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Supra-nutritional vitamin E supplementation for 28 days before slaughter maximises muscle vitamin E concentration in finisher pigs

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Abstract

A 4 × 3 factorial experiment (*n*=8 pigs per treatment combination) was conducted with 96 female Landrace x Large White pigs to examine the required level of dietary vitamin E and optimum feeding duration before slaughter to maximise muscle vitamin E content in the *Longissimus thoracis et lumborum* (LTL) muscle. The respective factors were four dietary levels of vitamin E (supplemented as *dl*-α-tocopheryl acetate; 35, 300, 500, and 700 IU/kg) and three feeding durations (14, 28 and 42 days before slaughter). Vitamin E concentration in the LTL was maximised at 6 mg/kg, which was achieved by feeding a 700 IU vitamin E diet for 28 days before slaughter (P<0.001). There was no further increase in the vitamin E content of the LTL by feeding the high vitamin E diet more than 28 days before slaughter.

Keywords: Intramuscular fat; Meat quality; Muscle vitamin E; Pork; Vitamin E

1. Introduction

Vitamin E is a well-known antioxidant and free-radical scavenger heavily involved in the immune functions of mammals (Chew, 1996), with a study by de Oliveria Otto *et al* (2012) finding that the risk of metabolic syndrome and cardiovascular disease could be reduced by 20-30% with an increased dietary intake of vitamin E. The recommended daily vitamin E intake for adult female and male are 7 and 10 mg of α-tocopherol per day, respectively (Nutrient Reference Values for Australia and New Zealand, 2006). In general, meat is a poor source of vitamin E as supplementation of vitamin E in feeds for production animals has been to meet the minimum requirement of the animal and not to enhance the nutrient composition of the meat. The NRC (2012) recommends a level of 34 IU/kg of vitamin E in a finisher pig diet and typically, a cut of loin pork contains approximately 2 mg vitamin E/kg. For a human nutrition claim to be made for a particular product, a serving (135g fresh meat) should contain no less than 10% of the recommended daily intake (Food Standards Australia & New Zealand, 2014). This would require pork vitamin E levels to be increased to 5.1 and 7.4 mg/kg for female and male, respectively. Several pig studies have investigated the effect of increased dietary vitamin E supplementation on muscle vitamin E concentration and reported a linear increase in its

content. For example, Hasty, van Heugten, See, & Larick (2002) reported a linear increase in muscle vitamin E concentration from 1.99 mg/kg to 4.83 mg/kg when pigs were fed a diet containing 351 IU vitamin E/kg for 42 days before slaughter in comparison to pigs fed a diet containing 12 IU vitamin E/kg. Jensen *et al.* (1997) showed that finishing pigs fed supplemented diets containing 700 IU of vitamin E increased their muscle concentration to 11 mg/kg in cuts of loin. In a meta-analysis, Trefan et al (2012) reported that vitamin E supplementation increased muscle vitamin E content to a maximum of 6.4 mg/kg in the loin. These findings indicate that it is possible to increase the muscle vitamin E concentrations in pork. However vitamin E is one of the more expensive components of a mineral-vitamin premix and therefore the optimal inclusion rate as well as duration of feeding needs to be determined to minimise cost while maximising vitamin E deposition. Furthermore, it has not been investigated whether supplementation with higher than 400 IU of vitamin E for less than 42 days (e.g. 14-28 days) would increase muscle vitamin E level at the same, or greater, extent to supplementing at a lower rate for 42 days.

Improvements in the quality of pork may also be addressed by the addition of vitamin E. Increasing muscle vitamin E concentration is known to increase the storage life of pork products (Hoving-Bolink *et al*., 1998). Asghar *et al.* (1991) reported reduced oxidation in pork chops after storing for 10 days when pigs were supplemented with 10, 100 or 200 IU Vitamin E/kg for approximately 14 weeks. Thiobarbituric acid reactive substances (TBARS), an indicator of lipid oxidation by-products in the muscle, were significantly lower in pork from pigs fed a high vitamin E diet. These authors also found decreased drip loss in frozen pork derived from pigs supplemented with high levels of vitamin E. Moreover, Duroc pigs selected for intramuscular fat (IMF) accumulated more monounsaturated fatty acids in the IMF compared with a control line (Burkett, 2009), and six generations of selection for IMF in Duroc pigs increased their total IMF content in the LD muscle by 88% (Schwab, Baas, Stalder & Nettleton, 2009). As vitamin E is fat soluble and is absorbed with fatty acids and triglycerides (Linder, 1991), total amounts of IMF deposition may have implications for the way the vitamin E is deposited in the muscle. However, such an investigation and reports are scarce in

the pork meat literature and worthy of investigation to better understand possible background mechanisms for the kinetics of muscle vitamin E saturation.

The aim of this experiment was to investigate the potential for Vitamin E supplementation in finisher pigs to enrich the vitamin E content of muscle and improve indices of eating quality. The hypotheses tested were: (1) muscle (*Longissimus thoracis et lumborum*, LTL) vitamin E concentrations can be maximised by feeding a high vitamin E (700 IU/kg) diet for 14 days, compared with feeding a low vitamin E (35 IU/kg) diet for 42 days before slaughter; (2) increasing muscle vitamin E concentrations will decrease the concentration of TBARS in fresh pork when measured 24 hours after slaughter; and (3) muscle vitamin E concentration will be positively correlated with IMF concentration.

2. Materials and Methods

2.1. Care of animals

This experiment was approved by the Animal Ethics Committee of the Department of Agriculture and Food, Western Australia (AEC 4-13-4). Animals were handled according to the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013).

2.2. Experimental design

The experiment was a 4×3 factorial design with the respective factors being four levels of vitamin E supplementation in the form of *dl*-α-tocopheryl acetate (35, 300, 500, and 700 IU/kg, DSM Nutritional Products Australia), and three feeding durations (14, 28 and 42 days before slaughter). Ninety-six female Landrace × Large White pigs were acquired from a commercial farm at three different live weights, to meet the requirements of feeding durations of 14, 28 and 42 days before slaughter, and allocated to different supplementation groups based on these weights [14 days (32 pigs at 80.0 ± 0.19 kg, mean ± SEM), 28 days (32 pigs at 62.4 ± 0.20 kg), and 42 days (32 pigs at 49.3 ± 0.15 kg)]. Pigs were blocked and stratified to the factorial design based on the blocking criteria of age

and live weight, such that there were 8 pigs per treatment (4 vitamin E levels × 3 feeding durations × 8 pigs = 96 pigs; n=8 per treatment). Pigs were housed individually in a pen fitted with a nipple drinker and a metal feed trough.

2.3. Diets and sample collection

Diets were formulated to contain 14.0 MJ digestible energy (DE)/kg and 0.6 g standardised ileal digestible lysine/MJ DE (Table 1). The experimental diets are similar in major ingredients and nutrient specifications to the diets commercially used in the source farm. As these diets were fed from 50 kg live weight, the diets were formulated to contain moderate amount of fat (75 g/kg). The analysed vitamin E values were different from formulated values due most likely to variations in vitamin E contents in the individual ingredient and to sampling and analytical errors. Diets were produced in a single mix and vitamin E content was analysed in duplicate. Variation between duplicate analyses was less than 5%. Especially the control diet contained 60 IU vitamin E/kg instead of formulated value of 35 IU/kg. The pigs were fed these diets *ad libitum* for the designated feeding duration and performance indices (average daily gain, average daily feed intake and feed conversion ratio) were recorded. Blood samples were collected at days 0, 14, 28 and 42 from the 42-day feeding duration group only (8 pigs x 4 dietary treatments x 4 collections = 128 samples) to measure plasma vitamin E content. Blood samples were collected from the jugular vein into a heparinized tube and immediately centrifuged at 2000 *g* for 15 min. at 4 ˚C. Plasma samples were then stored at -20 ˚C for subsequent chemical analyses. At the end of the supplementation period, all pigs were sent to a commercial abattoir and slaughtered by CO_2 stunning and exsanguination. Hot carcass weight was measured based on AUSMEAT trim 13 (head off, flare off, fore trotters off, hind trotters on; AUS-MEAT Ltd, South Brisbane, Qld, Australia). Dressing percentage was calculated as the ratio between live weight and hot carcass weight. P2 back fat depth was measured between the $12^{th}/13^{th}$ ribs of the left side of each carcass. At 24 hour after slaughter a 2 kg sample of LTL muscle was collected between the 10^{th} and 15^{th} ribs and objective measures of meat quality (colour, drip loss, pH, shear force), muscle vitamin E concentration, and IMF concentrations were subsequently measured. The

LTL samples collected from the 42-day feeding group (8 samples x four dietary vitamin E treatments = 32 samples) were measured for TBARS.

2.4. Objective meat quality measures

Objective measures of meat quality were conducted in the 14-day and 42-day feeding duration groups only (2 feeding durations x 4 dietary treatments x 8 samples = 64 samples) as the range of muscle vitamin E would be covered sufficiently. Muscle pH was determined at 45 min, 24, 48 and 72 hours after slaughter in the LTL. The pH was measured using a pH 300 hand-held pH/mV/temperature meter (Cyberscan pH 300, Eutech Instruments, Singapore) fitted with a temperature probe and IJ44C intermediate junction pH probe (Ionode, Tennyson). The pH meter was calibrated on two standards (pH 4.01 and 7.0) as per the manufacturer's instructions. At 24 hours the probe was inserted into the LTL of each carcass between the 3rd and 4th ribs 7.5 cm from the ventral edge of the split pork carcass (pH24 measurement). At 48 and 72 hours the pH was recorded directly into the removed piece of loin. The pH at 72 hours was considered the ultimate pH (pHu).

Loin colour was measured at 48 hours after slaughter. Muscle samples were cut and the surface was exposed to air at room temperature for 10 min. Meat colour was determined using a Minolta Chromameter Model CR-400 (Minolta, Osaka, Japan) (calibrated on a white tile) set on the L*, a* and b* system where L* denotes relative lightness (higher L* values = paler meat), a* relative redness (higher a* values = more red) and b* relative yellowness (higher b* values = more yellow), using D65 illumination and a 2° standard observer.

Drip loss was determined at 24 hours after slaughter using a modified method of Honikel (1998). A sample of pork loin was cut to a 40 g cube, weighed, and the weight recorded. The sample was then wrapped in a piece of square netting. The wrapped sample was then suspended in a sealed 500 ml plastic container and left to stand at 4°C for 24 hours, after which the sample was removed from the container, gently rolled in paper toweling and reweighed to determine percentage drip loss.

To prepare the samples for Warner-Bratzler shear force (WBSF, an index for tenderness), a rectangular block (80g ± 2 g) was cut from the loin where the length of the sample followed the length

of the muscle fibres. Samples were trimmed of all external fat and epimysium, weighed, vacuum packaged into individual bags and frozen at -20^{o}C until cooking. Samples were cooked from a frozen state within a week from the sampling days in a water bath preheated to 70°C until an internal temperature of 70°C was attained. Samples were then cooled in an iced water bath for 30 min. Five 1 cm^2 replicate samples were cut parallel to the orientation of muscle fibres (approximately 5 cm long) and WBSF was measured using a Lloyd Texture Analyser (TA-2, United Kingdom) fitted with a Warner Bratzler shear blade.

2.5. Chemical analyses

Intramuscular fat content was measured using the Ankom method for measurement of crude fat (extraction of crude fat using petroleum ether). The α-tocopherol content in the feed and meat was measured using the method of McMurray, Blanchflower, & Rice (1980). Briefly, 1 g of sample was homogenised in 10 ml of 6% pyrogallol by ultraturrex. One mL of 60% KOH in water was added and the sealed tubes were heated at 70°C for 30 min. After cooling, 5 mL of water and 20 mL of hexane was added. After extraction by vortexing, 5 ml of the hexane layer was evaporated under nitrogen and made up in 0.5 mL of methanol (0.1% butylated hydroxytoluene). The chromatographic separation was performed with an Agilent HPLC system (1100) using a Zorbax SB-C18 column (3 mm x 150 mm, 3.5 µm, Agilent). Alpha tocopherol was quantified using fluorescence detection (ex. 296 nm and em. 330 nm). The α-tocopherol content in plasma was analysed using the method of McMurray & Blanchflower (1979). Briefly, 1 mL of plasma was deproteinised with 1 mL of 1% pyrogallol in ethanol and 5 mL of hexane was added. After extraction by vortexing, 4 mL of the hexane layer was evaporated under nitrogen and made up in 0.5 ml of methanol (0.1% butylated hydroxytoluene). The chromatographic separation was performed with an Agilent HPLC system (1100) using a Zorbax SB-C18 column (3 mm x 150 mm, 3.5 µm) (Agilent) with a methanol mobile phase. Alpha tocopherol was quantified using fluorescence detection (ex. 296 nm and em. 330 nm).

The TBARS content in muscle tissue was measured using the modified method of AMSA (2012) and Jo, & Ahn (1998). Tissue samples were stored at −20 ^{o}C prior to analysis, but with the

following modifications to the colour reagent; inclusion of sodium docecylsulphate (to improve extraction of TBARS) and butylated hydroxytoluene (to prevent TBARS formation during assay) to final concentrations of 0.42% and 0.09%, respectively. Homogenised tissue was added at the rate of 0.3 ml to 1.5 ml of colour reagent. The TBARS were quantified using the standard 1,1,3,3-tetramethoxypropane with spectrofluorometric measurement (Jo, & Ahn 1998) at 510 nm excitation and 560 nm emission using a POLARstar Omega plate reader (BMG Labtech Pty. Ltd. Mornington, Victoria).

2.6. Statistical analysis

Data were analysed using one-way analysis of variance for pig growth performance and TBARS in the LTL muscle. All other data were analysed using two-way analysis of variance (Genstat 16th edition; VSN International Ltd, Hemel Hempstead, UK). When there was a significant treatment effect, means were separated using Fisher's-protected least significant difference (PLSD) test. The pig was the experimental unit for all analyses. As final weight was significantly different between feeding duration groups, final weight was used as a covariate for all statistical analyses when final weight was a significant source of variation (indicated in each presented data). Pearson's correlation analysis was conducted between muscle vitamin E concentration, IMF, and objective meat quality measures to investigate relationships. In addition, linear regression analyses were conducted to establish linear relationships between measured variables that showed significant correlations.

3. Results

3.1. Vitamin E contents in plasma and muscle

The effect of dietary vitamin E concentration and feeding duration on plasma and LD muscle vitamin E concentrations are presented in Figures 1 and 2, respectively. Vitamin E and feeding duration affected ($P<0.001$) plasma vitamin E concentration, however there was a vitamin E x feeding duration interaction ($P<0.001$) such that increasing the feeding duration of vitamin E-supplemented

diets significantly increased plasma vitamin E content, while increasing the feeding duration of the 35 IU vitamin E diet did not increase plasma vitamin E concentration. Plasma vitamin E content reached a peak concentration of 6 mg/L when pigs were fed a 700 IU diet for 28 days.

Vitamin E concentration in the LTL followed a similar pattern to the plasma vitamin E concentration (Figure 2). Vitamin E concentration in the LTL was maximised at 6 mg/kg, which was achieved by supplementing 700 IU vitamin E for 28 days before slaughter. Vitamin E and feeding duration affected ($P<0.001$) muscle vitamin E concentration and the vitamin E x feeding duration interaction was significant ($P<0.023$). The interaction occurred as there was no linear increase in muscle vitamin E content with increasing dietary vitamin E levels in samples collected from the 14-day feeding group, however muscle vitamin E content linearly increased in the samples collected from the 28 and 42 day feeding groups.

A linear regression analysis was conducted to establish a prediction equation for plasma vitamin E concentration from daily vitamin E intake (Figure 3). Plasma vitamin E concentration could be predicted from the daily vitamin E intake using the following equation: $y=0.002304+2.593x$, with a prediction accuracy of 59.6%, where x=daily vitamin E intake in milligrams (Figure 3). Further linear regression analysis was conducted to establish a prediction equation for vitamin E concentration in the LTL from plasma vitamin E concentration (Figure 4). Vitamin E concentration in the LTL could be predicted from the plasma vitamin E concentration using the following equation: $y=0.827+0.805x$, with a prediction accuracy of 82.5%, where x= plasma vitamin E in mg/L.

3.2. Carcass traits, objective meat quality and performance of pigs

The main effects of dietary vitamin E supplementation and feeding duration before slaughter on carcass traits are presented in Table 2. Feeding duration was confounded with live weight of pigs as the final weights of the pigs fed the experimental diets for 14 days were heavier ($P<0.001$) compared with the final weights of pigs fed for 28 or 42 days. Therefore, final live weight was used as a covariate for analysis of the other carcass data. Carcass weight and dressing percentage were higher ($P<0.05$) in pigs fed the experimental diets for 14 days compared with those fed for 28 and 42 days.

Back fat thickness was lower (P<0.05) in pigs fed a diet supplemented with 500 IU vitamin E/kg compared with pigs fed a diet supplemented with 700 IU vitamin E/kg. The IMF content was not affected (P>0.05) by vitamin E supplementation or feeding duration.

Effects of vitamin E supplementation and feeding duration on objective meat quality measures are presented in Table 3. Pigs fed the experimental diets for 42 days had a lower pH at both 48 (P<0.05) and 72 hours (P<0.001) after slaughter. An interaction between diet and feeding duration (P<0.05) occurred for pH in the LTL measured 48 hours after slaughter such that there was no effect of feeding duration on pH 48 for pigs supplemented with 35, 300, and 500 IU vitamin E, while pH 48 was significantly lower when pigs were fed 700 IU vitamin E diet for 42 days than for 14 days (5.40 vs. 5.50).

The performance of pigs measured over 42 days is presented in Table 4. Increasing dietary vitamin E concentration and increasing feeding duration from 14 to 42 days had no effect (P<0.05) on daily gain, feed intake and feed conversion ratio.

3.3. Correlation between muscle vitamin E and objective meat quality measures

Pearson's correlation coefficients are presented in Table 5. Drip loss was negatively correlated with pH in the LTL measured at 45 min (P<0.01), 24 hours (P<0.01), 48 hours (P<0.05) and 72 hours (P<0.05). Vitamin E concentration in the LD muscle was negatively correlated with pH measured at 48 hours (P<0.01) and 72 hours (P<0.01).

3.4. Dietary vitamin E and TBARS concentration at 24-hour after slaughter

The effect of dietary vitamin E content on TBARS measured at 24 hour after slaughter is presented in Figure 5. Increasing dietary vitamin E levels to 300 IU/kg and above decreased (P<0.001) TBARS concentration measured 24 hours after slaughter. Further linear regression analysis

established a negative relationship between muscle vitamin E concentration in the LTL and TBARS concentration measured 24 hours after slaughter in the LTL (P<0.01, Figure 6).

3.5. Relationships between muscle vitamin E and meat quality traits

Linear regression analysis was conducted and a significant negative linear relationship was observed between muscle vitamin E content and the pH in the LTL measured at 48 hours after slaughter [intercept -0.01624 (SE 0.0059), slope 5.5082 (SE 0.0259), R^2=0.102, RSD=1.01, n=64, *P*=0.006]). A significant negative linear relationship was also observed between muscle vitamin E content and the pH in the LTL measured at 72 hours after slaughter [intercept -0.0151 (SE 0.00535), slope 5.5414 (SE 0.0243), R^2=0.099, RSD=1.00, n=64, *P*=0.006]. Moreover, a positive linear relationship between muscle vitamin E content and relative redness of the LTL muscle was observed [intercept -0.2313 (SE 0.0918), slope 5.427 (SE 0.418), R^2=0.078, RSD=1.01, n=64, *P*=0.014]. However, the R^2 values for the relationships between the muscle vitamin E and meat quality traits were low.

4. Discussion

The initial hypothesis tested in this experiment was that muscle vitamin E concentration can be maximised by dietary supplementation with a high level of vitamin E for a short period (14 days), compared to dietary supplementation with a low level of vitamin E for a longer period (42 days) before slaughter. The results of this experiment indicate that the LTL muscle was maximised with vitamin E when pigs were fed a diet supplemented with 700 IU vitamin E for 28 days before slaughter. Feeding the 700 IU diet for 42 days did not further increase muscle vitamin E concentration compared to the 28-day feeding duration. Furthermore, no other diets fed for longer than 28 days reached the same vitamin E muscle concentration that the pigs supplemented with 700 IU reached in 28 days.

Equivocal results for muscle concentrations, however, have been reported in the literature regarding levels and duration of feeding of vitamin E. For example, Monahan *et al.* (1990) fed Landrace x Large White pigs either 30 IU or 200 IU vitamin E diet for 14 days and reported an increase of vitamin E in the longissimus dorsi (LD) muscle from 3.2 mg to 7.0 mg/kg tissue. Similarly, Jensen *et al.* (1997) fed diets containing either 132 IU or 722 IU of vitamin E in pigs between 50 – 90 kg live weights and reported an increase in the vitamin E concentration of the LD muscle from 5.4 mg to 11.4 mg/kg, which are seemingly much higher muscle vitamin E concentrations compared to other reported values. In contrast, more recent studies have reported lower vitamin E concentrations in LD muscle with and without supra-nutritional vitamin E supplementation (Rosenvold *et al*., 2002; Boler *et al.*, 2009). Rosenvold *et al*. (2002) supplemented diets with either 90 or 600 IU of vitamin E for 22 days before slaughter and reported an increase from 4.7 to 6.5 mg/kg of vitamin E in the LD muscle, a similar concentration to that reported in the present study. Moreover, Boler *et al*. (2009) fed pigs a series of vitamin E-supplemented diets, from 13.6 IU to 272 IU/kg for 95 days before slaughter (live weight of 119 kg), and reported an increase in vitamin E concentration of the LD muscle from 1.69 mg to 3.61 mg/kg.

It is unclear why there is such a huge variation in LD muscle vitamin E concentration between studies. However, reports of the greatest LD muscle vitamin E concentrations occurred in earlier publications and from this time on analytical techniques and (or) the genetic potential of pigs to deposit vitamin E may have changed and/or have been altered. Nevertheless, the variable LD muscle vitamin E concentrations reported in the present study and several recent reports (Hasty, van Heugten, See & Larick, 2002; Rosenvold *et al.*, 2002; Boler *et al.*, 2009) clearly demonstrates that increasing dietary vitamin E concentrations increased both plasma and LTL/LD muscle vitamin E concentrations. However, other studies did not examine the feeding duration required to maximise muscle vitamin E concentration. Thus, our finding that supra-nutritional vitamin E diets should be fed for at least 28 days to maximise vitamin E in the LTL has important implications if this nutritional strategy is to be adopted in pork production systems.

The second hypothesis, that increasing muscle vitamin E content will decrease the concentration of TBARS at 24 hours after slaughter, was accepted. As anticipated, increasing dietary vitamin E above 300 IU significantly reduced TBARS concentrations in the LTL, however no further reduction in TBARS concentration by supplementation of vitamin E over 300 IU was seen. This is consistent with results reported by Monahan *et al.* (1990), Jensen *et al.* (1997), Lahucky *et al.* (2007) and Boler *et al.* (2009), and suggests that at least in the LTL measured 24 hours after slaughter, dietary supplementation of 300 IU vitamin E reduces the formation of TBARS. In the study by Jensen *et al.* (1997), TBARS remained lower over the 6 days of retail display in LD muscle from pigs fed either 200 IU or 700 IU vitamin E compared with the LD muscles from pigs fed 100 IU vitamin E. This particular study by Jensen *et al.* (1997) suggests that the prevention of TBARS formation is likely to carry over during the retail display for up to 6 days. In a review by Dunshea, D'Souza, Pethick, Harper, & Warner (2005), the reduction in TBARS in the LD muscle due to supplementation of 100 or 200 mg vitamin E/kg diet ranged from 20% to 80% compared to the control sample. This consistent effect of vitamin E in reducing muscle concentration of TBARS suggests that dietary vitamin E supplementation between 200 to 300 IU will suppress TBARS in LD muscle, although levels higher than 300 IU vitamin E will prove beneficial when longer retail display periods are required (Jensen *et al.*, 1997).

The current study found increased redness of the LTL as muscle vitamin E concentration was increased, although the relationship was reasonably weak. When the effect of dietary vitamin E was examined for fresh meat colour of the LTL, no treatment effect was observed. However, when individual variation was taken into account by analysing the relationship between the individual muscle vitamin E concentration and fresh meat colour, there was a significant positive relationship between muscle vitamin E concentration and the redness of the LTL. Several studies have reported no effect of dietary vitamin E on colour of the LD muscle (Hasty, van Heugten, See & Larick, 2002; Rosenvold *et al.*, 2002; Swiger, McKeith, Carr, Brewer, & Culberston, 2004). However, no reports have investigated the relationship between individual vitamin E concentration and redness of this muscle. The findings in the current study indicate that actual muscle vitamin E levels should be used

rather than dietary level of vitamin E to predict colour expression in fresh pork meat. Given that different dietary vitamin E supplementation results in significant variation in the accumulation of muscle vitamin E, it may be inappropriate to draw a conclusion and further research is warranted to investigate (1) the source of variation of muscle vitamin E deposition, and (2) relationships between muscle vitamin E and fresh meat colour expression.

An unanticipated finding in the current experiment was the statistically significant negative relationships, albeit weak with only 10.2 and 9.9% of the total variations being explained respectively, between muscle vitamin E and pH at 48 and 72 hours after slaughter. It is well documented that a lower muscle pH significantly increases drip loss and decreases tenderness of meat, and hence reduces the overall eating quality of pork (Huff-Lonergan, Zhang, & Lonergan, 2010; Jose, Trezona, Channon, & D'Souza, 2013). However, Hasty, van Heugten, See & Larick (2002) reported no pH change 24 hours following slaughter by increasing dietary vitamin E supplementation from 12 IU to 351 IU, whilst Swigert, McKeith, Carr, Brewer, & Culberston (2004) reported an increase in pH in barrows but no difference in gilts when the LD muscle pH was measured 48 hours after slaughter from pigs fed with 500 IU vitamin E in the diet. In agreement with the finding in the present study, however, Rosenvold *et al.* (2002) reported decreased LD muscle pH at 24 hours after slaughter when pigs were supplemented with 500 IU vitamin E. These authors also reported a 10% increase in muscle glycogen for the vitamin E-supplemented pigs 24 hours before slaughter, although there were no differences in glycogen content on the slaughter day. Vitamin E supplementation, therefore, has potential to manipulate the muscle glycogen content before slaughter that in turn might impact negatively on the ultimate pH in muscle. However further research, including any gender-specific effects, of muscle vitamin E on muscle glycogen content and ultimate pH warrants investigation before firm conclusions can be drawn.

The final hypothesis tested in this experiment was that muscle vitamin E content would be correlated to IMF content. This is because vitamin E is transported to the muscle similarly to dietary fats. We used moderately high fat diet (75g/kg) throughout the experiment to facilitate vitamin E

transport and to increase IMF content in the LTL. Use of moderately high fat diet in the finishing phase is strategically used in Australian commercial farms to improve growth performance. In support of this notion, almost 2.5 times more vitamin E was reported in fat tissue and pork sausage compared with LD muscle (Harms, Fuhrmann, Nowak, Wenzel, & Sallmann, 2003). The IMF content of the LTL in the present study was very low, less than 0.75%, thus it is unclear whether such low IMF levels hindered the relationship between IMF and muscle vitamin E content. However, Lahucky *et al*. (2007) reported similar levels of IMF in LD muscles in pigs fed with and without 500 IU vitamin E supplementation (2.52% versus 2.50%, respectively), while vitamin E content in the LD muscle was significantly higher in the vitamin E-supplemented pigs (3.22 versus 1.60 mg/kg). Although it is premature to draw a firm conclusion, the study of Lahucky *et al*. (2007) together with our findings suggests that IMF content is not the cause for the variation of muscle vitamin E deposition in LD muscle.

5. Conclusions

This study clearly demonstrated that (1) vitamin E concentration in the LTL maximised within 28 days of feeding with supra-nutritional levels of vitamin E in the late finishing diet, (2) increasing muscle vitamin E minimized the concentration of TBARS at 24 hours after slaughter, and (3) IMF content of the LTL is not the cause of variation in muscle vitamin E deposition in this muscle. The weak relationships between muscle vitamin E concentration and ultimate pH and fresh meat colour expression need further investigation before a firm conclusion can be drawn.

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References

AMSA. (2012). AMSA Meat Color Measurement Guidelines, TBARS for Oxidative Rancidity—Rapid, Wet Method (pp. 0–124). Champaign, Illinois, American Meat Science Association.

Asghar, A., Gray, J. L., Booren, A. M., Gomaa, E. A., Abouzied, M. M., Miller, E. R., & Buckley, D. J. (1991). Influence of supra-nutritional dietary vitamin E levels on subcellular deposition of alpha-tocopherol in the muscle on pork quality. *Journal of the Science of Food and Agriculture, 57,* 31-41.

Boler, D. D., Gabriel, S. R., Yang, H., Balsbaugh, R., Mahan, D. C., Brewer, M. S., McKeith, F. K., & Killefer, J. (2009). Effect of different dietary levels of natural-source vitamin E in grow-finish pigs on pork quality and shelf life. *Meat Science, 83,* 723-730.

Burkett, J. L. (2009). The effect of selection for intramuscular fat on fatty acid composition in Duroc pigs. Ph. D. dissertation, Iowa State University.

Cheah, K. S., Cheah, A. M., & Jrausgrill, D. I. (1995). Effect of dietary supplementation of vitamin E on pig meat quality. *Meat Science, 39,* 255-264.

Chew, B. P. (1996). Importance of antioxidant vitamins in immunity and health in animals. *Animal Feed Science and Technology, 59,* 103-114.

De Oliveria Otto, M. C., Alonso, A., Lee, D. K., Delclose, D. L., Bertoni, A. G., Jiang, R., Lima, J. A., Symanski, E., Jacons, D. R., & Nettleton, J. A. (2012). Dietary intakes of zinc and heme iron from red meat, but not from other sources, are associated with greater risk of metabolic syndrome and cardiovascular disease. *Journal of Nutrition, 142,* 526-533.

Dunshea, F. R., D'Souza, D. N., Pethick, D. W., Harper, G. S., & Warner, R. D. (2005). Effect of dietary factors and other metabolic modifiers on quality and nutritional value of meat. *Meat Science, 71,* 8-83.

Food Standards Australia & New Zealand (2014). Australia and New Zealand Food Standards Code Standard 1.2.7. Nutrition, health and related claims. Canberra, Australia. Available at http://www.comlaw.gov.au/Details/F2014C01191/Download. Accessed on 02 April 2015.

Harms, C., Fuhrmann, H., Nowak, B., Wenzel, S., & Sallmann, H. -P. (2003). Effect of dietary vitamin E supplementation on the shelf life of cured pork sausage. *Meat Science, 63,* 101-105.

Hasty, J. L., van Heugten, E., See, M. T., & Larick, D.K. (2002). Effect of vitamin E on improving fresh pork quality in Berkshire- and Hampshire-sired pigs. *Journal of Animal Science, 80,* 3230-3237.

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat Science, 49,* 447-457.

Hoving-Bolink, A. H., Eikelenboom, G., van Diepen, J., Jongbloed, A. W., Houben, J. H., & van Diepen, J. M. T. (1998). Effect of dietary vitamin E supplementation on pork quality. *Meat Science, 49,* 205–212.

Huff-Lonergan, E., Zhang, W., & Lonergan, S. M. (2010). Biochemistry of postmortem muscle – Lessons on mechanisms of meat tenderization. *Meat Science, 86,* 184-195.

Jensen, C., Guider, J., Skovgaar, I. M., Staun, H., Skibsted, L. H., Jensen, S. K., Møller, A. J., Buckley, J., & Bertelsen, G. (1997). Effects of dietary α-tocopheryl acetate supplementation on α-tocopherol deposition in porcine m. psoas major and m. longissimus dorsi and on drip loss, colour stability and oxidative stability of pork meat. *Meat Science, 45,* 491-500.

Jo, C., & Ahn, D. U. (1998). Fluorometric analysis of 2-thiobarbituric acid reactive substances in turkey. *Poultry Science 77,* 475-480.

Jose, C. G., Trezona, M., Channon, H. A., & D'Souza, D. (2013). Eating quality linked to ultimate pH and tenderness in Australian pork. In J. R. Pluske & J. M. Pluske (Eds), *Manipulating Pig Production XIV* (p. 34), Werribee, Victoria, Australasian Pig Sceince Association.

Lahucky, R., Bahelka, I., Kuechenmeister, U., Vasickova, K., Neurnberg, K., Ender, K., & Nuernberg, G. (2007). Effects of dietary supplementation of vitamins D3 and E on quality characteristics of pigs and *longissimus* muscle antioxidative capacity. *Meat Science, 77,* 264-268.

Linder, M. C. (1991). Nutrition and metabolism of vitamins. In M. C. Linder (Ed.), *Nutritional biochemistry and metabolism with clinical applications, 2nd ed.* (pp. 111-189). Connecticut, Appleton & Lange.

McMurray, C. H., Blanchflower, W. J., & Rice, D. A. (1980). Influence of extraction techniques on determination of alpha-tocopherol in animal feedstuffs. *Journal of the Association of Official Analytical Chemists, 63,* 1258-1261.

McMurray, C. H., & Blanchflower, W. J. (1979). Application of a high-performance liquid chromatographic fluorescence method for the rapid determination of alpha-tocopherol in the plasma of cattle and pigs and its comparison with direct fluorescence and high-performance liquid chromatography-ultraviolet detection methods. *Journal of Chromatography, 178,* 525-31.

Monahan, F. J., Buckley, D. J., Gray, J.I., Morrissey, P. A., Asghar, A., Hanrahan, T.J., & Lynch, P.B. (1990). Effect of dietary vitamin E on the stability of raw and cooked pork. *Meat Science, 27,* 99-108.

MHMRC, (2013). Australian code for the care and use of animals for scientific purposes. (8th ed.). Canberra, National Health and Medical Research Council.

NRC, (2012). Nutrient requirements of swine. Washington DC, National Research Council of the National Academies.

Nutrient Reference Values for Australia and New Zealand (2006). Nutrient reference values for Australia and New Zealand including recommended dietary intakes. Canberra, Australian Health and Medical Research Council. Availabe at https://www.nrv.gov.au/sites/default/files/page_pdf/n35-vitamine_0.pdf. Accessed on 02 April 2015.

Rosenvold, K., Lærke, H. N., Jensen, S. K., Karlsson, A. H., Lundstrom, K., & Andersen, H. J. (2002). Manipulation of critical quality indicators and attributes in pork through vitamin E supplementation, muscle glycogen reducing finishing feeding and pre-slaughter stress. *Meat Science, 62,* 485-496.

Schwab, C. R., Baas, T. J., Stalder, K. J., & Nettleton, D. (2009). Results from six generations of selection for intramuscular fat in Duroc swine using real-time ultrasound. I. Direct and correlated phenotypic responses to selection. *Journal of Animal Science*, *87*, 2774-2780.

Swigert, K. S., McKeith, F. K., Carr, T. C., Brewer, M. S., & Culberston, M. (2004). Effects of dietary vitamin D3, vitamin E, and magnesium supplementation on pork quality. *Meat Science*, *67*, 81-86.

Trefan, L., Bunger, L., Bloom-Hansen, J., Rooke, J. A., Salmi, B., Larzul, C., Terlouw, C., & Doeschl-Wilson, A. (2011). Meta-analysis of the effects of dietary vitamin E supplementation on α-tocopherol concentration and lipid oxidation in pork. *Meat Science*, *87*, 305-314.

Zanardi, E., Novelli, E., Ghiretti, G. P., Dorigoni, V., & Chizzolini, R. (1999). Colour stability and vitamin E content of fresh and processed pork. *Food Chemistry*, *67*, 163-171.

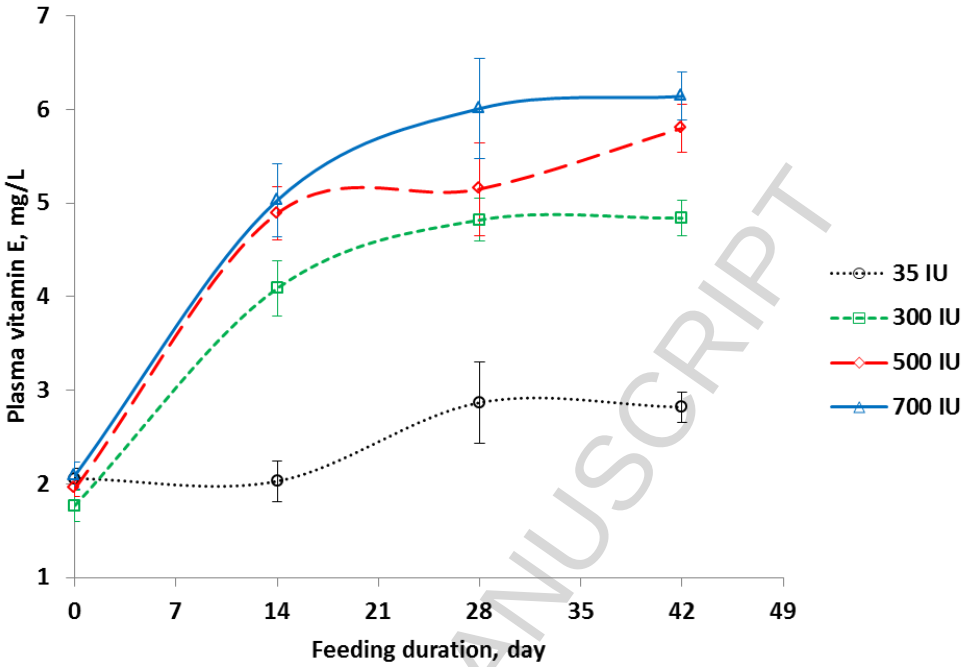

Figure 1. Effect of feeding duration before slaughter and dietary vitamin E level on plasma vitamin E concentrations in finisher pigs (*n*=8).

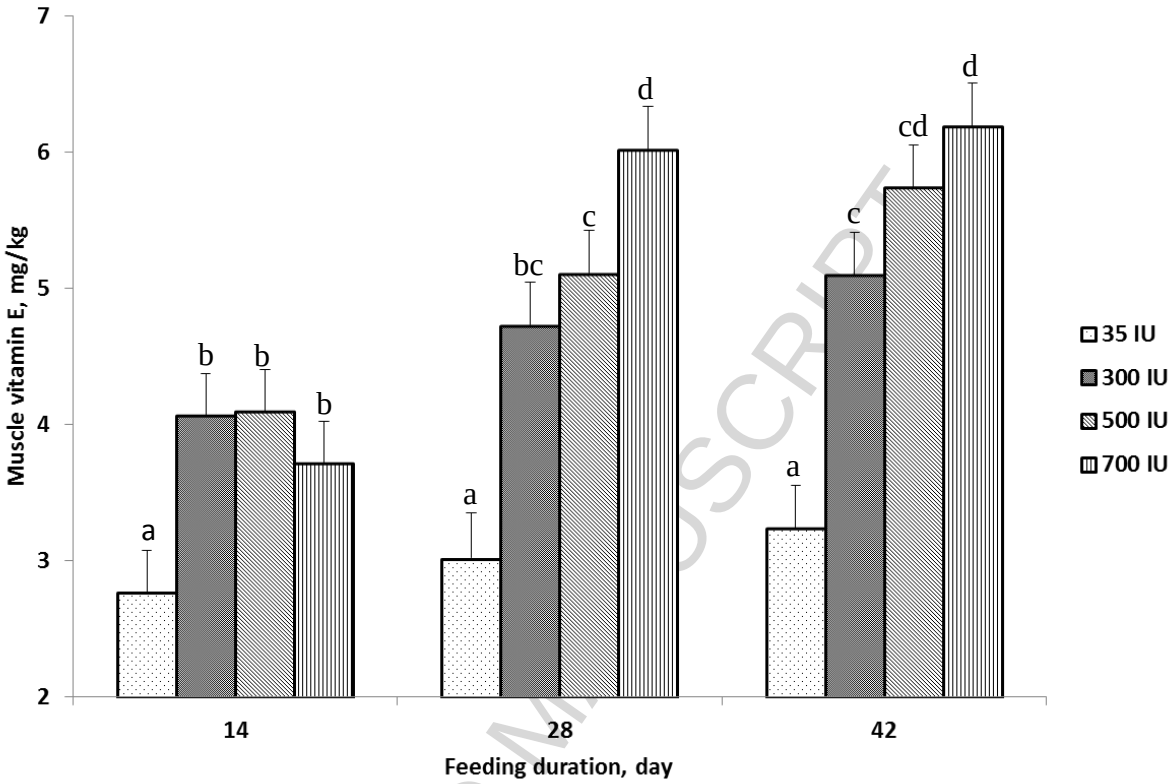

Figure 2. Effect of feeding duration before slaughter and dietary vitamin E level on vitamin E concentration in the LTL muscle (*n*=8). Means not having the same alphabetical letter are significantly different (P<0.05).

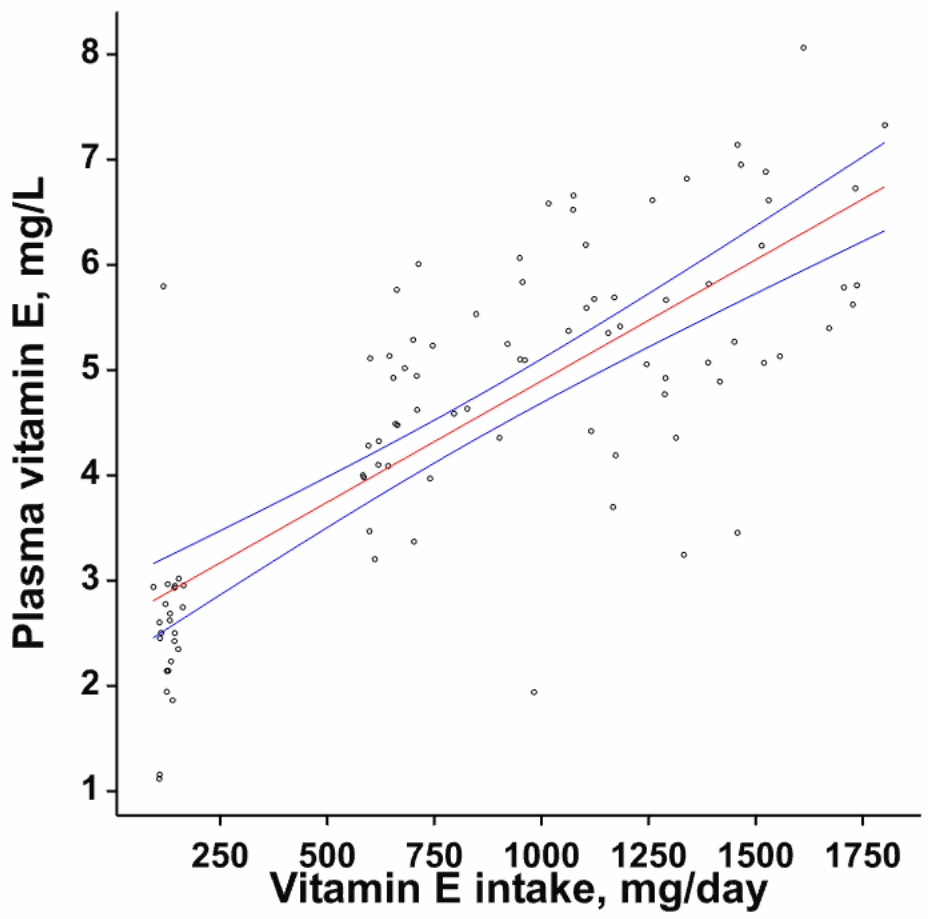

Figure 3. Linear relationship with 95% confidence interval between daily vitamin E intake and plasma vitamin E content. [Intercept 0.002304 (SE 0.000194), slope 2.593 (SE 0.193), R^2 = 0.59.6, RSD=0.973, n=64, P=0.001].

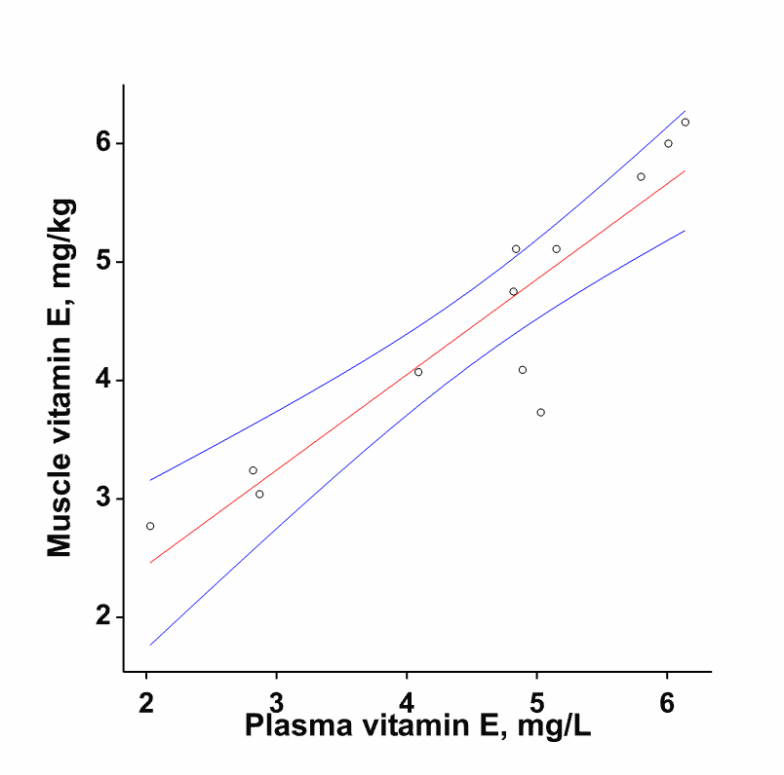

Figure 4. The relationship between vitamin E contents in the plasma and LTL muscle [intercept 0.827 (SE 0.523), slope 0.805 (SE 0.11), R^2 = 0.825, RSD=1.02, n=12, P<0.001].

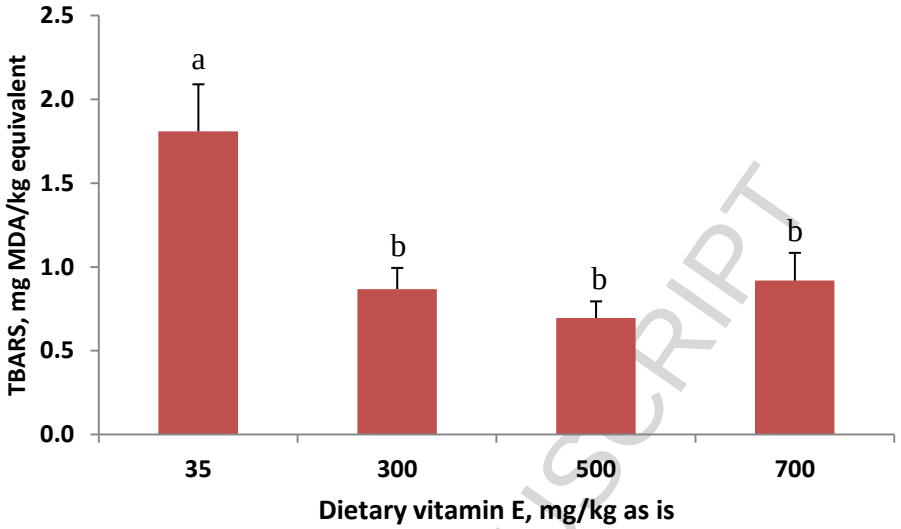

Figure 5. Effect of feeding diets with varying levels of vitamin E for 42 days before slaughter on TBARS in the LTL muscle measured 24 hours after slaughter (*n*=8). Means not having the same alphabetical letter are significantly different (P<0.001).

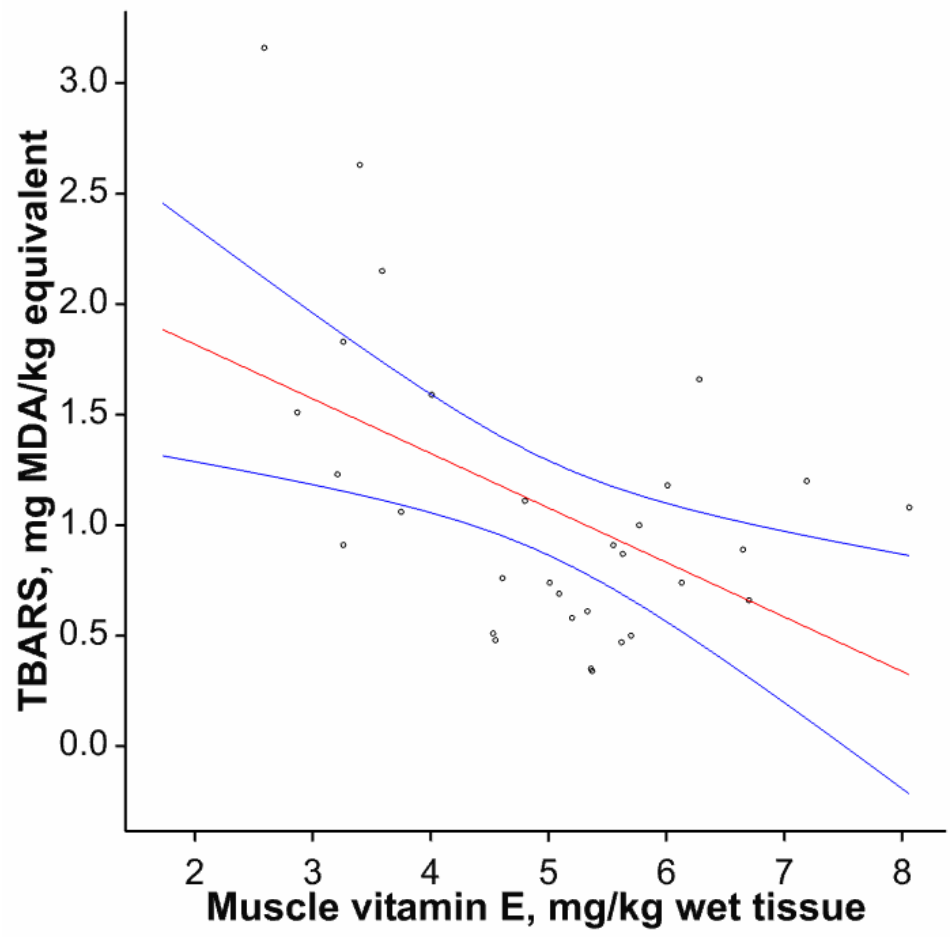

Figure 6. Linear relationship with 95% confidence interval between muscle vitamin E content and TBARS in the LTL muscle measured 24 hours after slaughter [intercept -0.2467 (SE 0.0792), slope 2.312 (SE 0.41), R^2 = 0.225, RSD=1.03, n=32, P=0.004].

Table 1. Ingredients and composition of the experimental diets (g/kg as isair-dry basis).

Ingredient Name	Vit E 35	Vit E 300	Vit E 500	Vit E 700
Barley	604603.8	603602.9	602.20	602601.6
Wheat	100	100	100	100
Australian sweet lupin	100	100	100	100
Canola Meal	100	100	100	100
Full fat soya	30	30	30	30
Meat Meal	14.8	14.8	14.8	14.8
Canola Oil	37.0	37.3	37.6	37.8
L-Lysine	3.51	3.52	3.52	3.52
DL-Methionine	0.97	0.97	0.98	0.98
L-Threonine	1.09	1.10	1.10	1.10
L-Tryptophan	0.21	0.21	0.21	0.21
Vitamin/mineral Premix[1]	0.7	0.7	0.7	0.7
Vitamin E[2]	0	0.6	1	1.4
Limestone	5.45	5.44	5.44	5.44
Salt	2	2	2	2
Choline Chloride 60%	0.47	0.47	0.47	0.47
Ingredient Total:	1000	1000	1000	1000
Calculated composition				
DE, MJ/kg	14.0	14.0	14.0	14.0
NE, MJ/kg	10.0	10.0	10.0	10.0
Crude protein	157	157	157	157
Crude fat	70	70	70	70
ND Fibre	183	182	182	182
AD Fibre	58	58	58	58
Calcium	5.0	5.0	5.0	5.0

Digestible phosphorus	2.0	2.0	2.0	2.0
Analysed chemical composition				
Lysine	10.1	10.3	10.3	9.7
Methionine	3.6	3.8	3.5	3.3
Cysteine	3.8	3.9	3.8	3.7
Threonine	8.1	8.3	7.6	7.6
Isoleucine	6.4	6.8	6.6	6.4
Leucine	12.6	13.2	12.9	12.5
Valine	8.2	8.5	8.4	7.9
Vitamin E, IU/kg	60	305	483	656

[1]Provided the following nutrients (per kg of air-dry diet): Vitamins: A 4900 IU, D_3 980 IU, E 14 mg, K 0.7 mg, B_1 0.7 mg, B_2 2.1 mg, B_6 1.05 mg, B_{12} 10.5 μg, Calcium pantothenate 7.5 mg, Folic acid 0.13 mg, Niacin 8.4 mg, Biotin 21 μg; Minerals: Co 0.14 mg (as cobalt sulphate), Cu 7 mg (as copper sulphate), Iodine 0.35 mg (as potassium iodine), Iron 42 mg (as Ferrous sulphate), Mn 28 mg (as Manganese oxide), Se 0.21 mg (as Sodium Selenite), Zn 70 mg (as zinc oxide), Antioxident 14 mg. (BJ Grower 1, BioJohn Pty Ltd., WA, Australia).

[2]Promix 50, providing 500 g *dl*-α-tocopheryl acetate per kg product (DSM Nutritional Products Australia Pty Ltd).

Table 2. Main effects of supplemented dietary vitamin E (VE) level and feeding duration (FD) on carcass traits and intramuscular fat (IMF) content in the LTL muscle.

	Vitamin E, IU[1]				Feeding duration, day[2]			
	35	300	500	700	14	28	42	SE
Final weight, kg	88.5	88.0	88.2	89.0	91.4[a]	87.3[b]	86.6[b]	1.2
Carcass weight, kg[13]	60.1	60.2	59.9	60.2	60.9[a]	59.7[b]	59.7[b]	0.5
Dressing, %[13]	67.9	68.1	67.7	68.1	68.8[a]	67.5[b]	67.6[b]	0.6
P2 Backfat, mm[13]	9.5[ab]	8.9[ab]	8.8[a]	10.1[b]	9.3	9.1	9.7	0.6
IMF, %[13]	0.64	0.63	0.62	0.61	0.75	0.50	0.62	0.15

[1]Values are means of 24 observations per treatment.

[2]Values are means of 32 observations per treatment.

[3]Final weight was used as a covariate for statistical analysis.

Table 3. Main effects of supplemented dietary vitamin E (VE) level and feeding duration (FD) on objective meat quality measurements in the LTL.

	Vitamin E, IU[1]				Feeding duration, day[2]			
	35	300	500	700	14	42	SEM	
Colour								
Relative lightness (L*)	55.3	55.3	56.4	54.8	55.8	55.0	0.77	0
Relative redness (a*)	6.17	6.43	6.34	6.81	6.22	6.65	0.349	0
Relative yellowness (b*)	12.96	13.00	12.95	12.95	13.07	12.86	0.246	0
pH								
45 min	6.24	6.34	6.32	6.39	6.31	6.34	0.089	0
24 hours	5.42	5.39	5.41	5.40	5.40	5.42	0.021	0
48 hours[13]	5.44	5.42	5.44	5.45	5.46[a]	5.42[b]	0.020	0
72 hours	5.47	5.46	5.48	5.48	5.50[a]	5.45[b]	0.018	0
Drip loss, %	8.13	8.22	7.44	7.34	7.81	7.76	0.806	0
Cook loss, %	23.0	22.2	24.4	22.7	22.5	23.7	1.08	0
WA Shear force, kg	46.8	45.0	47.8	42.9	47.3	43.9	3.24	0

[1]Values are means of 16 observations per treatment.

[2]Values are means of 32 observations per treatment.

[31]Interaction occurred due to significantly higher pH in pigs fed a 700 IU vitamin E diet for 14 days (5.50) compared with pigs fed a 700 IU vitamin E diet for 42 days (5.39).

Table 4. Performance of pigs fed the experimental diets containing varying concentrations of vitamin E[1].

	Vitamin E, IU					
	35	300	500	700	SEM	*P*=
Pigs fed diets for 14 days before slaughter						
Initial weight	79.6	80.0	80.2	80.3	0.18	0.104
Final weight	90.3	91.8	90.8	92.2	0.70	0.222
ADG, kg	0.76	0.85	0.76	0.86	0.044	0.284
ADFI, kg	2.27	2.27	2.32	2.42	0.076	0.466
FCR, kg/kg	3.03	2.76	3.15	2.85	0.181	0.442
Pigs fed diets for 28 days before slaughter						
Initial weight	62.3	62.5	62.3	62.5	0.19	0.894
Final weight	87.7	87.5	86.0	88.1	1.22	0.641
ADG, kg	0.91	0.89	0.85	0.91	0.042	0.646
ADFI, kg	2.23	2.22	2.21	2.37	0.075	0.429
FCR, kg/kg	2.46	2.50	2.66	2.61	0.087	0.364
Pigs fed diets for 42 days before slaughter						
Initial weight	49.3	49.2	49.2	49.4	0.15	0.766
Final weight	87.4	84.8	87.5	86.7	1.32	0.435
ADG, kg	0.91	0.85	0.91	0.89	0.032	0.474
ADFI, kg	2.15	2.22	2.28	2.25	0.055	0.397
FCR, kg/kg	2.40	2.65	2.51	2.57	0.092	0.316

ADG: average daily gain; ADFI: Average daily feed intake; FCR: feed conversion ratio. Feed intake was not different between treatment.

[1]Values are means of 24 observations.

Table 5. Pearson's correlation coefficients between muscle vitamin E content and objective meat quality measures (*n*=64).

	Muscle vitamin E	Drip loss	Cook loss	Shear force	pH 45 min	pH 24 hours	pH 48 hours
Drip loss	0.010						
Cook loss	0.185	0.181					
Shear force	-0.203	-0.102	0.361**				
pH 45 min	0.159	-0.329**	-0.218	-0.144			
pH 24 hours	-0.111	-0.401**	0.007	0.094	0.115		
pH 48 hours	-0.350**	-0.320*	-0.077	0.159	0.058	0.728***	
pH 72 hours	-0.352**	-0.295*	-0.108	0.116	0.010	0.646***	0.910***

Significance: *P<0.05, **P<0.01, ***P<0.001

Title: **Supra-nutritional vitamin E supplementation for 28 days before slaughter maximises muscle vitamin E concentration in finisher pigs**

Research Highlights

- Vitamin E concentration in the LTL was saturated maximised at 6 mg/kg.
- Feeding a 700 IU vitamin E diet for 28 days saturated maximised LTL vitamin E content.
- Increasing muscle vitamin E suppresses minimised lipid oxidation as measured by TBARS concentration in the LTL.
- IMF content of the LTL is not the cause of variation in muscle vitamin E deposition.
- LTL vitamin E negatively correlated with pHu but positively correlated with redness.