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Short term supplementation rates to optimise vitamin E concentration for retail colour stability of Australian lamb meat

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Abstract

The relationship between vitamin E supplementation rate and colour stability was investigated using 70 mixed sex 6-8 month old crossbred lambs. An initial group of 10 were slaughtered, while the remainder were fed a pellet ration containing either 30, 150, 275 or 400IU Vitamin E/kg ration or on green pasture for 56 days. After slaughter, carcases were halved; one side packed fresh (5 days) and the other in CO_2 (21 days), both at 2^{o}C. Five muscles were set for retail display for 96 hours. The oxy/metmyoglobin ratio was measured every 12 hours. Colour stability increased with increasing muscle vitamin E until an apparent maximum effect for vitamin E concentration (3.5-4.0mg α-tocopherol/kg tissue) was reached beyond which no further response was evident. This was reached within 3-4 weeks (275IU treatment), and meat from these lambs should reach 60 hours retail display with a satisfactory surface colour. This effect was most apparent in aerobic muscle types and meat aged post slaughter.

Keywords: Colour stability; Vitamin E; Alpha tocopherol; supplementation; lamb.

1. Introduction

During retail display the surface of lamb meat will turn from red to brown. Consumers' choose to buy meat primarily by visual appearance. A cut with a bright red colour is more likely to be

sold than a cut that appears brown, forcing the retail sector to discount meat that fails to sell quickly, resulting in a substantial economic loss (Williams, Frye, Frigg, Schaefer, Scheller & Liu, 1992).

The browning of meat is due to the oxidation of the muscle pigment myoglobin, from red oxymyoglobin to brown metmyoglobin. Colour stability of meat is defined by the rate of this colour change. The formation of metmyoglobin, can be slowed by the use of antioxidants such as vitamin E (Faustman, Chan, Schaefer & Havens, 1998; Wulf, Morgan, Sanders, Tatum, Smith & Williams, 1995). In Western Australia vitamin E deficiency causes nutritional myopathy in grazing lambs due to a lack of green pasture in the summer season. Coincidentally meat retailers observe a decline in colour stability of meat during this period (Pearce, Masters, Smith, Jacob & Pethick, 2005), with meat appearing brown before the retail shelf life bench mark of 48 hours is reached (a benchmark commonly used in Australian retail).

Vitamin E (α-tocopherol) is a lipid soluble antioxidant that protects lipids from peroxidation. However the causal relationship between lipid peroxidation and formation of metmyoglobin in meat during retail display has yet to be proven (Faustman *et al.*, 1998). Thus while the mechanism is not yet proven it is assumed that vitamin E protects myoglobin in meat against oxidation by inhibition of lipid peroxidation (Faustman, Sun, Mancini & Suman, 2010).

Meat is often aged before sale because of logistical considerations of transport to market or strategically to enhance tenderness of premium cuts. A common practice by Australian processors is to age carcasses in CO_2 atmospheres, which allows for longer storage of 3-4 weeks. However, lipid peroxidation during retail display will increases with ageing and thus aging leads to a decline in the colour stability of meat after the product has been cut for retail display (Ponnampalam, Norng, Burnett, Dunshea, Jacobs & Hopkins, 2014; Wulf *et al.*, 1995). This is

particularly evident in more oxidative muscle types such as the *m. gluteus medius* (GM) (O'Keefe & Hood, 1982), where the free-radical load is likely greater. Therefore aged meat from the more oxidative cuts is likely to have greater potential for a protective effect of vitamin E. Vitamin E is available to grazing livestock from green pasture, and grain based rations need to be supplemented with vitamin E to meet nutritional requirements for animal health. The majority of colour stability studies have been done with cattle, whereby vitamin E supplementation of grain-based feed lot rations improved the colour stability of beef (Faustman *et al.*, 1998). Similar studies have shown positive effects in sheep (Guidera, Kerry, Buckley, Lynch & Morrissey, 1997; Turner, McClure, Weiss, Borton & Foster, 2002; Wulf *et al.*, 1995) over the supplementation range of 300IU to 1000IU of vitamin E/kg of feed, with these effects all similar in magnitude. Kasapidou, Wood, Richardson, Sinclair, Wilkinson & Enser (2012), found that the optimum level of vitamin E supplementation for decreasing lipid peroxidation was 250IU/kg. Alternatively, Turner *et al.* (2002) found no effect of vitamin E when supplemented at the rate of 15IU/kg. This implies that the rate of vitamin E supplementation must exceed a threshold concentration to impact on sheep meat colour stability which appears to lie between 15 and 250IU/kg. The authors are not aware of any work testing dose responses within this range in Australian lambs.

Threshold muscle concentrations of vitamin E have been described as a concentration in which a maximum response in maintaining colour stability is observed. These levels have been reported in sheep to be 1.9 (Kasapidou *et al.*, 2012), 2.26 (Álvarez, De la Fuente, Díaz, Lauzurica, Pérez & Cañeque, 2008) and 5.6mg/kg (Lopez Bote, Daza, Soares & Berges, 2001), while in beef, muscle vitamin E concentrations of between 3.0 (Faustman, Cassens, Schaefer, Buege, Williams & Scheller, 1989) and 3.3 mg/kg (Arnold, Arp, Scheller, Williams & Schaefer,

1993) have been reported to improve colour stability. More recently, threshold concentrations of 3.45mg/kg (Ponnampalam *et al.*, 2014) and 3.76mg/kg (Hopkins, Lamb, Kerr, van de Ven & Ponnampalam, 2013) have been reported for Australian sheep, however these studies did not involve any dietary supplementation and any dietary vitamin E was obtained from grazing green pastures. Any muscle concentrations beyond these threshold levels were shown to have no added benefit to the colour stability. Our objective was to confirm the concentration of vitamin E required in muscle to reach the maximum response in colour stability of lamb meat. Furthermore to determine the rate and period of time required for supplementation to achieve this level; to provide a practical solution for colour stability to the Australian prime lamb industry during periods of grain feeding over summer.

This experiment examined the effect on meat colour stability of the *ad lib* supplementation rate of vitamin E in the range of 30-400IU/kg fed to lambs for 8 weeks. Several hypotheses were tested:

(i) Colour stability will increase with vitamin E dose rate to a maximum response threshold value obtained in the supplementation range tested.

(ii) The positive effect of vitamin E supplementation on colour stability will be most apparent when meat is aged and in more oxidative type muscles.

(iii) Lambs from green pasture will have a higher muscle vitamin E concentration compared to those supplemented with 30IU (minimum nutritional requirement) and thus will have a better colour stability.

2. Methods and materials

2.1. Lambs and dietary treatments

This experiment was performed under the guidelines of animal ethics and all procedures were given approval via the animal ethics committee at Murdoch University.

70 mixed sex 6-8 month old crossbred lambs with an average live weight of 38.0±0.38 kg (mean±sem) were used for this experiment. Lambs were sourced in summer from a commercial farm where they grazed dry pasture supplemented with lupin grain at the rate of 600g/lamb/day, conditions in which tissue vitamin E levels are rapidly depleted (Fry, Smith, McGrath, Speijers & Allen, 1993). Initial plasma measurements, confirmed that all animals entering the experiment were deficient or close to deficient in vitamin E (< 0.5mg/L; (Menzies, Langs, Boermans, Martin & McNally, 2004)). Lambs were transported to Murdoch University farm, stratified for weight and a group of the 10 heaviest lambs (mean live weight 38.8 ± 0.47 kg) were sent immediately to a commercial abattoir for slaughter in order to measure colour stability of meat from non-supplemented lambs (pre-treatment level) coming straight off the dry pasture. From the remaining 60 lambs the heaviest 12 were allocated to the pasture group (assuming subsequent growth rates would be lower) and the remainder randomly allocated to 1 of 4 groups (n=12), where the animals would be supplemented with either 30, 150, 275 or 400 IU of vitamin E/kg of pelleted feed. They were drenched with an anthelmintic that contained selenium (Cydectin© 0.5mg/ml sodium selenate; administered at the rate of 1ml/5kg of body weight) and as suggested by the manufacturer would offer 8 weeks of protection. Groups 1 to 4 were held in pens under shade and fed a pelleted ration for 56 days whilst pasture group grazed an irrigated pasture

(mixed sward of kikuyu (*Pennisetum clandestinum*)/clover (*Trifolium)*/ryegrass *(Lolium perenne))* for the same period. The clover contained 97mg/kg of vitamin E and the kikuyu grass pasture 127 mg/kg on a dry matter basis (as measured by methods described below). No estimate was made for the ryegrass as this was present in very small amounts. The pelleted feed contained synthetic α-tocopherol acetate (ROVIMIX® E50) at the rate of 30, 150, 275 and 400 IU/kg DM for groups 1 to 4 accordingly. The pellets consisted of 30% barley, 3.5% oats, 28% lupin grain and 38.5% hay, with an energy content of 11 MJ/kg ME and 18% crude protein.

Muscle biopsies were performed on all animals upon arrival at Murdoch University, then every 14 days thereafter (a total of 4 times), to measure muscle vitamin E concentrations in the *m. semimembranosus* (SM) and *m. semitendinosus* (ST). Biopsies were performed by the method of Gardner, Jacob and Pethick (2001). Briefly, the sampling site was located in the groove between the two muscles, approximately 5 - 10cm distal to the anus. This location allowed clear access to both muscles through a single incision. Following biopsy, muscle samples were placed into liquid nitrogen after "blotting" away blood and removal of visible fat. Muscle samples were stored at -80 °C until assayed. At the time of each biopsy lamb live weights were measured and venous blood was taken for vitamin E analysis. Blood was taken from the jugular vein using lith-heperan vacutubes and placed on ice before centrifugation to allow for the sampling of plasma.

After 56 days the animals were transported for 2 hours to the same commercial abattoir used to slaughter the initial 10 lambs and slaughtered after a 24h lairage period. Liver samples were collected immediately post slaughter for Vitamin E analysis.

Each carcase was divided longitudinally into halves and 1 entire half was packaged in a modified atmosphere package containing greater than 99% Carbon Dioxide with a 2:1 gas to muscle headspace ratio and aged for 21 days. Carbon Dioxide flushing was carried out as per

industry standards highlighted by Meat and Livestock Australia. Briefly, carcase sides were stored in bags that are not permeable to oxygen or other gases. Target oxygen levels inside the pack were to be less than 0.1% and no greater than 0.15% and thus the bags were flushed and evacuated three times with CO_2 before the bags were finally sealed and stored. This group was labelled as "Aged". The other carcase half was packed into a loose and unsealed plastic bag for 5 days before muscles were dissected, and the group was labelled as "Fresh". Both the "Aged" and "Fresh" samples were stored together in the same cool room at 2^{o}C for the respective time period before 5 muscles (*m. gluteus medius* (GM), *m. longissimus lumborum* (LL), SM, ST and *m.rectus femoris* (RF)) were dissected for sampling. The LL, SM and ST were taken from all carcases, while the RF and GM were sampled in half the carcases only, due to some carcases not having RF or GM large enough to provide an appropriate sized steak.

2.2. Retail display and Colour stability measurements

Prior to commencing measurements each muscle was sliced into 2cm thick slices, visible fat removed, placed on black Styrofoam trays. Samples were allowed to bloom for 30 minutes at 4^{o}C beforebeing over wrapped with chloride cling wrap (Resinite "DHW" Meat AEP, 3µ thickness, oxygen transmission rate of 2300 – 3000 cc/100 sq in/24hrs) and stored at 4^{o}C in a display refrigerator fitted with cool white fluorescent lights (OSRAM L36W/20, Germany). Colour measurements were taken every 12 hours for 96 hours using a Hunter Lab Mini Scan XE Plus (model No. 45/0-L, Hunter Associates Laboratory Inc., Reston VA, USA, aoerture of 3.18 cm), with the light source set to "D65" and the observer angle set to 10^{o}.

Light reflectance was measured and the oxy/metmyoglobin ratio calculated as the ratio of light reflectance at 630 and 580 nm. Hunt (1980) noted that very little metmyoglobin was present

in meat with an oxy/metmyoglobin ratio above 4, while at a ratio approaching 1, metmyoglobin was the only remaining form. Thus the perceived surface colour of meat will increase in brownness as the oxy/metmyoglobin ratio decreases from 4 to 1. At an oxy/metmyoglobin ratio of 3.5 consumers perceive meat to be brown in colour, influencing purchasing choices (Morrissey, Jacob & Pluske, 2008). Khliji, van de Ven, Lamb, Lanza & Hopkins (2010) reported this level to be 3.3, however, the bench mark by Morrissey *et al.* (2008) was calculated using the same lab and materials as the current experiment so the value of 3.5 was used as a benchmark for consumer acceptance of meat colour in this study. Hunter L*a*b* colour scores were also recorded.

2.3. Assay procedure, Ultimate pH and Driploss

The vitamin E content of muscle, blood, liver and muscle biopsies were measured using high performance liquid chromatography with fluorescence detection (McMurray & Blanchflower, 1979). Muscle and feed samples were saponified by the method of Bieri *et al.*(1961), before extraction with hexane. Plasma was deprotienized before the assay using ethanol. The assay was standardized against an α-tocopherol standard (0.496g per 50ml; added at the commencement of assay) and the recovery rates for tissues and feed samples were 94% and 91% respectively.

Drip loss was measured by taking samples of SM, LL and RF. At the beginning of the retail display period a piece of muscle weighing about 15g (approximately a 3cm cube) was weighed and recorded and noted as the weight at display time zero. Samples were wrapped in mesh netting before being hung in a plastic bag at 2^{o}C for 24 hours. These samples were weighed again after 24h, the weight loss calculated.

Ultimate pH (pHu) was measured in each muscle at the time the meat was cut for retail display, using a TPS WP-80 pH meter (TPS Australia, Qld, Cat no: 121180) equipped with a glass-tipped Ionode pH electrode (Ionode Pty Ltd, Qld, Cat no: IJ 44) and a temperature probe (TPS Australia, Cat no: 121249) calibrated at ambient temperature using buffers at pH 4.00 and 6.88.

2.4. Data analysis

The software package SAS® was used for all statistical analyses (SAS Institute, 2001). Oxy/metmyoglobin ratio data was analysed using a linear mixed effects model using display time as a covariate, diet, muscle and packaging type as fixed effects, and animal within package as a random term The initial L* value was analysed in a similar model excluding time as a covariate. In a separate analysis, the oxy/metmyoglobin ratio at 48 and 60 hours for each individual sample was predicted for the grain fed animals only by fitting the following exponential function:

$$y_t = x_u + (x_i - x_u)^{-xkt}$$

where y_t = oxy/metmyoglobin ratio at time t, x_u = final oxy/metmyoglobin ratio reached, x_i = starting ratio, xk is a constant and t is time. This function was fitted to the data using a non linear model in SAS. This enabled the impact of muscle Vitamin E concentration on the predicted oxy/metmyoglobin ratios at 48 and 60 hours to be tested, and to estimate the threshold concentration of muscle Vitamin E that yielded the least change in oxy/metmyoglobin ratio during the display period. Linear mixed effects models were used, with packaging type and muscle as fixed effects, muscle Vitamin E concentration as a covariate, and animal within package as the random term.

The oxy/metmyoglobin ratio first reading was taken 30 minutes after cutting to allow bloom time returned consistently lower values than the subsequent readings. This was most likely due to an interaction between the time allowed for the blooming process to occur and the commencement of myoglobin oxidation. Therefore the values at time zero were excluded from all model fitting processes.

All mean data is presented with plus or minus the standard error of the mean (±sem).

3. Results

3.1. Liveweight and growth

Lambs on the pelleted rations consumed about 1.6 kg per head per day. Given that Vitamin E was supplemented at 30, 150, 275 and 400 IU per kg of this ration this resulted in total Vitamin E intakes of 48, 240, 440 or 640 IU/lamb/day of α-tocopherol acetate for the 4 treatment groups accordingly. No estimate could be made for the green pasture group. Lambs grew at an average of 1.35±0.143, 1.15±0.143, 1.29±0.149, 0.99±0.143 and 0.39±0.143 kg/week (±sem) for the 30, 150, 275, 400IU and green pasture diets respectively. Those fed the grain diets grew faster than the pasture lambs ($P<0.05$). Mean slaughter weights were 38.8 ± 0.47, 47.63±0.896, 44.83±0.896, 47.27±0.936, 43.69±0.896 and 43.69±0.896 (±sem) for the Pre-treatment, 30, 150, 275, 400IU and green pasture diets respectively. The 30 and 275IU groups were the heaviest at slaughter ($P<0.05$).

3.2. Tissue Vitamin E concentration

The concentration of vitamin E in both SM (Figure 1a) and ST (Figure 1b) increased with time during the feeding period ($P<0.05$) across all treatments groups except the 30IU treatment. The rate that muscle accumulated vitamin E increased as the level of supplementation increased ($P<0.05$). Muscle Vitamin E concentrations in the 400IU group increased nearly 3-fold over 6 weeks compared to only 2 fold for the 150IU group. Until week 4, the muscle vitamin E of the pasture fed and 400IU group animals increased similarly, after which the concentration for pasture fed animals seemed to reached a plateau.

Vitamin E concentration changed in plasma in a similar trend to muscle but to a greater magnitude. In the 400IU group the value at 6 weeks, was as much as 4 fold than the starting value (Figure 1c). In contrast to muscle, the 30IU group showed some increase ($P<0.05$) doubling over the supplementation period (Figure 1c). The levels of vitamin E in the plasma from the Pre-treatment lambs (Table 1) were sufficiently low enough to be at high risk of developing nutritional myopathy (< 0.5mg/L; (Menzies *et al*., 2004)) and these lambs can be regarded as being deficient in vitamin E.

At slaughter, muscle, plasma and liver vitamin E concentration generally increased according to the supplementation rate of α-tocopherol acetate ($p<0.05$) (Table 1). The exception being the pre-treatment group, which had the lowest vitamin E concentration in liver and plasma; but muscle values were no different to the 30IU group in all muscles except the LL (Table1). Vitamin E concentrations differed between muscles ($P<0.05$), however this also varied within diet treatments and no one muscle constantly had the highest vitamin E concentration. However the ST consistently contained the lowest concentration of vitamin E across all diets (Table 1).

Strong positive correlations existed ($P<0.05$) between all tissues for vitamin E concentration at slaughter, although the correlation between plasma and the rest of the tissues were relatively weaker (Table 2). During the growing period, plasma levels correlated well with the muscle concentrations (biopsy data) at week 0 and 2 of the feeding period (Table 2). However, at week 6 the correlation between plasma and muscle concentrations was weaker.

3.3. Colour Stability

The oxy/metmyoglobin ratio was significantly affected by diet ($P<0.01$; Table 3). All animals supplemented with α-tocopherol acetate had higher muscle oxy/metmyoglobin ratios ($P<0.05$) in the later periods of display when compared to pre-treatment animals (Figure 2). Pre-treatment animals had relatively poor colour stability, and in some cases (SM Aged; Figure 2e) cuts from pre-treatment animals reached a ratio of 3.5, 40 hours before cuts from the vitamin E treated animals. The colour of meat from green pasture animals declined in ratio at a similar rate to those supplemented with Vitamin E, however due to a lower starting point they reached a ratio of 3.5 earlier resulting in the shorter shelf life observed (Figure 2). Similar rates were also observed in the pre-treatment samples but in the aged treatments only, while fresh pre-treatment samples had a notably more rapid decline in colour ($P<0.05$).

Muscle vitamin E concentration had a positive effect on the oxy/metmyoglobin ratio however this was only seen in meat packaged/aged in CO_2 (Figure 2). Increasing the muscle vitamin E concentration improved the oxy/metmyoglobin ratio (maintaining an oxy/metmyoglobin ratio above 3.5 for 60 hours retail display) at all time points and for all

muscles except the GM (Figure 3; only LL, SM and GM shown). The GM muscle did not maintain a ratio of 3.5 at 48 hours for any vitamin E concentration (Figure 3). Furthermore, when muscle concentrations reached between 3.0 - 3.5mg α-tocopherol/kg tissue (4mg/kg in the SM) no further improvement of colour was observed in any muscle.

When vitamin E levels were deficient (from an animal health perspective), as observed in the pre-treatment animals, aging resulted in a lower oxy/metmyoglobin ratio over the display period in the GM, LL and SM muscles (Figure 2), and all muscles reached an oxy/metmyoglobin ratio of 3.5, 15-20 hours earlier than the fresh product for the pre-treatment group of lambs. However there was no difference observed in the ST between aging treatments in the pre-treatment group, and aging extended the shelf life of the RF by approximately 15 hours (Figure 2). Aged cuts supplemented with Vitamin E maintained a higher oxy/metmyoglobin ratio over the display period when compared to the fresh products of the same dietary treatments ($P<0.05$; Table 3), this trend was observed across all muscles except the SM (Figure 2e and f). In the GM this increase was worth 10 hours retail display, while an estimate in other muscles could not be made as a ratio of 3.5 was not reached over 96 hours of display.

There was a significant effect of muscle type ($P<0.05$; Table 3) on the oxy/metmyoglobin ratio, having a similar trend across all diets. The oxy/metmyoglobin ratio of the SM, LL and GM declined rapidly (2-3 ratio units) when compared to the RF and ST muscles (1-1.5 ratio units). Although the ST and RF declined slowly, the initial oxy/metmyoglobin ratio was low and thus sometimes reached a ratio of 3.5 early in the display period. The LL muscle consistently had the highest initial oxy/metmyoglobin ratio; this often resulted in LL samples not reaching a ratio of 3.5 over the display period. The effect of vitamin E were most evident in the LL, SM and GM

muscles, where pre-treatment samples declined about 1.5 units more over the display period than the other samples.

Generally freshly cut meat initially resulted in lower L* values (darker) than the aged product ($P<0.05$) in all diets except the pre-treatment group (37.39±0.63, 36.96±0.61, 36.61±0.58, 36.44±0.61, 37.66±0.58, 37.35±0.61 compared to the aged values 37.69±0.64, 37.99±0.61, 38.51±0.59, 38.11±0.61, 38.65±0.59, 38.71±0.61 for the 30, 150, 275, 400 IU and pasture diets respectively). In the LL, SM and GM the aged product became darker while the fresh product would become lighter over the display period ($P<0.05$; data not shown). Pre-treatment animals showed a more rapid decline in the a* value compared to Vitamin E treated animals (Figure 4; SM shown only); this effect was amplified when the cuts were aged. Meat from pasture fed animals had a lower a* value than any other fresh packaged cuts ($P<0.05$; Table 3). However, aging the product improved the a* value within the pasture animals during early display hours ($P<0.05$; Figure 4 SM shown only).

3.4. Ultimate pH and Driploss

Ultimate pH (pHu) was significantly affected by diet and muscle type ($P<0.05$). Diet only affected the pHu of the GM and the RF muscles, resulting in a higher pHu in the GM of pre-treatment animals, while the pre-treatment RF was low when compared to Vitamin E grain fed animals (Table 1). The ST and the RF had significantly higher pHu when compared to the other muscles (Table 1).

Meat from carcase sides aged in CO_2 packs lost half a percentage more moisture over 24 hours ($P<0.05$; CO_2 2.79±0.049; Fresh 2.35±0.071). The RF muscle lost about 0.66 and 0.55 % less weight over 24 hours than both the LL and SM muscles respectively ($P<0.05$; RF 2.23±0.081; LL 2.89±0.073; SM 2.78±0.074). Meat from carcases fed diets containing Vitamin E lost at least 1.3 % less moisture than the pre-treatment animals and meat from pasture animals retained the most weight over 24 hours ($P<0.05$; Pre-treatment 3.9±0.191; 30IU 2.59±0.095; 150IU 2.49±0.098; 275IU 2.31±0.098; 400IU 2.37±0.093; Pasture 2.16±0.095).

4. Discussion

4.1. Colour Stability

The results supported our hypothesis that a threshold concentration exists for vitamin E in muscle above which colour stability is not improved any further. This threshold concentration was 3.5-4.0mg/kg of vitamin E (figure 3), similar to those reported in beef, 3.0-3.3 mg α-tocopherol/kg tissue (Arnold *et al.*, 1993; Faustman *et al.*, 1989), and in the range previously reported for lamb, 2.26-5.3 α-tocopherol/kg tissue (Ponnampalam *et al*., 2014; Hopkins *et al*., 2013; Álvarez *et al.*, 2008; Lopez Bote *et al.*, 2001). By comparison the maximum concentration expected for muscle is probably 6-10 mg/kg (Wulf *et al.*1995; Chan, Hakkarainen, Faustman, Schaefer, Scheller & Liu, 1996) so the threshold level for colour stability occurred at about half

the maximum concentration possible. Wulf *et al.*(1995) reported concentrations of 5.79 mg/kg in the LL after feeding for 56 days at the rate of 1000IU. Concentrations as high as 10 mg α-tocopherol/kg tissue in beef *m. psoas major* have been achieved after dietary supplementation (Chan *et al.* 1996).

The optimal supplementation level in feed to achieve meat colour stability depends on the length of the feeding time available to reach the threshold level in muscle. It appears that when feeding for 8 weeks, about 150IU of vitamin E is required (Table 1). However, a muscle vitamin E concentration of 3.5-4.0mg/kg can be reached in 1 or 2 weeks when supplementation rates of 400 and 275IU/kg feed are used (figure 1). Supplementing lambs an equivalent of about 440mg of vitamin E/sheep.day (275IU treatment group) for a period of 3 to 4 weeks will guarantee muscle vitamin E concentrations of 3.5-4.0mg/kg will be reached. This indicates that with higher levels of dietary Vitamin E a meat colour stability optimum can be reached over a shorter duration of supplementation, this being of practical benefit where the labour costs of supplementary feeding are high.

The greatest effect of increased muscle vitamin E concentrations was observed in the aged samples and the oxidative type muscles, supporting our second hypothesis. The colour stabilizing mechanisms of vitamin E seem to have a greater effect under conditions of increased oxidative stress and have little benefit when oxidation within the cell is at a minimum. The SM and LL muscles are more oxidative muscle types, thus the effect of vitamin E was more apparent as shown by a steeper increase in the oxy/metmyoglobin ratio when increasing muscle α-tocopherol concentration. The effect of muscle Vitamin E concentration on the oxy/metmyoglobin ratio increased between muscles in the same order as expected for myoglobin

concentration from low in the ST, to medium in the LL and high in the SM (Gardner, Hopkins, Greenwood, Cake, Boyce & Pethick, 2007). Muscles can be categorised in to colour stability ranges by considering how quickly that muscle would reach an unacceptable colour for consumers (ratio of 3.5)(Morrissey *et al.*, 2008). Using this criterion both the SM and GM were classed as unstable muscles, the RF and ST muscles were classed intermediate and the LL a stable muscle (figure 2). However, the categories the muscles are placed in does not necessarily reflect the effect vitamin E has on the oxidative stability of the respective muscle, as shown in the LL in which vitamin E has a large effect on the stability, yet it is a very colour stable muscle. Further investigation is needed to establish a greater understanding of the difference in colour stabilities between muscles such as the SM and LL. However it must be noted that the location on the muscle where the sample was taken from may result in different levels of heterogeneous fibre contents, with greater proportion of "red" fibres differing from greater proportions of "white" fibres.

As well as muscle type, aging conditions will likely increase the oxidative stress in meat systems (Wulf *et al.*, 1995). Many researchers have shown that aging of beef causes a faster deterioration of surface colour once on display (Lanari, Brewer, Yang & Tume, 2002; Liu, Scheller, Arp, Schaefer & Frigg, 1996; Lynch, Kerry, Buckley, Faustman & Morrissey, 1999). Additionally, this effect of aging can be slowed by the supplementation of vitamin E (Liu *et al.*, 1996; Sanders, Morgan, Wulf, Tatum, Williams & Smith, 1997), and as demonstrated in the current studies the colour stability can be improved in aged samples when threshold levels of vitamin E have been achieved. Recently Ponnampalam, Butler, Burnett, McDonagh, Jacobs & Hopkins (2013) found that 4 week aged vacuum packed lamb LL was more colour stable than

the unaged/fresh product when muscle vitamin E concentrations were greater than 3.4mg/kg. Further investigation into different aging periods and vitamin E is required

The relative importance of colour at the commencement of display and the change in the oxy/metmyoglobin ratio over time during display is an important consideration for describing colour stability. Pasture fed animals had the same rate of decline (slope) of oxy/metmyoglobin as the grain fed animals, however the pasture group had a lower initial ratio and therefore were browner in colour when first cut, thus reaching an unacceptable colour much earlier. This difference in initial colour, within the same muscle type, is not well understood given that metmyoglobin formation occurs below the meat surface at the junction of the deoxy and oxy layers.

Meat from pasture fed lambs was less stable in colour than those fed supplemented grain diets, even though pasture fed animals had relatively high muscle vitamin E concentrations, disproving our final hypothesis. This result aligns with work by both Lanari *et al.* (2002) and Yang *et al.* (2002) who found supplemented cattle had a better colour stability than pasture fed animals. Different muscles may vary in initial colour due to differing pigment concentrations (myoglobin), associated with variation in fibre type (Gardner *et al.*, 2007). However in this study, the fibre type of pasture fed lambs is unlikely to have changed significantly compared to other treatments over such a short feeding period. Additionally the starting a* values, which correlate with redness and therefore oxymyoglobin (Hunt, 1980), did not differ between diet groups, further supporting the assertion that myoglobin concentration and fibre type was not the cause of the lower starting ratio in the pasture fed lambs. Further work is required to elucidate the mechanism explaining the difference in initial colour of the pasture fed animals.

4.2. Meat quality

All supplemented animals had a lower drip loss than that of the pre-treatment animals and supports previous evidence that Vitamin E lowers drip loss in pork (Monahan *et al.*, 1994). However, this could also be due to the pre-treatment animals being slightly less mature at the time of slaughter. Pasture animals had the least drip loss out of all the treatments, possibly due to a combination of vitamin E and other nutritional factors delivered through green feed, however no further explanation can be offered and further investigation could be warranted. The RF muscle retained the highest amount of moisture. This muscle also had a higher ultimate pH (Table 4) than the other muscles tested, and it has been well established that water holding capacity is increased with a higher pH (Huff-lonergan & Lonergan, 2005), thus the decreased drip loss is likely a result of a higher ultimate pH .

5. Conclusions

Retail colour stability of lamb meat can be improved by supplementing grain finished lambs with sufficient vitamin E. Lamb muscle has a threshold for vitamin E concentration of 3.5-4.0 mg α-tocopherol/kg tissue, above which no added benefit can be expected. This threshold concentration can be easily reached by supplementing lambs with 275IU α-tocopherol acetate/kg of feed (daily requirement being 440mg α-tocopherol acetate/sheep.day) for a period of 3 to 4 weeks. Because the depletion of vitamin E in the muscle can occur rapidly (Fry *et al.*, 1993), it is important that the supply of α-tocopherol be continual for this period, up until

slaughter, for added benefits on colour stability. Meat from lambs supplemented in this way will reach 60 hours retail display in air without an undesirable level of metmyoglobin accumulating on the surface of the meat under the display conditions currently tested. Vitamin E can also be supplied via green pasture although the effect on retail display period may not be as advantageous.

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Figure 1. **a)** SM, **b)** ST and **c)** Plasma Vitamin E concentration (VitE) over the feeding period for lambs fed different diets as measured by fortnightly biopsy and plasma analysis. The broken line in a and b represents the "threshold" vitamin E concentration level of 3.5mg/kg, while for c the line represents a plasma vitamin E concentration level typically used to indicate a risk of nutritional myopathy. Values are means ± standard error of the mean

Figure 2. The oxy/met ratio of meat from lambs from the pre-treatment group, and lambs fed pasture, and diets containing 30IU and 275IU of added vitamin E over 96 hours of retail display. Both aged and fresh *m. gluteus medius* (GM), *m. longissimus lumborum* (LL), *semimembranosus* (SM), *m.rectal femoris* (RF) and *m. semitendinosus* (ST) are presented. The discrimination point of 3.5 is clearly marked as a horizontal broken line. Values are means ± standard error of the mean

Figure 3. The effect of muscle vitamin E concentration (VitE) on the Oxy/metmyoglobin ratio at 60 hours in aged *m. longissimus lumborum* (LL), *m. semimembranosus* (SM) and *m. gluteus medius* (GM). Empty circles present individual data points adjusted by the model. The discrimination point of 3.5 is marked as a horizontal broken line

Figure 4. a* value of the SM muscle from lambs from the pre-treatment group, and lambs fed pasture, and diets containing 30IU and 275IU of added vitamin E over 96 hour retail display. Values are means ± standard error of the mean

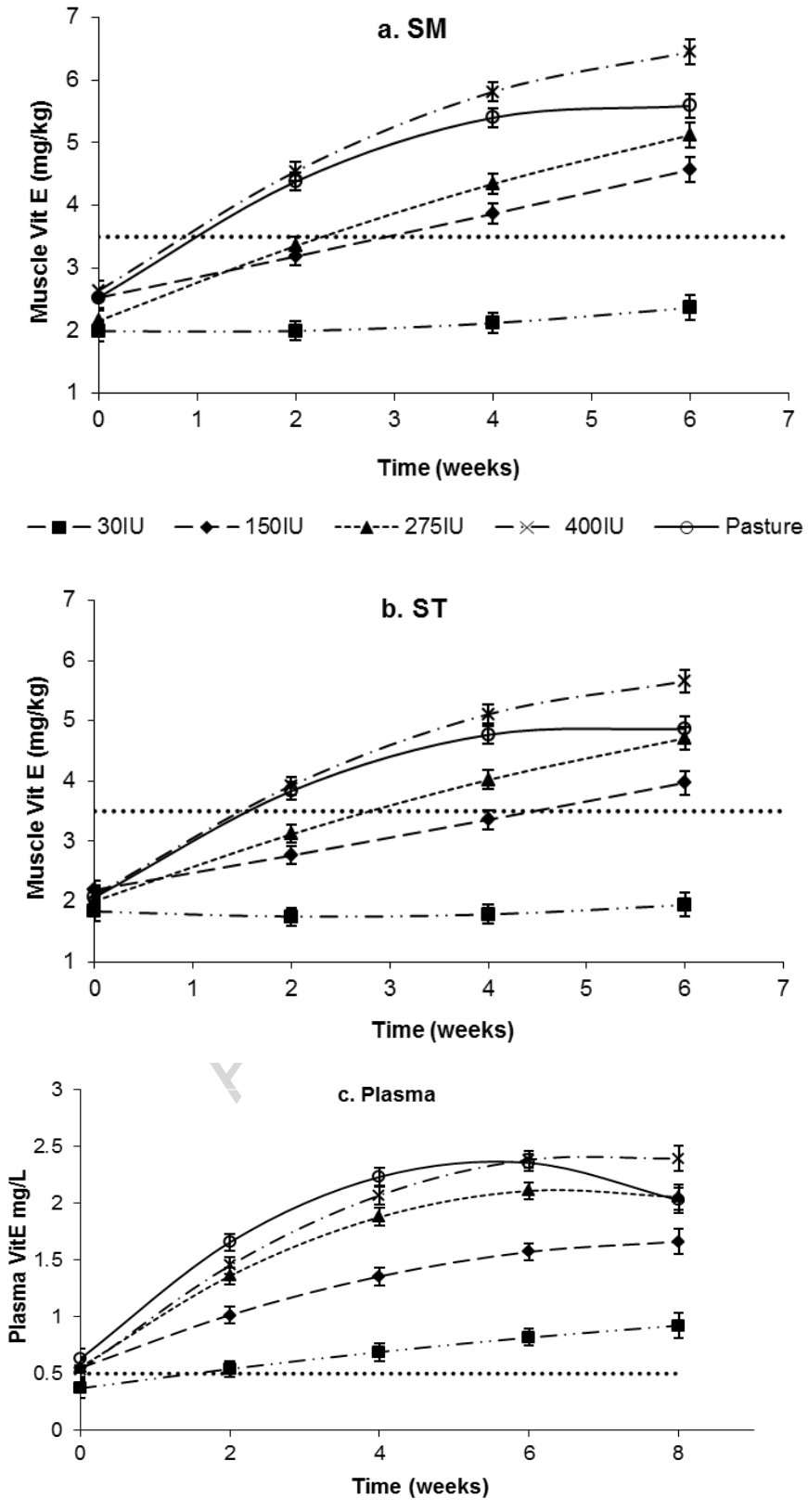

Fig. 1

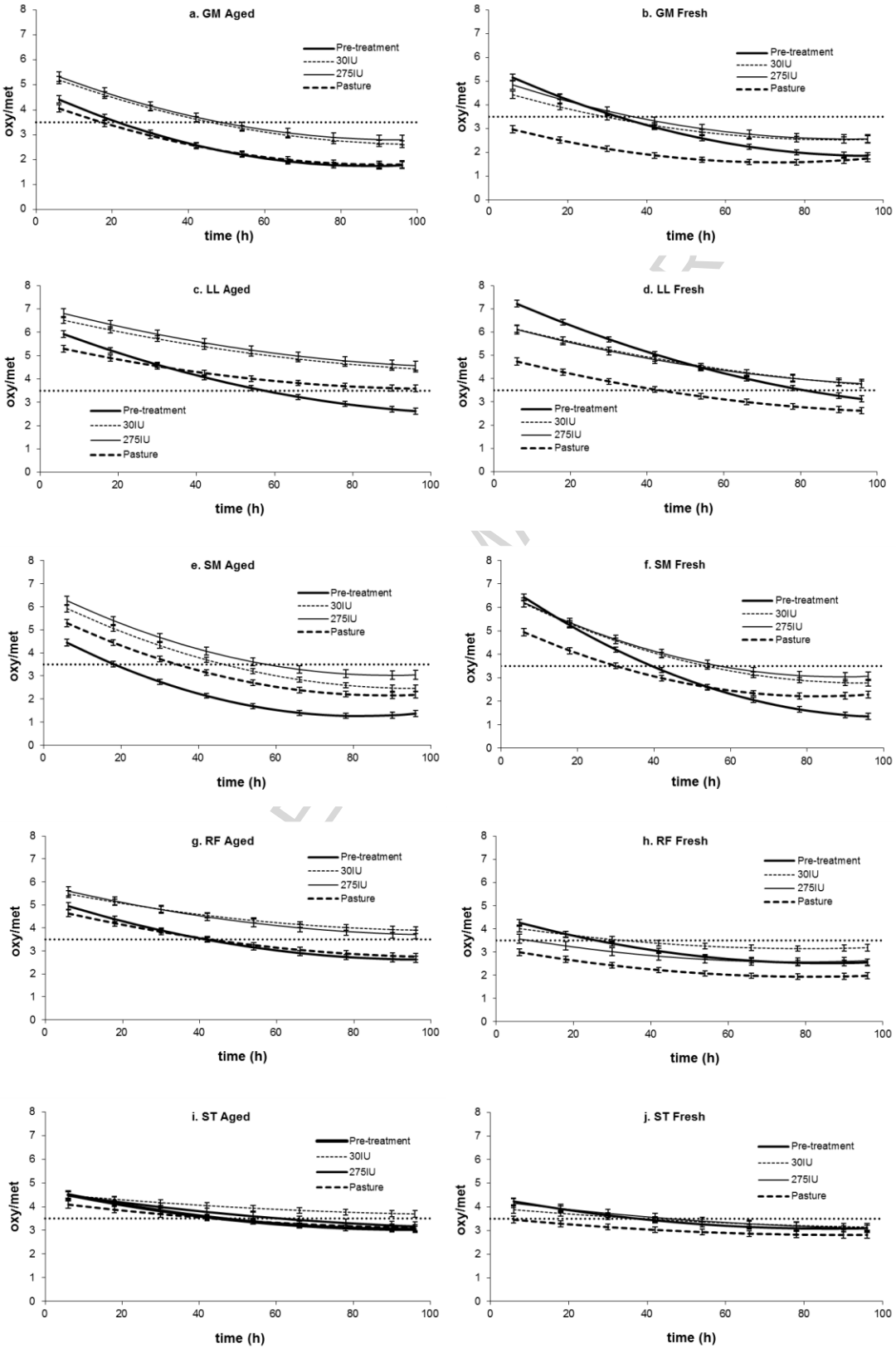

Fig. 2

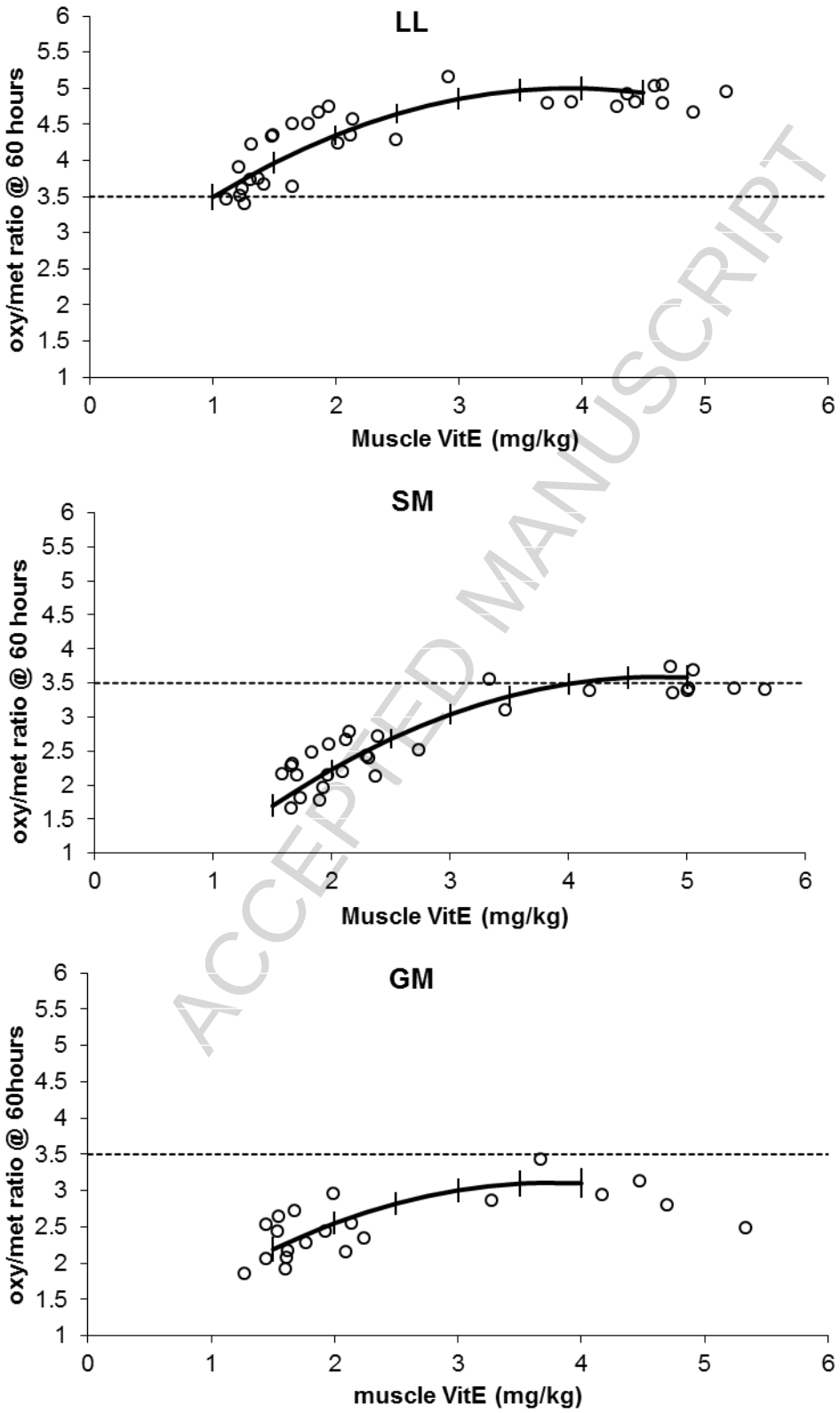

Fig. 3

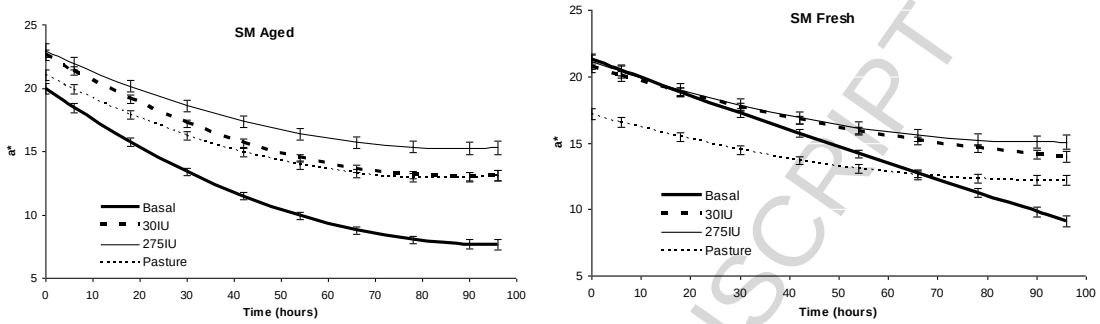

Fig. 4

Table 1. Vitamin E concentrations at slaughter, for muscles (mg/kg), liver (mg/kg) and plasma (µg/ml), and ultimate pH for muscle within lambs maintained on the different dietary treatments. Values are least square means ± standard error of the mean.

Table 2. Correlations coefficients for vitamin E concentrations between tissue types during the growing stage for muscle biopsy and plasma concentrations at weeks 0, 2 and 6; and at slaughter for muscle, plasma and liver concentrations.

Table 3. F values for the statistical model of the oxy/met ratio, a* value and L* value

Table 1

	GM	LD	RF	SM	ST	Plasma	Liver
Diet	Vitamin E concentration						
Pre-treatment	1.64±0.05^{bz}	1.31±0.05^{cy}	1.58±0.05^{bz}	1.86±0.05^{az}	1.36±0.05^{cz}	0.36±0.09^{z}	1.93±1.68^{z}
30IU	2.02±0.26^{az}	2.12±0.18^{az}	1.87±0.26^{az}	2.38±0.18^{az}	1.93±0.18^{az}	0.85±0.13^{w}	5.61±1.53^{z}
150IU	4.1±0.24^{abx}	4.04±0.18^{abx}	3.63±0.24^{bcx}	4.35±0.18^{ax}	3.51±0.18^{cx}	1.63±0.13^{y}	13.53±1.53^{y}
275IU	4.81±0.24^{aw}	4.79±0.18^{aw}	4.5±0.26^{abw}	5.07±0.18^{aw}	4.12±0.18^{bw}	2.32±0.13^{x}	18.59±1.60^{x}
400IU	5.99±0.24^{ay}	5.09±0.17^{bw}	5.58±0.25^{aby}	5.48±0.17^{abw}	4.6±0.17^{cw}	2.49±0.13^{x}	22.56±1.53^{x}
Pasture	4.24±0.26^{abxw}	3.68±0.18^{bcx}	4.35±0.26^{aw}	3.98±0.18^{abx}	3.35±0.18^{cx}	2.25±0.13^{x}	12.49±1.53^{y}
	Ultimate pH						
Pre-treatment	5.62±0.015^{bcw}	5.60±0.021^{cx}	5.71±0.026^{ax}	5.57±0.016^{cx}	5.66±0.03^{abx}	-	-
30IU	5.58±0.015^{cxw}	5.63±0.02^{cx}	5.78±0.025^{awzy}	5.60±0.016^{cx}	5.72±0.029^{bx}	-	-
150IU	5.58±0.015^{bxw}	5.61±0.02^{bx}	5.73±0.025^{axy}	5.56±0.016^{bx}	5.73±0.029^{ax}	-	-
275IU	5.57±0.015^{bx}	5.63±0.02^{bx}	5.81±0.026^{az}	5.58±0.016^{bx}	5.74±0.029^{ax}	-	-
400IU	5.61±0.014^{bxw}	5.62±0.019^{bx}	5.72±0.024^{axw}	5.60±0.015^{bx}	5.71±0.028^{ax}	-	-
Pasture	5.59±0.014^{cxw}	5.64±0.02^{bx}	5.71±0.025^{ax}	5.57±0.016^{cx}	5.71±0.028^{ax}	-	-

Differences are denoted by letters ‘abc’ between muscle types within diets; and by letter ‘wxyz’ between diets within muscles, plasma and liver.

Table 2

Growth values

		Plasma		
Muscle Biopsy		Week 0	Week 2	Week6
SM	week 0	0.361*		
	week2		0.614	
	week6			0.369*
ST	week 0	0.495		
	week2		0.545	
	week6			0.412^

Slaughter values

	Liver	Plasma	SM	ST	LD
Liver	1	0.592	0.776	0.766	0.789
Plasma	0.592	1	0.575	0.652	0.623
SM	0.776	0.575	1	0.875	0.909
ST	0.766	0.652	0.875	1	0.905
LD	0.789	0.623	0.909	0.905	1

All values less than 1 are significant to a level of P< 0.01, except those marked with ^ P<0.05 and * P>0.05.

Table 3

Effect	Ratio		a* value		L* value	
	NDF;DDF	F Value	NDF;DDF	F Value	NDF;DDF	F Value
Time	1;5676	2160.37***	1;6376	1086.59***	-	-
Diet	5;5676	30.12***	5;6376	36.78***	5;664	4.04***
Muscle	4;5676	341.52***	4;6376	513.07***	5;664	360.38***
Package	1;110	18.55***	1;6376	1154.56***	1;664	45.11***
Time^2	1;5676	675.87***	1;6376	220.71***	-	
Time x Diet	5;5676	8.8***	5;6376	1.97*	-	-
Time x Muscle	4;5676	102.42***	4;6376	139.16***	-	-
Time x Package	1;5676	9.23***	1;6376	174.52***	-	-
Diet x Muscle	20;5676	9.6***	20;6376	5.59***	24;664	1.97***
Muscle x Package	4;5676	97.39***	4;6376	38.77***	5;664	16.79***
Diet x Package	5;5676	38.1***	5;6376	15.91***	5;664	2.5**
Time^2 x Diet	5;5676	2.52**	5;6376	0.72	-	-
Time^2 x Muscle	4;5676	41.74***	4;6376	48.1***	-	-
Time x Diet x Muscle	20;5676	3.59***	20;6376	5.08***	-	-
Time^2 x Package	-	-	1;6376	79.94***	-	-
Time x Muscle x Package	4;5676	21.22***	4;6376	2.38**	-	-
Time x Diet x Package	5;5676	8.76***	5;6376	9.19***	-	-
Diet x Muscle x Package	20;5676	5.41***	20;6376	5.09***	-	-
Time^2 x Muscle x Package	-	-	4;6376	2.5**	-	-
Time^2 x Diet x Package	-	-	5;6376	7.9***	-	-
Time x Diet x Muscle x Package	20;5676	2.57***	20;6376	1.98***	-	-

Level of significance denoted by *** P< 0.01; ** P< 0.05; * P< 0.1. NDF, numerator degrees of freedom; DDF, denominator degrees of freedom

Highlights

No 1 Colour stability of lamb meat is improved by nutritional supplementation with vitamin E

No 2 Maximum effect threshold of muscle vitamin E for lamb colour stability was 3.5-4mg/kg

No 3 The threshold level is required to improve the shelf life of lamb meat up to 60 hours

No 4 lamb meat aged for 21 days had improved shelf life when a sufficient muscle vitamin E concentration was met.