Vitamin E and fatty acid content of lamb meat from perennial or annual pasture systems with supplements.

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Abstract:

This study investigates the effect of a perennial lucerne and phalaris pasture, or annual pasture with lucerne hay and a range of supplements provided as pellets (700 g/day) on vitamin E and fatty acid content of skeletal muscle and oxidative stability of lamb meat post-farm gate. Treatments were lambs grazing perennial pasture only (T1); lambs grazing annual pasture with lucerne hay/oat grain pellet supplement (T2); lambs grazing annual pasture with lucerne hay/oat grain/cracked flaxseed pellet supplement (T3); and lambs grazing annual pasture with lucerne hay/oat grain/flaxmeal pellet supplement (T4). After seven weeks of feeding, lambs were slaughtered after an overnight fast. At 24 h post-slaughter samples of muscle *longissimus lumborum* (LL) were collected for determination of fatty acid profile and antioxidant status as measured by vitamin E content. Samples were also collected for retail colour assessment of fresh and aged (vacuum-packed and stored at 2°C for 4 weeks) meat at 0 h, 24 h, 48 h and 72 h from the time display commenced. Vitamin E content of the LL from perennial pasture (T1) was higher (5.9 vs 3.4 mg α-tocopherol/kg, *P* < 0.01) compared with lambs grazing other treatments. Long chain n-3 and total n-3 fatty acid content in the muscle were similar between treatment groups and adequate to claim as a source of n-3. Inclusion of oat grain at 245 g (T2) or at 175 g with flaxseed (T3) or 175 g with flaxmeal (T4) per day in the diet of lambs increased the linoleic acid content (*P*<0.05) and the ratio of n-6/n-3 (*P*<0.007) in the LL compared with lambs grazing perennial pasture (T1). Oxidative stability of meat post-farm gate evaluated by retail shelf life (colour stability) and formation of lipid oxidative substance showed no differences between treatment groups and values were within the range for quality meat over the time frame used in this study. These results provide some evidence that inclusion of perennial pasture in the diets of lambs during dry seasons (late summer to autumn) is an effective tool in improving the vitamin E content of muscle tissues that contributes to maintaining the oxidative stability of meat post-farm gate.

Keywords: Lamb; perennial pasture; vitamin E; fatty acids; colour; oxidative stability of meat.
Introduction

A consistent supply of premium quality lamb meat with improved nutritive characteristics and retail colour post-farm gate is desirable for profitable marketing through consumer attraction and longer retail shelf life. The stability of colour and fatty acid oxidation (functionality) of meat is influenced by the composition of muscle tissues which is in turn governed by the level and type of fats, antioxidant status and haem pigments (haem iron), all of which are tightly related to feed offered (Ponnampalam et al., 2001a and 2001b; Luciano et al., 2009; Daley et al., 2010).

Annual ryegrass (Lolium rigidum Gaud.) is widely used as a pasture to finish lambs born in the autumn (March-May) and slaughtered in spring in Southern Australia. This grass establishes quickly in autumn with high productivity in winter and in the early spring season. However, its annual growth cycle means senescence in late spring rapidly reduces its nutritive characteristics such as essential fatty acids, antioxidant status, vitamins and trace elements (Moure et al., 2001; Van Ranst et al., 2009). Such pasture, when grazed as senesced pasture by lambs born in spring (September-November) and finished in autumn, is generally of low nutritive value and requires supplementary feeding to meet the nutrient requirements of growing animals. Victoria produces flax as an oil seed crop during spring-summer and the use of flaxmeal by-product, high in lipids and protein, in the lamb industry may be a suitable and alternative supplement for lupins or soybean in improving lamb productivity and nutritive characteristics of meat.

Length of supplementary feeding can vary from 4-8 weeks depending on the quality and availability of the basal pasture diet and the cost of supplement. The aim was to achieve a finished liveweight of at least 40-45 kg. A companion study investigated the use of flaxseed and flaxmeal on weight gain and carcass
characteristics, used as a supplement to lambs grazing annual pasture (Burnett et al., 2011). This study investigated the effect of feeding a perennial pasture (lucerne/phalaris) compared with grazing senesced annual pasture with different supplements including lucerne hay with -oat grain, -crushed flaxseed or -flaxseed meal, on muscle composition and functional status (retail colour and lipid oxidation) of lamb meat.

Materials and methods

Experimental design, animals and diets

Details of feeding strategies, liveweight gain and carcass characteristics of lambs are described elsewhere (Burnett et al., 2011). In brief, fifty four second cross lambs (Border Leicester × Merino ewe × Dorset) weighing 35.84 ± 2.03 kg were randomly allocated to four dietary treatments by live weight. They were mixed of wethers and ewes, divided into treatments approximately in equal numbers. The treatments were:

Perennial pasture (n = 9, T1); Annual pasture with lucerne hay/annual hay plus oat grain pellet supplement (n = 15, T2); Annual pasture with lucerne hay/annual hay plus oat grain and cracked flaxseed pellet supplement (n = 15, T3); Annual pasture with lucerne hay/annual hay and oat grain plus flaxmeal pellet supplement (n = 15, T4). Lambs grazed each treatment for seven weeks.

Lucerne hay (60%) and grass hay (5%) were used with oat grain (35%), flaxseed or flaxmeal for the formulation of pellets, all offered on an air dry basis. In T3 and T4, 10% of oat grain was replaced by flaxseed (T3) and flaxmeal (T4). At the start of the experimental period, lambs on T 2, 3 & 4 were offered 500 g of pellets daily for two weeks and 700 g of pellets daily for the remaining 5 weeks. Treatments 2, 3 and 4 were balanced for the quantity of lucerne and annual hay contained in the supplement,
and T3 and T4 were balanced for crude protein. Pasture (annual and perennial) and supplements (pellets) were collected weekly over the seven week feeding period, bulked into one sample, homogenous samples (2 × 100 g) were ground for the determination of chemical composition of the diets as shown in Table 1. The concentrations of alpha-linolenic acid (ALA, 18:3n-3), linoleic acid (LA, 18:2n-6), total fat, ratio of ALA/total fat and LA/total fat of the diets used for the feeding of lambs is given in Table 2.

Slaughter of lambs and sample collection

At the completion of the seven week period, animals were divided proportionally across treatments and transported 300 km (Monday & Wednesday) to the Meat Research & Training Centre, Werribee for slaughter and muscle sample collection. After an overnight fast (12 h), lambs were slaughtered on Tuesday (12 May 2009) and Thursday (14 May 2009). Lambs were allocated in equal number across treatments and slaughtered in random order. Animals were restrained in a V-restrainer and electrically stunned with a dual point electrode placed to the head, exsanguinated and euthanized via cervical dislocation. Carcasses were trimmed according to the specifications of AUSMEAT (Anon, 1992) and chilled overnight at 2-3°C.

At 24 h post-mortem, the pH_{24} (model TPS WP-80 pH meter with ionode probe IJ44c attached, TPS Pty Ltd., 4 Jamberoo Street, Springwood, Qld. 4127, Australia) of the longissimus lumborum (LL) muscle was recorded at the lumber site of the intact carcass (left side) before removal of loin muscle for sample collection. The entire loin muscle was removed from the left side of the carcass i.e., 5^{th}/6^{th} rib to lumber. The loin was cut in half and the cranial section was used for fresh colour, vitamin E and
fatty acid determination. Duplicate samples (~25 g) were collected and stored at -20°C for the determination of fatty acid profile. Another set of duplicate samples (~25 g) were collected from the same region and stored at -80°C for determination of antioxidant status (Vitamin E). The rest of the cranial section was used to evaluate the retail colour of fresh meat. The caudal section of the loin was vacuum-packed and stored at 2°C for 4 weeks.

**Analysis of vitamin E and fatty acid in muscle tissues**

The vitamin E content of muscle tissue was determined as described by Ball (1988). Samples for fatty acid analysis were freeze dried and 0.5 g of homogenised dry muscle sample was used for fatty acid extraction using a slightly modified method from O'Fallon et al. (2007) as described by Ponnampalam et al. (2010a). Total fat is the sum of all fatty acid fractions determined in the extraction procedure.

**Assessment of colour stability and lipid oxidation**

The remainder of the cranial section (LL) was cut into 3 transverse slices (3 chops, 2.5 cm thickness), placed on a plastic tray, over-wrapped with a 15 micron PVC film and displayed under refrigerated conditions (3-4°C) with fluorescent lights set at 1500 Lux. The colour of the meat was measured at 0 h, 24 h, 48 h and 72 h from the time samples were displayed, using a HunterLab meter (Hunterlab Miniscan, TM XE Plus 45/10, Reston, USA) with light source set at D65/10 (i.e., 0 h measurement was taken at 24 h post mortem). After 4 weeks, vacuum packs were opened and loin chops were sliced for the colour evaluation of aged meat over 3 days as explained above. Colour stability of fresh and aged meat was assessed by measuring the change in redness of the meat (a*-value) and the formation of brownness (ratio of
oxymyoglobin and metmyoglobin (oxy/met) in the meat surface as determined by the reflectance ratio 630/580 nm wavelength). The reflectance ratio of 630 and 580 nm was used as an indirect measure of metmyoglobin formation (brownness) on the meat surface as described by MacDougall (1995). On the day of preparation for both fresh and aged meat, colour was measured after a 30 min bloom at 3°C.

After 72 h of display, the meat samples from all lambs (fresh and aged) were analysed for the concentration of malondialdehyde (MDA) using the 2-thiobarbituric acid reactive substances (TBARS) procedure (Witte et al., 1970), as an indicative assessment of the development of lipid oxidative substances during retail display.

**Statistical analysis**

All statistical analyses were conducted in the GenStat statistical package (GenStat 2009). Data on muscle fatty acid composition, pH$_{24}$, vitamin E and TBARS on each lamb were analysed by ANOVA. The treatment structure was specified with the effect for Treatment nested within the factor “PType”. The factor, “PType”, functioned to separate the treatments that were on annual plots from the single treatment that was on the perennial plot. The blocking structure was specified as Animals nested within Group (SubPlots) nested within Plots. Redness (a*-value) and brownness formation (reflectance at 630/580nm = oxy/met) variables that were measured repeatedly on the same unit, were analysed using a repeated measures ANOVA, adopting the same treatment structure and blocking structure as the muscle composition data. All analyses were followed by graphs of residuals vs. fitted values, histograms, and normal probability plots to check the usual constant variance and normal distribution assumptions. Least significant differences (5% level) were used to separate treatment means, subject to significant F-tests.
Results

Vitamin E and fatty acid content of diets

The concentration of α-tocopherol and γ-tocopherol of diets used for the feeding of lambs in the present study is given in Figures 1a and 1b. The estimated daily intakes of α-tocopherol for T1, T2, T3 & T4 were 57.2, 8.3, 8.0 & 8.1 mg, respectively (refer Burnett et al., 2011 for estimation of daily feed intakes). Daily intakes (estimated) of ALA content for T1, 2, 3 & 4 were 1885, 1795, 19197 & 4618 mg, respectively. Although the concentration of ALA was much higher in flaxseed than flaxmeal, the ratio of ALA/total fat and LA/total fat were similar for both supplements (Table 2). Among the supplements used, oat grain had the lowest ratio of ALA to total fat (0.01) and the highest ratio of LA to total fat (0.36), which was similar to the ratio of LA to total fat found in annual pasture (0.39) used. The ratio of both ALA and LA to total fat were similar for perennial (0.24 & 0.29) pasture and grass hay (0.12 & 0.15) (Table 2). The ratio of ALA to total fat for annual pasture was similar (0.11) to that in the grass hay (0.12) used as part of the supplement, which was half as much as the ratio of ALA to total fat present in perennial pasture (0.24). Lucerne hay used as part of the supplement had the highest ratio of ALA to total fat (0.42) which was 2-fold higher than the amount of LA to total fat (0.21) present.

Vitamin E and fatty acid contents in muscle tissues

When compared with the concentration of α-tocopherol in meat, the concentration of γ-tocopherol was negligible (Ponnampalam EN, unpublished results) and therefore
\( \gamma \)-tocopherol concentrations was not determined in this study. Lambs grazing perennial pasture had higher \( (P<0.01) \) vitamin E concentration in meat (5.9 vs 3.4 mg/kg of meat) compared to lambs on annual pasture with supplements (Table 3). Fatty acid analysis showed no differences in health claimable n-3 fatty acids (eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA)), long chain n-3 (EPA + DHA + docosapentaenoic acid (DPA)), total n-3, polyunsaturated fatty acid (PUFA), saturated, monounsaturated or total fatty acid contents between treatment groups. Linoleic acid \( (P<0.05) \) concentration of meat was lower with perennial pasture (T1) than those on annual pasture with supplements (Table 4). There were no differences in the concentrations of ALA \( (P = 0.07) \) and total n-3 fatty acid \( (P = 0.16) \) in meat from lambs fed treatments 2, 3 or 4. The pH of the LL muscle measured at 24 h post-mortem showed no difference between treatment groups and values are within the range (5.4-5.7) for quality sheep meat (Table 3).

Colour and lipid oxidative stability of fresh and aged meat

Colour stability of fresh and aged meat assessed by the redness of meat \( (a^*-value; \) Figures 2a & 2b) and the formation of brownness \( (oxy/met indicated by the reflectance ratio at 630/580nm; \) Figures 3a & 3b) of meat showed no differences between treatments. As noted for \( a^*-values \) (redness), the oxy/met ratio (brownness) for aged meat were greater than the values for fresh meat over the 4 days of retail display. There were no significant differences between the treatments with respect to the TBARS of fresh meat, but there was a tendency towards higher TBARS for aged meat from supplemented lambs (Table 3).
Discussion

Diet and vitamin E content in muscle

Actively growing grass, as opposed to senesced grass, is rich in natural antioxidants such as vitamin E in the form of $\alpha$-tocopherol compared to processed grains or conserved forages (Moure et al., 2001). Figures 1a & 1b show the variation in $\alpha$-tocopherol and $\gamma$-tocopherol concentrations between perennial pasture, grains, grass hay and senesced annual pasture used in this study. The highest value for $\alpha$-tocopherol was observed in perennial pasture despite a high proportion of the perennial pasture being senesced as evidenced by the low ME content. Whilst no measures of dietary selection were made, it is likely that lambs would have preferentially selected the green components of feed on offer and therefore consumed even higher amounts of $\alpha$-tocopherol compared to the completely senesced annual pasture as reflected by the results in Table 3. The effect of $\gamma$-tocopherol in flaxmeal and flaxseed on blood antioxidant status is reported by Burnett et al. (2011). Since vegetable oils and nuts are rich sources of $\gamma$-tocopherol (Jiang et al., 2001), the present study showed that although lambs fed flaxseed and flaxmeal treatments received greater amounts of $\gamma$-tocopherol, the concentrations in meat were very low (as determined by a pilot study). This would indicate that there is preference for absorption of $\alpha$-tocopherol over $\gamma$-tocopherol at the peripheral tissue system of the body and this is in line with Handelman et al. (1985) who showed plasma $\gamma$-tocopherol is replaced by $\alpha$-tocopherol when the intake of $\alpha$-tocopherol is high. Others have reported $\alpha$-tocopherol is the predominant form of vitamin E in most human and
animal tissues (Behrens and Madere, 1987) and the concentration in human plasma is generally 4-10 times higher than that of \( \gamma \)-tocopherol (Handelman et al., 1994).

The increase in \( \alpha \)-tocopherol in meat from T1 compared with T2-T4 was due to the greater concentrations of \( \alpha \)-tocopherol in the perennial pasture stand. The estimated daily consumption of \( \alpha \)-tocopherol from T1 was 57.2 mg/day. This compared with lambs in T2-T4 consuming approximately 8.0-8.3 mg \( \alpha \)-tocopherol/day (1.44 mg/day from annual pasture, 5.6 mg/day from the lucerne hay and the rest is from other ingredients used in the pellets). T1 lambs consumed seven times the amount of \( \alpha \)-tocopherol compared with T2-T4 lambs. It is suggested that the lucerne hay in T2, T3 and T4 contributed a significant proportion of \( \alpha \)-tocopherol to the \( \alpha \)-tocopherol concentration in the muscles. Results also indicate that the efficiency of conversion of \( \alpha \)-tocopherol from the diet (8.0-8.3 vs 57.2 mg/day) to the muscle tissue (3.2-3.6 vs 5.9 mg/kg meat) was higher in T2, T3 and T4 than those grazing perennial pasture (T1) alone.

The vitamin E content of muscle in lambs fed the senesced annual pasture treatments (average 3.39 mg \( \alpha \)-tocopherol) was similar to that found in another experiment (3.46 mg \( \alpha \)-tocopherol) where lambs grazed low quality annual pasture (ryegrass/barley grass) during late spring (Ponnampalam et al., 2011). Eighteen month-old Merino sheep grazing dry pasture with 200 g of barley grain had 2.4 mg \( \alpha \)-tocopherol/ kg meat (Pearce et al., 2005) while 7-month old lambs receiving 800 g barley/lentils (air dry 80:20) with capeweed hay produced meat with 1.67 mg \( \alpha \)-tocopherol/ kg meat (Ponnampalam et al., 2011). The present study indicates that perennial pasture finishing is useful to elevate the vitamin E content of muscles in
lamb finishing systems on-farm. Recent findings support our latter statement where
winter drop lambs grazing lucerne pasture or perennial ryegrass pasture as a single
stand through spring to summer had muscle vitamin E concentration of 3.8 or 4.1 mg/
kg meat, respectively (Ponnampalam et al., 2012, unpublished data).

Diet and fatty acid composition in muscle

Alpha linolenic acid and its longer chain derivatives

Meat from all treatments provide long chain EPA+DHA equal or above the level
(> 30 mg/135 g portion) to claim lamb meat as a source of omega-3 (Pannier et al.,
2010). The estimated level of ALA intake from T3 (19.20 g/day) and T4 (4.62 g/day)
was much greater than the level of ALA in T1 (1.89 g/day) or T2 (1.79 g/day). Given
the concentrations of ALA provided by flaxseed and flaxmeal in T3 & T4, one might
expect a significant increase in ALA and total n-3 content of meat compared to T1
and T2, but this did not occur. It would appear that the ALA provided by flaxseed or
flaxmeal supplements was converted into other forms during the process of digestion
and absorption. This might have been due to biohydrogenation and saturation of fatty
acids in the rumen via degradation/hydrolysis process of lipids from flax (Demeyer
and Doreau, 1999). Other studies have also reported no differences in ALA in meat or
milk when fattening lambs or dairy ewes were supplemented with 12.5% and 111
g/day extruded flaxseed, respectively compared to pasture feeding (Bessa et al.,
2007).

Alternatively, it is reasonable to state that the selection of green leafy materials (in
this case, lucerne) or lucerne hay might have contributed a significant amount of
ALA, protected from biohydrogenation and reaching the circulatory system through
digestion/absorption processes and therefore masking any effects of ALA from flax in T3 and T4 groups. The protection of ALA may be associated with the amount of tannin or secondary metabolites present in the grass. Results from others (Bessa et al., 2007) support the latter statement. In their study when lucerne hay pellets as a basal diet were compared with 10% of added soybean oil or concentrate diet with 10% of soybean oil, ALA and its longer chain derivatives (EPA, DHA & DPA) in the meat were significantly higher (2-3 fold) in lambs fed a lucerne hay pellet diet. Bessa et al. (2005) also showed when lucerne hay pellets were compared with 2.5% flaxoil plus 5% sunflower oil (2:1 ratio) or 7.5% sunflower oil in the pellets, the pelleted lucerne diet produced meat with significantly higher amounts of ALA and its longer chain n-3 derivatives than other groups. A recent study investigating the genetics and environmental effects on ALA and its long chain n-3 derivatives (EPA, DHA, DPA) in meat from 2000 lambs has shown that animals grazing perennial pasture, mainly lucerne, had higher concentrations of ALA and its longer chain n-3 derivatives than animals grazing annual pasture alone or grazing grass hay/grain supplements (Pannier et al., 2010; Ponnampalam et al., 2010b).

**Linoleic acid and ratio of n-6 to n-3 fats in muscle tissue**

Previous studies show that provision of grain supplements or concentrates in the diets of sheep and cattle can elevate the concentration of n-6, ratio of n-6/n-3 and ratio of saturates/polyunsaturate content in lamb and beef, which is detrimental to the nutritional value of meat (Enser et al., 1998; Nuernberg et al., 2005; Daley et al., 2010). Inclusion of oat grain at 245 g/day (T2) or 175 g/day (T3 & T4) significantly increased LA, which in turn increased the ratio of n-6/n-3 in meat compared with lambs in T1. Partial replacement of oats by flaxseed or flaxmeal in T3 and T4 groups
reduced the ratio of n-6/n-3 compared with T2 lambs receiving oat grain alone. However, meat from all lambs in this study had a n-6/n-3 ratio below 4. Levels of this ratio above 4 are considered detrimental to health (Department of Health, 1994). This was similar to the findings of Ponnampalam et al. (2010a) where lambs fed barley grain/lentils (800 g/day, 80:20) with a grass hay diet had a higher content of LA, total n-6 and ratio of n-6/n-3 in meat compared with lambs grazing low quality annual pasture in late spring to early summer.

Oxidative stability of meat post-farm gate

Retail colour stability (Shelf life of meat)

Vitamin E, mainly in the form of α-tocopherol, is a powerful antioxidant (Brigelius-Flohe and Traber, 1999; Jiang et al., 2001) which is thought to prevent oxidation of oxymyoglobin to metmyoglobin, which contributes to colour deterioration directly (Greene, 1969; Faustman et al., 1998). There was no threshold value reported in the literature for vitamin E in lamb muscle to maintain better retail colour stability. Wulf et al. (1995) investigated the discolouration of lamb meat but those assessments were subjective and cannot be compared with objective measures of meat colour in the present study. Studies conducted in beef cattle have shown that the threshold level of muscle vitamin E concentration is 3.0-3.5 mg of α-tocopherol per kg of meat (Faustman et al., 1998, Arnold et al., 1993), to provide optimum beef colour at retail display. There was evidence from the current study when vitamin E concentration of lamb muscle is above 3.1 mg/kg muscle, as assessed by the redness (a*-value) or the brownness formation (HunterLab reflectance at 630/580 nm = oxy/met), the retail colour stability of fresh or aged meat was not affected as shown. Vitamin E was supplied via the pasture and hay supplement, lambs in T2-T4 had 3.1-
3.6, whilst lambs in T1 had 5.9 mg α-tocopherol/kg of muscle. Whether the vitamin E content was 3.1 or 3.6 or 5.9, there was no deviation between the mean values of redness for fresh and/or aged meat at 24, 48, 72 and 96 h retail display for all T1, T2, T3 and T4 treatments. Similarly, the brownness formation in meat, which is another measure of retail colour stability used by researchers, did not differ between all treatments for both fresh and aged meat over the same display period.

Previous studies conducted in sheep have shown that at 6.3 mg α-tocopherol/kg of muscle, the colour stability of meat from 18 month old sheep (hogget) fed saltbush/barley grain was higher than the meat from sheep fed dry pasture/barley grain having 2.4 mg α-tocopherol/kg of meat (Pearce et al., 2005). Jose et al. (2008) have shown that supplementation with synthetic vitamin E at 175.7 mg/kg feed can result in better meat colour of aged meat (LL muscle) after 30 days. Another study in agreement with this statement (Ponnampalam et al., 2011) found that 3.46 mg α-tocopherol in the muscle tissues of lambs grazing annual pasture (ryegrass/barley grass) during late spring led to a greater colour stability compared to lambs fed barley grain/lentils with grass hay having only 1.69 mg α-tocopherol/kg muscle tissue. The results suggest that when the muscle vitamin E concentration is above 3.1 mg/kg of meat, the retail colour in lambs would not be affected over 96 h post slaughter.

Khliji et al., (2010) reported that for meat colour being considered acceptable by consumers, redness (a*-value) of meat should be equal or above 9.5 and 14.8 for fresh and aged meat, respectively. The current study shows when vitamin E concentration of meat was above 3.1 mg/kg muscle, the redness of fresh and aged meat after 96 h post slaughter was 13.1 and 15.2, respectively for all treatment groups and suggests
lambs can be finished on annual pasture with supplements. It is noted that the redness (a*-values) and formation of brownness (reflectance at 630/580 nm) were higher for aged meat than for fresh meat. This could be due to several reasons; 1. the anaerobic condition due to vacuum packaging might have increased the redness through biochemical reaction; 2. the oxygen consumption and/or the oxygen diffusion into the muscle surface may have been changed or 3. the changes in pH may have influenced the colour, as increase in pH may increase the redness of meat. This is an area that warrants further investigation.

**Formation of oxidative substance as assessed by TBARS and lipid stability of meat**

The TBARS values of lamb meat at 24 h and 7 day post slaughter are reported to range from 0.1-0.8 mg and 0.6-3.7 MDA per kg of meat, respectively (Kennedy et al., 2005; Linares et al., 2007). The TBARS values at 92 h post slaughter for lamb meat from the current study ranged from 0.31-0.35 MDA per kg of meat, values within current published ranges. Verma and Sahoo (2000) and Green and Cumuze (1981) reported that from 0.6 up to 2 mg TBARS levels is a threshold amount for rancidity or off-flavour development in meat. While others have reported TBARS levels of 4.2-7.5 mg of MDA/kg of meat, the acceptability of lamb meat decreased because slight off-odours were detected by the sensory panellists (Berruga et al., 2005).

In a study, Campo et al. (2006) promoted lipid oxidation in beef loins (73) containing different concentrations of LA and ALA collected from different feeding, via 10 days of conditioning at 1°C then freezing, thawing and storing. The later procedure produced TBARS up to 12 MDA per kg of meat and the study identified TBARS at 2.3 is the point where rancid or other abnormal flavour dominate beef
flavour to make beef having unacceptable flavour. In the current study, the highest
and lowest TBARS for the 54 aged (vacuum packed and stored at 2°C for 4 weeks)
lamb loins at 72 h display were 0.60 and 4.59. The mean TBARS of aged meat for all
treatment groups ranged from 1.2-2.3 mg of MDA/kg of meat.

The present study indicates that when the concentration of vitamin E in lamb is
above 3.1 mg/kg of muscle, it is unlikely that the colour stability and lipid oxidative
stability would be detrimentally affected and as such is sufficient to prevent meat
from deterioration through oxidation process during retail display or storage over the
time frame used in this study. Results suggest that the higher concentration of ALA
present in lucerne may have provided greater amounts of antioxidants available in the
intestine for absorption as vitamin E and be incorporated into skeletal muscle tissues
with fats intact because vitamin E is fat soluble. Perennial pasture, in the diet of lambs
either as grazing pasture or lucerne hay in the form of pellets, may be beneficial and
an effective tool in improving the antioxidant status and essential fatty acid content of
muscle tissues, and to maintain oxidative stability of meat post-farm gate. These
effects in muscle components and oxidative stability may be seen with feeding other
perennial pastures when used in sheep feeding systems during dry seasons. However,
further study is needed to confirm whether the use of improved or native pastures can
offer similar outcomes.

Conclusions

Feeding perennial pasture increased the vitamin E (α-tocopherol) concentration of
skeletal muscle compared to lambs grazing annual pasture with supplements. Flaxseed
and flaxmeal supplementation with senesced annual pasture/oat grain/lucerne hay did
not improve long chain n-3 fatty acid composition and vitamin E content compared with oat grain supplement alone with senesced annual pasture/lucerne hay. However, oat grain significantly increased linoleic acid (LA) and ratio of n-6/n-3 in meat compared to lambs fed perennial pasture alone. Replacing oat grain partly by flaxseed or flaxmeal in T3 & T4 reduced n-6/n-3 ratio in meat compared with oat grain alone in T2 diet. With vitamin E content of muscle tissues above 3.1 mg α-tocopherol/kg of fresh meat, the oxidative stability of lamb meat as fresh and aged meat was not affected over the time frame used in this study. Further investigation is needed to identify pathways to produce lambs with higher muscle antioxidant status and essential fatty acids using perennial and annual pasture systems.

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References


Burnett VF, Seymour GR, Norng S, Jacobs JL and EN Ponnampalam (2011) Lamb growth performance and carcase yield from perennial or annual pasture systems with supplements. *(submitted to Animal Production Science).*


Ponnampalam EN, Warner RD, Kitessa S, McDonagh MB, Pethick DW, Allen D and Hopkins DL (2010a) Influence of finishing systems and sampling site on fatty


Table 1. Dry matter (%), crude protein, neutral detergent fibre, dry matter digestibility (%DM), metabolisable energy (MJ/kg DM) and crude fat (%DM) content of dietary components.

<table>
<thead>
<tr>
<th>Type of diets</th>
<th>Basal pasture*</th>
<th>Supplement pellets</th>
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<tbody>
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<td></td>
<td>Perennial</td>
<td>Annual</td>
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<td>Dry matter</td>
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<td>10.6</td>
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<tr>
<td>Neutral detergent fibre</td>
<td>67.0</td>
<td>69.9</td>
</tr>
<tr>
<td>Dry matter digestibility</td>
<td>34.6</td>
<td>36.0</td>
</tr>
<tr>
<td>Metabolisable energy</td>
<td>4.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Crude fat</td>
<td>1.4</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Samples were collected weekly for analysis and the means across all 7 collection periods are reported on dry matter basis.

*Annual pasture was senesced stage and perennial pasture were dormant (matured) stem stage during autumn season.
Table 2. Concentrations (mg/100 g DM) of α-linoleic acid (ALA, 18:3n-3), linoleic acid (LA), total fat, ratio of ALA/total fat and LA/total fat in the diets used for the feeding of lambs

<table>
<thead>
<tr>
<th></th>
<th>ALA</th>
<th>LA</th>
<th>Total fat</th>
<th>ALA/total fat</th>
<th>LA/total fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual pasture</td>
<td>52</td>
<td>189</td>
<td>488</td>
<td>0.11</td>
<td>0.39</td>
</tr>
<tr>
<td>Perennial pasture</td>
<td>145</td>
<td>175</td>
<td>595</td>
<td>0.24</td>
<td>0.29</td>
</tr>
<tr>
<td>Lucerne hay</td>
<td>377</td>
<td>186</td>
<td>891</td>
<td>0.42</td>
<td>0.21</td>
</tr>
<tr>
<td>Grass hay</td>
<td>36</td>
<td>45</td>
<td>299</td>
<td>0.12</td>
<td>0.15</td>
</tr>
<tr>
<td>Oat grain</td>
<td>60</td>
<td>1,754</td>
<td>4,870</td>
<td>0.01</td>
<td>0.36</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>24,920</td>
<td>6,024</td>
<td>39,985</td>
<td>0.61</td>
<td>0.15</td>
</tr>
<tr>
<td>Flaxmeal</td>
<td>4,094</td>
<td>1,162</td>
<td>7,397</td>
<td>0.55</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Values are means of two samples expressed in mg/100 g dry matter, from the homogenate sample obtained over the seven week period of feeding.

*xAnnual and perennial pastures were at senesced and dormant stage, respectively during autumn season.
Figure 1a & 1b. Concentration of α-tocopherol (mg/kg DM) (Fig. 1a) and γ-tocopherol (mg/kg DM) (Fig. 1b) in feeds used in the experimental study. Values are means of two samples from the homogenate sample obtained over the seven week period of feeding. Feed type, PP = perennial pasture, AP = annual pasture, GH = grass hay, LH = lucerne hay, OG = oat grain, FM = flaxmeal, FS = flaxseed.
Figure 2a & 2b. Changes in the redness (a* value) of the meat surface during simulated retail display of fresh (3a) and aged (3b) meat from lambs fed perennial pasture (T1), annual pasture with oat grain, lucerne hay and annual hay (T2), annual pasture with oat grain, lucerne hay, annual hay and flaxseed (T3) and annual pasture with oat grain, lucerne hay, annual hay and flaxmeal (T4).

Figure 3a & 3b. Changes in brownness formation (oxy/met ratio as measured by reflectance at 630/580 nm) of the meat surface during simulated retail display of fresh (4a) and aged (4b) meat from lambs fed perennial pasture (T1), annual pasture with oat grain, lucerne hay and annual hay (T2), annual pasture with oat grain, lucerne hay, annual hay and flaxseed (T3) and annual pasture with oat grain, lucerne hay, annual hay and flaxmeal (T4).
Table 3. Vitamin E (α-tocopherol) content of meat and concentration of thiobarbituric acid reactive substances (TBARS) in fresh meat and aged meat from lambs fed perennial pasture (T1), annual pasture with oat grain, lucerne hay and annual hay (T2), annual pasture with oat grain, lucerne hay, annual hay and flaxseed (T3) and annual pasture with oat grain, lucerne hay, annual hay and flaxmeal (T4).

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Perennial Pasture T1</th>
<th>Annual Pasture</th>
<th>s.e.d</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T2 T3 T4</td>
<td></td>
<td>Perennial vs Other</td>
</tr>
<tr>
<td>Vitamin E (mg/kg meat)</td>
<td>5.88^b</td>
<td>3.43^a 3.10^a 3.63^a</td>
<td>0.57 0.51</td>
<td>0.05 0.91</td>
</tr>
<tr>
<td>TBARS-Fresh (mg MDA/kg meat)</td>
<td>0.24</td>
<td>0.36 0.33 0.37</td>
<td>0.08 0.06</td>
<td>0.15 0.67</td>
</tr>
<tr>
<td>TBARS-Aged (mg MDA/kg meat)</td>
<td>1.17</td>
<td>2.11 2.30 2.31</td>
<td>0.51 0.49</td>
<td>0.09 0.63</td>
</tr>
<tr>
<td>pH_24</td>
<td>5.53</td>
<td>5.59 5.59 5.57</td>
<td>0.035 0.028</td>
<td>0.12 0.79</td>
</tr>
</tbody>
</table>

Number of lambs in the perennial pasture treatment = 9 and number of lambs in treatment 2, 3 & 4 having annual pasture with supplements = 15. a,b,c Within a raw, means without a common superscript differ significantly (P<0.05).
Table 4. Eicosapentaenoic acid (EPA, 20:5n-3) plus docosahexaenoic acid (DHA, 22:6n-3), long chain (LC) n-3, total n-3, total n-6 polyunsaturated fatty acids (PUFA) and other major fatty acids in mg/100 g of meat from lambs fed perennial pasture (T1), annual pasture with oat grain, lucerne hay and annual hay (T2), annual pasture with oat grain, lucerne hay, annual hay and flaxseed (T3) and annual pasture with oat grain, lucerne hay, annual hay and flaxmeal (T4).

<table>
<thead>
<tr>
<th>Fatty acid type</th>
<th>Perennial Pasture T1</th>
<th>Annual Pasture</th>
<th>s.e.d</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T2</td>
<td>T3</td>
<td>T4</td>
<td>Perennial vs Other</td>
</tr>
<tr>
<td>Alpha-linolenic acid (ALA)</td>
<td>61.5</td>
<td>51.8</td>
<td>61.0</td>
<td>58.9</td>
</tr>
<tr>
<td>Linoleic acid (LA)</td>
<td>137&lt;sup&gt;a&lt;/sup&gt;</td>
<td>160&lt;sup&gt;b&lt;/sup&gt;</td>
<td>154&lt;sup&gt;b&lt;/sup&gt;</td>
<td>158&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>EPA + DHA</td>
<td>33.5</td>
<td>30.1</td>
<td>31.5</td>
<td>29.4</td>
</tr>
<tr>
<td>LC n-3 PUFA</td>
<td>56.1</td>
<td>51.3</td>
<td>52.5</td>
<td>50.2</td>
</tr>
<tr>
<td>Total n-3 fatty acid</td>
<td>117</td>
<td>103</td>
<td>114</td>
<td>109</td>
</tr>
<tr>
<td>Total n-6 fatty acid</td>
<td>215</td>
<td>242</td>
<td>238</td>
<td>239</td>
</tr>
<tr>
<td>Saturated fats (SFA)</td>
<td>1364</td>
<td>1425</td>
<td>1502</td>
<td>1484</td>
</tr>
<tr>
<td>Monounsaturated fats</td>
<td>1343</td>
<td>1199</td>
<td>1219</td>
<td>1253</td>
</tr>
<tr>
<td>Polyunsaturated fats (PUFA)</td>
<td>333</td>
<td>345</td>
<td>351</td>
<td>348</td>
</tr>
<tr>
<td>Ratio of n-6 to n-3</td>
<td>1.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ratio of PUFA to SFA</td>
<td>0.25</td>
<td>0.25</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>Total muscle fat</td>
<td>3032</td>
<td>2959</td>
<td>3063</td>
<td>3076</td>
</tr>
</tbody>
</table>

Number of lambs in the perennial pasture treatment = 9 and number of lambs in treatment 2, 3 & 4 having annual pasture with supplements = 15.

<sup>a</sup>Total omega-6 (n-6) fats include 18:2cis, 18:2trans, 18:3, 20:3, 20:4 and 22:4. Total omega-3 (n-3) fats include 18:3 (ALA), 18:4, 20:3, 20:5 (eicosapentaenoic acid, EPA), 22:5 (docosapentaenoic acid, DPA) and 22:6 (docosahexaenoic acid, DHA). Saturated fats (SFA) include 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 and 22:0. Polyunsaturated fats (PUFA) include total omega-6 and total omega-3 fats. Monounsaturated fats (MUFA) include 14:1, 15:1, 16:1, 17:1, 18:1, 20:1. Total muscle fat includes SFA + MUFA + PUFA.

<sup>a,b,c</sup>Within a raw, means without a common superscript differ significantly (P<0.05).