The influence of rate of dietary vitamin E supplementation on the shelf life and retail display quality of Australian Pork
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Report prepared for the Co-operative Research Centre for High Integrity Australian Pork

By

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Executive Summary

This experiment tested the ability that increased muscle vitamin E concentrations had on improving the retail shelf life and storage time(0, 14 and 28 days storage) of pork loin. Thirty-two finisher pigs were fed one of four diets supplemented with increasing vitamin E concentrations (35, 300, 500 and 700IU) for 42 days before slaughter. The loin and plasma vitamin E content increased with increasing level of supplementation. Increasing the muscle vitamin E concentration did not affect growth or any carcass composition, nor did it have an impact on water loss and shear force measurements. The storage time, however did improve the sheer force values in the 14 and 28 day stored products and thus aging pork loin passed 14 days improved tenderness compared to an unaged product.
Increased muscle vitamin E concentrations improved the shelf life of pork loin as measured by TBARS, but in the non-stored product only. Storage past 14 days resulted in a non-significant trend of improved TBARS concentrations with increased vitamin E concentrations. Although this trend was non-significant, adding vitamin E through supplementation seemed to keep the product from reaching the TBARS spoilage threshold even in the long aged product after 6 days of retail display. It is suggested that vitamin E improves shelf life under a threshold mechanism, as to where a level must be reached and no further benefit is observed. In the current experiment muscle vitamin E concentrations were relatively high even in the lowest supplementation level, thus it is suggested that the lack of impact of vitamin E in the long stored product was due to the presence of sufficient vitamin E concentrations. Vitamin E did not seem to improve colour measurements and as a result, it is suggested that colour measurements are not a beneficial measurement for the shelf life of pork.
Increasing levels of intramuscular fat in the muscle resulted in a large increase in the development of TBARS in the long stored product only and thus resulted in a shorter shelf life and reaching the spoilage threshold for TBARS after 4 days of display (28 day stored) at the high end of the intramuscular fat range. This is a significant finding as the level of intramuscular fat is often associated with increased eating quality, and the current loin levels are considered low (range of 0.1-1.9%). Thus if the industry is to increase intramuscular fat to improve eating quality and consistency more investigation is required between the interactions of muscle vitamin E and intramuscular fat during long stored pork product.

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1. Introduction

Extended storage times of fresh meat products are becoming more common and thus the shelf life of these products is an important issue to be addressed by the industry. A product that is considered fresh may come directly from the abattoir, however this is not always of a better eating quality due to the aging process improving tenderness (Hopkins & Thompson, 2001). Extending the shelf life of products will also allow for greater confidence in exported product as well being able to keep the product on retail display for longer. Once the product is placed on retail display the shelf life of that product is determined by the rate of spoilage. In red meat this is easily determined by a change in surface colour from red to brown, however in pork, due to the white nature of muscle fibres, determination of spoilage is through the development of off flavours due to increased lipid peroxidation (Jensen, Lauridsen & Bertelsen, 1998).

Supplementing livestock with vitamin E has shown to be effective in improving the shelf life of meat products, particularly in improving the colour stability of red meat. The levels required to improve the shelf life of red meat products has been extensively documented and was found to be between 2.26-5.3mg/kg in lamb (Álvarez, De la Fuente, Díaz, Lauzurica, Pérez & Cañeque, 2008; Jose, Gardner, Pethick & Jacob, 2008b; Lopez Bote, Daza, Soares & Berges, 2001) and 3.0-3.3mg/kg in beef (Arnold, Arp, Scheller, Williams & Schaefer, 1993; Faustman, Cassens, Schaefer, Buege, Williams & Scheller, 1989). Additionally, the positive effects of vitamin E on colour stability seem to be most apparent in aged products and more oxidative muscle types (Calnan, Jacob, Pethick & Gardner, 2014; Jose, Gardner, Pethick & Jacob, 2008a).

The benefit of vitamin E supplementation on colour stability in pork seems to be unclear, with authors reporting little to no improvement in shelf life as determined by colour, while some authors have reported colour shelf life benefits in *M.longissimus dorsi* of pigs fed vitamin E supplemented diets (O'Sullivan, Byrne, Nielsen, Andersen & Martens, 2003). However the colour stability does not seem to be a limiting factor in the shelf life of pork and lipid peroxidation products are a better indication of spoilage. Vitamin E supplementation in pigs has been shown to decrease lipid peroxidation in pork products (Boler *et al.*, 2009; Houben, Eikelenboom & Hoving-Bolink, 1998; Jensen *et al.*, 1997; Wang, Wang, Shi & Shan, 2012; Zanardi, Novelli, Ghiretti, Dorigoni & Chizzolini, 1999), however this has not been extensively studied in long aged products.

This experiment aimed to investigate the impact of increasing levels of muscle vitamin E and extended storage time on several measures of shelf life in pork *M.longissimus dorsi*. The experimental hypothesis, that Vitamin E will stabilize the shelf life of pork under extended storage periods (14 and 28 days), thus allowing lengthened storage periods, was tested.

2. Methodology

2.1. Experimental design

The experiment tested four levels of vitamin E supplementation, in the form of *dl*-□-tocopherol acetate (35, 300, 500, and 700 IU/kg, DSM), fed over a period of 6 weeks. Thirty-two female Landrace x Large White pigs were acquired from a commercial farm at live weights of 49.3 ± 0.15 kg. Pigs were randomly allocated

across four treatment groups and housed individually in a pen fitted with a nipple drinker and a metal feed trough (n=8/treatment).

Diets were formulated to contain 14.0 MJ DE and 0.6 g standardized ileal digestible lysine/MJ DE. Actual vitamin E content was 60, 305, 483 and 656 IU for the 35, 300, 500 and 700IU treatments. The pigs were fed these diets 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 weeks 0, 2, 4 and 6 weeks to measure plasma vitamin E content. Blood samples were collected from the jugular vein into a heparinized tube and were immediately centrifuged at 2000 *g* for 15 minutes at 4 $^{\circ}$C. Plasma samples were then stored at -20 $^{\circ}$C until chemical analysis was performed. At the end of the supplementation period, all pigs were slaughtered in a commercial abattoir and carcass composition was recorded. Hot carcass weight was measured based on AUSMEAT trim 13 (head off, flare off, fore trotters off, hind trotters on). Dressing percentage was calculated as the ratio between live weight and hot carcass weight. P2 back fat depth was measured 65 mm from the dorsal midline and level with the posterior edge of the last rib of the left side of each carcass. At 24 hour post-slaughter 2 kg of *longissimus dorsi* (*LD*) muscle samples were collected between the 10th and 15th ribs in the chiller. The samples were divided into 3 equal proportions and randomly allocated to one of the 3 storage treatments (0, 14 and 28 days). Samples were vacuum packaged and stored at 2-4^{o}C for the respective period.

Once storage was complete, 2 x 2cm thick steaks were cut, visible fat removed, and placed on black Styrofoam trays. Samples were allowed to bloom for 30 minutes at 4^{o}C before being 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 second day for 6 days on one of the steaks. At the same time a small 5g sample was removed from the other steak, vacuum packaged and frozen until Thiobarbituric acid reactive substances (TBARS) were measured.

2.2. **Objective measures**

Muscle pH was determined at 45 min, 24, 48 and 72 hours after slaughter in the LD. 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 LD 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).

Meat colour was measured 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}. L*, a* and b* system were recorded (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)) as well as the reflectance values from 400 to 700nm. The hue angle was calculated by the following equation

Hue angle = [arctangent (b*/a*)]

The percentage metmyoglobin was calculated by first converting reflectance (R) converted to reflex attenuance (A) by the following equation

$$A = \log(1/R)$$

Where R = reflectance at a specific wavelength expressed as a decimal rather than a percentage (i.e. 0.3 rather than 30%).
The percentage metmyoglobin could then be calculated using the following equation

$$\%MMB = 1.395 - \left\{\left[\frac{(A572-A730)}{(A525-A730)}\right]\right\} \times 100$$

Drip loss was determined at 24 hours after slaughter (0 day stored samples only) 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.
Purge loss was measured in the 14 and 28 day stored samples only. Proportions were pre-weighed before being vacuum packed and stored. Once the storage period had finished samples were removed from the vacuum bag and towel dried and re-weighed. The percentage difference between the two weights was determined as the purge loss.

To determine cooking loss and prepare the samples for Warner-Bratzler shear force (WBSF), 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°C until cooking. It's been reported that moisture loss between fresh and frozen meat samples are not statistically different (Leygonie et al., 2012). 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 minutes. Samples were dried and weighed to determine cook loss (expressed as a percentage of weight lost due to cooking) and then stored at 4°C for 24 hours. From each sample, 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.

2.3. Chemical measurements

Intramuscular fat content was measured using the Ankom method for measurement of crude fat (extraction of crude fat using petroleum ether).
Alpha tocopherol content in the feed and meat was measured using the method of McMurray, Blanchflower and 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). Alpha tocopherol content in the plasma sample was analysed using the method of McMurray and 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 ºC 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 *et al.*, 1998)at 510 nm excitation and 560 nm emission using a POLARstar Omega plate reader (BMG Labtech Pty. Ltd. Mornington, Victoria).

2.4. Data analysis

The software package SAS® was used for all statistical analyses (SAS Institute, 2001). Data were analysed using a linear mixed effects model. In the first analysis, the plasma/carcass/slaughter data were analysed using dietary treatment as a fixed effect. The analysis of drip loss, purge, cook loss and shear force used dietary treatment and storage time as fixed effects, while the remaining analysis of shelf life used day (on retail display) and storage time as a fixed effects and muscle vitamin E as a continuous effect. The overall effect of storage time for each measurement of shelf life was significant and thus the analysis was broken down into the aging treatments and each result will be discussed based on the aging group with muscle vitamin E concentration as a covariate. Additionally, the IMF concentration was used as a covariate for the analysis of TBARS.

3. Outcomes

3.1. Tissue Vitamin E levels

Increasing the level of dietary vitamin E supplementation increased the plasma vitamin E concentration (figure 1; P<0.01). The plasma vitamin E concentration increased with time on feed for all supplementation rates other than 35 IU in which no increase was observed over the 42 day feeding period (figure 1). With all supplemented groups there was very little increase in plasma concentration after 14 day supplementation.

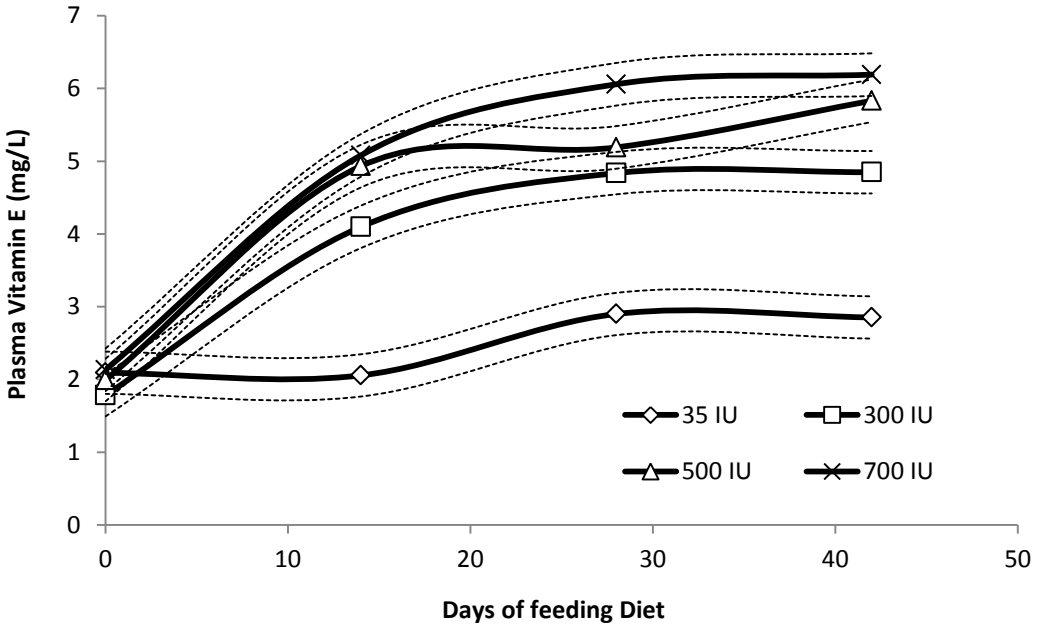

Figure 1: The plasma vitamin E content over time during supplementation with ¤-tocopherol acetate at different rates for 42 days.

Muscle vitamin E concentrations increased with increasing rate of supplementation (table 1; P<0.0001). After 6 weeks of supplementation, the 700IU group reached a mean muscle concentration of 6.18mg/kg in the LD compared to only 3.24mg/kg in the 35IU treatment group. The muscle vitamin E concentrations for all groups combined ranged from 2.59 to 8.06 mg/kg with an overall mean of 5.06 ±0.24 mg/kg.

3.2. Carcass measurements

The carcass and performance measurements are given in Table 1. There was no effect of dietary treatment on any carcass measurements other than the pH at 24 hours post mortem. The pH at 24 hours for the 700IU treatment was lower than other treatments (P<0.05), however no other pH measurements differed between dietary treatments. Across all treatments the mean pH 24, 48 and 72 hours was 5.42± 0.011, 5.42± 0.01 and 5.45±0.009 respectively.

The different dietary levels did not influence the growth rates, daily gains and feed conversion rates of the animal. Additionally, there was no difference in fat depth, carcass weights and dressing percentage. The mean intramuscular fat content was 0.61% ±0.09 with a range of 0.1 to 1.9%.

3.1. Objective measurements

There were no significant effects of increasing muscle vitamin E concentration on improving objective measures of water holding capacity (Table 2). Drip loss was not improved when muscle vitamin E was increased in the short stored products, while there was also no effect of vitamin E in purge loss in the 14 or 28 days stored products. Additionally, there was no effect of vitamin E on cooking loss or shear force values. However the length of storage time reduced the shear force values ($P<0.001$; Table 2) indicating improved tenderness. Samples stored for 14 days required 37 % (16.2N) less force to shear through compared to the 0 day stored samples. Samples stored for 28 days required 26% (4.7N) less force than the 0 day stored samples, thus tenderness did not improve pass 14 day of storage.

3.1. Shelf life measurements

The effects of vitamin E on shelf life measurements are given in Table 3.

TBARS increased with days on retail display ($P<0.001$) and at a greater rate in the product stored for 14 and 28 days. Increasing vitamin E content decreased the TBARS concentration in the 0 day-stored product by 0.02g MDA eq./kg for every 1mg/kg of vitamin E in the muscle ($P<0.001$; figure 2). However, there was no significant effect of increased vitamin E in the meat stored for 14 and 28 days (Figure 2). When adjusted for IMF content no products reached the off-flavour threshold (0.5 MDA eq. /kg) even in the 28 day stored product displayed for 6 days.

There was no effect of increasing IMF concentration on TBARS in 0 day stored product (figure 3). However an increase of 1% IMF increased the production of TBARS by 0.085 and 0.12g MDA eq. /kg for the 14 and 28 day stored products, respectively (Figure 3). This was despite a small range in IMF content (0.1 to 1.9%). As a result under long-stored (28d) conditions the higher IMF pork samples had reached the off-flavour threshold (0.5 MDA eq. /kg) after 4 days of retail display (figure 3).

Greater vitamin E concentrations resulted in a higher a* value (more red; figure 4) and increased metmyoglobin percentage (Figure 5) in the 0 day stored product only ($P<0.01$). No other measurement was influenced by vitamin E concentration at any storage treatment (Table 3). However, the hue did trend towards becoming redder with increasing dietary vitamin E supplementation (figure 6). Samples would initially become more red (higher a* value; lower hue) from day 0 to day 2, after which the redness would decrease with days on display ($P<0.01$; figure 4 and 6). This was also observed with the b* value as yellowness peaked at 2 days of display but decreased thereafter (figure 7). Percentage metmyoglobin increased as

display period increased, while the product got lighter (increased L* value, figure 8).

Increased storage time resulted in increased L* values, hue and percentage metmyoglobin while the a* value decreased (P<0.01). After 28 days of storage the percentage metmyolglobin exceeded 40% after 6 days on display (figure 5). The b* value was unaffected by storage other than a lower b* value in the day 0, 0 day stored product.

Table 1 Effects of dietary Vitamin E level on carcass measurements, growth performance and postmortem pH and muscle vitamin E content in the *Longissimus dorsi*

	Vitamin E, IU					Statistics		
	35	*300*	*500*	*700*	*SEM*	*NDF;DDF*	*F value*	*P value*
Final weight, kg	87.43	84.75	87.53	86.73	1.86	3;28	0.48	0.698
ADG, kg/day	0.89	0.84	0.91	0.9	0.04	3;28	0.88	0.465
Feed conversion rate	2.40	2.65	2.51	2.57	0.118	3;28	0.77	0.519
Carcass weight, kg	58.95	58.45	59.34	58.13	1.21	3;28	0.2	0.898
Dressing, %	67.47	69.02	67.79	67.03	0.57	3;28	2.21	0.109
P2 Back fat, mm	9.75	9.5	9.25	10	0.56	3;28	0.34	0.798
Intramuscular fat, %	0.5	0.66	0.75	0.51	0.19	3;28	0.39	0.761
pH								
45 min	6.30	6.31	6.33	6.41	0.042	3;92	1.3	0.280
24 hours	5.43[a]	5.42[a]	5.44[a]	5.39[b]	0.012	3;92	3.61	0.016
48 hours	5.43	5.42	5.44	5.40	0.011	3;92	2.58	0.058
72 hours	5.45	5.46	5.47	5.43	0.011	3;92	1.76	0.160
Vitamin E, mg/kg	3.24[a]	5.11[b]	5.72[bc]	6.18[c]	0.28	3;28	21.84	<.0001

Superscript letters represent differences between treatments

Table 2 Effects of dietary Vitamin E level and storage time on moisture loss and Warner Bratzler shear force measures in the *Longissimus dorsi*

	Vitamin E, IU					Storage Time , Days				Statistics	
	35	*300*	*500*	*700*	*SEM*	*0*	*14*	*28*	*SEM*	*VE*	*ST*
Drip loss, %	7.75	8.16	7.54	7.58	0.817		Na			0.947	na
Cook loss, %	25.60	22.12	23.64	22.69	1.546	25.80	22.29	22.46	1.339	0.4077	0.1182
Purge loss %	8.74	6.97	8.78	8.05	0.631	na	8.13	8.14	0.446	0.156	0.9984
WB Shear force, N	36.31	31.43	36.04	34.90	1.477	43.91[a]	27.70[c]	32.41[b]	1.279	0.0824	<.0001

Superscript letters represent differences between treatments

Table 3 Effects for shelf life measurement of TBARS and colour. Interacted with days on retail display, muscle vitamin E concentration and IMF content (TBARS analysis only)

		Day of retail display			Muscle vitamin E			Intramuscular fat		
Measure	*Storage*	*NDF;DDF*	*F value*	*P value*	*NDF;DDF*	*F value*	*P value*	*NDF;DDF*	*F value*	*P value*
TBARS	**0**	3;118	80.79	<.0001	1;118	19.56	<.0001	1;118	0.17	0.6806
	14	3;118	40.2	<.0001	1;118	2.48	0.1182	1;118	19.8	<.0001
	28	3;119	11.09	<.0001	1;119	0.29	0.5886	1;119	7.52	0.0071
L	**0**	3;119	6.06	0.0007	1;119	0.03	0.8537			
	14	3;120	16.23	<.0001	1;120	0.05	0.8157			
	28	3;121	7.38	0.0001	1;121	0.29	0.5941			
a*	**0**	3;119	46.9	<.0001	1;119	8.02	0.0055			
	14	3;120	26.35	<.0001	1;120	0.36	0.5501			
	28	3;121	69.8	<.0001	1;121	1.82	0.1801			
b*	**0**	3;119	66.67	<.0001	1;119	1.5	0.2227			
	14	3;120	6.11	0.0007	1;120	1.25	0.2664			
	28	3;121	18.92	<.0001	1;121	0.2	0.6545			
Hue	**0**	3;119	15.4	<.0001	1;119	2.93	0.0896			
	14	3;120	20.56	<.0001	1;120	0.17	0.6837			
	28	3;121	82.38	<.0001	1;121	3.43	0.0666			
%MMB	**0**	3;119	232.13	<.0001	1;119	18.54	<.0001			
	14	3;120	161.23	<.0001	1;120	0.2	0.6537			
	28	3;121	137.99	<.0001	1;121	0	0.9831			

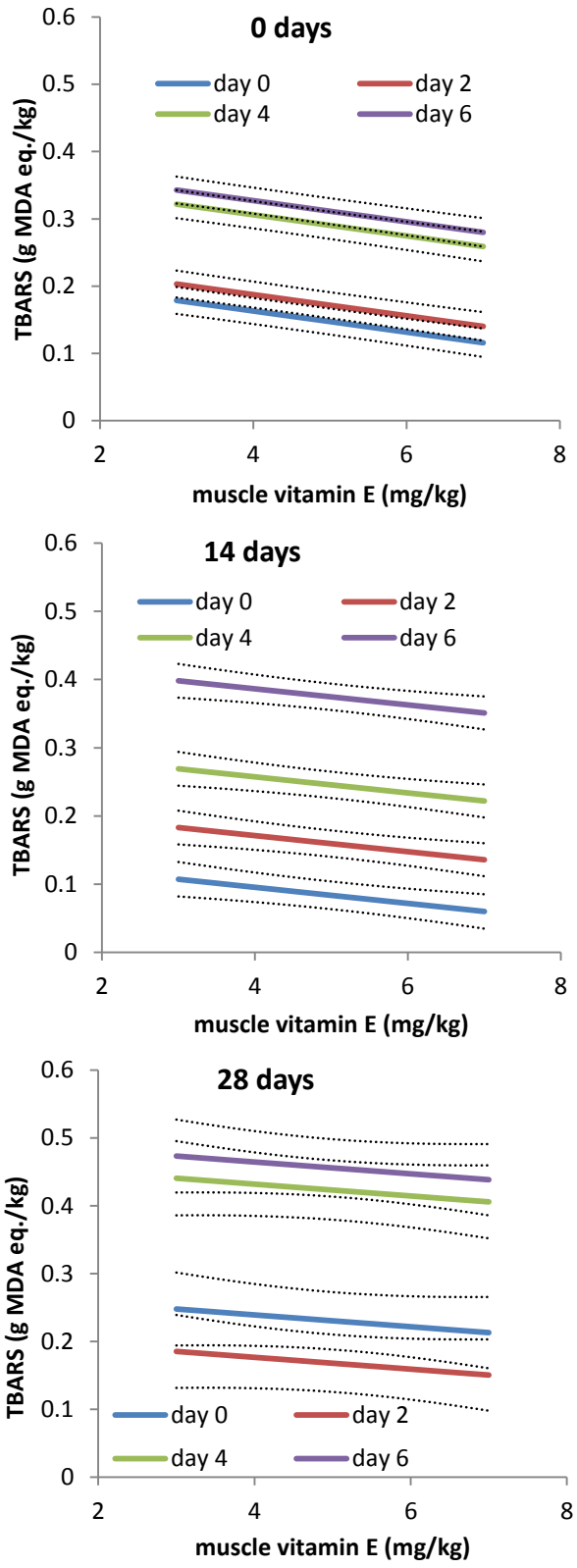

Figure 2. The impact of muscle vitamin E concentration on the production of TBARS in 0, 14 and 28 day stored loin during days of retail display ± SEM.

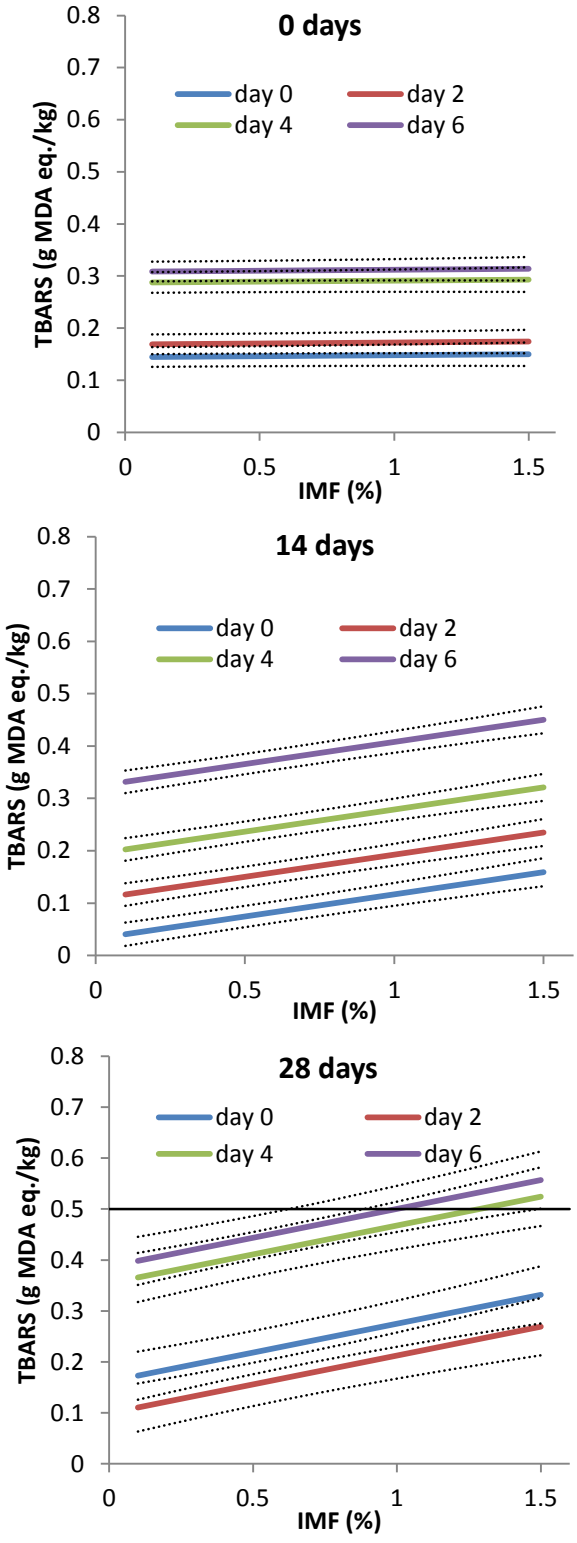

Figure 3. The impact of intramuscular fat on the production of TBARS in 0, 14 and 28 day stored loin during days of retail display ± SEM. The horizontal line at 0.5 is the TBARS threshold.

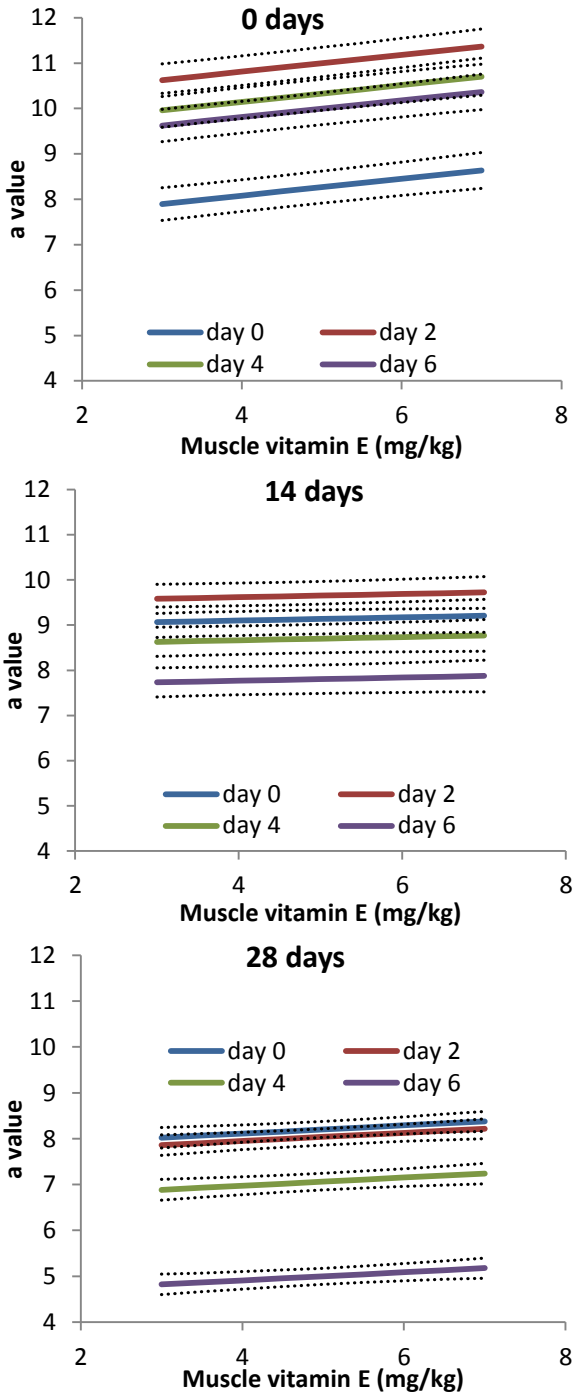

Figure 4. The impact of muscle vitamin E concentration on the a*value in 0, 14 and 28 day stored loin during days of retail display ± sem.

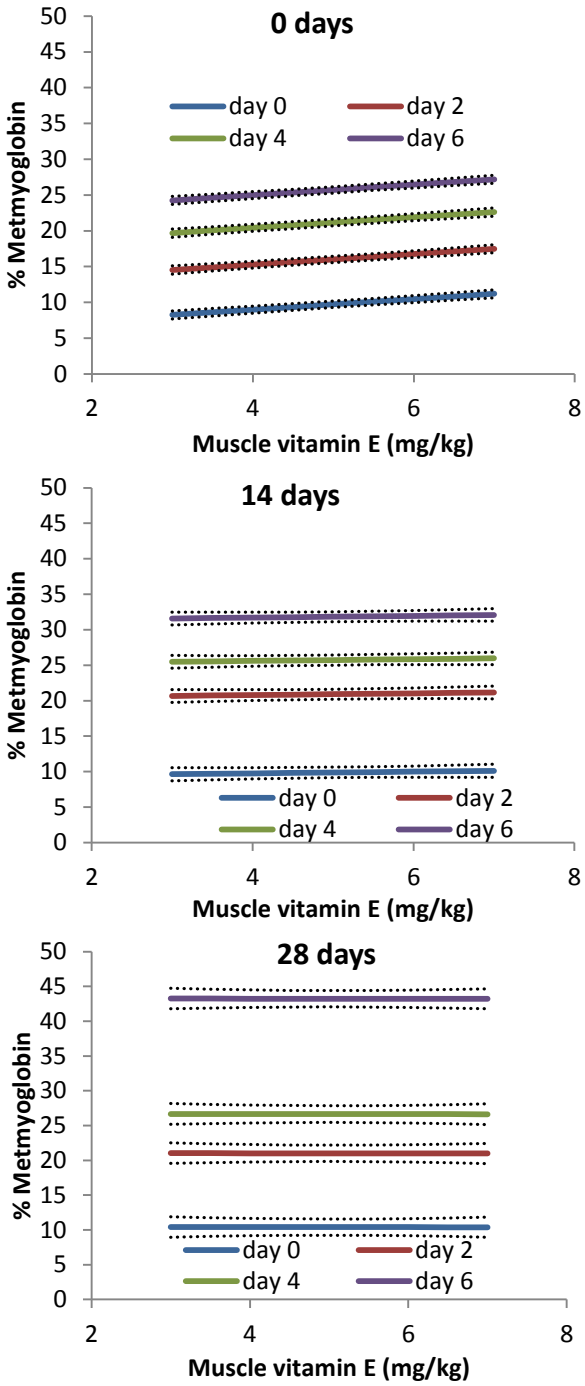

Figure 5 The impact of muscle vitamin E concentration on the percentage metmyoglobin in 0, 14 and 28 day stored loin during days of retail display ± SEM.

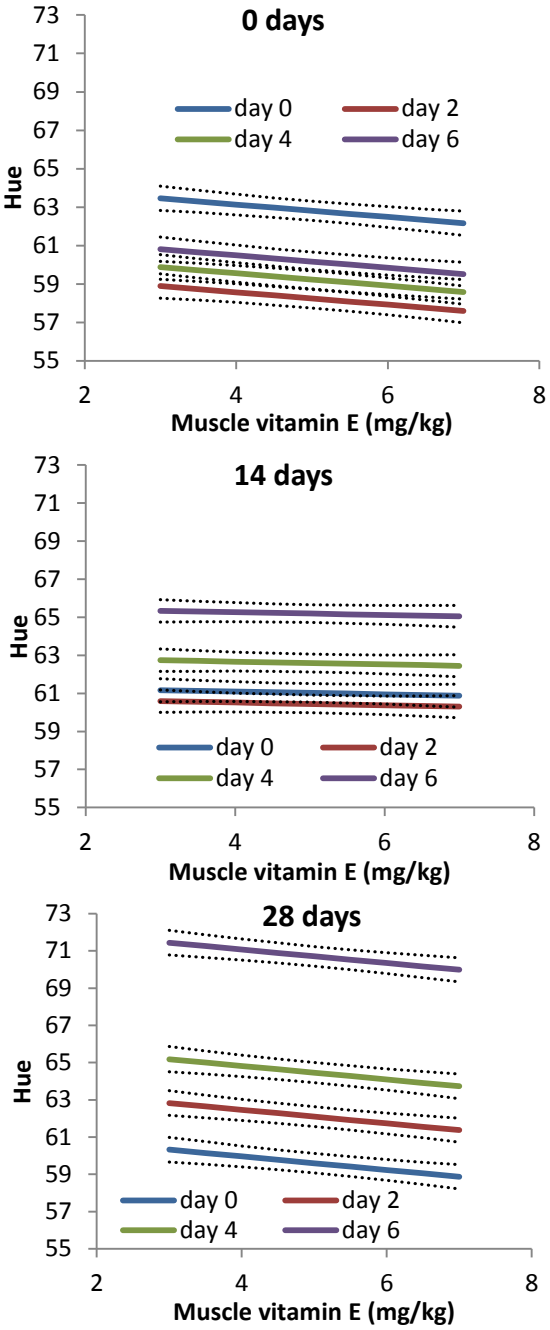

Figure 6 The impact of muscle vitamin E concentration on the Hue in 0, 14 and 28 day stored loin during days of retail display ±SEM.

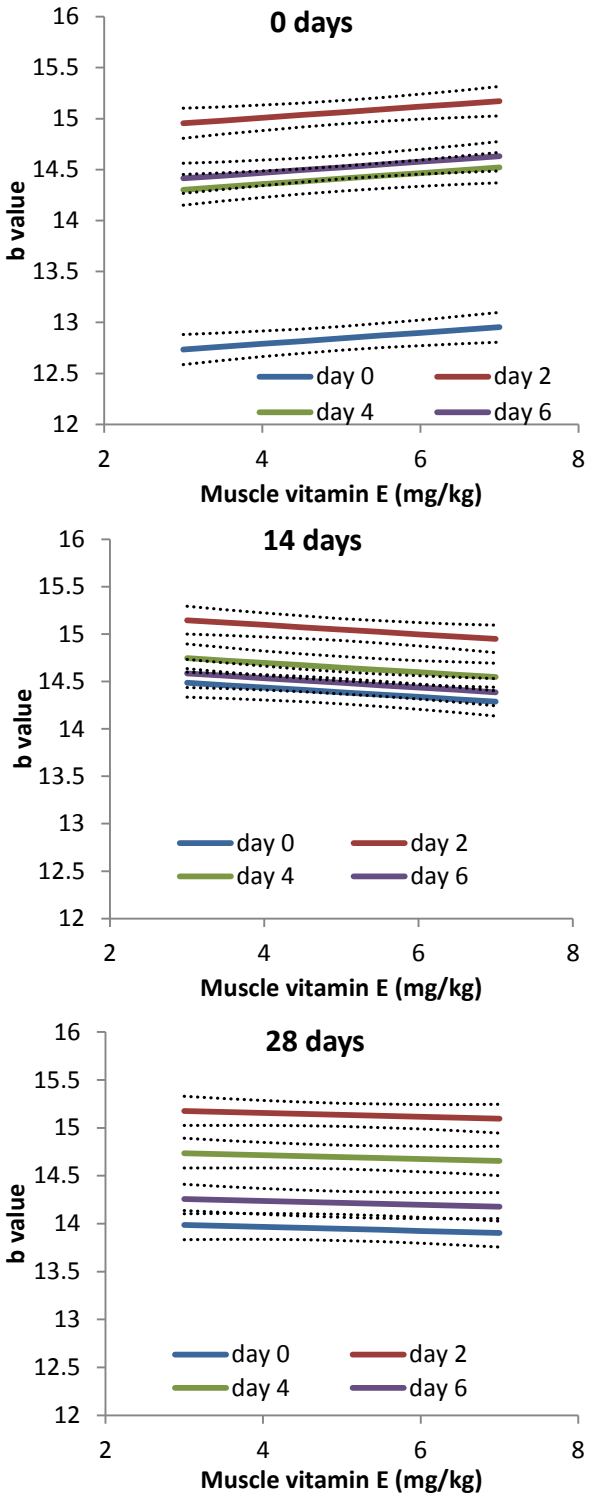

Figure 7 The impact of muscle vitamin E concentration on the b* value in 0, 14 and 28 day stored loin during days of retail display ±SEM.

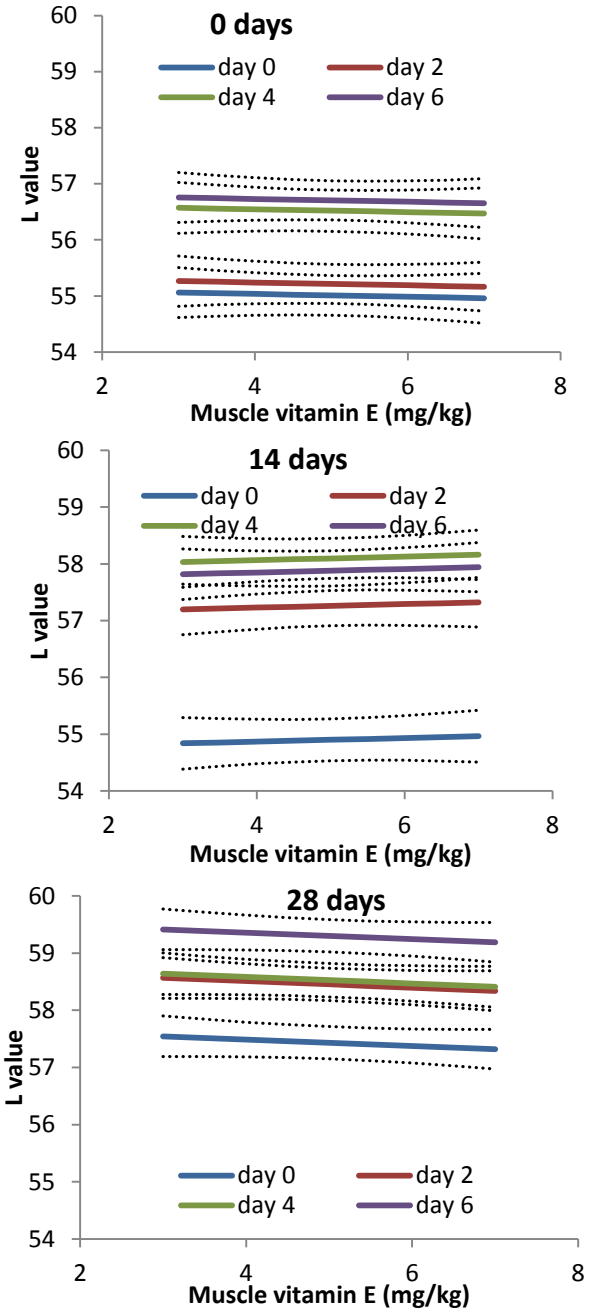

Figure 8. The impact of muscle vitamin E concentration on the L* value in 0, 14 and 28 day stored loin during days of retail display ±SEM. A higher L value means increased lightness.

4. Application of Research

Increased storage time of pork loin muscle was associated with an increase in the development of TBARS. Although increasing the muscle vitamin E concentration decreased the development of TBARS in the samples that were not subjected to extended storage. The fact that there was no relationship between muscle vitamin E concentration and TBARS in the 14 and 28 day stored product disproves the hypothesis tested. However across the range of vitamin E tested (adjusted for IMF), no samples reached the off-flavour cut-off point even in the long stored product on display for 6 days. When considering vitamin E/shelf life literature from other species, the current muscle vitamin E concentrations might have been too high to warrant a difference along the continuum. Several authors have described a threshold concentration of vitamin E that once reached no further benefit of vitamin E on shelf life will be observed. These thresholds vary between authors but in lamb it is stated to be between 2.26-5.3mg/kg (Álvarez *et al.*, 2008; Lopez Bote *et al.*, 2001) while in beef 3.0-3.3mg/kg is required (Arnold *et al.*, 1993; Faustman *et al.*, 1989). As far as the authors are aware no threshold has been described for pork muscle, and thus it is possible that the current range limited the observed effect of vitamin E, thus it is still likely that Vitamin E supplementation should be considered for product that will have extended storage times. If this is the case, muscle vitamin E concentrations as high as the current study would not need to be reached and thus costs of supplementation would be considerably lower. Our results also suggest the time of feeding higher levels of Vitamin E can be reduced. Currently, the NRC (2012) recommend that finisher diets for pigs contain 11 IU/kg of feed, a value almost 50IU lower than what was measured in the lowest diet in this study. In this case, it is possible a greater effect of vitamin E on shelf life would be observed.

Increasing IMF concentration, across the range tested, proved to have a significant impact on the shelf life of long stored pork loin. At the higher end of the IMF range the product would reach the off-flavour threshold after only 4 days on display when stored for 28 days, when adjusted for muscle vitamin E concentrations. When considering the trend of vitamin E to decrease the production of TBARS, it is likely that this effect of IMF could be worse if vitamin E concentration was at a minimum. Furthermore, eating quality is improved as IMF is increased (Huff-Lonergan, Baas, Malek, Dekkers, Prusa & Rothschild, 2002), however the concentrations in the current study are still considerably low. The findings have implications if genetic selection for increased IMF is applied and therefore measures would need to be incorporated to manage the detrimental effects on shelf life. Although vitamin E was not currently shown to stabilize long stored pork loin most likely because vitamin E content was not limiting, the interactions between vitamin E and IMF in long stored products needs further investigation and incorporation of vitamin E in the diet is likely necessary in long stored pork.

There was very little effect of vitamin E on any colour measurements in the current study, other than an increased redness (a*) in the 0 day stored products and an increased %MMB. Vitamin E is well known to increase redness in red meat products but in the current study any extended storage of the product diminished this effect. However, it may be important to consider an increased redness as this may have implications for certain export markets. An increase in the % MMB is currently not understood, however, because it was only apparent in the 0 day stored product and that the browning of pork is not currently a limiting factor on

the shelf life of pork, and increased %MMB is not likely an important finding. All other colour parameters were not affected by vitamin E or increased IMF concentration. This is in support of previous literature that vitamin E had no effect on colour in pork (Houben *et al.*, 1998; Jensen *et al.*, 1997; Zanardi *et al.*, 1999), and thus colour is not likely to be a good indicator of shelf life in pork.

Supplementing with vitamin E had no impact on animal performance or on water holding capacity and tenderness. Although, supplementing with vitamin E did not improve these aspects, it also did not decrease the quality. Previous literature has shown that vitamin E supplementation can improve drip loss (Asghar *et al.*, 1991), however the lack of effect may also be due to no samples being considered low in muscle vitamin E.

Current research by the Pork CRC has highlighted a lack of aging on the eating quality of pork. In this study shear force value was reduced 37% when the product was stored for 14 days or more. No further improvement was observed when aged greater than 14 day. The improvement observed would likely have a significant effect on tenderness. However, because no further aging was observed past 14 days storage, it isn't possible to say if all 14 days is required to optimize the aging process. It is suggested that increased pH declines effectively "knock out" the activity of enzyme responsible for the aging process. Although there is not extensive data presented for pH declines in the current data set, the values of the pH measurements at 45 minutes suggest that the pH decline was not excessively rapid. However further investigation into the rates of decline, what is causing them and how this may affect the aging process would seem warranted

5. Conclusion

Although vitamin E did not stabilize the long stored product against development of TBARS, the current study indicates that more investigation into vitamin E supplementation is required. It is still not understood if a threshold level for vitamin E exists in pork muscle, which would help manage supplementation rates, nor is it understood if an impact of vitamin E would have been observed if lower concentrations were present in the current study. Thus further study would be warranted. Additionally, the interaction of IMF and vitamin E during storage needs to be investigated. Increasing IMF could be an important factor for the Australian pork industry, as it is associated with improved eating quality. If IMF has such a great impact on the shelf life in pork, then this needs to be further investigated. Currently, a high IMF product stored for 28 days will have a retail shelf life of no greater than 4 days. This is not necessarily troublesome, compared to the shelf life of some red meat products.

6. Limitations/Risks

There is no risk associated with these outcomes

7. Recommendations

It is currently difficult to recommend a supplementation rate for vitamin E as there are still a number of unknowns that warrant further investigation. The first is the possible interactions between muscle vitamin E concentrations and increased IMF content. Increasing IMF should be an important target for the Australian pork industry, if it is going to focus on improved eating quality. However, this may limit the amount of time the product can be stored. The storage of product is extremely important for the export and even domestic markets as longer transport times generally come at a cheaper cost. Thus it is imperative that once the product reaches the market it still maintains a high quality and shelf life. The impact of elevated IMF levels on this is not known, and if vitamin E supplementation will have a positive impact. Thus, in this case, further studies structured around ranges of IMF are necessary.

At the current rates of supplementation, there are no detrimental effects on the meat quality or animal performance. However, the current range in muscle vitamin E was not great enough to statistically decrease the production of TBARS in the longer stored products. This could mean there was no effect of vitamin E, or that the low treatment rate was enough to facilitate an effect. Thus currently it would be suggested that finisher pigs be supplemented with at least 35IU of vitamin E per kg of feed until further research is carried out.

8. References

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