AUSTRALIAN MEAT PROCESSOR CORPORATION

Enhancing Retail Colour Stability and Shelf Life of Lamb Meats for Key Markets

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Prepared by:	Professor Frank Dunshea, Dr Surinder Chauhan and Dr Eric Ponnampalam
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1.0 Executive Summary

Consumers choose to buy meat primarily by visual appearance. A meat cut red in colour is more likely to be sold than a cut brown in color forcing the retailers to discount the prices of discolored meat leading to economic losses. The appearance of brown on the meat surface is an oxidative process which reflects the antioxidant potential of muscles. Antioxidants have been reported to influence both lipid and protein oxidation and Vit E (α-tocopherol) and have been extensively documented to minimize lipid oxidation and improve meat colour stability (Ponnampalam et al., 2012; Suman et al., 2014a). However, there is not sufficient work published on the oxidative status of finisher lambs during hot summer days. Considering that growth is a trait that is affected by the intake of dietary antioxidants (Catoni et al., 2008) and that the high metabolic rate of growing tissues produces elevated levels of free radicals (Rollo, 2002), we hypothesize that antioxidant supplementation may help to counter the negative effects of oxidative stress (OS) associated with growth and short term heat stress (HS) which is often experienced by the finisher lambs in Australia. Therefore, the present project investigated the impact of dietary vitamin E (Vit E) and selenium (Se) supplementation during the finishing period on the oxidative status and meat colour stability of lambs exposed to short term hot conditions during the finishing period. The specific questions investigated were whether meat colour stability and shelf life can be enhanced by inclusion of high levels of antioxidants in finishing diets to reduce the negative impacts of heat stress on lambs. This project also compared the retail colour stability and shelf life of lambs finished on different levels of dietary antioxidants, under a grain based vs pasture system, under heat stress and thermoneutral conditions during finishing, transportation and in lairage.

The first study investigated the dose response to dietary Vit E and Se supplementation on live weight gain, oxidative status, carcass quality and meat retail colour stability of lambs finished under hot conditions before transport and subsequent lairage. Forty-eight lambs (crossbred; 42 ± 2 kg body weight, 7 mo age) were allocated to one of three groups (16 per group) and fed 3 different doses of Vit E and Se. A standard finisher pellet ration (17% CP and 12.57 MJ ME/kg DM) was used as the basis of treatment diets. The doses of Vit E and Se for control (CON), moderate (MOD), and supranutritional (SUP) diets were 28, 130, 228 mg/kg DM as α- tocopherol acetate and 0.16, 0.66, 1.16 mg Se as SelPlex™ kg/DM, respectively. After 4 weeks feeding in individual pens, including 1 week of adaptation, lambs were moved to metabolism cages for 1 week and allocated to one of 2 heat regimes (8 per feeding group): thermoneutral (TN) (18-21°C and 40-50% relative humidity) or heat stress (HS) (28-40°C and 30-40% relative humidity) conditions. Dietary Vit E and Se supplementation increased average daily feed intake ($P = 0.009$) and average daily gain ($P = 0.057$) linearly (CON to SUP) during the 3 week finisher period before thermal treatments were applied. During thermal treatment, HS elevated ($P < 0.001$) both respiration rate (RR) and rectal temperature (RT). However, there were reductions in both RR ($P = 0.05$) and RT ($P = 0.08$) in lambs fed SUP levels of Vit E and Se. The concentration of plasma reactive oxygen metabolites (ROM) increased ($P < 0.001$) under HS, but were reduced by the SUP diet (117 CARR U) as compared to CON (128 CARR U). The concentration of plasma advanced oxidation protein products (AOPP) were increased ($P < 0.001$) during HS. Again, lambs fed the SUP diet had 35% lower (P = 0.005) AOPP levels than maximum levels in CON lambs when exposed to heat. Both RR and RT of lambs finished under HS were normalized after 14 h in lairage under TN conditions.

Supranutritional antioxidant supplementation improved the hot standard carcass weights and decreased the GR depth at moderate levels. Furthermore, moderate levels of antioxidant supplementation for 4 weeks during the finishing phase, increased the concentration of vitamin E in the muscle of lambs given dietary vitamin E @130 mg/kg as compared with control (vitamin E @ 27.6 mg/kg) group (2.13 vs 1.72 mg/kg, respectively). However, the vitamin E concentration achieved in the muscle was not sufficient to improve meat colour stability or shelf life. It was concluded that either higher levels of dietary vitamin E or a longer duration (>3 weeks) of supplementation is required to improve the colour stability of fresh and aged meat of lambs finished under hot conditions.

The second study compared the effects of a lucerne-based diet versus a grain-based diet supplemented with VitE on muscle VitE concentration and the retail colour stability of lamb meat. Forty-one Lambs (n=41) were fed for 8 weeks either a lucerne-based diet (37 mg/kg of VitE) or a grain-based control (CON; 42 mg/kg of VitE) or supranutritional VitE (SUP; 285 mg/kg of VitE) diet. Loin muscle samples were assessed for VitE concentration and retail colour. Lambs fed the SUP diet had a higher muscle VitE concentration (5.1 mg/100g meat; $p<0.001$, SED = 0.44) compared to CON (2.5 mg) or LUC (3.4mg). No difference was detected for meat colour evaluated as redness (a*-value) at day 0 of display (LUC=17.5, CON=17.7; SUP=17.5) while at day 4 of display redness of meat tended to be higher for LUC (16.9; $p<0.1$, SED = 0.65) than CON (15.6) or SUP (15.7). Although the SUP group had greater muscle VitE status, the lucerne-based diet maintained retail colour of meat better than the VitE supplemented grain-based diet. It was concluded that higher incorporation of VitE can be achieved in muscle through supranutritional supplementation in the diet during finishing period of 3-4 weeks while to improve the retail colour stability of lamb meat for key markets, lambs may be finished on lucerne-based diet as other micronutrients may also influence the antioxidant activity and retail colour stability.

2.0 Introduction

Consumer's decision to buy meat is strongly influenced by meat colour. The stability of meat colour is affected by biochemical components (lipids & iron) and antioxidant content of muscle on farm, processing at the abattoir and retail display practices. The appearance of brown on the meat surface is an oxidative process which reflects the antioxidant potential of muscles; therefore, increasing the concentration of antioxidants in the muscle has an influence on meat colour. Under a changing climate there will be more frequent heat waves and increased exposure of animals to prolonged periods of high environmental temperature and relative humidity. For lambs born in spring this means they are finished in summer increasing the likelihood of heat stress and this coincides with reduced nutrient (antioxidant) availability in grazed pasture or forage. This can lead to excessive free radical production under heat stress, impairing the antioxidant content of muscles thus affecting retail colour stability and the shelf life of meat. The problem is likely to be exacerbated by exposure of animals to heat stress during the transportation to abattoirs and during lairage.

Environmental heat stress is traditionally thought to reduce animal performance due to reduced nutrient intake, however recently, this notion has been challenged with observations that there are direct effects of heat stress on animal physiology that markedly alters post absorptive carbohydrate, lipid, and protein metabolism (Baumgard and Rhoads, 2012). Heat stress induced economic losses, are not only associated with the decline in production, but are also contributed by the poor health status, increased incidence of diseases and high mortality.

The health status of the animals is argued to be affected because of the increased oxidative stress in the animals under exposure to heat stress (Bernabucci et al., 2002; Mitchell and Russo, 1983). The increased oxidative stress leads to the peroxidation of lipids and may thus compromise the retail colour stability and shelf life of meat. With the changing climatic conditions, there will be more frequent heat waves and increased exposure of animals to prolonged periods of higher environmental temperature and solar radiation leading to heat stress. In our recent experiments on sheep under controlled conditions, we have found that heat stress leads to increased reactive oxygen metabolites (ROMs) levels in plasma and the biological antioxidant potential was reduced.

The importance of sufficient levels of antioxidants in meat, especially vitamin E as it is the major lipid soluble chain breaking antioxidant present in the lipid membranes and selenium because of its vitamin E sparing effect, is accentuated by the changing practices to increase the intramuscular fat (marbling) content, PUFA and the iron content in ruminant meats. In the presence of highly reactive oxygen metabolites, PUFA are the prime targets for these radicals because of the presence of double bonds. Also, iron as a potent pro-oxidant, can react with less reactive hydrogen peroxide and lead to the production of metmyoglobin and highly reactive hydroxyl radicals leading to the further damage of PUFA and colour deterioration.

As suggested by Ponammpalam et al., 2012 high levels of essential fatty acids such as PUFA can only be maintained in muscle tissues by accessing vitamin E that is already present in the muscle. They further suggested that vitamin E can be maintained through dietary supplementation or pasture feeding to improve stability of colour, lipid oxidation and nutritional value of meat. However, it is unknown what the optimum levels of dietary antioxidants to be incorporated in the muscle to be sufficient to optimise the functionality and nutritional value of meat under heat stress conditions. In some meat markets, it is a common practice to apply natural antioxidants to the surface of meat or blend antioxidants into ground meat; however, such practices are not permitted for fresh meat sold in Australia and New Zealand or for export to the Middle East and other countries under vacuum packaging.

Furthermore, it is traditionally known that lambs become deficient in vitamin E during summer and autumn period in Southern and Western Australia (Sheep CRC) due to the lower availability of green feed, however with our recent heat stress experiments we hypothesize that excessive free radical production under heat stress consumes the antioxidants further reducing the antioxidant levels of lambs during summer. More interesting were the findings that supranutritional levels of vitamin E and selenium have the potential to reverse the negative effects of heat stress on sheep oxidative and antioxidant potential. Heat stress also may influence the collagen content in muscles so that objective measures of tenderness of meat are essential.

Therefore, the question is whether meat colour stability and shelf life can be enhanced by inclusion of high levels of antioxidants in finishing diets to reduce the negative impacts of heat stress on lambs. This project will compare the retail colour stability and shelf life of meat from lambs finished on different levels of dietary antioxidants, under a grain based vs pasture system, under heat stress and thermoneutral conditions during finishing, transportation and in lairage.

3.0 Project Objectives

1. The premise of proposed research project is to demonstrate the effect of heat stress on blood lipid metabolites and antioxidant potential in live animal and the colour of fresh and stored lamb meat and to exploit the potential of dietary antioxidants at supranutritional levels to improve the meat colour stability, shelf life and nutritive value.
2. Measurement of heat stress (Respiration rate, rectal temperature) and oxidative stress (Free radical estimation, antioxidant potential in blood samples) in lambs during the finishing phase, transportation and in lairage.
3. Assess meat quality on fresh as well packed meat samples after slaughter (muscle quality, colour, retail colour and shelf life) from animals reared under heat stress and thermoneutral conditions.
4. Estimation of oxidant and antioxidant levels in the fresh and aged muscle samples after slaughter from animals reared under heat stress and thermoneutral conditions.

4.0 Methodology

The present research was completed by undertaking two experiments at two different locations.

4.1 Experiment 1

4.1.1 Experimental Animals

Forty-eight crossbred lambs (White Suffolk x Merino x Border Leicester; 42 ± 2 kg Bw, 7 mo old), previously reared on pastures were allocated to receive one of three doses of Vit E and Se incorporated in a standard finisher pellet. The doses of Vit E and Se for control (n = 16: CON), moderate (n = 16: MOD), and supranutritional (n = 16: SUP) diets were 28, 128, 228 IU/ kg DM as α- tocopherol acetate and 0.16, 0.66, 1.16 mg/kg Se as selenized yeast, respectively. The control diet was a standard lamb finisher pellet (Rivalea Australia Pty Ltd, Albury Road Corowa NSW 2646) and supplemented with Vit E and Se to achieve different levels of antioxidants to formulate MOD and SUP diets. All three diets contained 17% CP and 12.57 MJ ME/kg on DM basis.

After 4 weeks, including 1 week of adaptation, of antioxidant feeding in individual pens, the lambs were moved to metabolism cages for 1 week and randomly allocated to either thermoneutral (TN; 18-21°C and 40-50% relative humidity: n = 24) or heat stress (HS; 28-40°C and 30-40% relative humidity: n = 24) conditions. The temperature was set to rise daily at 0900 h to eventually reach a maximum of 40°C by 1400h and then maintained at 39 ± 1ºC until 1700 h followed by a decline to reach 28°C at 2000 h. The temperature was set to 28°C overnight from 2000h to 0900h and varied from 26 to 29°C. Relative humidity was set at 35% and varied from 30 to 40%. When the temperature was between 38 and 40°C, the RH dropped to 30% and varied between 36 to 40% overnight (2000h to 0700h). These conditions were selected based on Bureau of Meteorology records for the region, for the period of the study. The temperature in the thermoneutral room was maintained between 18-21°C during each 24 h period and the average RH was 48%. The metabolism crates were approximately 1.0 x 0.5 m and stood 1.0 m off the ground with metal mesh floors and metal walls. Access to feed and water was provided by troughs and buckets attached to the front and side of the cages, respectively.

Lambs were weighed on the first day of the experiment at 0800 h before feeding to record their initial live weight. During three weeks of antioxidant feeding, lambs were weighed at weekly intervals, and during the last week of the study (when thermal treatments were applied) they were weighed on days 1 and 7 of the thermal treatment. Lambs were fed twice daily at 0900h and 1600h throughout the study with refusals recorded daily at 0800 h and average daily feed intake (ADFI) was calculated. Freshwater was provided daily. For lambs exposed to HS during the thermal treatment the water was replenished 2-3 times per day or as needed.

4.1.2 Physiological Measures

Respiration rate was recorded four times per day at 0900, 1300, and 1700 hr by counting the number of breaths per 10 second period and extrapolating to one minute. Heart rate was recorded four times per day at 0900, 1300, and 1700 hr by aide of a stethoscope and counting the number of beats per 20 second period and extrapolating to one minute.

Rectal temperature was measured using a digital thermometer at the same time intervals as respiration rate (0900, 1300, and 1700) for the duration of the study. Skin temperature was measured using a handheld thermometer similar to the rectal thermometer by placing the reader end of the measuring device in between the wool folds and pressed gently against the body of the animal. Skin temperature measurements were taken at the same time as rectal temperatures.

4.1.3 Blood Sampling

During the study, blood samples were taken on day 1 and d 7 of heat stress during each replication at 1300, hr. At each sample time 2 x 10ml sodium EDTA blood tubes (vacutainers, BD) were collected via vein puncture (jugular vein). Blood samples were then processed for white blood cell collection and plasma sample collection. Once processed, these samples have since been stored at 4°, -20°, or -80° C depending on assay procedure requirements until assay can be initiated.

4.1.4 Transportation, Lairage and Slaughter

At the end of heat stress (d 7) in each replication (12 lambs x 4 replications), the lambs were transported from Dookie Campus, University of Melbourne to JBS Australia Pty Limited, Brooklyn. The lambs were kept in lairage for 15 hrs and respiration rates and rectal temperature were recorded in the morning and one blood sample was collected before the animals were sent for slaughter. Animals were slaughtered as per the standard procedures followed at the abattoir and muscle samples were collected for colour assessment on fresh muscle and also stored at 2^0C for 6 weeks for the assessment of retail colour stability and shelf life. Muscle tissues were stored in liquid nitrogen and later in – 80 ^{0}C for gene expression and – 20 ^{0}C for antioxidant (Vit E) and fatty acid composition analysis. At 30 min post-slaughter, muscle (longissimus lumborum) samples (5 g) were collected for gene expression (associated with oxidative potential) and then carcass weight, GR were recorded. At 24 h post-mortem, muscle (longissimus lumborum) samples were collected for colour assessment (fresh & aged) and vitamin E concentration.

4.1.5 Laboratory Analysis

Plasma obtained on the last day of the thermal treatment (day 7) and after 14 h in lairage (d 8) was analyzed for reactive oxygen metabolites (ROMs), biological antioxidant potential (BAP), advanced oxidation protein products (AOPP) and cortisol. The concentration of plasma ROMs and the BAP were measured by commercial kits (Diacron International, Grosseto, Italy) by means of a dedicated spectrophotometer (FREE Carpe Diem, Diacron International, Grosseto, Italy).

The degree of OS was expressed as an OS Index (OSI), which was estimated using the ratio of ROMs/BAP x 100. The plasma concentration of AOPP was estimated according to WitkoSarsat et al. (1996) and was expressed as µmoles/L of chloramine-T equivalents. Plasma cortisol concentrations were estimated using a RIA technique as per the method reported by Bocking et al. (1986). The limit of detection of the cortisol assay was 0.20 ng/ml and the inter-assay coefficient of variation was 5.4%.

4.1.6 Gene Expression Analysis

Total RNA was extracted from skeletal muscle using Trizol (Invitrogen, California, USA) and PureLink® RNA Mini Kit (Ambion® by Life technologies) as per the manufacturer's protocol. The quality and quantity of RNA extracted from the tissue samples was determined using the Experion System, automated electrophoresis station (Bio-Rad Laboratories Inc, California, USA) with the Experion StdSens Analysis Kit (Bio-Rad Laboratories Inc. California, USA). The electropherograms for each sample were analyzed for the concentration of RNA and the ratio of 28S to 18S. All the samples had RNA Quality Indicator (RQI) greater than 9.0. Following quality and quantity evaluation of the RNA, total RNA (8 µL) was transcribed to cDNA using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, USA).

The genes evaluated were Glutathione peroxidase 1 (GPX 1), Mitochondrial superoxide dismutase (SOD - 2), and beta actin as a house keeper gene. Primer sets for all genes evaluated were designed using Invitrogen's OligoPerfect™ Designer, based on ovine nucleotide sequences obtained from National Centre for Biotechnology Information (NCBI) nucleotide database and synthesized by GeneWorks, Thebarton SA 5031, Australia. Real-time quantitative PCR reaction and amplification was performed in twenty-five µl reactions (including 1-3 µL of forward and reverses primer; depending on optimized concentration, and 2 µL of sample cDNA) prepared as per manufacturer's instructions using SYBR® GreenER™ Supermix (Life Technologies, USA). PCR quantification of each sample was performed in triplicate and SYBR green fluorescence was quantified in iQ5 Single Colour Real Time PCR Detection System (BioRad Laboratories INC, California, USA). Each assay plate contained non template control and a positive control (pooled cDNA), and a standard curve (five serial dilutions of a pooled cDNA sample) was run for each gene to determine amplification efficiency of the respective primer pair.

The thermo cycling conditions employed for each assay run for all genes were: initial denaturation at 95 °C for 3 minutes followed by 40 cycles of 95 °C for 10 seconds and 52 to 60 °C for 45 seconds (annealing temperature optimised depending upon primers), and 72 °C for 1 minute. Optical detection was performed at 72 °C. After amplification, a melt curve (95 °C for 1 minute, 55 °C for 1 minute and then 40 cycles of 55 °C for 10 seconds) was included in the protocol to validate primer specificity and amplification of a single PCR product. Analyses of amplification plots were performed with the iQ5 Optical System Software version 2.0 (Bio-Rad Laboratories, INC.). The threshold cycles for each sample were normalized to house keeper gene (*β-actin*) and relative expression of the target gene was quantified as fold change in the expression of target gene relative to the expression of thermoneutral control according to $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001)

4.1.7 Statistical Analysis

Data were analysed by conducting analysis of variance using Genstat® 14th edition (VSN International, Hemel Hempstead, UK). Feed intake and daily gain data were analysed for the main effect of dose of antioxidants with animal as a random effect. To examine the effects of heat stress on RR, HR, RT and ST, data were analysed for the main and interactive fixed effects of time of day (0900, 1300, 1700), d (1-7), thermal treatment and linear and quadratic effects of dose of antioxidants with animal as random effect. While there were highly significant ($P < 0.006$) effects of day of HS, these effects were inconsistent and so data were pooled across days for analysis. Plasma data were analysed for the main and interactive effects of thermal treatment and linear and quadratic effects of dose of antioxidants with data from d 7 (thermal treatment) and d 8 (lairage) analyzed separately. Where there was no evidence of a linear effect of dose of antioxidants the data were also analysed with supplemental antioxidants pooled (MOD and SUP) as a fixed effect. Multiple comparisons between the means were estimated by conducting the Bonferroni test and results were reported as means with pooled standard errors except where specified.

4.2 Experiment 2

The second experiment was conducted at the Victorian Department of Economic Development, Jobs, Transport and Resources (DEDJTR), Rutherglen. All procedures undertaken in this study were approved by the DEDJTR Ethical committee, AEC 2014-19. The experiment was conducted from February to April 2015 at Rutherglen DEDJTR research center (Rutherglen, VIC, Australia).

4.2.1 Animal Background

Sixty [Border Leicester x Merino] X Poll Dorset weather and ewe lambs with similar live weight and age were selected at weaning (3 months) from the DEDJR Rutherglen Research Farm Flock and allowed to graze a perennial improved pasture without lucerne until the beginning of the study. 6 months old lambs were then collected, weighed and allocated in individual pens (1.25 m^2) with mashed floor and walls and monitored during ten days of adaptation to the new diet and environment. Adaptation diet consisted of perennial hay plus pellet with a moderate amount of antioxidant (MOD), with the former gradually reduced and the latter gradually increased in the first 7 days. *Ad libitum* MOD pellet was thus provided for the latter 3 days of adaptation and individual feed intake during these days was recorder. Fresh water was provided daily *ad libitum*. Forty eight animals were selected to be enrolled for the feeding trial, based on health status and average pellet intake in the latest three days of adaptation. Animals were blocked by sex (weathers or females) and feed intake in the latest three days of adaptation (4 feed intake blocks/sex) and randomly assigned to one of the three dietary treatments (16 lambs each): moderate level of antioxidant (MOD, 42 mg·kg^{-1} vitamin E as *all-rac* α-tocopheryl acetate and 0.1-0.15 mg·kg-1 mg of organic Se as Selenosource® (Diamond V Mills, Inc. USA), supranutritional level of antioxidant (SUP, 285 mg·kg^{-1} of vitamin E *all-rac* α-tocopheryl acetate and 0.5-0.6 mg·kg-1 of organic Se as Selenosource® or lucerne based diet (LUC, 37 mg/kg vitamin E). All diets were made by Farm Balance (Kerang, VIC) (Tab. 1). CON and SUP diets were pelleted (3 mm diameter) to avoid sorting behavior, potentially impairing antioxidant intake, while LUC was provided as a total mixed ration. Vitamin E inclusion of MOD pellet was designed to achieve similar content of VitE as in LUC diet, as lucerne vitamin E concentration was determined prior to the beginning of the study. 100 g of feed samples were collected weekly and pooled to determine chemical composition (Table 1).

Temperature (T°) and relative humidity (RH) was recorded every 20 min for the entire experimental period using data logger (Tynitag®, Gemini Data Loggers, Chichester, UK) placed in 5 different point of the experimental chamber. THI was calculated according to Marai et al., (2007): THI = db °C -[(0.31 – 0.31RH) (db °C – 14.4)] where db°C is dry bulb temperature in C and RH = relative humidity percentage/100.5. The average THI was 25.14 in the first 4 weeks (summer) and 19.20 in the latter 4 weeks (autumn) of the experimental period. Animals were fed twice a day and the amount of feed refused was recorded prior to morning feed to calculate feed intake. Lambs were weighed weekly prior to morning feeding with a portable scale (SO 33 Manual Weigh Crate, ProWay- Prattley Pty Ltd, Wagga Wagga, NSW, Australia) with an accuracy of 0.1 kg.

4.2.2 Slaughtering and Meat Quality Analysis

At the end of the feeding trial, animals were transported (300 km) to a commercial abattoir where they were kept in lairage (in a separate pen without commingling with the other lambs at the abattoir) for seventeen hours overnight. The lambs were slaughtered after head only stunning. All carcasses were electrically stimulated approximately 30 minutes after stunning before being chilled.

Carcasses were chilled over 24 h periods. Hot carcass weight (HCW) was recorded and the tissue depth (muscle and fat) from the surface of the carcass to the lateral surface of the twelfth rib 110mm from the midline was measured using a GR knife (GR). 24 h post-mortem the LL muscle was removed from the left side loin, from the 9^{th} and 10^{th} lumbar vertebrae to the caudal end, trimmed from external fat and connective tissue and sampled as reported in Figure 1 for meat quality analysis. Aged samples were vacuumed packed, stored for 42 days at 2-4°C and then frozen at -20°C.

For retail color stability, the designed portion of LL was sliced to create a fresh surface, packed on a black foam tray and over wrapped with a PVC food film (15 µm thickness) as retail packs. Trays were maintained at 3–4 °C under fluorescent light (1000 lux) for 72 hours. Lightness (L*-value), redness (a*-value) and yellowness (b*-value) of meat at 0 (allowed to bloom 60 minutes), 24, 48 and 72 h of display life were measured in duplicate on each sample using a Hunter Laboratory Mini Scan XE Plus meter with a 5-mm aperture, light source set to illuminant D-65 with a 10 degree standard observer (model 45/0-S, Hunter Associates Laboratory Inc., Reston VA, USA) as described by Ponnampalam et al. (2010) An estimate of the oxy/met myoglobin ratio was calculated by dividing the percentage of light reflectance at wavelength 630 nm, by the percentage of light reflectance at wavelength 580 nm ($R_{630/580}$). Lipid oxidation was assessed by measuring the concentration of malondialdehyde (MDA, expressed in mg/kg of muscle) using the thiobarbituric acid reactive substances (TBARS) procedure (Witte et al., 1970) on samples at 24 h post mortem and at the end of display life. A homogeneous sample of freeze dried ground material (approximately 0.5 g) was used for the determination of fatty acid composition with a method described by Ponnampalam et al. (2014). Fatty acid levels in the experimental diets and in the muscle were determined in mg/100 g of fresh sample. The vitamin E content of both experimental diets and muscle tissue was determined as described by Ball (1988).

4.2.3 Statistical Analysis

Statistical analysis was carried out using SAS 9.3 (SAS Inc, Cary, NC, USA). Data distribution was tested with Sahpiro-Wilk test and abnormally distributed data were Log_{10} transformed. For feed and vitamin E intake, live weight gain, carcass traits (HCW and GR), muscle FAME and vitamin E a REML procedure (PROC MIXED) was carried out with a mixed model that accounted for the fixed effect of dietary treatment and the random effect of animal within dietary treatment.

For TBARS, color coordinates (L*, a* and b*) and Met myoglobin (R580/630) of fresh and aged samples, a REML procedure (PROC MIXED) was carried out with a mixed model that accounted for the fixed effects of dietary treatment and display time and the random effect of the animal within dietary treatment. Bonferroni post hoc test applied for multiple comparisons.

5.0 Project Outcomes

5.1 Experiment 1

There was a linear effect ($P = 0.009$) of dietary antioxidants on ADFI, such that lambs on the SUP treatment consumed more feed over the 3 weeks prior to the thermal treatments as compared to the lambs given CON or MOD diets (935, 936 and 1,111 ± 61.4 (mean ± SED) g/day for CON, MOD and SUP lambs, respectively). Similarly, there tended to be a linear effect ($P = 0.057$) of dietary antioxidants on average daily gain (ADG), such that lambs on the SUP treatment grew faster over the 3 weeks prior to the thermal treatments as compared to lambs consuming the CON or MOD diets (94, 91 and 178 ± 47.4 (mean ± SED) g/day for CON, MOD and SUP lambs, respectively). Under HS conditions the value of Temperature Humidity Index (THI) ranged from 30 - 34.1 between 09.00 and 17.00 h and was 25.0 between 17.00 and 19.00 h. During TN conditions the average THI was 19.0. The lambs were exposed to extreme severe heat stress during day time in the heat stress room while there was no stress in thermonutral room as per THI values (THI < 22.2 = absence of heat stress; 22.2 to < 23.3 = moderate heat stress; 23.3 to < 25.6 = severe heat stress and 25.6 and more = extreme severe heat stress (Marai et al., 2001; Marai et al., 2007).

Respiration rate (pooled across days) increased in response to HS (68 vs 155 breaths per minute for TN and HS conditions, respectively, $P < 0.001$) and increased over the day (74, 131, and 139 breaths per minute at 0900, 1300, and 1700 respectively, $P < 0.001$) (Figure 1a). However, there was an interaction ($P < 0.001$) between thermal treatment and time such that RR increased to a greater extent over the day in the lambs exposed to HS compared to those housed under TN conditions. While there were no linear ($P = 0.99$) or quadratic ($P = 0.20$) main effects of antioxidant supplementation on RR, there was an interaction between the diet and thermal treatments and time ($P = 0.009$) such that RR increased to a lesser extent during the HS in the lambs receiving the MOD and SUP doses of dietary antioxidants than in the CON lambs (Figure 1a).

Rectal temperature increased in response to HS (39.5 vs 39.9 °C for TN and HS conditions, respectively, $P < 0.001$) and increased over the day from 0900 h to 1700 h (39.5, 39.8 and 39.8 °C at 0900, 1300, and 1700 h respectively, $P < 0.001$) (Figure 1b). Again, there was an interaction ($P < 0.001$) between thermal treatment and time such that RT increased to a greater extent over the day when sheep were exposed to HS compared to those housed under TN conditions.

While there were no linear (P = 0.19) or quadratic (P = 0.35) main effects of antioxidant supplementation on RT, there was a trend (P = 0.08) for an interaction between antioxidant and thermal treatments such that RT increased to a lesser extent in the lambs receiving antioxidants (MOD and SUP) (39.5 vs 39.8 °C for TN and HS conditions, respectively) as compared to the control lambs (39.5 vs 40.0 °C for TN and HS conditions, respectively) under HS conditions.

Skin temperature increased in response to heat stress (37.5 vs 38.5 °C for TN and HS conditions, respectively, P < 0.001) and increased over the day (37.6, 38.1 and 38.2 °C at 0900, 1300, and 1700 h respectively, P < 0.001) (Figure 1c). Again, there was an interaction (P < 0.001) between thermal treatment and time such that ST increased to a greater extent over the day in the sheep exposed to HS compared to those housed under TN conditions. There were no linear (P = 0.93) or quadratic (P = 0.90) effects of antioxidant supplementation on ST.

There was no main effect of HS on HR (98.1 vs 96.7 beats per min for TN and HS conditions, respectively, P = 0.70) (Figure 1d). Heart rate increased over the day (91, 101 and 101 beats per min at 0900, 1300, and 1700 h respectively, P < 0.001), but there was an interaction (P = 0.021) between time and thermal treatment such that HR increased to a greater extent over the day in those lambs kept under TN conditions. There was also an indication of a linear interaction (P = 0.055) between level of antioxidant and time such that the increase in HR over the day decreased with increasing dietary antioxidants.

During the thermal treatment period there was no main effect of HS on the ADFI (1,250 vs 1,176 g/day for TN and HS lambs respectively, P = 0.36) (Table 1.1). While there was a linear (P = 0.002) increase in ADFI with increasing dietary antioxidants there was also a linear interaction with thermal treatment such that HS decreased feed intake in the lambs fed the CON diet and the linear effect of dose of antioxidants was only evident under HS.

Plasma ROMs were increased (120 vs 136 CARR U for TN and HS, respectively, P = 0.013) on d 7 of HS and decreased in a linear manner as supranutritional levels of antioxidant increased (137, 129, 118 CARR U, for CON, MOD and SUP diets, respectively, P = 0.021) supplementation (Table 1). There were no effects of heat stress (P = 0.85) or linear (P = 0.46) or quadratic (P = 0.47) effects of dietary antioxidants on plasma BAP concentration (Table 1). OSI was increased by HS (3.41 vs 3.92 arbitrary units for TN and HS respectively, P = 0.015), but not significantly altered by dietary antioxidant supplementation (Table 1). Plasma AOPP tended to be increased on d 7 of HS (35.0 vs 40.2 mol/L for TN and HS respectively, P = 0.069) and decreased linearly with increasing level of antioxidants (40.8, 38.8 and 33.3 mol L^{-1}, for CON, MOD and SUP respectively, P = 0.032) (Table 1). However, there was an interaction (P = 0.037) between thermal and dietary treatments; HS increased AOPP most markedly in those lambs fed the CON diet and the linear decrease in AOPP only occurred in the HS treated lambs. Plasma cortisol tended to be increased in lambs exposed to HS (7.6 vs 12.3 ng mL^{-1} for TN and HS respectively, P = 0.080), but was not altered by dietary antioxidant supplementation (Table 1.1).

During lairage there were no effects (all P > 0.26) of either previous thermal treatment or dietary antioxidants on respiration rate or rectal temperature (Table 1.2). On the other hand, plasma ROMs were greater during lairage in lambs that had previously been exposed to HS (106 vs 125 CARR U for TN and HS, respectively, P < 0.001) and were decreased by supranutritional levels of antioxidant (120, 123, 105 CARR U for CON, MOD and SUP diets, respectively, P = 0.019) supplementation (Table 2).

Plasma BAP was greater during lairage in lambs previously exposed to TN (3,386 vs 3,187 mmol/L for TN and HS respectively, $P < 0.012$) and tended to increase linearly with increasing level of antioxidants (3224, 3,248 and 3,388 mmol/L, for CON, MOD and SUP respectively, $P = 0.082$) (Table 2).

However, there was an indication of an interaction ($P = 0.059$) such that previous exposure to HS decreased BAP most markedly in those lambs fed the CON diet and the linear increase in BAP only occurred in the HS treated lambs. Plasma OSI was higher (3.15 vs 3.96 for TN and HS respectively, $P < 0.001$) during lairage in lambs previously exposed to HS. The OSI also responded in a linear ($P = 0.001$) and quadratic ($P = 0.026$) manner with increasing dietary antioxidants (3.75, 3.81 and 3.10 for CON, MOD and SUP respectively) (Table 1.2). Plasma AOPP concentration was greater during lairage in lambs previously exposed to HS (33.4 vs 44.1, mol/L for TN and HS respectively, $P = 0.004$) and tended to decrease linearly with increasing level of antioxidants (42.1, 40.5 and 33.7 mol L^{-1}, for CON, MOD and SUP respectively, $P = 0.056$) (Table 2). However, there was an interaction ($P = 0.021$) between thermal and dietary treatments such that HS increased AOPP most markedly in those lambs fed the CON and MOD diets and the decrease in AOPP only occurred in the HS treated lambs fed the SUP diet. There was no effect of either previous thermal treatment or dietary antioxidants on plasma cortisol in lairage (Table 1.2).

Vitamin E concentration in the *Longissimus lumborum* (LL) muscle tended ($P = 0.15$) to be increased by the higher antioxidant supplementation such that lambs fed moderate or supranutritional levels of antioxidants had greater concentration as compared with control lambs (Table 1). There was no effect of heat stress on Vitamin E concentration in the muscle and nor was there any interaction between heat stress and antioxidants. There was significant ($P = 0.006$) effect of diet on the soft tissue depth at GR site such that lambs fed moderate antioxidants had leaner meat as compared to control (8 vs 10 mm for moderate and control, respectively), however, there was no effect of heat stress on GR depth. Hot standard carcass weight (HSCW) was also significantly ($P = 0.047$) affected by diet such that lambs supplemented with supranutritional levels of antioxidants had higher HSCW as compared with lambs fed control diets (22.7 vs 20.7 kg, for SUP and CON lambs, respectively) (Table 1.3).

The expression of skeletal muscle GPx-1 mRNA abundance was increased by dietary antioxidant (supplement) supplementation under thermoneutral conditions (2.42 fold; $P < 0.050$) while chronic heat stress tended to ($P = 0.070$) decrease muscle GPx-1 mRNA abundance (Figure 3). However, there was an interaction between the thermal and dietary treatments such that although the decrease in GPx-1 mRNA expression in response to HS was significant in antioxidant supplemented sheep, it was still 30% greater than the sheep on control diet under HS (Figure 1.2). Muscle GPx4 mRNA expression was increased by 100% following supplementation of high antioxidants ($P = 0.015$), whereas there was interaction between heat treatment and dietary antioxidants ($P = 0.055$) such that the decline in GPx4 expression was greater in sheep on the control diet compared to sheep supplemented with antioxidants (Figure 1.2).

Dietary treatment did not affect growth performance and carcass quality (Table 2.3). Although having a lower dietary energy content, LUC fed animals grew and delivered carcasses similar to those fed MOD and SUP. Muscle fatty acids (FA) composition has been presented in Table 2.4. Dietary treatment did not affect total FA, C14:0, C16:0, ALA, EPA, DPA, DHA, as well as total n-3, SFA and MUFA concentration. LUC fed animals showed a higher C18:0 and CLA content and a lower C18:2n-6 and C20:4n-6 content. These differences led to a lower total n-6 and PUFA content for the LUC group, leading to lower n-6/n-3 and PUFA/SFA ratios compared with other treatments.

Dietary treatment affected average daily vitamin E intake (P<0.001) and SUP fed animals had a higher vitamin E intake compared to the others two experimental groups (P<0.01), while no differences were observed between MOD and LUC groups (Figure 2.1). There was significant (P<0.001) effect of dietary treatment on muscle vitamin E content. Higher dietary intake (SUP groups) resulted in a higher (P<0.01) muscle vitamin E concentration compared to both MOD and LUC groups (Figure 2.2).

5.2 Experiment 2

Dietary treatment affected overall color traits of both fresh and aged meat during display life (Table 2.5). Although treatment*time effect only tended to be significant for redness (P=0.08) and metmyoglobin (P=0.05), a different pattern of a* and $R_{630/580}$ evolution during display life was evident (Table 2.5). In aged meat no differences were observed in redness and metmyoglobin accumulation during display life within group (Table 2.6). A diet effect was evident for a* value (P=0.04) and SUP group showed a lower overall a* compared to MOD group (14.71 vs 16.54, P=0.04), without differ from LUC group. A diet*time effect was evident for metmyoglobin formation (P=0.02), but no differences within display time were evident between the three groups.

Oxidative stability of both fresh and aged loin (Table 2.7) did not significantly differ between dietary treatments, although TBARS were numerically lower in SUP group compared to LUC and MOD for aged samples at the end of display life.

6.0 Discussion

6.1 Experiment 1

The present study indicates that supranutritional dietary Se and Vit E supplementation during the finishing phase improved ADFI, ADG, and oxidative status under HS in finisher lambs, thereby supporting our hypothesis. High environmental temperatures have a detrimental effect on sheep production in many parts of the world. During HS, a series of physiological, metabolic and immunological responses are invoked to defend the thermal load resulting in lowered production (Fuquay, 1981; Silanikove, 2000; Marai et al., 2007; Rhoads et al., 2013). In ruminants, HS is associated with increased OS and reduced concentrations of blood antioxidants (Bernabucci et al., 2002; Saker et al., 2004; Padilla et al., 2006; Chauhan et al., 2014b). This study indicated that lambs exposed to HS for 7 days during the finishing period, were also exposed to OS due to the lowered availability of antioxidants and increased oxidant production.

During the three week of antioxidant feeding under ambient temperatures, the lambs fed supranutritional levels (SUP) of Se and Vit E, had greater ADFI and gained more body weight than those on control (CON) or moderate (MOD) levels of these antioxidants. Vitamin E and Se are essential components of the antioxidant defense system and play an important role in growth through their participation in critical enzymes and enzyme reactions (Surai, 2002; Willshire and Payne, 2011).

Selenium is a component of glutathione peroxidases, while Vit E is a fat soluble vitamin that neutralizes reactive oxygen species before they can oxidise unsaturated membrane lipids thus helping to maintain the integrity of lipid membranes. Selenium also plays important roles in metabolism and growth via expression of the iodothyronine deiodinases that regulate production of active T3 hormone in the thyroid gland and peripheral tissues (Rooke et al., 2004).

The effect of SUP feeding was also evident during HS such that lambs fed SUP levels of Se and Vit E continued to have greater feed intake compared to lambs given CON or MOD levels during HS. Generally, feed intake is expected to decline under HS in an effort to reduce metabolic heat production. For instance, a decline in DM intake was reported in Sardinian ewes housed in a climatic chamber for 49 days under elevated THI (ETHI: THI = 82.0 +/- 2.5) (Bernabucci et al., 2009). More recently, decreased feed intake was reported in Malpura sheep following 35 days of simulated HS under semiarid tropical environment (Indu et al., 2014). In the present study, feed intake was reduced under HS in the lambs fed the CON diet (-24%), but importantly feed intake was maintained in lambs fed increased levels of dietary antioxidants. This is consistent with our previous study where exposure to HS (THI = 34 between 0900 h to 1700 h) resulted in a 13 % decline in feed intake in ewes fed recommended requirements for Se and Vit E whereas antioxidant supplementation at a similar level to the SUP diet used in the present study prevented the decline in feed intake under restricted feeding (Chauhan et al., 2014b).

In the present study, HS resulted in a THI of 30 and an average increase of 0.5 °C in RT of the lambs. Similar to our previous study with sheep (Chauhan et al., 2014b), supranutritional antioxidant supplementation decreased RT by 0.2 °C under HS compared to control lambs. Our results are also consistent with those reported by Alhidary et al. (2012), who observed a 0.3 °C decrease in RT following injection of 5 mg Se (sodium selenite) in sheep during HS. It is anticipated that an increase in heat load will increase RR in order for the lambs to lose heat via the respiratory route (Silanikove, 2000). This is especially relevant to sheep where heat loss via the respiratory system is the primary mechanism as heat loss via sweating is prevented by presence of the wool (Marai et al., 2007). The lower RR observed in supplemented lambs in the current study confirmed the ability of supranutritional antioxidants to mitigate the effects of HS on RR of the lambs. These effects of antioxidants may be explained by decreased the RT and reduced OS observed in the present investigation, and lower cortisol levels observed in previous studies during HS (Magdub et al., 1982; Surai, 2002; Sivakumar et al., 2010). A similar decrease in RR and RT following antioxidant supplementation during HS was also reported in Malpura sheep and a favorable effect of antioxidants on thermoregulation was suggested (Sejian et al., 2014). Our results have further shown that high levels of antioxidants have consistent ameliorative effects on HS such as decreased RT and RR and can be successfully used for HS mitigation purposes.

Holding lambs in lairage is another management strategy that can be employed to reduce stress levels in lambs before slaughter (Liste et al., 2011). Accordingly, we observed the normalizing effect of lairage on both RT and RR of lambs previously finished under hot conditions (both values returned to normal). While there was no effect of antioxidant supplementation on either of these parameters, 14 h of lairage under TN conditions was enough to reduce RT by 1°C and RR by 150%.

The greatest decline in RT and RR was observed in lambs previously subjected to HS conditions reinforcing the importance of the management of lambs in lairage, particularly when lambs have been finished and transported under hot summer conditions. Although it is difficult to compare directly because of different sampling times, it appears that plasma cortisol levels were approximately doubled during lairage irrespective of whether lambs were finished under hot or TN conditions. This difference was most likely a result of mixing and handling during transport, lairage and blood sampling (Hopster et al., 1999; Miranda-de la Lama et al., 2012).

Handling of lambs before slaughter can have important implications on the stress levels in lambs at slaughter and hence transportation and handling during pre-slaughter and lairage are critical points of pre-slaughter logistic chain that may compromise lamb welfare and meat quality (Miranda-de la Lama et al., 2010; Miranda-de la Lama et al., 2011), especially during hot summer finishing when lambs are more likely to experience HS. Antioxidant supplementation did not appear to reduce the impact of transportation and handling stresses response to adrenocorticotropin hormone administration (Chauhan et al. 2014b).

Heat stress has also been implicated in increased OS in ruminants. In agreement with the previous reports (Bernabucci et al., 2002; Di Trana et al., 2006 and Chauhan et al., 2014b), the observed increase in plasma ROMs levels in lambs exposed to HS was expected. The increase was particularly evident for the lambs that received CON or MOD levels of antioxidants, while the lambs receiving SUP levels of antioxidants exhibited the lowest ROMs during HS. In agreement with our previous findings (Chauhan et al., 2014b), supranutritional levels of Vit E and Se are necessary to prevent oxidant challenge induced by HS. For optimum cellular functions, both Vit E and Se are important as they help to maintain low cellular and tissue concentrations of ROM (McDowell et al., 1996; Chauhan et al., 2014b). While it is not clear whether plasma BAP decreased as a direct consequence of elevated ROMs under HS conditions or vice versa, supplementation of combined Vit E and Se tended to increase BAP concentrations in a dose-dependent manner during HS. Recently, Alhidary et al, (2015) also reported that plasma total antioxidant status were greatest in sheep receiving Se and vitamin E supplementation for 50 d as compared to non-supplemented sheep. While the molecular mechanisms responsible for excessive ROM production or lowered antioxidant defenses during HS are still largely unknown in ruminants, there is some evidence of the involvement of the mitochondrial electron transport chain or terminal oxidation, at least in poultry (Mujahid et al., 2005; Sahin et al., 2013).

Antioxidant supplementation maintained redox homeostasis during HS as reflected by the observed OSI value in lambs fed the SUP diet under HS. The lower AOPP in lambs fed the SUP diet under HS is indicative of protective role of Vit E and Se against the oxidative damage of proteins induced by HS and reinforces the need for higher levels of antioxidants than the current recommended levels under conditions such as HS. On the other hand, the higher concentration of AOPP observed in lambs fed the CON diet under HS suggests that the recommended levels of antioxidant may not be sufficient to scavenge the ROS leading to oxidative stress and oxidative damage of proteins during HS. The elevation of AOPP following HS is of particular interest as it is a marker of protein oxidation and also considered to mediate pro-inflammatory responses (Celi, 2011a; Celi et al., 2011c) and may have important implications on protein accretion and oxidative stability in lamb muscles which is very important from a meat quality perspective. Recent work has demonstrated that HS leads to oxidative stress in skeletal muscles of pigs (Montilla et al., 2014), but these effects need to be tested in the skeletal muscle of lambs.

We have recently shown that HS leads to up-regulation of pro-inflammatory genes mRNA expression, especially TNF- α, which may also lead to OS, however supranutritional levels of antioxidant supplementation during HS down regulated TNF- α gene expression (Chauhan et al., 2014a).

The oxidative status of lambs before slaughter may have important implications for lamb meat quality, as the discoloration of meat for example is an oxidative process (Faustman and Cassens, 1990; Faustman et al., 2010; Suman et al., 2014b) and can be affected by muscle antioxidant concentrations (Faustman et al., 1998; Ponnampalam et al., 2012; Suman et al., 2014a).

Quite clearly, the increase in ROM, OSI and AOPP and the reduction in BAP induced by HS are still present when the lambs were slaughtered as were the protective effects of supranutritional antioxidants. As expected, supranutritional antioxidant supplementation improved the hot standard carcass weights and decreased the GR depth at moderate levels. Furthermore, moderate levels of antioxidant supplementation for 4 weeks during finishing phase, increased the concentration of vitamin E in the muscle of lambs given dietary vitamin E @130 mg/kg as compared with control (vitamin E @ 27.6 mg/kg) group (2.13 vs 1.72 mg/kg, respectively). However, the vitamin E concentration achieved in the muscle was not sufficient to improve the meat colour stability or shelf life. It is indicated that either higher levels of dietary vitamin E or longer duration (> 4 weeks) of supplementation is required to improve the colour stability of fresh and aged meat of lambs finished under hot conditions. This finding may have substantial benefits in the finishing lambs during summer or even feed lot industry in warmer climates or even enroute to key markets. Antioxidants have been reported to influence both lipid and protein oxidation and Vit E (α-tocopherol) has been extensively used to minimize lipid oxidation and improve meat color stability (Ponnampalam *et al.* 2012b; Suman *et al.* 2014a). A consumer's decision to buy meat is influenced by meat colour and their preference is for fresh meat to be bright red or bright pink and any deviation from this may influence the purchase. The heme-containing proteins, hemoglobin and myoglobin, are the primary pigments in muscle tissue associated with color (McDowell *et al.* 1996). Oxidation manifests as a conversion of the red muscle pigment myoglobin to brown metmyoglobin and the development of rancid odours and flavors from the degradation of the PUFA in tissue membranes (Wood and Enser 1997). Appearance of brown on the meat surface limits its display life and costs the meat industry millions of dollars per year. There is insufficient published evidence on the oxidative status of lambs finished during summer. Considering that growth is a trait that is affected by the intake of dietary antioxidants (Catoni *et al.* 2008) and that the high metabolic rate of growing tissues produces large amounts of free radicals (Rollo 2002), it is conceivable that antioxidant supplementation may help to counter negative effects of OS associated with growth and HS in finisher lambs.

In the present investigation, high antioxidant supplementation improved the oxidative status of lambs under hot conditions, however fresh meat colour and retail colour stability was not affected. It can be explained based on the insufficient incorporation of vitamin E achieved in muscle. It has been recently suggested in aged (vacuum packed for 4 weeks) muscle displayed for 4 days that when muscle vitamin E concentration was below 2.95 mg/kg muscle, PUFA and heme iron concentration affected lipid oxidation. When vitamin E concentration was above 2.95 mg/ kg muscle, the lipid oxidation was controlled by vitamin E not by the level of PUFA or iron present in the muscle (Ponnampalam *et al.*2014b). As the muscle vitamin E concentration in the present study was well below 2.95 mg/kg muscle, meat colour stability was not improved. Previously lamb feeding perennial pastures (lucerne and phalaris) increased the Vitamin E concentration of skeletal muscle compared with the lambs grazing annual pastures with some supplements (Ponnampalam *et al.* 2012a). Kasapidou (2012) also showed that vitamin E supplementation at levels of 250 IU/kg DM are required to ensure sufficient deposition in the muscle in sheep fed on concentrate based diets during the finishing period, whereas the similar results could be achieved at supplementation levels of only 60 IU/kg DM provided some green is included the sheep diets. The authors also found that a minimum 1.9 µg/g muscle vitamin E levels are required to prevent oxidation of muscles. Therefore, more research is required to optimize the levels and duration of antioxidant supplementation to incorporate high levels of vitamin E in the muscles to improve its colour and oxidative stability.

6.2 Experiment 2

A lack of differences in growth rate and carcass quality feeding lambs a grain based diet or lucerne has been reported by Ponnampalam et al. (2014). Moreover, several authors reported that increasing dietary vitamin E supplementation, ranging from 0 to 1020 mg/kg of synthetic vitamin E, did not affect lamb performance (Macit et al., 2003a, b; Lauzurica et al. 2005; de la Fuente et al., 2007; Chen et al., 2008; Kasapidou et al., 2009 and 2012; Kott et al; Berthelot et al., 2014). LUC fed animals showed a higher C18:0 and CLA and a lower C18:2n-6, C20:4n-6 content. These differences led to a lower total n-6 and PUFA content for LUC group, reducing therefore n-6/n-3 and PUFA/SFA ratios. The higher C18:0 can be explained by the higher C18:0 content of LUC diet. Regarding C18:2n-6, is possible to suppose that LUC diet has been retained in the rumen for longer, determining therefore a higher biohydrogenation of the unsaturated FA as C18:2, reducing therefore its content in the meat despite the small difference in feed concentration. Moreover, the biohydrogenation of linoleic acid can concurred to elevate muscular C18:0 and CLA content in the meat compared to grain based diet. Indeed, 70 to 95% of the dietary linoleic acid is biohydrogenated into the rumen (Lock and Baumann, 2004) and FA biohydrogenation increases following increase in feed particle size (Gerson et al., 1988). Higher CLA after feeding lucerne compared to a grain based diet has been previously reported by Gonzaléz-Calvo et al. (2015). Higher dietary vitamin E did not affect muscle FA profile in accordance with Kasapidou et al. (2012). However, other oauthors have reported an increase in SFA (Chen et al., 2008; Kott et al., 2010; Berthelot et al., 2014) and a decrease in MUFA (Kott et al., 2010; Muíño et al., 2014), following increase in dietary vitamin E intake. The reported inconsistencies of the effects of dietary vitamin E on muscle FA across studies could be due to the different composition of the administered diets.

Despite a similar VitE intake, muscle vitamin E content was higher ($P<0.05$) in LUC compared to MOD group. Indeed, the concentration of muscle vitamin E per unit of vitamin E consumed was 1.37 times greater for LUC compared to CON group, underlining therefore a difference in bioavailability between natural and synthetic form, similar to the conversion factor for international unit (IU) calculation from *all-rac* α-tocopheryl acetate to RRR α-tocopherol of 0.73 proposed by EFSA FEEDAP PANEL (EFSA, 2010). Higher transfer rate of natural vitamin E compared to *all-rac* α-tocopheryl acetate has been previously reported in pork (Boler et al. 2009, turkey (Rey et al., 2015), lamb muscle (Kasapidou et al., 2009), in sheep (Gallardo et al., 2015 and cow milk (Weiss et al., 2009; Vagni et al., 2011).

Meat from LUC fed animals was characterized by higher redness stability as shown by less decline in redness during display life, instead was evident for both MOD and SUP diets. Furthermore, metmyoglobin formation was stable from day 2 to day 4 in LUC and SUP group, while a progressive decline was evident until day 4 of display life for MOD group. Taken together the results of color and oxidative stability, LUC group showed a better color stability of fresh meat only compared to MOD and SUP, without any difference in oxidative stability of both fresh and aged meat. As expected a decline of L*, a*, b* and $R_{630/580}$ ($P<0.0001$) was evident during display life in all of the three dietary treatment.

A lack of effects of increasing dietary synthetic vitamin E up to 500 mg/kg of feed on lamb meat color stability during the first 4 days of display has been reported by Macit et al. (2003a,b), Kasapidou et al., 2012 (under modified atmosphere with 75% CO_2 and 25% of O_2) and Ripoll et al., 2013, while a positive effect of increasing dietary vitamin E was evident in extending display life over the time tested as in the present study, excluding the reports of Macit et al. (2003a,b).

Meat from lambs that received supplemental vitamin E up to 1000 mg/kg of feed showed a reduction of metmyoglobin formation in the studies of Guidera et al. (1997); De la Fuente et al., and Jose et al., under natural atmosphere and Lauzurica et al. (2005)., under modified atmosphere (70% $O_2$and 30% CO_2). The improvement of color stability in LUC group despite a lower muscle vitamin E concentration compared to the animals fed SUP diet could be attributed to the additive effect of other micronutrients other than vitamin E exerting antioxidant activity, as carotenoids, flavonoid and phenolic compounds (Ferreira et al., 2015). The reported data seems to confirm that nutrients other than vitamin E in lucerne also help to improve antioxidant activity in the muscle. Other than the presence of several antioxidants in lucerne, it is possible that some other factors have concurred to determine the lack of effect of supranutritional level of dietary vitamin E and organic selenium and a better effect of LUC fed diet on color and oxidative stability. Warner et al (2010) reported that predicted pH at 18°C, intramuscular fat, copper and iron represent significant sources of variation in retail color stability of lamb meat. In the present study pH at 18°C did not differ between groups (data not shown) and the intramuscular fat is not expected to be different as total fatty acids were similar. Due to the fact that LUC diet contained different components compared to both MOD and SUP (lower amount of barley, no canola expeller) is possible that the different composition has led to a different muscle iron and copper level, and the previous authors demonstrated that muscle iron and copper concentrations are negatively correlated with oxy/met ratio. However, iron and copper concentration in the muscle were not available for the present study. Regarding oxidative stability of lamb meat, no difference on short term display under simulated retail conditions increasing dietary vitamin E has been reported by Macit et al (2003b), De la Fuente et al. (2007) and Kott et al. (2010). On the contrary, the majority of the studies reported an improvement of oxidative stability increasing dietary vitamin E (Guidera et al., 1997; Macit et al., 2003a; Kasapidou et al., 2012; Ripoll et al., 2013; Muíño et al., 2014). Ripoll et al. (2013) reported no differences in oxidative stability of meat from lamb grazing lucerne pasture or grain diet supplemented with 500 mg of dl-α-tocopheryl acetate/kg of feed since 10 days before slaughter.

Results of the present research have confirmed that increasing dietary vitamin E increases muscle vitamin E content in lambs thus improving the nutritional value of lamb meat. However, finishing lambs on Lucerne, can help to improve color stability of fresh lamb meat possibly due to the higher bioavailability of vitamin E provided through natural sources compared to the artificial form and the presence of other micronutrients influencing antioxidant activity in the muscle.

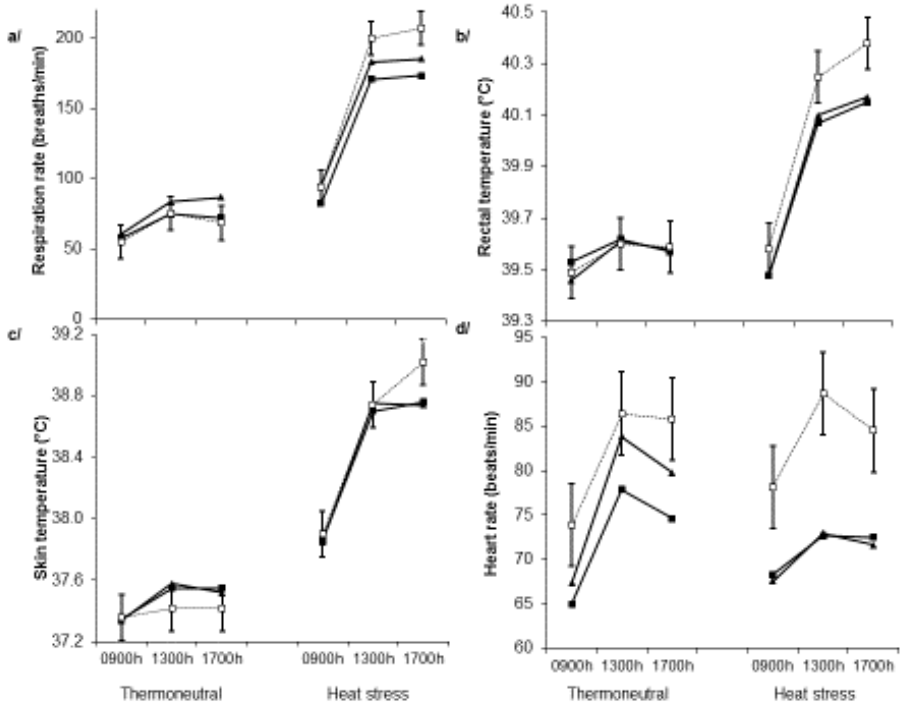

Figure 1.1. Relationships between a) respiration rate, b) rectal temperature c) skin temperature and d) heart rate and time of day in finisher lambs fed either 0.16 mg Se and 28 mg/kg DM Vitamin E, (CON, □), 0.66 mg Se and 130 mg/kg DM Vitamin E (MOD, ■) or 1.16 mg Se and 228 mg/kg DM Vitamin E (SUP, ▲) under thermoneutral or heat stress conditions. The *P*-values for the effects of temperature, linear and quadratic effects of antioxidants, time, temperature x time and linear antioxidant x temperature x time interactions were 0<.001, 0.99, 0.20, 0<.001, ,0.001 and 0.009 for respiration rate, 0<.001, 0.19, 0.35, 0<.001, <0.001 and 0.20 for rectal temperature, <0.001, 0.93, 0.90, <0.001, <0.001 and 0.34 for skin temperature and 0.70, 0.38, 0.38, <0.001, 0.021 and 0.65 for heart rate, respectively. There were no other significant interactions unless stated in the text. Data are mean and standard error of the differences for temperature x time x antioxidant displayed on the CON treatment.

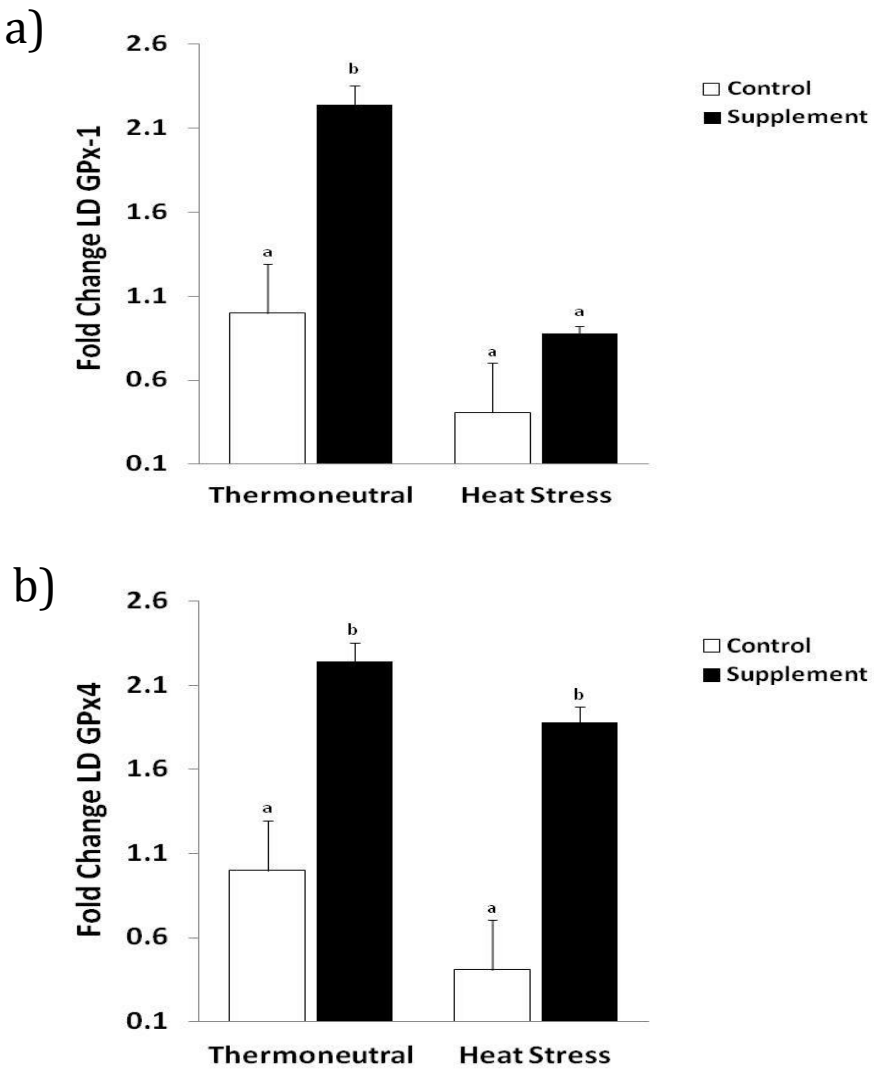

Figure 1.2 Effect of dietary vitamin E and selenium (doses of Vit E and Se for control (CON) and (Supplement) diets were 27.6 and 130-227.5 IU/kg DM as α- tocopherol acetate and 0.16 and 0.66-1.16 mg Se as SelPlex™ kg^{-1}DM, respectively) on the expression of a) Glutathione peroxidase-1(GPx1), b) Glutathione peroxidase- 4 (GPx4) gene in longissimus muscle of lambs exposed to thermoneutral (TN) or heat stress (HS) during finishing phase.

Table 1.1 Effect of dietary vitamin E and selenium on feed intake, plasma oxidative stress biomarkers and cortisol levels of lambs exposed to hot conditions for 7 days during the finishing phase:

	Thermoneutral			Heat stress				P-value			
	CON[1]	MOD[2]	SUP[3]	CON	MOD	SUP	SE	Temp[4]	Linear	Quadratic	Temp x Linear
Feed intake[5], g day^{-1}	1266^{a}	1129^{a}	1356^{a}	959^{b}	1094^{ab}	1476^{a}	137.2	0.36	0.002	0.13	0.037
ROMs, CARR U[6]	124^{a}	123^{a}	113^{a}	150^{b}	136^{ab}	123^{a}	10.9	0.013	0.021	0.77	0.3
BAP, mmol L^{-1}	3677^{a}	3722^{a}	3284^{b}	3471^{a}	3519^{a}	3613^{a}	241.5	0.85	0.47	0.46	0.13
OSI, arbitrary units	3.42^{a}	3.38^{a}	3.43^{a}	4.34^{b}	3.89^{ab}	3.53^{a}	0.349	0.015	0.11	0.85	0.1
AOPP, mol L^{-1}	33.1^{a}	38.8^{a}	33.1^{a}	48.4^{b}	38.8^{a}	33.4^{a}	4.78	0.069	0.032	0.54	0.032
Cortisol[7], ng mL^{-1}	0.74^{a}	0.98^{ab}	0.88^{ab}	1.1^{b}	1.05^{b}	1.11^{b}	0.211	0.08	0.64	0.61	0.73
	(-5.51)	(-9.73)	(-7.6)	(-12.6)	(-11.3)	(-13)					

Values within the same row with different superscripts were significantly different ($P < 0.05$).

[1] Control = Control diet contained Vit E 28 mg/kg DM as α- tocopherol acetate, and 0.16 mg/kg DM Se as SelPlex™

[2] Moderate = Moderate diet contained Vit E 130 mg/kg DM as α- tocopherol acetate, and 0.66 mg/kg DM Se as SelPlex™

[3] Supranutritional = Supranutritional diet contained Vit E 227.5 mg/kg DM as α- tocopherol acetate, and 1.16 mg/kg DM Se as SelPlex™

[4] Temperature = Lambs were exposed to either thermoneutral (18- 21°C and 40-50% relative humidity (RH) or Heat stress (HS) (28-40°C and 30-40% RH) conditions before holding in lairage for 14 h under thermoneutral conditions.

[5] Feed intake = average of feed intake over 7 d thermal treatment period.

[6] CARR U = Carratelli Unit (1 CARR U = 0.08mg/dl of H_2O_2)

[7] Values were log transformed

Table 1.2 Effect of dietary vitamin E and selenium on physiological measures, plasma oxidative status biomarkers and cortisol levels of lambs finished under thermoneutral or hot conditions and kept in lairage for 18 h

	Thermoneutral			Heat stress				P-value				
	CON[1]	MOD[2]	SUP[3]	CON	MOD	SUP	SE	Temp[4]	Linear	Quadratic	Temp x Linear	Temp x diet x lairage
Respiration rate, breaths min[1]	57.6	59.1	59.6	55.5	58.1	54.8	3.99	0.26	0.82	0.47	0.62	0.96
Rectal temperature, °C	39.31	39.36	39.34	39.33	39.14	39.31	0.225	0.55	0.97	0.61	0.91	0.66
ROMs, CARR U[5]	106^{a}	115^{a}	97.4^{a}	133^{b}	130^{b}	113^{a}	8.46	<0.001	0.019	0.057	0.35	0.9
BAP, mmol L^{-1}	3410^{a}	3352^{a}	3395^{a}	3038^{b}	3143^{ab}	3381^{a}	129.9	0.012	0.082	0.46	0.059	0.16
OSI, arbitrary units	3.13^{a}	3.45^{a}	2.88^{a}	4.38^{b}	4.17^{b}	3.32^{a}	0.271	<0.001	0.001	0.026	0.043	0.34
AOPP, mol L^{-1}	33.3^{a}	31.8^{a}	35.2^{a}	50.8^{b}	49.2^{b}	32.2^{a}	6.02	0.004	0.056	0.48	0.021	0.85
Cortisol[6], ng mL^{-1}	1.16	1.26	1.26	1.42	1.2	1.31	0.149	0.33	0.99	0.51	0.34	0.78
	(-14.3)	(-18)	(-18.3)	(-26.5)	(-15.7)	(-20.3)						

Values within the same row with different superscripts were significantly different (P < 0.05).

[1] Control = Control diet contained Vit E 28 mg/kg DM as α- tocopherol acetate, and 0.16 mg/kg DM Se as SelPlex™

[2] Moderate = Moderate diet contained Vit E 130 mg/kg DM as α- tocopherol acetate, and 0.66 mg/kg DM Se as SelPlex™

[3] Supranutritional = Supranutritional diet contained Vit E 227.5 mg/kg DM as α- tocopherol acetate, and 1.16 mg/kg DM Se as SelPlex™

[4] Temperature = Lambs were exposed to either thermoneutral (18- 21°C and 40-50% relative humidity (RH) or Heat stress (HS) (28-40°C and 30-40% RH) conditions before holding in lairage for 14 h under thermoneutral conditions before holding in lairage for 14 h under thermoneutral conditions.

[5] CARR U = Carratelli Unit (1 CARR U = 0.08mg/dl of H_2O_2)

[6] Values were log transformed

Table 1.3. Effect of dietary vitamin E and selenium supplementation on muscle vitamin E concentration and carcass quality parameters of lambs exposed to hot conditions during finishing phase

	Thermoneutral			Heat stress				P-value		
	CON[A]	MOD[B]	SUP[C]	CON	MOD	SUP	SED	Temp[D]	Diet	Temp x diet
Fat Score	3	3	3	3	3	3	0.1	0.387	0.023	0.123
GR, mm	10	8	10	10	8	10	0.6	0.568	0.006	0.932
HSCW[E], kg	20.9	21.4	22.7	21.6	20.7	22.2	0.46	0.695	0.047	0.428
Vitamin E, mg/kg	1.73	1.92	1.96	1.72	2.13	2.07	0.141	0.476	0.152	0.797

[A] Control,
[B] Moderate,
[C] Supranutritional,
[D] Temperature,
[E] Hot standard carcass weight

Table 2.1: Chemical composition of diets fed to lambs consuming grain based diet with moderate (MOD) or supranutritional (SUP) level of antioxidant or lucerne based diet (LUC).

Components, g·kg^{-1}	MOD	SUP	LUC
Oat hay	300	300	-
Lucerne hay	-	-	600
Barley	530	530	390
Canola expeller	150	150	-
Limestone	10	10	-
Acid Buf® (Celtic Sea Minerals)[1]	10	10	10
Chemical composition			
DM	91.6	91.8	89.2
CP, g·kg DM^{-1}	156	138	150
NDF, g·kg DM^{-1}	230	246	222
ADF, g·kg DM^{-1}	140	145	462
Estimated IVDOMD[2], %	76.7	76.6	67.3
EE, g·kg DM^{-1}	60	71	
Ash, g·kg DM^{-1}	87	81	36
Estimated ME, MJ·kg DM^{-1}	13.1	13.4	10.6
Vitamin E, mg·kg AF^{-1}	41.92	284.79	37.19

[1] DM 95% ¸Ca 30%, Mg 5.5%, K0.7%, P 500 mg·kg^{-1}, Bo 10 mg·kg^{-1} Fe 800 mg·kg^{-1}, Co 0.1mg·kg^{-1}, Cu 10mg·kg^{-1}, Zn 10mg·kg^{-1}, Mn 50 mg·kg^{-1}, Mo 0. 2mg·kg^{-1} Se 1.8 mg·kg^{-1}, I 30 mg·kg^{-1}

[2] In vitro digestibility of organic matter.

Table 2.2 Fatty acid composition of the experimental diets (mg FAME·100g^{-1} AF)

Fatty acids	MOD	SUP	LUC
C12:0	7.91	1.31	12.29
C13:0	2.60	2.61	2.57
C14:0	22.07	29.40	20.78
C14:1	1.30	2.61	2.57
C15:0	2.60	2.61	7.48
C15:1	2.60	2.61	2.57
C16:0	848.59	1117.26	847.99
C16:1	20.38	27.38	14.99
C17:0	5.75	7.56	9.21
C17:1	2.60	2.61	2.57
C18:0	198.22	166.95	261.35
C18:1n9c	4658.98	3208.90	6785.61
C18:2n6c	1714.48	2011.91	1733.03
C20:0n6	24.69	26.90	33.69
C18:3n6	2.60	2.61	2.57
C18:3n3 (ALA)	241.89	248.56	333.98
C18:2 9c,11t (CLA)	2.60	2.61	2.57
Total n3	241.89	248.56	333.98
Total n6	1722.28	2174.37	1740.73
n6/n3	6.76	8.23	4.88
Saturated Fatty Acids (SFA)	1165.17	1390.95	1307.11
Mono Unsaturated Fatty Acids (MUFA)	4688.46	3246.74	6810.89
Polyunsaturated Fatty Acids (PUFA)	1979.60	2440.72	2099.85
PUFA/SFA	2.42	2.50	2.29
Total fatty acids	7831	7075	10215

Table 2.3 Growth performance, feed intake and carcass characteristics

	MOD	SUP	LUC	SEM	P
Number of animals	13	13	15		
Initial weight, kg	38.04	38.22	37.30	0.53	0.40
Final weight, kg	44.13	44.59	45.35	1.62	0.87
Average daily gain, $g \cdot d^{-1}$	102	106	129	29	0.76
Average daily feed intake (ADFI), $g \cdot d^{-1}$	903	1033	1045	90	0.46
Estimated ME intake, $MJ \cdot d^{-1}$	11.83	13.85	11.08	1.12	0.19
CP intake, $g \cdot d^{-1}$	141	143	157	18.36	0.61
Average daily vitamin E intake, $mg \cdot d^{-1}$	41.14 A	232.33 B	41.89 B	20.75	<0.001
Number of carcasses	13	13	14		
Hot Carcass weight (HCW), kg	21.30	22.15	22.16	0.79	0.68
GR, mm	13.08	13.46	15.50	1.19	0.30

Table 2.4 Loin fatty acid composition (mg FAME·100g^{-1} of fresh meat)

	MOD	SUP	LUC	SEM	P
C12:0	6.41	7.34	7.33	0.85	0.67
C13:0	0.69	0.70	0.68	0.01	0.49
C14:0	104.53	112.6	114.10	10.57	0.79
C14:1	2.76	2.75	2.72	0.44	1.00
C15:0	14.26	14.65	13.97	1.17	0.92
C15:1	2.44	2.45	2.21	0.13	0.27
C16:0	804.46	845.59	853.36	53.60	0.78
C16:1	41.71	42.13	40.74	3.64	0.96
C17:0	39.10	40.95	38.81	2.62	0.82
C17:1	6.81 AB	7.79 A	5.99 B	0.48	0.03
C18:0	452.69 B	520.44 b	609.84 aA	29.96	0.0023
C18:1n-9c	1083.21	1257.24	1308.10	79.42	0.12
C18:2n-6c	198.42 A	179.12 A	124.33 B	7.88	<0.0001
C20:0	2.60	3.24	3.50	0.24	0.03
C18:3n-6	0.68	0.70	0.68	0.01	0.49
C18:3n-3	31.25	33.24	30.69	2.13	0.67
C18:2 9c,11t (CLA)	7.17 a	7.70 ab	10.48 b	1.16	0.10
C21:0	1.27	1.43	1.73	0.24	0.37
C20:2n-6	1.27 A	1.00 AB	0.68 B	0.14	0.02
C22:0	0.68	0.70	0.68	0.01	0.49
C20:3n-6	4.67 A	4.83 A	3.90 B	0.15	0.0001
C20:3n-3	0.70	0.70	0.68	0.01	0.49
C20:4n-6	49.08 A	49.68 A	40.33 B	1.89	0.001
C23:0	1.36	1.89	1.51	0.25	0.30
C22:2n-6	0.69	0.70	0.68	0.01	0.49
C20:5n-3 (EPA)	14.26	15.54	13.77	0.61	0.11
C24:0	0.69	0.70	0.68	0.01	0.49
C24:1	0.69	0.70	0.68	0.01	0.49
C22:5n-3 (DPA)	24.29	24.64	23.40	0.76	0.48
C22:6n-3 (DHA)	6.78	6.97	7.58	0.44	0.40
EPA+DHA	21.04	22.51	21.36	0.89	0.48
EPA+DPA+DHA	45.33	47.15	44.76	1.49	0.49
n-3	77.27	81.09	76.14	3.10	0.49
n-6	254.81 A	236.03 A	170.61 B	8.43	<0.0001
n-6/n-3	3.30 a	2.91 bB	2.47 C	0.13	<0.0001
SFA	1428.74	1550.24	1646.21	93.63	0.26
MUFA	1137.62	1313.08	13.60.44	82.81	0.14
PUFA	339.24 A	324.83 A	257.23 B	10.43	<0.0001
PUFA/SFA	0.24 A	0.22 A	0.16 B	0.01	<0.0001
TOTAL FAT	2905.61	3188.16	3263.90	177.25	0.32

Table 2.5 Retail color stability of fresh loin

	MOD	SUP	LUC	SEM	P diet	P time	P diet*time
L*							
Day 1	31.76 X	31.55 X	33.36 X	0.35	0.07	<0.0001	0.87
Day 2	35.02 Y	34.97 Y	36.70 Y				
Day 3	34.10 Y	34.00 YZ	35.41 YZ				
Day 4	33.66 y	32.76 XZ	34.61 XZ				
a*							
Day 1	17.74 Xx	17.48 x	17.46	0.27	0.18	<0.0001	0.08
Day 2	16.52 xy	16.30 xy	17.10				
Day 3	16.11 y	16.43 xy	17.79				
Day 4	15.57 Y	15.70 y	16.94				
b*							
	MOD	SUP	LUC	SEM	P diet	P time	P diet*time
Day 1	14.98 X	14.62 xX	14.90 X	0.18	0.05	<0.0001	0.61
Day 2	16.67 Y	15.86 xy	16.50 Y				
Day 3	16.67 Y	16.44 Y	17.28 Y				
Day 4	16.78 Y	16.04 y	17.12 Y				
R630/580							
Day 1	6.25 X	6.02 X	5.80 X	0.13	0.66	<0.0001	0.05
Day 2	3.99 Yy	4.07 Y	4.25 Y				
Day 3	3.36 yz	3.66 Y	3.98 Y				
Day 4	3.12 z	3.57 Y	3.59 Y				

	MOD	SUP	LUC	SEM	P diet	P time	P diet*time
L*							
Day 1	37.82 X	38.28 X	37.64 X	0.48	0.76	<0.0001	0.55
Day 2	35.23 Y	35.31 Y	34.85 Y				
Day 3	34.74 Y	35.22 Y	34.19 Y				
Day 4	34.62 Y	35.07 Y	33.82 Y				
a*							
Day 1	20.08 X	19.06 X	19.40 X	0.31	0.04	<0.0001	0.07
Day 2	17.83 Y	15.55 Y	17.16 Y				
Day 3	15.08 Z	12.90 Z	14.76 Z				
Day 4	13.16 W	11.35 W	12.86 W				
b*							
Day 1	17.43 X	16.98 X	17.02 X	0.24	0.24	<0.0001	0.53
Day 2	17.92 X	16.89 X	17.37 X				
Day 3	16.60 Y	15.53 Y	15.82 Y				
Day 4	15.49 Z	14.36 Z	14.77 Z				
R630/580							
Day 1	5.59 X	5.27 X	5.36 X	0.13	0.13	<0.0001	0.02
Day 2	4.39 Y	3.63 y	4.38 Y				
Day 3	3.25 Z	2.63 Z	3.37 X				
Day 4	2.53 W	2.16 Z	2.66 W				

Table 2.6 Retail color stability of 6 weeks-aged loin

Table 2.7 Lipid oxidative substances (TBARS, expressed in mg MDA/kg meat) in meat displayed for 0 day and 4 days at simulated retail condition in loin stored at 2-3°C for 1 (fresh) or 42 days (aged).

	MOD	SUP	LUC	SEM	P diet	P time	P diet*time
Fresh							
Day 1	0.09	0.07	0.06	0.04	0.22	<0.0001	0.93
Day 4	0.18	0.15	0.14				
Aged							
Day 1	0.32	0.26	0.31	0.07	0.34	<0.0001	0.70
Day 4	1.93	1.38	1.48				

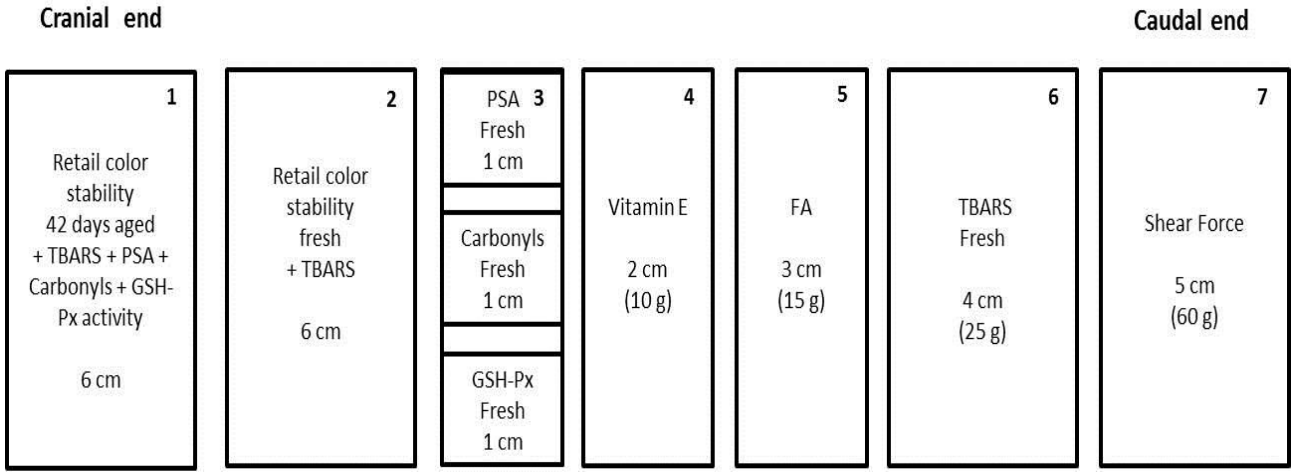

Figure 2.1 Sampling diagram of the left loin for meat quality analysis (samples 1, 2, 4, 5 and 6 have bee processed to obtain the data reported in the present milestone).

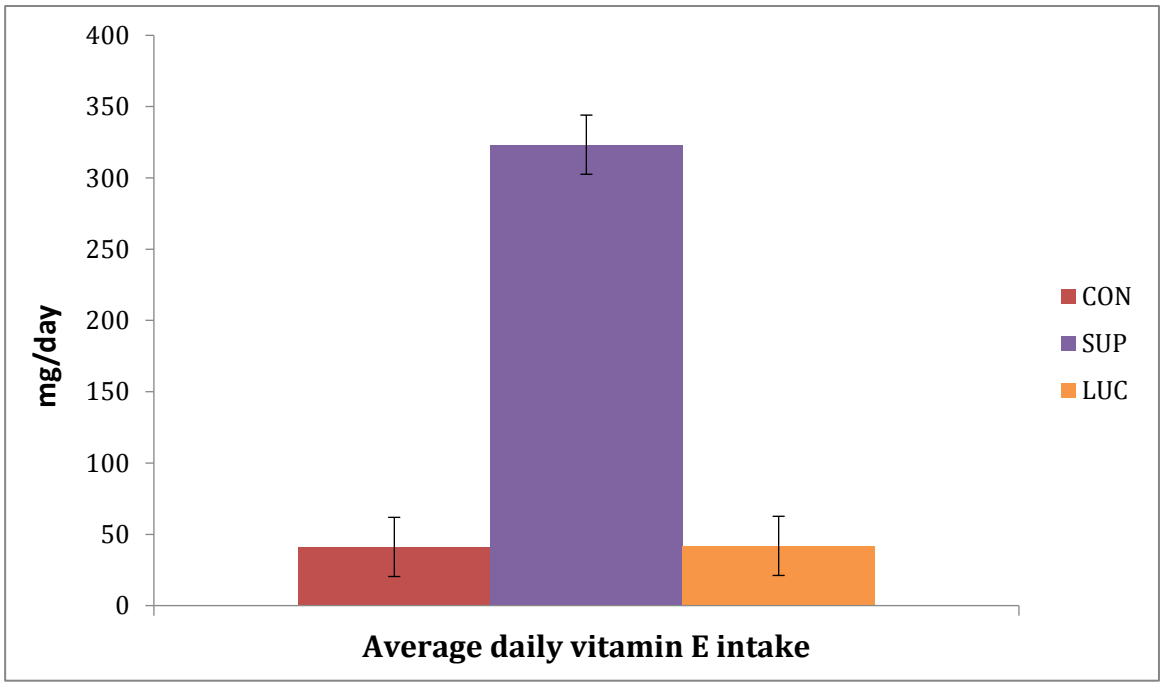

Figure 2.2 Average daily vitamin E intake of lambs fed CON or SUP or LUC diet

A,B P≤0.01

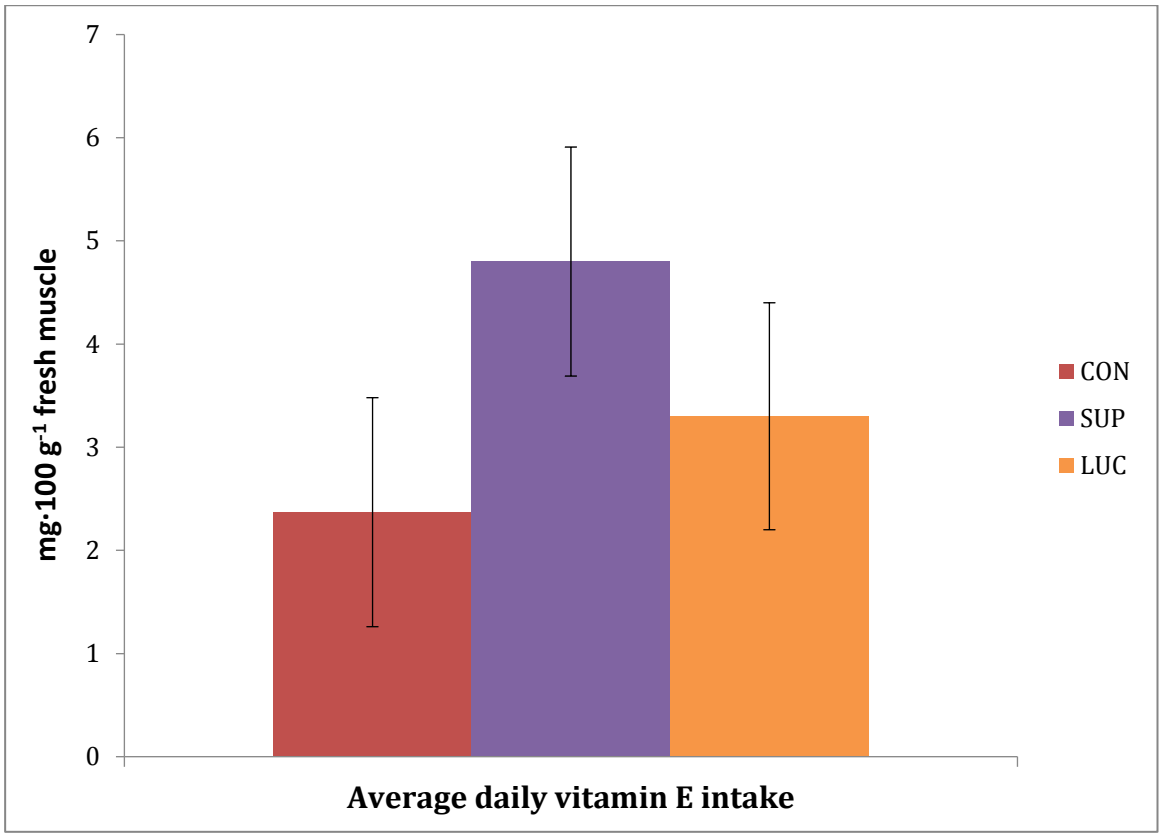

Figure 2.3 Muscle vitamin E concentration of lambs fed CON or SUP or LUC diet

7.0 Conclusion/Recommendations

The present research project has indicated that heat stress increases the rectal temperature and respiration rate of lambs finished under hot conditions resulting in increased oxidative stress and decreased feed intake compromising the growth rate. This research has also demonstrated that antioxidant supplementation during the finishing phase help the lambs to ameliorate heat stress impacts. Supplementation of vitamin E and Se for 3-4 weeks before the expected heat exposure help the lambs to reduce oxidative stress induced by heat stress and helps to maintain their feed intake resulting in enhanced growth rate and increased carcass weight. Further, this project has also shown that antioxidant intake of lambs can be increased either by finishing lambs on good quality pastures such as Lucerne or by supplementing antioxidants in concentrate diets. Pasture finishing is helpful to improve the colour stability of aged meat and oxidative muscles while the feed lotting with antioxidant supplementation improves the colour of fresh meat. Therefore, it is recommended to finish lambs on quality pastures if the meat is intended for sale after aging. The lambs that have to be transported to key markets under hot conditions should preferably be finished on concentrate diet with antioxidant supplementation for at least 3-4 weeks prior to transportation. To achieve the threshold of vitamin E (3.5 mg/kg) in muscle to improve colour stability, vitamin E supplementation @ 285 mg/kg of diet/ day for 4 weeks is required.

In this project, HS was of short duration and hence investigation of HS during the full duration of a finishing period is needed as applied to other hotter climates. Further research is also required to develop economic lamb finishing systems to achieve higher levels of antioxidants in lamb muscles and to test the effects of high antioxidant feeding on the oxidative stability of lamb meat and meat products. In this project, duration of transportation to slaughter was short and hence further research is also required to investigate the impact of long duration of transportation under hot conditions on meat quality (such as live export to middle east) and how the antioxidant supplementation during finishing period may help to reduce theses negative effects. In this project, impact of heat stress and antioxidant supplementation on different muscle type was not investigated and hence more research is also required to investigate the impact of heat stress on different muscle types and how the antioxidant supplementation improve the colour stability of particular muscles so as to develop muscle specific marketing strategies for meat and meat products.

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9.0 Appendix

9.1 Lamb Feeding Trial

At Dookie Campus, The University of Melbourne

9.2 Lamb Feeding Trial

At the Victorian Department of Economic Development, Jobs, Transport and Resources (DEDJTR), Rutherglen.

9.3 Appendix 3. Research Paper 1

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High dietary vitamin E and selenium improves feed intake and weight gain of finisher lambs and maintains redox homeostasis under hot conditions

S.S. Chauhan[a,1], E.N. Ponnampalam[a,b], P. Celi[a,c], D.L. Hopkins[d], B.J. Leury[a], F.R. Dunshea[a,*]

[a] Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010, Australia
[b] Agriculture Research and Development, DEDJTR, Attwood, VIC 3049, Australia
[c] Faculty of Veterinary Science, University of Sydney, Narellan, NSW 2567, Australia
[d] NSW Department of Primary Industries, Centre for Red Meat and Sheep Development, PO Box 129, Cowra, NSW 2794, Australia