted in complete cessation of feeding in the Large White and a 90% decrease in feed intake in the Kolbroek and Windsnyer pigs during the 8 h post injection period (i.e., from 10:00 to 18:00). There were no significant differences ($P$ > 0.05) in the percentage change in voluntary feed intake between the three breeds of pigs after they received poly I:C.

After the injection of LPS all three breeds of pigs exhibited a significant reduction in voluntary feed intake during the 8 h post injection period (i.e. from 10:00 to 18:00) compared to when pigs received saline ($F_{3,12}$ = 18.34; $P$ = 0.0004; Figure 4-4 middle panel). There were no significant differences ($P$ > 0.05) between the breeds of pigs in the reduction of voluntary feed intake after they received LPS.

Only the Large White pigs showed a significant reduction ($F_{3,12}$ = 17.32; $P$ = 0.00048) in voluntary feed intake after they received *S. aureus* ($P$ = 0.0097) whereas the reduction

in voluntary feed intake for Kolbroek and Windsnyer pigs was not significantly different from when the pigs received saline ($P > 0.05$; Figure 4-4 lower panel). The reduction in feed intake in Windsnyer pigs was significantly less than the feed intake reduction observed in both Kolbroek ($P = 0.0067$) and Large White pigs ($P = 0.00063$).

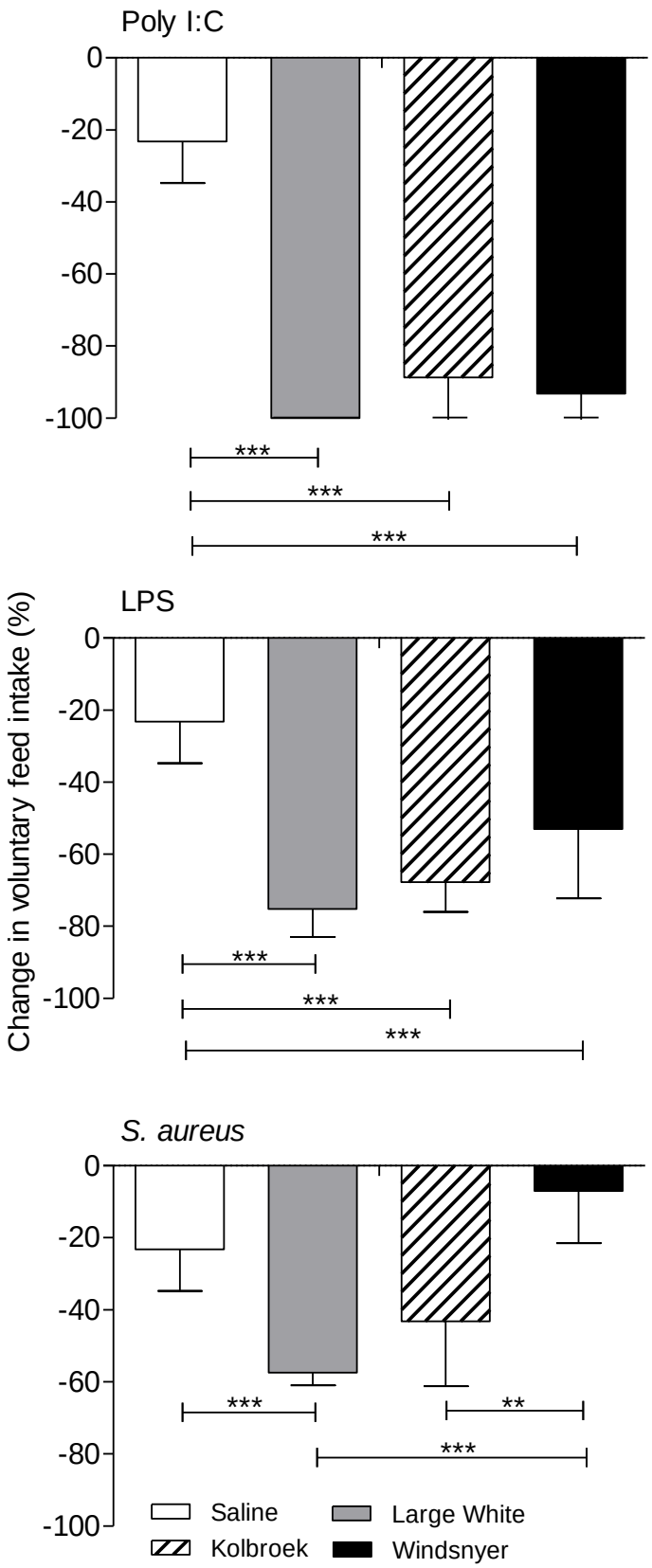

Poly I:C
LPS
S. aureus
Change in voluntary feed intake (%)
0
-20
-40
-60
-80
-100
***
***
***
**
Saline
Large White
Kolbroek
Windsnyer

**Figure 4-3**: Change in voluntary feed intake in the three breeds in pigs after injecting them with lipopolysaccharide, polycytidylic acid: polyinosinic acid, *Staphylococcus aureus* and saline.

Change in voluntary feed intake was calculated as the difference between voluntary feed intake between 10:00 and 18:00 on the day of injection and voluntary feed intake for the same time period on the day prior to the injection and expressed as a percentage of the voluntary feed intake for the same time period on the day prior to the injections of poly I:C (0.5 mg/kg), LPS (2 µg/kg), s. aureus ($1.7 \pm 0.1 \times 10^{10}$ cell walls/kg) and saline. Negative changes represent a reduction below pre-injection levels. Asterisks represents statistical differences (one-way repeated measures ANOVA: ** $P < 0.01$; *** $P < 0.0001$). Data are mean ± SD.

#### 4.6.2.3 Body mass changes

Figure 4-5 shows the body mass changes over approximately 22 h for the three breeds of pigs after they received intravenous injections of poly I:C, LPS, *S. aureus* or saline. Following the injection of poly I:C, all three breeds of pigs exhibited a significant reduction in body mass when compared to when they received saline ($F_{3,27} = 18.24$; $P < 0.0001$; Figure 4-5 upper panel). There were no significant difference ($P > 0.63$) between the breeds in the reduction in body masses after they received poly I:C.

Only the Windsnyer pigs ($P = 0.046$) displayed a significant reduction in body mass 22 h after they received LPS compared to saline ($F_{3,28} = 4.23$; $P = 0.017$; Figure 4-5 middle panel). The Kolbroek pigs maintained a positive body mass gain while the average gain in body mass of the Large White pigs was close to zero. The reduction in body mass of the Kolbroek pigs was significantly different ($P = 0.015$) to that of the Windsnyer pigs, but not the Large White pigs ($P = 0.13$), after they received LPS.

The *S. aureus* injection caused a significant reduction in body mass of Windsnyer pigs ($P = 0.028$) compared to when pigs received saline ($F_{3,27} = 3.65$; $P = 0.029$; Figure 4-5 lower panel). However, the Large White and Kolbroek pigs continued to gain body mass

following injection of *S. aureus*. There was no significant difference ($P > 0.39$) between the breeds in the percentage change in body masses after they received *S. aureus*.

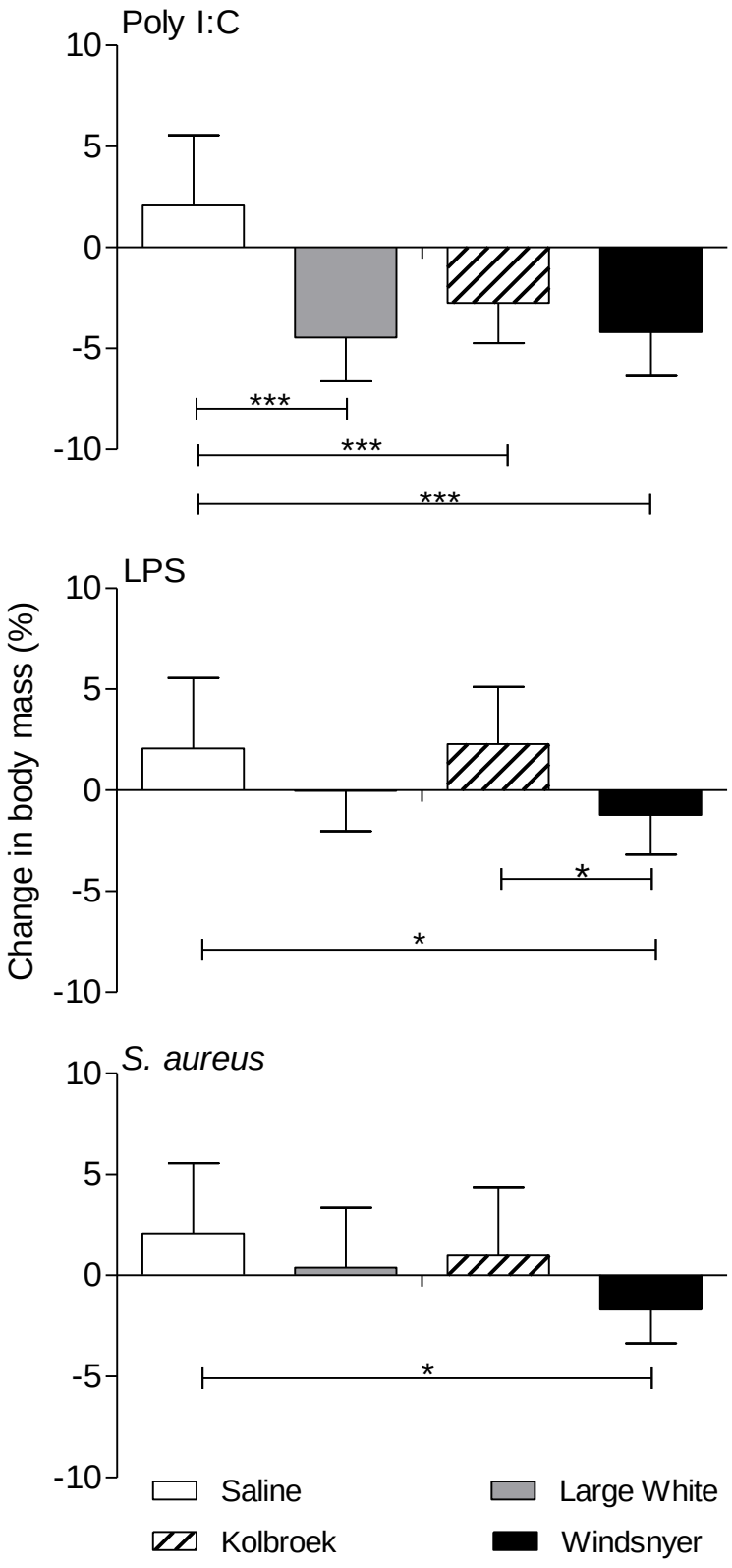

Poly I:C
LPS
S. aureus
Change in body mass (%)
10
5
0
-5
-10
***
***
***
*
*
*
Saline
Large White
Kolbroek
Windsnyer

**Figure 4-4**: Change in body mass in the three breeds in pigs after injecting them with lipopolysaccharide, polycytidylic acid: polyinosinic acid, *Staphylococcus aureus* and saline.

Change in body mass was calculated as the difference between the body mass measured 22 h following the injections and the body mass measured 2 h before the injections and expressed as a percentage of the body mass measured 2 h before the intravenous injection of poly I:C (0.5 mg/kg), LPS (2 µg/kg), *S. aureus* (1.7 ± 0.1 x 1010 cell walls/kg) and saline. Asterisks represent statistical differences (One-way repeated measures ANOVA; * $P < 0.05$; *** $P < 0.0001$). Data are mean ± SD.

#### 4.6.2.4 Clinical signs

Table 4-2 shows the clinical signs over ~8 h (10:00-18:00) for the three breeds of pigs that received intravenous injections of poly I:C, LPS, *S. aureus* or saline. The Large White and, to a lesser degree, the Kolbroek pigs showed severe clinical signs (seizures, cyanosis and hyperventilation; Table 4-2) after receiving *S. aureus*. The injection had to be halted midway and the remaining pyrogen was then injected after the pigs had recovered (~8 min). Although the Windsnyer pigs did not display seizure, cyanosis and hyperventilation during the injection of *S. aureus*, they were the only breed to vomit shortly after the injection. Injection of poly I:C induced vomiting in all three breeds of pigs, it also resulted in more severe diarrhoea, hyperventilation and increased sleeping in the post-injection period in the Large White pigs than it did in the Kolbroek and Windsnyer pigs (Table 4-2). The Large White and Kolbroek pigs started vomiting within an hour of the poly I:C injection which was followed by the development of watery diarrhoea which lasted for 2 h. The Large White pigs also experienced watery diarrhoea, hyperventilating and increased sleeping to a greater extent than the local breeds in response to LPS injection (Table 4-2).

**TABLE 4-2**: Behavioural scores of Windsnyer, Kolbroek and Large White pigs during an 8 h period after intravenous injection with polyinosinic acid: polycytidylic acid (poly I:C) (0.5 mg/kg); lipopolysaccharide (LPS) (2 µg/kg) and *Staphylococcus aureus* (*S. aureus*) (1.7 x $10^{10}$ cell walls/kg)

| | Poly I:C | | | LPS | | | *S. aureus* | | | Saline | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | WN | KB | LW | WN | KB | LW | WN | KB | LW | WN | KB | LW |
| Seizures | – | – | – | – | – | – | – | ++ | +++ | – | – | – |
| Cyanosis | – | – | – | – | – | – | – | ++ | +++ | – | – | – |
| Sleeping | + | ++ | +++ | + | ++ | +++ | – | + | ++ | – | – | – |
| Vomiting | + | + | + | – | – | – | + | – | – | – | – | – |
| Diarrhoea | – | + | ++++ | – | + | ++ | – | + | – | – | – | – |
| Hyperventilation | – | + | +++ | + | + | +++ | – | + | ++ | – | – | – |

**Note:** WN – Windsnyer; KB – Kolbroek; LW – Large White. Negative (-) symbol means the parameter was absent; positive (+) symbol indicates the presence of the parameter with an increasing intensity denoted by the number of the positive (+) symbols. **Seizures and cyanosis: +** = up to 3 minutes; **++** = 4 to 5 minutes; **+++** = 5 to 8 minutes; **Sleeping: +** = up to 2 h; **++** = 2 to 4 h; **+++** = 4 to 6 h; **Diarrhoea and vomiting: +** = up to 30 minutes; **++** = 30 minutes to 1 h; **+++** = 1 to 2 h; **++++** = > 2 h; **Hyperventilation: +** = up to 1 h; **++** = > 1 to 2 h; **+++** = > 2 h

## 4.7 Discussion

To my knowledge, this is the first study to compare the febrile responses and sickness behaviours in the local Kolbroek and Windsnyer pigs and the exotic Large White pigs after being injected with poly I:C (a viral mimetic), LPS (a gram-negative bacterial derivative) and *S*. *aureus* (gram-positive bacteria) cell walls. All the pigs in the present study responded to simulated infection with the three mimetics by developing fever and sickness behaviours. The present results show that the febrile responses induced by the poly I:C and LPS in the exotic pigs had different characteristics to those in local pigs but all three breeds had similar fever indices in responses induced by *S. aureus*. Yet the febrile responses were not consistently longer for the larger large White pigs. The Large White pigs developed a longer fever in response to poly I:C (~150 minutes), a higher

thermal response index and took a longer time to attain peak core body temperature compared to the local breeds. In contrast, the Large White had a lower thermal response index after receiving LPS compared to the local breeds of pigs.

In terms of sickness behaviours, poly I:C resulted in a decrease in activity, voluntary feed intake and body mass in all the pigs. LPS reduced voluntary feed intake in all the pigs, but reduced activity in the Large White and Windsnyer pigs, and led to a reduction in body mass in the Windsnyer pigs only. It is important to note that voluntary feed intake was measured over 8 h whereas body mass was measured 22 h after injection, so there may have been time to increase voluntary feed intake overnight to compensate for reduced food intake during the day. Indeed, crossbred barrows injected intraperitoneally with LPS, at 0.5 µg/kg BM, compensated for a reduced voluntary feed intake over the first four hours post-injection by increasing feeding between 4 and 8 h post injection (Johnson and von Borell (1994). The ability to compensate for reduced voluntary feed intake in the Large White and Kolbroek pigs might have important implications for a pig production system primarily interested in growth rate. A compensatory increase in feed intake suggests that the Large White and Kolbroek pigs would maintain their growth rates and may attain the desirable marketing body mass at the same time as their uninfected counterparts, provided the infection is mild. *Staphylococcus aureus* resulted in reduced activity in the Large White and Windsnyer pigs, reduced voluntary feed intake in the Large White pigs and body mass in the Windsnyer pigs only. Although the Windsnyer pigs showed no significant change in voluntary feed intake following *S. aureus* injection, they lost body mass which probably was due to the extensive vomiting observed in this breed only. In response to poly I:C, the Large White pigs demonstrated more severe clinical signs, such as diarrhoea and vomiting, than did the local pigs. These observations might be the reason why farmers anecdotally attribute greater disease resistance to local breeds.

Despite differences in sickness behaviours and clinical signs, the febrile responses were remarkably similar between the three breeds of pigs and slight differences may simply be the result of size differences between the pigs. Because the Large White grew much faster than the local breeds of pigs I was not able to control for size. I age matched the

pigs because there are differences in responses to infection between young and old animals. Younger pigs develop more severe clinical signs and symptoms with higher morbidities and mortalities than their older counterparts (Klinge et al., 2009) probably due to their underdeveloped immune system. By age-matching the different breeds of pigs, the faster feed conversion efficiency of the exotic Large White pigs resulted in individuals that were almost twice as large as the local breeds at the time of the mimetic administration. Larger animals display significantly longer duration of fever and higher core body temperatures than smaller animals, as observed in guinea pigs injected with *Pasteurella multocida* (Blatteis, 1974) and rats injected with *Salmonella typhosa* (Ford and Klugman, 1980). Although body size may account for the larger thermal response index for the Large White pigs in response to poly I:C, there was no difference in thermal response index between the breeds following injection of *S. aureus* and the Large White pigs actually displayed a lower thermal response index compared to the local Kolbroek and Windsnyer pigs in response to LPS. Thus slight differences in the febrile response could not consistently be attributed to differences in body mass.

Although mimetics have limitations, currently they present the best way to assess immune responses under controlled conditions as they allow for the characterisation of the febrile and sickness behavioural responses and reproduce results (Fortier et al., 2004). However, non-replicating bacterial or viral mimetics do not completely replicate the progression of disease and physiological changes that would occur. Thus, I could not assess whether the differences in febrile responses and sickness behaviours observed in my study might have had survival consequences.

Fever may be beneficial to the host in that high body temperatures inhibit the growth of viruses (Lwoff, 1959, Lwoff, 1969) and kill bacteria (Bennett and Nicastri, 1960, Carpenter et al., 1933). It may also enhance immune functions that promote the removal of pathogens (Hasday et al., 2000). However, in a recent review "*Fever and sickness behaviors: Friend or Foe*", Harden et al. (2015) argued that fever may not be beneficial all the time. The host animal must defend itself against pathogens in order to survive, while the pathogen has to evade or suppress the host animal immune response in order to proliferate, resulting in host-pathogen evolutionary arms race (Lim, 2012, Schulze-

Lefert and Panstruga, 2011), whereby the host is not always the victor. Although fever likely evolved as an adaptive host-defense response to infection, not all fevers may be beneficial to the infected animal (Kluger, 1986), particularly if the magnitude of the fever exceeds a threshold. High magnitude of fevers reduced survival in rats (Banet, 1981) and rabbits (Kluger and Vaughn, 1978).

Similarly, different breeds of pigs have shown differences in fever magnitudes that influenced survival rates. In a study where Large White crosses and Mao Laat pigs were experimentally infected with Classical Swine Fever virus, the Large White crosses developed a higher fever (mean rectal temperature, 40.1°C vs 39.2°C) and had a shorter survival time (11 days vs 18 days) than the Mao Laat pigs (Blacksell et al., 2006). Conversely, I found no differences in magnitude of the febrile response between the Windsnyer, Kolbroek and Large White breeds when they were exposed to bacterial and viral mimetics. Notably, the magnitudes were similar to what other researchers have found for poly I:C (Dilger and Johnson, 2010) and LPS (Johnson and von Borell, 1994, Warren et al., 1997) previously. I was unable to find literature on the febrile response to *S. aureus* in pigs but it is similar to that in goats (Mphahlele et al., 2004). The results from my study are comparable to what has been reported previously in literature (Dilger and Johnson, 2010, Johnson and von Borell, 1994).

In addition to the febrile response, sickness behaviours can facilitate the immune response and inhibit pathogen proliferation (Spencer et al., 2011). For example, a reduction in activity may allow an animal to conserve energy for the energetically costly immune response (Borderas et al., 2008, Hart, 1988) and is believed to increase the individual's survival prospects (Bonneaud et al., 2003).

All breeds decreased activity in response to poly I:C, and only the Large White and Windsnyer to LPS and none of the breeds reduced activity in response to *S. aureus*. LPS administration depressed activity levels and provoked lethargy and anorexia in the pigs (Johnson and von Borell, 1994, Llamas Moya et al., 2008, Warren et al., 1997, Klir et al., 1997) as well as poly I:C (Dilger and Johnson, 2010). The Kolbroek breed of pigs did not show lethargy in response to bacterial mimetics. Although a decrease in physical

activity may have immunological advantages, it may restrict feeding time and subsequently reduce voluntary feed intake. Thus, from a production point of view, the Kolbroek pigs may be better able to maintain a positive growth rate than the other two breeds during bacterial infections.

Yet reducing feed intake may be advantageous as it “may conserve energy for immune activation as gut activity is reduced (Broom and Fraser, 2007) and also reduces the availability of essential nutrients such as iron, that is essential for the proliferation of the pathogens (Hart, 1988)”. All breeds decreased voluntary feed intake in response to poly I:C and LPS, but only the Large White voluntary decreased feed intake in response to *S. aureus*. The reduction in voluntary feed intake following the injection of poly I:C and LPS concurs with the results previously reported in pigs in response to poly I:C (Dilger and Johnson, 2010) and LPS (Elander et al., 2007, Johnson and von Borell, 1994, Kim et al., 2007, Wright et al., 2000, Klir et al., 1997). The reduction in voluntary feed intake in the Large White in response to a gram-positive bacterial mimetic might show that they might not resist the gram-positive bacterial mimetic. Although the reduction in voluntary feed intake might be beneficial in the fight against pathogens it may have long term consequences on animal production. If the pigs cannot compensate for the reduced feed intake associated with infection, it would lead to a reduction in the body mass and farmers may start to suffer economic losses. The reduction in productivity cost the farmers an estimated USD120 million annually in the United States alone (Kennedy, 1960).

All the breeds decreased body mass in response to poly I:C but only the Windsnyer pigs decreased body in response to both LPS and *S. aureus*. Both the Kolbroek and Large White were able to compensate for the reduced feed intake following LPS administration and within 22 h of injection showed no change in body mass. I did not find a decrease in body mass in the Large White pigs. At a low dose (0.5 and 5 µg/kg) administered intraperitoneally. the negative effects on body mass and feed intake only lasted for the first 8 h whereas at a higher dose (50 µg/kg) the LPS continued to suppress voluntary feed intake for up to 24 h (Johnson and von Borell, 1994).

The Large White pigs also compensated for the decreased feed intake following *S. aureus* injection with no change in body mass within 22 h. The Windsnyer pigs however, lost body mass despite no decrease in feed intake, probably as a result of the vomiting following *S. aureus* injection. All pigs vomited following poly I:C, but none vomited following the injection of LPS. Previously similar results have been recorded in the Large White pigs following poly I:C injection (Dilger and Johnson, 2010) as well as LPS (Norimatsu et al., 1995, Klir et al., 1997) as well as when LPS was administered orally (Schaumberger and Schatzmayr, 2012). The result in my study was contrary to what was reported previously in pigs (Girod et al., 2000), who used a higher dose of LPS and it seemed to induce vomiting in the Large White pigs. The Windsnyer pigs appeared to be more resistant to the development of diarrhoea in response to all three mimetics, whereas the Kolbroek developed a short-lived (<30 min) diarrhoea in response to all three mimetics and Large White developed a serious (1-2h) diarrhoea following poly IC and LPS administration. Previously the Large White pigs were shown to develop diarrhoea in response to LPS (Olson et al., 1985, Parrot et al., 1995, Klir et al., 1997) and poly IC (Dilger and Johnson, 2010). The dose 5 mg/kg BM poly I:C (Dilger and Johnson, 2010) induced a secretory diarrhoea that inhibited the absorption of sodium ions and water in the small intestine and, probably only secondarily through an increased volume, by promoting intestinal motility so that it could flush out the toxins recognised by the system. However the mechanisms accounting for why some animals do not develop diarrhea are not known but the injection of LPS is known to up regulate the levels of pro-inflammatory cytokine IL-1β and serum amyloid A when an animal develops diarrhoea (Kruse et al., 2008). The differences observed in responses could be due to the use of a lower dose of 2 µg/kg LPS in the current study compared to the higher doses of between 0.5 and 8 mg/kg LPS used in a previous study.

The LPS induced hyperventilation in all the breeds but with the poly I:C and *S. aureus* inducing hyperventilation in the Large White (> 2 h) and Kolbroek pigs (between > 1 to 2 h) only. Previously the Large White pigs were shown to hyperventilate in response to a higher dose of LPS (200 µg/kg BM given intravenously) (Schaumberger and Schatzmayr, 2012). The Windsnyer pigs appeared to be more resistant to the development of seizures and cyanosis in response to all three mimetics. The Kolbroek

pigs developed short-lived (between 4-5 min) seizures and cyanosis while the Large White pigs developed serious seizures and cyanosis (between 5 and 8 min) following *S. aureus* administration. The greater sensitivity to *S. aureus* of Large White and Kolbroek pigs could be related to their genetic make-up.

The difference in the responses between the local and exotic pigs could be related to their genotypes as well as endemic stability. An online medical dictionary defines endemic stability as a situation in which all factors influencing disease occurrence are relatively stable, resulting in little fluctuation in disease incidence over time; changes in one or more of these factors (for example, reduction in proportion of individuals with immunity from exposure to infectious agent) can lead to an unstable disease conditions (Farlex Partner Medical Dictionary, 2012). In the current study, the personal communication with Dr Arnold Kanengoni, indicated that the Large White pigs were vaccinated while the local pigs were not vaccinated. Pigs that were not vaccinated might have been exposed to pathogens as well as having acquired immunity from the antibodies obtained from the sows (Penrith et al., 2004), Evidence of genetic control of disease resistance to bacterial and viral challenges are well documented in pigs (Zanga et al., 2003, Nguyen et al., 1998, Gibbons et al., 1977), cattle (Carroll et al., 2011) and poultry (Bishop et al., 2002). The variation is caused either by single genes with big effects or multiple genes each with additive effects (Hoffmann, 2010). A large variety of genes associated with variation in host defense have been reported using genomic studies such as microarray and other technology (Hammamieh et al., 2003, Moser et al., 2004). Genetic polymorphisms alter the immune response of animals to pathogens or immune system challenges (Duchet-Suchaux et al., 1991). For example, the variation in severity of clinical disease symptoms among infected pigs within a herd and between herds suggests that genetic differences for resistance or susceptibility to PRRSV exist (Warner et al., 1987). Gene mutations in horses (Horin et al., 2004), turkeys (Tsai et al., 1992) and pigs (Uenishi et al., 2010) were also noted to alter the resistance and susceptibility of these animals to diseases. Alternatively epigenetics could play a role in the development of the disease resistance or tolerance to cope with the new diseases. Epigenetics is defined as heritable changes in gene function from cell to its descendants that occur without altering the DNA sequence (Sinclair et al., 2007, Zhao et al., 2010).

These changes arise principally as a consequence of specific covalent modifications to DNA and associated histone proteins which act in concert with chromatin structure to define the transcriptome associated with a specific cell lineage (Sinclair et al., 2007). In several mammalian species, early life exposure to pathogens pre-program immune responses in later life (Bilbo et al., 2010, Galic et al., 2009).

### 4.8 Conclusion

Although we currently do not know the possible genetic contribution to the observation that the different pig breeds seem to respond differently to pathogen challenges, our understanding of how the local Windsnyer and Kolbroek pigs and the exotic Large White pigs respond to both bacterial and viral pathogens has been enhanced. The local pigs had a lower fever intensity than the Large White pigs after injection of poly I:C but the local pigs had a higher fever intensity following injection of LPS compared to the Large White pigs while there were no differences in the response to the *S. aureus*. The Large White showed severe clinical signs to both the viral and the bacterial mimetics with local Windsnyer pigs showing less adverse responses to both bacterial mimetics. All breeds were sensitive to simulated viral infection (poly I:C) and displayed evident and quantifiable sickness behaviours, including reduction in activity, voluntary feed intake and body mass. However the breeds showed variable responses to bacterial mimetics. Only the Large White and Kolbroek pigs decreased activity in response to LPS and none decreased activity following *S. aureus*. All breeds were sensitive to simulated gram-negative infection (LPS) but only the Large White decreased voluntary feed intake in response to *S. aureus*. Only the Windsnyer pigs decreased body mass in response to LPS and *S. aureus*.

Further studies on measurements of the different cytokines in the local and exotic pigs after challenging them with the different mimetics are necessary to investigate some of the possible mechanisms that will help to understand the immune response of the different pigs to the viral and bacterial mimetics. It is predicted that climate change will alter the epidemiology of diseases causing geographical and seasonal changes in disease distribution thus exposing naïve livestock to new diseases. The newly exposed

populations may lack resistance or acquired immunity leading to the development of serious clinical disease.

The chapter above described the febrile responses and sickness behaviours of the both local and exotic pigs using viral and bacterial mimetics. Climate change will result in changes in the diseases patterns which will lead to new diseases arising in some areas and the old ones re-emerging. Animals not adapted to the viral and bacterial disease may suffer severe consequences leading to losses in productivity. In addition to diseases climate change may also result in high temperatures and severe droughts in areas already water stressed. In the next chapter I investigated the thermoregulatory and physiological abilities of pigs in response to simulated environmental conditions in a climatic chamber. These traits will be important when confronted with hotter and drier conditions that are likely to accompany climate change. The next chapter is a description of the laboratory based trials undertaken to evaluate the physiological responses of pigs under various simulated conditions.

# CHAPTER 5: EVALUATION OF THERMOREGULATORY AND BEHAVIOURAL RESPONSES OF THE LOCAL KOLBROEK, WINDSNYER AND EXOTIC LARGE WHITE PIGS DURING EXPOSURE TO COLD AND HOT AMBIENT TEMPERATURES, HIGH RELATIVE HUMIDITY AND WATER DEPRIVATION

## 5.1 Introduction

The pork industry in the United States loses over USD450 million annually due to heat stress (Pollmann, 2010). The losses from heat stress are primarily due to reduced fertility, reduced feed to meat conversion efficiency, increased health problems as well as increased costs of maintaining pig production facilities within the thermoneutral zone for pigs (St-Pierre et al., 2003). Industrialised pig farming, which is the mainstay of commercial production systems, is a relatively energy intensive (e.g. maintaining optimal temperatures) production system compared to smallholder/subsistence farming where pigs tend to remain outdoors all year long with rudimentary shelters to protect them from predators and inclement weather. Due to limited resources, smallholder farmers don't have access to housing facilities with controllable environmental variables, such as temperature and humidity. Consequently pigs reared in the smallholder sector may be adversely affected by high ambient temperatures unless the breeds of pigs used are better adapted to the widely varying ambient environment.

Most subsistence farmers in southern Africa are located in areas marginal for profitable agriculture. These areas are characterised by very dry winters and extremely hot summers. For example, in the Limpopo Province of South Africa, the maximum temperatures in summer are generally between 30°C and 34°C but temperatures as high as 45°C have been recorded and rainfall can be as low as 200 mm per year (FAO, 2004). Increasing air temperatures are expected to exacerbate the impact of summer weather extremes on the ability of vulnerable farm animals to thermoregulate (Hahn, 1995).

Pigs are particularly sensitive to high environmental temperatures because they have a limited capacity to cool themselves evaporatively. Pigs are relatively poor at panting and have a limited ability to sweat (Ingram, 1965, Ingram, 1967), although they can lose some heat by transepidermal water loss (Cunningham, 1968). Although evaporative cooling helps to maintain core body temperature in hot environments, it comes at a cost as it can deplete body water if there is inadequate water to replenish water lost. The use of evaporative cooling during the hot and dry season can be potentially costly to the animal leading to dehydration and death if there is inadequate water to replenish the losses. Both local and exotic pigs have limited evaporative cooling and will have to trade water conservation off against thermoregulation. Consequently, it is predicted that smallholder livestock farmers will experience the greatest impacts from the emerging climate change-related problems due to their limited use and access to modern technologies in their farming practice (Hoffmann, 2010, Kurukulasuriya et al., 2006) to minimise the adverse effects of heat related stress, feed and water shortages. In the smallholder sector in South Africa, subsistence farmers use two local breeds, the Kolbroek and Windsnyer pigs, while in the commercial sector the Large White breed is the most dominant. Anecdotally, the local breeds are adapted to the harsh conditions of subsistence farming, yet there is little scientific evidence to back up the claim. Consequently it is also unclear whether the local breeds are better able (compared to the exotic breeds) to withstand the thermal and water challenges associated with the predicted climate change in the region. Hence I set out to investigate the differences in the physiological responses to varied thermal and relative humidity challenges as well as water deprivation between the local and exotic breeds of pigs.

## 5.2 Aims

The aim of the experiment described below was to investigate, characterise and describe the physiological responses of the two local breed of pigs and an exotic breed of pig after exposing them to short term cold or high temperatures, high and low humidity and partial water deprivation.

## 5.3 Hypothesis

I hypothesised that the Kolbroek and Windsnyer pigs would be better able to tolerate water deprivation and varied thermal environmental conditions than the Large White pigs. To test this hypothesis I measured changes in physical activity, core body temperature and respiration rates. I also used the van Beaumont's equation (van Beaumont, 1972), to determine the ability of the three pig breeds to defend their plasma volume when exposed to high temperatures and deprived of water.

## 5.4 Materials and Methods

All experimental procedures used in this study were approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, Johannesburg, South Africa (AESC Clearance Certificate Number 2010/58/04 – see Appendix 9.1).

### 5.4.1 Animals and housing

Kolbroek (n=6; 40 ± 1.3 kg); Windsnyer (n=7; 46 ± 7.7 kg) and Large White (n=7; 60 ± 1.3 kg) pigs (*Sus scrofa domesticus*) were used in this study. The piglets of the three breeds were sourced from the Agricultural Research Station, Irene, Pretoria, South Africa. All pigs were seven month old boars at the start of the experimental protocol. The pigs were injected intramuscularly (i.m.) with a 0.03 mg/kg multivitamin preparation (Vitamins A, D and E, Centaur Labs, Johannesburg, South Africa) for the prevention of vitamin deficiencies and to maintain their health and wellbeing during the study. A maximum of four pigs per breed with similar body masses were placed in the same subgroup to prevent bullying. The subgroups of the three different breeds of pigs were always kept in separate pens.

When not undergoing experimental treatments, the pigs were housed in holding pens measuring 1.5 m x 2.0 m in the temperature-controlled farm animal unit of the Central Animal Services (CAS), University of the Witwatersrand. The holding pens were cleaned each morning and disinfected once a week with Chloroxylenol (Microsept®, Johnson

Diversey, Racine, Wisconsin, USA) for hygiene purposes. In the holding pens, water was made available *ad libitum* through nipple drinkers. The pigs were fed once daily at 06:30, with a 15% crude protein maize/soybean meal-based pig grower diet enriched with vitamins and minerals (Epol, Johannesburg, South Africa) at 2% of total body mass of the pigs in each pen for maintenance of body mass. The food was moistened with water in the ratio of 2 feed: 1 water. Dry wheat straw provided bedding and was changed daily before feeding. A 12:12 light-dark cycle was used throughout the experimental protocol, with lights on at 06:00. The ambient temperature in the farm animal unit was 23.0 ± 2.0°C and relative humidity was 45.0 ± 5.0%; both were recorded every 5 min with a data logger (Hobo U12-013 Temp/RH/2 External Data Logger, Onset Computer Corporation, Pocasset, MA, USA).

### 5.4.2 Surgery

The surgical procedure was described under the fever study (see section 4.4.2 in Chapter 4). The same pigs used in the fever study were used in this experiment.

### 5.4.3 Core body temperature

The procedure for measuring core body temperature was the same as that described under the fever study (see section 4.4.3 in Chapter 4).

### 5.4.4 Activity

The procedure for measuring activity was the same as that described under the fever study (see section 4.4.4 in Chapter 4).

## 5.5 Experimental procedures

Each subgroup of each breed of pig was exposed to five experimental treatments, namely cold temperature, thermoneutral temperature, high temperature, high temperatures and water deprivation, and high temperature and high humidity in a randomised cross over design. Cold temperature treatments were conducted in a

climatic chamber which measured 1.2 m x 4.2 m while the thermoneutral and high temperature treatments were conducted in a climatic chamber which measured 2.8 m x 2.3 m. A minimum of two and a maximum of four pigs per subgroup were placed in the climatic chamber at any one time.

On the first day of each treatment, the pigs were transported from the holding pens to the climatic chambers where the pigs were allowed three days to acclimate to their new environment. During the acclimation period the room conditions were maintained at 20.4 ± 0.5°C and 41.7 ± 1.9% relative humidity. Experimental treatments occurred on the fourth and fifth days. The pigs were weighed daily from day two to day five between 06:00 and 06:30 before feed was offered. A weighing cage was placed on top of a platform digital scale (50 g accuracy, Micro, Associated Scale Corporation, Johannesburg, South Africa) and the pigs were habituated to walking in and out of the weighing cage.

During the cold temperature treatment, the room temperature was lowered from 20.4 ± 0.5°C to 5.5 ± 0.5°C at 08:00 on the fourth day (the first day of the two days of the experimental treatment) and kept at that level for 48 h until 08:00 on the sixth day (Figure 5-1; panel F). We did not control relative humidity in the climatic chamber during the cold temperature treatment, but it stabilised at 94.5 ± 5.6%. For the thermoneutral treatment, the room conditions were maintained at 20.4 ± 0.5°C and 41.7 ± 1.9% relative humidity for 48 h (Figure 5-1; panel G). For the high temperature treatments, the room temperature was increased from 20.4 ± 0.5°C to 29.4 ± 0.2°C at 06:30 and took 90 ± 20 minutes to stabilise. At 16:00, the room temperatures were lowered from 29.4 ± 0.2°C to 23.2 ± 2.0°C, to mimic the changes in daytime temperatures (Figure 5-1; panel H). For the high ambient temperature treatments, relative humidity was maintained at 41.7 ± 1.9%. For the high temperature and high humidity treatment, the room temperature was increased as for the high temperature treatment and relative humidity was increased from 41.7 ± 1.9% to 62.5 ± 2.4% at 06:30 and took about 70 ± 30 minutes to stabilise and was then decreased from 62.5 ± 2.4% to 41.7 ± 1.9% at 16:00 (Figure 5-1; panel J). For the high temperatures and water deprivation treatment, pigs were deprived of drinking water for 48 h; ambient temperature and relative humidity were

maintained as for the high temperature treatment. The 48 h of water deprivation began on the third day at 14:00 when the bucket of water was removed from the climatic chamber. Pigs were not given water until after 14:00 on the fifth day after pigs had been weighed and bled (Figure 5-1; panel I). During all other experimental treatments, drinking water was made available in a bucket on day four and day five at 06:30 and 16:00 for an hour and then the bucket was removed when all pigs had imbibed water. The pigs were not given access to water during the experimental treatments to prevent water spillage onto the floor and subsequent wetting of the skin. If the pigs spilled the drinking water during the morning or afternoon drinks the wet wheat straw was removed and replaced with dry straw. On the morning of day six the pigs were returned to the holding pens, except during the high temperature and water deprivation treatment where the pigs were returned to the holding pens at 15:00 on the fifth day. Pigs had a nine-day recovery period between treatments in the holding pens of the farm animal unit.

### 5.5.1 Measurement of haematocrit, plasma osmolality and water intake post high temperature and water deprivation

To determine the effects of high temperature and water deprivation on haematocrit and plasma osmolality, the pigs were bled at 14:00 on day one, before transportation to the climatic chamber, and again at 14:00 on day five, after exposure to the high temperature and 48 h of water deprivation. To obtain blood samples, the pigs were placed in a sling and 1 mL of blood was drawn on each occasion through the marginal ear vein and placed in sterile heparinised tubes (BD-Vacutainer, BD, Plymouth, United Kingdom). To determine haematocrit, blood was drawn into microcapillary tubes in triplicates, sealed at one end and centrifuged using a micro-haematocrit centrifuge (Centrolit II-BL; Abrera, Barcelona, Spain) at 5 000 revolutions per minute for 10 min at room temperature and then read off a micro-haematocrit reader. Plasma for osmolality determination was separated by centrifugation of blood in sterile heparinised tubes (WIFUG Lab Centrifuges, Pary Lane, Bradford, England) at 5 000 revolutions per minute for 10 min at room temperature. Plasma osmolality was determined in triplicate using an osmometer (5600 Vapor Pressure Osmometer, Wescor Biomedical Systems, Elitech Group, Logan Utah, USA).

After blood collection on the fifth day, each pig was given drinking water in a pre-weighed bowl. Each pig was allowed to drink water continuously. Once the pig stopped drinking the bowl of water was removed and reweighed to determine the amount of water drunk by each pig.

### 5.5.2 Respiratory rate and behavioural observations

The respiratory rates were determined visually by counting the flank movements per pig over a period of one minute in triplicate per animal at 12:00 on day four of the treatment. Baseline respiratory rate data were collected on day 3 before the pigs were exposed to the experimental treatments. To avoid disturbing the pigs, the respiration rates were observed through a transparent perspex window measuring 0.4 m x 0.4 m in the door. Disturbance that interfered with the systematic flank movement, for example chewing, swallowing or movement, resulted in recounting. In addition to counting of respiratory rates at 12:00, I also recorded behavioural observations such as huddling together, sprawling, nest building and burrowing under the straw on the fourth and fifth days of each treatment.

### 5.5.3 Data Analysis

Activity sensors on the tags in one Large White and one Kolbroek pig failed to record activity which reduced the number of animals included in the analysis of activity.

To assess the effect of different experimental treatments on the thermoregulatory responses of the three breeds of pigs I calculated the mean, maximum, minimum and the amplitude of core body temperature for each pig over the 8 h period when the pigs were exposed to different experimental treatments. The amplitude was calculated as the difference between the maximum core body temperature and the minimum core body temperature during the 8 h period when the pigs were exposed to the experimental treatments. The mean ($t > 0.0.047$, $df = 6$; $P > 0.12$), minimum ($t > 1.03$, $df = 6$; $P > 0.20$), maximum ($t > 0.36$, $df = 6$; $P > 0.33$) and amplitude ($t > 0.30$, $df = 6$; $P > 0.29$) of core body temperature did not differ between day four and day five when the pigs were exposed to the experimental treatments therefore I report core body temperature

responses data from day four in further analyses. A repeated measures two-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison *post-hoc* Test to assess if there were significant differences in the core body temperature profiles, between the three breeds of pigs in response to the different experimental treatments was used. Data was tested for normality and homoscedasticity before being analysed.

Change in activity was calculated as the difference between the sum of activity during an 8 h period (08:00-16:00) of exposure to experimental treatment on day four and the average sum of activity during an equivalent time period in the two days prior to exposure to experimental treatment and expressed as a percentage of the average sum of activity during the equivalent period in the two days prior to exposure to experimental treatment. A repeated measures two-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison *post-hoc* Test to assess if there were differences in percentage change in activity between the three breeds of pigs in response to the different experimental treatments was used. Data was tested for normality and homoscedasticity before being analysed.

Change in the respiratory rate was calculated as the difference between the average of the three respiratory rate counts taken at 12:00 when the pigs were exposed to the experimental treatments during day four and the average of the three respiratory rate counts recorded at 12:00 when pigs were kept at TNZ on day 3, the day prior to exposure to the experimental treatment, and expressed as a percent change from the average of the three respiratory rate counts recorded at 12:00 when pigs were kept at TNZ on day 3. A repeated measures two-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison *post-hoc* Test to assess if there were differences in percentage change in respiratory rates between the three breeds of pigs in response to the different experimental treatments was used. Data was tested for normality and homoscedasticity before being analysed.

Change in haematocrit, plasma osmolality and body mass was calculated as the difference between the variable after and before exposure to the high temperature and water deprivation treatment and expressed as a percentage of the variable before

exposure to the high temperature and water deprivation treatment. Body mass measurements were taken at 06:30 in the morning before the pigs were offered feed and exposed to the experimental treatments. The percentage water intake was calculated by expressing the water intake of each pig after exposure to high temperature and 48h water deprivation as a percentage of the body mass of the pig measured at the same time. One-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison *post-hoc* Test to assess if the percentage change in haematocrit, plasma osmolality and body mass and percentage water intake was different between the three breeds of pigs following exposure to the high temperature and water deprivation treatment was used. Data was tested for normality and homoscedasticity before being analysed.

To assess whether the pigs stored red blood cells in their spleen and released them during the high temperature and water deprivation treatment, I estimated the plasma volume from the haematocrit measured before and after exposure to the high temperature and water deprivation treatment using the equation below.

$$\frac{\boldsymbol{P_2}}{\boldsymbol{P_1}} = \frac{H_1{}^*(100\text{-}H_2)}{H_2{}^*(100\text{-}H_1)} \qquad \text{…… (van Beaumont, 1972)}$$

*Where:* $H_1$ is the percentage of haematocrit obtained before and $H_2$ is the percentage of haematocrit obtained after exposure to the high temperature and water deprivation. The equation assumes that the red cell volume remains unchanged (van Beaumont, 1972).

I then compared using a paired Student t-test the value I obtained after substituting the percentages of haematocrit to the value of the ratio obtained between plasma osmolality before and after dehydration ($\mathbf{Osm_1/Osm_2}$) (measured value). If $\boldsymbol{P_2/P_1}$ is not equal to $\mathbf{Osm_1/Osm_2}$, then the pigs might have stored the red blood cells and then released them from the spleen during the dehydration and thermal challenge. This relationship checks if its only water that moved out of the plasma due to dehydration. $\boldsymbol{P_2/P_1}$ has to be equal

to $\mathbf{Osm_1/Osm_2}$. If $\boldsymbol{P_2/P_1}$ is higher or lower than $\mathbf{Osm_1/Osm_2}$ it therefore means that either the calculation was wrong or some electrolytes were lost from the cells.

The data was analyzed according to the linear model for repeated measures below:

$$y_{ijkl} = \mu + \alpha_i + b_j + c_k + (\alpha c)_{ik} + e_{ijk}$$

where: $y_{ijk}$ is the measured response (core body temperature and activity counts) on the $j^{th}$ breed of pig when injected with the $i^{th}$ treatment (one of the five treatments),

$\mu$ is the overall mean effect,

$\alpha_i$ is the $i^{th}$ fixed breed of pig (Windsnyer, Kolbroek and Large White) effect;

$b_j$ is the random effect of the $j^{th}$ breed of pig within the $i^{th}$ treatment,

$c_k$ is a fixed effect of environmental condition $k$,

$(\alpha b)_{ij}$ is the fixed interaction effect between breed of pig and treatment

$e_{ijk}$ is the random error associated with the $j^{th}$ breed of pig assigned to the $i^{th}$ treatment at time $t$

Statistical analyses were performed using Statistica (version 12 for windows, StaSoft Inc, Tulsa OK, USA). Values are reported as mean ± SD and $P < 0.05$ was considered statistically significant.

## 5.6 Results

### 5.6.1 Core body temperature rhythm

The 24 h rhythm of core body temperature was higher during exposure to the 5°C and exaggerated during exposure to high ambient temperatures compared to TNZ in all three breeds of pig (Figure 5-1; panels A to E). The core body temperature rhythms during exposure to high ambient temperatures matched the changes in ambient temperature in the climatic chamber (Figure 51). Large White pigs generally maintained a lower core body temperature than the local pig breeds throughout all treatment (Figure 5-1; panels A to E).

There was a significant difference in mean core body temperature between the breeds ($F_{2,17}$ = 9.3; $P$ = 0.0019) with the Large White pigs displaying a significantly lower mean core body temperature than the Kolbroek ($P$ = 0.00035) and Windsnyer ($P$ = 0.0069) pigs during the 8 h period of exposure on day four (Figure 5-2A). There were no differences in mean core body temperature between the experimental treatments ($F_{4,68}$ = 2.1; $P$ = 0.092) and no interaction between breed of pig and experimental treatment ($F_{8,68}$ = 1.3; $P$ = 0.28). There was a significant difference in maximum core body temperature between the breeds of pigs ($F_{2,17}$ = 12.5; $P$ = 0.00047) with the Large White pigs displaying a significantly lower maximum core body temperature than both the Kolbroek ($P$ = 0.0037) and Windsnyer ($P$ = 0.00076) pigs during the 8 h period of exposure on day four (Figure 5-2B). Even though there was a significant effect of experimental treatment overall ($F_{4,68}$ = 3.0; $P$ = 0.026) on maximum core body temperature, the Tukey's *post-hoc* test revealed no significant difference between the experimental treatments. There was no significant interaction between the breed of pig and experimental treatment ($F_{8,68}$ = 3.0; $P$ = 0.69).

The minimum core body temperature was significantly different between the breeds of pigs ($F_{2,17}$ = 7.1; $P$ = 0.0058) with the Large White pigs having a significantly lower minimum core body temperature than both the Kolbroek ($P$ = 0.0067) and Windsnyer pigs ($P$ = 0.031) during the 8 h period of exposure on day four (Figure 5-2C). There were no significant differences in minimum core body temperature between the treatments ($F_{4,68}$ = 2.1; $P$ = 0.85) and there was no significant interaction between the breed of pig and experimental treatment ($F_{8,68}$ = 1.5; $P$ = 0.16).

The amplitude of core body temperature was significantly different between the three breeds of pigs ($F_{2,17}$ = 5.54; $P$ = 0.014) with the Large White pigs having a larger amplitude of core body temperature than the Windsnyer pigs ($P$ = 0.011) during the 8 h period of exposure on day four (Figure 5-2D). There were significant differences in the amplitude of core body temperature between the experimental treatments ($F_{4,68}$ = 2.81; $P$ = 0.032). A larger amplitude of core body temperature was recorded during exposure to 30°C when drinking water was not available ($P$ = 0.015) than when the pigs were

exposed to 20°C (Figure 5-2D), otherwise there were no differences in amplitude between other experimental treatments. There was no significant interaction ($F_{8,68}$ = 1.06; $P$ = 0.40) between breed of pig and experimental treatments.

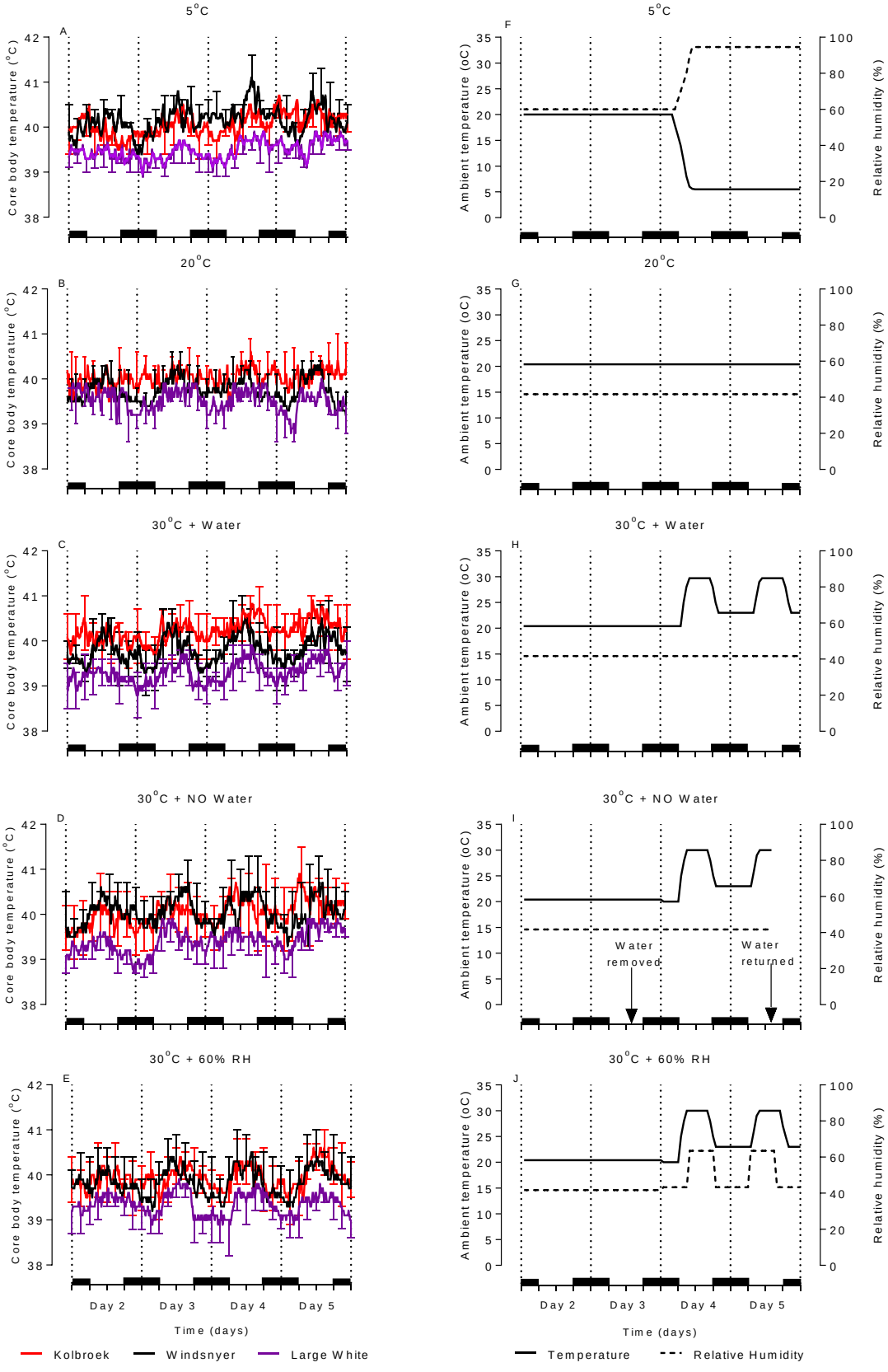

5°C
A
F
20°C
B
G
30°C + Water
C
H
30°C + NO Water
D
I
Water removed
Water returned
30°C + 60% RH
E
J
Core body temperature (°C)
Ambient temperature (oC)
Relative humidity (%)
Day 2
Day 3
Day 4
Day 5
Time (days)
Kolbroek
Windsnyer
Large White
Temperature
Relative Humidity

**Figure 5-1:** Core body temperature rhythm of the three breeds of pigs (left panels) under the five different experimental treatments (right panels.

Core body temperature rhythm of the three breeds of pigs (left panels) under the five different experimental treatments: cold temperatures treatment (5°C), thermoneutral temperatures (20°C), high temperature treatment (30°C + water), water deprivation treatment (30°C + no water) and high relative humidity treatment (30°C + 60% RH). Ambient temperature (solid line) and relative humidity (broken line) variations during experimental treatments (right panels). The error bars denote the SD from the mean. The black bars denote night time.

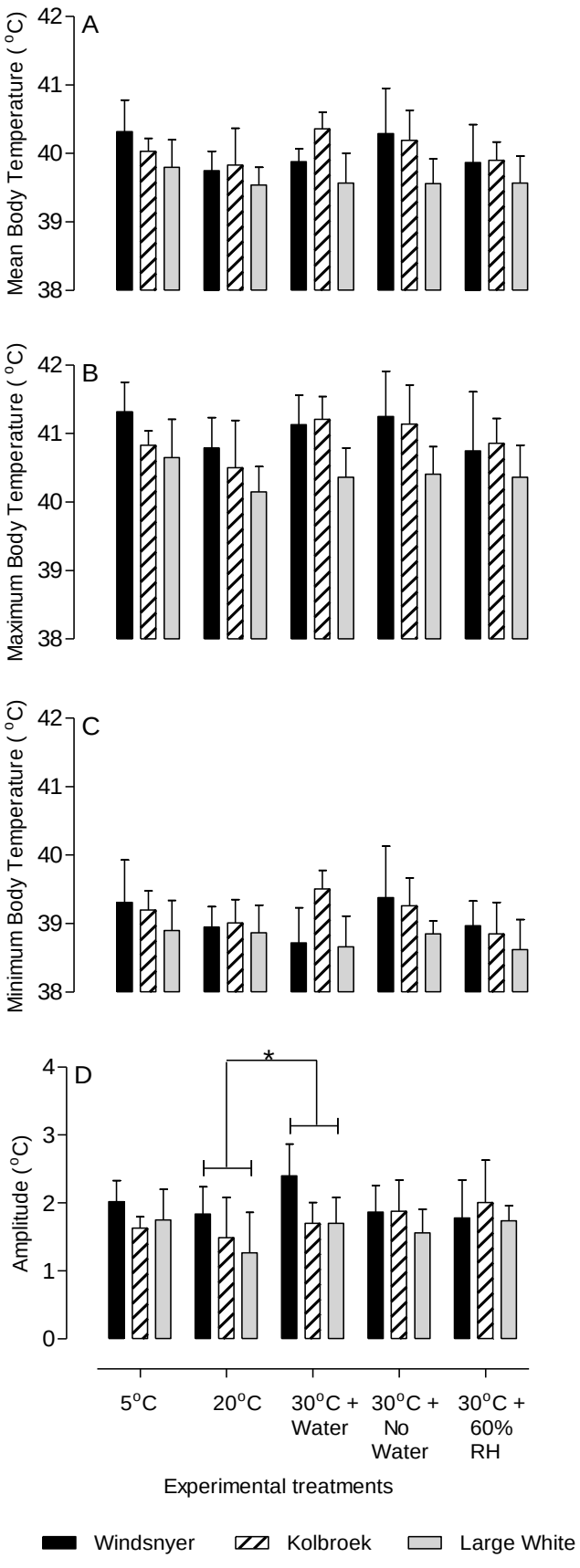

A
Mean Body Temperature (°C)
42
41
40
39
38
B
Maximum Body Temperature (°C)
C
Minimum Body Temperature (°C)
D
Amplitude (°C)
4
3
2
1
0
*
5°C
20°C
30°C + Water
30°C + No Water
30°C + 60% RH
Experimental treatments
Windsnyer
Kolbroek
Large White

**FIGURE 5-2**: The 8 h mean (panel A), maximum (panel B), minimum (panel C), and amplitude (panel D) of core temperature of three breeds of pigs during exposure to the five experimental treatments on day 4.

### 5.6.2 Activity

There was a significant difference between the breeds in the change of activity ($F_{2,15} = 5.58$; $P = 0.015$) following exposure to the treatments on day four. The Large White pigs had a significantly greater increase in activity than the Windsnyer pigs ($P = 0.015$) but there was no difference in the percentage change in activity between the Large White and Kolbroek pigs ($P = 0.84$) and the Windsnyer and the Kolbroek pigs ($P = 0.072$). There was a significant difference in the change of activity between experimental treatments ($F_{4,60} = 12.65$; $P < 0.0001$), with a greater reduction in activity during exposure to high ambient temperatures than when the pigs were exposed to 5°C ($P < 0.0001$) and 20°C ($P < 0.002$) treatments. There was a significant interaction between breed of pig and experimental treatment on the change of activity ($F_{8,60} = 4.61$; $P = 0.0002$; (Figure 5-3A). The Large White pigs were the only breed to increase their activity when exposed to 5°C.

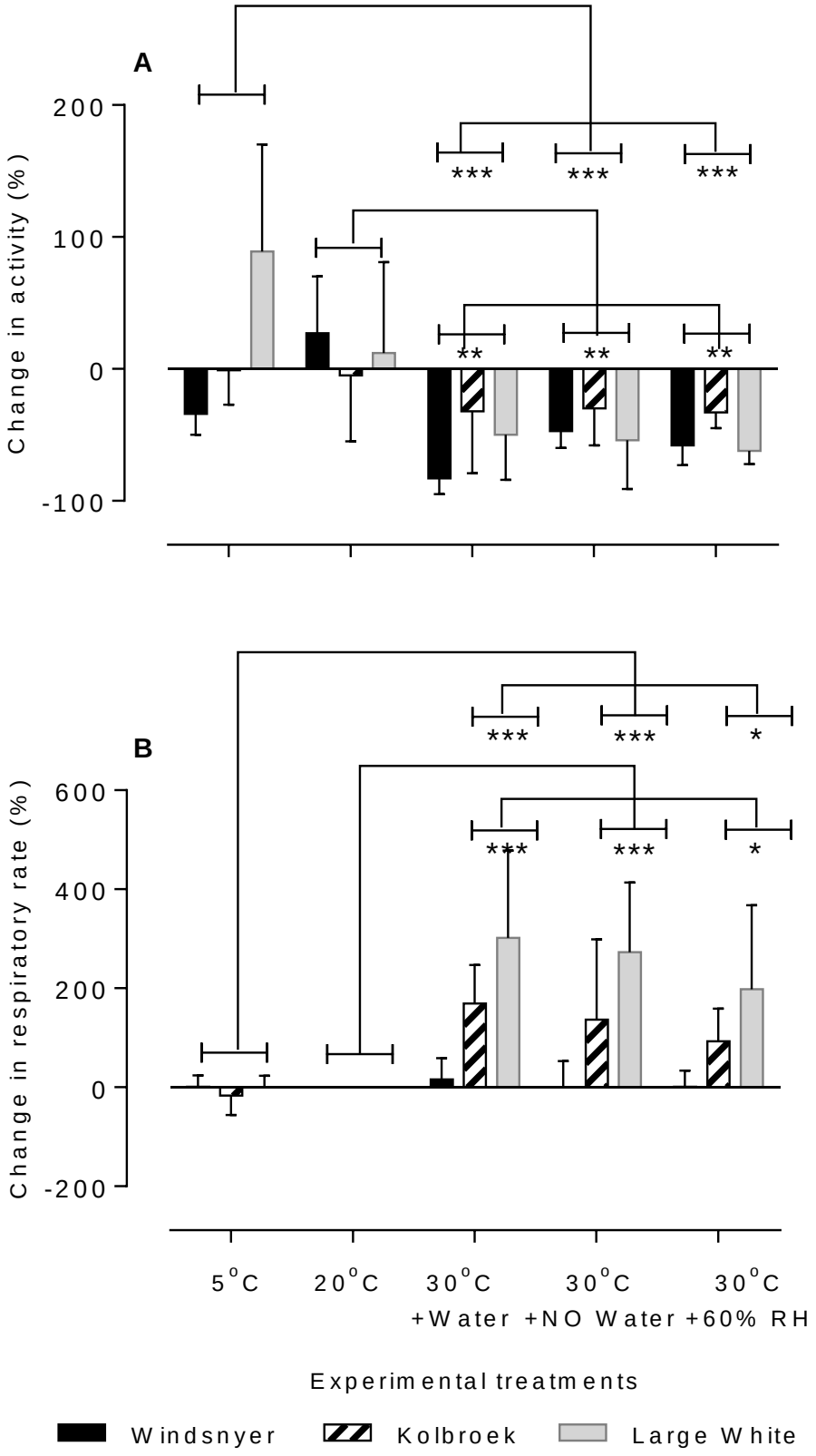

A
200
100
0
-100
Change in activity (%)
***
***
***
**
**
**
B
600
400
200
0
-200
Change in respiratory rate (%)
***
***
*
***
***
*
5°C
20°C
30°C
30°C
30°C
+Water
+NO Water
+60% RH
Experimental treatments
Windsnyer
Kolbroek
Large White

**Figure 5-3**: Change in activity (panel A) and respiratory rates (panel B) of the three breeds of pigs following exposure to the five experimental treatments.

Change in activity was calculated as the difference between the sum of activity during an 8 h period (08:00-16:00) of exposure to experimental treatment and the average sum of activity during an equivalent time period in the two days prior to exposure to experimental treatment and expressed as a percentage of the average sum of activity during the equivalent period in the two days prior to exposure to experimental treatment. Change in the respiratory rate was calculated as the difference between the average of the three respiratory rates counts recorded at 12:00 when pigs were kept at were exposed to the experimental treatments and the average of the three respiratory rates counts taken at 12:00 when the pigs TNZ on the day prior to exposure to the experimental treatment and expressed as a percent change from the average of the three respiratory rates counts recorded at 12:00 when pigs were kept at TNZ on the day prior to exposure to the experimental treatment. (*$P < 0.01$; **$P < 0.001$; *** $P < 0.0001$)

### 5.6.3 Respiratory rates

The Large White pigs displayed a significantly larger increase in respiratory rate ($F_{2,17} = 42.13$; $P < 0.0001$) than the Windsnyer ($P = 0.00016$) and Kolbroek ($P = 0.0012$) pigs, and Kolbroek pigs displayed a larger increase in respiratory rates than Windsnyer pigs ($P = 0.011$), on day four of the experimental treatments compared to day 3 when pigs were kept at TNZ (Figure 5-3; panel B).

The increase in respiratory rate occurred during high temperature treatments ($F_{4,68} = 14.96$; $P < 0.0001$) when compared to exposure of 5°C ($P < 0.001$) and 20°C ($P < 0.005$). There were no significant ($P = 0.346$) differences in the change in respiratory rates at 5°C and 20°C. Combining high temperatures with water deprivation and high relative humidity, did not significantly affect the change in respiratory rates of the breeds of pigs compared to the high temperature treatment (Figure 5-3; panel B).

The significant interaction between breed of pig and experimental treatment ($F_{8,68} = 5.56$; $P = 0.00002$) for the percentage change in respiratory rate, revealed that

the Large White pigs had significantly larger increases ($P < 0.001$) in respiratory rates compared to the local breeds of pigs when exposed to high temperature treatments (Figure 5-3; panel B).

### 5.6.4 Plasma osmolality, body mass and water intake

The Large White pigs had a significantly larger increase in plasma osmolality ($F_{2,17} = 9.53$; $P = 0.00017$) compared to the Windsnyer ($P = 0.0016$) and Kolbroek ($P = 0.028$) pigs (Table 5-1) following the high ambient temperature and water deprivation treatment. All breeds showed an increase in haematocrit following high ambient temperatures and water deprivation (Table 5-1). However there was no significant difference between the breeds ($F_{2,17} = 1.54$; $P = 0.24$) in the percentage change in the haematocrit following the high ambient temperature and 48 h water deprivation treatment (Table 5-1). The comparison of the plasma osmolality and the plasma volume ratios revealed that these two values were significantly different in the Kolbroek pigs (t-test; t = 4.14; df = 5, $P = 0.009$) and in the Windsnyer pigs (t-test; t = 2.45, df = 6, $P = 0.04$) but the two values were not significantly different in the Large White (t-test; t = 0.73; df = 6, $P = 0.49$) (Table 5-1).

**TABLE 5-1:** Mean ± SD of plasma osmolality, osmolality ratio, haematocrit and plasma volume ratio in the three breeds of pigs before and after exposure to the 48 h water deprivation at 30ºC

| Variable | | Kolbroek (n=6) | Windsnyer (n=7) | Large White (n=7) |
|---|---|---|---|---|
| Osmolality (mOsmol/kg $H_2O$) | Before | 301 ± 13 | 297 ± 6 | 290 ± 5 |
| | After | 325 ± 15 | 313 ± 10 | 328 ± 5 |
| | % Change | $8.1^{a}$ ± 4.8 | $5.6^{a}$ ± 1.7 | $13.1^{b}$ ± 2.6 |
| Osmolality ratio (***$Osm_1/Osm_2$***) | | $0.93 \pm 0.04^{a}$ | $0.94 \pm 0.02^{a}$ | $0.88 \pm 0.02^{a}$ |
| Haematocrit (%) | Before | 47 ± 1 | 47 ± 0.5 | 49 ± 1 |
| | After | 52 ± 1 | 50 ± 2.1 | 53 ± 1 |
| | % Change | $11.5^{a}$ ± 4.5 | $7.5^{a}$ ± 4.5 | $8.0^{a}$ ± 4.0 |
| Plasma volume ratio * (***$P_2/P_1$***) | | $0.81 \pm 0.06^{b\#}$ | $0.87 \pm 0.07^{b\#}$ | $0.86 \pm 0.08^{a}$ |

**Note**: $^{ab}$ Within a row, means without a common superscript differ (Student t-test)

$^{\#}$Indicates significant difference in osmolality ratio and plasma volume ratio within the same column

*** $P_2/P_1$** = $H_1*(100-H_2)/(H_2*(100-H_1)$

The percentage change in body masses of all of the pig breeds were not significantly different ($F_{2,17} = 0.33$; $P = 0.72$; Figure 5-4A) after the high temperature and water deprivation treatment. Water intake was not significantly different ($F_{2,17} = 1.2$; $P = 0.33$; Figure 5-4B) between the breeds of pigs when they were allowed access to water following the high temperature and water deprivation treatment.

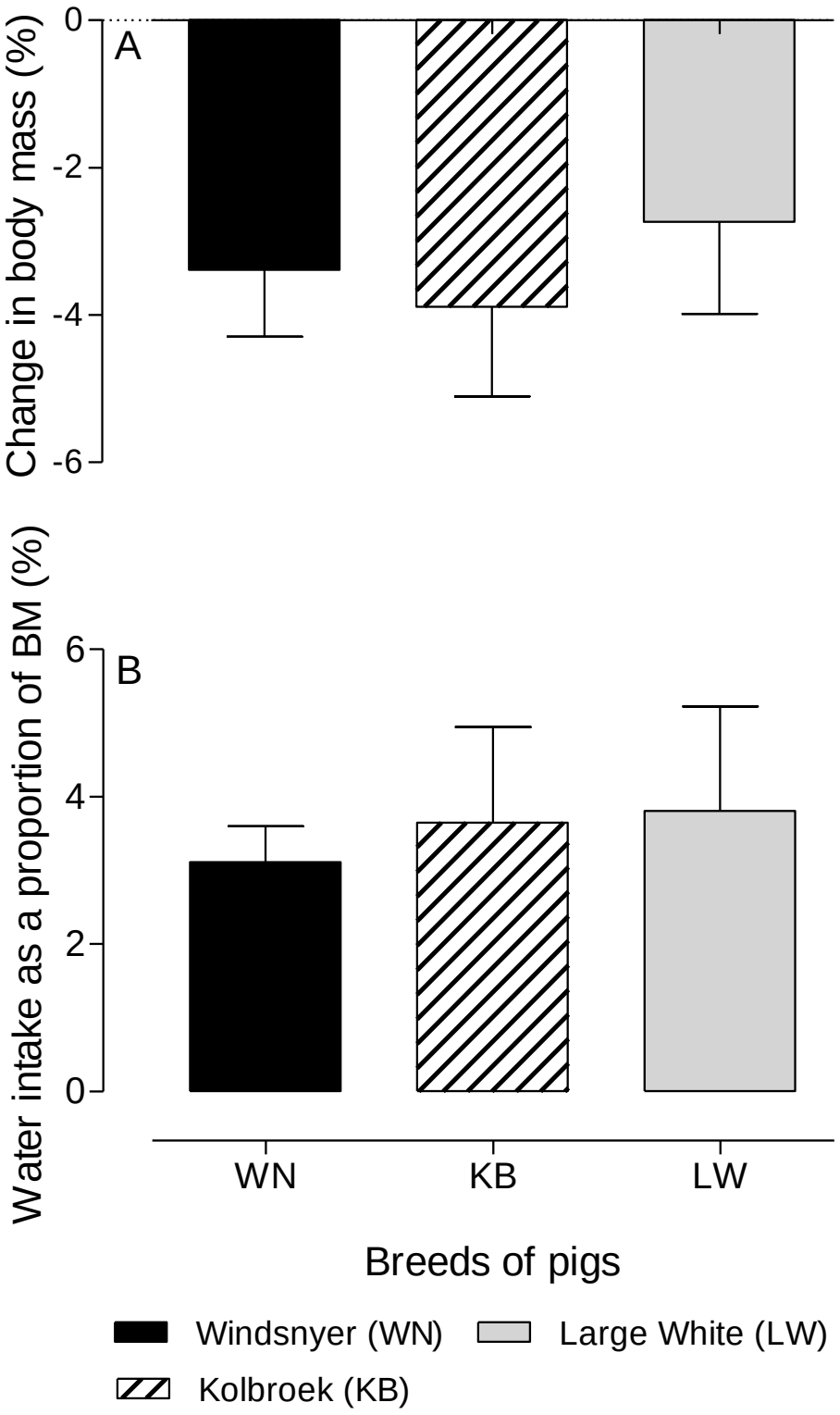

A
Change in body mass (%)
0
-2
-4
-6
B
Water intake as a proportion of BM (%)
6
4
2
0
WN
KB
LW
Breeds of pigs
Windsnyer (WN)
Large White (LW)
Kolbroek (KB)

**Figure 5-4**: Comparison of the percentage change in body mass (%) (panel A) and water intake (as a % of body mass) (panel B) following 48h of water deprivation and exposure to high temperatures (30ºC) for three breeds of pigs.

Change in body mass was calculated as the difference between the body mass after and before the pigs were exposed to the high temperature and water deprivation treatment and expressed as a percentage of the body mass before the pigs were exposed to the high temperature and water deprivation treatment. Percentage water intake was calculated as water intake after 48 h of water deprivation expressed as a percentage of body mass after 48 h of water deprivation.

### 5.6.5 Behavioural responses

During exposure to cold temperatures only the Windsnyer pigs exhibited primitive adaptive traits such as burrowing under the straw and nest building. Both the local pig breeds huddled together when in the cold room while the Large White pigs did not. Instead the Large White pigs increased physical activity to generate body heat and to maintain their core body temperature instead. In the climatic chamber, when exposed to high temperatures, all breeds of pigs reduced their physical activity. All the breeds were observed sprawling out after they had pushed the wheat straw aside to lie in direct contact with the floor to increase heat loss through conduction.

## 5.7 Discussion

This study is the first to investigate the thermoregulatory responses of Kolbroek, Windsnyer and Large White pigs following exposure to low and high temperatures, water deprivation and varying relative humidity. All three breeds of pig had a similar core body temperature rhythm across the five experimental treatments indicating that the pigs regulated core body temperature effectively. The Large White pigs had a lower core body temperature than the Windsnyer and Kolbroek pigs during all experimental treatments. On exposure to 5°C, the Kolbroek and Windsnyer pigs reduced activity and employed behavioural strategies such as huddling together and burrowing under the straw bedding to maintain core body temperature while the Large White pigs increased

their physical activity. During exposure to the cold temperatures the respiratory rates in all the three breeds of pigs were not significantly different to the respiratory rates under TNZ. When exposed to high ambient temperatures, the Large White and Kolbroek pigs increased their respiratory rates while the Windsnyer pigs did not increase their respiratory rate compared to TNZ. The Windsnyer might have had a different strategy to prevent core body temperature from increasing in the heat. When exposed to high ambient temperatures, restricting access to water and increasing humidity had no effect on respiratory rates of the pigs. Exposure to high temperatures and water deprivation resulted in an increase in haematocrit in all the pigs. There was a significantly greater increase in plasma osmolality in the Large White pigs than in the local pigs (13% vs 6%). The Windsnyer and Kolbroek pigs were able to protect their plasma volume (i.e. preserve body water) better than the exotic Large White pigs when exposed to the high temperature and the 48 h water deprivation treatment.

The differences in thermoregulatory responses between the breeds may be the result of differences in body sizes. All the breeds of pigs in this study had different body masses at the start of the experiment due to innate differences in growth rates. Consequently I was unable to control for body mass, instead I controlled for age. Age may also impact on thermoregulation in that piglets have little subcutaneous fat (Berthon et al., 1994, Lossec et al., 1988, Herpin et al., 2002) and that makes them susceptible to cold, whereas the well-nourished adult pigs tend to have a thick fat layer that makes them susceptible to heat because they are unable to sweat (Huynh, 2005). Furthermore the large body size of adult pigs reduces the surface area to volume ratio and lowers rates of heat loss thereby reducing the effectiveness of transepidermal water loss (TEWL), particularly for species dependent on sweating. The susceptibility of large animals to heat is further exacerbated by their lower tissue conductance relative to small species (Finch, 1985). These differences in heat transfer between large and small animals may result in differences in core body temperature. Although there has been some debate as to whether core body temperature increases or decreases with body mass (Rodbard, 1950, Morrison and Ryser, 1952, McNab, 1970, Scholander et al., 1950), the most recent meta-analyses which included phylogenetic corrections indicated a negative relationship (Clarke and Rothery, 2008), particularly for *artiodactyls*. In the current study,

the larger Large White pigs had a lower core body temperature than the local pigs which had a lower body mass, these results concurred with the meta-analyses conducted by Clarke and Rothery (2008) for the species within the order *Artiodactyla*.

An alternative explanation for the higher core body temperature in local pig breeds compared to the Large white might be that they are better adapted to hot and dry environments. The high core body temperature observed in pigs might be adaptive, as has been observed in sheep and goats (Kay, 1997) and camels (Schmidt-Nielsen et al., 1967, Schmidt-Nielsen et al., 1957) because it (high core body temperature) has been found to reduce the thermal gradient between the animal and the environment and results in the conservation of body water. Indeed, in kangaroos when water was available, arid adapted species had higher core body temperature than mesic species (McCarron et al., 2001), but when water was limited mesic species showed larger fluctuations in core body temperature (Dawson et al., 2007). The large variations in core body temperature observed in the mesic kangaroos could be indicating that they are struggling to cope with high ambient temperatures and water deprivation. The pigs in the current study showed small variations in body temperature indicating that they were able to thermoregulate efficiently when they were mildly dehydrated. Differences in core body temperature have also been observed between heat stressed farm animals of tropical and temperate origins. Conversely the Large White pigs of temperate origin had a lower mean, maximum, and minimum core body temperature than the Windsnyer and the Kolbroek pigs of tropical origin when they were exposed to all experimental treatments. The maintenance of a low core body temperature by the Large White pigs could be disadvantageous in that it increases the temperature gradient between the animal and environment leading to an increased heat gain thereby increasing the need of evaporative heat loss (i.e. increased respiratory rates). In the study on kangaroos highlighted earlier, the mesic species were more dependent on evaporative cooling (i.e. increased licking) than in the arid species (McCarron et al., 2001). However, pigs do not lick themselves like the kangaroos and do not sweat so they must rely on panting and non-evaporative heat loss (such as conductance).

Previous studies have shown that when pigs are exposed to ambient temperatures below the thermoneutral zone, they may increase heat production through either muscular shivering thermogenesis (Berg et al., 2006) or conserve heat through changing posture to reduce the body-surface exposed to cold (Van der Hel et al., 1986, Hayne et al., 2000), build nests (Olczak et al., 2015, Berg et al., 2006), huddling together and select favourable microhabitats (Ingram and Legge, 1970a). Unlike the Large White pigs, the physical activity of the Kolbroek and Windsnyer pigs in the cold was not significantly different to activity when the pigs were placed in TNZ instead the Windsnyer and Kolbroek pigs huddled together in an attempt to conserve body heat. In addition only the Windsnyer pigs exhibited primitive adaptive traits such as burrowing under the straw and nest building, which is a trait that is seen in the wild boar (Berg et al., 2006) whereas the Kolbroek and Large White pigs did not display those behaviours. In a study conducted by Hayne et al. (2000), the Yorkshires, Landrace and Duroc pigs have been seen to display some of these primitive behaviours such as nest building and burrowing under the straw when they have access to bedding when temperatures were lower than 5°C (Hayne et al., 2000). Despite similar ambient temperatures and comparable amounts of straw, the Large White pigs in this study did not burrow. The pigs in the study conducted by Hayne et al. (2000) had a straw density between 1.1 and 10.9 kg/m while in the current study a straw density of 8.43 kg/$m^2$ in the pens was used. The differences in the responses between the Large White pigs in my study and that conducted by Hayne et al. (2000) could be attributed to the differences in body mass. The pigs in the study by Hayne et al. (2000) were smaller than the pigs used in this study (20 kg vs 60 kg) and could have lost more heat than the bigger Large White pigs hence the need to employ behavioural thermoregulation to preserve heat. Also the Windsnyer and Kolbroek pigs were smaller than the Large White pigs hence possibly accounting for the differences in behavioural responses. On the contrary, in the current study in the cold the Large White pigs increased physical activity to generate body heat and maintain their core body temperature. The increase in the physical activity by the Large White pigs in the current study concurs with the findings recorded by Quiniou et al. (2001) in which the Large White x Pietrain pigs increased activity when exposed to 12°C compared to when they were exposed to 22°C.

When ambient temperatures were increased to 30°C, all pigs sprawled out in the climatic chamber after pushing the wheat straw aside in order to lie in direct contact with the floor to increase heat loss through conduction. When temperature increases, sensible heat loss (radiation, conduction and convection) decreases rapidly and evaporative heat loss becomes the important route for pigs to eliminate heat load (Huynh et al., 2005b). When the pig cannot maintain the thermal balance through use of non-evaporative physical processes, it increases evaporative respiratory heat loss, to prevent its core body temperature from increasing (Huynh, 2005) and the same response has been observed in cattle (Hahn, 1999). In the current study the Large White and Kolbroek pigs significantly increased their respiratory rates when they were exposed to high ambient temperatures compared to at 5°C and 20°C while the Windsnyer pigs maintained a constant respiratory rate throughout the five experimental treatments. The Windsnyer had little increase in the respiratory rates in the heat, yet they have a thin dermis (see Chapter 6) so they may be more dependent on conductance to lose heat. On the other hand, the Large White and Kolbroek pigs have a thick dermis and showed a huge increase in the respiratory rates in the heat indicating that they were more dependent on respiratory evaporative cooling to lose heat. An increase in respiratory rates has been reported in heat stressed Large White pigs (Aberle et al., 1974, Renaudeau et al., 2010, Renaudeau et al., 2007). However, panting in pigs, is not as efficient in dissipating excess heat as in sheep (Ingram and Legge, 1969), where it accounts for heat loss of between 60-80% of the total heat dissipated by the sheep (Hales and Brown, 1974) compared to 33% in pigs (Morrison et al., 1967). When in the thermoneutral zone [temperature range from 18 to 26°C; (Muirhead and Alexander, 1997), Large White pigs lose 0.29 L per day for a 20 kg pig and 0.58 L per day for a 60 kg pig through respiratory evaporative losses (Holmes and Mount, 1967). In the Duroc breed of pig, increasing temperatures from 15°C to 29°C increased the pigs respiratory evaporative water loss from 12% to 33% of the total evaporative water loss (Morrison et al., 1967).

The majority of evaporative water loss occurs through the respiratory tract (Mount, 1962, Ingram, 1964, Ingram and Legge, 1969). Pigs don't sweat, but they can use transepidermal water loss (Justino et al., 2014). Transepidermal water loss (TEWL) is

defined as the normal, constitutive loss of water vapour from the skin through diffusion and evaporation processes in the absence of sweat gland activity (Pinson, 1942). In the current study the TEWL might have been reduced as animals were dehydrated, as was observed in a study by Haggarty et al. (1994). At ambient temperatures similar to those that used in this study, pigs can evaporate 30-40 g/$m^2$.h through TEWL (Ingram, 1964). The total evaporative heat loss is the sum of the heat lost through panting/sweating, and through the transepidermal water loss route. During heat stress, an increase in relative humidity lowers the efficiency of evaporative cooling since the vapor pressure gradient between the animal and the environment decreases (Silanikove, 2000). Morrison et al. (1967) noted that respiratory evaporative cooling represented 67% of the total evaporative heat loss. The transepidermal water loss was high even at high relative humidity. So it would be interesting to assess transepidermal water loss of pigs at different relative humidity. The increase in plasma osmolality post water deprivation was highest in Large White compared to both Windsnyer and Kolbroek pigs. Therefore it is important to measure transepidermal water loss in the Windsnyer and Kolbroek pigs.

Under hot and low humidity conditions pigs tend to develop dry skin (Bissett and McBride, 1983) and the dry skin allows for effective evaporative cooling. Increasing relative humidity from 40% to 60% resulted in no increase in core body temperature and respiratory rates indicating the relative humidity might not have been different enough to cause changes. Dehydration and high relative humidity in my study did not appear to adversely affect evaporative water loss. In the current study, high humidity had no effect on the respiratory rates between all the breeds of pigs exposed to high ambient temperatures. In previous studies that exposed pigs to mild conditions and restricted water intake, pigs have been shown to reduce transepidermal water loss and evaporative respiratory water losses (Haggarty et al., 1994, Cunningham, 1968). This study confirms that pigs have some form of control over body water levels when water intake is limited. If a pig has free access to water it can use both panting and transepidermal water loss to rid itself of excess heat without any concern for dehydration. In the current study pigs were deprived of water for 48 h and exposed to 30°C with 40% RH. However dehydration might have resulted in a trade-off between use of evaporative cooling and conservation of body water as has been noted in other

mammals (Cain et al., 2006) that were facing water shortages prompting an animal to reduce the amount of water lost through evaporative cooling and the skin.

The ability to conserve water and protect the plasma volume when deprived of drinking water is a trait of mammals inhabiting hot and dry environments (Carmi et al., 1993, Horowitz and Samueloff, 1979). The ability to protect of the plasma volume is vital in the maintenance of the circulatory function and thermoregulation (Degen, 1997). Animals that protect their plasma volume when deprived of drinking water tolerate dehydration better than animals that do not (Carmi et al., 1993) and this allows them to tolerate heat stress (Degen, 1997). The ability to protect their plasma volume by the local pigs suggests that they have the potential to tolerate hot and dry conditions unlike the Large White pigs that might lose a lot of water and might be prone to dehydration. In the current study, the local pigs lost approximately 6% of their plasma volume compared to the 13% reduction in the plasma volume shown by the Large White pigs. The local pigs demonstrated that they were better conservers of plasma volume than the Large White pigs. Based on the comparisons ($\boldsymbol{Osm_1/Osm_2}$ versus $\boldsymbol{P_2/P_1}$), which relate changes in osmolality and haematocrit as an aid in the assessment of hydration status it could be inferred that an increase in haematocrit in the Windsnyer and Kolbroek pigs might have been due to the release of the red blood cells from the contractile spleen into the circulatory system. The release of red blood cells from the spleen during periods of stress enables an increased oxygen-carrying capacity of the blood when cardiac output offsets the increased viscosity (Stewart, 2002). It is thus likely that in our study, when the pigs were exposed to high temperatures and deprived of water, the pigs might have activated the sympathetic-adrenal medullary system that resulted in an increased production, and release of endogenous catecholamines into circulation just like in the pigs that were stressed in a study by McCarty et al. (1988). Interestingly the Large White pigs had a significantly greater increase in plasma osmolality compared to the local pigs suggesting that their increase in haematocrit might have mainly been due to dehydration. Dehydration results in haemoconcentration which is characterised by an increase in plasma osmolality and haematocrit (Takamata, 2012).

Animals are known to adapt to or cope with the changing environmental conditions either through natural selection which can impact the genotype (Orr, 2009) or employing phenotypic plasticity. Phenotypic plasticity is defined as 'the ability of individual genotypes to produce different phenotypes when exposed to different environmental conditions" (Pigliucci et al., 2006). Phenotypic plasticity confers adaptability to an individual animal. Through personal communication with Dr. Arnold Kanengoni (Research Officer at the Agricultural Research Station, Irene Pretoria, South Africa) the Windsnyer pigs were sourced from the Limpopo Province, which is a predominantly hot and dry region in South Africa, between 2007 and 2008. The Kolbroek breeding herd at the Agricultural Research Station at Irene, Pretoria, South Africa was established in 2004. The breeding herd of the Large White pigs at the Agricultural Research Station at Irene, Pretoria, South Africa was outsourced from other commercial breeders in 2006. Probably through many previous generations having existed for many years in this area the progeny of the local pigs might have acquired genes of thermotolerance through transgenerational epigenetic inheritance and as such the local pigs are most likely to have undergone natural selection than phenotypic plasticity. The local pigs at the Agricultural Research Station at Irene, Pretoria, South Africa, were produced using an outdoor production system where they are exposed to much greater environmental variations compared to the Large White pigs which were kept under indoors under semi-controlled conditions. Nevertheless, there is a need to study the role of genes and epigenetics in adaptive thermoregulation in local breeds of pigs.

## 5.8 Conclusion

The different breeds showed similarities in core body temperature rhythms across treatments all demonstrating that all breeds were efficient in their thermoregulation both at low and at high air temperatures. At low temperatures, the three breeds employed different thermoregulatory behavioural responses; the local breeds huddled together, built nests and burrowed under the straw whereas the Large White pigs increased physical activity. When exposed to the high temperatures the Windsnyer pigs did not increase respiratory rate while the Kolbroek and Large White pigs significantly increased their respiratory rates. The Kolbroek and Large White used evaporative cooling to

prevent the core body temperature from increasing while the Windsnyer might have relied on a different mechanism. When the pigs were deprived of water at high temperatures the local pigs protected their plasma volume while the Large White failed to protect their plasma volume resulting in statistical significant change in osmolality. These data have lent credence to claims that the local pigs are better adapted to their hot arid environment. With climate change predicted to result in increased ambient temperatures and decreased water availability and feed resources, the Windsnyer breed of pig appear to have the physiological responses to cope with these predicted changes. It is thus recommended that breeding programmes to preserve these valuable genetic resources should be initiated and encouraged.

Having determined the physiological responses to thermally challenging conditions in the three breeds of pigs the reasons for the Windsnyer pigs not to increase their respiratory rates may be explained by them using other thermoregulatory mechanisms. In species like cattle the skin is an important organ in thermoregulation (Nay and Hayman, 1956). I therefore decided to investigate the skin morphology (thickness of the skin layers and the size and depth of the sweat glands) to determine if differences in skin characteristics could have played a role in thermoregulation of the Windsnyer pigs. The next chapter gives a detailed description of the experiment I conducted to evaluate the full thickness skin samples from the three body regions using standard staining techniques to investigate the differences in skin characteristics. The differences in skin characteristics might be become important in pigs when dealing with high temperatures and solar radiation which are expected to accompany climate change.

# CHAPTER 6: COMPARISON OF THE HISTOLOGICAL CHARACTERISTICS OF THE SKIN OF THE WINDSNYER, KOLBROEK AND LARGE WHITE PIGS

*The results in the chapter were submitted as part of an article to the journal* ***Animal***

## 6.1 Introduction

In Chapter 5, I observed that the Windsnyer pigs did not increase their respiratory rate when exposed to high temperatures while the Kolbroek and Large White pigs did. This observation was intriguing and led to the investigation of the differences in the skin characteristics between the breeds. The skin consists of two main layers, namely the epidermis and the dermis which lies between the epidermis and hypodermis.

The skin which is an interface between an animal and its environment (Costin and Hearing, 2007) has several important functions that include protection, homeostasis and sensation. The skin provides a physical barrier that protects underlying tissues from physical abrasion, bacterial invasion, dehydration and ultraviolet (UV) light (Haake et al., 2001, Costin and Hearing, 2007). It also contributes to controlling the loss of water and solutes for the body (Meyer et al., 2011, Żak et al., 2011).

The skin is involved in the regulation of body temperature (Haake et al., 2001). It has thermoreceptors that detect thermal changes (hot and cold) (Singh et al., 2013). Once the information from the thermoreceptors is detected in the hypothalamus, signals are sent to the blood vessels to either constrict or dilate and also to sweat glands to either decrease or increase sweat production. When environmental temperatures venture above the thermoneutral zone (TNZ), the skin blood flow increases due to increased vasodilation, and thermal sweating increases in species with functional sweat glands. These two processes increase heat loss from the skin. When environmental temperatures venture below the TNZ increased vasoconstriction and reduced sweat production occur to conserve heat. The thickness of the skin layers (particularly fat deposition), body mass, hair-coat properties, number, depth and size and functional

activity of the sweat glands also has an effect on the rate of heat transfer from an animal to the environment (Collier and Gebremedhin, 2015).

In pigmented animals the basal layers of the skin contain melanin  The melanin protects the skin, underlying tissues and organs against UV-induced skin damage through its optical and chemical filtering properties (Ahene et al., 1995). The traits of the skin may play an important role in the capacity of animals to adapt to the changing increased thermal loads due to climate change.

Globally, climate change is predicted to result in spatial increases/decreases in surface temperatures and high solar radiation in the summer months. Based on the currently available data, the average world surface temperature increased by 0.8 °C [0.72 to 0.85°C] when comparing the 1850-1900 and the 2003-2012 periods (IPCC, 2013). South Africa lies partly in the tropics and already seems to be experiencing an increase in annual temperatures. It is reported that the average annual temperatures in South Africa have increased by at least twice the observed global average of 0.8°C per 100 years reported in the Intergovernmental Panel on Climate Change (IPCC) Assessment Report No. 4 between 1960 and 2010 (DEA, 2013). Solar radiation considerably increases the environmental thermal load on animals during summer months (Spiers, 2012). Studies on skin characteristics demonstrated that indigenous breeds of cattle (*Bos indicus*) in Australia (Pan, 1962); sheep in Brazil (McManus et al., 2011) and the indigenous Creole pigs in the Caribbean (Renaudeau et al., 2006) tolerated heat stress better than the exotic breeds of livestock. It is not known whether the local pigs in South Africa also possess traits that may give them an advantage to cope with the high thermal loads.

Pig production is important to the livelihoods of subsistence farmers in South Africa where the pigs contribute to nutrition (protein), food security, poverty alleviation, enhanced livelihood and employment creation for the rural community (Antwi and Seahlodi, 2011, Dietze, 2011, Mergenthaler et al., 2009).The subsistence farmers mainly use two local breeds of pig, the Windsnyer and the Kolbroek pigs (Halimani et al.,

2010) whereas the commercial pig farming sector relies mainly on exotic breeds such as the Large White and Landrace pigs.

Despite the roles of the skin in thermoregulation, and as a protective barrier, there is limited information on the characteristics of the skin of local pigs in southern Africa. Madzimure et al. (2012) in a study on the gross morphometry of the skin of Windsnyer and Large White pigs found that the local Windsnyer pigs had longer hair, greater hair density and a thicker fat layer than the Large White pigs. The study by Madzimure et al. (2012) is limited in that it did not investigate the histological structure of the skin of the Windsnyer pigs thus there is no information on the thickness of the skin layers, sweat gland size and distribution as well as melanin all of which are major important parameters in the physiological functions of the skin. In addition no studies were found on the microstructure of the skin of Kolbroek pigs.

In light of the changing climate, gaining an understanding of the anatomical traits in local pigs has become imperative to allow predictions of physiological responses of the pigs in light of the changing climate. According to Dowling (Dowling, 1955), if animals are adapted to a set of environmental conditions they will have skin morphological features that will allow them to survive under a given environmental conditions.

The objective of this study was to determine whether there were any differences in the thickness of the skin layers, position and size of the sweat glands, and presence of melanin of the local (Kolbroek and Windsnyer) pigs and the exotic (Large White) pig breeds in order to draw inferences on how the different breeds might be able to cope with the increased temperatures and solar radiation predicted to accompany climate change in the southern African region.

## 6.2 Material and methods

All experimental procedures used in this study were approved by the Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand, Johannesburg,

South Africa (AESC Clearance Certificate Number 2010/58/04 – see Appendix 9.1 and M&E – see Appendix 9.2).

### 6.2.1 Animals

Uncastrated male pigs [Large White (n=7), Windsnyer (n=5) and Kolbroek (n=4)] aged between six and eight months old were used to investigate the skin characteristics. All the three breeds of pigs (Large White, Kolbroek and Windsnyer) were purchased from the Agricultural Research Council (ARC) Station in Irene, Pretoria, South Africa (GPS Coordinates: -28.165430 S 28.306129 E). The Large White and Kolbroek were purchased as 4-week old weaners. The Windsnyer were purchased at 6 months of age. All pigs were kept in the Central Animal Services Farm Animal Unit and fed a standard soybean/maize meal-based ration. The pigs were fed once daily at 08:30, with a 15% crude protein soybean/maize meal-based pig grower diet enriched with vitamins and minerals (Epol, Johannesburg, South Africa) at 2% of total body mass of the pigs in each pen for maintenance of body mass. The food was moistened with water in the ratio of 2 feed: 1 water. Dry wheat straw provided bedding and was changed daily before feeding. A 12:12 light-dark cycle was used throughout the experimental protocol, with lights on at 06:00. The ambient temperature in the farm animal unit was 23.0 ± 2.0$^{o}$C and relative humidity was 45.0 ± 5.0%; both were recorded every 5 min with a data logger (Hobo U12-013 Temp/RH/2 External Data Logger, Onset Computer Corporation, Pocasset, MA, USA). At the time of termination, the pigs were between six and eight months old with a body mass of 58.8 ± 3.1 kg (Kolbroek), 59.6 ± 13.3 kg (Windsnyer) and 115.8 ± 18.7 kg (Large White). The pigs were first sedated by a deep intramuscular (i.m.) injection of 11 mg/kg ketamine (Bayer Animal Health Division, Isando, Johannesburg, South Africa) and 0.3 mg/kg midazolam (Roche Products, Isando, Johannesburg, South Africa) and then killed by injecting a lethal dose of sodium pentobarbitone (Euthapent, 200 mg/kg intravenously (i.v.); Kyron Laboratories (Pty) Ltd, Benrose, Johannesburg, South Africa).

### 6.2.2 Skin sample collection and processing for histology

A scalpel blade was used to excise full thickness skin samples, measuring approximately 20 mm × 20 mm, from the dorsal interscapular region, lateral thoraco-abdominal region and ventral abdominal region of the bodies of all the pigs. After excision, the skin samples were immediately fixed in 10% buffered formalin for at least seven days. The fixed tissue samples were then transferred into plastic cassettes and processed using an automatic tissue processor (Shandon Citadel 1000; Thermo Scientific, Germany). A 17 h processing schedule which consisted of 11 stages was followed. The first stage, following the fixation, was immersion of the tissue in 70% ethanol for 1 h. The tissue was then immersed in three changes of 95% ethanol (2, 1½ and 1½ h respectively) followed by three changes of 100% ethanol (1, 2 and 1 h respectively) (Absolute Ethanol (v/v); VWR Prolabo Chemicals – International Ltd, Lutterworth, United Kingdom). Subsequently, the tissue was immersed in two changes of 100% chloroform (Associated Chemical Enterprises, Johannesburg, South Africa) for 1 and 2 h respectively. The final two stages involved the immersion of the tissue in molten paraffin wax (Paramat extra pastilles, Merck KGaA, Darmstadt, Germany) for 2 h each at stage. On removal from the automatic tissue processor the samples were embedded in paraffin wax with a melting point of 60°C using a Tissue-Tek TEC 5 Tissue Embedding Console System (Sakura Finetek Europe B.V., Amsterdam, The Netherlands).

After embedding, thin vertical sections measuring 6 µm in thickness were cut using a manually operated rotary microtome (Leica RM2125RT, Leica Biosystems, Nussloch, GmbH, Germany). The sections were floated in a water bath (Electrothermal MH8504, Stachwell Sunvic Ltd, London, Great Britain) set at 50ºC and containing tissue section adhesive (Sta-On, Surgipath Medical Industries Inc, Winnipeg Manitoba Canada). The sections were then placed onto frosted glass slides. The slides were then placed onto a drying plate (Slide Warmer, Precision Scientific Co, Chicago, USA) maintained at 50°C to dry overnight. The sections were dewaxed in xylene (Merck KGaA, Darmstadt, Germany) for 5 minutes and passed through graded alcohol solutions (100%, 100% and 95%) (Merck KGaA, Darmstadt, Germany), agitated for 30 seconds and rehydrated in

distilled water for 30 seconds. Two sections from each of the three regions were stained for 5 minutes in an aqueous One-Step Mallory-Heidenhain stain (Cason, 1950), for the evaluation of general morphology. To show the melanin in the skin, the two sections from each of the three regions was stained with Fontana stain (Renaudeau et al., 2006) for 2 h in an oven at 60°C. After staining, the slides were washed under running tap water until the water became clear and placed in 95% alcohol for 30 seconds, transferred to two changes of absolute alcohol for 30 seconds, followed by clearing in two changes of xylene for 2 minutes each. The slides were then mounted with a cover slip using Entellan (Merck, KGaA, Darmstadt, Germany) as a mounting medium.

Sections stained with the One-Step Mallory-Heidenhain stain were photographed under a low-power [0.75x (objective lense) and 10x (eye piece lense)] using a Nikon SMZ1500 Zoom Stereomicroscope (Nikon, Japan). The microscope was coupled with a digital colour camera (Nikon model DS-Fi1, Nikon, Japan) and a computer with board for digital capture Pixel View Play TV (1280×980 pixels) for image capture to allow for the measurement of the thickness of the dermis, epidermis and hypodermis. The morphometric software (Image J, NIH, http://rsb.info.nih.gov/ij/) was used to measure the thickness of the epidermis (µm), dermis and hypodermis (mm). The epidermis was measured using Image J software at two different points; above the dermal papillae (the thinnest part of the epidermis) and from below the stratum corneum to the end of the rete peg (thickest part of the epidermis). Sections stained with the One-Step Mallory-Heidenhain stain were also photographed under a low-power [4x (objective lense) and 10x (eye piece lense)] using a Nikon microscope. The microscope was coupled with a digital colour camera TV-Lens C-06X (Nikon, Japan) and computer with ‘board for image capture’ to allow for measurement of the perimeter and depth of the sweat glands using the morphometric software Image J. Sections that were stained with the Fontana Stain were photographed under a low-power [(20x (objective lense) and 10x (eye piece lense)] using a Nikon microscope for the observation of the melanin.

### 6.2.3 Data analyses

The thickness of the epidermis in this study is reported for the thin part of the epidermis (this is the area of the epidermis which lies above the dermal papillae) and the thick part of the epidermis (this is the measurement from the top of the epidermis to the tip of the rete peg). The thickness of the layers was determined by calculating the average of four measurements of the thinnest and of the thickest parts (Table 1-1) (Sathar et al., 2010). The height of the dermal papillae (or dermal pegs) was calculated as the difference between the thickest part of the epidermis and the thinnest part of the epidermis. The size of the sweat glands was calculated by determining the perimeter (mm) of the sweat glands (Carvalho et al., 1995) using the Image J software. The depth of the sweat glands was determined by measuring the distance between the epidermis and the top end of the sweat glands in each body region and then four measurements per region were averaged. The amount of melanin was assigned either a negative sign (for the absence of melanin) or a positive sign (for the presence of melanin). The greater the number of positive signs the more the melanin present (See Table 6-3).

Statistical analyses were performed using the GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA). The data was analysed using repeated measures two-way analysis of variance (ANOVA) at 5% level. Data was tested for normality and homoscedasticity before being analysed. The thickness of the epidermis, dermis, hypodermis, height of the dermal papillae (or dermal pegs), size (perimeter) and depth of the sweat glands were used compared between breeds across body regions. When the ANOVA revealed significant differences between the means, the Tukey's Multiple Comparison *post-hoc* Test was performed to detect the differences in the anatomical traits between the breeds and between the three body regions within a breed. Values are reported as mean ± SD. $P < 0.05$ was considered statistically significant.

## 6.3 Results

### 6.3.1 Epidermis

The thin part of the epidermis was significantly different ($F_{2,13}$ = 7.31; $P$ = 0.0075) between the breeds with the Kolbroek having a significantly thicker epidermis than the Large White ($P$ < 0.01) and the Windsnyer ($P$ < 0.001) pigs. There were no significant differences ($P$ > 0.05) in the thickness of the thin part of the epidermis between the Large White and Windsnyer pigs. When considered by specific region, in the dorsal interscapular region, the Windsnyer had a significantly different thin part compared to the Large White ($P$ < 0.001) and the Kolbroek pigs ($P$ < 0.01) with the Kolbroek pigs having a significantly ($P$ < 0.01) thicker part compared to the Large White pigs. In the lateral thoraco- abdominal region the Kolbroek had a significantly thicker section than the Large White pigs ($P$ < 0.01) while no differences ($P$ > 0.05) were detected in the thickness of the thin part between the Large White and Windsnyer pigs and the Kolbroek and the Kolbroek pigs. There were no differences in the thickness of the thin part between the breeds in the ventral abdominal region ($P$ > 0.05). The thin part of the epidermis was also significantly different ($F_{2,26}$ = 11.08; $P$ = 0.0003) between the three body regions (Table 6-1) within the breeds, with the ventral abdominal region having a significantly thinner epidermis than the dorsal interscapular ($P$ = 0.002) and lateral thoraco-abdominal ($P$ = 0.0001) regions. The thin part of the epidermis was not significantly different ($P$ = 0.68) between the dorsal interscapular and lateral thoraco-abdominal regions. There was a significant interaction between the breed of pig and body region ($F_{4,26}$ = 3.19; $P$ = 0.030). The differences between breeds were not consistent across body regions. The epidermis of the Windsnyer pigs was particularly thin on the dorsal interscapular while in the Large White and Kolbroek pigs it was thin in ventral abdominal region.

The thick part of the epidermis was significantly different ($F_{2,13}$ = 15.34; $P$ = 0.0004) between the breeds with the Windsnyer breed of pig having a significantly thinner part than the Large White ($P$ < 0.0001) and Kolbroek ($P$ < 0.001) pigs. The thickness of the

thick part of the epidermis was not significantly different ($P > 0.05$) between the Large White and Kolbroek pigs. In the dorsal interscapular region, there was no significant difference in the thickness between the Large White and Kolbroek ($P > 0.05$). However the thick part in the Windsnyer pigs was significantly thinner than in the Large White ($P < 0.001$) and the Kolbroek pigs ($P < 0.01$). In the lateral thoraco- abdominal region there were no differences in the thickness of the thick part of the Large White compared to the Kolbroek pigs ($P > 0.05$) and Windsnyer pigs ($P > 0.05$). However, the Windsnyer pigs had a significantly thinner thick part of the epidermis compared to the Kolbroek pigs ($P < 0.01$). In the ventral abdominal region, the thick part of the epidermis of the Windsnyer pigs was significantly thinner compared to the Large White ($P < 0.0001$) and the Kolbroek pigs ($P < 0.001$) while there was no significant difference in the thickness between the Large White and Kolbroek ($P > 0.05$). There were no significant differences ($F_{2,26} = 0.78$; $P = 0.47$) in the thickness of the thick part of the epidermis across the three body regions. There was no significant interaction between the breed of pig and body region ($F_{4,26} = 1.58$; $P = 0.21$).

The height of the dermal papillae was significantly different ($F_{2,13} = 9.98$; $P = 0.0024$) between the breeds with the Windsnyer pigs having a significantly shorter dermal papillae than the Large White ($P < 0.001$) and Kolbroek ($P < 0.01$) pigs. The height of the dermal papillae was not significantly different ($P > 0.05$) between the Large White and Kolbroek pigs. In the dorsal interscapular region, the Windsnyer had a significantly ($P < 0.01$) shorter dermal papillae than the Large White. There were no significant differences in the height of the dermal papillae between the Kolbroek and the Large White ($P > 0.05$) and between the Kolbroek and Windsnyer pigs ($P > 0.05$). In the lateral thoraco- abdominal region there were no significant ($P > 0.05$) differences in the height of the dermal papillae between the three breeds of pigs. In the ventral abdominal region, the Windsnyer had a shorter dermal papillae than the Large White pigs ($P < 0.001$) and the Kolbroek pigs ($P < 0.01$) while there was no significant difference in the height of the dermal papillae between the Large White and Kolbroek ($P > 0.05$). There were no significant differences ($F_{2,26} = 0.98$; $P = 0.39$) in the height of the dermal papillae across the three regions within the breeds. There was no significant interaction between the breed of pig and body region ($F_{4,26} = 1.94$; $P = 0.13$). On subjective assessment, the

dermal papillae in the Windsnyer pigs appeared widely spaced and where they occurred they were in clusters (Figure 6-2) compared the Large White and Kolbroek pigs.

### 6.3.2 Dermis

The thickness of the dermis was significantly different ($F_{2,13}$= 59.29; $P$ < 0.0001) between the breeds, with the Windsnyer pigs having a significantly thinner dermis across the three body regions than the Kolbroek pigs ($P$ < 0.001) and the Large White ($P$ < 0.0001). The Kolbroek had a thinner dermis than the Large White pigs ($P$ < 0.001) (Table 6-1). In the dorsal interscapular region, the Windsnyer pigs had a significantly thinner dermis than the Large White ($P$ < 0.0001) and the Kolbroek ($P$ < 0.0001) while the Kolbroek had a thinner dermis than the Large White pigs ($P$ < *0.01*) pigs. In the lateral thoraco- abdominal region, the Windsnyer also had a significantly thinner dermis than the Large White ($P$ < 0.0001) and the Kolbroek ($P$ < 0.001) while the Kolbroek had a thinner dermis than the Large White pigs ($P$ < 0.0001) pigs. In the ventral abdominal region, the Windsnyer pigs had a significantly thinner dermis than the Large White ($P$ < 0.0001) and Kolbroek ($P$ < 0.001). However, the thickness of the dermis was not significantly ($P$ > 0.05) different between the Large White and Kolbroek.

The thickness of the dermis was significantly different ($F_{2,26}$ = 28.83; $P$ < 0.00001) across the body regions with the ventral abdominal region having a significantly thinner dermis ($P$ = 0.0001) than the dorsal interscapular and lateral thoraco-abdominal regions ($P$ = 0.0001). The thickness of the dermis was not significantly different ($P$ = 0.17) between the dorsal interscapular and lateral thoraco-abdominal regions. There was a significant interaction between the breed of pig and body region ($F_{4,26}$ = 5.57; $P$ = 0.0022). The differences between breeds were not consistent across body regions. The dermis of the Windsnyer and Kolbroek pigs was particularly thin on the ventral abdominal region while in the Large White pigs it was thin on the dorsal interscapular and the ventral abdominal region.

### 6.3.3 Hypodermis

The thickness of the hypodermis was significantly different ($F_{2,13} = 17.75$; $P = 0.0002$) between the breeds with the Windsnyer pigs having a significantly thinner hypodermis than the Kolbroek pigs ($P < 0.0001$) and Large White ($P < 0.0001$) while there was no significant difference in the thickness of the hypodermis between the Large White and Kolbroek ($P > 0.05$) (Table 6-1). In the dorsal interscapular region, the Windsnyer pigs had a significantly thinner hypodermis than the Large White ($P < 0.001$) and the Kolbroek ($P < 0.0001$). In the lateral thoraco- abdominal region, the Windsnyer had a significantly thinner hypodermis than the Large White ($P < 0.0001$) and the Kolbroek ($P < 0.001$). The thickness of the hypodermis was not significantly different ($P > 0.005$) between the Large White and Kolbroek pigs in both the dorsal interscapular and lateral thoraco- abdominal regions. In the ventral abdominal region, the Windsnyer had a significantly thinner hypodermis than the Kolbroek ($P < 0.01$) while there was no significant difference in the thickness between the Large White pigs and the Kolbroek ($P > 0.05$). There was also no significant difference in the thickness of the hypodermis in the ventral abdominal region of the Large White compared to the Windsnyer pigs ($P > 0.05$).

There were significant differences ($F_{2,26} = 14.32$; $P < 0.0001$) in the thickness of the hypodermis across the body regions, with the ventral abdominal region having a significantly thinner hypodermis than the lateral thoraco-abdominal ($P = 0.002$) and dorsal interscapular ($P = 0.001$) regions. There were no significant differences ($P = 0.94$) in the thickness of the hypodermis between the dorsal interscapular and lateral thoraco-abdominal regions. There was a significant interaction between the breed of pig and body region ($F_{4,26} = 4.78$; $P = 0.051$). The differences between breeds were not consistent across body regions. The hypodermis of the Windsnyer pigs was thin on the lateral thoraco-abdominal regions while in the Large White and Kolbroek pigs it was thin in the ventral abdominal region.

**TABLE 6-1**: Effect of the breed on the thickness of the epidermis, dermis and hypodermis of the local Kolbroek and Windsnyer pigs and the exotic Large White pigs.

| | Large White (n=7) | Kolbroek (n=4) | Windsnyer (n=5) |
|---|---|---|---|
| **T*hin part of epidermis* (µm)** | | | |
| Dorsal interscapular region | 46.5 ± 11.0$^{a,1}$ | 58.4 ± 7.2$^{b,1}$ | 35.5 ± 3.7$^{c,1}$ |
| Lateral thoraco-abdominal region | 43.9 ± 6.5$^{a,1}$ | 55.8 ± 5.4$^{b,1}$ | 48.1 ± 9.0$^{a,b,1}$ |
| Ventral abdominal region | 36.9 ± 5.9$^{2}$ | 41.8 ± 9.2$^{2}$ | 36.7 ± 1.9$^{2}$ |
| ***Thick part of epidermis* (µm)** | | | |
| Dorsal interscapular region | 145.9 ± 40.6$^{a}$ | 137.0 ± 28.1$^{a}$ | 75.3 ± 24.0$^{b}$ |
| Lateral thoraco-abdominal region | 108.5 ± 24.4$^{a,b}$ | 139.3 ± 36.3$^{a}$ | 79.1 ± 37.3$^{b}$ |
| Ventral abdominal region | 140.1 ± 46.3$^{a}$ | 123.6 ± 19.4$^{a}$ | 54.8 ± 2.9$^{b}$ |
| ***Height of dermal papillae* (µm)** | | | |
| Dorsal interscapular region | 104.4 ± 39.9$^{a}$ | 90.3 ± 34.1$^{a,b}$ | 50.6 ± 20.3$^{b,1}$ |
| Lateral thoraco-abdominal region | 64.7 ± 27.2 | 83.0 ± 36.1 | 54.2 ± 22.4$^{,1}$ |
| Ventral abdominal region | 103.2 ± 47.4$^{a}$ | 80.5 ± 19.6$^{a}$ | 23.2 ± 1.8$^{b,2}$ |
| **Dermis (mm)** | | | |
| Dorsal interscapular region | 4.5 ± 1.0$^{a,1}$ | 3.3 ± 0.2$^{b,1}$ | 1.1 ± 0.1$^{c,1}$ |
| Lateral thoraco-abdominal region | 5.4 ± 1.3$^{a,1}$ | 3.3 ± 0.5$^{b,1}$ | 1.3 ± 0.3$^{c,1}$ |
| Ventral abdominal region | 2.6 ± 0.5$^{a,2}$ | 2.3 ± 0.3$^{a,2}$ | 0.5 ± 0.1$^{b,2}$ |
| **Hypodermis (mm)** | | | |
| Dorsal interscapular region | 8.3 ± 2.3$^{a,1}$ | 10.4 ± 4.7$^{a,1}$ | 1.5 ± 0.2$^{b,1}$ |
| Lateral thoraco-abdominal region | 10.5 ± 3.3$^{a,1}$ | 8.6 ± 5.2$^{a,1}$ | 1.3 ± 0.4$^{b,1}$ |
| Ventral abdominal region | 4.4 ± 1.7$^{a,b,2}$ | 5.4 ± 0.5$^{a,2}$ | 1.1 ± 0.5$^{b,2}$ |

**Note**: $^{a,b,c}$ Within a row, means without a common superscript differ at $P < 0.05$

$^{1,2}$ Values within a column with different superscripts differ significantly at $P < 0.05$ between the body regions. Data are shown as mean ± SD.

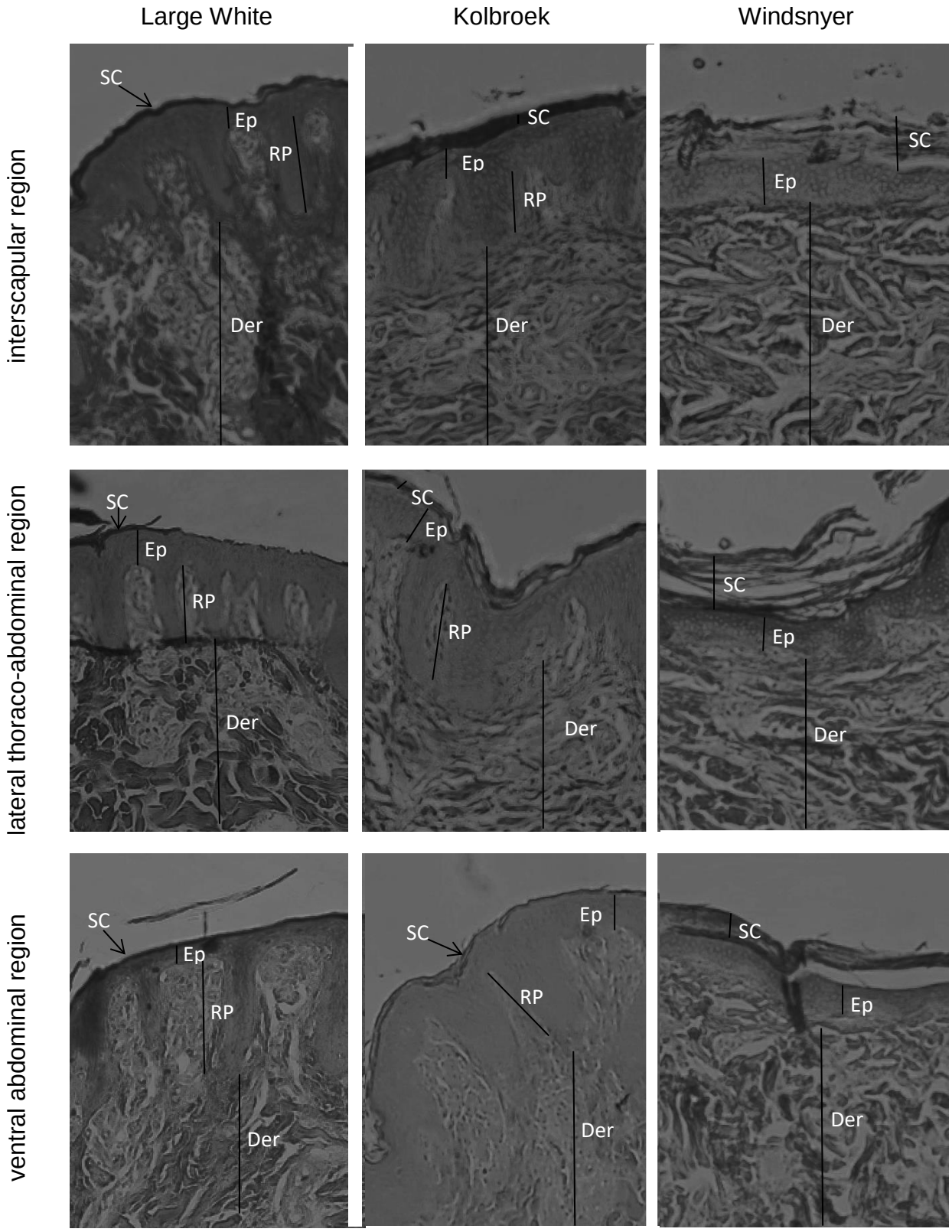



**FIGURE 6-1:** Representative vertical skin sections from the dorsal interscapular region, lateral thoraco-abdominal region and ventral abdominal region of

Windsnyer, Kolbroek and Large White pigs. The three sections from the different body regions of the three breeds of pigs show the epidermis (Ep), dermis (Der) and stratum corneum (SC) and rete pegs (RP) (Mallory-Heidenhain stain; objective: 10x).

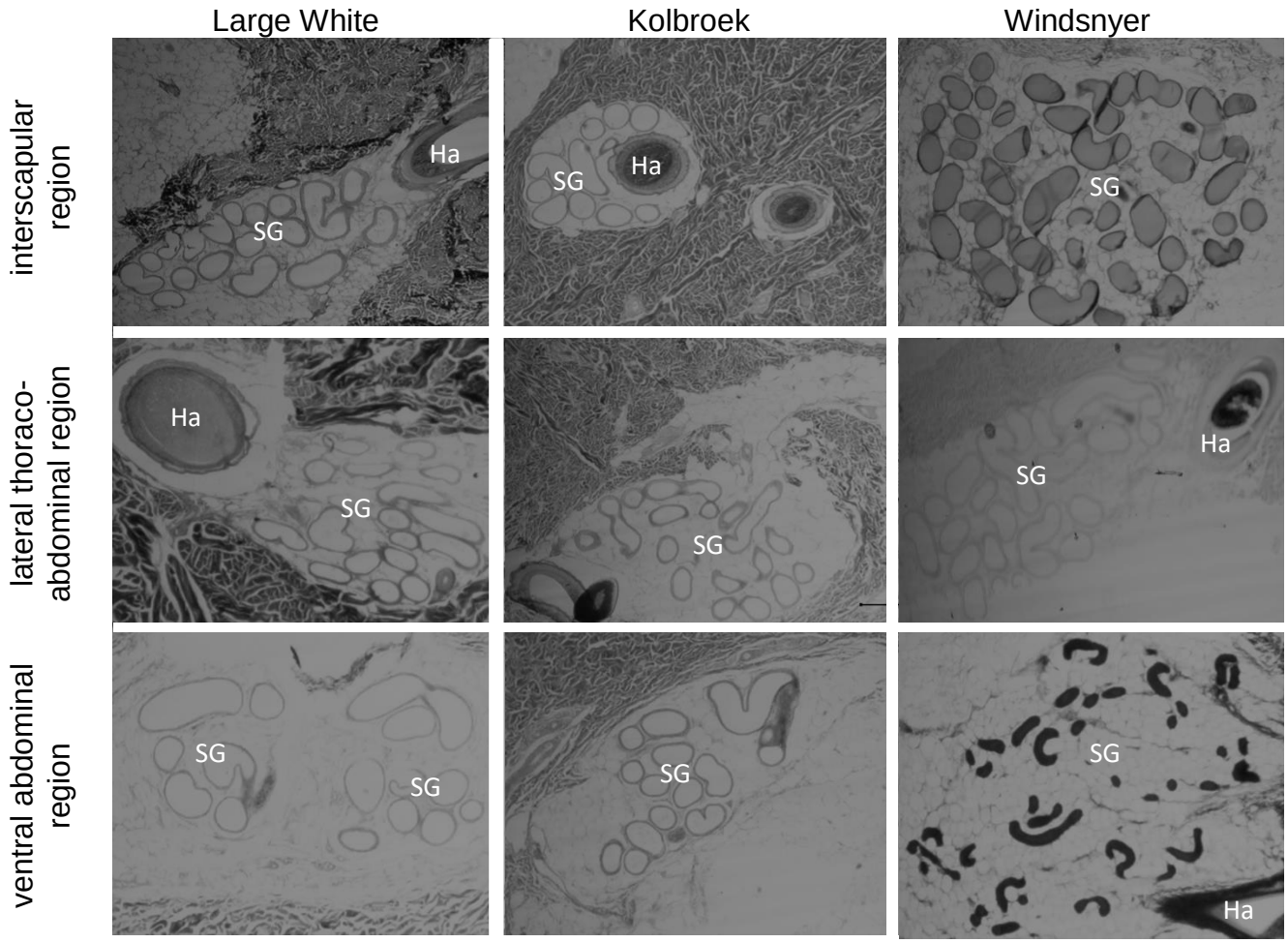



**FIGURE 6-2:** Representative vertical skin sections from the dorsal interscapular region, lateral thoraco-abdominal region and ventral abdominal region of Windsnyer, Kolbroek and Large White pigs showing the relative sizes of the sweat glands in the three body regions. (Sweat gland (SG) and hair structure (Ha)) (Mallory-Heidenhain stain; objective: 4x).

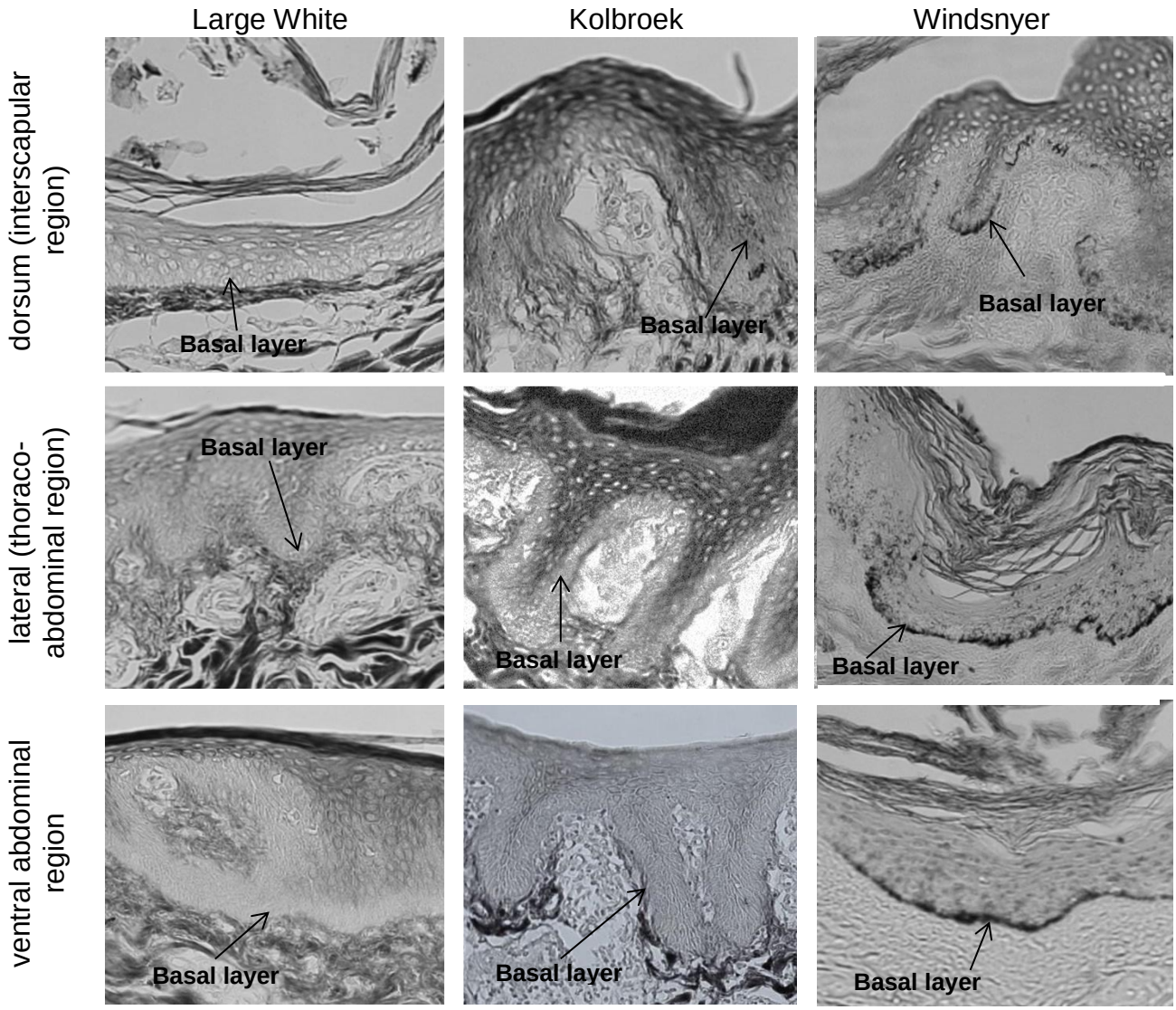



**Figure 6-3**: Representative vertical skin sections from the dorsal interscapular region, lateral thoraco-abdominal region and ventral abdominal region of Windsnyer, Kolbroek and Large White pigs. The figure shows a large amount of melanin pigments in the basal layer of the epidermis in the Windsnyer pigs, very little in the interscapular region in the Kolbroek pigs and no melanin pigments in Large White pigs (Fontana staining; objective: 20x).

### 6.3.4 Sweat glands

Figure 6-2 shows photomicrographs indicating the relative sizes of the sweat glands from the interscapular, lateral thoraco-abdominal and the ventral abdominal regions in the different breeds of pigs. In all the regions the sweat glands were distributed in the dermis and some sweat glands were associated with a hair follicle.

#### 6.3.4.1 Perimeter of the sweat glands

The average perimeter (size) of the sweat glands was significantly different ($F_{2,13}$ = 52.48; $P$ < 0.0001) between the breeds of pigs with the Windsnyer pigs having a significantly larger average perimeter than in the Large White ($P$ < 0.0001) and Kolbroek ($P$ < 0.001) pigs in all three body regions (Table 6-2). There were no significant ($P$ > 0.05) differences in the perimeter of the sweat glands between the Large White and Kolbroek pigs in all the three body regions. The average perimeter of the sweat glands was not significantly different ($P$ = 0.68) between the Large White and Kolbroek pigs. There were significant differences ($F_{2,26}$ = 9.45; $P$ = 0.0008) in the average perimeter of the sweat glands across the body regions with the average perimeter of the sweat glands in the ventral abdominal region being significantly smaller ($P$ = 0.034) than those in the lateral thoraco-abdominal region and dorsal interscapular in all the three breeds of pigs. There were no significant differences in average perimeter of the sweat glands between the body regions ($P$ = 0.94) in the Large White and Kolbroek pigs. However, in the Windsnyer pigs the average perimeter of the sweat glands in the ventral abdominal region was significantly larger than ($P$ = 0.029) those in the dorsal and lateral thoraco-abdominal regions. There was no significant interaction between the breed of pig and body region ($F_{4,26}$ = 2.32; $P$ = 0.084).

#### 6.3.4.2 Depth of sweat glands

The depth of the sweat glands was significantly different ($F_{2,13}$ = 125.60; $P$ < 0.0001) between the breeds of pigs, with the Windsnyer pigs having a significantly more superficial sweat glands than the Large White ($P$ < 0.0001) and Kolbroek ($P$ < 0.0001)

pigs in all the three body regions (Table 6-2). The Kolbroek pigs had sweat glands which were more superficial ($P < 0.001$) than in the Large White pigs in all the three body regions. There were no significant differences ($F_{2,26} = 0.40$; $P = 0.67$) in the mean depth of sweat glands within the three regions within the breeds. There was no significant interaction between the breed of pig and body region ($F_{4,26} = 0.093$; $P = 0.98$).

**TABLE 6-2**: A comparison of the sweat gland characteristics of the local Kolbroek and Windsnyer pigs and the exotic Large White pigs.

| | Large White (n=7) | Kolbroek (n=4) | Windsnyer (n=5) |
|---|---|---|---|
| **Perimeter of sweat glands (mm)** | | | |
| Dorsal interscapular region | $2.1 \pm 0.8^{a,1}$ | $2.1 \pm 0.1^{a,1}$ | $4.7 \pm 1.1^{b,1}$ |
| Lateral thoraco-abdominal region | $2.2 \pm 0.4^{a,1}$ | $2.5 \pm 0.6^{a,1}$ | $5.0 \pm 0.5^{b,1}$ |
| Ventral abdominal region | $1.6 \pm 0.4^{a,1}$ | $2.1 \pm 0.2^{a,1}$ | $3.5 \pm 0.7^{b,2}$ |
| **Depth of sweat glands (mm)** | | | |
| Dorsal interscapular region | $3.9 \pm 0.9^{a,1}$ | $2.8 \pm 0.7^{b,1}$ | $1.1 \pm 0.2^{c,1}$ |
| Lateral thoraco-abdominal region | $4.0 \pm 0.4^{a,1}$ | $2.9 \pm 0.7^{b,1}$ | $1.0 \pm 0.2^{c,1}$ |
| Ventral abdominal region | $3.7 \pm 0.4^{a,1}$ | $2.7 \pm 0.5^{b,1}$ | $1.0 \pm 0.1^{c,1}$ |

**Note**: $^{a, b, c}$ Within a row, means without a common superscript differ at $P < 0.05$

$^{1, 2}$ Values within a column with different superscripts differ significantly at $P < 0.05$ between the body regions

Data are shown as mean ± SD.

### 6.3.5 Melanin

Figure 6-3 shows representative photomicrographs of sections of the three sampled regions of the epidermis showing the distribution of melanin in the basal layers. The Windsnyer pigs had visibly more melanin in the basal layer (Table 6-3), than the Kolbroek which had little melanin. The Large White pigs had no visible melanin.

**TABLE 6-3**: A comparison of the presence of melanin in the basal layers of the skins of the local Kolbroek and Windsnyer pigs and the exotic Large White pigs.

| | Large White (n=7) | Kolbroek (n=4) | Windsnyer (n=5) |
|---|---|---|---|
| **Melanin** | | | |
| Dorsal interscapular region | - | + | ++ |
| Lateral thoraco-abdominal region | - | - | ++ |
| Ventral abdominal region | - | + | ++ |

**Note**: Negative sign (-) no visible melanin present in the basal layer; Positive sign (+) little melanin present in the basal layer; (++) plenty of melanin present in the basal layer

## 6.4 Discussion

There were regional anatomical differences in the thickness of the skin layers, size and depth of the sweat glands in the interscapular, lateral thoraco-abdominal and ventral abdominal regions in the three breeds. The Windsnyer pigs had the thinnest epidermis, dermis, hypodermis layer and more superficial sweat glands across the three body regions than in the Large White and Kolbroek pigs. In addition, the Windsnyer pigs had more visible melanin in the basal layer while the Kolbroek pigs had very little. The Large White did not have melanin across all body regions.

### 6.4.1 Epidermis

In the current study, there were regional differences in the thickness of the epidermis between the three breeds of pigs. The epidermis thickness of the pig is reportedly quite variable. For example, a thickness of 30 to 100 µm (Morris and Hopewell, 1990) and 70 to 140 µm (Meyer et al., 1978) in the Yorkshire and miniature pigs respectively has been reported. We found that the thickness of the epidermis in both the local Windsnyer and Kolbroek and the exotic Large White pigs in the current study ranged between 35 and 146 µm which is comparable to the values previously noted in Yorkshire and miniature pigs. The thickness of the epidermis plays an important role in heat tolerance. Previous

studies on cattle and Indian buffaloes showed that those animals with a thinner epidermis were found to tolerate heat stress better than animals with a thicker epidermis (Saravanakumar and Thiagarajan, 1992). Consequently a thin epidermis appears to confer improved heat tolerance in cattle and buffaloes. The Windsnyer pigs in the current study had a thinner epidermis than the Large White and Kolbroek pigs which may confer increased heat tolerance than the Large White and Kolbroek pigs as has been shown in cattle and buffaloes with different thickness of the epidermis.

### 6.4.2 Dermis and hypodermis

In the current study the thickness of the dermis in the Large White pigs ranged between 2.6 and 4.5 mm; between 2.3 and 3.3 mm in the Kolbroek pigs and between 0.5 and 1.3 mm in the Windsnyer. Compared to literature on other pigs, the thickness of the dermis in both local pigs was less than the 3.6 mm reported for local Caribbean Creole pigs by Renaudeau et al. (2006). However, the thickness of the dermis in all the three sampled body regions of the Large White and Kolbroek pigs were within the range recorded by Andrews et al. (2013) in the Large White pigs, while the dermis in all the body regions of the Windsnyer pigs was lower. However the thickness of the dermis in the Large White pigs was greater in the dorsal (4.5 mm) and lateral thoraco-abdominal regions (5.4 mm) than that obtained (3.6 mm) in the Large White pigs by Renaudeau et al. (2006). In all the three breeds the dermis was thinner in the ventral abdominal region compared to the other two body regions sampled. The thicker skin on the dorsal/lateral-thoraco abdominal areas is probably an anatomical adaptation to offer protection as pigs normally rub their bodies against rough structures. It may also be to offer more of a physical protective barrier to trauma as they are mostly likely to be attacked or injured on the sides and back which are also more exposed to the elements than the ventrum. The thickness of the dermis also plays an important role in heat tolerance of some animals with those having a thinner dermis tending to be more heat tolerant than those with a thicker dermis (Daghash et al., 1999, Wang et al., 2012). In this current study, the Windsnyer pigs had a thinner dermis than the Large White and Kolbroek pigs in all the three body regions suggesting that the Windsnyer pigs might have a better heat tolerance than both the Large White and Kolbroek pigs. This thinner dermis that I

observed in the pigs in the current study might explain the differences in the thermoregulatory responses between the pigs in the hot climatic chamber when exposed to high temperatures (Chapter 5).

In the current study the hypodermis which encompasses the subcutaneous fat layer was thinner in the Windsnyer compared to the Large White and Kolbroek pigs in the three body regions. Unlike our findings, Madzimure et al. (2012) found that the Windsnyer had a thicker subcutaneous fat layer than the Large White pigs. A thicker hypodermis can hinder heat transfer from the body to the environment (Sokolov, 1982). Differences in the thickness of the hypodermis between the Windsnyer pigs in the current study could be attributed to nutrition of the pigs. In their study, Madzimure *et al.* (2012) fed the pigs *ad libitum* while in our study we fed the pigs at a maintenance rate hence they were more likely to have an increased fat accretion. A previous study has also shown that age and sex have an effect on fat deposition in pigs wherein females pigs had a thicker fat layer than males as they grew older (Bollen et al., 2004). Madzimure et al. (2012) used 3 months old females whereas in this study boars aged between 6 and 8 months were used. These factors (nutrition, sex and age) may explain the differences in thickness of hypodermis noted between our study and that of Madzimure et al. (2012).

### 6.4.3 Sweat glands

In this study, the size and depth of the sweat glands differed between the breeds and between the body regions. The Windsnyer pigs had sweat glands that were twice as big and more superficial than those in the Large White and Kolbroek pigs in all the three sampled regions of the body. The larger perimeter and closeness to the surface of the sweat glands in the Windsnyer pigs might suggest that they had a greater capacity through which moisture can be lost hence a constant respiratory rate when exposed to high temperatures (Chapter 5). In cattle the sweating efficiency appears to be affected by the size, the density, number and depth of sweat glands (Nay and Hayman, 1956). *Bos indicus* cattle (which sweat more efficiently than *Bos taurus*) have been shown to have sweat glands that were two and a half times as large and one and a half times as numerous than those in *Bos taurus* cattle (Pan, 1962, Carvalho et al., 1995). **However,**

**it is important to note that most of the studies on domestic pigs have found that sweat glands in the pigs are not functionally effective for thermoregulation** (Ingram, 1967, Curtis, 1981, Bracke, 2011). In a chapter in a book, "*Mammal Skin"* by Sokolov (1982), he states that of the *artiodactyla* studied "the sweat glands were best developed in the wild boar". The reason is that the wild boar has a thick subcutaneous fat layer that hinders heat transfer through the skin (Sokolov, 1982). Given the findings in the wild boars by Sokolov (1982), it is feasible that the local Windsnyer pigs might have active sweat glands especially considering the assertion by other researchers that the Windsnyer pig is closely related to the wild boar that has origins in Europe (Swart, 2010). It is imperative that studies on the sweat glands in the Windsnyer pigs be undertaken.

### 6.4.4 Melanin

Melanin is important in the absorption, scattering, and reflection of different wavelengths of light (Jablonski, 2004). Specifically it absorbs ultraviolet (UV) light that could damage DNA and other biological molecules. It also scavenges free radicals, regulates vitamin D3 biosynthesis by influencing the penetration of UV light through the skin. Melanin plays a role in thermoregulation and detoxification wherein it binds to some organic molecules, drugs and heavy metals (Patel and Forsythe, 2008). The amount and distribution of melanin in the skin is influenced by genetic, environmental, and endocrine factors (Costin and Hearing, 2007).

In the current study, as expected the Large White pigs had no visible melanin. The lack of melanin in the Large White is attributed to a double mutation of the KIT gene, which was shown to correspond to the Dominant white (I) coat colour locus on chromosome 8 (Pielberg, 2004, Marklund et al., 1998). Melanin in animals occurs in two broad groups the eumelanin ('eu' means good) which are dark and range from brown to black and the pheomelanin ('pheo' means cloudy or dusty) are reddish or yellowish (Solano, 2014). The eumelanin is the important type as it has a photo protective role (Thody et al., 1991). The Fontana-Masson silver stain used in the current study contains silver nitrate which is reduced by melanin and melanin-like pigments to metallic silver which appears

black (Bishop et al., 2012). Although this method has a high specificity for melanin this stain seems to react with all melanin and melanin-like pigments and it is not easy to distinguish between the types of melanin present in the pigs. I further recommend that future studies be conducted to distinguish between the types of melanin present in the local Windsnyer and Kolbroek pigs. Genetically, two loci *Extension* and *Agouti* regulate the relative quantities of eumelanin and pheomelanin produced by the cells. The *Extension* locus encodes the melanocortin 1 receptor (MC1R) that interacts with α melanocyte-stimulating hormone (αMSH) resulting in the production of eumelanin. The *Agouti* loci antagonises MC1R, blocking its interaction with the αMSH-receptor causing a production of more pheomelanins than eumelanins (Lu et al., 1994, Ollmann et al., 1998) hence a white colour. Molecular variations to MC1R produces variation in pigmentation in many domesticated mammals originating in different areas, including cattle (Klungland et al., 1995, Rouzaud et al., 2000, Adalsteinsson et al., 1995), goats (Fontanesi et al., 2009), pigs (Kijas et al., 1998, Kijas et al., 2000), and sheep (Fontanesi et al., 2010, Royo et al., 2008, Våge et al., 2003).

A surprising observation was the presence of very little melanin in the Kolbroek pigs. From a distance the Kolbroek were observed to have black and white hair patches (See Figures 2-1, 2-2 and 2-3 for images of the breeds of pigs). However on close inspection of the skin, it was notable that the Kolbroek pigs had black and white hairs, whereas the underlying skin had a uniform light coloured appearance. The Windsnyer pigs had entirely black hairs and as was the case with the Kolbroek, the skin underlying had a uniform light appearance superficially, but on histological examination was found to contain a lot of melanin in the basal layer of the skin. The substantial amount of melanin in the basal layer suggests that the Windsnyer pigs might have better protective, physiological and functional properties than the other breeds. As the ozone layer gets depleted, animals will have increased exposure to ultraviolet radiation leading to increased susceptibility to its detrimental effects such as suppressed cell immunity (de Gruijl et al., 2003). Continued exposure to UV radiation reduces lymphocytes (Krueger et al., 1995) and animals become susceptible to infection by viruses and bacteria. Thus continued depletion of the ozone layer might impact the epidemiology of some animal diseases in future (Kimaro and Chibinga, 2013) with the less pigmented pig breeds such

as the Kolbroek and Large White pig breeds being more likely to be affected than the pigmented Windsnyer. However it is important to note that most of the studies on effects of increased exposure to UV radiation were conducted in mice and humans (van der Leun and de Gruijl, 1993) and there is need for further investigation in other animal cells (Kimaro and Chibinga, 2013) and particularly pigs because of the nature of their skin because it is sparsely protected by hairs unlike in other animals.

## 6.5 Conclusion

In South Africa climate change is predicted to result in increased environmental temperatures that will be higher than the world average and consequently animals in the region will experience a higher heat load than animals in other regions in the world. The Windsnyer pigs appear to possess skin anatomical traits that might buffer the effects of the changing climate such as the increased temperatures. It is thus prudent that conservation of this important genetic resource be encouraged in the region.

# CHAPTER 7: CONCLUSIONS AND FUTURE DIRECTION

The economic impact of climate change will likely be catastrophic on the livelihoods of smallholder subsistence agriculture farmers due to their vulnerability as they have a low adaptive capacity. As the environmental temperatures rise and water shortage worsens they will have a secondary effect on the geographical and seasonal distribution of diseases. These stressors will adversely impact on the productivity of livestock and hence food security.

The aim of this thesis was to contribute to the understanding of the physiological adaptability traits of local pigs that are likely to be required to withstand the effects of climate change. This was achieved by investigating the febrile and thermoregulatory responses that could be helpful in buffering the effects of bacterial and viral diseases (Chapter 4) and hot and dry environmental conditions (Chapter 5) respectively as well as the morphology of the skin in the three breeds (Chapter 6).

The local pig breeds are a vital genetic resource we wish to conserve, yet we know so little about them. To date the majority of studies that sought to understand the response to the effects of climate change in the agricultural production systems primarily focused on crops and very few on livestock (Leclère et al., 2014). My study has started to answer some questions regarding local pig breeds responses to bacterial and viral mimetics and the differing thermal environments, but the differences appear to be more subtle than anecdotally reported.

## 7.1 Fever

The hypothesis underlying this component of the study was that “the local Kolbroek and Windsnyer pigs will show a dampened febrile response and sickness behavioural responses (physical activity, voluntary feed intake and body mass) compared to the exotic Large White pigs when injected with bacterial and viral mimetics”. The increase in animal diseases is threatening animal health and subsequently food security. As highlighted in Chapter 4, I characterised the febrile responses of the local pigs to viral,

gram-negative bacterial and gram-positive bacterial mimetics for the first time. I noted that there were slight differences in the febrile responses that were not biologically significant. With the spread of pathogens as predicted under climate change, there could be new or re-emergence of old diseases that would have various effects on animals.

There are no previous studies that have investigated the febrile responses and sickness behavioural responses in the local pigs using the three mimetics. However studies have been done on the Large White pigs using LPS (Johnson and von Borell, 1994, Webel et al., 1997) with emphasis on the acute phase response where they investigated febrile responses and sickness behaviours. The development of sickness behaviours accompanied by severe clinical signs in the pigs will be counterproductive and might lead to reduced productivity and hence decreased profitability. The development of sickness behaviours is important for farmers to be able to identify and either treat, isolate or cull the sick individual animals and prevent the spread of the pathogens within the herds. Consequently, a better understanding of the fever in the local breeds might be important in that it will help the farmers to know how the diseases will manifest so that they can manage their animals better and minimise the negative effects of the diseases on productivity. It might therefore be more profitable to strategically farm particular selected breeds in different regions of the world as animals have shown different adaptation abilities. Studies in different regions are already suggesting which breeds of pigs should be prioritised in the respective regions based on their resistance to diseases (Russo et al., 2004, Zanga et al., 2003). Hence it was important to establish the adaptability of the local Kolbroek and Windsnyer pigs through studies on their febrile responses. However, long term studies assessing the reproductive performance of the local breeds of pigs in comparison with the exotic breeds of pigs following infection with different pathogens are required in the different regions. These aspects can be assessed by investigating the traits of economic importance such as fertility, litter size, litter birth mass, finishing target body mass, voluntary feed intake and growth rates when the pigs are exposed to multiple stressors at the same time. Computation of the economic impact assessment of the effects of climate change induced effects on the different breeds to measure breed success and adaptability would be vital in this assessment.

## 7.2 Thermoregulation

Climate change is expected to exacerbate the conditions in southern Africa in regions that are already water stressed as well as experiencing high environmental temperatures (IPCC, 2007). In Chapter 5, I investigated the thermoregulatory and behavioural responses between the local Windsnyer and Kolbroek breeds of pigs and the exotic Large White pigs when exposed to different simulated environmental conditions for the first time. In this study I found that the different breeds efficiently thermoregulated when exposed to both cold and hot ambient temperatures despite having different body masses. In the cold the local Kolbroek and Windsnyer pigs used more of primitive behaviours to maintain their core body temperatures due to their smaller body size (larger surface area relative to body mass) compared to the Large White that increased physical activity. There was, however a difference in the percentage change in the respiratory rates between the Windsnyer pigs and the Large White and the Kolbroek pigs. When exposed to the hot temperatures the Large White and Kolbroek pigs relied more on evaporative cooling to prevent the body temperature from rising probably because of the thicker skin layers and the hypodermis (Chapter 6) that impeded heat loss. On the contrary the Windsnyer pigs had a thinner skin layers and hypodermis (Chapter 6) and might have relied on conductance to lose heat. Although both the Large White and Kolbroek pigs appeared sensitive to high environmental temperatures, that did not result in high core body temperatures when compared to TNZ. In a previous study, it was found that the Windsnyer and Large White pigs had similar rectal temperatures throughout the day (Madzimure et al., 2012). However, in the present study I found that the local breeds maintained a higher core body temperature than the exotic Large White pigs and that might be related to body size as was previously found in the artiodactyl by Clarke and Rothery (2008). The higher body temperature might be beneficial to the pigs as was found in the camels (Schmidt-Nielsen et al., 1957) and kangaroos (McCarron et al., 2001).

Increased environmental temperatures and water shortages will be common in most areas in southern Africa. To test how the pigs will respond to those situations in future, simulated conditions of increased ambient temperatures and drinking water deprivation

were used. I found that the local pigs had a remarkable ability to conserve their body water. The Large White pigs failed to conserve their plasma volume indicating that they might be prone to dehydration more than the local pigs. The local pigs showed that they can have control over their body water when drinking water is limited. The ability to protect the plasma volume has been found to be useful in animals that inhabit dry environments where access to drinking water is constantly a problem throughout the year (Carmi et al., 1993).

Indeed a study is already suggesting which breed of pigs based on heat tolerance should be prioritised for use in the smallholder production systems for the Eastern Cape Province, South Africa (Madzimure et al., 2012). This province is an area that experiences high maximum temperatures in summer. The smallholder livestock systems are vulnerable to climate change and need to adapt and adjust the production system to continue producing at the current levels (Muller, 2013, Thornton and Herrero, 2014) without undermining food security. My study further confirms the importance of this local breed as it may require much less water for survival and ensured productivity than the Large White pigs hence reducing the demand for water in pig production. In some regions people spend a lot of time 'looking for/collecting water' hence use of a breed with low water requirements puts less pressure on the people to find/collect water for livestock.

Normally it is believed that adaptation to heat stress will have occurred when an animal has developed physiological, morphological and/or behavioural traits subsequent to repetitive exposure to high ambient temperatures leading to better resistance to heat stress (Curtis, 1981). The morphological trait that has been found to be important for animals in hot environments is the skin, as it has been used to respond to climate change (Barnosky et al., 2003). The skin has been found to have attributes that assisted cattle (Carvalho et al., 1995, Dowling, 1956, Nay and Hayman, 1956) and buffaloes (Saravanakumar and Thiagarajan, 1992) to adapt and tolerate heat. Pigs of different genetic origins have been reported to have different skin characteristics (Renaudeau et al., 2006).

## 7.3 Future Research

This thesis contains research which is the first to describe and characterise the acute febrile responses and sickness behaviours in response to viral and bacterial mimetics as well as the thermoregulatory responses. In addition, the microscopic skin details of the Windsnyer and Kolbroek and Large White pigs were investigated. These are the physiological responses and the skin characteristics that the pigs might use to buffer the effects of climate change. Thus it is important to support the current efforts by the Food and Agriculture Organization of the United Nations to characterise and preserve the local pig genetic resources (FAO, 2007a). In light of the above studies, the future research questions that have to be investigated further are described below.

### 7.3.1 Spread of diseases

As a result of changes in temperatures and moisture, the epidemiology of diseases has changed in some natural and agro-systems (Altizer et al., 2013). Consequently free-living pigs are exposed to multiple stressors simultaneously in environments that are far hotter than what the pigs in the current study were exposed to. In pigs, infections tend to reduce appetite, leading to decreased feed intake and subsequent reduction in daily growth rates (Balaji et al., 2000). I therefore recommend the investigation of effects of multiple stressors on the production variables such as voluntary feed intake, litter size, growth rates, body mass changes and reproductive performance as well as the physiological and behavioural responses.

The use of mimetics does not necessarily give a clear picture of what will actually happen in the pigs should the infection persist or a new pathogenic microorganism invade before the previous infection is cleared. I thus also recommend the investigation of disease tolerance in the local pig breeds after repeated infections which are likely to occur with the spread of bacterial and viral pathogens due to climate change. Knowledge of how local pigs cope with different pathogens is valuable to understanding their potential responses to the predicted increase in the geographical and seasonal changes in the distribution of new and re-emerging diseases under climate change. This

knowledge will empower the subsistence smallholder farmers to select animals that will help them adapt to climate change taking into account the epidemiology of diseases at the same time being able to realise some meaningful productivity.

I also recommend the investigation of the acute phase response, which is part of the immune system that is triggered by infection (Cray et al., 2009) in the free-living local pigs after injecting them with live pathogens. Measurements should focus on the extent and duration of the febrile responses as well as sickness behavioural responses. During the acute phase response we also need to measure the cytokines to elucidate the mechanisms of the febrile responses in the local pigs when injected with live pathogens. Knowing the mechanisms behind the bacterial and viral fevers will shed more light on the most appropriate time to intervene as the illness progresses. During this critical phase of infection, changes in voluntary feed intake, water intake, body mass and physical activity need to be measured. An improved understanding of which behaviours change as illness progresses will assist the smallholder subsistence farmers to detect the illness early and intervene appropriately.

### 7.3.2 Exposure to high temperatures

Free-living pigs are exposed to higher ambient temperatures than those that the pigs in this study were exposed to. For example the pigs in the Limpopo Province experience maximum summer temperatures of around 34°C and at times even as high as 45°C during heat waves (FAO, 2004). The current study could not use temperatures higher than 30°C and also deprive pigs of water for more than 48 h because of ethical and welfare considerations. I recommend further investigation of the contribution of the transepidermal water loss in the Windsnyer pigs as well as the sweating rates in the local pigs especially in the Windsnyer pigs that did not increase their respiratory rates when exposed to high temperatures and deprived of drinking water. Furthermore the thermoregulatory and physiological responses (haematocrit and plasma osmolality) in the local pigs when exposed to temperatures higher than those used in the current study and with complete water deprivation should be assessed. Since the pigs did not appear to be particularly vulnerable to high ambient temperatures (30°C), as was initially

proposed, future studies should investigate the effects of temperatures higher than those used in the current study and with complete water deprivation.

The potential benefits of the proposed research to the smallholder subsistence local pig farmers are that it helps with the understanding of the biophysical impacts of high temperatures and diseases and how well the local pigs will be able to adapt to the changing environmental conditions. At the same time it gives the smallholder subsistence local pig farmers an option that can allow them to adapt to climate change without serious disruption to productivity while maintaining their livelihoods under harsh conditions.

According to Vignola et al. (2015), the most promising adaptation practices “take advantage of the existing ecological processes and biological diversity to provide adaptation benefits to agricultural producers and can be potentially incorporated in many of the increasing number of initiatives that are promoting ecosystem-based responses to climate change and variability”.

# CHAPTER 8: LITERATURE CITED


ABERLE, E. D., MERKEL, R. A., FORREST, J. C. & ALLISTON, C. W. 1974. Physiological responses of stress susceptible and stress resistant pigs to heat stress. *Journal of Animal Science,* 38**,** 954-959.

ADALSTEINSSON, S., BJARNADOTTIR, S., VAGE, D. I. & JONMUNDSSON, J. V. 1995. Brown coat color in Icelandic cattle produced by the loci Extension and Agouti. *The Journal of Heredity,* 86**,** 395-398.

AHENE, A. B., SAXENA, S. & NACHT, S. 1995. Photoprotection of solubilized and microdispersed melanin particles. *In:* ZEISE, L., CHEDEKEL, M. & FITZPATRICK, T. B. (eds.) *Melanin, Its Role in Human Photoprotection.* Overland Park, KS: Valendmar.

ALEXOPOULOU, L. 2001. Recognition of double stranded RNA and activation of NF-κB by toll-like receptor 3. *Nature Australia,* 413**,** 732-738.

ALTIZER, S., OSTFELD, R. S., JOHNSON, P. T. J., KUTZ, S. & HARVELL, C. D. 2013. Climate change and infectious diseases: From evidence to a predictive framework. *Science,* 341**,** 514-519.

ALTMANN, J. 1974. Observational study of behaviour: sampling method. *Behaviour,* 49**,** 227-267.

ANDREWS, S. N., JEONG, E. & PRAUSNITZ, M. R. 2013. Transdermal delivery of molecules is limited by full epidermis, not just stratum corneum. *Pharmaceutical Research,* 30**,** 1099–1109.

ANTWI, M. & SEAHLODI, P. 2011. Marketing constraints facing emerging small-scale pig farmers in Gauteng province, South Africa. *Journal of Human Ecology (Delhi, India),* 36**,** 37-42.

ARC. 1993. Pig production in South Africa. Pretoria South Africa. Irene Animal Production Institute. 19-97.

ARMITAGE, K. B. 2009. Fur color diversity in marmots. *Ethology Ecology and Evolution,* 21**,** 183-194.

AU-IBAR. 2013. *Impact of livestock diseases in Africa* [Online]. Addis Ababa, Ethiopia African Union InterAfrican Bureau for Animal Resources. Available: http://www.au-ibar.org/vacnada-livestock-diseases?showall=&limitstart= [Accessed 12 December 2015].

AYALEW, W., DANBARO, G., DOM, M., AMBEN, S., BESARI, F., MORAN, C. & NIDUP, K. 2011. Genetic and cultural significance of indigenous pigs in Papua New Guinea and their phenotypic characteristics. *Animal Genetic Resources,* 48**,** 37-46.

BAILEKAE, M. M. 2012. *Development of cryopreservation strategies for improved reproductive competence in South African pig genotypes.* Magister Technologiae: Agriculture, Central University of Technology, Free State.

BALAJI, R., WRIGHT, K. J., HILL, C. M., DRITZ, S. S., KNOPPEL, E. L. & MINTON, J. E. 2000. Acute phase response of pigs challenged orally with *Salmonella typhimurium*. *Journal of Animal Science,* 78**,** 1885-1891.

BALDWIN, B. A. & INGRAM, D. L. 1967. The effect of heating and cooling the hypothalamus on behavioural thermoregulation in the pig. *Journal of Physiology,* 191**,** 375-392.

BALDWIN, B. A. & INGRAM, D. L. 1968. The influence of hypothalamic temperature and ambient temperature on thermoregulatory mechanisms in the pig. *Journal of Physiology,* 198**,** 517-529.

BALE, J. S., MASTERS, G. J., HODKINSON, I. D., AWMACK, C., BEZEMER, T. M., BROWN, V. K., BUTTERFIELD, J., BUSE, A., COULSON, J. C., FARRAR, J., GOOD, J. E. G., HARRINGTON, R., HARTLEY, S., JONES, T. H., LINDROTH, R. L., PRESS, M. C., SYMRNIOUDIS, I., WATT, A. D. & WHITTAKER, J. B. 2002. Herbivory in global climate change research: Direct effects of rising temperature on insect herbivores. *Global Change Biology,* 8**,** 1-16.

BANET, M. 1981. Fever and survival in the rat. Metabolic versus temperature response. *Cellular and Molecular Life Sciences,* 37**,** 1302-1304.

BANHAZI, T., AARNINK, A. T., PEDERSEN, S., HARTUNG, J., MALTZ, E., PAYNE., MULLAN, B. & BERCHMANS, D. Issues related to livestock housing under hot climatic conditions including the animal responses to high temperatures. CIGR Workshop “Animal Housing in Hot Climate”, April 1 - 4 2007 Cairo, Egypt. 4-24.

BARNOSKY, A. D., HADLY, E. A. & BELL, C. J. 2003. Mammalian response to global warming in varied temporal sclaes. *Journal of Mammalogy,* 84**,** 354-368.

BAUMGARD, L. H. & RHOADS, R. P. 2013. Effects of heat stress on postabsorptive metabolism and energetics. *Annual Review of Animal Biosciences,* 1**,** 311-337.

BAY, A., GARBONE, L. A., FRANK, W. A. & QUIMBY, F. 1995. Swine. *In:* ROLLIN, B. E. & KESEL, M. L. (eds.) *The Experimental Animal in Biomedical Research. Volume II: Care, Husbandry, and Well-being, An Overview by Species.* Boca Raton, Florida.: CRC Press.

BAYLIS, N. & GITHEKO, A. K. 2006. The effects of climate change on infectious diseases of animals. T 7.3.: Foresight. Infectious disease. Preparing for the future. Office of science and innovation.

BEATTY, D. T., BARNES, A., TAYLOR, E., PETHICK, D., MCCARTHY, M. & MALONEY, S. K. 2006. Physiological responses of *Bos taurus* and *Bos indicus* cattle to prolonged, continuous heat and humidity. *Journal of Animal Science,* 84**,** 972-985.

BEHL, R., BEHL, J. & JOSHI, B. 2010. Heat tolerance mechanisms in cattle-status in zebu cattle: a review. *The Indian Journal of Animal Sciences,* 80**,** 1-12.

BENNET, J. W. 1964. Thermal insulation of cattle coats. *Proceedings of the Australian Society of Animal Production,* 5**,** 160-166.

BENNETT, I. L. J. & NICASTRI, A. 1960. Fever as a mechanism of resistance. *Bacteriological Reviews,* 24**,** 16-34.

BERG, F., GUSTAFSON, U. & ANDERSSON, L. E. D. J. P. 2006. The uncoupling protein 1 gene (UCP1) is disrupted in the pig lineage: A genetic explanation for poor thermoregulation in piglets. . *PLoS Genetics,* 2**,** 1178-1181.

BERTHON, D., HERPIN, P. & LE DIVIDICH, J. 1994. Shivering thermogenesis in the neonatal pig. *Journal of Thermal Biology,* 19**,** 413-418.

BEST, P. 2012. *Global pig production: Trends shaping the pork industry* [Online]. WATTAgNET.com. Available: http://www.wattagnet.com/articles/13056-global-pig-production-trends-shaping-the-pork-industry [Accessed 8 March 2016].

BILBO, S. D., WIESELER, J. L., BARRIENTOS, R. M., TSANG, V., WATKINS, L. R. & MAIER, S. F. 2010. Neonatal bacterial infection alters fever to live and simulated infections in adulthood. *Psychoneuroendocrinology,* 35**,** 369-381.

BISHOP, J. A., NELSON, A. M., MERZ, W. G., ASKIN, F. B. & RIEDEL, S. 2012. Evaluation of the detection of melanin by the Fontana-Masson silver stain in tissue with a wide range of organisms including Cryptococcus. *Human Pathology,* 43**,** 898-903.

BISHOP, S., DE JONG, M. & GRAY, D. 2002. Opportunities for incorporating genetic elements into the management of farm animal diseases: Policy issues. *In:* FAO (ed.) *CGRFA Background Study Paper No.18.* Rome: FAO.

BISSETT, D. L. & MCBRIDE, J. F. 1983. The use of the domestic pig as an animal model of human dry skin and for comparison of dry and normal skin properties. *Journal of Society Cosmetic and Chemistry,* 34**,** 317-326.

BLACKSELL, S. D., KHOUNSY, S., VAN AKEN, D., GLEESON, L. J. & WESTBURY, H. A. 2006. Comparative susceptibility of indigenous and improved pig breeds to Classical Swine Fever Virus infection: Practical and epidemiological implications in a subsistence-based, developing country setting. *Tropical Animal Health and Production,* 38**,** 467-474.

BLATTEIS, C. M. 1974. Influence of body weight and temperature on the pyrogenic effect of endotoxin in guinea pigs. *Toxicology and Applied Pharmacology,* 29**,** 249-258.

BOCHSLER, P. N. & SLAUSON, D. O. 2002. Inflammation and repair of tissue. *In:* BOCHSLER, P. N. & SLAUSON, D. O. (eds.) *Mechanism of Disease. A Textbook of Comparative General Pathology.* St. Louis: Mosby.

BOLLEN, P. J. A., MADSEN, L. W., MEYER, O. & RITSKES-HOITINGA, J. 2004. Growth differences of male and female Göttingen minipigs during ad libitum feeding: a pilot study. *Laboratory Animals,* 39**,** 80-93.

BONNEAUD, C., MAZUC, J., GONZALEZ, G., HAUSSY, C., CHASTEL, O., FAIVRE, B. & SORCI, G. 2003. Assessing the cost of mounting an immune response. *American Naturalist,* 161**,** 367-379.

BORDERAS, T. F., DE PASSILLÉ, A. M. & RUSHEN, J. 2008. Behavior of dairy calves after a low dose of bacterial endotoxin. *Journal of Animal Science,* 86**,** 2920-2927.

BOTO, I. & LA PECCERELLA, C. 2012. The role of livestock for ACP countries: Challenges and opportunities ahead. Brussels, Belgium. 39. https://brusselsbriefings.files.wordpress.com/2012/10/br-12-livestock-eng.pdf. [4 March 2016]

BRACKE, M. B. M. 2011. Review of wallowing in pigs: Description of the behaviour and its motivational basis. *Applied Animal Behaviour Science,* 132**,** 1-13.

BRAND, T. S., CLOETE, S. W. P. & FRANCK, F. 1991. Wheat-straw as roughage component in finishing diets of growing lambs. *South African Journal of Animal Science,* 21**,** 184-188.

BROOM, D. M. & FRASER, A. F. 2007. *Domestic animal behavior and welfare,* Oxfordshire, UK, CABI International.

BROWN-BRANDL, T. M., YANAGI, T., XIN, H., GATES, R. S., BUCKLIN, R. A. & ROSS, G. S. 2003. A new telemetry system for measuring core body temperature in livestock and poultry. *Applied Enginnering in Agriculture,* 19**,** 583 - 589.

BROWNE, A. 1994. *Production traits of selected purebred and crossbred pigs.* MSc (Agric) Thesis, University of Pretoria.

CABANAC, M. C. 1996. Heat stress and behaviour. *Handbook of Physiology, Environmental Physiology.* American Physiological Society.

CAIN, J. W., KRAUSMAN, P. R., ROSENSTOCK, S. S. & TURNER, J. C. 2006. Mechanisms of thermoregulation and water balance in desert ungulates. *Wildlife Society Bulletin,* 34**,** 570-581.

CARDELLINO, R. A. 2003. Animal genetic resources conservation and development: The role of FAO. *Archivos de Zootecnia,* 52**,** 185-192.

CARMI, N., PINSHOW, B., HOROWITZ, M. & BERNSTEIN, M. H. 1993. Birds conserve plasma volume during thermal flight-incurred dehydration. *Physiological Zoology,* 66**,** 829-846.

CARPENTER, C. M., BOAK, R. A., MUCCI, L. A. & WARREN, S. L. 1933. Studieson the physiological effects of fever temperatures. The thermal death time of *Neisseria gonorrhoeae* in vitro with special reference to fever temperatures. *Journal of Laboratory and Clinical Medicine,* 18**,** 981-990.

CARROLL, J. A., BURDICK, N. C., REUTER, R. R., CHASE JR, C. C., SPIERS, D. E., ARTHINGTON, J. D. & COLEMAN, S. W. 2011. Differential acute phase immune responses by Angus and Romosinuano steers following an endotoxin challenge. *Domestic Animal Endocrinology,* 41**,** 163-173.

CARVALHO, F. A., LAMMOGLIA, M. A., SIMOES, M. J. & RANDEL, R. D. 1995. Breed affects thermoregulation and epithelial morphology in imported and native cattle subjected to heat stress. *Journal of Animal Science,* 73**,** 3570-3573.

CASON, J. E. 1950. A rapid one-step Mallory-Heidenhain stain for connective tissue. *Stain Technology,* 25**,** 225-226.

CENA, K. & MONTEITH, J. L. 1975. Transfer processes in animals coats I. Radiative transfer. *Proceedings of the Royal Society of London, Series B: Biological Sciences,* 188**,** 377-393.

CHIDUWA, G., CHIMONYO, M., HALIMANI, T. E., CHISAMBARA, S. R. & DZAMA, K. 2008. Herd dynamics and contribution of indigenous pigs to the livelihoods of rural farmers in a semi-arid area of Zimbabwe. *Tropical Animal Health and Production,* 40**,** 125-136.

CHIMONYO, M., BHEBHE, E., DZAMA, K., HALIMANI, T. E. & KANENGONI, A. 2005. Improving smallholder pig production for food security and livelihood of the poor in Southern Africa. *African Crop Science Conference Proceedings,* 7**,** 569-573.

CHIMONYO, M., DZAMA, K. & BHEBHE, E. 2006. Genetic determination of individual birth weight, litter weight and litter size in Mukota pigs. *Livestock Science,* 105**,** 69-77.

CHIMONYO, M., DZAMA, K. & BHEBHE, E. 2008. Genetic determination of mothering ability and piglet growth in indigenous Mukota sows of Zimbabwe. *Livestock Science,* 113**,** 74-80.

CHIMONYO, M., DZAMA, K. & MAPIYE, C. 2010. Growth performance and carcass characteristics of indigenous Mukota of Zimbabwe. *Tropical Animal Health and Production,* 42**,** 1001-1007.

CHOE, J.-H., YANG, H.-S., LEE, S.-H. & GO, G.-W. 2015. Characteristics of pork belly consumption in South Korea and their health implication. *Journal of Animal Science and Technology,* 57**,** 22-28.

CHRISTON, R. 1988. The effect of tropical ambient temperature on growth and metabolism in pigs. *Journal of Animal Science,* 66**,** 3112-3123.

CLARKE, A. & ROTHERY, P. 2008. Scaling of body temperature in mammals and birds. *Functional Ecology,* 22**,** 58-67.

COLLIER, R. J. & GEBREMEDHIN, K. G. 2015. Thermal biology of domestic animals. *Annual Review of Animal Biosciences,* 3**,** 513-532.

COLLIN, A., VAN MILGEN, J., DUBOIS, S. & NOBLET, J. 2001. Effect of high temperature on feeding behaviour and heat production in group-housed young pigs. *British Journal of Nutrition,* 86**,** 63-70.

COSTIN, G.-E. & HEARING, V. J. 2007. Human skin pigmentation: melanocytes modulate skin color in response to stress. *FASEB Journal,* 21**,** 976-994.

COX, R. M., MUÑOZ-GARCIA, A., JURKOWITZ, M. S. & WILLIAMS, J. B. 2008. β-Glucocerebrosidase activity in the *Stratum corneum* of house sparrows following acclimation to high and low humidity. *Physiological and Biochemical Zoology,* 81**,** 97-105.

CRAY, C., ZAIAS, J. & ALTMAN, N. H. 2009. Acute Phase Response in Animals: A Review. *Comparative Medicine,* 59**,** 517-526.

CRESTANI, F., SEGUY, F. & DANTZER, R. 1991. Behavioral effects of peripherally injected interleukin-1: Role of prostaglandins. *Brain Research,* 542**,** 330-335.

CUMMING, G. S. & VAN VUUREN, D. P. 2006. Will climate change affect ectoparasite species ranges? *Global Ecology and Biogeography,* 15**,** 486-497

CUNNINGHAM, H. M. 1968. Use of tritiated water to determine the effect of water restriction on the insensible water loss of pigs. *Journal of Animal Science,* 27**,** 412-417.

CURTIS, S. E. 1981. *Environmental management in animal agriculture,* Mahomet, Illinois, USA, Iowa State University Press.

D'ALLAIRE, S., DROLET, R. & BRODUER, D. 1996. Sow mortality associated with high ambient temperatures. *Canadian Veterinary Journal,* 37**,** 237-239.

DAFF. 2013. A profile of the South African pork market value chain. . Available: http://www.nda.agric.za/doaDev/sideMenu/Marketing/Annual%20Publications/Commodity%20Profiles/Livestock/Pork%20market%20value%20chain%20profile%202013.pdf [Accessed 22 February 2016].

DAGHASH, H. A., SALEM, I. A., ZENHOM, M. & SALIMA, A. E. 1999. Skin thickness and hair coat characteristics of water Buffaloes and Jersey cows under subtropical conditions. *Buffalo Journal,* 1**,** 81-91.

DANTAS-TORRES, F. 2015. Climate change, biodiversity, ticks and tick-borne diseases: The butterfly effect. *International Journal for Parasitology: Parasites and Wildlife,* 4**,** 452-461.

DARCAN, N. K., CANKAYA, S. & KARAKOK, S. G. 2009. The effects of skin pigmentation on physiological factors of thermoregulation and grazing behaviour of dairy goats in a hot and humid climate. *Asian-Australian Journal of Animal Science,* 22**,** 727-731.

DAS, R., SAILO, L., VERMA, N., BHARTI, P., SAIKIA, J., KUMAR, I. & KUMAR, R. 2016. Impact of heat stress on health and performance of dairy animals: A review. *Veterinary World,* 9**,** 260-268.

DAVIDS, P., JOOSTE, A. & MEYER, F. H. 2014. Evaluating the South African pork value chain. *IFAMA 2014 Symposium.* Cape Town, South Africa: IFAMA.

DAWSON, T. J., BLANEY, C. E., MCCARRON, H. C. K. & MALONEY, S. K. 2007. Dehydration, with and without heat, in kangaroos from mesic and arid habitats: different thermal responses including varying patterns in heterothermy in the field and laboratory. *Journal of Comparative Physiology B,* 177**,** 797-807.

DE GROOT, J., KRANENDONK, G., FILLERUP, M., HOPSTER, H., BOERSMA, W., HODGSON, D., VAN REENEN, K. & TAVERNE, M. 2007. Response to LPS in female offspring from sows treated with cortisol during pregnancy. *Physiology and Behavior,* 90**,** 612-618

DE GRUIJL, F. R., LONGSTRETH, J., NORVAL, M., CULLEN, A. P., SLAPER, H., KRIPKE, M. L., TAKIZAWA, Y. & VAN DER LEUN, J. C. 2003. Health effects from stratospheric ozone depletion and interactions with climate change. *Photochemical and Photobiological Sciences,* 2**,** 16-28.

DEA. 2013. Long-Term Adaptation Scenarios Flagship Research Programme (LTAS) for South Africa. Climate Trends and Scenarios for South Africa. Pretoria, South Africa. Department of Environmental Affairs. http://www.sanbi.org/sites/default/files/documents/documents/ltasclimate-trends-and-scenarios-tech-report2013low-res.pdf. [26 January 2016]

DEGEN, A. A. 1997. *Ecophysiology of the small desert animals,* Berlin & New York, Springer.

DEHOVE, A., COMMAULT, J., PETITCLERC, M., TEISSIER, M., MACÉ, J. & 31(2): 2012. Economic analysis and costing of animal health: A literature review of methods and importance. *Revue Scientifique et Technique Office International des Epizooties,* 31**,** 591-617.

DIETZE, K. 2011. Pigs for Prosperity. *In:* FAO (ed.) *Diversification Booklet Number 15.* Rome: Food and Agriculture Organization of the United Nations, Rome.

DILGER, R. N. & JOHNSON, R. W. 2010. Behavioural assessment of cognitive function using a transitional neonatal piglet model. *Brain, Behavior, and Immunity,* 24**,** 1156-1165.

DINARELLO, C. A. 1984. Interleukin 1. *Review of Infectious Diseases,* 6**,** 51-95.

DOWLING, D. F. 1955. The thickness ofcattle skin. *Australian Journal of Agricultural Research,* 6**,** 776-785.

DOWLING, D. F. 1956. An experimental study of heat tolerance of cattle. *Australian Journal of Agricultural Research,* 7**,** 469-481.

DRUCKER, A. G., EMELINE BERGERON, E., LEMKE, U., THUY, L. T. & ZÁRATE, A. V. 2006. Identification and quantification of subsidies relevant to the production of local and imported pig breeds in Vietnam *Tropical Animal Health and Production,* 38**,** 305-322.

DUCHET-SUCHAUX, M. F., BERTIN, A. M. & MENANTEAU, P. S. 1991. Susceptibility of Chinese Meishan and European Large White pigs to enterotoxigenic *Escherichia coli* strains bearing colonization factor K88, 987P, K99, or F41. *American Journal of Veterinary Research,* 52**,** 40-49.

EDWARDS, R. L., OMTVEDT, I. T., TURMAN, E. J., STEPHENS, D. F. & MAHONEY, G. W. A. 1968. Reproductive performance of gilts following heat stress prior to breeding and in early gestation. *Journal of Animal Science,* 27**,** 1634-1637.

EINARSSON, S., BRANDT, Y., LUNDEHEIM, N. & MADEJ, A. 2008. Stress and its influence on reproduction in pigs: a review. *Acta Veterinaria Scandinavica,* 50**,** 48-55.

ELANDER, L., ENGSTRÖM, L., HALLBECK, M. & BLOMQVIST, A. 2007. IL-1b and LPS induce anorexia by distinct mechanisms differentially dependent on microsomal prostaglandin-E synthase-1. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,* 292**,** R258-R267.

ELBERS, A. R. W., KOENRAADT, C. J. M. & MEISWINKEL, R. 2015. Mosquitoes and Culicoides biting midges: vector range and the influence of climate change. *Revue Science et Technique,* 34**,** 123-137.

ELIN, R. J. & WOLFF, S. M. 1976. Biology of endotoxin. *Annual Review of Medicine,* 27**,** 127-141.

EPSTEIN, P. R. 2001. Climate change and emerging infectious diseases - Review. *Microbes and Infection,* 3**,** 747-754.

ERRIDGE, C., BENNETT-GUERRERO, E. & POXTON, I. R. 2002. Structure and function of lipopolysaccharides. *Microbes and Infection,* 4**,** 837-851.

FAO. 2004. Drought impact mitigation and prevention in the Limpopo River Basin: A Situational Analysis. Rome, Italy. Food and Agriculture Orgnisation of the United Nations.

FAO. 2007a. Global Plan of Action for Animal Genetic Resources and the Interlaken Declaration. Available: ftp://ftp.fao.org/docrep/fao/010/a1404e/a1404e00.pdf) [Accessed 16 January 2016].

FAO. 2007b. The state of the world's animal genetic resources for food and agriculture. Rome, Italy. Commission on genetic resources for food and agriculture. 39. http://www.fao.org/3/92137630-88e6-50c7-a60d-64e5c04efbef/a1260e00.pdf. [25 January 2016]

FAO. 2009. Farmer's Hand Book on Pig Production (For the small holders at village level). Rome, Italy. Food and Agriculture Organization of the United Nations. http://www.fao.org/ag/AGAInfo/themes/documents/pigs/Handbook%20on%20Pig%20Production_English%20layout-Vietanm-Draft.pdf. [22 February 2016]

FAO. 2012. Report of a consultation on the definition of breed categories. Rome, Italy. Food and Agriculture Organisatio of the United Nations. http://www.fao.org/docrep/meeting/026/me588e.pdf. [12 January 2015]

FAO FOOD OUTLOOK. 2015. Food Outlook: Biannual Report On Global Food Markets Rome, Italy. Food and Agriculture Organization of the Unite Nations (FAO). 140. http://www.fao.org/3/a-i5003e.pdf. [18 February 2016]

FAOSTAT. 2015. *Production - Live Animals* [Online]. Rome, Italy: Food and Agriculture Organization Corporate Statistical Database (FAOSTAT). Available: http://faostat.fao.org/site/573/DesktopDefault.aspx?PageID=573#ancor [Accessed 9 February 2016].

FARLEX PARTNER MEDICAL DICTIONARY 2012. Farlex Partner Medical Dictionary.

FAS/USDA. 2015. Livestock and Poultry: World Markets and Trade. USA. United States Department of Agriculture. 21.

FAS/USDA. 2016. *Production, Supply and Distribution Online* [Online]. USA: United States Department of Agriculture, Foreign Agricultural Services (USDA/FAS),. Available: http://apps.fas.usda.gov/psdonline/psdQuery.aspx [Accessed 6 March 2016].

FINCH, V. A. 1985. Comparison of non-evaporative heat transfer in different cattle breeds. *Australian Journal of Agricultural Research,* 38**,** 497.

FINCH, V. A., BENNET, I. L. & HOLMES, C. R. 1984. Coat colour in cattle: effect on thermal balance, behaviour and growth, and relationship with coat type. *The Journal of Agricultural Science,* 102**,** 141-147.

FONTANESI, L., BERETTI, F., RIGGIO, V. & DALL'OLIO, S. 2009. Missense and nonsense mutations in melanocortin 1 receptor (MC1R) gene of different goat breeds: association with red and black coat colour phenotypes but with unexpected evidences. *BMC Genetics,* 10**,** 47-56.

FONTANESI, L., BERETTI, F., RIGGIO, V. & DALL'OLIO, S. 2010. Sequence characterization of the melanocortin 1 receptor (MC1R) gene in sheep with different coat colours and identification of the putative e allele at the ovine extension locus. *Small Ruminant Research,* 914**,** 200-207.

FOOD AND AGRICULTURE ORGANISATION (FAO). 1998. Biodiversity for Food And Agriculture: Farm Animal Genetic Resources. Rome, Italy. Food and Agriculture Organisation of the United Nations. ftp://ftp.fao.org/docrep/fao/010/a1250e/a1250e.pdf. [11 December 2015]

FORD, D. M. & KLUGMAN, K. P. 1980. Body mass and sex as determining factors in the developement of fever in rats. *Journal of Physiology,* 304**,** 43-50.

FORTIER, M. E., KENT, S., ASHDOWN, H., S., P., BOSKA, P. & G.N., L. 2004. The viral mimic, polyinosinic: polycytidylic acid, induces fever in rats via an interleukin-1-dependent mechanism. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,* 287**,** R759-R766.

FOURNIER, B. & PHILPOTT, D. J. 2005. Recognition of *Staphylococcus aureus* by the innate immune system. *Clinical Microbiology Reviews,* 18**,** 521-540.

FRATTO, M. A. & DAVIS, A. K. 2011. Do black-furred animals compensate for high solar absorption with smaller hairs? A test with a polymorphic squirrel species. *Current Zoology,* 57**,** 731-736.

GABAY, C. & KUSHNER, I. 1999. Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine,* 340**,** 448-454.

GAINER, J. H. & GUARNIERI, J. 1985. Effects of poly I:C in porcine iron-deficient neutropenia. *Cornell Veterinarian,* 75**,** 454-465.

GALE, P., DREW, T., PHIPPS, L. P., DAVID, G. & WOOLDRIDGE, M. 2009. The effect of climate change on the occurrence and prevalence of livestock diseases in Great Britain: a review. *Journal of Applied Microbiology,* 106**,** 1409–1423.

GALIC, M. A., SPENCER, S. J., MOUIHATE, A. & PITTMAN, Q. J. 2009. Postnatal programming of the innate immune response. *Integrative and Comparative Biology,* 49**,** 237-245.

GANDHI, R., HAYLEY, S., GIBB, J., MERALI, Z. & ANISMAN, H. 2007. Influence of poly I:C on sickness behaviours, plasma cytokines, corticosterone and central monoamine activity: Moderation by social stressors. *Brain, Behavior, and Immunity,* 21**,** 477-489.

GIBBONS, R. A., SELWOOD, R., BURROWS, M. & HUNTER, P. A. 1977. Inheritance of resistance to neonatal *Escherichia* diarrhea in the pig: examination of the genetic system. *Theoretical and Applied Genetics,* 55**,** 65-70.

GIROD, V., BOUVIER, M. & GRELOT, L. 2000. Characterization of lipopolysaccharide-induced emesis in conscious piglets: effects of cervical vagotomy, cyclooxygenase inhibitors and a 5-$HT_3$ receptor antagonist. *Neuropharmacology,* 39**,** 2329-2335.

GREGORY, N. G. 2010. How climatic changes could affect meat quality. *Food Research International,* 43**,** 1866-1873.

GRIMBEEK, P. J., DAVIDS, T. & HUMAN, M. 2016. *South Africa: Challenges in an isolated industry* [Online]. Pig Progress. Available: http://www.pigprogress.net/Finishers/Articles/20141/12/South-Africa-Challenges-in-an-isolated-industry-1649951W/ [Accessed 2 March 2016].

HAAKE, A., SCOTT, G. A. & HOLBROOK, K. A. 2001. Structure and function of the skin: overview of the epidermis and dermis. *In:* FREINKEL, R. K. & WOODLEY, D. T. (eds.) *The Biology of the Skin.* New York, USA: The Parthenon Publishing Group Inc.

HADLEY, N. F. 1991. Integumental lipids of plants and animals: comparative function and biochemistry. *Advances in Lipid Research,* 24**,** 303-320.

HAGGARTY, P., FRANKLIN, M. F., FULLER, M. F., MCGAW, B. A., MILNE, E., DUNCAN, G., CHRISTIE, S. L. & S., S. J. 1994. Validation of the doubly labeled water method in growing pigs. *American Journal of Physiology,* 36**,** R1574-R1588.

HAHN, G. L. Global warming and potential impacts on cattle and swine in tropical and temperate areas. 1st Brazilian Congress on Biometeorology, 1995 Jaboticabal, SP, Brazil. 136−173.

HAHN, G. L. 1999. Dynamic responses of cattle to thermal heat loads. *Journal of Animal Science,* 77**,** 10-20.

HALES, J. R. S. & BROWN, G. D. 1974. Net energetic and thermoregulatory efficiency during panting in the sheep. *Comparative Biochemistry and Physiology Part A: Physiology,* 49**,** 413-422.

HALIMANI, T. E., MUCHADEYI, F. C., CHIMONYO, M. & DZAMA, K. 2010. Pig genetic resource conservation: The Southern African perspective. *Ecological Economics,* 69**,** 944-951.

HAMILTON, W. J. 1973. *Life's colour code,* New York, McGraw-Hill Book Company.

HAMMAMIEH, R., BI, S. & MANI, S. 2003. Genetic variation in PBMC in piglets used as an animal model for staphylococcal entertoxin exposures. *OMICS: A Journal of Integrative Biology,* 7**,** 401-409.

HARDEN, L., PLESSIS, I. D., POOLE, S. & LABURN, H. 2008. Interleukin (IL)-6 and IL-1B act synergistically within the brain to induce sickness behaviour and fever in rats. *Brain, Behavior, and Immunity,* 22**,** 838-849.

HARDEN, L. M., KENT, S., PITTMAN, Q. J. & ROTH, J. 2015. Fever and sickness behavior: Friend or foe? *Brain, Behavior, and Immunity,* 50**,** 322-333.

HART, B. L. 1988. Biological basis of the behaviour of sick animals. *Neuroscience & Biobehavioral Reviews,* 12**,** 123-137.

HARVELL, C. D., MITCHELL, C. E., WARD, J. R., ALTIZER, S., DOBSON, A. P., OSTFELD, R. S. & SAMUEL, M. D. 2002. Ecology - climate warming and disease risks for terrestrial and marine biota. *Science,* 296**,** 2158-2162.

HASDAY, J. D., FAIRCHILD, K. D. & SHANHOLTZ, C. 2000. The role of fever in the infected host. *Microbes and Infection,* 2**,** 1891−1904.

HASHIMOTO, M., BANDO, T., IRIKI, M. & HASHIMOTO, K. 1988. Effect of indomethacin on febrile response to recombinant human interleukin 1-a in rabbits. *American Journal of Physiology,* 24**,** R527-R533.

HAUGEN, M. J., TIELEMAN, B. I. & WILLIAMS, J. B. 2003. Phenotypic flexibility in cutaneous water loss and lipids of the stratum corneum. *The Journal of Experimental Biology,* 206**,** 3581-3588

HAYNE, S. M., TENNESSEN, T. & ANDERSON, D. M. 2000. The responses of growing pigs exposed to cold with varying amounts of straw bedding. *Canadian journal of animal science. Ottawa ON,* 80**,** 539-546.

HELMUTH, B., KINGSOLVER, J. G. & CARRINGTON, E. 2005. Biophysics, physiological ecology and climate change. Does mechanism matter? . *Annual Review of Physiology,* 67**,** 177-201.

HERPIN, P., DAMON, M. & LE DIVIDICH, J. 2002. Development of thermoregulation and neonatal survival in pigs. *Livestock Production Science,* 78**,** 25-45.

HETEM, R. S., DE WITT, B. A., FICK, L. G., FULLER, A., KERLEY, G. I. H., MEYER, L. C. R., MITCHELL, D. & MALONEY, S. K. 2009. Body temperature, thermoregulatory behaviour and pelt characteristics of three colour morphs of springbok (*Antidorcas marsupialis*). *Comparative Biochemistry and Physiology, Part A: Molecular & Integrative Physiology,* 152**,** 379-388.

HETEM, R. S., STRAUSS, W. M., FICK, L. G., MALONEY, S. K., MEYER, L. C. R., SHOBRAK, M., FULLER, A. & MITCHELL, D. 2012. Does size matter? Comparison of body temperature and activity of free-living Arabian oryx (*Oryx leucoryx*) and the smaller Arabian sand gazelle (Gazella subgutturosa marica) in the Saudi desert. *Journal of Comparative Physiology B Biochemical, Systems, and Environmental Physiology,* 182**,** 437-449.

HOFFMAN, L. C., STYGER, W. F., BRAND, T. S. & MULLER, M. 2005. The growth, carcass yield, physical and chemical characteristic of two South African indigenous pig breeds. *South African Journal of Animal Science,* 6**,** 25-35.

HOFFMANN, I. 2010. Climate change and the characterization, breeding and conversation of animal genetic resources. *Animal Genetics,* 41**,** 32-46.

HOLMES, C. W. & MOUNT, L. E. 1967. Heat loss from groups of growing pigs under various conditions of environmental temperature and air movement. *Animal Production,* 9**,** 435-452.

HOLNESS, D. H. 1973. The role of indigenous pigs as a source of protein in Africa: - A review. *Rhodesian Agricultural Journal,* 73**,** 59-63.

HOLNESS, D. H. 1995. *The Tropical Agriculturalist-Pigs,* The Netherlands, McMillian Education Ltd Publishers, .

HOLTKAMP, D., KLIEBENSTEIN, J. B., NEUMANN, E. J., ZIMMERMAN, J. J., ROTTO, H., YODER, T. K., WANG, C., YESKE, P. E., MOWRER, C. L. & HALEY, C. A. 2013. Assessment of the economic impact of porcine reproductive and respiratory syndrome viris on United States pork producers. *Journal of Swine Health and Production,* 21**,** 72-84.

HORIN, P., SMOLA, J., MATIASOVIC, J., VYSKOCIL, M., LUKESZOVA, L., TOMANOVA, K., KRALıK, P., GLASNAK, V., SCHROFFELOVA, D., KNOLL, A., SEDLINSKA, M., KRENKOVA, L. & JAHN, P. 2004. Polymorphisms in equine immune response genes and their associations with infections. *Mammalian Genome,* 15**,** 843-850.

HOROWITZ, M. & SAMUELOFF, S. 1979. Plasma water shifts during thermal dehydration. *Journal of Applied Physiology: Respiration, Environment and Exercise Physiology,* 47**,** 738-744.

HOUGHTON, J. T., MEIRA FILHO, L. G., CALLANDER, B. A., HARRIS, N., KATTENBERG, A. & MASKELL, K. 1995. The Science of Climate Change. Contribution of WGI to the Second Assessment Report of the Intergovernmental Panel on Climate Change. *In:* LAKEMAN, J. A. (ed.) *Climate Change 1995.* Cambridge University Press: Intergovernmental Panel on Climate Change.

HUTCHINSON, J. C. D. & BROWN, G. D. 1969. Penetrance of cattle coats by radiation. *Journal of Applied Physiology,* 26**,** 454-464.

HUYEN, L. T. T., ROESSLER, R., LEMKE, U. & ZÁRATE, A. V. 2005. Impact of the use of exotic compared to local pig breeds on socio-economic development and biodiversity in Vietnam. VERLAG GRAUER, Beuren, Stuttgart,. Institute of Animal Production in the Tropics and Subtropics of the University of Hohenheim. 48. https://www.academia.edu/9934967/Impact_of_the_use_of_exotic_compared_to_local_pig_breeds_on_socio-economic_development_and_biodiversity_in_Vietnam. [22 February 2016]

HUYNH, T. T. T. 2005. *Heat stress in growing pigs.* PhD, Wageningen University.

HUYNH, T. T. T., AARNINK, A. J. A., GERRITS, W. J. J., HEETKAMP, M. J. H., CANH, T. T., SPOOLDER, H. A. M., KEMP, B. & VERSTEGEN, M. W. A. 2005a. Thermal behaviour of growing pigs in response to high temperature and humidity. *Applied Animal Behaviour Science,* 91**,** 1-16.

HUYNH, T. T. T., AARNINK, A. J. A., VERSTEGEN, M. W. A., GERRITS, W. J. J., HEETKAMP, M. J. H. & KEMP, B. 2005b. Pigs' physiological responses at different relative humidity and increasing temperatures *Journal of Agricultural Science and Technology,* 4**,** 57-63.

HUYNH, T. T. T., AARNINK, A. J. A., VERSTEGEN, M. W. A., GERRITS, W. J. J., HEETKAMP, M. J. H., KEMP, B. & CANH, T. T. 2005b. Effect of increasing temperatures on physiological changes in pigs at different relative humidities. *Journal of Animal Science,* 83**,** 1385-1396.

IDSO, S. & IDSO, C. 2013. Observations: Temperature Records. *In:* IDSO, C. D., CARTER, R. M. & S.F., S. (eds.) *Climate Change Reconsidered II: Physical Science.* The Heartland Institute, Chicago, IL: Non-Governmental International Panel on Climate Change.

INGRAM, D. L. 1964. The effect of environmental temperature on heat loss and thermal insulation in the young pig. *Research Veterinary Science,* 5**,** 357-364.

INGRAM, D. L. 1965. Evaporative cooling in the pig. *Nature,* 207**,** 415-416.

INGRAM, D. L. 1967. Stimulation of cutaneous glands in the pig. *Journal of Comparative Pathology,* 77**,** 93-98.

INGRAM, D. L. 1977. Adaptations to ambient temperature in growing pigs. *Pflügers Archiv European Journal of Physiology,* 367**,** 257-264.

INGRAM, D. L. & LEGGE, K. F. 1969. The effect of environmental temperature on respiratory ventilation in the pig. *Respiratory Physiology,* 8**,** 1-12.

INGRAM, D. L. & LEGGE, K. F. 1970a. The thermoregulatory behavior of young pigs in a natural environment. *Physiology and Behavior,* 5**,** 981-987.

INGRAM, D. L. & LEGGE, K. F. 1970b. Variations in deep body temperature in the young unrestrained pig over the 24 hour period. *Journal of Physiology,* 210**,** 989-998.

IPCC. 2007. Fourth Assessment Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Geneva, Switzerland. Intergovernmental Panel on Climate Change.

IPCC 2013. Summary for Policymakers. *In:* STOCKER, T. F., QIN, D., PLATTNER, G.-K., TIGNOR, M., ALLEN, S. K., BOSCHUNG, J., NAUELS, A., XIA, Y., BEX, V. & M., M. P. (eds.) *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

IPCC. 2014. Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. Intergovernmental Panel on Climate Change. 1454.

ISLAM, M. M., AHMED, S. T., MUN, H.-S., BOSTAMI, A. B. M. R., Y.-J., K. & YANG, C.-J. 2015. Use of thermal imaging for the early detection of signs of disease in pigs challenged orally with *Salmonella typhimurium* and *Escherichia coli*. *African Journal of Microbiology Research,* 9**,** 1667-1674.

IUPS (ed.) 2001. *Glossary of terms for thermal physiology 3rd Edition*: The Commission for Thermal Physiology.

JABLONSKI, N. G. 2004. The evolution of human skin and skin color. *Annual Review of Anthropology,* 33**,** 585-623.

JESSEN, C. 2000. Changes in set-point in Temperature regulation in humans and other mammals. *In:* JESSEN, C. (ed.) *Temperature Regulation in Humans and Other Mammals.* Springer-Verlag Berlin Heidelberg New York.

JOHNSON, R. W. & VON BORELL, E. 1994. Lipopolysaccharide-induced sickness behaviour in pigs is inhibited by pre-treatment with indomethacin. *Journal of Animal Science,* 72**,** 309-314.

JUSTINO, E., DE A. NÄÄS, I., CARVALHO, T. M. R., NEVES, D. P. & SALGADO, D. D. 2014. The impact of evaporative cooling on the thermoregulation and sensible heat loss of sows during farrowing. *Journal of the Brazilian Association of Agricultural Engineering,* 34**,** 1050-1061.

KANENGONI, A. T., CHIMONYO, M., ERLWANGER, K. H., NDIMBA, B. K. & DZAMA, K. 2014. Growth performance, blood metabolic responses, and carcass characteristics of grower and finisher South African Windsnyer-type indigenous and Large White × Landrace crossbred pigs fed diets containing ensiled corncobs. *Journal of Animal Science,* 92**,** 5739-5748.

KANENGONI, A. T., DZAMA, K., CHIMONYO, M., J., K. & MASWAURE, S. M. 2004. Growth performance and carcass traits of Large White, Mukota and Large White

x Mukota F1 crosses given graded levels of maize cob meal. *Animal Science,* 78**,** 61-66.

KANENGONI, A. T., DZAMA, K., CHIMONYO, M., KUSINA, J. & MASWAURE, S. M. 2002. Influence of level of maize cob meal on nutrient digestibility and nitrogen balance in Large White, Mukota and LW x MF1 crossbred pigs. *Animal Science,* 74**,** 127-134.

KANITAKIS, J. 2002. Anatomy, histology and immunohistochemistry of normal human skin. *European Journal of Dermatology,* 12**,** 390-401.

KAY, R. N. B. 1997. Responses of African livestock and wild herbivores to drought. *Journal of Arid Environments,* 37**,** 683-694.

KENNEDY, G. 1960. Virus pneumonia of pigs. *Iowa State University Veterinarian,* 22**,** 81-84.

KESHAV, V., POTGIETER, N. & BARNARD, T. G. 2010. Detection of *Vibrio cholerae* O1 in animal stools collected in rural areas of the Limpopo Province *Water SA,* 36**,** 167-171.

KIJAS, J. M., MOLLER, M., PLASTOW, G. & ANDERSSON, L. 2000. A frameshift mutation in MC1R and a high frequency of somatic reversions cause black spotting in pigs. *Genetics,* 158**,** 779-785.

KIJAS, J. M., WALES, R., TORNSTEN, A. & CHARDON, P. 1998. Melanocortin receptor 1 (MC1R) mutations and coat color in pigs. *Genetics,* 150**,** 1177-1185.

KIM, Y. W., KIM, K. H., AHN, D. K., KIM, H. S., KIM, J. Y., LEE, D. C. & PARK, S. Y. 2007. Time course change of hormones and cytokines by lipopolysaccharide and its relation with anorexia. *Journal of Physiological Sciences,* 57**,** 159-165.

KIMARO, E. G. & CHIBINGA, O. C. 2013. Potential impact of climate change on livestock production and health in East Africa: A review. *Livestock Research for Rural Development,* 25**,** 116.

KLINGE, K. L., VAUGHN, E. M., ROOF, M. B., BAUTISTA, E. M. & MURTAUGH, M. P. 2009. Age-dependent resistance to porcine reproductive and respiratory syndrome virus replication in swine. *Virology Journal,* 6**,** 177-186.

KLIR, J. J., SHAHBAZIAN, L. M., MATTERI, R. L., FRITSCHE, K. L. & BECKER, B. A. 1997. Effects of thermal environment on response to acute peripheral lipopolysaccharide challenge exposure in neonatal pigs. *American Journal of Veterinary Research,* 58**,** 364-369.

KLUGER, M. J. 1986. Is fever beneficial? *Yale Journal of Biology and Medicine,* 59**,** 89-95.

KLUGER, M. J. & VAUGHN, I. K. 1978. Fever and survival in rabbits injected with *Pasteurella multocida*. *Journal of Physiology,* 282**,** 243-251.

KLUNGLAND, H., VAGE, D. I., GOMEZ-RAYA, L. & ADALSTEINSSON, S. 1995. The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. *Mammalian Genome,* 6**,** 636-639.

KOVARIK, M. 1964. Flow of heat in an irradiated protective cover. *Nature,* 201**,** 1085-1087.

KRUEGER, J. G., WOLFE, J. T., NABEYA, R. T., VALLAT, V. P., GILLEAUDEAU, P., HEFTLER, N. S., AUSTIN, L. M. & GOTTLIEB, A. B. 1995. Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. *Journal of Experimental Medicine,* 182**,** 2057-2068

KRUSE, R., ESSÉN-GUSTAVSSON, B., FOSSUM, C. & JENSEN-WAERN, M. 2008. Blood concentrations of the cytokines IL-1beta, IL-6, IL-10, TNF-alpha and IFN-gamma during experimentally induced swine dysentery. *Acta Veterinaria Scandinavica,* 50**,** 32-38.

KURUKULASURIYA, P., MENDELSOHN, R., HASSAN, R., BENHIN, J., DERESSA, T., DIOP, M., EID, H. M., FOSU, K. Y., GBETIBOUO, G., JAIN, S., MAHAMADOU, A., MANO, R., KABUBO-MARIARA, J., EL-MARSAFAWY, S., MOLUA, E., OUDA, S., OUEDRAOGO, M., SE´NE, I., MADDISON, D., NIGGOL SEO, S. N. & DINAR, A. 2006. Will African agriculture survive climate change? *The World Bank Economic Review,* 20**,** 367-388.

LECLÈRE, D., HAVLÍK, P., FUSS, S., SCHMID, E., A., M., WALSH, B., VALIN, H., HERRERO, M., KHABAROV, N., OBERSTEINER, M. & 124018 2014. Climate change induced transformations of agricultural systems: insights from a global model. *Environmental Research Letters,* 9**,** 124018.

LILLYWHITE, H. B. 2006. Water relations of tetrapod integument. *Journal of Experimental Biology,* 209**,** 202-226.

LILLYWHITE, H. B., MENON, J. G., MENON, G. K., SHEEHY, C. M. & TU, M. C. 2009. Water exchange and permeability properties of the skin in three species of amphibious sea snakes ( Laticauda spp.). *The Journal of Experimental Biology,* 212**,** 1921-1929

LIM, E. S. 2012. *Fundamentals of the Host-Virus Evolutionary Arms Race.* PhD, University of Washington.

LIPTON, J. M. & TICKNOR, C. B. 1979. Influence of sex and age on febrile responses to peripheral and central administration of pyrogens in the rabbit. *Journal of Physiology (London),* 295**,** 263-272.

LIU, Y. L., LI, D. F., GONG, L. M., FENG, Z. Y., YI, G. F., GAINES, A. M. & CARROLL, J. A. 2003. Effects of fish oil supplementation on performance as well as immunological, adrenal and somatotropic responses of weaned pigs after *Escherichia coli* lipopolysaccharide challenge. *Journal of Animal Science,* 81**,** 2758-2765.

LLAMAS MOYA, S., BOYLE, L. A., LYNCH, P. B. & ARKINS, S. 2008. Surgical castration of pigs affects the behavioural response to a low-dose lipopolysaccharide (LPS) challenge after weaning. *Applied Animal Behaviour Science,* 112**,** 40-57.

LOEWEN, K. G. & DERBYSHIRE, J. B. 1986. Interferon induction with polyinosinic: polycytidylic acid in the newborn piglet. *Canadian Journal of Veterinary Research,* 50**,** 232 - 237.

LOPES, P. C. 2014. When is it socially acceptable to feel sick? *Proceedings of the Royal Society B,* 281**,** 1-8.

LOSSEC, G., HERPIN, P. & LE DIVIDICH, J. 1988. Thermoregulatory responses of the newborn pig during experimentally induced hypothermia and rewarming. *Experimental Physiology,* 83**,** 667-678.

LU, D., WILLARD, D., PATEL, I. R., KADWELL, S., OVERTON, L., KOST, T., LUTHER, M., CHEN, W., WOYCHIK, R. P., WILKISON, W. O. & CONE, R. D. 1994. Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. *Nature,* 371**,** 799-802.

LWOFF, A. 1959. Factors influencing the evolution of viral diseases at the cellular level and in the organism. *Bacteriological Reviews,* 23**,** 109-124.

LWOFF, A. 1969. Death and transfiguration of a problem. *Bacteriological Reviews,* 33**,** 390-403.

MACHADO, P. R. L., ARAÚJO, M. I. A. S., CARVALHO, L. & CARVALHO, E. M. 2004. Immune response mechanisms to infections. *Anais Brasileiros de Dermatologia.* November/December 2004 ed. Rio de Janeiro Nov./Dec. 2004: Continuing Medical Education.

MACLEOD, M., GERBER, P., MOTTET, A., TEMPIO, G., FALCUCCI, A., OPIO, C., VELLINGA, T., HENDERSON, B. & STEINFELD, H. 2013. Greenhouse gas emissions from pig and chicken supply chains - A global life cycle assessment.

Rome, Italy. Food and Agriculture Organization of the United Nations (FAO). 196. http://www.fao.org/docrep/018/i3460e/i3460e.pdf.

MADER, T. L., FRANK, K. L., HARRINGTON, J., J. A., HAHN, G. L. & NIENABER, J. A. 2009. Potential climate change effects on warm-season livestock production in the Great Plains. *Climatic Change,* 97**,** 529-541.

MADZIMURE, J., CHIMONYO, M., ZANDER, K. K. & DZAMA, K. 2012. Diurnal heat-related physiological and behavioural responses in South African indigenous gilts. *Journal of Arid Environments,* 87**,** 29-34.

MADZIMURE, J., CHIMONYO, M., ZANDER, K. K. & DZAMA, K. 2013. Potential for using indigenous pigs in subsistence-oriented and market-oriented small-scale farming systems of Southern Africa. *Tropical Animal Health and Production,* 45**,** 135-142.

MAES, M., BERK, M., GOEHLER, L., SONG, C., ANDERSON, G., GAŁECKI, P. & LEONARD, B. 2012. Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. *BMC Medical Education,* 10**,** 1-19.

MAJDE, J. A. 2000. Viral double-stranded RNA, cytokines, and the flu. *Journal of Interferon & Cytokine Research,* 20**,** 259-272.

MALONEY, S. K. & DAWSON, T. J. 1995. The heat load from solar radiation on a large, diurnally active bird, the emu (*Dromaius novaehollandiae*). *Journal of Thermal Biology,* 20**,** 381-387.

MANI, V., WEBER, T. E., BAUMGARD, L. H. & GABLER, N. K. 2012. Growth and Development Symposium: Endotoxin, infl ammation, and intestinal function in livestock. *Journal of Animal Science,* 90**,** 1452-1465.

MARKLUND, S., KIJAS, J., RODRIGUEZ-MARTINEZ, H., RO¨NNSTRAND, L., FUNA, K., MOLLER, M., LANGE, D., EDFORS-LILJA, I. & ANDERSSON, L. 1998. Molecular basis for the dominant white phenotype in the domestic pig. *Genome Research,* 8**,** 826-833.

MARUYAMA, M., NISHI, M., KONISHI, M., TAKASHIGE, Y., NAGASHIMA, K., KIYOHARA, T. & KANOSUE, K. 2003. Brain regions expressing Fos during thermoregulatory behavior in rats. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,* 285**,** 1116-1123.

MATHIAS, E. & MUNDY, P. 2005. Herd movements: The exchange of livestock breeds and genes between North and South. Ober-Ramstadt, Germany: League for Pastoral Peoples and Endogenous Livestock Development.

MCCARRON, H. C. K., BUFFENSTEIN, R., FANNING, F. D. & DAWSON, T. J. 2001. Free-ranging heart rate, body temperature and energy metabolism in eastern grey kangaroos (*Macropus giganteus*) and red kangaroos (*Macropus rufus*) in the arid regions of south east Australia. *Journal of Comparative Physiology B: Biochemical, Systemic and Environmental Physiology,* 171**,** 401-411.

MCCARTY, R., HORWATT, K. & KONARSKA, M. 1988. Chronic stress and sympathetic-adrenal medullary responsiveness. *Social Science and Medicine,* 26**,** 333-341.

MCGLONE, J. & POND, W. 2003. Reproductive biology. *Pig Production: Biologocal Principles and Applications.* New York, USA: Thomson Delmar Learning.

MCGLONE, J. J. 1999. Managing heat stress in the outdoor pig breeding herd. *Symposium on Outdoor Pig Production in Brazil, September, 1999. .* Brazil.

MCMANUS, C., LOUVANDINI, H., GUGEL, R., SASAKI, L. C. B., BIANCHINI, E., BERNAL, F. E. M., PAIVA, S. R. & PAIM, T. P. 2011. Skin and coat traits in sheep in Brazil and their relation with heat tolerance. *Tropical Animal Health and Production,* 43**,** 121-126.

MCNAB, B. K. 1970. Body weight and the energetics of temperature regulation. *Journal of Experimental Biology,* 53**,** 329-348.

MCORIST, S., KHAMPEE, K. & GUO, A. 2011. Modern pig farming in the People's Republic of China: growth and veterinary challenges. *Revue scientifique et technique (International Office of Epizootics),* 30**,** 961-968.

MEJIA-MARTINEZ, K., LEMUS-FLORES, C., ZAMBRANO-ZARAGOZA, J. F., RAMIREZ-NECOECHEA, R., MOTA-ROJAS, D. & ALONSO-SPILSBURY, M. 2008. Cytokine immune response in Mexican Creole and commercial pigs under controlled conditions. *Journal of Animal and Veterinary Advances,* 7**,** 625-629.

MERGENTHALER, M., WEINBERGER, K. & QAIM, M. 2009. The food system transformation in developing countries: A disaggregate demand analysis for fruits and vegetables in Vietnam. *Food Policy,* 34**,** 426-436.

MEYER, W., SCHMIDT, J., KACZA, J., BUSCHE, R., NAIM, H. Y. & JACOB, R. 2011. Basic structural and functional characteristics of the epidermal barrier in wild mammals living in different habitats and climates. *European Journal of Wildlife Research,* 57**,** 873–885

MEYER, W., SCHWARZ, R. & NEURAND, K. 1978. The skin of domestic mammals as a model for the human skin, with special reference to the domestic pig. *Current Problems in Dermatology,* 7**,** 39-52.

MHLANGA, F. N., KHOMBE, C. T. & MAKUZA, S. M. 1999. *Indigenous livestock genotypes of Zimbabwe* [Online]. Addis Ababa, Ethiopia: International Livestock Research Institute. Available: https://www.ilri.org/InfoServ/Webpub/fulldocs/AnGenResCD/docs/IndiLiveGenoZimbabwe/CURRENTKNOWLEDGE.htm [Accessed 11 February 2016].

MORRIS, G. M. & HOPEWELL, J. W. 1990. Epidermal cell kinetics of the pig: a review. *Cell and Tissue Kinetics,* 23**,** 271-282.

MORRISON, P. R. & RYSER, F. A. 1952. Weight and body temperature in mammals. *Science,* 116**,** 231-232.

MORRISON, S. R., BOND, T. E. & HEITMAN, H. J. 1967. Skin and lung moisture loss from swine. *Transactions of American Society of Agricultural and Biological Engineers,* 10**,** 691-92.

MOSER, R. J., REVERTER, A., KERR, C. A., BEH, K. J. & LEHNERT, S. A. 2004. A mixed model approach for the analysis of cDNA microarray gene expression data from extreme-performing pigs after infection with *Actinobacillus pleuropneumoniae*. *Journal of Animal Science,* 82**,** 1261-1271.

MOUNT, L. E. 1962. Evaporative heat loss in the new-born pig. *Journal of Physiology (London),* 164**,** 274-281.

MOUNT, L. E. 1979. Pigs. *In:* BARRINGTON, E. J. W., WILLIS, A. J. & SLEIGH, M. A. (eds.) *Adaptation to the Thermal Environment: Man and His Productive Animals.* Bedford Square, London, UK: Edward Arnold.

MPHAHLELE, N. R., FULLER, A., ROTH, J. & KAMERMAN, P. R. 2004. Body temperature, behavior, and plasma cortisol changes induced by chronic infusion of *Staphylococcus aureus* in goats. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,* 287**,** R863-R869.

MUELLER, M., LINDNER, B., DEDRICK, R., SCHROMM, A. B. & SEYDEL, U. 2005. Endotoxin: physical requirements for cell activation. *Journal of Endotoxin Research,* 11**,** 299-303.

MUIRHEAD, M. R. & ALEXANDER, T. J. L. 1997. Managing pig health and the treatment of disease: a reference for the farm. Sheffield 5M Enterprises.

MULLER, C. 2013. African lessons on climate change risks for agriculture. *Annual Reviews of Nutrition,* 33**,** 395-411.

MURAKAMI, N. & ONO, T. 1987. Sex-related differences in fever development of rats. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,* 252**,** R284-R289.

MUSHANDU, J., CHIMONYO, M., DZAMA, K., MAKUZA, S. M. & MHLANGA, F. N. 2005. Influence of sorghum inclusion level on performance of growing local Mukota, Large White and their F1 crossbred pigs in Zimbabwe. *Animal Feed Science and Technology,* 122**,** 321-329.

MUYS, D. & WESTENBRINK, G. 2004. *Keeping pigs in the tropics,* Wageningen, The Netherlands, Digigrafi, Wageningen, The Netherlands.

NARDONE, A., RONCHI, B., LACETERA, N., RANIERI, M. S. & BERNABUCCI, U. 2010. Effects of climate changes on animal production and sustainability of livestock systems. *Livestock Science,* 130**,** 57-69.

NAY, T. & HAYMAN, R. H. 1956. Sweat glands in Zebu (*Bos indicus* L) and European (*Bos taurus* L.) cattle: I. Size of individual glands, the denseness of their population, and their depth below the skin surface *Australian Journal of Agricultural Research,* 7**,** 482-496.

NCUBE, M., DZAMA, K., CHIMONYO, M., KANENGONI, A. & HAMUDIKUWANDA, H. 2003. Effect of boar genotype on reproductive performance of the local sows of Zimbabwe. *Livestock Research for Rural Development.* Cali, Colombia: Fundación CIPAV.

NGUYEN, V. P., GILL, H. S. & HINCH, G. N. Variation in the immune responsiveness of three pig breeds in Vietnam. Proceedings of the Australian Society of Animal Production, 1996. 359.

NGUYEN, V. P., WONG, C. W., HINCH, G. N. & COLDITZ, I. G. 1998. Variation in the immune status of two Australian pig breeds. *Australian Veterinary Journal,* 76**,** 613-617.

NOAA. 2015. *Global Analysis - Annual 2015* [Online]. USA: National Oceanic and Atmospheric Administration (NOAA), National Centers for Environmental Information. Available: https://www.ncdc.noaa.gov/sotc/global/201513 [Accessed 6 March 2016].

NORIMATSU, M., ONO, T., AOKI, A., OHISHI, K., TAKAHASHI, T., WATANABE, G., TAYA, K., SASAMOTO, S. & TAMURA, Y. 1995. Lipopolysaccharide-induced apoptosis in swine lymphocytes in vivo. *Infection and Immunity,* 63**,** 1122-1126.

OLCZAK, K., NOWICKI, J. & KLOCEK, C. 2015. Pig behaviour in relation to weather conditions - a review. *Annals of Animal Science Journal,* 15**,** 601-610.

OLIVEIRA, E. A., BERTOL, T. M., COLDEBELA, A., SANTOS FILHO, J. I., SCANDOLERA, A. J. & WARPECHOWSKI, M. B. 2015. Live performance, carcass quality, and economic assessment of over 100kg slaughtered pigs *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* 67**,** 1743-1750.

OLLMANN, M. M., LAMOREUX, M. L., WILSON, B. D. & BARSH, G. S. 1998. Interaction of Agouti protein with the melanocortin 1 receptor *in vitro* and *in vivo*. *Genes and Development,* 12**,** 316-330.

OLSON, N. C., BROWN, J., T. T. & ANDERSON, D. L. 1985. Dexamethasone and indomethacin modify endotoxin-induced respiratory failure in pigs. *Journal of Applied Physiology,* 58**,** 274-284.

OLWOCH, J. M., RAUTENBACH, D. W. C. J., ERASMUS, B. F. N., ENGELBRECHT, F. A. & VAN JAARSVELD, A. S. 2003. Simulating tick distributions over sub-Saharan Africa: the use of observed and simulated climate surfaces. *Journal of Biogeography,* 30**,** 1221-1232.

OPAL, S. M. & COHEN, J. 1999. Clinical gram-positive sepsis: does it fundamentally differ from gram-negative bacterial sepsis? *Critical Care Medicine,* 27**,** 1608-1616.

OPIO, C., GERBER, P., MOTTET, A., FALCUCCI, A., TEMPIO, G., MACLEOD, M., VELLINGA, T., HENDERSON, B. & STEINFELD, H. 2013. Greenhouse gas emissions from ruminant supply chains - A global life cycle assessment. Rome, Italy. Food and Agriculture Organisation of the United Nations. 214. http://www.fao.org/docrep/018/i3461e/i3461e.pdf. [2 February 2016]

ORR, H. A. 2009. Fitness and its role in evolutionary genetics. *Nature Reviews Genetics,* 10**,** 531-539.

OWEN-ASHLEY, N. T., TURNER, M., HAHN, T. P. & WINGFIELD, J. C. 2006. Hormonal, behavioural and thermoregulatory responses to bacterial lipopolysaccharide in captive and free-living whitecrowned sparrows (*Zonotrichia leucophrys gambelii*). *Hormones and Behavior,* 49**,** 15-29.

PAN, Y. S. 1962. Quantitative and morphological variation of sweat glands, skin thickness, and skin shrinkage over various body regions of Sahiwal Zebu and Jersey cattle. *Australian Journal of Agricultural Research,* 14**,** 424-437.

PARROT, R. F., VELLUCCI, S. V., GOODE, J. A., M., L. D. & FORSLING, M. L. 1995. Cyclo-oxygenase mediation of endotoxin-induced fever, anterior and posterior pituitary hormone release and hypothalamic c-Fos expression in the prepubertal pigs. *Experimental Physiology,* 80**,** 663-674.

PARROT, R. F., VELLUCCI, S. V., GOODE, J. A., M., L. D. & FORSLING, M. L. 1997. Interrelated adrenocortical and neurohypophysial responses associated with in endotoxin-treated pigs. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,* 273**,** R1046-R1052.

PATEL, A. & FORSYTHE, P. 2008. *Small Animal Dermatology,* Beijing, China, Saunders Elsevier.

PENRITH, M. L., THOMSON, G. R., BASTOS, A. D. S., PHIRI, O. C., LUBISI, B. A., DU PLESSIS, E. C., MACOME, F., PINTO, F., BOTHA, B. & ESTERHUYSEN, J. 2004. An investigation into natural resistance to African swine fever in domestic pigs from an endemic area in southern Africa. *Revue scientifique et technique (International Office of Epizootics),* 23**,** 965-977.

PICCIONE, G. & REFINETTI, R. 2003. Thermal chronobiology of domestic animals. *Frontiers in Bioscience,* 8**,** 258-264.

PIELBERG, G. 2004. *Molecular Coat Colour Genetics. Dominant White in Pigs and Greying with Age in Horses.* PhD, Swedish University of Agricultural Sciences.

PIGLIUCCI, M., MURREN, C. J. & SCHLICHTING, C. D. 2006. Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology,* 209**,** 2362-2367.

PILING, D. & HOFFMAN, I. 2011. Climate change and animal genetic resources for food and agriculture: State of knowledge, risks and opportunities. *In:* UNITED NATIONS, F. A. A. O. (ed.) *Background Study Paper No. 53* Italy, Rome: Food and Agriculture Organisation.

PINSON, E. A. 1942. Evaporation from human skin with sweat glands inactivated. *American Journal of Physiology,* 187**,** 492-503.

POLLMANN, D. S. 2010. Seasonal effects on sow herds: industry experience and management strategies. *In:* POLLMANN, D. S. (ed.) *Seasonal effects on sow herds: industry experience and management strategies.* Des Moines, IA: Midwest American Society of Animal Science conference proceedings.

PORTNER, H. O. & KNUST, R. 2007. Climate change affect marine fishes through the oxygen limitation of thermal tolerance. *Science,* 315**,** 95-97.

PURSE, B. V., MELLOR, P. S., ROGERS, D. J., SAMUEL, A. R., MERTENS, P. P. C. & BAYLIS, M. 2005. Climate change and the recent emergence of bluetongue in Europe. *Natural Reviews Microbiology,* 3**,** 171-181.

QUINIOU, N., NOBLET, J., VAN MILGEN, J. & DUBOIS, S. 2001. Influence of low ambient temperatures on heat production and energy balance of single-housed growing pigs fed ad libitum: a comparison with group-housed pigs. *Animal Research,* 50**,** 325-333.

RAETZ, C. R. & WHITFIELD, C. 2002. Lipopolysaccharide endotoxins. *Annual Review of Biochemistry,* 71**,** 635-700.

RAITH, K. & NEUBERT, R. H. 2000. Liquid chromatography-electrospray mass spectrometry and tandem mass spectrometry of ceramides. *Analytica Chimica Acta,* 403**,** 295-303.

RAMSAY, K., HARRIS, L. & KOTZE, A. 1998. *Landrace breeds: South Africa's indigenous and locally developed farm animals: Farm Animal Conservation,* Irene, Pretoria, Farm Animal Conservation Trust.

RENAUDEAU, D. 2005. Effects of short term exposure to high ambient temperature and relative humidity on thermoregulatory responses of European (Large White) and Caribbean (Creole) restrictively-fed growing pigs. *Animal Research,* 54**,** 81-93.

RENAUDEAU, D., ANAIS, C., TEL, L. & GOURDINE, J. L. 2010. Effect of temperature on thermal acclimation in growing pigs estimated using a nonlinear function1. *Journal of Animal Science,* 88**,** 3715-3724.

RENAUDEAU, D., HUC, E., KERDONCUFF, M. & GOURDINE, J. L. Acclimation to high ambient temperature in growing pigs: effects of breed and temperature level. 2006 Symposium COA/INRA Scientific Cooperation in Agriculture, November 7 - 10, 2006 2007 Tainan (Taiwan, R. O. C.). 177-182.

RENAUDEAU, D., LECLERCK-SMEKENS, M. & HERIN, M. 2006. Difference in skin characteristics in European (Large White) and Caribbean (Creole) growing pigs with reference to thermoregulation. *Animal Research,* 55**,** 209–217.

RISCHKOWSKY, B. & PILLING, D. 2007. The state of the world's animal genetic resources for food and agriculture. *In:* RISCHKOWSKY, B. & PILLING, D. (eds.). Food and Agriculture Organisation, Rome: Food and Agriculture Organization of the United Nations.

ROCKSTROM, J. 2000. Water resources management in smallholder farms in eastern and southern Africa, an overview. *Physics and Chemistry of the Earth Part B: Hydrology Oceans and Atmosphere,* 25**,** 275-283.

RODBARD, S. 1950. Weight and body temperature. *Science,* 111**,** 465-466.

ROGERS, D. J. & RANDOLPH, S. E. 2000. The global spread of malaria in a future, warmer world. *Science,* 289**,** 1763-1766.

ROMANOVSKY, A. A. & BLATTEIS, C. M. 1995. Biphasic fever: what triggers the second temperature rise? *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,* 269**,** 280-286.

ROSS, J. W., HALE, B. J., GABLER, N. K., RHOADS, R. P., KEATING, A. F. & BAUMGARD, L. H. 2015. Physiological consequences of heat stress in pigs. *Animal Production Science,* 55**,** 1381-1390.

ROUZAUD, F., MARTIN, J., GALLET, P. F. & DELOURME, D. 2000. A first genotyping assay of French cattle breeds based on a new allele of the extension gene encoding the melanocortin-1 receptor (Mc1r). *Genetic, Selection and Evolution,* 32**,** 511-520.

ROWLINSON, P. Adapting livestock production systems to climate change - temperate zones. *In:* ROWLINSON, P., STEELE, M. & NEFZAOUI, A., eds. Proceedings of an International Conference on Livestock and global change, 17-20 May 2008 2008 Hammamet, Tunisia. Cambridge, UK, Cambridge University Press, 61-63.

ROYO, L. J., ALVAREZ, I., ARRANZ, J. J. & FERNANDEZ, I. 2008. Differences in the expression of the ASIP gene are involved in the recessive black coat colour pattern in sheep: evidence from the rare Xalda sheep breed. *Animal Genetics,* 39**,** 290-293.

RUSSO, V., FONTANESI, L., DAVOLI, R., CHIOFALO, L., LIOTTA, L. & ZUMBO, A. 2004. Analysis of single nucleotide polymorphisms in major and candidate genes for production traits in Nero Siciliano pig breed. *Italian Journal of Animal Science,* 3**,** 19-29.

SADC. 2016. *Livestock Production* [Online]. Gaborone, Botswana: SADC. Available: http://www.sadc.int/themes/agriculture-food-security/livestock-production/ [Accessed 9 February 2016].

SAKUMOTO, R., KASUYA, E., KOMATSU, T. & AKITA, T. 2003. Central and peripheral concentration of tumour necrosis factor-α in Chinese Meishan pigs stimulated with lipopolysaccharide. *Journal of Animal Science,* 81**,** 1274-1280.

SANDBERG, F. B., EMMANS, G. C. & KYRIAZAKIS, I. 2007. The effects of pathogen challenges on the performance of naive and immune animals: the problem of prediction. *Animal,* 1**,** 67-86.

SARAVANAKUMAR, V. R. & THIAGARAJAN, M. 1992. Comparison of sweat glands, skin characteris and heat tolerance coefficients amongst Murrah, Surti and non-descript buffaloes. *Indian Journal of Animal Sciences,* 62**,** 625-628.

SATHAR, F., BADLANGANA, N. L. & MANGER, P. R. 2010. Variations in the thickness and composition of the skin of the giraffe. *The Anatomical Record,* 293**,** 1615-1627.

SCARPA, R., DRUCKER, A. G., ANDERSON, S., FERRAES-EHUAN, N., GOMEZ, V., RISOPATRÓN, C. R. & RUBIO-LEONEL, O. 2003. Valuing genetic resources in peasant economies: the case of 'hairless' creole pigs in Yucatan. *Ecological Economics,* 45**,** 427-443.

SCHAUMBERGER, S. & SCHATZMAYR, G. 2012. Effort on an oral endotoxin challenge in pigs. *Journal of Animal Science,* 90**,** 389-390.

SCHMIDT-NIELSEN, K., CRAWFORD, E. C., JR., NEWSOME, A. E., RAWSON, K. S. & HAMMEL, H. T. 1967. Metabolic, rate of camels : effect of body temperature and dehydration. *American Journal of Physiology,* 212**,** 341-346.

SCHMIDT-NIELSEN, K., SCHMIDT-NIELSEN, B., JARNUM, S. A. & HOUPT, T. R. 1957. Body temperature of the camel and its relation to water economy. *American Journal of Physiology,* 188**,** 103-112.

SCHOLANDER, P. F., HOCK, R., WALTERS, V., JOHNSON, F. & IRVING, L. 1950. Heat regulation in some arctic and tropical animals and birds. *Biological Bulletin, Marine Biological Laboratory, Woods Hole,* 99**,** 237-258.

SCHULZE-LEFERT, P. & PANSTRUGA, R. 2011. A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends in Plant Science,* 16**,** 117-125.

SCOLARI, S. C., CLARK, S. G., KNOX, R. V. & TAMASSIA, M. A. 2011. Vulvar skin temperature changes significantly during estrus in swine as determined by digital infrared thermography. *Journal of Swine Health and Production,* 19**,** 151-155.

SEHIC, E. & BLATTEIS, C. M. 1996. Blockade of lipopolysaccharide-induced fever by subdiaphragmatic vagotomy in guinea pigs. *Brain Research,* 726**,** 160 - 166.

SHIELDS, S. & ORME-EVANS, G. 2015. The impacts of climate change mitigation strategies on animal welfare. *Animals,* 5**,** 361-394.

SILANIKOVE, I. 2000. Effects of heat stress on the welfare of extensively managed domestic animals. *Livestock Production Science,* 67**,** 1-18.

SINCLAIR, K. D., LEA, R. G., REES, W. D. & YOUNG, L. E. 2007. The developmental origins of health and disease: current theories and epigenetic mechanisms. *Society of Reproduction and Fertility Supplement,* 64**,** 426-443.

SLENNING, B. D. 2010. Global climate change and implications for disease emergence. *Veterinary Pathology,* 47**,** 28-33.

SOKOLOV, V. E. 1982. Mammal Skin. *In:* MATVEYEV, B. S. (ed.) First ed. Berkeley/Los Angeles, USA: University of California Press, Ltd.

SOLANO, F. 2014. Melanins: Skin pigments and much more—types, structural models, biological functions, and formation routes. *New Journal of Science***,** 1-28.

SOSZYNSKI, D., KOZAK, W. & SZEWCZENKO, M. 1991. Course of fever response to repeated administration of sublethal doses of lipopolysaccharides, polyinosinic: polycytidylic acid and muramyl dipeptide to rabbits. *Experientia,* 47**,** 43-47.

SOUTH AFRICAN PORK PRODUCERS ORGANISATION, S. 2014. Evaluating the competitiveness of South African pork production within the global context Available: http://www.bfap.co.za/documents/research%20reports/Agribenchmark%20pork%20progress%20report%201_2014.pdf [Accessed 19 February 2016].

SPENCER, S. J., GALIC, M. A. & PITTMAN, Q. J. 2011. Neonatal programming of innate immune function. *American Journal of Physiology, Endocrinology and Metabolism,* 300**,** E11-E18.

SPIERS, D. E. 2012. Physiological basics of temperature regulation in domestic animals. *In:* COLLIER, J. & COLLIER, J. L. (eds.) *Environmental Physiology of Livestock.* USA: John Wiley and Son.

ST-PIERRE, N. R., COBANOV, B. & SCHNITKEY, G. 2003. Economic losses from heat stress by US Livestock Industries. *Journal of Dairy Science,* 86**,** E52-E77.

STATISTICS SOUTH AFRICA. 2013. Census 2011 Agricultural households: Key highlights. Pretoria, South Africa. Statistics South Africa. 27 pp. http://www.statssa.gov.za/publications/Report-03-11-01/Report-03-11-012011.pdf. [25 March 2016]

STEINFELD, H., GERBER, P., WASSENAAR, T., CASTEL, V., ROSALES, M. & DE HAAN, C. 2006. Livestock's long shadow: environmental issues and options. Rome. Food and Agriculture Organization of the United Nations.

STEPHENSON, J. 2010. Livestock and climate policy: Less meat or less carbon? Organisation for Economic Co-operation and Development http://www.oecd.org/sd-roundtable/papersandpublications/46512036.pdf. [13 January 2016]

STEWART, I. B. 2002. *Spleenic contractions, catecholamine release and blood volume redistribution during exercise in man.* PhD, The University of British Columbia.

STEYN, W. J., CASEY, N. H. & VAN RENSBURG, C. J. 2012. Effects of different penning conditions, feeding regimens and season on growth and carcass attributes of boars of a selected genetic line. *South African Journal of Animal Science,* 42**,** 178-188.

STOTT, G. H. & WIERSMA, F. 1973. Climatic thermal stress, a cause of hormone depression and low fertility in bovine. *International Journal of Biometeorology,* 5**,** 115-122.

STRAUSS, W. M., HETEM, R. S., MITCHELL, D., MALONEY, S. K., MEYER, L. C. R. & FULLER, A. 2015. Selective Brain Cooling Reduces Water Turnover in Dehydrated Sheep. *PloS One,* 10**,** e0115514.

SURICO, G. 2013. The concepts of plant pathogenicity, virulence/avirulence and effector proteins by a teacher of plant pathology *Phytopathologia Mediterranea,* 52**,** 399−417.

SURIYASOMBOON, A., LUNDEHEIM, N., KUNAVONGKRIT, S. & EINARSSON, S. 2004. Effect of temperature and humidity on sperm production in Duroc boars under different housing systems in Thailand. *Livestock Production Science,* 89**,** 19-31.

SWART, H. 2010. *Micro-satellite-based characterisation of southern African domestic pig (Sus scrofa domestica) breeds.* MSc (Zoolgy) Research Dissertation, University of Limpopo.

TAKAMATA, A. 2012. Modification of thermoregulatory response to heat stress by body fluid regulation. *Journal of Physical Fitness and Sports Medicine,* 1**,** 479-489.

TAKEDA, K. & AKIRA, S. 2005. Toll-like receptors in innate immunity. *International Immunology,* 17**,** 1-14.

TAKEUCHI, O. & AKIRA, S. 2007. Recognition of viruses by innate immunity. *Immunological Reviews,* 220**,** 214-224.

TAYLOR, G., ROESE, G. & HERMESCH, S. 2005. Breeds of pigs—Large White. *In:* INDUSTRIES, N. D. O. P. (ed.). Australia: NSW Department of Primary Industries.

THATCHER, W. W., FLAMENBAUM, I., BLOCK, J. & BILBY, T. R. Interrelationships of heat stress and reproduction in lactating dairy cows. 2010 High Plains Dairy Conference 2010. 45-60.

THODY, A. J., HIGGINS, E. M., WAKAMATSU, K., ITO, S., BURCHILL, S. A. & MARK, J. M. 1991. Pheomelanin as well as eumelanin is present in human epidemis. *Journal of Investigative Dermatology,* 97**,** 340-344.

THORNTON, P., VAN DE STEEG, J., NOTENBAERT, A. & HERRERO, M. 2013. Climate change: do we know how it will affect smallholder livestock farmers? *The Futures of Agriculture, Brief No. 43 - English, Future Studies*.

THORNTON, P. K. & HERRERO, M. 2014. Climate change adaptation in mixed crop-livestock systems in developing countries. *Global Food Security,* 3**,** 99-107.

THORNTON, P. K., HERRERO, M., FREEMAN, A., MWAI, O., REGE, E., JONES, P. & MCDERMOTT, J. 2007. Mapping climate vulnerability and poverty in Africa. *SAT eJournal,* 4**,** 1-23.

TOPPS, J. H. 1975. Behavioural and physiological adaptation of wild ruminants and their potential for meat production. *Proceedings of the Nutrition Society,* 34**,** 85-93.

TROSTLE, R. & SEELEY, R. 2013. *Developing Countries Dominate World Demand for Agricultural Products* [Online]. USA: United States Department of Agriculture, Economic Research Services Available: http://www.ers.usda.gov/amber-waves/2013-august/developing-countries-dominate-world-demand-for-agricultural-products.aspx#.VtQOJpx97IU [Accessed 22 February 2016].

TSAI, H. J., SAIF, Y. M., NESTOR, K. E., EMMERSON, D. A. & PATTERSON, R. A. 1992. Genetic variation in resistance of turkeys to experimental infection with Newcastle Disease Virus. *Avian Diseases,* 36**,** 561-565.

UEHARA, A., ISHIKAWA, Y., OKUMURA, T., SEKIYA, C., TAKASUGI, Y. & NAMIKI, M. 1989. Indomethacin blocks the anorexic action of interleukin-1. *European Journal of Pharmacology,* 170**,** 257-260.

UENISHI, H., SHINKAI, H., MOROZUMI, T., MUNETA, Y., JOZAKI, K., KOJIMA-SHIBATA, C. & SUZUKI, E. 2010. Polymorphisms in pattern recognition receptors and their relationship to infectious disease susceptibility in pigs. *International Symposium on Animal Genomics for Animal Health (AGAH 2010).* Paris, France.

UMBOH, J. F. & TULUNG, B. 2001. Performance of the pigs maintained in a highland and cosatl area of Minahasa Region, North Sulawesi. *Asian-Australia Journal of Animal Science,* 14**,** 1014-1018.

UMESIOBI, D. O. 2010. Enhanced testicular development and fertilizing capacity of boars following sexual stimulation at different ages. *Journal of Applied Animal Research,* 37**,** 105-108.

UPAH, N., PEARCE, S., GABLER, N. K. & BAUMGARD, L. H. 2011. Effects of heat stress and plane of nutrition on production and metabolism in growing pigs. Iowa State University. Iowa State University. 3. http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1702&context=ans_air.

UPTON, M. 2004. The role of livestock in economic development and poverty aleviation. FAO, Rome, Italy. Food and Agriculture Organisation of the United Nations. pp. 66. http://www.fao.org/ag/againfo/programmes/en/pplpi/docarc/wp10.pdf. [7 February 2016]

USDA. 2015. Production and Trade of All Meats to Expand in 2016 *Livestock and Poultry: World Markets and Trade* [Online]. Available: http://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.PDF [Accessed 17 February 2016].

VÅGE, D. I., FLEET, M. R., PONZ, R. & OLSEN, R. T. 2003. Mapping and characterization of the dominant black colour locus in sheep. *Pigment Cell Research,* 16**,** 693-697.

VAN BEAUMONT, W. 1972. Evaluation of hemocontration from haematocrit measurements. *Journal of Applied Physiology,* 32**,** 712-713.

VAN DER HEL, W., DUIJGHUISEN, R. & VERSTEGEN, M. W. A. 1986. The effect of ambient temperature and activity on the daily variation in heat production of growing pigs kept in groups. *Netherlands Journal of Agricultural Science,* 34**,** 173-184.

VAN DER LEUN, J. C. & DE GRUIJL, F. R. 1993. Influences of ozone depletion on human and animal health. *In:* TEVINI, M. (ed.) *UV-B radiation and ozone depletion: Effects on humans, animals, plants, microorganisms, and materials.* 3rd ed. Ann Arbor: Lewis Publishers.

VAN DER VALK, P. G. M., KUCHAREKOVA, M. & TUPKER, R. A. 2004. Transepidermal water loss and its relation to barrier function and skin irritation. *In:* FLUHR, J., ELSNER, P., BERARDESCA, E. & MAIBACH, H. I. (eds.) *Bioengineering of the skin: Water and the stratum corneum.* 2nd Edition ed. Washington D. C., USA: CRC Press.

VENGRIS, V. E. & MARE, C. J. 1972. Swine Interferon 1: Induction in porcine cell cultures with viral and synthetic inducers. *Canadian Journal of Contemporary Medicine,* 36**,** 282-287.

VIGNOLA, R., HARVEY, C. A., BAUTISTA-SOLIS, P., AVELINO, J., RAPIDEL, B., DONATTI, C. & MARTINEZ, R. 2015. Ecosystem-based adaptation for smallholder farmers: Definitions, opportunities and constraints. *Agriculture, Ecosystems & Environment,* 211**,** 126-132.

VISSER, D. P. 2004. *Structuring of breeding objectives in the pork supply chain in South Africa* PhD, University of Pretoria.

WALSBERG, G. E., CAMPBELL, G. S. & KING, J. R. 1978. Animal coat color and radiative heat gain: A re-evaluation. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology,* 126**,** 211-222.

WALSBERG, G. E. & WOLF, B. O. 1995. Effects of solar-radiation and wind-speed on metabolic heat-production by two mammals with contrasting coat colors. *Journal of Experimental Biology,* 198**,** 1499-1507.

WANG, J., S., K., SUKLERD, S. & PONGTHAISONG, P. 2012. Skin morphology of Thai native cattle. *Khon Kaen Agriculture Journal (Supplement),* 2**,** 392-394.

WARNER, C. M., MEEKER, D. L. & ROTHSCHILD, M. F. 1987. Genetic control of immune responsiveness: A review of its use as a tool for selection for disease resistance. *Journal of Animal Science,* 64**,** 394-406.

WARREN, E. J., FINCK, B. N., ARKINS, S., KELLEY, K. W., SCAMURRA, R. W., MURTAUGH, M. P. & JOHNSON, R. W. 1997. Coincidental changes in behavior and plasma cortisol in unrestrained pigs after intracerebroventricular injection of tumor necrosis factor-α. *Endocrinology,* 138**,** 2365-2371.

WEBEL, D. M., FINCK, B. N., BAKER, D. H. & JOHNSON, R. W. 1997. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. *Journal of Animal Science,* 75**,** 1514-1520.

WEBER, F., WAGNER, V., RASMUSSEN, S. B., HARTMANN, R. & PALUDAN, S. R. 2006. Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses. *Journal of Virology,* 80**,** 5059-5064.

WEISS, F. & LEIP, A. 2012. Greenhouse gas emissions from the EU livestock sector: a life cycle assessment carried out with the CAPRI model. *Agriculture, Ecosystems & Environment,* 149**,** 124-134.

WETTEMANN, R. P., WELLS, M. E., OMTVEDT, I. T., E., P. C. & TURMAN, E. J. 1976. Influence of elevated ambient temperature on reproductive performance of boars. *Journal of Animal Science,* 42**,** 664-669.

WHITNEY, M. 2014. Minimizing heat stress in pigs during the summer.

WICKEDFOOD EARTH. 2016. *Indigenous pigs* [Online]. Wickedfood Earth. Available: http://www.wickedfoodearth.co.za/farming/animals/pigs/indigenous-pigs/ [Accessed 6 March 2016].

WMO. 2016. *Climate change breaches symbolic thresholds, fuels extreme weather* [Online]. Geneva, Switzerland: World Metereological Organiztion (WMO),. Available: https://www.wmo.int/media/content/wmo-2015-likely-be-warmest-record-2011-2015-warmest-five-year-period [Accessed 6 March 2016].

WOLF, B. O. & WALSBERG, G. E. 2000. The role of the plummage in heat transfer processes of birds. *American Zoologist,* 40**,** 575-584.

WON, S. J. & LIN, M. T. 1988. Pyrogenicity of interferon and its inducer in rabbits. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology,* 254**,** R499-R507.

WORLEY PARSONS ANNUAL REPORT. 2015. Corporate Responsibility Definition & Clarifications. Sydney, Australia. Worley Parsons. 120. http://www.worleyparsons.com/InvestorRelations/corporateresponsibility/Documents/CRDefinitions.pdf. [4 April 2016]

WRIGHT, K. J., BALAJI, R., HILL, C. M., DRITZ, S. S., KNOPPEL, E. L. & MINTON, J. E. 2000. Integrated adrenal, somatotropic, and immune responses of growing pigs to treatment with lipopolysaccharide. *Journal of Animal Science,* 78**,** 1892-1899.

ZABRISKIE, J. & VISVANATHAN, K. 2007. *Peptides and mimetics for reducing symptoms of toxic shock syndrome and septic shock* United States patent application US 10/555,370. 1 February 2007.

ŻAK, M., KUROPKA, P., KOBIELARZ, M., DUDEK, A., KALETA-KURATEWICZ, K. & SZOTEK, S. 2011. Determination of the mechanical properties of the skin of pig foetuses with respect to its structur. *Acta Bioengineering and Biomechanics,* 13**,** 37-43.

ZANGA, J., CHIMONYO, M., KANENGONI, A., DZAMA, K. & MUKARATIWA, S. 2003. A comparison of the susceptibility of growing Mukota and Large White pigs to infection with *Ascaris suum*. *Veterinary Research Communications,* 27**,** 653-660.

ZHAO, J., WHYTE, J. & PRATHER, R. S. 2010. Effect of epigenetic regulation during swine embryogenesis and on cloning by nuclear transfer. *Cell Tissue Research,* 341**,** 13-21

ZHAO, S., ZHU, M. & CHEN, H. 2012. Immunogenomics for identification of disease resistance genes in pigs: a review focusing on Gram-negative *bacilli*. *Journal of Animal Science and Biotechnology,* 3**,** 34-46.

# CHAPTER 9: APPENDICES

## 9.1 Ethical Clearance certificate

AESC 3

**UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG**

**STRICTLY CONFIDENTIAL**

**ANIMAL ETHICS SCREENING COMMITTEE (AESC)**

**CLEARANCE CERTIFICATE NO. 2010//58/04**

**APPLICANT:** Mr D Moyo

**SCHOOL:** Physiology
**DEPARTMENT:**
**LOCATION:**

**PROJECT TITLE:** **Comparison of thermoregulation and febrile responses of the Kolbroek Windsnyer and the Landrace breeds of pigs**

**Number and Species**

2 pigs in the initial instance and then a further 24pigs if the pilot study is successful.

Approval was given for the use of animals for the project described above at an AESC meeting held on 30.11.2010. This approval remains valid until 30.11.2012

The use of these animals is subject to the AESC guidelines for the use and care of animals, limited to the procedures described in the application form, subject to the establishment of a financial agreement with either the Research Office or the Wits Health Consortium in the case that the research has potential commercial outputs, subject to arrangements negotiated with the Central Animal Services personnel restricted to the applicant and / or listed co-workers and subject to the following:

- That further evidence from the existing scientific literature is provided to the committee to ensure that the period of fluid deprivation and heat exposure will indeed be tolerated by pigs.
- That the pilot study provides additional evidence that the pigs can tolerate the period of fluid deprivation and heat exposure
- That the applicant considers drawing repeated blood samples from the ear or jugular vein rather than using an implanted catheter to obtain blood.
- Animals should not be returned to the farm of origin, but rather euthanized or sent to a petting farm.

Signed: _____ Date: 20/12/2010
(Chairperson, AESC)

I am satisfied that the persons listed in this application are competent to perform the procedures therein, in terms of Section 23 (1) (c) of the Veterinary and Para-Veterinary Professions Act (19 of 1982)

Signed: _____ Date: 03/01/2011
(Registered Veterinarian)

## 9.2 Modifications and Extensions

AESC 2012 M&E

**Please note that only typewritten applications will be accepted.**

# UNIVERSITY OF THE WITWATERSRAND

### ANIMAL ETHICS SCREENING COMMITTEE
### MODIFICATIONS AND EXTENSIONS TO EXPERIMENTS

a. Name: DAVISON MOYO

b. Department: PHYSIOLOGY

c. Experiment to be modified / extended

| | | AESC NO | | |
|---|---|---|---|---|
| Original AESC number | 2010 | 58 | 04 | |
| Other M&Es : To replace the pigs that died (No number allocated) | | | | B |

d. Project Title:

| | | No. | Species |
|---|---|---|---|
| e. | Number and species of animals originally approved: | 24 | *Sus scrofa (pigs)* |
| f. | Number of additional animals previously allocated on M&Es: | 4 | *Sus scrofa (pigs)* |
| g. | Total number of animals allocated to the experiment to date: | 28 | *Sus scrofa (pigs)* |
| h. | Number of animals used to date: | 28 | *Sus scrofa (pigs)* |

i. Specific modification / extension requested:

I would like to request for permission to:
a. collect tissue samples (for gross anatomy and histology) from all the 24 pigs at the termination stage.
b. Include additional co-workers on the project.

j. Motivation for modification / extension:

To investigate differences in the three breeds of pigs and further characterise them as part of the study. Initially I had indicated that at the end of the study I would euthanize the pigs and incinerate their carcasses after retrieval of the implanted tele-transmitters and biologgers (Stowaway). The pigs represent an important genetic resource which requires further investigations. In an effort to maximise use of the animals I would like to collect tissues at the end of the study after euthanasia.

Samples collected will help explain some of the differences observed in the fever and thermoregulation experiments.

To assist with the investigations i would like to include the following as co-workers/co-investigators:

i. Miss Sediba Tjale (BSc Honours student), School of Physiology

ii. Assoc Prof Amadi Ihunwo, School of Anatomical Sciences: for neurogenesis and histologic examinations (0117172767)

iii. Mr Pedzisai Mazengenya, School of Anatomical Sciences: for neurogensis and histologic examinations (011 7172204)

Date: 17 May 2012

Signature:

**RECOMMENDATIONS**
Approved.

Date: 23 May 2012

Signature:

**Samples to be collected from each pig**

i. **Heads** – for the determination of neurogenesis. After Euthanasia the pigs heads will be decapitated and perfused for removal of the brain for further analysis by colleagues in the School of Anatomical Sciences.

ii. **Liver** – for the determination of glycogen and lipid storage differences in the different breeds

iii. **Skin biopsies** – for histological examination of the thickness of the different layers and for other possible indicators that could explain the breed differences in thermoregulation which I have found

iv. **Blood** – I would, like to collect 10 ml of blood by cardiac puncture for:
   a. Erythrocyte Fragility tests
   b. Routine haematology
   c. Hormones regulating metabolism (Insulin and leptin)

v. **Clinical biochemistry profile** I would also like to make measurements (mass, length and histology where applicable) of the abdominal viscera and to analyse the GIT content

## 9.3 DATA USED FOR DRAWING GRAPHS THAT ARE APPEARING IN CHAPTERS 4 AND 5.

**PLEASE NOTE**: The data is available on request. You may send me a request on davison.moyo@gmail.com so that I forward the data set