

GROWTH PERFORMANCE, BLOOD PARAMETERS, AND MEAT QUALITY IN FINISHING DOHNE MERINO LAMBS REARED UNDER SINGLE- OR PAIRED- PEN HOUSING

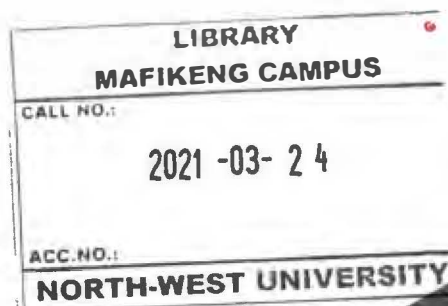
K.P Maraba

orcid.org/0000-0001-5372-1985

BSc Animal Health

Dissertation submitted in fulfilment of the requirements for the
Master of Science degree in Agriculture (Animal Science) at
Mafikeng Campus of the North-West University

Supervisor: Prof. V. Mlambo
Co:Supervisor: Dr. A.O Yusuf



Graduation October 2017

Student number: 24055476



**GROWTH PERFORMANCE, BLOOD PARAMETERS, AND MEAT QUALITY IN
FINISHING DOHNE MERINO LAMBS REARED UNDER SINGLE- OR PAIRED-PEN
HOUSING**

Maraba Kgadi Paulina

(24055476)

Thesis is presented in fulfilment of the requirements for the degree of Master of Science in
Agriculture (Animal Science) at Mafikeng Campus of the North-West University

Supervisor: Prof. V. Mlambo

Co-supervisor: Dr. A.O. Yusuf

Department of Animal Science

Faculty of Agriculture, Science and Technology

North-West University

P Bag x2046

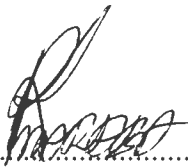
Mmabatho 2735

Mafikeng

November 2017

DECLARATION

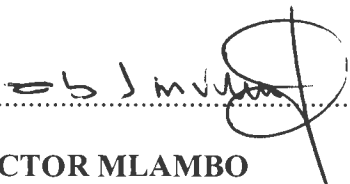
I, Kgadi Paulina Maraba, student number 24055476, at North-West University (Mafikeng Campus) hereby submit this thesis for the degree of Master of Science in Animal Science. I declare that the entirety of the work contained herein is my own, original work and that I have not previously, in its entirety or in part, submitted it for any qualification. The work has been done under the supervision of Prof. V.Mlambo and Dr A. O Yusuf.

Signed: 

Date: 10/10/2017

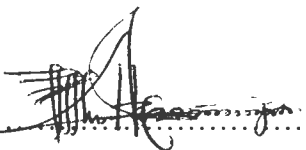
KGADI P. MARABA

This dissertation has been submitted with my approval as a University supervisor and would certify that the requirements for the applicable Master of Science degree rules and regulations have been fulfilled.

Signed: 

Date: 10/10/2017

PROF. VICTOR MLAMBO

Signed: 

Date: 2017/10/10

DR AZEEZ. O. YUSUF

ABSTRACT

The study was designed to evaluate the effect of dietary vitamin E and selenium on growth performance, blood parameters, and meat quality of finishing Dohne Merino lambs reared under two types of confinement. Twenty four lambs with an average body weight of 25.8 ± 2.7 kg were used. The lambs were randomly assigned to four treatment combinations: single-pen housing with no extra vitamin E-selenium supplementation, single-pen housing with extra vitamin E-selenium supplementation, paired-pen housing with no extra vitamin E-selenium supplementation, and paired-pen housing with extra vitamin E-selenium supplementation, in a completely randomized design. The results showed no significant differences ($P > 0.05$) on the overall weight gain and overall feed conversion ratio across the four treatments. Lambs supplemented with vitamin E-Se consumed more feed (65.01 kg) over the entire feeding period as compared to the lambs that were not offered the supplements (60.37 kg). An interaction between supplement and housing condition was observed on rectal temperature on the last day of the experiment where animals housed in pairs had a higher mean rectal temperature (39.3°C) as compared to those housed individually (38.8°C). The results showed that most of the hematological indices were not significantly ($P < 0.05$) affected by the dietary supplement and housing type, with the exception of red blood cell (RBC), hematocrit/packed cell volume (HCT/PCV), and mean corpuscular haemoglobin concentration (MCHC). For these parameters, the supplemented lambs had higher RBC ($12.61 \times 10^{12}/\text{L}$), HCT/PCV (28.88 %) and MCHC (32.24 g/dL) counts/levels as compared to the non-supplemented animals (RBC, $11.17 \times 10^{12}/\text{L}$; HCT/PCV, 24.87 %; and MCHC, 15.96 g/dL) and irrespective of the housing type. Superoxide dismutase and glucose levels were significantly ($P < 0.05$) affected by housing type only, with the higher means of 0.05 u/mL and 3.95 mmol/L, respectively, being observed in companion

group as compared to the solitary confinement group (0.03 u/mL and 3.65 mmol/L, respectively). At the end of feeding trial, the animals were slaughtered to determine meat quality characteristics. Results from this analysis showed that most of the carcass measurements and meat qualities were not significantly ($P < 0.05$) influenced by the dietary vitamin E-selenium supplementation and housing type with the exception of slaughter weight, dressing percentage and pHu (on shelf life). These parameters were higher in lambs fed with the dietary vitamin E-selenium than in the control group. A significant ($P < 0.05$) interaction between supplementation $\times$ housing was observed for drip loss percentage and carcass length. All in all, the results of this study provided a valuable insight into the impact of dietary vitamin E and selenium on lamb performance, physiological parameters, blood parameters and meat quality. Dietary vitamin E and selenium induced more appetite, as the supplemented group consumed more feed than the control group. On the other hand, the RBC, HCT/PCV, MCHC, slaughter weight, dressing percentage and pHu were higher in the supplemented group than the control group. It can be concluded that the companion housing and solitary confinement caused different levels of stress, as an interaction was observed on the rectal temperature, drip loss percentage and carcass length.

Keywords: Vitamin E, selenium, physiological response, carcass characteristics, sheep, housing effect.

ACKNOWLEDGEMENTS

No work is possible without the help of many. There are many praise-worthy people that have helped me from the beginning till the completion of this research work, either directly or indirectly. I am deeply indebted to my supervisors Prof: V. Mlambo and Dr. A.O Yusuf for their generous academic guidance and other support throughout my study. I would like to extend my sincere gratitude to Prof: U. Marume who assisted me with meat quality analysis, Prof: M. Mwanza and Mrs M. Tsheole for providing materials and equipment and their assistance in blood collection, ration formulation and laboratory analysis and Mr T.F Buthane for assisting with all the materials and infrastructure needed during my feeding trial. I am also indebted to my family, friends and colleagues for encouraging me consistently, for their endless support and always being the source of encouragement and moral support for me from the very beginning of this study.

DEDICATION

This work is dedicated to my mother Dikeledi Mary Maraba, my daughter Fortunate Ledile Maraba, my granny Eunice Ledile Maraba and my brother Howard Machaka Maraba.

LIST OF ABBREVIATION/ACRONYMS

CREA	Creatinine
GSHpx	Glutathione peroxide
Hb	Haemoglobin
Hct	Haematocrit
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
RBC	Red blood cells
ROM	Reactivated oxygen metabolism
ROS	Reactivated oxygen species
SOD	Superoxide dismutase
TP	Total protein
WBC	White blood cells

TABLE OF CONTENTS

DECLARATION	I
ABSTRACT	II
ACKNOWLEDGEMENTS	IV
DEDICATION	V
LIST OF ABBREVIATION/ACRONYMS.....	VI
TABLE OF CONTENTS	VII
LIST OF TABLES.....	X
LIST OF FIGURES.....	XI
1 CHAPTER ONE - GENERAL INTRODUCTION.....	1
1.1 BACKGROUND.....	1
1.2 PROBLEM STATEMENT	2
1.3 JUSTIFICATION	3
1.4 THE OBJECTIVES OF THE STUDY	3
1.5 HYPOTHESES.....	4
1.6 REFERENCES	5
2 CHAPTER TWO - LITERATURE REVIEW	7
2.1 SOUTH AFRICAN SHEEP PRODUCTION	7
2.2 THE DOHNE MERINO BREED	7
2.3 PRODUCTION SYSTEMS (EXTENSIVE VS. INTENSIVE)	8
2.4 ANIMAL WELFARE IN SHEEP PRODUCTION.....	9
2.3 SPACE ALLOCATION	10
2.3.1 <i>Space allowance for intensive housing</i>	11
2.3.2 <i>Space allowance during transportation</i>	12
2.4 SOLITARY CONFINEMENT VS. GROUP HOUSING	12
2.5 NUTRITION AND WELFARE	13
2.5.1 <i>Vitamin E</i>	14
2.5.2 <i>Selenium</i>	14
2.5.3 <i>Interrelationship between vitamin E and selenium</i>	15
2.6 EFFECT OF ENVIRONMENTAL CONDITIONS ON SHEEP PRODUCTIVITY	15
2.7 OXIDATIVE STRESS IN SMALL RUMINANTS	16
2.8 ANTIOXIDANTS	17
2.9 NON-ENZYMATIC ANTIOXIDANTS	18
2.9.1 <i>Bilirubin and vitamin E-Se</i>	18
2.10 CORTISOL AND FERRITIN	18
2.11 ENZYMATIC ANTIOXIDANTS.....	19
2.11.1 <i>Superoxide dismutase</i>	19
2.11.2 <i>Glutathione peroxidases GSH-px</i>	20
2.11.3 <i>Protein thiol</i>	21
2.12 GROWTH PERFORMANCE AND STRESS.....	21
2.13 BLOOD PARAMETERS AND STRESS	22
2.13.1 <i>Red blood cells</i>	22

2.13.2	White blood cells	23
2.14	MEAT QUALITY AND STRESS	23
2.15	REFERENCES	25
3	CHAPTER THREE - GROWTH AND PHYSIOLOGICAL PARAMETERS OF THE DOHNE MERINO SHEEP REARED UNDER SINGLE OR PAIRED-PEN HOUSING: EFFECT OF EXTRA DIETARY VITAMIN E-SELENIUM SUPPLEMENTATION	44
3.1	INTRODUCTION	45
3.2	MATERIALS AND METHODS.....	46
3.2.1	Study site.....	46
3.2.2	Management of experimental animals	47
3.2.3	Housing and dietary treatments.....	47
3.2.4	Growth performance, body condition and physiological parameters.....	48
3.2.5	Statistical analysis	49
3.3	RESULTS	50
3.4	DISCUSSION	54
3.5	CONCLUSION	56
3.6	REFERENCES	57
4	CHAPTER FOUR - HAEMATOLOGICAL INDICES AND SERUM BIOCHEMISTRY IN FINISHING DOHNE MERINO SHEEP REARED UNDER SINGLE- OR PAIRED-PEN HOUSING: EFFECT OF EXTRA DIETARY VITAMIN E-SELENIUM SUPPLEMENTATION	64
4.1	INTRODUCTION	65
4.2	MATERIALS AND METHODS.....	67
4.2.1	Blood sample collection.....	67
4.2.2	Analytical procedures.....	68
4.2.3	Statistical analysis	68
4.3	RESULTS	69
4.4	DISCUSSION	71
4.5	CONCLUSION	74
4.6	REFERENCES	75
5	CHAPTER FIVE - CARCASS CHARACTERISTICS AND MEAT QUALITY IN DOHNE MERINO SHEEP REARED UNDER SINGLE- OR PAIRED-PEN HOUSING: EFFECT OF EXTRA DIETARY VITAMIN E-SELENIUM SUPPLEMENTATION.....	79
5.1	INTRODUCTION	81
5.2	MATERIALS AND METHODS.....	83
5.2.1	Slaughtering procedure	83
5.2.2	Meat quality analysis.....	83
5.2.3	Statistical analysis	84
5.3	RESULTS	85
5.4	DISCUSSION	89
5.5	CONCLUSION	91
5.6	REFERENCES	92
6	CHAPTER SIX: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS	97
6.1	GENERAL DISCUSSION.....	97
6.2	CONCLUSION	99
6.3	RECOMMENDATIONS.....	100

6.4 REFERENCES 101

LIST OF TABLES

TABLE 2.1: THE RECOMMENDED SPACE ALLOWANCE VALUES FOR HOUSED SHEEP. 10

TABLE 3.1: STATISTICAL SIGNIFICANCE (*P* VALUES) OF THE EFFECT OF THE MAIN FACTORS (HOUSING AND VITAMIN E-SE SUPPLEMENT) AND THEIR INTERACTIONS ON THE OVERALL FEED INTAKE (KG), WEIGHT GAIN (KG), AND FEED CONVERSION RATIO (FCR) OF THE DOHNE MERINO LAMBS.....50

TABLE 3.2: STATISTICAL SIGNIFICANCE (*P* VALUES) OF THE EFFECT OF THE MAIN FACTORS (DIETARY VITAMIN E-SE AND HOUSING CONDITIONS) ON RECTAL TEMPERATURE, HEART RATE AND RESPIRATORY RATE IN LAMBS AT THE END OF THE FEEDING TRIAL (WEEK 8). 53

TABLE 3.3: EFFECT OF DIETARY VITAMIN E-SE AND HOUSING CONDITIONS ON RECTAL TEMPERATURE LEVELS OF LAMBS AT THE END OF THE FEEDING TRIAL.....53

TABLE 4.1: STATISTICAL SIGNIFICANCE (*P* VALUES) OF EFFECTS OF THE MAIN FACTORS (HOUSING AND VITAMIN E-SE) AND THEIR INTERACTION ON HEMATOLOGICAL INDICES AND STRESS BIOMARKERS. 69

TABLE 4.2: EFFECT OF DIETARY VITAMIN E-SE SUPPLEMENTATION ON HEMATOLOGICAL INDICES AND SERUM ANTIOXIDANTS LEVELS OF THE DOHNE MERINO LAMBS.....70

TABLE 5.1: STATISTICAL SIGNIFICANCE (*P* VALUES) OF EFFECTS OF THE MAIN FACTORS (HOUSING AND VITAMIN E-SE SUPPLEMENT) AND THEIR INTERACTIONS ON CARCASS MEASUREMENT AND MEAT QUALITY PARAMETERS OF THE DOHNE MERINO LAMBS.....85

TABLE 5.2: EFFECTS OF VITAMIN E-SELENIUM SUPPLEMENTATION ON CARCASS AND MEAT QUALITY OF THE DOHNE MERINO LAMBS. 86

LIST OF FIGURES

FIGURE 3.1: EFFECT OF DIETARY VITAMIN E AND SELENIUM SUPPLEMENTATION ON WEEKLY BODY WEIGHT GAIN OF THE DOHNE MERINO LAMBS.....51

FIGURE 3.2: EFFECT OF DIETARY VITAMIN E AND SELENIUM SUPPLEMENTATION ON WEEKLY FEED INTAKE OF THE DOHNE MERINO LAMBS.....52

FIGURE 5.1: INTERACTIONS BETWEEN THE HOUSING AND DIETARY SUPPLEMENTATION EFFECTS ON DRIP LOSS AND CARCASS LENGTH. 88

FIGURE 5.2: INTERACTIONS BETWEEN THE HOUSING AND DIETARY SUPPLEMENTATION EFFECTS ON DRIP LOSS. 89

1 CHAPTER ONE - GENERAL INTRODUCTION

1.1 Background

Livestock production plays a significant role in the South African economy. South Africa has approximately 8 000 commercial and about 5 800 communal sheep farmers (Abstract of Agricultural Statistics, 2012). The estimated total number of sheep in South Africa is 28.8 million (Abstract of Agricultural Statistics, 2012). Therefore, sheep farming makes a major contribution with respect to animal fiber, meat and dairy products. However, there are many factors which influence sheep productivity in South Africa. These include temperature, nutrition, water availability, disease, transportation, group size, and heredity. Indeed, productivity, health and quality of meat from sheep are easily affected by rearing conditions as well as nutrition. Finishing lambs are the major source of meat products and they are often intensively fed in large groups. These rearing conditions may induce stress in the animals resulting in suboptimal welfare. These rearing conditions may lead to oxidative stress either through the production of excessive reactive oxygen species (ROS) or decreased antioxidant defenses (Bernabucci *et al.*, 2002; Saker *et al.*, 2004; Trana *et al.*, 2006). Excessive reactive oxygen metabolites (ROM) overwhelm the antioxidant defenses leading to oxidative damage of biological molecules including proteins, lipids and DNA (Machlin & Bendich, 1987; Halliwell & Gutteridge, 1990), resulting in diseased cells and endangering the survival of the cells.

The damage to DNA, proteins and lipids play a key role in the development of diseases such as cancer, arteriosclerosis, arthritis, mastitis, neurodegenerative disorders and other conditions (Ames & Shigenaga 1992; Dizdaroglu *et al.*, 2002; Emerit *et al.*, 2004 ; Scott 2004; Galle *et al.*, 2006). The action of these free radicals can be neutralized by several metalloenzymes (e.g.,

glutathione peroxidase, catalase, superoxide dismutase) and by the non-enzymatic antioxidant defense system (e.g., tocopherol, β -carotene, ubiquinol, vitamin C, glutathione, lipoic acid, uric acid, metallothionein, selenium and bilirubin) (Onyema *et al.*, 2006). Antioxidant vitamins like vitamin C and E have been shown to protect biological membranes against ROM damage. Dietary supplementation with vitamin E plays a vital role in reducing lipid peroxidation, increasing antioxidant enzyme activity and largely reversing the depression in growth rate and feed efficiency caused by corticosterone. Vitamin E interacts with a number of antioxidants such as vitamin C, GSH, β -carotene or lipoic acid to combat stress resulting from elevated levels of corticosterone and other stress factors (Coombes *et al.*, 2001). Like vitamin E, selenium acts as an antioxidant. In fact, selenium is even more effective as an antioxidant through its production of the high density lipoprotein, when combined with vitamin E (Cheung *et al.*, 2001).

1.2 Problem statement

Sheep are a gregarious animal species that thrive in loosely organized flocks; as such, social isolation is likely to constitute a major psychosocial stress (Kilgour & De Langen, 1970). The impact of isolation stress in sheep reared in South Africa on growth performance, stress biomarkers, and meat quality is largely unknown but could have profound effects on the productivity and meat quality. Indeed, finishing lambs are often fed in confinement, which may compromise their welfare. During the intensive feeding of finishing lambs, social factors and space allocation influence weight gain, health and quality of meat. Suboptimal welfare may reduce contribution of the lamb to the animal meat industry and also affects the quality of meat supplied to the consumers. The use of antioxidants, such as vitamin E and selenium, has the potential to mitigate stress induced by suboptimal housing, and thereby enhancing productivity, health and meat quality. Therefore, this research was aimed at evaluating the effectiveness of

extra vitamin E and selenium as dietary antioxidants in finishing lambs reared under confinement. The main objective of the study was to evaluate the effect of extra dietary vitamin E and selenium on growth performance, blood parameters, and meat quality of finishing Dohne Merino lambs reared under two types of housing; individual and paired housing.

1.3 Justification

Oxidative stress is a significant concern among livestock owners because it results in losses in animal performance and quality of animal products, which negatively affects profit margins. Suboptimal social housing is one of many factors that may cause oxidative stress in sheep, leading to poor reproduction, performance, and meat quality. Indeed, Costa *et al.*, (2015) report that social isolation may be associated with negative effects such as abnormal behavior and developmental problems, in a number of animal species. Therefore, it was important, in this study that the effects of social housing on growth performance, stress biomarkers and meat quality be defined under the South African production systems since such types of research had not yet been done. Information generated thereof would be important as ‘proof of principle’ as well as to guide sheep producers on the possible production outcomes of varying housing systems.

1.4 The objectives of the study

The main objective of the study was to evaluate the effect of extra dietary vitamin E and selenium on growth performance, blood parameters, and meat quality of finishing Dohne Merino lambs reared under two types of confinement.

The specific objectives were as follows:

1. To evaluate the effect of extra vitamin E and selenium on growth performance, haematology, oxidative status, and meat quality of lambs reared under confinement: and
2. To determine the effect of extra vitamin E and selenium supplementation on transportation stress on lambs.

1.5 Hypotheses

Lambs housed individually (solitary confinement with limited space) will experience a suboptimal welfare resulting in a relatively poor growth performance, health, and meat quality. On the other hand, lambs housed in pairs and, therefore, able to socialize will have a better welfare resulting in a better growth performance, health and meat quality. It was also hypothesized that an extra dietary supplementation with vitamin E and selenium could reduce the effects of stress induced by individual housing.

1.6 References

- Ames, B. N & Shigenaga, M.K., 1992. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U S A.* 90, 7915–7922.
- Bernabucci, U., Ronchi, B., Lacetera, N. & Nardone, A., 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J. Dairy Sci.* 85, 2173 - 2179.
- Cheung, F.M., Leung, K., Song, W.Z & Zhang, J.X., 2001. The Cross-Cultural (Chinese) Personality Assessment Inventory-2 (CPAI- 2) [Available from F. M. Cheung, Department of Psychology, The Chinese University of Hong Kong, Shatin, Hong Kong].
- Coombes J.S., Powers S.K. & Rowell B., 2001. Effects of vitamin E and α -lipoic acid on skeletal muscle contractile properties. *J. Appl. Physiol.* 90, 1424-1430.
- Costa, J. H. C., von Keyserlingk, M. A. G. & Weary, D. M., 2015. Effects of group housing of dairy calves on behavior, cognition, performance, and health. *J. Dairy Sci.* 99, 2453–2467.
- Dizdaroglu, M., Jaruga, P & Birincioglu, M., 2002. Free-radical-induced damage to DNA: mechanisms and measurement. *Free Rad Bio Med.* 32, 15-1102.
- Emerit, J., Edeas, M., & Bricaire F., 2004. Neurodegenerative diseases and oxidative stress. *Biomed Pharmacother.* 58, 39–46.
- Galle. J., Hansen-Hagge, T., Wanner, C & Seibold, S., 2006. Impact of oxidized low density lipoprotein on vascular cells. *Atherosclerosis.* 185, 219–26.

- Halliwell, B & Gutteridge, J.M., 1990. The antioxidants of human extracellular fluids. *Arch Biochem Biophys.* 280, 1-8.
- Kilgour, R. & De Langen H., 1970. Stress in sheep resulting from management practices. *Proc. N. Z. Soc. Anim. Prod.* 30, 65–76.
- Machlin, L.J. & Bendich, A., 1987. Free radical tissue damage: protective role of antioxidant nutrients. *FASEB. J.* 1, .441-445.
- Onyema, O.O., Farombi, E.O., Emerole, G.O., Ukoha, A.I. & Onyeze, G.O., 2006. Effect of vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. *Indian. J. Biochem. Biophys.* 43, 20-24.
- Saker, K. E.; Fike, J. H.; Veit, H.; Ward, D. L., 2004. Brown seaweed- (Tasco™) treated conserved forage enhances antioxidant status and immune function in heat-stressed wether lambs. *J. Anim. Physiol. Anim. Nutr.*, 88, 122-130.
- Scott, J., 2004. Pathophysiology and biochemistry of cardiovascular disease. *Curr Opin Genet Develop.* 14, 271–9.
- Trana A., Celi P., Claps S., Fedele V & Rubino R., 2006: The effect of hot season and nutrition on the oxidative status and metabolic profile in dairy goats during mid lactation. *J. Ani. Sci*, 82, 717–722.

2 CHAPTER TWO - LITERATURE REVIEW

2.1 South African sheep production

Livestock industry contributes over 49% of the total agricultural output in South Africa and is, therefore, regarded as the largest agricultural industry in South Africa (FAO, 2013). Extensive livestock production is suitable for approximately 80% of agricultural land in South Africa (FAO, 2013), thus making the red meat industry one of the most significant sub-sectors of South African agriculture. Approximately 590 000 km² of land in South Africa is occupied by sheep and goat farming, representing 53% of all agricultural land in the country (FAO 2008). Sheep and goat industries contribute a total of 8.06% to the gross value of total animal production, subdivided into 5.26% for sheep and goat meat and 2.80% for wool, compared to beef, pigs and poultry, which contribute 37%, 4.47% and 37.04%, respectively (DAFF,2009). According to the Department of Agriculture, Forestry and Fisheries, South Africa normally produces 85% of its meat requirements while the remainder (15%) is imported from Australia, New Zealand and the Southern African Customs Union (SACU) represented by Botswana, Lesotho, Namibia and Swaziland (NDA, 2012).

2.2 The Dohne Merino breed

Sixty per cent of the sheep population in South Africa is dominated by the Merino sheep. The Dohne Agricultural Research Station near Stutterheim in the Eastern Cape initiated a program in 1939 under the guidance of Mr JJJ Kotze, to produce a dual purpose (meat and wool) sheep breed (Dohne Merino) by breeding the cross breed German Mutton Merino rams (Elsenburg College of Agriculture) with the South African Merino ewes to meet the required characteristics (Kotze, 1951; McMaster, 1991) such as excellent adaption to sourveld (climate and pastures),

ability to produce high quality meat and wool (without hair or coloured fibers), exceptionally good reproduction characteristics, high fertility rate, lambing at regular intervals (autumn & spring), rapid lamb growth rate and ability to produce good quality slaughter lambs (BKB, 2010; SAIB, 2010). This breed fulfills all the prerequisites mentioned and adapts exceptionally well to both the extensive and intensive production systems (SAIB, 2010).

2.3 Production systems (extensive vs. intensive)

Rearing of sheep in an intensive production system is estimated by the Compassion in World Farming group (CIFW, 2010) to be 1% of the sheep flock globally. South Africa is predominantly by extensive sheep farming found in the arid regions of the country (AGRISTATS, 2008), even though, no official statistics are currently available on the occurrence of intensive and extensive livestock production systems (AGRISTATS, 2008). The intensive rearing production system is when animals are housed in a confined area (indoors or outdoors) and fed a specifically formulated diet with limited or no physical activity, whereas in an extensive production system, animals roam freely without being confined but they are exposed to extreme weather conditions, predator, inadequate supply of feed especially during dry seasons and theft, and all these effects are more likely to reduce production (Guesdon *et al.*, 2015). Intensive production systems has more advantages as compared to the extensive production system because it is the fastest growing sector in meat production systems and produces approximately 40% of meat (all species) globally (FAO, 2002; Nierenberg, 2005; reviewed by Dickson-Hoyle & Reenberg, 2009). The implementation of intensive livestock production systems benefits (efficiency, high productivity, high yields, high turn-over time, low production cost, high profit, low risk, minimal land needed) the farmer/producer and environmental changes have no direct impact on the productivity of this system, but may influence the availability of

grains. In extensive rearing production system, animals are exposed to extreme weather conditions, predator, and inadequate supply of feed especially during dry seasons, and theft; and all these effects are more likely to reduced production (Guesdon *et al.*, 2015).

2.4 Animal welfare in sheep production

In 2012, ten general principles were put forward by the 178 member nations of the World Organization for Animal Health, to guide the development of specific standards for various animal species (OIE, 2012). The reason for formulating these general principles was because of the public concern over the welfare of animals. The public's concerns include: veterinary epidemiology, environmental physiology, environmental design (housing types), comparative psychology and behaviour of animal and animal's handlers (Fraser, 2008; Mellor *et al.*, 2009; Appleby *et al.*, 2011). Of all these public concerns, confinement production systems (housing types) and nutrition are of the utmost importance since they are within the direct control of animal producers. Concerns regarding housing types and nutrition put farmers in a difficult situation as they have to balance between minimizing cost, maximizing animal performance and ensuring optimum animal health and welfare (Webster, 1994). The Commission Regulations (EC) (834/2007 and 889/2008) prescribe space allowance for young sheep weighing 15-25 kg and 25-40 kg as 0.60 and 1 m²/head, respectively, while for ewes and rams, 1.5-2 and 2.2-2.5 m²/head, respectively, is recommended (Table 2.1).

When farmers select a housing system, they should prioritize protecting animals from heat or cold stress, ensure an adequate supply of feed (Berge, 1997; Brosh *et al.*, 1998), and facilitate social interaction between animals (Guesdon *et al.*, 2015) in order to maintain the welfare and health of livestock. There are a number of factors that farmers should take into account such as

restriction to external areas, thermal extremes protection, adequate space allocation, optimal indoor climate and hygiene, adequate ventilation and light regimes, and the availability of nutritious feed (Curtis, 1983; Hartung, 1994; Sevi *et al.*, 1999; Casamassima *et al.*, 2001; Sevi *et al.*, 2001a). Companionship plays a vital role because animals are able to learn together how to handle potentially dangerous situations (Kavaliers *et al.*, 2003). Socializing also stimulates motor and social skills and reinforces positive behavioural development and coping abilities (Fagen, 1981; Newberry *et al.*, 1988; Thomson, 1998). These factors are vital for improvement of the health and welfare of housed sheep. Space allowance is also closely related to the amount of feed to be consumed in group-fed animals. The fulfilling of the basic needs such as resting, eating, drinking, and socialization are imperative for maintenance of the animals' health and welfare (Estevez *et al.*, 2007; Petherick, 2007; Petherick & Phillips, 2009).

Table 2.1: The recommended space allowance values for housed sheep.

Young animals (15-25 kg body weight)	0.60 m ² /head
Young animals (25-40 kg body weight)	1 m ² /head
Adult animals (ewe)	1.5-2 m ² /head
Adult animals (ram)	2.2-2.5 m ² /head
Feeder space	0.2 m/head
Airspace (adult animals)	7 m ³ /head

Adopted from Sevi *et al.*, (2009)

2.3 Space allocation

Space allocation is an important aspect of animal production. It ultimately determines which behaviour animals will be able to perform and for how long they can perform them. Critical surviving activities such as feeding, drinking, excretion and resting must be optimized, whilst

other behaviours, such as locomotion/exercise, self-grooming and social behaviour are only essential for longer-term health and welfare (Petherick, 2007).

2.3.1 Space allowance for intensive housing

Space allowance increases with specific weight. For sheep weighing no more than 60 kg and reared on straw litter or slatted floor, a minimum space allowance of 0.7 m²/head and 1 m²/head must be maintained (Loynes, 1983). For sheep weighing from 60 to 90 kg, the space allowance should increase by 30% and a further 30% during the suckling of lambs. A 10% reduction for recently sheared sheep is recommended (Dickson & Stephenson, 1979). Intensive housing is advantageous since animals are protected from extreme climate and inadequate nutrition. This is because animals do not experience unpredictable and unfavourable environments and thus intensive housing is believed to improve welfare and health of livestock (Berge, 1997; Brosh *et al.*, 1998). Published evidence challenges these advantages since there are several factors that are essential to ensure welfare and health of the animal such as space allocation (Sevi *et al.*, 1999) and climate and hygiene (Curtis, 1984; Hartung, 1994) that may easily be compromised under the intensive housing systems. A predictable and structured environment can be depressing to sheep, because their behaviour, physiology and production performance activities are not optimal. Sheep might suffer due to restriction to animal behaviour (Hughes & Duncan 1988) as they are unable to perform their natural behaviours and are unable to socialize or interact (Petherick & Rushen 1997). When sheep are reared intensively (in confinement), they tend to be highly reactive to human handling, exhibit poor adaptation to their environment and experience high levels of stress, which reduces their level of welfare (Bickell *et al.*, 2009).

Different studies have investigated the effects of space allowance on behaviour, health, and production measures. In a semi-intensive system, animals are free to exhibit their natural

behaviours (e.g. grooming, licking each other, etc.). Behavioural plasticity allows animals to change strategies and adapt more easily to the varying social and physical conditions. Gonyou *et al.* (1985) discovered lower daily weight gains when sheep were reared under a reduced space allowance. Space reduction also increases aggressive behaviours resulting in animals fighting for dominance (e.g. over feed, space etc.) (Dove *et al.*, 1974; Lynch *et al.*, 1985).

2.3.2 *Space allowance during transportation*

Transportation is considered a major stressor for animals (Tarrant, 1990; Lambert *et al.*, 1998). This might be because often the animals are crowded on long trips with unfavourable conditions at loading and lairage (Van Putten & Elshof, 1978; Shaw & Tume, 1992; Hemsworth & Coleman, 1998; Terlouw, 2008; Ferguson & Warner, 2008; Miranda-de la Lama *et al.*, 2010). Under these suboptimal conditions, animals experience fear, dehydration and hunger, physical injury and fatigue, and these events may ultimately have deleterious impacts onto the animal's health and quality of meat (Ferguson & Warner, 2008; Adenkola & Ayo, 2010). Exposing an animal to stressors will trigger reactions in the organism, with activation of the nervous sympathetic-adrenomedullary system and the hypothalamic–pituitary–adrenocortical axis, causing an increase in levels of catecholamines and glucocorticoids, respectively (Chrousos, 1998). When environmental factors are unfavourable in the final phases of an animal's life, meat quality will be negatively influenced (Ferguson & Warner, 2008; Ekiz *et al.*, 2010).

2.4 **Solitary confinement vs. group housing**

Social isolation has a greater impact onto the livelihood of animals causing abnormal behavior, developmental problems such as poor growth performance, poor carcass and meat quality and the development of diseases in a range of animal species (Bouissou *et al.*, 2001; Fisher &

Matthews., 2001). Social learning allows individuals to learn from the positive or negative effects on other individuals (Bandura., 1977; Conte and Paolucci., 2001). This type of learning is thought to be important in the development of feeding behavior in many farmed species (Keeling & Hurnik, 1996; Launchbaugh & Howery, 2005). Companionship is considered a basic need because it reduces fear of novel situations and non-harmful stimuli, reduces heat loss, and maintains a healthy pelage. Grooming, which is common among animals housed as a group, has a calming effect that allows animals to engage in natural behaviours. An animal that is individually housed is limited in terms of animal activities. Socially isolated animals tend to suffer from boredom, do not benefit from grooming behaviours and have restricted animal behaviours (Fraser *et al.*, 2013), resulting in suboptimal growth performance, production and welfare. Sheep are very unique creatures that easily experience fear, anxiety and frustration, which can be caused by husbandry operations or by poor adaptability to indoor confinement, because they are very gregarious and non-aggressive (Lynch *et al.*, 1992; Fitzpatrick *et al.*, 2006).

2.5 Nutrition and welfare

Livestock production requires essential nutrients for the well-being of animals. It is important to provide animals with adequate nutrients to maintain their growth performance and health. Conventional ideas regarding the levels of essential nutrients in farm animal nutrition are based on experimental findings on the quantities necessary to prevent deficiency diseases and to support rapid growth and high productivity (Clifford, 2001). Research on vitamins has made extensive progress over the last 20 years. The intensive research interest in vitamin E stems from its potential role as an antioxidant that is able to prevent free-radical mediated tissue damage, and as a consequence to prevent or delay the development of certain degenerative and inflammatory

diseases (Baldi, 2005). Studies have also shown that combining both Se and vitamin E may result in a synergistic enhancement of the immune response and disease resistance (Surai, 2002; Singh *et al.*, 2006).

2.5.1 Vitamin E

Vitamin E is a fat soluble antioxidant and one of the micronutrients with a positive influence on the immune function, health, growth, reproduction, prevention of various diseases, and protection of the integrity of tissues. It is well-known to protect the biological membrane from oxidative damage by acting as a scavenger of the reactive oxygen species (ROS) (Negre-Salvayre *et al.*, 2008). It protects the aqueous-phase cell components by increasing the concentration of reduced glutathione in red blood cells (Lii *et al.*, 1998). Vitamin E is a poor inhibitor of reactions, i.e., it can react with other reactive oxidants, including alkoxyl radicals, singlet oxygen, nitrogen dioxide (NO₂), peroxynitrite (ONOO⁻), ozone and superoxide (O₂⁻). An increase in the activities of the total anti-oxidation competence (T-AOC), superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX), and a decreased content of nitric oxide (NO), malondialdehyde (MDA) and activity of the nitric oxide synthase (NOS) have been reported when vitamin E is added to the diet (Zhu *et al.*, 2010).

2.5.2 Selenium

Selenium (Se) is a component of at least 25 selenoproteins, which maintain a redox balance by regulating the various functions of the body such as antioxidant defence mechanism. It is a mineral that is essential for human and animal nutrition (Surai & Fisinin, 2014). Selenium plays a vital role as an essential part of the glutathione peroxidases (GSH-Px), which provide defence against oxidative stress by catalysing the reduction of hydrogen peroxide and lipid peroxides to

less harmful hydroxides (Baltić *et al.*, 2015). Previous studies have shown that selenium affects body condition, reproduction, survival and antioxidant protection levels (Dean & Combs, 1981; Hoffman & Heinz, 1998; De Vink *et al.*, 2008; Franson *et al.*, 2011). Therefore, a small quantity should be given for a normal biological function because too much of it can have a negative effect onto the vertebrae (Heinz *et al.*, 1989; Spallholz & Hoffman, 2002; Ohlendorf, 2003). In addition, dietary Se will improve the oxidative stress of meat products, since it can prevent myopathesis in sheep (Daun & Akesson, 2004; Negre-Salvayre *et al.*, 2008).

2.5.3 *Interrelationship between vitamin E and selenium*

The relationship between Se and vitamin E is to protect against cellular damage by reactive oxygen species (Schriner *et al.*, 2005). The metabolic function of Se is closely linked to the Se-containing enzyme GSH-Px functions in the cytosol of the cell, while vitamin E is an integral component of lipid membranes (Suraï, 2002). Studies reported that the combined supplementation of vitamin E and Se increases GSH-Px activity (Suraï & Dvorska, 2002; Ebeid, 2012). Glutathione peroxidase is an antioxidant enzyme, which plays a vital role in the defence system of cells since it reduces a number of peroxides and improves the lipid stability of meat (Skřivanová *et al.*, 2007). Studies have shown that combining both Se and vitamin E may result in a synergistic enhancement of the immune response and disease resistance (Suraï, 2002; Singh *et al.*, 2006).

2.6 **Effect of environmental conditions on sheep productivity**

Environmental conditions that may be responsible for inducing stress may include handling by humans, air temperature changes, high ambient temperature (heat stress) and relative humidity, (Mitchell & Kettlewell, 1998; Joachim *et al.*, 2010). Animals that are housed must have enough

ventilation for thermal exchanges between their body surfaces and the environment and also to avoid an excessive increase in the relative humidity and reducing the levels of noxious gases and airborne particles. This will help in maintaining the welfare and performance of lambs (Sevi, 2005). Heat stress plays a vital role onto the animal's well-being, because of the several physiological, molecular and immunological responses that are invoked to minimize its adverse effects (Collier *et al.*, 2006; Gaughan *et al.*, 2009). For example, the increased rectal temperature, respiration rate, reductions in growth rate and impaired antioxidant defence system (Miller *et al.*, 1993; Bernabucci, 2012) may have a direct effect onto the health status of animals such as the animals' immunosuppression system, which may increase susceptibility to the various diseases and even an increased mortality rate (Griffin, 1989; Dahl *et al.*, 2012). In turn, this leads to some extreme economic losses for producers, as a result of the poor performance of animals.

2.7 Oxidative stress in small ruminants

Free radicals are usually referred to as "reactive oxygen species", known to be chemical species containing unpaired electrons and they are also commonly referred to as paramagnetic (they are unstable, short-lived and highly reactive). They are essential for several physiological roles such as protein phosphorylation, transcription factors activation, cell differentiation, apoptosis, oocyte maturation, steroidogenesis, cell immunity and cellular defence against microorganisms (Miller *et al.*, 1993; Dröge, 2002; Agarwal *et al.*, 2005) and also for the induction of a mitogenic response when they are at a low concentration (Polli *et al.*, 2004). A higher concentration due to excessive production "termed oxidative stress" can result in the damaging of cell structures, including lipids and membranes, proteins and nucleic acids (Miller *et al.*, 1993; Polli *et al.*, 2004; Sugino, 2006). Oxidative stress remains a major stressor amongst livestock owners across the

world, because it is a mechanism that leads to biological damage, and is regarded as one of the causes of several pathologies that affect the growth and production in living animals. This negative effect of ROS can be scavenged by the enzymatic antioxidant system (e.g., glutathione peroxidase (GPx), thiol, superoxide dismutase (SOD)) and the non-enzymatic antioxidant defence system (e.g., α -tocopherol, β -carotene, ubiquinol, vitamin C, glutathione, lipoic acid, uric acid, metallothionein, and bilirubin), which literally inhibit the ROS activity (Halliwell 1996; Yavuz *et al.*, 2004; Onyema *et al.*, 2006).

2.8 Antioxidants

Antioxidants are substances that interact with free radicles and willing to offer their electrons to such free radicles to prevent cellular damage. Intracellular, extracellular, lipophilic and aqueous antioxidant mechanisms are found throughout the body and work together to prevent the generation of ROS (preventative antioxidants); destroy or inactivate ROS which are formed (scavenging antioxidants); terminate chains of ROS-initiated peroxidation (chain-breaking antioxidants) (Strain & Benzie, 1998). Enzymatic and non-enzymatic antioxidants aid in preventing and ameliorating the harmful effects of oxidative stress by forming the antioxidant defense mechanisms. Vitamin E, β -carotene and coenzyme Q serve as the first line of defense which can be found in the fat-soluble cellular membrane while the second line of antioxidant scavengers include vitamin C and enzymatic antioxidants such as glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase, glutathione (GSH) and protein thiol (Kao *et al.*, 2010).

2.9 Non-enzymatic antioxidants

2.9.1 Bilirubin and vitamin E-Se

Bilirubin is a bile pigment formed as a result of the catabolism of haemoglobin from aged red blood cells in animals. Bilirubin is transported to the liver as a complex with albumin where it is normally conjugated and excreted into the bile. It serves as a chain breaking antioxidant. Its antioxidant activity has the ability to scavenge free radicals such as the superoxide anion and peroxide radical (Farrera *et al.*, 1994). Approximately 400 µg/kg of Se in the diet has shown to increase the function of the glutathione peroxidase, glutathione reductase and the red blood cell catalase (Rao *et al.*, 2013). Whereas, the values >0.5 mg/L of vitamin E is considered to be adequate (Menzies *et al.*, 2004). Therefore, a dietary supplement of vitamin E-Se is significant to optimize the non-enzymatic and enzymatic antioxidants. Apart from a supplementation of vitamins and minerals, the naturally occurring antioxidants (such as sea Buckthorn, grape seed extract, Moringa leaves, *Lippia javanica*, red clover) can also be used as substitutes in scavenging ROS and reducing lipid peroxides. Oyedemi *et al.* (2010) states that Moringa leaves increase the levels of GSH that aids in the protection of mammalian cells via the maintenance of a thiol-redox status and the detoxification of exogenous and endogenous reactive molecules.

2.9.2 Cortisol and Ferritin

Cortisol is a stress inhibitor particularly when the stressor evokes a negative effect or a social threat (Dickerson & Kemeny, 2004); inducing an increase in the cortisol secretion, which then mobilizes the metabolic energy to cope with stressors. Blood glucocorticoid (GC) concentrations have been used as an indicator of stress in many studies (e.g. Boonstra *et al.*, 1998; Hopster *et al.*, 2002; Hackländer *et al.*, 2003; Romero & Reed, 2005; Kitaysky *et al.*, 2007

Sheriff *et al.*, 2009a). Intracellular ferritin is a means of storing iron safely (Balla *et al.*, 1992) and its synthesis is increased as a result of oxidative stress (Tsuji *et al.*, 2000; Orino *et al.*, 2001; Hailemariam *et al.*, 2010; Huang *et al.*, 2013). Serum ferritin originates from the cell leakage as a result of the cellular damage (Theil, 2013). The increase in serum ferritin indicates a reduction in stored body iron and is regarded as one of the oxidative stress markers.

2.10 Enzymatic antioxidants

2.10.1 Superoxide dismutase

Superoxide dismutase (SOD) enzymes catalyse the superoxide radicals by adding or removing an electron from the superoxide molecules it encounters, thus changing the O_2^- into one of the two less damaging species (oxygen (O_2) and hydrogen peroxide (H_2O_2)) (Halliwell & Chirico, 1993). There are three different forms of SODs, the copper zinc superoxide dismutase (CuZn-SOD), the manganese superoxide dismutase (MnSOD) and the extracellular superoxide dismutase (ECSOD) (Fridovich, 1997)). The CuZn-SOD is found in the cytoplasm and other cell organelles, weighing approximately 32000 kilodaltons (kDa), with two subunit proteins, each containing the catalytically active copper and zinc atoms. The MnSOD is found in the mitochondria of most cells, weighing 40000 kDa. It is believed to contain four protein subunits each with an individual manganese atom (Fridovich, 1997). Karlsson *et al.* (1993) reported that ECSOD is the major SOD detectable in extracellular fluids and is released into circulation from the surface of the vascular endothelium following an injection of heparin. Superoxide dismutase acts both as an antioxidant and anti-inflammatory. It is also considered the most significant enzymatic antioxidant because of its ability to mitigate ROS and radical chain reactions. It functions by repairing cells and retarding the damage by converting superoxide radicals to more stable

products (Oyedemi *et al.*, 2010) through the help of a catalase or glutathione peroxidase reaction (Curtis & Mortiz, 1972).

2.10.2 Glutathione peroxidases GSH-px

Glutathione peroxidases (GPx) prevent the oxidation of glutathione by catalysing the conversion of lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water (Ursini *et al.*, 1995). The highest concentration of GPx is found in the liver and is mainly synthesized in the kidney. It is widely distributed in almost all tissues and sub-distributed in the cytosol and mitochondria (Rotruck *et al.*, 1973). Pavlata *et al.* (2001) proved a high correlation between Se content in the whole blood and GSH-Px activity in the whole blood ($r = 0.93$). In ewes with an intake of 100 μg Se/kg dietary DM, Olivera *et al.* (2004) reported the activity of GSH-Px to be in the range of 194.38 to 236.51 $\mu\text{kat/l}$ of blood while a simultaneous content of selenium in the blood plasma ranged from 83.44 to 183.90 $\mu\text{g/L}$.

Glutathione (GSH) is a major cellular antioxidant responsible for protecting protein sulphydryl groups from oxidation by serving as a biological redox reagent. It is also referred to as a master antioxidant because it does more than fighting free radicals; it also has the ability to regenerate other antioxidants such as vitamin E and C and participates in the detoxification of xenobiotics that cause cellular injury by generating free radicals (Meister *et al.*, 1988; Beutler *et al.*, 1989; Shi *et al.*, 1994). Blood plasma serves as a transport medium for glutathione from liver to the other various body tissues that are consumers of glutathione and cysteine (Abbott & Meister, 1986).

2.10.3 Protein thiol

Proteins are one of the major cellular constituents, targeted by ROS (Davies, 2005). The problem arises when proteins are oxidized as the oxidation may affect almost all the important functions of the cell. The oxidation is irreversible and when cells experience an excessive oxidation of their proteins, this is often used as a crucial indicator of oxidative stress (Møller *et al.*, 2007). They are different types of protein modifications; of importance being the oxidation of a protein thiol, which may be caused by the various environmentally induced oxidative stresses (Braconi *et al.*, 2011). On the other hand, a thiol antioxidant acts through a variety of mechanisms. It acts as a component of the general thiol/disulphide redox buffer, a metal chelator, a radical quencher, a substrate for specific redox reactions (GSH) and as a specific reductant of individual protein disulfate bonds (thioredoxin) (Koolman & Roehm, 2005).

2.11 Growth performance and stress

Growth performance is controlled genetically and environmentally. The environmental factors that can influence average daily gain are the availability of nutrients, space allocation, hormones, enzymes, and elevated ambient temperatures (Hafez, 1987; Habeeb *et al.*, 1992). Environmental factors should be controlled such that the animals might not be exposed to high ambient temperatures that increase respiration rate, body temperature and consumption of water and a decline in feed intake (results in weight loss) (Marai *et al.*, 2001). Hence, an appropriate rearing system should be carefully selected to optimized performance.

2.12 Blood parameters and stress

2.12.1 Red blood cells

Red blood cells (RBCs) or erythrocytes are anucleate, biconcave, discoid cells filled with a reddish protein called hemoglobin (HGB), which transports oxygen from the lungs to the cells and carbon dioxide from the cells back to the lungs. It is through this protein that the blood appears red. An erythrocyte measures about 7.61μ in thickness and about 8.8μ in diameter (Kerr, 2002) making up 30-40% of the formed element of the blood (Seeley *et al.*, 1996). The biconcave shape of RBCs enables them to squeeze through the smallest blood vessels (capillaries), whereby they readily exchange O_2 and CO_2 because they have flexible membranes for successful distribution (Percy & Lappin, 2008; Bull & Herman, 2010). All the required array of enzymes, proteins, carbohydrates, lipids, anions and cations are balanced by matured RBCs for effective metabolism and functioning of the cells.

When an animal experiences oxidative stress, this causes an imbalance between the erythrocyte's components, and with the erythrocytes having a poor ability to cope with oxidative stress, this can result in a degenerative change in the hemoglobin, cell membrane and enzyme levels required for the normal erythrocyte function (Nagababu & Rifkind, 1998). Hemoglobin oxidation triggers the formation of several intermediates: methemoglobin (MetHb), reversible hemichromes (rHCRs), irreversible hemichromes (iHCRs) and hemin (Liu *et al.*, 1988; Jarolim *et al.*, 1990). Erythrocytes have three basic parameters (RBC count, HGB, and HCT) that are used to compute the RBC indices; mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). The MCV reflects the RBC diameter on a Wright-stained blood film. The MCHC, expressed in g/dL, reflects the RBC staining intensity and the amount of central pallor. The MCH in picograms (pg) expresses the mass of hemoglobin

and parallels the MCHC. Hematocrit, also called the packed cell volume (PCV), and the packed cells referring to RBCs, is another parameter used to measure the ratio of the volume of the packed RBCs to the volume of the whole blood (Perkins, 2009).

2.12.2 White blood cells

Leukocytes (white blood cells) and thrombocytes (platelets) make up the remaining 5% the formed element blood (Secley *et al.*, 1996). Leukocytes (WBCs) are a loosely related category of cell types dedicated to fighting diseases and removing harmful substances from the body. They are transported in the blood from their source, usually bone marrow or the lymphoid tissue; to where they are required or body cavity destination (Ravandi & Hoffman, 2005). An increased number of leucocytes indicate that the body is fighting an infection. White blood cells may be counted visually using a microscope and a hemacytometer. Leucocyte counts are about 5,000 - 10,000 per microliter. A decreased WBC count (fewer than 4500/mL) is called leukopenia, and an increased WBC count (more than 11,500/mL) is called leucocytosis. There are two types of leucocytes namely, granulocytes (neutrophils, eosinophils and basophils) and agranulocytes (monocytes and lymphocytes). The agranulocytes and agranulocytes are produced in the bone marrow except from the lymphocyte, which develops from the lymph nodes (Kerr, 2002; Scanlon & Sanders, 2007). The lymphocyte is the first line of defense. An increased number of lymphocytes are an indication of stress or stress response (Tomquist & Rigas, 2010). Neutrophils are cells that play an important role in immune responses (Ayub & Hallett, 2004).

2.13 Meat quality and stress

Consumers demand good quality meat and for any food product to be successful, it largely depends on the perception of quality (Cardello, 1995; Acebron & Dopico, 2000; Napolitano *et*

al., 2007). Therefore, meat appearance is important for a consumer who is to make a purchase decision (Faustman & Cassens, 1990). Colour is one of the most influential factors in helping the consumer purchase the meat (Faustman & Cassens, 1990). ‘Red’ or ‘bright red’ is a guide to freshness of the raw product and any brownish colour is an indicator of the meat being stale or spoiled (Forbes *et al.*, 1974). Stress factors such as lack of feed, excessive or insufficient exercise, sudden changes in temperature, management practices, stress during transportation (e.g. to the abattoir), regrouping of the animals, inhumane treatment, space allocation, diet, pollution, sunlight etc., which trigger the production of ROM, leading to browning in meat (dark cutting) (Rams bottom *et al.*, 1949; Lawrie, 1958; Ashmore *et al.*, 1973). Flavour, texture, and colour of displayed meat are strongly affected by the oxidative processes, which cause the degradation of meat lipids and proteins (Ripoll *et al.*, 2013). When this process of oxidation is prolonged, meat colour changes from red to brown due to metmyoglobin formation (Renner, 1990). Lipid oxidation and microbial growth are related to the oxidation process that determines meat quality loss and shelf-life reduction (Fernández-López *et al.*, 2005).

2.14 Summary

The modern red meat consumer considers meat quality as the most important characteristic but also demands healthier products that are environmentally friendly, promote sustainability and comply with animal welfare guidelines. Livestock production systems are being intensified to improve efficiency and productivity. It is crucial to provide the consumer with a product that is consistent in quality in order to increase the consumer’s confidence in the product and encourage repurchasing, therefore it is essential to investigate the effect of dietary vitamin E-selenium on growth performance, blood parameters and meat quality, to ensure the quality is maintained and

consumer expectations are met. The review of literature reveals a gap in terms of linking suboptimal welfare to production and meat quality parameters in South African sheep.

2.15 References

- Abbott, W. A & Meister, A., 1986. intrahepatic transport and utilization of biliary glutathione and its metabolites. *Proc. Natl. Acade. Sci. U.S.A.* 83, 1246-1250.
- Acebron, L. B & Dopico, D. C., 2000. The importance of intrinsic and extrinsic cues to expected and experienced quality: an empirical application for beef. *Food Quality and Preference*, 11, 229-238.
- Adenkola, A.Y & Ayo, J.O., 2010. Physiological and behavioural responses of livestock to road transportation stress: A review. *Afri. J. Biotech.* 9, 4845-4856.
- Agarwal, A., Gupta, S & Sharma, R., 2005. Role of oxidative stress in female reproduction. *Reprod. Biol. Endocrin.* 28, 23 - 29.
- AGRICULTURAL STATISTICS., 2008. Directorate: Agricultural Statistics of the National Department of Agriculture, Private Bag X144, Pretoria, 0001.
- Appleby, M.C., Mench, J.A., Olsson, I.A.S & Hughes, B.O. (Eds.), 2011. *Animal Welfare*, Second Ed. CABI, Wallingford, England, UK, 336.
- Arther, R., 2000. *Compositions for enhanced acaricidal activity*. U.S. Patent Application 09, 727,117.
- Ashmore, C. R., Addis, P.B., Doerr, L & Stokes, H., 1973. Development of muscle fibers in the complexus muscle of normal and dystrophic chicks. *J. Histochem, Cytochem.* 21, 266.
- Ayub, K., & Hallett, M.B., 2004a. Ca^{2+} influx shutdown during neutrophil apoptosis: importance and possible mechanism. *Immun.* 111, 8–12.
- Baldi, A., 2005. Vitamin E in dairy cows. *Livest. Prod. Sci.* 98, 117–122.

- Balla, G., Jacob, H.S, Balla, J., Rosenberg, M & Nath K., 1992. Ferritin: a cytoprotective antioxidant strategem of endothelium. *J Biol Chem.* 267, 18148–18153.
- Baltić, M.Ž., Starčević, M.D., Bašić, M., Zenunović, A., Ivanović, J., Marković, R., Janjić, J. and Mahmutović, H., 2015. Effects of selenium yeast level in diet on carcass and meat quality, tissue selenium distribution and glutathione peroxidase activity in ducks. *Ani. Feed. Sci. Techno.* 210, 225-233.
- Bandura, A., 1977. Self-efficacy: toward a unifying theory of behavioral change. *Psych. rev.* 84, 191.
- Berge, E., 1997. Housing of sheep in cold climate. *Livest. Prod. Sci.* 49, 139–149.
- Bernabucci, U., 2012. Chronic heat stress up-regulates leptin and adiponectin secretion and expression and improves leptin, adiponectin and insulin sensitivity in mice. *J. molecular endocrinology*, 48, 129-138.
- Bickell, N.A., Weidmann, J., Fei, K., Lin, J.J. & Leventhal, H., 2009. Underuse of breast cancer adjuvant treatment: patient knowledge, beliefs, and medical mistrust. *J. Clin. Oncol.* 27, 5160-5167.
- BKB. (2010). The history of wool and mohair in South Africa: Dohne Merino.
- Boonstra, M.J., Tjeerdsma B.F & Groeneveld H.A.C., 1998. Thermal modification of non-durable wood species. Part 1, The Plato technology: thermal modification of wood. *Intl. Res. Grp. Wood. Pres. Doc no. IRG/WP 98-40123*

- Bouissou, MF., Boissy, A., Le Neindre, P & Veissier, I., 2001. The social behaviour of cattle. In: Keeling L, Gonyou H, editors. Social behaviour in farm animals. Wallingford, UK: CABI Publishing; 2001, 113–45.
- Braconi, D., Bernardini, G & Santucci A., 2011. Linking protein oxidation to environmental pollutants: redox proteomic approaches. *J. Proteomics*. 74, 2324–37.
- Branconi D., Amato L., Bernardini G., Arena S., Orlandini M., Scaloni A & Santucci A., 2011. Surfome analysis of a wild-type wine *Saccharomyces cerevisiae* strain. *Food. Microbiol.* 28, 1220-1230.
- Brosh, A., Aharoni, Y., Degen, A.A., Wright, A & Young, B.A., 1998. Effects of solar radiation, dietary energy and time of feeding on thermoregulatory responses and energy balance in cattle in a hot environment. *J. Anim. Sci.* 76, 2671–2677.
- Cardello, A. V. (1995). Food quality: relativity, context and consumer expectations. *Food Quality and Preference*, 6, 163-170.
- Casamassima, D., Sevi, A., Palazzo, M., Ramacciato, R., Colella, G.E. and Bellitti, A., 2001. Effects of two different housing systems on behavior, physiology and milk yield of Comisana ewes. *Small. Rum. Res.* 41, 151-161.
- Chrousos, G.P., 1998. Stressors, stress, and neuroendocrine integration of the adaptive response: The 1997 Hans Selye Memorial Lecture. *Ann. NY. Acad Sci.* 851, 311-335.
- CIWF. (2010). The Compassion in World Farming Group. [WWW document]. URL <http://www.ciwf.org.uk>. 16 March 2010.

- Clifford, A.A. 2001. Total nutrition Feeding Animals for Health and Growth, Nottingham University Press, Nottingham
- Collier, R.J., Dahl, G.E & Van Baale, M.J., 2006. Major advances associated with environmental effects on dairy cattle. *J. Dairy Sci.* 89, 1244-1253.
- Conte, R. & Paolucci, M., 2001. Intelligent social learning. *J. Art. Soc. Soc. Sim.* 4, U61-U82.
- Curtis, J.J., & Mortiz, M., 1972. Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. *Gastroenterology*. 62, 84–92.
- Curtis, S.E., 1983. Environmental Management in Animal Agriculture. Iowa State Press, Ames.
- Dahl, G.E., Tao, S & Thompson I.M., 2012. Effects of photoperiod on mammary gland development and lactation. *J. Anim. Sci.* 90, 755-760.
- Daun, C & Åkesson, B., 2004. Comparison of glutathione peroxidase activity, and of total and soluble selenium content in two muscles from chicken, turkey, duck, ostrich and lamb. *Food. Chem.* 85, 295-303.
- Davies, M.J., 2005. The oxidative environment and protein damage. *Biochimica. Biophysica. Acta.* 1703, 93 – 109.
- De Vink, R., Binder, K., Horbach, J & De Virgiliis, A., 2008. Confinement effects on phase behavior of soft matter systems. *Soft Matter*, 4, 1555-1568.
- Dean, W.F. & Combs Jr, G.F., 1981. Influence of dietary selenium on performance, tissue selenium content, and plasma concentrations of selenium-dependent glutathione peroxidase, vitamin E, and ascorbic acid in ducklings. *Poultry. Sci.* 60, 2655-2663.

- Dickerson, S. S & Kemeny, M. E., 2004. Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychl. Bulletin.* 130, 355-391.
- Dickson, I.A & Stephenson, D.E., 1979. The housing of ewes. West of Scotland Agricultural College, Auchincruive, Scotland, Technical Note no. 63.
- Dickson-Hoyle, S. & Reenberg, A. 2009. The shrinking globe: Globalisation of food system and the changing geographies of livestock production. *Danish. J. Geo.* 109, 105- 112.
- Dove, H., Beilharz, R.G & Black, J.L., 1974. Dominance patterns and positional behaviour of sheep in yards. *Ani. Sci.* 19, 157-168.
- Dröge, W., 2002. Free radicals in the physiological control of cell function. *Physiological Reviews* 82: 47 - 95.
- Ebeid, E.N., El-khodary, N.M., El-haggar, S.M & Eid, M.A., 2012. Study of the pharmacokinetic and pharmacogenetic contribution to the toxicity of high-dose methotrexate in children with acute lymphoblastic leukemia. *Med. Oncol.* 29, 2053-2062.
- Ekiz, B., Yilmaz, A., Ozcan, M., Kaptan, C., Hanoglu, H., Yildirim, M. & Kocak, O., 2010. Carcass quality characteristics of Hair Goat and Saanen× Hair Goat crossbred kids from intensive production system. *J. Ani. Feed. Sci.* 19, 368-378.
- Estévez, Z.C., Betancourt, A.A., Muzio González, V., Baile, NF & Silva, C.V., 2007. Immunogenicity and safety assessment of the Cuban recombinant hepatitis B vaccine in healthy adults. *Biologicals.* 35(2): 115–122.
- Fagen, R., 1981. *Animal Play Behaviour.* Oxford University Press, New York, 3–69.

- FAO, (2008). FAO Food Outlook. [WWW document]. URL <http://www.fao.org/docrep/011/ai474e/ai474e09.htm>. 02 April 2010.
- FAO, (2013). Food and Agriculture Organization of the United Nations. **FAOSTAT database**. URL. <http://faostat3.fao.org/browse/Q/QA/E>
- FAO. (2002). Some Issues associated with livestock industries of the Asia-pacific region. Regional Office for Asia and the Pacific Publication 2002/06. Bangkok, FAO RAP & Animal Production & Health Commission for Asia and the Pacific.
- FAO. (2009). Livestock's Long Shadow: Environmental Issues and Options. [WWW document]. URL <http://www.fao.org/ag/magazine/0612sp1.htm>. 02 April 2010.
- Faustman, C & Cassens, R.G., 1990. The biochemical basis for discoloration in fresh meat: a review. *J. foods*. 10.1745-4573.
- Ferguson, D.M. & Warner, R.D., 2008. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants. *Meat. Sci.* 80, 12-19.
- Fernández-López, J., Zhi, N., Aleson-Carbonell, L., Pérez-Alvarez, J. A & Kuri, V., 2005. Antioxidant and antibacterial activities of natural extracts: Application in beef meatballs. *Meat. Sci.* 69, 371–380.
- Fisher, A & Matthews, L., 2001. The social behaviour of sheep. In: Keeling L, Gonyou H, editors. *Social behaviour in farm animals*. Wallingford, UK: CABI Publishing; 211–45.
- Fitzpatrick, J., Scott, M & Nolan, A., 2006. Assessment of pain and welfare in sheep. *Small. Rumin. Res.* 62, 55–61.

- Forbes, E.A., Posner, A.M & Quirk, J.P., 1974. The specific adsorption of organic Hg (II) species and Co(III) complex ions on goethite. *J. Colloid. Interface. Sci.* 49, 403 – 409
- Forbes, S. M. C., Vaisey, M., Diamant, R & Cliplef, R., 1974. Relationships between consumer criteria for choosing beef and beef quality. *Canadian. Inst. Food. Sci. Techno. J.* 7, 130–135
- Franson, N., Chiang, C.Y., Aroh, K., Satsangi, V.R., Dass, S & Ehrman, S., 2011. Copper oxide nanoparticle made by flame spray pyrolysis for photoelectrochemical water splitting–Part II. Photoelectrochemical study. *Int. J. Hydrogen Energy*, 36(24), 15519-15526.
- Fraser, D., 2008. *Understanding Animal Welfare: The Science in its Cultural Context*. Wiley-Blackwell, Oxford, England, UK, 324
- Fraser, G.L., Barr, J., Puntillo, K., Ely, E.W., Gélinas, C., Dasta, J.F., Davidson, J.E., Devlin, J.W., Kress, J.P., Joffe, A.M. & Coursin, D.B., 2013. Clinical practice guidelines for the management of pain, agitation, and delirium in adult patients in the intensive care unit. *Critical Care Medicine* 41, 263-306.
- Fridovich, I., 1997. Superoxide anion radical (O₂⁻), superoxide dismutases, and related matters. *J. Biol. Chem.* 272, 18515-7.
- Gaughan, J.P., Katari, S., Turan, N., Bibikova, M., Erinle, O., Chalian, R., Foster, M., Coutifaris, C. and Sapienza, C., 2009. DNA methylation and gene expression differences in children conceived in vitro or in vivo. *Human Mol. Gen.* 18, 3769-3778.

- Gonyou, H.W., Vanderwert, W., Berger, L.L., McKeith, F.K., Baker, A.M & Bechtel, P.J., 1985. Influence of zeranol implants on growth, behavior and carcass traits in Angus and Limousin bulls and steers. *J. Ani. Sci.* 61, 310-319.
- Griffin, W.S., Stanley, L.C., Ling, C.H.E.N., White, L., MacLeod, V., Perrot, L.J., White, C. & Araoz, C., 1989. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proceedings of the National Academy of Sciences*, 86, 7611-7615.
- Guesdon, A., Ciuffa, R., Lamark, T., Tarafder, A.K., Rybina, S., Hagen, W.J., Johansen, T. & Sachse, C., 2015. The selective autophagy receptor p62 forms a flexible filamentous helical scaffold. *Cell Reports*, 11, 748-758.
- Habeeb, A.A., Marai, I.F.M & Kamal, T.H., 1992. Heat stress. In *Farm Animals and the Environment*, edited by C. Philips and D. Piggins. C.A.B. International, U.K., 27-47.
- Hafez, E.S.E., 1987. *Transport and survival of gametes*. In: *Reproduction in farm animals* (ed. E. S. E. Hafez). pp. 168-188.
- Halliwell, B & Chirico, S., 1993. Lipid peroxidation: its mechanism, measurement, and significance. Am. J. Clin. Nutr. 57, 715S-724S
- Halliwell, B., 1996. Antioxidants in human health and disease, *Ann. Rev. Nutr.* 16: 33–50.
- Hartung, J., 1994. Environment and animal health. In: Wathes, C.M., Charles, D.R. (Eds.), *Livestock Housing*. CAB International, Wallingford, 25–48.

- Heinz, G.H., 1996. Selenium in birds. *Environmental contaminants in wildlife: interpreting tissue concentrations*. Lewis Boca Raton. 9, 447-458.
- Hemsworth, P.H. & Coleman, G.J., 1998. Predicting stockperson behaviour towards pigs from attitudinal and job-related variables and empathy. *App. Ani. Beh. Sci.* 58, 63-75.
- Hoffman, D.J. & Heinz, G.H., 1998. Effects of mercury and selenium on glutathione metabolism and oxidative stress in mallard ducks. *Environ. Toxic Chem.* 17, 161-166.
- http://www.bkb.co.za/content.asp?PageID=27&_History%20of%20Wool%20&%20Mohair. 16 March 2010.
- Huang, Z., Chen, K., Zhang, J., Li, Y., Wang, H., Cui, D., Tang, J., Liu, Y., Shi, X., Li, W., Liu, D., Chen, R., Sugang, R.S & Pan, X., 2013. A functional variomics tool for discovering drug-resistance genes and drug targets. *Cell. Rep.* 3, 577-85.
- Hughes, B.O., & Duncan, I.J.H., 1988. The notion of ethological 'need', models of motivation and animal welfare. *Anim. Behav.* 36, 1696-1707.
- Jarolim, P., Lahav, M., Liu, S.C & Palek, J., 1990. Effect of hemoglobin oxidation products on the stability of red cell membrane skeletons and the associations of skeletal proteins: correlation with a release of hemin. *Blood* 76, 2125-31.
- Kao, S.Y., Yang, M.H., Hsu, D.S.S., Wang, H.W., Wang, H.J., Lan, H.Y., Yang, W.H., Huang, C.H., Tzeng, C.H., Tai, S.K. & Chang, S.Y., 2010. Bmi1 is essential in Twist1-induced epithelial-mesenchymal transition. *Nat. Cel. Bio.* 12, 982.

- Kavaliers, M., Colwell, D.D & Choleris, E., 2003. Learning to fear and cope with a natural stressor: individually and socially acquired corticosterone and avoidance responses to biting flies. *Horm. Behav.* 43, 99–107.
- Keeling, L.J & Hurnik, F.J., 1996. Social facilitation acts more on the appetitive than the consummatory phase of feeding behaviour in domestic fowl. *Ani. Bev.* 52, 11-15.
- Kerr, M. G., 2002. *Veterinary laboratory medicine (Clinical biochemical and haematology)*. 2nd edition. Blackwell science Ltd. Oxford, London. 386.
- Koolman, J & Röhm, K., 2005. *Color Atlas of Biochemistry Medical*. 467
- Kotze, J.J.J., 1951. The development of a mutton woolled sheep for the sour - grass veld area. *Farming in South Africa* 26, 110-113.
- Lacetera, N., 2012. Heuristic thinking and limited attention in the car market. *Amer. Econ. Rev.* 102, 2206-2236.
- Lambert, M.G., Knight, T.W., Cosgrove, G.P., Anderson, C.B., Death, A.F & Fisher, E.F., 1998. Exercise effects on muscle glycogen concentration in beef cattle. *NZ. Soc. Ani. Prod.* 60, 243-244
- Launchbaugh, K.L & Howery, L.D., 2005. Understanding landscape use patterns of livestock as a consequence of foraging behavior. *Rangeland. Ecol. Manag.* 58, 99-108.
- Lawrie, R. A., 1958. Physiological stress in relation to dark-cutting beef. *J. Sci. Food. Agric.* 9DOI: 10.1002/jsfa.2740091106

- Lii, K.H., Huang, Y.F., Zima, V., Huang, C.Y., Lin, H.M., Jiang, Y.C., Liao, F.L. and Wang, S.L., 1998. Syntheses and structures of organically templated iron phosphates. *Chem. Mat.* 10. 2599-2609.
- Liu, S.C., Zhai, S & Palek, J., 1988. Detection of heme release during hemoglobin S denaturation. *Blood* 71,1755.
- Loynes, I.J., 1983. Sheep house design. In: *Housing Sheep*. FarmBuilding Information Centre, Stoneleigh. 4–9.
- Lynch, J.J., Hinch, G.N & Adams, D.B., 1985. The Behaviour of Sheep. In: *Biological Principles and Implications for Production*. CAB International, Wallingford.
- Marai, I. F. M., Ayyat, M. S & El-Monem, U. M., 2001. Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation, under Egyptian conditions. *Trop. Anim. Health. Prod.*, 33, 457–462.
- McMaster, J.C., Kotze, J.J. & vander van der., 1991. Dohne Merino. Dohne Merino Breed Society of South Africa, Souvenir Brochure, 25th Annual Council Report, 2-4.
- Meister, B., Hökfelt, T., Steinbusch, H. W. M., Skagerberg, G., Lindvall, O., Geffard, M., Joh, T. H., Cuello, A. C & Goldstein, M., 1988. Do tyrosine hydroxylase-immunoreactive neurons in the ventrolateral arcuate nucleus produce dopamine or only L-DOPA? *J. Chem. Neuroanat.* 1, 59–64.
- Mellor, D.J., Patterson-Kane, E & Stafford, K.J., 2009. *The Sciences of Animal Welfare*. Wiley-Blackwell, Oxford, England, UK, 198.

- Menzies, D., Dial, S., Alrasadi, K., Manoukian, C & Huang, A., 2004. Risk of *Clostridium difficile* diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. *Canadian. Med. Assoc. J.* 171, 33-38.
- Miller, J.K., Brzezinska-Slebodzinska, E & Madsen, F.C., 1993. Oxidative stress, antioxidants, and animal function. *J. Dairy. Sci.* 76, 2812 - 2823.
- Miranda-De la Lama, G.C., Rivero, L., Chacón, G., Garcia-Belenguer, S., Villarroel, M. and María, G.A., 2010. Effect of the pre-slaughter logistic chain on some indicators of welfare in lambs. *Livest. Sci.* 128, 52-59.
- Mitchell, M.A & Kettlewell, P.J., 1998. Physiological stress and welfare of broiler chickens in transit: solutions not problems. *Poultry Sci*, 77, 1803-1814.
- Møller , I.M., Jensen, P.E & Hansson, A., 2007. Oxidative modifications to cellular components in plants. *Annu. Rev. Plant. Biol.* 58, 459–81.
- Nagababu, E & Rifkind, J.M., 1998. Formation of fluorescent heme degradation products during the oxidation of hemoglobin by hydrogen peroxide. Biochem. Biophys. Res .Commun. 29, 592-6.
- Napolitano, F., Braghieri, A., Caroprese, M., Marino, R., Girolami, A. & Sevi, A., 2007. Effect of information about animal welfare, expressed in terms of rearing conditions, on lamb acceptability. *Meat. Sci.* 77, 431-436.
- Negre-Salvayre, A., Coatrieux, C., Ingueneau, C & Salvayre, R., 2008. Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *Br. J. Pharmacol.* 153, 6-20.

- Newberry, R.C., Wood-Gush, D.G.M & Hall, J.W., 1988. Playful behaviour of piglets. *Behav. Process.* 17, 205–216.
- Nierenberg, D. (2005). *Happier meals: Rethinking the global meat industry*. Washington, D. C., World Watch Institute.
- Ohlendorf, H.M., 2003. Ecotoxicology of selenium. *Handbook of Ecotoxicology* 2, 465-500.
- OIE., 2012. Introduction to the recommendations for animal welfare. In: *Terrestrial Animal Health Code*, 21st Ed. World Organisation for Animal Health (OIE), Paris, France, Article 7.1.4.
- Olivera, P., Jovanovic, B., Gvozdic, D & Stojic, V., 2004. Selenium status of sheep and their lambs in the Northern Serbian Province of Vojvodina. *Acta Vet.-Beograd*, 54, 403–409.
- Onyema, O.O., Farombi, E.O., Emerole, G.O & Ukoha, A.I., 2006. Effect of Vitamin E on monosodium glutamate induced hepatotoxicity and oxidative stress in rats. *Ind. J. Biochem. Biophys.* 43: 20-24.
- Orino, K., Lehman, L., Tsuji, Y., Ayaki, H., Torti, S.V & Torti, F.M., 2001 Ferritin and the response to oxidative stress. *Biochem. J.* 357, 241-7.
- Oyedemi, S.O., Bradley, G & Afolayan, A.J., 2010. In vivo and in vitro antioxidant activities of aqueous stem bark extract of *Strychnos henningsii* (Gilg). *Afri. J. Pharm. Pharmacol*, 4, 70–78.
- Pavlatá, L., Pechová, A., Bečvář, O., Illek, J., 2001. Selenium status in cattle at slaughter: analyses of blood, skeletal muscle, and liver. *Acta Vet. Brno.* 70, 277–284.

- Percy, M.J & Lappin, T.R., 2008. Recessive congenital methaemoglobinaemia: cytochrome b (5) reductase deficiency. Br. J. Haematol. 141, 298-308.
- Perkins, J.G., 2009. An Evaluation of the Impact of Apheresis Platelets Used in the Setting of Massively Transfused Trauma Patients. J. Trauma. Injury. Infection. .Critical Care 66(4), S77-S85.
- Petherick, J. C., & Phillips, C. J. C. 2009. Space allowances for confined livestock and their determination from allometric principles. App. Anim. Behav. Sci. 117, 1–12.
- Poli, G., Leonarduzzi, G., Biasi, F & Chiarpotto, E., 2004. Oxidative stress and cell signaling. Curr. Med. Chem. 11: 1163–1182.
- Ramsbottom, J. M & Strandine, E. J., 1949. Comparative tenderness and identification of muscles in wholesale beef cuts. Food. Res. 13, 315–330.
- Ravandi, F & Hoffiman, R., 2005. Phagocytes. In: Postgraduate haematology. 5th edition. Ed: Hoffbrand, A.V., Catovsky, D. Tuddenham, E.G.D. Blackwell pub. Oxford, UK. 291.
- Renner, M., 1990. Factors involved in the discoloration of beef meat: Intl. J. Food. Sci. Technol. 25, 613 – 630.
- Ripoll, G., González-Calvo, L., Molino, F., Calvo, J. H & Joy, M., 2013. Effects of finishing period length with vitamin E supplementation and alfalfa grazing on carcass color and the evolution of meat color and the lipid oxidation in light lambs. Meat. Sci. 93, 906–913.

- Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G & Hoekstra, W.G., 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Sci.* 179, 588-90.
- Scanlon, V. C & Sanders, T., 2007. *Essentials of anatomy and physiology*. 5th edition . F.A. Davis co. Philadelpina. 622.
- Schriner, S.E., Linford, N.J., Martin, G.M., Treuting, P., Ogburn, C.E., Emond, M., Coskun, P.E., Ladiges, W., Wolf, N., Van Remmen, H. & Wallace, D.C., 2005. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Sci.* 308, 1909-1911.
- Sevi, A., Casamassima, D., Pulina, G & Pazzona, A., 2009. Factors of welfare reduction in dairy sheep and goats. *Ital. J. Anim. Sci.* 8, 81-101.
- Sevi, A., Marino, R., Considine, T., McSweeney, P.L.H & Kelly, A.L., 2005. Contribution of proteolytic activity associated with somatic cells in milk to cheese ripening. *Int. Dairy J.* 15, 1026-1033.
- Sevi, A., Massa, S., Annicchiarico, G., Dell'aquila, S & Muscio, A., 1999. Effect of stocking density on ewe's milk yield, udder health and microenvironment. *J. Dairy Res.* 66, 489–499.
- Sevi, A., Taibi, L., Albenzio, M., Muscio, A., Dell'Aquila, S & Napolitano, F., 2001a. Behavioral, adrenal, immune, and productive responses of lactating ewes to regrouping and relocation. *J. Anim. Sci.* 79, 1457–1455.

- Shaw, F.D & Tume, R.K., 1992. The assessment of pre-slaughter and slaughter treatments of livestock by measurement of plasma constituents—a review of recent work. *Meat. Sci.* 32, 311-329.
- Shi, X., Lenhart, A & Mao, Y., 1994. ERS spin trapping investigation on peroxy-nitrate decomposition: No evidence for hydroxyl radical production. *Bio. Chem. Biophys. Res. Commun.* 203, 1515-1521.
- Singh, A.K., Szczech, L., Tang, K.L., Barnhart, H., Sapp, S., Wolfson, M. and Reddan, D., 2006. Correction of anemia with epoetin alfa in chronic kidney disease. *NE J. Med.* 355, 2085-2098.
- Skřivanová, E., Marounek, M., De Smet, S & Raes, K., 2007. Influence of dietary selenium and vitamin E on quality of veal. *Meat Sci.* 76, 495-500.
- South African Indigenous Breeds (SAIB). (2010). Dohne Merino. [WWW document]. URL <http://www.indigenusbreeds.co.za>. 05 March 2010.
- Spallholz, J.E. & Hoffman, D.J., 2002. Selenium toxicity: cause and effects in aquatic birds. *Aqua. Toxic.* 57, 27-37.
- Strain, J.J & Benzie, I.F.F., 1998. Antioxidant nutrients. *Special. PUBL-ROY. SOC. CHEM.* 215, 74-79.
- Surai, P.F & Dvorska, J.E., 2002. Effect of selenium and vitamin E content of the diet on lipid peroxidation in breast muscle tissue of broiler breeder hens during storage. *Proc. Ausi. Poult. Sci. Sym.* pp.14.

- Surai, P.F & Fisinin, V.I., 2014. Selenium in poultry breeder nutrition: An update. *Anim. Feed. Sci. Tech.* *191*, 1-15.
- Surai, P.F., 2002. *Natural antioxidants in avian nutrition and reproduction* (pp. 5-9). Nottingham: Nottingham. Univ. Press.
- Tarrant, P.V., 1990. Transportation of cattle by road. *App. Anim. Behav. Sci.* *28*, 153-170.
- Terlouw, C., 2005. Stress reactions at slaughter and meat quality in pigs: genetic background and prior experience. A brief review of recent findings. *Livst. Prod. Sci.* *94*, 125–135.
- Terlouw, E.M., Arnould, C., Auperin, B., Berri, C., Le Bihan-Duval, E., Deiss, V., Lefèvre, F., Lensink, B.J., Mounier, L. 2008. Pre-slaughter conditions, animal stress and welfare: Current status and possible future research. *Animal*. *2*(10), 1501-1517.
- Theil, E.C., 2013, Ferritin: The protein nanocage and iron biomineral in health and in disease. *Inorg. Chem.* *52*, 10-1021.
- Thomson, K.V., 1998. Self assessment in juvenile play. In: Bekoff, M., Byers, J.A. (Eds.), *Animal Play: Evolutionary, Comparative and Ecological Perspectives*. Cambridge University Press, Cambridge. 183–204.
- Tornquist, S. J & Rigas, J., 2010. Interpretation of ruminant leukocyte responses In: Schalm, s veterinary hematology. Ed: Weiss. D.J and Wardrop, K.J. 6th edition. A John Wiley and sons, Ltd.USA. 307-313

- Tsuji T, Miyazaki M, Sakaguchi M, Inoue Y, Namba M. (2000). A REIC gene shows down-regulation in human immortalized cells and human tumor-derived cell lines. *Biochem. Biophys. Res. Commun.* 268, 20-24.
- Ursini, F., Matorino, M., Brigelius-Flohe', R., Aumann, K.D., Roveri, A., Schomburg, D & Flohe', L., 1995. Diversity of glutathione peroxidase. *Methods Enzymol.* 252: 38–53
- Van Putten, G & Elshof, W.J., 1978. Observations on the effect of transport on the well being and lean quality of slaughter pigs. *Animal Regulation Studies* 1, 247-271.
- Webster, A.J.F., 1994. Comfort and injury. In: Wathes, C.M., Charles, D.R. (Eds.), *Livestock Housing*. CAB International, Wallingford, 49–68.
- Yavuz, T., Altuntas, I., Delibas, N., Yildirim, B., Candir, O., Cora, A., Karahan, N., Ibrisim, E & Kutsal, A., 2004. Cardiotoxicity in rats induced by methidathion and ameliorating effect of vitamins E and C. *Hum Exp. Toxicol.* 23:323–329.
- Yavuz, T., Altuntas, I., Delibas, N & Kutsal, A., 2004. Cardiotoxicity in rats induced by methidathion and ameliorating effect of vitamins E and C. *Human. Experim. toxico*, 23, 323-329.
- Zhu, H.; Luo, HL.; Meng, H.; Zhang, GJ.; Yan, LY & Yue, DB. (2010). Effect of Vitamin E supplement in diet on antioxidant ability of testis in Boer Goat. *Anim. Reprod. Sci*, 117: 90-94.

3 CHAPTER THREE - GROWTH AND PHYSIOLOGICAL PARAMETERS OF THE DOHNE MERINO SHEEP REARED UNDER SINGLE OR PAIRED-PEN HOUSING: EFFECT OF EXTRA DIETARY VITAMIN E-SELENIUM SUPPLEMENTATION

ABSTRACT

The objective of the study was to compare single and paired-pen housing systems through growth performance and stress-related physiological parameters when extra dietary vitamin E-selenium was supplemented. Twenty four lambs with an average body weight of 25.8 ± 2.7 kg were used. The lambs were randomly assigned to four treatment combinations: single-pen housing with no extra vitamin E-selenium supplementation, single-pen housing with extra vitamin E-selenium supplementation, paired-pen housing with no extra vitamin E-selenium supplementation, and paired-pen housing with extra vitamin E-selenium supplementation, in a completely randomized design. Results indicated that dietary Se and vitamin E did not have any significant ($P > 0.05$) effect on the weight gain and feed conversion ratio of lambs, but a linear and steady increase in body weight was observed throughout the experiment for all the treatments. Lambs supplemented with vitamin E-Se consumed more feed (65.01 kg) over the entire feeding period as compared to the lambs that were not offered the supplements (60.37 kg). Vitamin E-Se dietary supplementation did not ($P > 0.05$) influence the physiological parameters throughout the feeding period (week 1 – week 8). However, a significant ($P < 0.05$) antioxidant supplement and housing condition interaction effect on rectal temperature was observed at week 8. Animals housed in pairs had higher rectal temperatures (39.3°C) compared to those housed individually (38.8°C). However, for the non-supplemented group, no differences ($P > 0.05$) were observed between the rectal temperatures of both the paired (39.3°C) and individually (39.0°C) housed sheep. It can be concluded that dietary vitamin E and selenium did not have an effect on

the weight gain and FCR except that it increased the feed intake in the supplemented group when compared to the control group. An interaction was observed on the rectal temperature on the last day of the experiment.

Keywords: vitamin E, selenium, performance, , sheep, housing effect.

3.1 Introduction

Sheep are reared in intensive farming systems for faster growth and to obtain a desired slaughter weight in the shortest possible time. This production system tends to induce stress in animals due to the novelty of the environment, social separation, the mixing of unfamiliar animals, confinement, or handling (Terlouw *et al.*, 2008). Such stress factors may have a major impact on the animal's immune responses (Bonnette *et al.*, 1990; Niekamp *et al.*, 2007), which may result in the development of diseases (Nash *et al.*, 1997; Brodgen *et al.*, 1998). Finishing lambs are mostly subjected to these factors because farmers often finish lambs in intensive feeding system where space allocation is less than recommended. Confinement is a type of environmental concern, which hinders growth performance, production and welfare of the animals by being too barren, inducing boredom, and by being too restrictive in terms of normal animal behaviours (Fraser *et al.*, 2013). In sheep rearing, isolation from conspecifics either to single or group pens, even for short periods of time, is known to be one of the most stressful and fear eliciting situations, inducing significant behavioural and physiological agitations (Vandenheede *et al.*, 1998; Viérin & Bouissou, 2003; Boissy *et al.*, 2005, Dodd *et al.*, 2012). Animals that are in groups are often competitive in terms of the limited resources such as feeding space, drinker

space, lying space and shade such that this may have implications for the performance and welfare of the intensively-finished lambs (Lindberg, 2001; Bøe *et al.*, 2006; Jongman *et al.*, 2008; Rice *et al.*, 2016). Animals housed in single-pens express more stereotypic behaviours compared to animals housed in a group (Done-Currie *et al.*, 1984; Fitzpatrick *et al.*, 2006; Guesdon *et al.*, 2015).

Vitamin E has been shown to play a role in growth, immune function enhancement, tissue integrity, reproduction, disease prevention, and as a biological antioxidant (Rooke *et al.*, 2004; Dalle Zotte & Szendrő, 2011). Chauhan *et al.* (2016) postulated that a supplementation of lambs with levels of vitamin E-Se during the finishing period improves ADG while the physiological responses and oxidative status are normalized. There is, therefore, a possibility that supplementing sheep with vitamin E and selenium may mitigate against stress factors that may negatively affect growth performance and meat quality. In this context, we hypothesized that growth performance and stress-related physiological parameters would be more negative in a single-pen housing condition but less negative in a social interaction condition when extra dietary vitamin E-selenium is supplemented. The present study thus investigated the influence of single- or paired-pen housing systems on growth performance and stress-related physiological parameters when extra dietary vitamin E-selenium is supplemented.

3.2 Materials and methods

3.2.1 Study site

The study was carried out at the North-West University farm animal experimental unit in Mafikeng (Molelwane), South Africa from September to December 2016. The farm is situated between latitude 25° S and longitude 25° E. The ambient temperatures in this area range from 25

to 37°C during summer and 10 to 20°C during winter. The area receives less than 300 mm of rainfall per annum. It has a uniform terrain with an altitude of 1400 metres above sea level. During the study, temperatures ranged from 30 to 40°C with chances of rainfall.

3.2.2 *Management of experimental animals*

A total of 24 wether Dohne Merino lambs aged between 9 to 12 weeks, with an average weight of 25.8 ± 2.7 kg, were purchased from a farmer around Bloemfontein. The animals were administered oxytetracycline LA (1 mL/10 kg) and Ivermectin LA (1 mL/50 kg) sub-cutaneously for prophylactic intervention. All experimental and animal management procedures were approved by the University Ethics Committee, North-West University, South Africa (ethical clearance: NWU-00100-14-S9). The welfare of the lambs was monitored by the National Society for the Prevention of Cruelty to Animals (NSPCA) in South Africa. The lambs were subjected to two types of housing. In the first group, a single animal was allocated to a 1.4 m² (Sevi *et al.*, 2009) pen with no view/sight of any other animal and thus no social interaction or contact with other animals (single-pen housing). In the second group (paired-pen housing), a pair of lambs were allocated to a 2.8 m² (Sevi *et al.*, 2009) thus allowing social interaction between the two animals. All the pens were housed in a shed with concrete floor and corrugated iron sheets. Paired lambs were separated by removable barrier only during feeding to facilitate individual feeding.

3.2.3 *Housing and dietary treatments*

Lambs were fed a finishing commercial ration (Nutri.Feeds, Lichtenburg) twice daily at 0800h and in the afternoon at 1400h at a rate of 4% of body weight. The diet was modified for the animals receiving extra vitamin E-selenium by adding a vitamin E (200 mg/kg)-selenium (0.2

mg) pre-mix to the finishing commercial ration. Each of the 24 experimental wether lambs were allocated to the following four diet × housing treatment combinations in a 60-day feeding trial:

1. Single-pen housing with no extra vitamin E-selenium supplementation
2. Single-pen housing with extra vitamin E-selenium supplementation
3. Paired-pen housing with no extra vitamin E-selenium supplementation
4. Paired-pen housing with extra vitamin E-selenium supplementation

Thus the study was executed using a 2 × 2 factorial treatment arrangement in a completely randomized design with 6 experimental replicates per treatment. Lambs were afforded 2 hours at each feeding time before collection and quantification of refusals. The animals were afforded a 7-day adaptation period before data collection commenced (Zhao *et al.*, 2013)

3.2.4 Growth performance, body condition and physiological parameters

Feed offered to the lambs and the refusals were weighed daily using a digital scale. Feed intake was calculated on daily basis as feed offered less feed refused. Lambs were weighed using a digital scale at the beginning of the experiment, and thereafter on a weekly basis till the end of the experiment. The weights were taken in the morning before feeding in order to avoid measuring gut-filled weight. Average daily weight gain (ADWG) was calculated as the interim weight (final weight – initial weight) divided by time taken to accumulate the weight (63 days):

$$ADWG = \frac{\text{interim weight}}{\text{time taken}}$$

Feed conversion efficiency (FCE) was calculated by dividing body weight gain by feed intake (FI).

Rectal temperature (RT), heart rate (HR), and respiration rate (RR), were recorded 4 hours after feeding at the onset of the experiment and weekly thereafter, until week 9 prior to slaughter. Rectal temperature was measured using a digital thermometer supplied by the NWU Animal Hospital (Dale Beighle Center for Animal Health Studies). Heart rate was recorded with a stethoscope supplied by the NWU Animal Hospital (Dale Beighle Center for Animal Health Studies). Heart beat was recorded for 15 s and then converted to beats per min (15s x 4). Respiration rate was recorded by counting the number of breaths per 15 s and then converting to breaths per min (Chauhan *et al.*, 2016).

3.2.5 Statistical analysis

The data obtained from this experiment were analysed using a two-way analysis of variance using the general linear model procedures as contained in the SAS (2010) software and according to the linear model:

$$Y_{ijk} = \mu + H_i + V_j + (HV)_{ij} + \epsilon_{ijk}$$

Where Y_{ijk} = the response variable (growth performance and physiological parameters); μ = the overall mean (general mean); H_i = the housing effect; V_j = the vitamin E-Se effect; HV_{ij} = the effect of interaction between the grouping and vitamin E-Se supplementation; ϵ_{ijk} = the experimental error associated with observation ijk , which is assumed to be normally and independently distributed.

Parameters measured repeatedly from the experimental subjects (weekly feed intake, growth rate, FCR were analysed using a repeated measure analysis as contained in the general linear

models procedures of SAS (2010). When the analysis of variance revealed the existence of a significant difference among treatment means, a probability of difference (Pdiff) option in the least square means (lsmean) statement of the GLM procedures of SAS (2010) was used to locate the treatments that were significantly different from one another. For all statistical tests, significance was declared at $P \leq 0.05$.

3.3 Results

The statistical significance of the effect of main factors (housing and vitamin E-Se supplement) and their interactions on the overall feed intake, weight gain and feed conversion ratio is summarized in Table 3.1.

Table 3.1: Statistical significance (P values) of the effect of the main factors (Housing and Vitamin E-Se supplement) and their interactions on the overall feed intake (kg), weight gain (kg), and feed conversion ratio (FCR) of the Dohne Merino lambs.

Factor	Overall feed intake	Overall weight gain	Overall FCR
Housing	NS	NS	NS
Vitamin E-Se supplement	*	NS	NS
Housing × Vitamin E-Se	NS	NS	NS

NS: $P > 0.05$; * $P \leq 0.05$

There was no significant effect ($P > 0.05$) of dietary supplementation with the antioxidants and housing conditions on the overall weight gain and feed conversion ratio. However, a significant

($P < 0.05$) supplementation effect was observed on the overall feed intake in lambs fed with a combination of VitE-Se. Lambs that were supplemented with vitamin E-Se consumed more feed (65.01 kg) over the entire feeding period as compared to those lambs that were not offered the supplements (60.37 kg).

The weekly feed intake of lambs supplemented with vitamin E-Se is presented in Figure 3.1. Lambs receiving the supplement had higher feed intake from the onset of the experiment to the end of the experiment as compared to the control group.

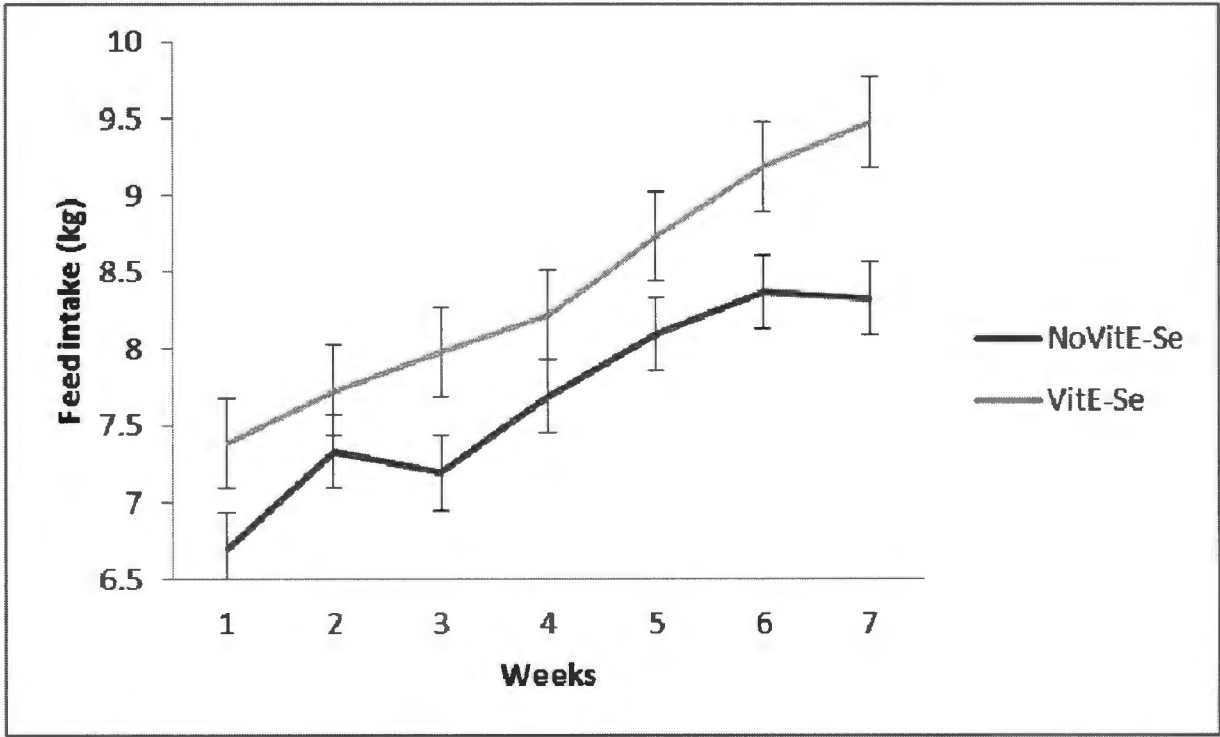


Figure 3.1: Effect of dietary vitamin E and selenium supplementation on weekly body weight gain of the Dohne Merino lambs.

The weekly weight changes of lambs supplemented with/without vitamin E-Se is presented in Figure 3.2. Supplemented lambs had higher weight changes than the non-supplemented group from week 3 to the end of the experiment.

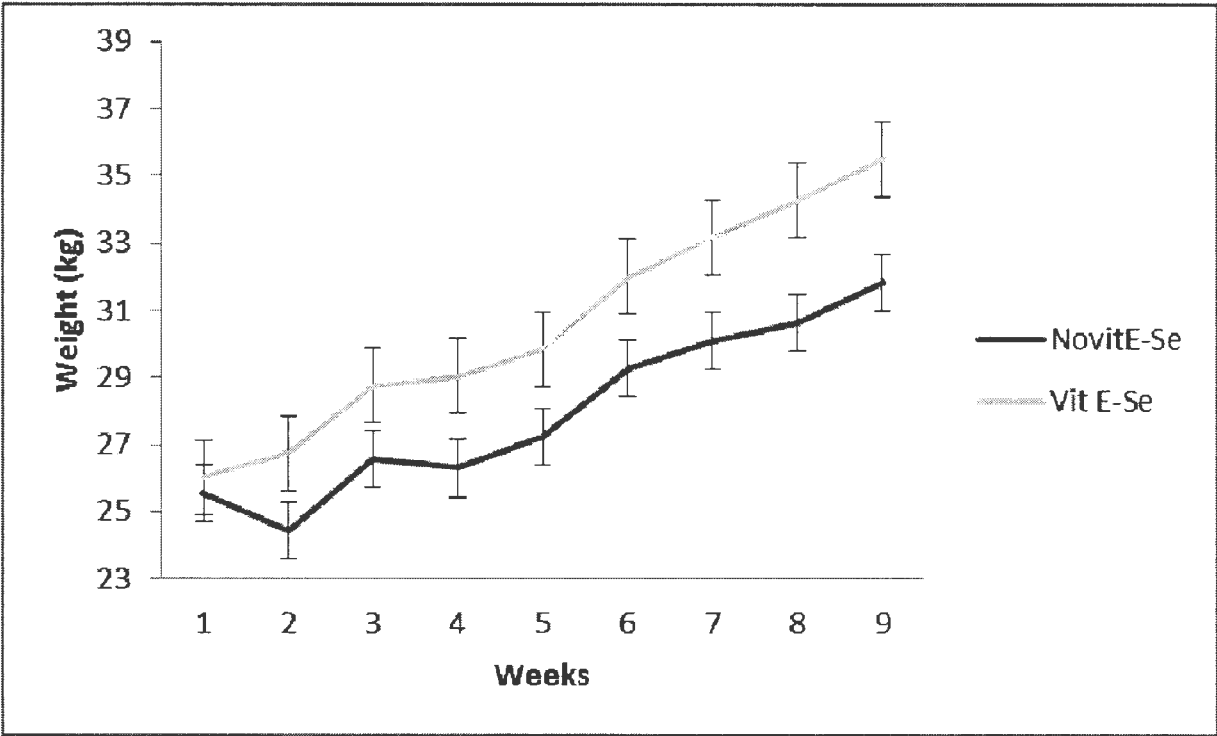


Figure 3.2: Effect of dietary vitamin E and selenium supplementation on weekly feed intake of the Dohne Merino lambs.

Table 3.2: Statistical significance (P values) of the effect of the main factors (dietary vitamin E-Se and housing conditions) on rectal temperature, heart rate and respiratory rate in lambs at the end of the feeding trial (Week 8).

Factor	Housing	Vitamin E - Se	Housing x Vitamin E -Se
Rectal temperature ($^{\circ}\text{C}$)	NS	NS	**
Heart rate (beats/min)	NS	NS	NS
Respiratory rate (breaths/min)	NS	NS	NS

NS: $P > 0.05$; * $P \leq 0.05$

Vitamin E-Se dietary supplementation did not ($P > 0.05$) influence the physiological parameters of the fed lambs throughout the feeding period (week 1 – week 8). However, a significant ($P < 0.05$) antioxidant supplement and housing condition interaction effect on week 8 rectal temperature was observed (Table 3.3).

Table 3.3: Effect of dietary vitamin E-Se and housing conditions on rectal temperature levels of lambs at the end of the feeding trial.

Housing	Dietary treatment	
	No Vitamin E & Se	Vitamin E & Se
Individual	$39.23 \pm 0.107^{\text{aA}}$	$38.83 \pm 0.107^{\text{bA}}$
Paired	$39.00 \pm 0.107^{\text{aA}}$	$39.30 \pm 0.107^{\text{aA}}$

^{ab}within a column, Lsmeans without a common lower case superscripts differ ($P < 0.05$).

Among the vitamin E-Se supplemented sheep, animals housed in pairs had higher rectal temperatures (39.3°C) compared to those housed individually (38.8°C). However, for the non-supplemented group, no differences ($P > 0.05$) were observed between the rectal temperatures of the paired (39.3°C) and individually (39.0°C) housed sheep.

3.4 Discussion

According to literature, isolation of animals in either single or group pens, even for short periods of time, is known to be one of the most stressful and fear eliciting situations, inducing significant behavioural and physiological agitations that may negatively affect feed intake and growth performance (Bouissou *et al.*, 2001; Fisher & Matthews., 2001). The results of the present study indicates that supplementing the diet of lambs with extra vitamin E and selenium has no significant effect on most growth performance traits, a finding which is consistent with observations made by other researchers (Dufrasne *et al.*, 2000; Macit *et al.*, 2003a; Juniper *et al.*, 2006; Maiorano *et al.*, 2007). This is despite the supplementation levels used in this study being higher than in the other previous studies. Vitamin E and selenium are essential components of the antioxidant defence system and play an important role in growth performance of animals through their participation in critical enzymes and enzyme reactions (Surai, 2002; Willshire & Payne, 2011). Selenium plays a vital role as an essential part of the glutathione peroxidases (GSH-Px), while vitamin E is a fat-soluble antioxidant that has a positive influence on the immune function and health of the body. Selenium also plays important roles in metabolism and growth via an expression of the iodothyronine deiodinases that regulate the production of the active T3 hormone in the thyroid gland and peripheral tissues (Rooke *et al.*, 2004). It is, therefore, important that animals are provided with adequate vitamin E and selenium to maintain

their growth performance and health, particularly when the animals are raised under highly stressful conditions.

In the present study, regardless of housing type, the extra dietary Se and vitamin E supplementation increased feed intake (65.01 kg) when compared to the un-supplemented animals over the 8-week feeding period but this observation is in contrast with the other previous studies that reported. An increase in feed intake with a commensurate increase in growth is not beneficial. (Rock *et al.*, 2001; Qin *et al.*, 2007; Kumar *et al.*, 2009) or dairy cattle (Lee *et al.*, 2007; Ebrahimi *et al.*, 2009; Covey *et al.*, 2010). The feed intake improvement may be due to protection of the ruminal epithelium against the ulcerative action of ruminal content in ruminant animals fed with dietary vitamin E and selenium (Brzezinska-Slebodzinska *et al.*, 1994; Vazquez-Anon *et al.*, 2008; Sahoo *et al.*, 2009). However, since the dietary Se and vitamin E increased feed intake, it was expected to see weight gain on the supplemented group, but the increased feed intake did not result into weight gain on the lambs. These findings might be due to the rearing conditions used in the current study that were not similar to those employed in the previous studies. In the current study, animals were subjected to stressful rearing conditions and thus it is conceivable that an extra vitamin E and selenium supplementation would have assisted the animal in dealing with the stressful rearing conditions and thus ameliorating the negative effects of stress on feed intake. Dietary Se and vitamin E did not have a significant ($P > 0.05$) effect on the weight gain and FCE of the lambs, but a linear and steady increase in body weights was observed throughout the experiment for all the treatments.

It is generally accepted that the upper critical body temperature of the sheep lies between 25°C and 30°C and that heat stress occurs when sheep are exposed to temperatures in excess of 30°C (Fuquay, 1981). The normal body temperature of sheep lies between 38.3 and 39.9. Among the

vitamin E-Se supplemented sheep, animals housed in pairs had higher rectal temperatures (39.3°C) compared to those housed individually (38.8°C). The RT of sheep housed in pairs were approximately 0.5°C higher than those housed individually and receiving a dietary supplement. The observations of our results are supported by Chauhan *et al.* (2014b). This ameliorating effect of the dietary vitamin E and selenium on RT during the house induced stress in sheep has not been well explained. Supplementation of dietary vitamin E and selenium could have been mediated by either reversing or inhibiting the reduction in antioxidant activity caused by the stress due to confinement stress (Bernabucci *et al.*, 2002; Surai, 2006) or it might have decreased the release of the adrenocorticotrophic hormone (ACTH), which is normally increased by confinement stress (Magdub *et al.*, 1982; Hirayama *et al.*, 2004). As a consequence of the thermal challenges associated with an increased variability in climate and the availability of quality fodder and pasture (Henry *et al.*, 2012), it is predicted that animal welfare and metabolic functions will be negatively impacted (Nardone *et al.*, 2010).

3.5 Conclusion

The results of the current study indicated that the dietary vitamin E and selenium did not have a significant effect onto the weight gain and FCR except that it increased the feed intake in the supplemented group when compared to the control group. An interaction was observed on the rectal temperature on the last day of the experiment. Therefore, dietary vitamin E and selenium can be supplemented into the lamb's diet without any negative effect onto the performance and physiological parameters. Even though the supplement increased only the feed intake and no significant difference in the weight gain, FCR and physiological parameters, all the lambs were in good health.

3.6 References

- Bernabucci, U., Ronchi, B., Lacetera, N. and Nardone, A., 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J. Dairy Sci.* 85, 2173-2179.
- Bøe, K.E., Berg, S & Andersen, I.L., 2006 Resting behaviour and displacements in ewes—effects of reduced lying space and pen shape. *Appl. Anim. Behav. Sci.* 98, 249–259.
- Boissy, A, Fisher, A.D, Bouix, J., Hinch, G.N & Le Neindre, P., 2005. Genetics of fear in ruminant livestock. *Livest Prod Sci.* 93, 23–32.
- Bonnette, E.D., Kornegay, E.T., Lindemann, M.D., & Hammerberg, C., 1990. Humoral and cell-mediated immune response and performance of weaned pigs fed four supplemental vitamin E levels and housed at two nursery temperatures. *J. Anim. Sci.* 68, 1337–1345.
- Bouissou, MF., Boissy, A., Le Neindre, P & Veissier, I., 2001. The social behaviour of cattle. In: Keeling L, Gonyou H, editors. *Social behaviour in farm animals*. Wallingford, UK: CABI Publishing; 2001, 113–45.
- Brodgen, K.A., Howard, D.L & Randall, C.C., 1998. *Pasteurella haemolytica* complicated respiratory infections in sheep and goats. *Vet. Res.* 29, 233–254.
- Brzezinska-Slebodzinska, E., Miller, J. K., Quigley, J. D., 3rd, Moore, J. R & Madsen, F. C., 1994. Antioxidant status of dairy cows supplemented prepartum with vitamin E and selenium. *J. Dairy. Sci.* 77, 3087-3095.

- Chauhan, S.S., Celi, P., Leury, B.J., Clarke, I.J & Dunshea, F.R., 2014b. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. *J. Anim. Sci.* 92, 3364–3374.
- Chauhan, S.S., Ponnampalana, E.N., Celi, P., Hopkins, D.L., Leury, B.J & Dunshea, F.R., 2016. High dietary vitamin E and selenium improves feed intake and weight gain of finisher lambs and maintains redox homeostasis under hot conditions. *Small. Rum. Res.* 137, 17–23.
- Covey, T. L., Elam, N.E., Carroll, J.A., Wester, D.B & Galyean, M.L., 2010. Supplemental selenium source in Holstein steers challenged with intranasal bovine infectious rhinotracheitis virus and in newly received beef heifers: Performance, morbidity, antibody titers, and blood cell counts. *Prof. Anim. Sci.* 26:82–92.
- Dalle Zotte, A & Szendrő, Z., 2011. The role of rabbit meat as functional food. *Meat. Sci.* 88, 319-331.
- Done-Currie, J.R., Hecker, J.F & Wodzicka-Tomaszewska, M., 1984. Behaviour of sheep transferred from pasture to an animal house, *Appl. Anim. Behav. Sci.* 12, 121–130.
- Dufresne, I., Marche, A., Clinquart, J.L., Hornick, C., van Eenaeme, C & Istasse, L., 2000. Effects of dietary vitamin E supplementation on performance and meat characteristics in fattening bulls from the Belgian Blue Breed. *Livest Prod Sci* 65, 197-201.
- Ebrahimi, M., Towhidi, A & Nikkhar, A., 2009. Effects of organic selenium (Sel-Plex) on thermometabolism, blood chemical composition and weight gain in Holstein suckling calves. *Asian-Australas. J. Anim. Sci.* 22, 984–992.

- Fisher, A & Matthews, L., 2001. The social behaviour of sheep. In: Keeling L, Gonyou H, editors. Social behaviour in farm animals. Wallingford, UK: CABI Publishing; 211–45.
- Fitzpatrick, J., Scott, M & Nolan, A., 2006. Assessment of pain and welfare in sheep. *Small Rumin. Res.* 62, 55–61.
- Fraser, D., 2008. Toward a global perspective on farm animal welfare. *Appl. Anim. Behav. Sci.*, 113, 330–339.
- Fraser, D., Duncan, I.J.H., Edwards, S.A., Grandin, T., Gregory, N.G., Guyonnet, V., Hemsworth, P.H., Huertas, S.M., Huzzey, J.M., Mellor, D.J., Mench, J.A., Špinka, M., & Whay, H.R., 2013. General principles for the welfare of animals in production systems: the underlying science and its application. *Vet. J.* 198, 19–27.
- Fuquay, J.W., 1981. Heat stress as it affects animal production. *J. Ani. Sci.* 52, 164–174.
- Garnier, J.P., Klont, R & Plastow, G., 2003. The potential impact of current animal research on the meat industry and consumer attitudes towards meat. *Meat Sci.*, 63, 79–88.
- Guesdon, V., Meurisse, M., Chesneau, D., Picard, S., Lévy, F & Chaillou, E., 2015. Behavioral and endocrine evaluation of the stressfulness of single-pen housing compared to group-housing and social isolation conditions. *Physio. Bev.* 147, 63–70.
- Henry, B., Charmley, E., Eckard, R., Gaughan, J.B & Hegarty, R., 2012. Livestock production in a changing climate: adaptation and mitigation research in Australia. *Crop. Past. Sci.*, 63, 191–202

- Hirayama, S., Hamazaki, T. and Terasawa, K., 2004. Effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder—a placebo-controlled double-blind study. *Euro. J. clinl. Nutri.* 58, 467-473.
- Jongman, E.C., Edge, M.K., Butler, K.L & Cronin, G.M., 2001. Reduced space allowance for adult sheep in lairage for 24 hours limits lying behaviour but not drinking behaviour. *Aust. J. Exp. Agric.*, 48, 1048–1051
- Kumar, N., Garg, A. K., Dass, R.S., Chaturvedi, V.K., Mudgal, V & Varshney, P., 2009. Selenium supplementation influences growth performance, antioxidant status and immune response in lambs. *Anim. Feed Sci. Technol.* 153,77–87.
- Lee, S. H., Park, B.Y., Yeo, J.M., Lee, S.S., Lee, J.H., Ha, J.K & Kim, W.Y., 2007. Effects of different selenium sources on performance, carcass characteristics, plasma glutathione peroxidase activity and selenium deposition in finishing Hanwoo steers. *Asian-Australas. J. Anim. Sci.* 20:229–236.
- Lindberg, A.C., Keeling, L.J & Gonyou, H.W., 2001. *Social Behavior in Farm Animals*, CABI, Wallingford, Oxfordshire, UK, 37–58
- Lusk, J.L & Norwood, J.L., 2008. A survey to determine the public opinion about the ethics and governance of farm animal welfare. *J. Am. Vet. Med. Assoc.*, 233, 1121–1123.
- Macit, M., Aksakal, V., Emsen, E., Aksu, M.İ., Karaoglu, M & Esenbuga, N., 2003a. Effects of Vitamin E supplementation on performance and meat quality traits of Morkaraman male lambs. *Meat. Sci* 63, 51-5.

- Magdub, A., Johnson, H.D. and Belyea, R.L., 1982. Effect of Environmental Heat and Dietary Fiber on Thyroid Physiology of Lactating Cows¹. *J. Dairy. Sci.* 65, 2323-2331.
- Maiorano, G., Cavone, C., McCormick, R.J., Ciarlariello, A., Gambacorta, M & Manchisi, A., 2007. The effect of dietary energy and vitamin E administration on performance and intramuscular collagen properties of lambs. *Meat. Sci* 76, 182-188.
- María, G.A., 2006. Public perception of farm animal welfare in Spain. *Lives. Sci.*, 103, 250–256.
- Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M.S & Bernabucci, U., 2010. Effects of climate changes on animal production and sustainability of livestock systems. *Livst. Sci.* 130, 57-69.
- Nash, M.L Hungerford, L.L., Nash, T.G & Zinn, G.M., 1997. Risk factors for respiratory disease mortality in lambs. *Small. Rum. Res.* 26, 53–60.
- Niekamp, S.R., Sutherland, M.A., Dahl, G.E & Salak-Johnson, J.L., 2007. Immune responses of piglets to weaning stress: impacts of photoperiod. *J. Anim. Sci.* 85, 93–100.
- Qin, S., Gao, J & Huang, K., 2007. Effects of different selenium sources on tissue selenium concentrations, blood GSH-Px activities and plasma interleukin levels in finishing lambs. *Biol. Trace Elem. Res.* 116, 91–102.
- Rice, M., Jongman, E.C., Butlera, K.L & Hemsworth, P.H., 2016. Relationships between temperament, feeding behaviour, social interactions, and stress in lambs adapting to a feedlot environment. *App. Ani. Bev. Sci.* 183, 42–50.

- Rock, M. J., Kincaid, R. L., & Carstens, G.E., 2001. Effects of prenatal source and level of dietary selenium on passive immunity and thermometabolism of newborn lambs. *Small Rumin. Res.* 40,129–138.
- Rooke, J.A., Robinson, J.J & Arthur, J.R., 2004. Effects of vitamin E and selenium on the performance and immune status of ewes and lambs. *J. Agric. Sci.*, 142, 253–262.
- Sahoo, S. S., Patra, R.C., Behera, P.C & Swarup,D., 2009. Oxidative stress indices in the erythrocytes from lactating cows after treatment for subclinical ketosis with antioxidant incorporated in the therapeutic regime. *Vet. Res. Commun.* 33,281- 290.
- Sevi, A., Casamassima, D., Pulina, G & Pazzona, A., 2009. Factors of welfare reduction in dairy sheep and goats. *Ital. J. Anim. Sci.* 8, 81-101.
- Surai, P.F., 2002. Natural antioxidants in avian nutrition and reproduction (pp. 5-9). Nottingham: Nottingham University Press.
- Surai, P.F., 2006. Selenium in nutrition and health (Vol. 974). Nottingham: Nottingham university press.
- Terlouw, E.M.C., Arnould, C., Auperin, B., Berri, C., Le Bihan-Duval, E., Deiss, V., Lefèvre, F., Lensink, B.J & Mounier, L., 2008. Pre-slaughter conditions, animal stress and welfare: current status and possible future research *Animal* 2, 1501–1517.
- Vandenheede, M., Bouissou, M.F. & Picard, M., 1998. Interpretation of behavioural reactions of sheep towards fear-eliciting situations. *Appl. Ani. Bev. Sci.* 58, 293-310.

- Vazquez-Anon, M., Nocek, J., Bowman, G., Hampton, T., Atwell, C., Vazquez, P & Jenkins, T., 2008. Effects of feeding a dietary antioxidant in diets with oxidized fat on lactation performance and antioxidant status of the cow. *J. Dairy. Sci.* 91, 3165-3172.
- Viérin, M & Bouissou, M.F., 2003. Responses of weaned lambs to fear-eliciting situations: Origin of individual differences. *Devlop. Psychbio.* 42, 131-147.
- Willshire, J.A & Payne, J.H., 2011. Selenium and vitamin E in dairy cows-a review. *Cattle. PRAC.* 19, 22-30.
- Winter, M., Fry, C & Carruthers, S.P., 1998. European agricultural policy and farm animal welfare. *Food Pol.*, 23, 305–323.
- Zhao, T., Luo, H., Zhang, Y., Liu, K., Jia, H., Chang, Y., Jiao, L. & Gao, W., 2013. Effect of vitamin E supplementation on growth performance, carcass characteristics and intramuscular fatty acid composition of Longissimus dorsi muscle in Tan'sheep. *Chilean J. Agric. Res.* 73, 358-365.

4 CHAPTER FOUR - HAEMATOLOGICAL INDICES AND SERUM BIOCHEMISTRY IN FINISHING DOHNE MERINO SHEEP REARED UNDER SINGLE- OR PAIRED-PEN HOUSING: EFFECT OF EXTRA DIETARY VITAMIN E-SELENIUM SUPPLEMENTATION

ABSTRACT

This study was designed to assess the effectiveness of extra dietary vitamin E-selenium supplementation in mitigating against housing-induced stress in Mutton Merino lambs as measured by the haematology, serum biochemistry and stress biomarkers. Twenty four lambs with an average body weight of 25.8 ± 2.7 kg were used. The lambs were randomly assigned to four treatment combinations: single-pen housing with no extra vitamin E-selenium supplementation, single-pen housing with extra vitamin E-selenium supplementation, paired-pen housing with no extra vitamin E-selenium supplementation, and paired-pen housing with extra vitamin E-selenium supplementation, in a completely randomized design. The results showed that most of the hematological indices were not significantly ($P < 0.05$) affected by the dietary supplement and housing type, with an exception of the RBC, HCT/PCV, and MCHC. For these

parameters, supplemented lambs had higher RBC ($12.61 \times 10^{12}/L$), HCT/PCV (28.88 %) and MCHC (32.24 g/dL) compared to the un-supplemented animals (RBC, $11.17 \times 10^{12}/L$; HCT/PCV, 24.87 %; and MCHC, 15.96 g/dL) irrespective of the housing type. Superoxide dismutase and glucose were significantly ($P < 0.05$) affected by the housing type only, with the higher means of 0.05 u/mL and 3.95 mmol/L, respectively, being observed in the companion group as compared to the solitary confinement group (0.03 u/mL and 3.65 mmol/L, respectively). The glutathione peroxide and most of the serum biochemical variables were not significantly affected by both the dietary supplementation and housing conditions. From this outcome, it can be deduced that the Dorne Merino sheep used in this study were exposed to moderate stress. The results of this study show that the addition of dietary vitamin E and selenium can be used to reduce or alleviate the consequences of housing-induced stress because supplementation increased SOD, RBC, HCT/PCV MCHC and glucose levels, which have important implications on sheep welfare.

Keywords: Vitamin E, selenium, blood parameters, serum biochemical parameters, sheep, housing effect.

4.1 Introduction

Several husbandry practices in intensive sheep production systems are known to induce stress (Knowles *et al.*, 1995) thus impacting onto the animals' physiological and production responses. These husbandry practices include weaning, transportation, classification, regrouping, novel environment and confinement (single or group housing) (Miranda-de la Lama *et al.*, 2010a). Single-housed animals are prone to more stressful conditions than animals that are reared under group housing conditions. The individual housing of animals is frequently used to control the

environment, facilitate biological sampling and limit social stress. However, this type of housing also prevents exploratory behaviors and could induce stereotypes that are non-compliant with welfare and possibly associated with a state of stress. Social learning allows individual animals to learn positive or negative effects from other animals (Bandura., 1977; Conte & Paolucci., 2001); this type of learning is thought to be important in the development of feeding behavior in many farmed species (Keeling & Humik., 1996; Launchbaugh & Howery, 2005). To our knowledge, no attempt has ever been made to summarize the available scientific information on the effects of isolation and social housing on lamb production systems, which may induce stress, leading to an excessive production of free radicals and an imbalance in the oxidant/antioxidant system (oxidative stress) (Khadija *et al.*, 2009).

Oxidative stress thus arises due to an enhanced reduction in enzymatic and non-enzymatic antioxidants. Reactive oxygen species (ROS) including the superoxide radicals, hydrogen peroxide and hydroxyl radicals have a negative effect on the normal function of biomolecules like nucleic acids, proteins, and cell membrane phospholipids (Singh *et al.*, 1999). Antioxidants such as the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), reduced glutathione (GSH) play a significant role in reducing the production of ROS. Superoxide dismutase is active in catalyzing the detoxification of superoxide radicals to hydrogen peroxide and water and also acts as the first line of defense against ROS (Gonzales *et al.*, 1984). While on the other side, the glutathione peroxidase (GPX) catalyses the reduction of hydrogen peroxides and organic hydrogen peroxides to water and alcohol. About 11.8% of the total Se in an organism is bound in GSH-Px together with vitamin E and other antioxidants, to prevent the oxidative damage of cells (Awadeh *et al.*, 1998). Therefore, we hypothesized that providing an extra dietary vitamin E-selenium to lambs subjected to housing stress (single-pen housing

isolation) would aid physiological responses to stress as measured by the haematological indices, serum biochemistry and stress biomarkers parameters. Thus the present study was designed to assess the effectiveness of an extra dietary vitamin E-selenium supplementation in mitigating housing-induced stress in Mutton Merino lambs as measured by the haematology, serum biochemistry and stress biomarkers.

4.2 Materials and methods

4.2.1 Blood sample collection

Blood samples were collected from all the 24 Mutton Merino lambs reared as described in Chapter 3. Blood collection was done on the last day of the experiment. The blood was drawn via the jugular venipuncture using a vacutainer holder and a vacutainer needle and stored in 10 mL glass red top serum tubes and 7 mL glass lavender top EDTA tubes (whole blood and plasma). The collected samples were analysed for haematology within 24 hours of collection. The full blood count included the white cell count (WBC), red blood cell (RBC) count, hematocrit/packed cell volume (PCV), hemoglobin, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets, platelet distribution width, and reticulocyte count. The serum biochemical components (creatinine, albumin, globulin and total protein) and non-enzymatic antioxidants (bilirubin and vitamin E) were also assayed for. Blood (2 ml) was also collected into some lithium heparin tubes placed on ice for determination of the activities of glutathione peroxidase (GSHpx) and superoxide dismutase (SOD). Blood tubes were stored at 4-25°C before the blood was centrifuged at 1000 - 2000 x g for 10 minutes in a refrigerated centrifuge and in order to get plasma and serum, and according to the guidelines of Thavasu *et al.* (1992).

4.2.2 Analytical procedures

Samples were analyzed using an IDEXX LaserCyte Haematology Analyzer for full blood count, and this full blood count included the WBC, RBC count, PCV, MCV, MCH, MCHC, RDW, platelets, platelet distribution width, and reticulocyte count. The associated differential count included the neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts. Glucose, creatinine, total protein, bilirubin and albumin were the serum biochemical parameters of interest analysed using the Catalyst Dx Chemistry Analyzer - IDEXX. Superoxide dismutase (SOD) activity in whole blood was determined by the method of Janknegt *et al.* (2007). The activity of glutathione peroxidase (GSH-px) was assayed by the method of Pascual *et al.* (1992). The results of the SOD and GPx were then tested by calculating the correlation coefficient (r) using Microsoft EXCEL 2010 software, and regression analysis and calculating the regression line equation allowing for the determination of the presumed values of y (GSH-Px) for the various values of x (standard concentration).

4.2.3 Statistical analysis

The haematological indices, serum biochemistry and stress biomarkers were analysed using a two-way analysis of variance as contained in the general linear models procedures of SAS (2010). When the analysis of variance revealed the existence of a significant difference among treatment means, the probability of difference (pdiff) option in least square means statement of the GLM procedure of SAS (2010) were then used to separate the treatment means. The statistical significance was declared at $P < 0.05$.

4.3 Results

Statistical significance of effects of the main factors (housing and vitamin E-Se) and their interaction on hematological indices, serum chemistry and antioxidants at the end of the experiment are presented in Table 4.1.

Table 4.1: Statistical significance (*P* values) of effects of the main factors (housing and vitamin E-Se) and their interaction on hematological indices and stress biomarkers.

Hematological indices	Housing	Vitamin E-Se	Housing x Vitamin E-Se
RBC ($9.45 \times 10^{12}/L$)	NS	*	NS
HCT/PCV (%)	NS	*	NS
Hgb (g/dL)	NS	NS	NS
MCV (fl)	NS	NS	NS
MCH (pg)	NS	*	NS
MCHC (g/dL)	NS	*	NS
RDW (%)	NS	NS	NS
WBC ($4.81 \times 10^9/L$)	NS	NS	NS
% NEU	NS	NS	NS
% LYM	NS	NS	NS
% MONO	NS	NS	NS
% EOS	NS	NS	NS
% BASO	NS	NS	NS
PLT ($\times 10^3/\mu L$)	NS	NS	NS
<i>Serum biochemical variables</i>			
Glucose (mmol/L)	*	NS	NS
Urea (mmol/L)	NS	NS	NS
Creatanine ($\mu\text{mol}/L$)	NS	NS	NS
Phosphorus (mmol/L)	NS	NS	NS
Calcium (mmol/L)	NS	NS	NS
Total protein (g/L)	NS	NS	NS
Albumin (g/L)	NS	NS	NS
Globumin (g/L)	NS	NS	NS
alanine aminotransferase (U/L)	NS	NS	NS
alkaline phosphatase (U/L)	NS	NS	NS
Amalyse (U/L)	NS	NS	NS
Lipase (U/L)	NS	NS	NS
<i>Enzymatic antioxidants</i>			
SOD (u/ml)	*	NS	NS
GPx (u/ml)	NS	NS	NS

NS = not significant; * $P < 0.05$ RBC = red blood cell, HCT = hematocrit, PCV = packed cell volume, Hgb = hemoglobin, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width, NEU = neutrophil, LYM = lymphocyte, MONO = monocyte; EOS = eosinophil, BASO = basophil, PLT = platelet.

The results show that most of the hematological indices were not significantly ($P > 0.05$) affected by the dietary supplement and housing type, with the exception of RBC ($P > 0.0442$), HCT/PCV ($P < 0.0183$), MCH ($P < 0.0316$), and MCHC ($P < 0.0203$). Superoxide dismutase and glucose were significantly ($P < 0.05$) affected by the housing type only, with the higher means of 0.05 u/mL and 3.95mmol/L, respectively, in the companion group as compared to the solitary confinement group (0.03 u/mL and 3.65, respectively), while the glutathione peroxide was not significantly affected by both the dietary supplementation and housing conditions.

As is shown in Table 4.2, sheep receiving the dietary vitamin E-Se supplements had higher RBC ($12.61 \times 10^{12}/L$), HCT/PCV (28.88 %) and MCHC (32.24 g/dL) as compared to the control group ($11.17 \times 10^{12}/L$), 24.87 %, 15.96 g/dL, respectively) and irrespective of the housing type. However, the MCH values followed an opposite trend (Control: 8.14 pg and Supplemented: 9.02 pg). Notably, most of the hematological parameters were within the expected normal range for the Dohne Merino sheep.

Table 4.2: Effect of dietary vitamin E-Se supplementation on hematological indices and serum antioxidants levels of the Dohne Merino lambs.

Parameters ¹	No Vitamin E-Se	Vitamin E-Se
<i>Hematological indices</i>		
RBC ($9.45 \times 10^{12}/L$)	11.17±0.478	12.61±0.456
HCT/PCV (%)	24.87±1.125	28.88±1.072

Hgb (g/dL)	9.89±0.200	10.22±0.191
MCV (fl)	22.25±0.278	22.90±0.265
MCH (pg)	9.02±0.273	8.14±0.260
MCHC (g/dL)	15.96±4.655 ^a	32.24±4.438 ^a
RDW (%)	21.04±0.368 ^a	21.02±0.351 ^a
WBC (4.81 × 10 ⁹ /L)	3.44±0.493	3.61±0.47
% NEU	34.65±3.891	37.41±3.710
% LYM	1.55±0.221	1.92±0.211
% MONO	0.34±0.060	0.17±0.057
% EOS	0.13±0.023	0.13±0.021
% BASO	0.03±0.004	0.03±0.003
PLT (× 10 ³ /μL)	17.73±6.833	11.08±6.515
<i>Serum biochemical variables</i>		
Glucose (mmol/L)	3.67±0.094	3.93±0.094
Urea (mmol/L)	4.13±0.195	4.65±0.195
Creatinine (μmol/L)	47.25±1.953	46.25±1.953
Phosphorus (mmol/L)	2.62±0.204	2.50±0.204
Calcium (mmol/L)	2.22±0.042	2.26±0.042
Total protein (g/L)	64.75±1.336	66.33±1.336
Albumin (g/L)	27.17±0.74	27.25±0.74
Globulin (g/L)	37.58±1.079	39.08±1.079
alanine aminotransferase (U/L)	10.00±0.00	10.00±0.00
alkaline phosphatase (U/L)	235.97±24.56	225.75±24.56
Amalyse (U/L)	21.83±1.639	23.58±1.639
Lipase (U/L)	149.97±12.65	144.17±12.650
<i>Enzymatic antioxidants</i>		
SOD (u/ml)	0.04±0.004	0.05±0.004
GPx (u/ml)	22.16±8.238	7.71±7.855

^aIn a row, different superscripts denote significant differences (P < 0.05) between the vitamin E-Se supplement means.

¹Parameters: RBC = red blood cell, HCT = hematocrit, PCV = packed cell volume, Hgb = hemoglobin, MCV = mean corpuscular volume, MCH = mean cell hemoglobin, MCHC = mean cell hemoglobin, RDW = red cell distribution width, NEU = neutrophil, LYM = lymphocyte, MONO = monocyte; EOS = eosinophil, BASO = basophil, PLT = platelet.

4.4 Discussion

There is little information on the haematological indices (WBC, RBC, PCV/HCT, MCV, MCH, MCHC, RDW, platelets) and serum biochemical variables (GLU, Urea, CREA, PHOS, CA, TP, ALB, GLOB, ALT, GGT, TBIL, AMYL, LIPA) of sheep fed with the dietary vitamin E and

selenium under different housing conditions in literature. This is despite the fact that housing conditions are some of the major sources of stress that affect the welfare of sheep and often manifested in the level of various blood parameters. The response of sheep to these conditions can be modulated by dietary supplementation, particularly vitamin E and selenium, which are potent antioxidants that reduce oxidative stress caused by the suboptimal housing conditions. However, in this study, vitamin E and selenium supplementation had no significant effect on most of the haematological indices, including the WBC as well as most of the serum biochemistry. Our findings, in this regard, corroborate with those of Lepherd *et al.* (2009) who reported that most of the blood hematological variables and serum biochemical variables measured were unaffected by vitamin E and Se injection but were within the normal reference ranges for clinically healthy sheep. Sheep supplemented with vitamin E and selenium had slightly higher RBC ($12.61 \times 10^{12}/L$), HCT/PCV (28.88 %) and MCHC (32.24 g/dL) values as compared to the control group ($11.17 \pm 9.45 \times 10^{12}/L$, 24.87 %, 15.96 g/dL, respectively). The MCH values followed an opposite trend with a slightly lower value (8.14 pg) in the un-supplemented sheep as compared to the supplemented group (9.02 pg).

Faixova *et al.* (2007) reported that sheep fed with a diet supplemented with 0.3 mg Se/kg and 150 mg/kg vitamin E, had greater RBC counts, HCT/PCV and MCHC as compared to the un-supplemented sheep when they were individually housed for 50 d in elevated (approximately 1 m above the floor) metabolism pens (1.5 m long by 0.5 m wide) in an environmental chamber. Confinement stress has a detrimental effect on sheep in many parts of the world. During periods of elevated stress, physiological and immunological responses are invoked to minimize adverse effects (Fuquay, 1981; Silanikove, 2000). Moreover, elevated stress conditions due to housing effects are associated with changes in the antioxidant status, haematological indices and serum

biochemical variables by promoting oxidative stress and reducing the blood concentrations of antioxidant micronutrients (zinc, selenium, and vitamin E) in ruminants (Bernabucci *et al.*, 2002; Saker *et al.*, 2004; Burke *et al.*, 2007) and poultry (Altan *et al.*, 2003; Bartlett and Smith, 2003; Sahin and Kucuk, 2007).

Serum concentrations of glucose were higher in the companion group (3.95 mM) compared to the solitary group (3.65 mM) and such findings are in line with those of Alhidary *et al.* (2015), who found an increase in the serum concentrations of glucose in sheep fed with diets supplemented with Se and vitamin E when they were individually housed for 50 d in elevated (approximately 1 m above the floor) metabolism pens (1.5 m long by 0.5 m wide) in an environmental chamber. Glucose is a significant component in animal's metabolism. It is an essential source of energy for the maintenance of many tissues, such as the nervous system and red blood cells (Sastradipradja, 1998). Superoxide dismutase levels were higher (0.05 u/mL) in the companion group compared to the solitary confinement group (0.03 u/mL), and this was in accordance with the finding of Barp *et al.* (2002), who found an increase in SOD levels when animals were placed in different age groups. Solitary confinement induces stress such that animals suffer from boredom, do not benefit from grooming behaviours and have restricted animal behaviours (Fraser *et al.*, 2013), resulting in suboptimal growth performance, production and welfare. Hence, companion animals were exposed to moderate stress due to their access to social interaction as compared to solitary confined, whereby they are socially excluded. Therefore, a dietary supplementation of vitamin E-Se is significant to optimize the SOD levels in blood (Zhu *et al.*, 2010) so as to meet up with their physiological needs.

Superoxide dismutase is considered to be the most significant enzymatic antioxidant because of its ability to mitigate ROS and radical chain reactions by repairing cells and retard the damage

done to them by the superoxide radical. The superoxide radical is also converted to more stable products (Oyedemi *et al.*, 2010) with the aid of catalase or the glutathione peroxidase reaction (Curtis & Mortiz, 1972). The glutathione peroxide was not significantly ($P < 0.05$) affected by both the dietary supplementation and housing conditions. These findings contradict with those of other studies (Suraï & Dvorska, 2002; Ebeid, 2012), which reported that the combined supplementation of vitamin E and Se increases GSH-Px activity. This contradiction might be due to the rearing conditions used in this study (solitary vs. companion housing). The SOD levels in the blood were sufficient enough to reduce the O_2 levels, such that the production of H_2O_2 from the SOD conversion was suppressed enough not to trigger an increased GPx production (Neill *et al.*, 2002).

4.5 Conclusion

From results of the hematological, biochemical, and enzymatic variables, it can be deduced that the Dohne Merino sheep used in this study were exposed to moderate stress. Results of this study show that the addition of a dietary feed supplemented with vitamin E and selenium can be used to reduce or alleviate the consequences of housing induced stress in relation to increased SOD as well as an increase in the RBC, HCT/PCV MCHC and glucose levels, which have important implications for the welfare and health of sheep. Moreover, the dietary vitamin E and selenium improved the ability of sheep to tolerate stress in terms of their haematological and biochemical profile and antioxidant status.

4.6 References

- Alhidary, I.A., Shini, S., Al Jassim, R.A.M., Abudabos, A.M. & Gaughan, J.B., 2015. Effects of selenium and vitamin E on performance, physiological response, and selenium balance in heat-stressed sheep. *J. Ani. Sci.* 93, 576-588.
- Altan, Ö., Pabuçcuoğlu, A., Altan, A., Konyalıoğlu, S. & Bayraktar, H., 2003. Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *Brit. poul. Sci.* 44, 545-550.
- Atakisi, O., Atakisi, E. & Kart A., 2009. Effects of dietary zinc and L-arginine supplementation on total antioxidants capacity, lipid peroxidation, nitric oxide, egg weight and blood biochemical values in Japanese quails. *Biol. Trace Elem. Res.*, 132, 136–143.
- Awadeh F.T., Kincaid R.L. & Johnson K.A., 1998. Effect of level and source of dietary selenium on concentrations of thyroid hormones and immunoglobulins in beef cows and calves. *J. Anim. Sci.* 76, 1204-1215.
- Bandura, A., 1977. Self-efficacy: toward a unifying theory of behavioral change. *Psych. Rev.* 84, 191.
- Barp J, AS Araujo, TR Fernands, KV Rigatto, S Llesuy, A Belloklein and P Singal, 2002. Myocardial antioxidant and oxidative stress changes due to sex hormones. *Brazilian J Med Biol Res*, 35: 1075-1081.

- Bernabucci, U., Ronchi, B., Lacetera, N & Nardone, A., 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J. dairy. Sci.* 85, 173-2179.
- Conte, R. and Paolucci, M., 2001. Intelligent social learning. *J. Artif. Soct. Socl. Simulation*, 4, U61-U82.
- Curtis, J.J., & Mortiz, M., 1972. Serum enzymes derived from liver cell fraction and response to carbon tetrachloride intoxication in rats. *Gastroenterology* 62, 84–92.
- Denli, M., Blandox, J.C., Guynot, M.E., Salado, S & Perez, J.F., 2009. Effects of dietary Afladetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B1. *Poult. Sci.*, 88, 1444–1451.
- Ebeid, T.A., Suzuki, T. and Sugiyama, T., 2012. High ambient temperature influences eggshell quality and calbindin-D28k localization of eggshell gland and all intestinal segments of laying hens. *Poul. Sci.* 91, 2282-2287.
- Emadi, M., Kaveh, K., Bejo, M.H., Ideris, A., Jahanshiri, F., Ivan, M & Alimon R.A., 2010. Growth performance and blood parameters as influenced by different levels of dietary arginine in broiler chickens. *J. Anim. Vet. Adv.*, 9, 70–74.
- Faixova, Z., Faix, Š., Leng, L., Vaczi, P., Makova, Z. & Szaboova, R., 2007. Haematological, blood and rumen chemistry changes in lambs following supplementation with Se-yeast. *Acta Veterinaria Brno.* 76, 3-8.
- Fuquay, J.W., 1981. Heat stress as it affects animal production. *J. Ani. Sci.* 52, 164-174.

- Gonzales R., Auclair, C., Voisin, E., Gautero, H., Dhermy, D & Boivin, P., 1984. Superoxide dismutase, catalase and glutathione peroxydase in red blood cells from patients with malignant diseases. *Cancer Res.* 44, 4137-4139.
- Janknegt, P.J., Rijstenbil, J.W., Van de Poll, W.H., Gechev, T.S. & Buma, A.G., 2007. A comparison of quantitative and qualitative superoxide dismutase assays for application to low temperature microalgae. *J. Photochem. Photobiol. B.* 87, 218-226.
- Keeling, L.J & Hurnik, F.J., 1996. Social facilitation acts more on the appetitive than the consummatory phase of feeding behaviour in domestic fowl. *Ani. Bev.* 52, 11-15.
- Knowles, T.G., Brown, S.N., Warriss, P.D., Phillips, A.J., Dolan, S.K., Hunt, P., Ford, J.E., Edwards, J.E & Watkins, P.E., 1995. Effects on sheep of transport by road for up to 24 h. *Vet. Rec.*, 136, 431–438.
- Launchbaugh, K.L & Howery, L.D., 2005. Understanding landscape use patterns of livestock as a consequence of foraging behavior. *Rangeland. Ecol. Manag.* 58, 99-108.
- Lepherd, M.L., Canfield, P.J., Hunt, G.B & Bosward, K.L., 2009. Haematological, biochemical and selected acute phase protein reference intervals for weaned female Merino lambs. *Austr. Vet. J.* 87, 5-11.
- Menzies, P., Langs, L., Boermans, H., Martin, J., & McNally, J., 2004. Myopathy and hepatic lipidosis in weaned lambs due to vitamin E deficiency. *Canadian. Vet. J.* 45, 244–247.
- Miranda-de la Lama, G.C., Rivero, L., Chacón, G., Garcia-Belenguer, S., Villarroel, M & Maria, G.A., 2010. Effect of the pre-slaughter logistic chain on some indicators of welfare in lambs. *Livst. Sci.*, 128, 52–59.

- Neill, S., Desikan, R & Hancock J., 2002. *Current Opinion in Plant Biology*, Volume 5, Issue 5, 388–395.
- Oyedemi, S.O., Bradley, G & Afolayan, A.J., 2010. In vivo and in vitro antioxidant activities of aqueous stem bark extract of *Strychnos henningsii* (Gilg). *Afri. J. Pharm. Pharmacol*, 4, 70–78.
- Pascual, P., Martinez-Lara, E., Barcena, J.A., Lopez-Barea, J. & Toribio, F., 1992. Direct assay of glutathione peroxidase activity using high-performance capillary electrophoresis. *J. Chromat. B: Biomed. Sci. Appl.* 581, 49-56.
- Rao SVR, Prakash B, Raju MVLN, Panda AK, Poonam S & Murthy O.K., 2013. Effect of supplementing organic selenium on performance, carcass traits, oxidative parameters and immune responses in commercial broiler chickens. *Asian Australas. J. Anim. Sci.* 26, 247–252.
- Sahin, N., Onderci, M., Balci, T.A., Cikim, G., Sahin, K & Kucuk, O., 2007. The effect of soy isoflavones on egg quality and bone mineralisation during the late laying period of quail. *Br. Poult. Sci.* 48, 363-369.
- Silanikove, N., 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livst. Prod. Sci.* 67, 1-18.
- Singh, SK., Dua, T.D., Tondon, A., Kumari, S., Ray, G & Batra, S., 1999. Status of lipid peroxidation and antioxidant enzymes in hypoxic ischemic encephalopathy. *Indian Ped.* 26, 659-668.

- Surai, P.F., Dvorska, J.E., 2002. Effects of selenium and vitamin E content of the diet on lipid peroxidation in breast muscle tissue of broiler breeder hens during storage. In: Proceedings of the Australian Poultry Science Symposium, 14, 187–192.
- Thavasu, P.W., Longhurst, S., Joel, S.P., Slevin, M.L. & Balkwill, F.R., 1992. Measuring cytokine levels in blood: importance of anticoagulants, processing, and storage conditions. J. Immunol. Methods. 153, 115-124.

5 CHAPTER FIVE - CARCASS CHARACTERISTICS AND MEAT QUALITY IN DOHNE MERINO SHEEP REARED UNDER SINGLE- OR PAIRED-PEN HOUSING: EFFECT OF EXTRA DIETARY VITAMIN E-SELENIUM SUPPLEMENTATION

ABSTRACT

This study compared the variation in carcass characteristics and meat quality of animals housed in single-pens (isolated) to those housed in paired-pens (socially interacting) when supplemented with extra dietary vitamin E-selenium. Twenty four lambs with an average body weight of 25.8 ± 2.7 kg were used. The lambs were randomly assigned to four treatment combinations: single-pen housing with no extra vitamin E-selenium supplementation, single-pen housing with extra vitamin E-selenium supplementation, paired-pen housing with no extra vitamin E-selenium

supplementation, and paired-pen housing with extra vitamin E-selenium supplementation, in a completely randomized design. The animals were slaughtered at the end of the feeding trial to determine the characteristics of meat quality. The results showed that most of the carcass measurements and meat quality parameters were not significantly ($P < 0.05$) influenced by the dietary vitamin E-selenium supplementation and housing type with an exception of the slaughter weight, dressing percentage and pHu (on shelf life). These parameters were higher in lambs fed with the dietary vitamin E-selenium than in the control group. A significant ($P < 0.05$) interaction between supplementation $\times$ housing was observed for the drip loss percentage and carcass length. But there were no differences observed ($P > 0.05$) on carcass length between the vitamin E-selenium supplemented groups and the non-supplemented groups irrespective of the housing conditions. The individuals in non-supplemented group showed longer carcass lengths as compared to the paired groups while the paired animals in Vit E-Se supplemented group showed longer carcass length as compared to the individually-housed group. Also no differences were observed ($P > 0.05$) on drip loss between the vitamin E-selenium supplemented groups and the non-supplemented groups, irrespective of the housing conditions. The dietary vitamin E and selenium supplementation reduced the drip loss percentage in the paired group but increased it in the individual group, while in the non-supplemented group; an increase in the drip loss percentage was observed in the paired group and decreased in the individual group. All in all, it can then be concluded that the dietary vitamin E and selenium are more favorably on slaughter weight and dressing out percentage, while an interaction between the housing type and vitamin E-selenium have a positive effect on drip loss and carcass length values.

Keywords: vitamin E, selenium, carcass characteristics, sheep, housing effect.

5.1 Introduction

Meat consumers make a purchase decision based on the visual appearance of meat products. Changes in meat colour are caused by oxidation of the muscle pigment myoglobin, from the red oxymyoglobin to the brown metmyoglobin (Wulf *et al.*, 1995; Faustman *et al.*, 1998). These attributes are influenced by muscle characteristics of the animal during its rearing periods and post-mortem biochemical reactions (Ouali 1990; Jurie *et al.*, 2007). Muscle characteristics are influenced by management factors such as rearing conditions (solitary or companion), environment conditions, breed, age, sex and diet (Cuvelier *et al.*, 2006; Jurie *et al.*, 2007). Consumers have a low preference for brown meat resulting in economic losses for the farmers (Williams *et al.*, 1993). Meat will become more susceptible to oxidation and consequently reduce its shelf life if the PUFA content in it is elevated. Increased levels of confinement stress can lead to increased lipid oxidation in the meat of stressed lambs. The confinement stress leads to a rapid ATP depletion and reduced nucleotides such as the NAD^+ and NADP^+ , which are involved in the regeneration of various pro-oxidant substances. The changes in meat colour; lipid oxidation and drip loss can be slowed down by a dietary supplementation of antioxidants like vitamin E and selenium (López-Bote *et al.*, 2001). These supplements are incorporated into cell membranes, increasing the stability, shelf life, and commercial value of meat (Kerry *et al.*, 1998). Indeed, vitamin E has been shown to delay the conversion of oxymyoglobin into metmyoglobin and increasing the shelf life of lamb meat (Ripoll *et al.*, 2013).

In addition, a dietary supplementation of α -tocopherol to finishing lambs in intensive systems (confinement) has attracted many researchers (Wulf *et al.*, 1995; Lauzurica *et al.*, 2005). López-Bote *et al.* (2001) reported that a concentration range of 5.3 to 5.6 mg of α -tocopherol/kg muscle, corresponding to dietary inclusion levels of 550 to 625 mg α -tocopheryl acetate/kg diet, was

sufficient to delay meat deterioration. McDowell *et al.* (1996) and the NRC (2007) recommended that 15 to 40 mg/kg of vitamin E is adequate for ruminants, however, levels above this recommendation can improve performance, meat quality and carcass characteristics. The carcass measures characterize the product and are highly correlated with weight (Wood *et al.*, 1980; El Karin *et al.*, 1988). Vitamin E has been shown to exert a protective effect on muscle membrane lipid peroxidation thereby increasing the PUFA content (Barja *et al.*, 1993; Wood *et al.*, 2003), thus improving the nutritional quality and also extending the shelf life of meat (Kasapidou *et al.*, 2012).

The beneficial effects of adding vitamin E to diets on production performance and meat quality characteristics have been extensively studied in lambs (Álvarez *et al.*, 2008; Kasapidou *et al.*, 2012), which is considered to have a shorter shelf life than beef, possibly due to its higher content of the long-chain PUFA (Enser *et al.*, 1996). However, no studies have been carried out to investigate the potential of extra dietary vitamin E supplementation to mitigate against the anticipated negative effects of stress induced by the suboptimal housing on meat quality and carcass characteristics. In this study, we tested the hypothesis that single-pen housed sheep would endure higher stress levels that lower carcass and meat quality as compared to sheep reared in paired-pens. We further hypothesized that an extra dietary vitamin E-selenium supplementation will mitigate against the negative changes in meat and carcass quality induced by the suboptimal housing conditions. This study, therefore, compared the variation in carcass characteristics and meat quality of animals housed in single-pens (isolated) to those housed in paired-pens (socially interacting) when supplemented with extra dietary vitamin E-selenium.

5.2 Materials and methods

5.2.1 Slaughtering procedure

At the end of the feeding trial (day 60) described in Chapter 3, all 24 lambs were transported over 60 km to Zeerust abattoir for slaughter after feeding in the morning. On arrival at the abattoir, off-loading activities were monitored. These included; transferring animals to the lairages where they then stayed for 24 hours for animals to rest and for the inspection to take place. They were given water ad-libitum prior to slaughter. The sheep were stunned using an electric stunner of 650 volts for five seconds. A sharp knife was used to cut the throat and they were allowed to bleed for 6 minutes. The dressing out percentage was determined as the proportion of carcass weight to the slaughter weight. Dressed carcasses were stored at 2°C for 24 h. After 24 h, the carcasses were dissected into two equal halves and according to a dissection method described by Fisher & de Boer (1994). The left hand side (LHS) of each carcass was used for linear measurements. Meat samples were collected on all the 24 animals from the *longissimus dorsi* muscle for analysis of the drip loss, white blood cell, meat tenderness, antioxidants (GSH-px, SOD, catalase and lipid peroxide) on meat. The muscle from the right thigh of each of the 24 lambs was used to analyse meat colour and pH. A small piece from the 4th to the 6th lumbar vertebrae was also collected for α -tocopherol analysis.

5.2.2 Meat quality analysis

A pH meter (Crison pH 25, Crison instruments, S.A., Alella, Spain) was used to measure the meat pH and temperature immediately after slaughter and also at 24 hours after slaughter. The temperature sampling (T24) and ultimate pH (pHu) of the carcass were measured 24 hours after slaughtering. Meat colour was determined immediately after slaughter, 24 hours, and for 5

consecutive days thereafter, using a Minolta colour-guide 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter measurement area and an illuminant D65-day light, 10° observation angle. Three readings were taken by rotating the Colour Guide 90° between each measurement, in order to obtain a representative average value of the colour. The guide was calibrated before each day's measurements using the green standard. Drip loss and water holding capacity were analysed by the method of Dufrasne *et al.* (2000). Following cooking, some sub-samples measuring 1x1x3 cm were obtained and sheared perpendicularly to the fibre direction using a Warner Bratzler (WB) shear device mounted onto a Texturer Analyzer (TA.XTplus, Surrey, UK) and the mean peak load (N) was then recorded for each sample.

5.2.3 Statistical analysis

The meat colour, pH, tenderness, drip loss, WHC, antioxidants, and fatty acids data obtained from this experiment was analysed using a two-way analysis of variance and the GLM procedures of SAS (2010) for a 2×2 factorial treatment arrangement in a completely randomized design and according to the following linear model:

$$Y_{ijk} = \mu + H_i + V_j + (HV)_{ij} + \varepsilon_{ijk}$$

Where Y_{ijk} is the response variable (carcass characteristics and meat quality); μ = overall mean (general mean); H_i = housing effect; V_j = vitamin E-Selenium effect; HV_{ij} = interaction between housing and vitamin E-Selenium supplementation; and ε_{ijk} = the experimental error associated with observation ijk , which is assumed to be normally and independently distributed. When the analysis of variance revealed the existence of a significant difference among treatment means, the probability of difference (pdiff) option in the least square means (lsmean) statement of the GLM procedure of SAS (2010) were used to locate those treatments that were

significantly different from one another. For all statistical tests, significance was declared at $P < 0.05$.

5.3 Results

The statistical significance of the main factors and their interactions are summarized in Table 5.1 below. The results show that most of the carcass measurements and meat qualities were not significantly ($P < 0.05$) influenced by the dietary vitamin E-selenium supplementation and housing type with an exception of the slaughter weight (SLW), dressing out percentage (%) and ultimate pH (pHu). A significant interaction between supplementation $\times$ housing was observed for the drip loss percentage ($P < 0.05$) and carcass length ($P < 0.05$).

Table 5.1: Statistical significance (P values) of effects of the main factors (Housing and Vitamin E- Se supplement) and their interactions on carcass measurement and meat quality parameters of the Dohne Merino lambs.

Meat quality parameters	Housing	Vitamin E supplement	Housing x Vitamin E supplement
<i>Carcass traits</i>			
SLW (kg)	NS	*	NS
Dressing out percentage	NS	*	NS
HCW (kg)	NS	NS	NS
CCW (kg)	NS	NS	NS

Carcass conformation	NS	NS	NS
Fat score	NS	NS	NS
Length (cm)	NS	NS	*
pHu	NS	NS	NS
Temperature (°C)	NS	NS	NS
<i>Meat traits</i>			
Drip loss (%)	NS	NS	*
Water holding capacity	NS	NS	NS
<i>Meat color</i>			
L*	NS	NS	NS
a*	NS	NS	NS
b*	NS	NS	NS
<i>Shelf life colour & pHu</i>			
L*	NS	NS	NS
a*	NS	NS	NS
b*	NS	NS	NS
pHu	NS	*	NS

NS: $P > 0.05$; * $P < 0.05$; ** $P < 0.01$

L* = lightness, a* = redness, b* = yellowish, HCW = hot carcass weight, CCW = cold carcass weight, pHu = ultimate pH.

Table 5.2: Effects of vitamin E-selenium supplementation on carcass and meat quality of the Dohne Merino lambs.

	Dietary supplement		Standard error
	No Vitamin E	Vitamin E	
<i>Carcass traits</i>			
SLW	32.00 ^b	37.97 ^a	1.442
Dressing out percentage	16.77 ^b	20.93 ^a	0.665
HCW (kg)	15.23	17.03	1.012
CCW (kg)	14.47	16.90	0.907
Carcass conformation	3	3	0
Fat score	2	2	0

Length (cm)	83.08	86.45	1.921
pHu	6.07	5.91	0.167
Temperature (°C)	45.27	45.66	0.613
<i>Meat traits</i>			
Water holding capacity	1.55	1.8	0.139
Meat color			
L*	3.22	2.44	0.626
a*	8.12	7.67	0.319
b*			
Shelf life colour & pHu			
L*	36.07	36.52	1.126
a*	8.98	9.12	0.448
b*	6.28	7.21	0.73
pHu	5.87 ^a	6.11 ^b	0.06

^{ab}In a row, different superscripts denote significant differences between the dietary means ($P < 0.05$).

L* = lightness, a* = redness, b* = yellowish, HCW = hot carcass weight, CCW = cold carcass weight, pHu = ultimate pH.

The slaughter weight, (37.97 kg), dressing percentage (20.93%) and pHu (on shelf life) (6.11) were significantly ($P < 0.05$) higher in lambs fed with the dietary vitamin E-selenium and lower in the control group (32.00 kg, 16.77% and 5.87, respectively) (Table 5.2).

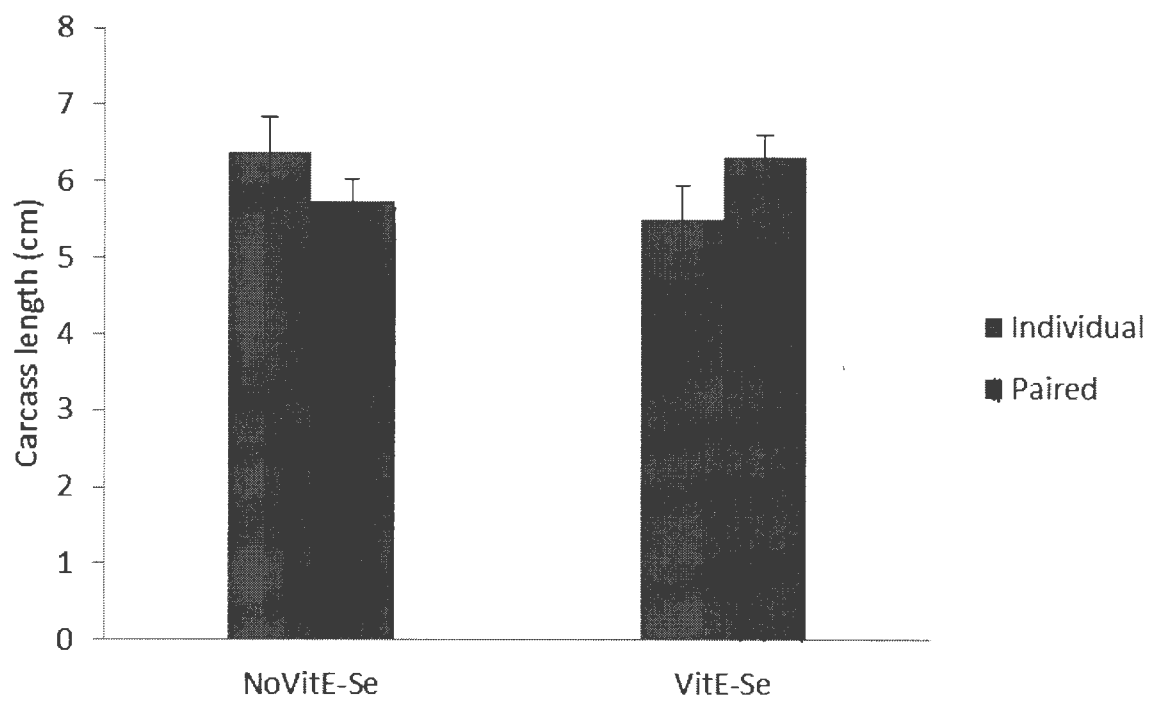


Figure 5.1: Interactions between the housing and dietary supplementation effects on drip loss and carcass length.

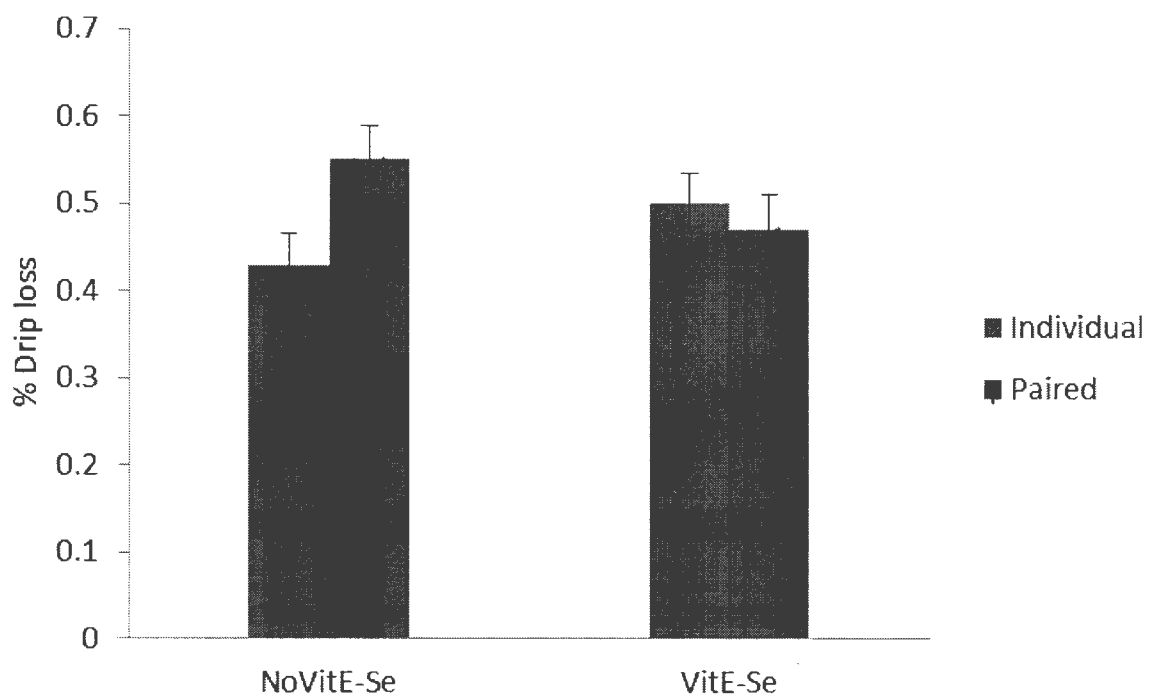


Figure 5.2: Interactions between the housing and dietary supplementation effects on drip loss.

There were no differences observed ($P > 0.05$) on drip loss, amongst the vitamin E-selenium supplemented groups and the non-supplemented groups, irrespective of the housing conditions. Both the dietary vitamin E and selenium supplementation reduced drip loss percentage in the paired group, but increased it in the individual group, while in the non-supplemented group, an increase in the drip loss percentage was observed in the paired group and decreased in the individual groups.

5.4 Discussion

Meat colour, flavour and odour can be altered during mechanical processing. During storage of meat and meat products, lipid oxidation is the main factor causing loss of quality. Hence the addition of vitamin E-selenium in ruminant diets in confinement systems can bring about benefits in terms of carcass characteristics, meat quality and the shelf-life of meat. Vitamin E plays a vital role in reducing lipid peroxidation, increasing the antioxidant enzyme activity and interacting with a number of antioxidants such as selenium to combat the damage of oxidation on meat quality (Coombes *et al.*, 2001). Selenium is even more effective as an antioxidant through the production of a high density lipoprotein when combined with vitamin E (Tapiero *et al.*, 2003).

Vitamin E-selenium supplementation in this study did not have a significant effect on most of the carcass characteristics, meat quality and shelf life of the meat. These findings are in agreement with those of Zhao *et al.* (2013), who found no significant differences in terms of main carcass

indices (hot carcass weight, LD muscle area (rib-eye area) and pH) in lambs allotted individually in shaded pens (1.0 m × 1.1 m) and allowed visual contact with each other. Similarly, Macit *et al.* (2003a) and Macit *et al.* (2003b) found no significant vitamin E supplementation effect on the carcass measurements except for the width of the gigots. Atay *et al.* (2009) also found no differences in the shelf life of meat in terms of its color (L*, a* and b* values) on the longissimus dorsi during a 12-day storage period. In contrast, Morrissey *et al.* (1994) indicated that the presence of vitamin E and selenium within muscle cell membranes reduced lipid oxidation, improving the quality characteristics of meat such as color, flavor, texture and nutritional value, and also extending its shelf-life. In addition, dietary vitamin E and selenium considerably reduce the drip loss and pH 24 h of the LD muscles (Zhao *et al.*, 2013). Other authors also found a significant difference for hot and cold carcass weights when evaluating lambs slaughtered at different ages and weights (Bueno *et al.*, 2000; Motta *et al.*, 2001). In reference to this report, the non-significant effects observed on most of the carcass indices might probably be attributed to the fact that the lambs used were of the same age and the commercial diet used had adequate crude protein energy levels.

The significantly higher slaughter weight and dressing out percentage observed for the supplemented group might be hinged onto the synergism aspect between Vit E and selenium, acting as an antioxidant, boosting the immunity of the experimental sheep as well as maintaining their physiological balance, hence, leading to the observed increased slaughter weights. These findings are in agreement with Motta *et al.* (2001); Abdullah & Qudsieh, (2008); Zhao *et al.* (2013). Zhao *et al.* (2013) found an increase in the dressing out percentage when he gave supplementation levels of below 100 IU vitamin E per lamb per day and those above 200 IU per lamb per day. A significant interaction between the housing type and vitamin E-selenium and

observed for the drip loss and carcass length is in contradiction with Zhao *et al.* (2013) who found that a vitamin E supplementation above 100 IU/lamb/day considerably reduced the drip loss. This reduction in meat drip losses has been attributed to the antioxidant activity of vitamin E- selenium, which may stabilize the membrane integrity post-mortem, thus retaining the sarcoplasmic protein in cells (Ashgar *et al.*, 1991). The mechanism by which the drip or purge is lost from meat is influenced by both the pH of the tissue and the amount of space in the muscle cell, and particularly the myofibril that exists for water to reside (Lagerstedt *et al.*, 2011; Vázquez *et al.*, 2004). However, in the present study, the pH and water holding capacity was not significantly affected by the dietary vitamin E and selenium hence no significant effect onto the drip loss. In the current study, no aspect of meat color was significant to any treatment. This was not expected due to the fact that vitamin E and selenium have been shown to delay the conversion of oxymyoglobin into metmyoglobin and increase the shelf life of lamb meat (Kasapidou *et al.*, 2012; Ripoll *et al.*, 2013). Nevertheless, the meat color values in the current study still fell within the acceptable ranges (Owens *et al.*, 2000).

5.5 Conclusion

In the current study, dietary vitamin E and selenium was shown to favorably affect slaughter weight and dressing out percentage. However, dietary vitamin E and selenium did not have any significant effect on most of the carcass measurements and meat quality characteristics. It can, therefore, be concluded that dietary vitamin E and selenium could not mitigate the effect of housing-induced stress on most carcass measurement and meat quality traits.

5.6 References

- Abdullah, A.Y & Qudsieh, R.I., 2008. Carcass characteristics of Awassi ram lambs slaughtered at different weights. *Livst. Sci.* 117, 165-175.
- Álvarez, I., J. De la Fuente, M.T. Díaz, S. Lauzerica, C. Pérez, and V. Cañeque. 2008. Estimation of α -tocopherol concentration necessary to optimize lamb meat quality stability during frozen storage in high-oxygen modified using broken-line regression analysis. *Ani.* 2, 1405-1411.
- Atay, O.K.A.N., Gökdal, O., Eren, V., Çetiner. Ş & Yikilmaz, H., 2006. Effects of dietary vitamin E supplementation on fattening performance, carcass characteristics and meat quality traits of Karya male lambs. *Arch Tierz*, 52, 618-626.

- Barja, G., Cadenas, S., Rojas, C., Lopez-Torres, M & Petez-Campo, R., 1994. A decrease of free radicle production near- critical targets as a cause of maximum longevity in animals. Comb. Biochem. Physiol. Biochem. Mol. Biol. 108, 501-512.
- Bueno, M.S., Cunha, E.A & Santos, L.E., 2000. Características de carcaça de cordeiros Suffolk abatidos em diferentes idades. Revista Brasileira de Zootecnia, 29, 1803-1810.
- Coombes J.S., Powers S.K. & Rowell B., 2001. Effects of vitamin E and α -lipoic acid on skeletal muscle contractile properties. J. Appl. Physiol. 90, 1424-1430.
- Cuvelier, C., Clinquart, A., Hocquette, J.F., Cabaraux, J.F., Dufrasne, I., Istasse, L. and Hornick, J.L., 2006. Comparison of composition and quality traits of meat from young finishing bulls from Belgian Blue, Limousin and Aberdeen Angus breeds. Meat. Sci, 74, 522-531.
- El Karin, A.I.A., Owens, J.B & Whitaker, C.J., 1988. Measurement on slaughter weight, side weight, carcass joints and their association with composition of two types of sudan desert sheep. J. Agric. Sci. 110, 65-69.
- Enser, M., Hallett, K., Hewitt, B., Fursey, G.A.J & Wood, J.D., 1996. Fatty acid content and composition of English beef, lamb and pork at retail. Meat. Sci. 42,443-456.
- Faustman, C., W. K. M. Chan, D. M. Schaefer, & A. Havens., 1998. Beef color update: The role for vitamin E. J. Anim. Sci. 76, 1019–1026.
- Fisher, A.V., & de Boer, H., 1994. The EAAP standard method of sheep carcass assessment.Carcass measurements and dissection procedures. Lives. Prod. Sci. 38, 149-159.

- Kasapidou, E., Wood, J. D., Richardson, R. I., Sinclair, L. A., Wilkinson, R. G., & Enser, M., 2012. Effect of vitamin E supplementation and diet on fatty acid composition and on meat colour and lipid oxidation of lamb leg steaks displayed in modified atmosphere packs. *Meat. Sci*, 90, 908–916.
- Kerry, J.P., Buckley, D.J., Morrissey, P.A., O'Sullivan, K & Lynch, P.B., 1998. Endogenous and exogenous alpha-tocopherol supplementation: Effects on lipid stability (TBARS) and warmed-over flavour (WOF) in porcine M-longissimus dorsi roasts held in aerobic and vacuum packs. *Food. Res. Intl.* 31, 211–216.
- Jurie, C., Picard, B., Hocquette, J.F., Dransfield, E., Micol, D. and Listrat, A., 2007. Muscle and meat quality characteristics of Holstein and Salers cull cows. *Meat. Sci*, 77, 459-466.
- Lauzurica, S., de la Fuente, J., Diaz, M.T., Alvarez, I., Perez, C & Caneque, V., 2005. Effect of dietary supplementation of vitamin E on characteristics of lamb meat packed under modified atmosphere. *Meat Sci.* 70, 639–646.
- López-Bote, C.J., Daza, A., Soares, M & Berges, E., 2001. Dose–response effect of dietary vitamin E concentration on meat quality characteristics in light-weight lambs. *Ani. Sci.* 73, 451–457.
- Lyan, B., Azais-Braesco, V., Cardinault, N., Tyssandier, V., Borel, P & Alexandre-Gouabau, M.C., 2001. Simple method for clinical determination of 13 carotenoids in human plasma using an isocratic high-performance liquid chromatographic method. *J. Chromatogr B.* 751, 297–303.

- Macit, M., Aksakal, V., Emsen, E., Aksu, M.İ., Karaoglu, M & Esenbuga, N., 2003a. Effects of Vitamin E supplementation on performance and meat quality traits of Morkaraman male lambs. *Meat Sci.* 63, 51-58.
- Macit, M., Aksakal, V., Emsen, E., Esenbuga, N & Aksu, M.I., 2003b. Effects of vitamin E supplementation on fattening performance, non-carcass components and retail cut percentages, and meat quality traits of Morkaraman male lambs. *Meat Sci.* 64, 1-6.
- Morrissey, P.A., D.J. Buckley, P.J.A. Sheehy, and F.J. Monahan. 1994. Vitamin E and meat quality. *Proc. Nutri. Soc.* 53, 289-295.
- Motta, O.S., Pires, C.C., Silva, J.H.S., Avaliação da carcaça de cordeiros da raça Texel sob diferentes métodos de alimentação e pesos de abate. *Ciência Rural* 31, 1051-1056.
- Ouali, A. and Talmant, A., 1990. Calpains and calpastatin distribution in bovine, porcine and ovine skeletal muscles. *Meat Sci.* 28, 331-348.
- Petherick, J.C & Phillips, C.J.C., 2009. Space allowances for confined livestock and their determination from allometric principles. *App. Anim. Behav. Sci* 117, 1–12.
- Ripoll, G., González-Calvo , L., Molino, F., Calvo , J.H & Joy, M., 2013. Effects of finishing period length with vitamin E supplementation and alfalfa grazing on carcass color and the evolution of meat color and the lipid oxidation of light lambs. *Meat. Sci.* 93, 906–913.
- Surai, P.F., 2002. Selenium in poultry nutrition 2. Reproduction, egg and meat quality and practical applications. *World's. Poult. Sci. J.* 58, 431-450.

- Tapiero, H., Townsend, D.M & Tew, K.D., 2003. The antioxidant role of selenium and seleno-compounds. *Biomed Pharm.* 57, 134–44.
- Turner, K.E., McClure, K.E., Weiss, W.P., Borton, R.J & Foster, J.G., 2002. Alpha-tocopherol concentrations and case life of lamb muscle as influenced by concentrate or pasture finishing. *J. Ani. Sci.* 80, 2513–2521.
- Wood, J.D., Macfie, H.J.H & Pomery, R.W., 1980. Carcass composition in four sheep breeds: The importance of breed and stage of maturity. *Ani. Prod.* 30, 135-152.
- Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P. R & Enser, M., 2003. Effects of fatty acids on meat quality: A review. *Meat Sci.* 66, 21-32.
- Wulf, D.M., Morgan, J.B., Sanders, S.K., Tatum, J.D., Smith, G.C & Williams, S., 1995. Effects of dietary supplementation of vitamin E on storage and case life properties of lamb retail cuts. *J. Ani. Sci.* 73, 399–405.
- Williams, G.T. & Smith, C.A., 1993. Molecular regulation of apoptosis: genetic controls on cell death. *Cell* 74, 777-779.
- Zhao, T., Luo, H., Zhang, Y., Liu, K., Jia, H., Chang, Y., Jiao, L. & Gao, W., 2013. Effect of vitamin E supplementation on growth performance, carcass characteristics and intramuscular fatty acid composition of Longissimus dorsi muscle in Tan'sheep. *Chilean J. Agric. Res.* 73, 358-365.

6 CHAPTER SIX: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

The study was designed to evaluate the effect of extra dietary vitamin E and selenium on growth performance, blood parameters, and meat quality of the finishing Dohne Merino lambs reared under two different types of housing confinement. Twenty four lambs with an average body weight of 25.8 ± 2.7 kg were used. The lambs were randomly assigned to four treatment combinations: single-pen housing with no extra vitamin E-selenium supplementation, single-pen housing with extra vitamin E-selenium supplementation, paired-pen housing with no extra vitamin E-selenium supplementation, and paired-pen housing with extra vitamin E-selenium supplementation, in a completely randomized design. In chapter 3, the growth performance and physiological parameters were determined. The overall weight gain and overall feed conversion ratio across the four treatments showed no significant differences ($P > 0.05$). A linear and steady increase in body weights was observed throughout the experiment for all the treatments. Our findings are consistent with those of other researchers (Dufrasne *et al.*, 2000; Macit *et al.*, 2003a; Juniper *et al.*, 2006; Maiorano *et al.*, 2007). Also a linear and steady increase was observed on feed intake. However, lambs supplemented with the vitamin E-Se consumed more feed (65.01 kg) over the entire feeding period as compared to those lambs that were not offered the supplements (60.37 kg). Findings of this current study nevertheless contradict those of other authors (Rock *et al.*, 2001; Qin *et al.*, 2007; Kumar *et al.*, 2009) and also with results reported in dairy cattle (Lee *et al.*, 2007; Ebrahimi *et al.*, 2009; Covey *et al.*, 2010). An interaction between dietary supplement and housing condition was observed on rectal temperature on the last day of the experiment. Among the vitamin E-Se supplemented sheep, animals housed in pairs had

higher rectal temperatures (39.3°C) compared to those housed individually (38.8°C). However, for the non-supplemented group, no differences ($P > 0.05$) were observed between the rectal temperatures of the paired (39.3°C) and individually (39.0°C) housed sheep (Chauhan *et al.*, 2014b).

Chapter 4 investigated the effectiveness of extra dietary vitamin E-selenium supplementation in mitigating housing-induced stress in the Mutton Merino lambs as measured by haematology, serum biochemistry and stress biomarkers. The extra dietary vitamin E and selenium supplementation had no significant effect on most of the haematological indices, including WBC as well as most of the serum biochemistry. Our findings corroborate with those of Lepherd *et al.* (2009) who reported that most of the blood haematological variables and serum biochemical variables measured were unaffected by the vitamin E and Se injection but were within the normal reference ranges for the clinically healthy sheep. In the present study, sheep supplemented with extra vitamin E and selenium had slightly higher RBC ($12.61 \times 10^{12}/L$), HCT/PCV (28.88 %) and MCHC (32.24 g/dL) values compared to the control group ($11.17 \pm 9.45 \times 10^{12}/L$, 24.87 %, 15.96 g/dL, respectively). Contrary, their MCH values followed an opposite trend with a slightly lower value (8.14 pg) in the un-supplemented sheep as compared to the supplemented group (9.02 pg) but still the findings are consistent with those of Faixova *et al.* (2007).

Chapter 5 reports the variation in carcass characteristics and meat quality of animals housed in single-pens (isolated) to those housed in paired-pens (socially interacting) when supplemented with extra dietary vitamin E-selenium. The results showed that most of the carcass measurements and meat quality characteristics were not influenced by the extra dietary vitamin E-selenium supplementation and housing type (Zhao *et al.*, 2013) with an exception of the slaughter weight,

dressing percentage and pHu (on shelf life). These parameters were higher in lambs fed with the dietary vitamin E-selenium than in the control group (Motta *et al.*, 2001; Abdullah & Qudsieh, 2008; Zhao *et al.*, 2013). An interaction between supplementation $\times$ housing was observed for the drip loss percentage and carcass length. But there were no differences observed for carcass length between the vitamin E-selenium supplemented groups and the non-supplemented groups, irrespective of the housing conditions. Individuals in the non-supplemented group showed longer carcass lengths as compared to those in the paired groups while the paired and vitamin E-Se supplemented group showed longer carcass lengths as compared to the individual VitE-Se supplemented group. Also, no differences were observed on drip loss between the vitamin E-selenium supplemented groups and the non-supplemented groups, irrespective of the housing conditions. The dietary vitamin E and selenium supplementation reduced drip loss % in the paired group, but not in the individual group, while in the non-supplemented group, an increase in the drip loss % was observed in the paired group and decreased in the individually-housed group. From this, it can be confidently concluded that the extra dietary vitamin E and selenium improved the slaughter weight and dressing out percentage, while an interaction between the housing type and vitamin E-selenium also had a positive effect onto the drip loss and carcass length values of meat than those of the non-supplemented group.

6.2 Conclusion

The extra dietary vitamin E and selenium induced more appetite, as sheep in the supplemented group consumed more feed than those in the control group. On the other hand, the RBC, HCT/PCV, MCHC, slaughter weight, dressing percentage and pHu (on shelf life) were higher in the supplemented group than in the control group. It can, therefore, be concluded that the

companion housing and solitary confinement induced different levels of stress, as an interaction was correspondingly observed on the rectal temperature, drip loss percentage and carcass length.

6.3 Recommendations

1. Extra dietary vitamin E and selenium can be supplemented into the lamb's diet without any negative effect on the performance, physiological parameters, blood parameters and meat quality.
2. Extra dietary vitamin E and selenium can be used to help in mitigating the effects associated with confinement stress. However more studies needs to be made.

6.4 References

- Abdullah, A.Y & Qudsieh, R.I., 2008. Carcass characteristics of Awassi ram lambs slaughtered at different weights. *Livest. Sci.* 117, 165-175.
- Chauhan, S.S., Celi, P., Leury, B.J., Clarke, I.J & Dunshea, F.R., 2014b. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. *J. Anim. Sci.* 92, 3364–3374.
- Covey, T. L., Elam, N.E., Carroll, J.A., Wester, D.B & Galyean, M.L., 2010. Supplemental selenium source in Holstein steers challenged with intranasal bovine infectious rhinotracheitis virus and in newly received beef heifers: Performance, morbidity, antibody titers, and blood cell counts. *Prof. Anim. Sci.* 26, 82–92.
- Dufresne, I., Marche, A., Clinquart, J.L., Hornick, C., van Eenaeme. C & Istasse, L., 2000. Effects of dietary vitamin E supplementation on performance and meat characteristics in fattening bulls from the Belgian Blue Breed. *Livest. Prod. Sci.* 65, 197-201.
- Ebrahimi, M., Towhidi, A & Nikkhah, A., 2009. Effects of organic selenium (Sel-Plex) on thermometabolism, blood chemical composition and weight gain in Holstein suckling calves. *Asian-Australas. J. Anim. Sci.* 22, 984–992.
- Faixova, Z., Faix, Š., Leng, Ľ., Vaczi, P., Makova, Z. & Szaboova, R., 2007. Haematological, blood and rumen chemistry changes in lambs following supplementation with Se-yeast. *Acta Veterinaria Brno.* 76, 3-8.
- Lee, S. H., Park, B.Y., Yeo, J.M., Lee, S.S., Lee, J.H., Ha, J.K & Kim, W.Y., 2007. Effects of different selenium sources on performance, carcass characteristics, plasma glutathione

peroxidase activity and selenium deposition in finishing Hanwoo steers. *Asian-Australas. J. Anim. Sci.* 20, 229–236.

Lepherd, M.L., Canfield, P.J., Hunt, G.B & Bosward, K.L., 2009. Haematological, biochemical and selected acute phase protein reference intervals for weaned female Merino lambs. *Austr. Vet. J.* 87, 5-11.

Macit, M., Aksakal, V., Emsen, E., Aksu, M.İ., Karaoglu, M & Esenbuga, N., 2003. Effects of Vitamin E supplementation on performance and meat quality traits of Morkaraman male lambs. *Meat. Sci.* 63, 51-5.

Maiorano, G., Cavone, C., McCormick, R.J., Ciarlariello, A., Gambacorta, M & Manchisi, A., 2007. The effect of dietary energy and vitamin E administration on performance and intramuscular collagen properties of lambs. *Meat. Sci.* 76, 182-188.

Motta, O.S., Pires, C.C., Silva, J.H.S., 2001. Avaliação da carcaça de cordeiros da raça Texel sob diferentes métodos de alimentação e pesos de abate. *Ciência Rural* 31, 1051-1056.

Qin, S., Gao, J & Huang, K., 2007. Effects of different selenium sources on tissue selenium concentrations, blood GSH-Px activities and plasma interleukin levels in finishing lambs. *Biol. Trace Elem. Res.* 116, 91–102.

Rock, M. J., Kincaid, R. L., & Carstens, G.E., 2001. Effects of prenatal source and level of dietary selenium on passive immunity and thermometabolism of newborn lambs. *Small Rumin. Res.* 40, 129–138.

Kumar, N., Garg, A. K., Dass, R.S., Chaturvedi, V.K., Mudgal, V & Varshney, P., 2009.

Selenium supplementation influences growth performance, antioxidant status and immune response in lambs. *Anim. Feed Sci. Technol.* 153, 77–87.

Zhao, T., Luo, H., Zhang, Y., Liu, K., Jia, H., Chang, Y., Jiao, L. & Gao, W., 2013. Effect of vitamin E supplementation on growth performance, carcass characteristics and intramuscular fatty acid composition of Longissimus dorsi muscle in Tan'sheep. *Chilean J. Agric. Res.* 73, 358-365.