

Meta-analysis of effects of dietary vitamin E and post slaughter storage conditions on changes of redness (a^*) of pork

LASZLO TREFAN¹, LUTZ BÜNGER¹, JOHN ROOKE¹, JESPER BLOM-HANSEN², BTISSAM SALMI³, CATHERINE LARZUL³, CLAUDIA TERLOUW⁴ and ANDREA DOESCHL-WILSON⁵

¹Sustainable Livestock Systems Group, Scottish Agricultural College, King's Buildings, Edinburgh, UK, ²Danish Meat Research Institute, Roskilde, Denmark, ³Station de Génétique Quantitative et Appliquée, Institut National de la Recherche Agronomique, Jouy-en-Josas, France, ⁴UR 1213 Herbivores, Institut National de la Recherche Agronomique Genès, St. Genès Champanelle, France, ⁵The Roslin Institute, University of Edinburgh, UK

Abstract

A meta-analysis was carried out to quantify the effects of dietary vitamin E and storage conditions on colour changes of pork from *M. longissimus dorsi*. After standardisation procedures, redness of pork (CIE colour specification a^*), one of the most important objective colour attributes, was used as an indicator for colour changes in this analysis. The analysis was based on results from five experiments, which met selection criteria. Analysis of changes of other objective colour attributes, lightness (L^*) and yellowness (b^*) was not possible due to lack of published data.

The statistical analysis (using mixed models) found significant effects of tissue α -tocopherol concentration in *M. longissimus dorsi*, simplified supplemented vitamin E levels as well as storage time and storage light on redness of pork and its changes over time. The relationship between redness and α -tocopherol concentration was found to be linear, and between redness and storage time was non-linear (third degree polynomial) in one model. This model suggested that an increase of 1 μg of α -tocopherol in the muscle led to an expected increase a^* value of 0.11. Another model identified significant interactions about 0.28 between α -tocopherol concentration and storage time in late storage periods. A third model found a significant difference of -0.48 between predicted a^* values at lower (≤ 50 IU/kg feed) and higher supplemented vitamin E levels (≥ 100 IU/kg feed). The models predicted an initial increase for 3 days, a stable period for 5 days and then a decrease for a^* values over storage time. The a^* values were significantly lower by about 1.4 when samples were exposed to light in the models, the effect of light found to be constant over time.

Further studies, carried out with standardized methods, are needed to increase the predictive power of the derived models and to validate the models for other muscles.

Keywords: pig, Vitamin E, α -tocopherol, colour, redness, pork quality, meta analysis

Zusammenfassung

Metaanalyse zur Prüfung des Einflusses von Vitamin E Futterzusatz und Fleischlagerung auf die Veränderung der Fleischfarbe beim Schwein

Um die Effekte von Vitamin E als Futterzusatz und der Lagerungsbedingungen auf die Farbstabilität von Schweinefleisch (*M. longissimus dorsi*) zu bewerten, wurde eine Metaanalyse durchgeführt. Nach der notwendigen Datenstandardisierung wurde die Fleischfarbe (a^*) als eine der wichtigsten objektiv messbaren Fleischeigenschaften ausgewählt und der Metaanalyse unterzogen. Die Analyse basierte auf Ergebnissen von fünf Experimenten, die gewisse Auswahlkriterien erfüllten. Die Analyse der Veränderung anderer objektiver Fleischfarbmerkmale wie Helligkeit (L^*) und Gelbverfärbung (b^*) war auf Grund mangelnder Daten nicht möglich.

Die statistische Analyse mit dem Mixed Modell zeigte signifikante Effekte der akkumulierten α -tocopherol Konzentration im Fleisch, der zugeführten Vitamin E Dosierung, sowie der Lagerzeit und der Lichtintensität während der Lagerung auf die Dynamik der Veränderung der Fleischfarbe. Die Abhängigkeit von der α -tocopherol Konzentration war linear und die von der Lagerzeit war nicht-linear (beschrieben durch ein Polynom dritten Grades). Laut dieses Modells entspricht eine α -tocopherol Erhöhung von 1 μg einer Erhöhung des a^* Wertes um 0.11. Ein weiteres Modell identifizierte signifikante Interaktionen zwischen der α -tocopherol Konzentration und der Lagerzeit. Ein drittes Modell fand einen signifikanten Unterschied von -0.48 in den a^* -Modellwerten, entsprechend einer niedrigen (≤ 50 IU/kg feed) oder einer hohen (≥ 100 IU/kg feed) Dosierung von Vitamin E Zusatz. Die Modelle berechneten eine Erhöhung des a^* -Wertes in den ersten drei Tagen nach der Schlachtung, gefolgt von einer stabilen Periode für etwa fünf Tage, und einer Verringerung über den restlichen Zeitraum.

Lichtverhältnisse während der Lagerung hatten einen signifikanten Einfluss auf die Fleischfarbe (a^*). Lagerung bei Licht reduzierte a^* um ca. 1.4. Die zeitliche Veränderung war nicht beeinflusst. Weitere Untersuchungen mit standardisierten Methoden wären von großem Nutzen für die Modell Validierung und um die Schätzgenauigkeit der abgeleiteten Modelle zu erhöhen.

Schlüsselwörter: Schwein, Vitamin E, α -tocopherol, Fleischfarbe, Röte, Schweinefleischqualität, Meta-Analyse

Introduction

Visual appearance is an important characteristic of meat quality, as it strongly influences the consumer's purchase decision. From the various factors which contribute to the visual appearance of meat, colour is the main factor that affects the acceptability of meat products (FAUSTMAN and CASSENS 1990). The rate of discolouration of meat is related to several factors including ultimate pH, storage temperature, lipid oxidation and illumination (FAUSTMAN and CASSENS 1990). To maintain acceptable fresh meat colour over prolonged periods of time, it is necessary to delay pigment oxidation and/or enhance reduction of oxidized myoglobin (GRAY *et al.* 1996). The supplementation of feed with vitamin E, a well known antioxidant was found to significantly reduce the oxidation process (BUCKLEY *et al.* 1995). For beef, vitamin E

supplementation resulted in an extension of retail display life from 1.6 to 5 days without compromising microbiological quality and similar trends have been reported for pork products (GRAY *et al.* 1996). There is strong evidence from various studies and review papers that dietary vitamin E supplementation decreases lipid oxidation in pork (BUCKLEY *et al.* 1995, LAHUCKY *et al.* 2000, PETTIGREW and ESNAOLA 2001, ROSENVOLD and ANDERSEN 2003, DUNSHEA *et al.* 2005, LAHUCKY *et al.* 2005, DIKEMAN 2007). However, results from individual studies on the effect of vitamin E on pork colour changes are ambiguous (ROSENVOLD and ANDERSEN 2003). While some papers have reported improved colour stability with vitamin E supplementation (e.g. ASGHAR *et al.* 1991, MONAHAN *et al.* 1992), several others found no significant effect (e.g. CANNON *et al.* 1996, JENSEN *et al.* 1997, PHILLIPS *et al.* 2001, KRŠKA *et al.* 2001). To resolve this ambiguity and to determine quantitatively whether vitamin E influences colour changes in pork using a cross-experimental approach, meta-analysis may be used.

In animal science, meta-analysis has recently been described as a useful statistical tool to objectively integrate the results of individual experiments and to establish general response laws that are valid over a range of conditions (ST-PIERRE 2001, PHILLIPS 2005 and SAUVANT *et al.* 2008).

The aim of this paper was to use meta-analysis to quantify the effects of different dietary vitamin E levels and post slaughter storage conditions (e.g. storage time, storage light) on the changes of pork colour during storage. This paper focuses on the analysis of redness (a^*) as an attribute of objective colour measurement of pork using the CIE L^*, a^*, b^* colour system (CIE 1976).

Material and methods

Data sourcing and construction of meta-analysis datasets

The literature search used published information written in English from 1970 to 2009, from various scientific sources including journals, conference proceedings, book articles and abstracts from various electronic databases. Indices used in the search were combinations of the terms ›vitamin E‹, ›alpha-tocopherol‹, › α -tocopherol‹, ›pork‹, ›dietary‹, ›pig‹, ›swine‹, ›hog‹ and plurals of these. In some cases, authors were contacted directly to complete missing information.

The literature research identified 60 initial references, related to the effect of dietary vitamin E supplementation on pork and pigs. Some studies reported aspects of dietary vitamin E utilization (e.g. blood α -tocopherol concentration), which were not relevant to the analysis and were therefore excluded. Since this study focused purely on the antioxidant effect of vitamin E, references using additional treatments which could possibly interfere with or modify the antioxidant effect of the vitamin E were also excluded. This was the case for experiments using diets with a fat to dry matter (DM) ratio of more than 5%, as this may change saturated and unsaturated fatty acid ratio in pork making it more susceptible to oxidation (BUCKLEY *et al.* 1995). Experiments using diets containing in addition to vitamin E other antioxidants (e.g. vitamin C) or minerals (e.g. Se) above the normal maintenance level, which may interact with the vitamin E (OLDFIELD 2003), were also excluded. These selection criteria together with the standardisation criteria outlined below led to a drastic reduction of

acceptable studies and the following references remained for inclusion in the meta-analysis (LANARI *et al.* 1995, DIRINCK *et al.* 1996, HOVING-BOLINK *et al.* 1998, PHILLIPS *et al.* 2001 and VAN HEUGTEN *et al.* 2002). Table 1 summarizes experimental details of these studies.

Table 1

References used for meta-analysis of changes of redness (a*) of pork

Literaturquellen, die für die Meta-Analyse der Veränderung der Fleischfarbe (a) verwendet wurden*

No. of pigs/ treatment group	Vitamin E levels (IU)*	Pig breed	Gender	Length of supplem., days prior to slaughter	Reference
24	50/200	Danis	gilt	105	DIRINCK <i>et al.</i> 1996
36/35	0/200	Y×(FL×DuL)	barrow & gilt	84	HOVING-BOLINK <i>et al.</i> 1998
4/4	0/200	0.5D×0.25LW×0.25L	gilt	105	LANARI <i>et al.</i> 1995
10	50/200	Y×Y	boar & gilt	42	PHILLIPS <i>et al.</i> 2001
48	0/100/150/300/600	Hybrid	barrow & gilt	42	VAN HEUGTEN <i>et al.</i> 2002

*Supplementary vitamin E levels rounded and expressed in IU/kg feed. Danis published in this form, no further information is available, Y×(FL×DuL) Yorkshire × (Finnish Landrace × Dutch Landrace), 0.5D×0.25LW×0.25L composite breed of 50 % Duroc × 25 % Large White × 25 % Landrace, Y×Y Yorkshire crossbred

Experimental data were entered into a database that had been constructed according to the conceptual and structural requirements for meta-analysis outlined by VERNET and ORTIGUES-MARTY 2005. The database includes a detailed description of all relevant references (e.g. type, name and date of publication, title, author names), animals used in the experiments (e.g. gender, breed, beginning or slaughter weight), diet (e.g. gross energy, crude protein, fat concentration), rearing conditions (e.g. indoor/outdoor, group size), slaughter processes (e.g. stunning method), pork quality traits studied, sample size and treatment (e.g. storage temperature) as well as results from statistical analyses performed on the datasets from individual experiments.

Dose definition and storage time standardiation

All experiments used in the analysis supplied vitamin E in form of all-rac- α -tocopheryl (DL- α -tocopheryl) acetate, which is an equimolar mixture of eight synthetic α -tocopherol stereoisomers in acetate form (LAURIDSEN *et al.* 2002). 1mg of this chemical compound has a bioactivity conversion factor of 1.0 and corresponds to 1 International Unit (IU) of α -tocopherol. For the three different terminologies referring to α -tocopherol that had been used in the studies to define vitamin E administration, i.e. vitamin E (e.g. HOVING-BOLINK *et al.* 1998), α -tocopherol (e.g. DIRINCK *et al.* 1996) or analysed fed α -tocopherol (e.g. LANARI *et al.* 1995) the amount of supplementation could thus be expressed in IU of α -tocopherol.

In one study (PHILLIPS *et al.* 2001) different amounts of vitamin E had been administered for different time periods throughout the supplementation time. We defined the administered vitamin E (in IU) in this case by calculating weighted averages with the individual time periods as weighting factors.

Furthermore the different experiments varied in their definition of control or treatment vitamin E levels and in the accuracy of the provided vitamin E measures. To minimise the potential bias introduced by this variation, we rounded administrated vitamin E in units of 50 IU/kg feed. For example amounts of supplementation below 25 IU/kg feed were defined as vitamin E dose 0 IU/kg feed, and supplementations between 25 and 75 IU/kg feed were defined as vitamin E dose 50 IU/kg feed. Henceforth the rounded measure is denoted as vitamin E dose.

Standardization procedures related to colour of pork

Objective colour parameters of meat based on reflectance method can be given in different systems, e.g. HUNTERLAB (2008) or CIE (1976). All references used in the present study provided pork colour in term of the CIE colour system, which describes colour by three parameters: lightness (L^*), redness (a^*), and yellowness (b^*) (CIE 1976). However only the redness (a^*) parameter of this colour system was assessed in this paper, because of lack of sufficient data for the other two colour attributes. The value as a^* value of this colour parameter is going to be equally referred as redness of pork in this paper.

The muscles used in the studies referred to *M. longissimus dorsi*, *M. longissimus lumborum* or *M. psoas major*. Pork redness data were available from *M. longissimus dorsi*/*M. longissimus lumborum* in all five references, whereas less data were available for the other muscle. Since *M. longissimus lumborum* is part of *M. longissimus dorsi*, the present study uses the term *M. longissimus dorsi* for both *M. longissimus dorsi* and *M. longissimus lumborum*.

As storage conditions affect the rate of colour changes (FAUSTMAN and CASSENS 1990) redness data were considered from studies in which samples were: (i) stored at 4-7 °C; (ii) wrapped in oxygen permeable material (PVC, plastic foil); and (iii) stored under atmospheric conditions i.e. non oxygen/carbon-dioxide elevated or vacuumed storage environment. If samples were exposed to illumination, redness data received the attribute ›light‹ and if samples were kept either in dark or where the description of storage conditions demonstrated that samples were not exposed to light, they received the attribute ›no-light‹ in the meta-analysis database.

In addition, it was necessary to standardize the time scales provided in different experiments to a uniform scale. The time of slaughter was considered as the starting point of storage (day 0) and expressed the storage time in units of days post slaughter.

Statistical analysis

The aim of this meta-analysis was to derive statistical models which describe how redness of pork changes over time, and how the trend is affected by factors e.g. dietary vitamin E dose, storage conditions.

The inputs for the meta-analysis were the results of the statistical models used in the original publications in the form of least square (LS) means or means (if LS means were not provided) and the corresponding standard errors of LS means (SEM) or standard deviations (SD) where SEM were not provided.

Meta-analysis was performed using the PROC MIXED procedure of SAS (SAS 2000) where the effects of different covariates and fixed effects as well as individual study effects and

autocorrelation between repeated measurements could be taken into account (VERBEKE and MOLENBERGHS 1997, WHITEHEAD 2002). The fitted models contained α -tocopherol concentration of *M. longissimus dorsi* as covariate, vitamin E dose, vitamin E supplementation time and storage light as fixed effects, as well as interaction terms between covariates and fixed effects. Effect of storage time was examined separately in the models either as covariate or as fixed effect. Including storage time in the model as a continuous covariate provides a description of the time trend of redness, whereas inclusion as a fixed effect lends itself better to the statistical comparison of redness associated with different stages during storage and of interactions between storage time and other factors influencing it. The random experimental effect was included as a »random intercept«. For one study (DIRINCK *et al.* 1996) that did not report muscle α -tocopherol concentrations, LS means predicted from a nonlinear model (TREFAN *et al.* 2010) were used. Autocorrelations between repeated measurements were examined using the repeat statement of PROC MIXED, where vitamin E dose was nested within experiment identifier and various residual covariance matrix structures (SAS 2000) were explored.

To compare the models, the following model fitting criteria were used: correlation between the predicted and observed values through Pearson correlation R; Akaike's information criteria (AIC) (SAS 2000); RSD (residual standard deviation)

$$RSD = [\sum \sum (\epsilon_{ij})^2 / (n-p)]^{1/2} \quad (1)$$

where ϵ_{ij} is the residual value of i -th experiment at j -th dose level, n is the number of observations and p is the number of parameters in the model; CD (coefficient of determination)

$$CD = 1 - \sum \sum (\epsilon_{ij})^2 / (\sum \sum (Y_{ij})^2) \quad (2)$$

where ϵ_{ij} is the same as in RSD and Y_{ij} is the predicted value i -th experiment at j -th dose level (BÜNGER and HERRENDÖRFER 1994). In addition, visual inspection of predicted values, residuals and normality tests of residuals were carried out (SAUVANT *et al.* 2008).

Since research designs and accuracy varied across studies, weighting factors based on the published experimental errors were included in the models. The weighting factor was the inverse of the square of these errors divided by their mean value (ST-PIERRE 2001). In one study (DIRINCK *et al.* 1996) neither SEM nor SD were published, and error terms were estimated based on the coefficient of variations of the other experiments. Weighting of models was performed using the WEIGHT statement of PROC MIXED procedure of SAS (SAS 2000).

Results

After stepwise removal of statistically non-significant factors influencing redness and comparison of different assumptions for storage time dependence (i.e. linear, quadratic, cubic), the statistical model (1) provided the best fit out of all models where storage time was a continuous variable (AIC: 227.8, R: 0.85, RSD: 0.96, CD: 0.99). According to this model accumulated α -tocopherol concentration in pork (at slaughter), storage time and storage light have significant effects on redness of pork:

$$a_{ijkl}^* = \beta_0 TOC_cont_{ij} + \beta_1 Time_{ijk} + \beta_2 Time_{ijk}^2 + \beta_3 Time_{ijk}^3 + StoreLight_l + b_i + e_{ijkl} \quad (3)$$

where a^*_{ijkl} is the a^* value of i -th experiment of j -th dose level at the k -th storage time point under l -th storage light condition; TOC_cont_{ij} is the α -tocopherol concentration in *M. longissimus dorsi* of the i -th experiment of j -th dose level and β_0 its slope; $Time_{ijk}$, $Time^2_{ijk}$, $Time^3_{ijk}$ are the storage time, square and cube of k -th storage time point of the i -th experiment of j -th dose level, respectively, β_1 , β_2 and β_3 their corresponding coefficients; $StoreLight_l$ is the effect of storage light ($l=0$ refers to 'no light' and $l=1$ refers to 'light'); b_i is the random effect ('random intercept') of the i -th experiment and e_{ijkl} denotes the residuals. There were no significant interactions between the different factors.

The slope β_0 for muscle α -tocopherol concentration and the coefficients β_1 , β_2 and β_3 for linear, quadratic and cubic storage time terms as continuous variables were significantly different from zero ($P<0.0309$, $P<0.0001$, $P<0.0001$ and $P<0.0011$, respectively). Muscle α -tocopherol concentration was found to have a positive effect on redness with a slope value $\beta_0=0.11$ (SE: 0.05). The linear, quadratic and cubic storage time coefficients β_1 , β_2 and β_3 , respectively were $\beta_1=1.04$ (0.21), $\beta_2=-0.13$ (0.03) and $\beta_3=0.004$ (0.001). A significant difference of 1.35 (SEM: 0.23) ($P<0.0001$) was found for the predicted redness with lower a^* values when light was present during storage.

Figure 1 shows the (LS) means of redness (a^*) measured in *M. longissimus dorsi* at different storage time points from the 5 selected experiments for the various doses of supplementary vitamin E.

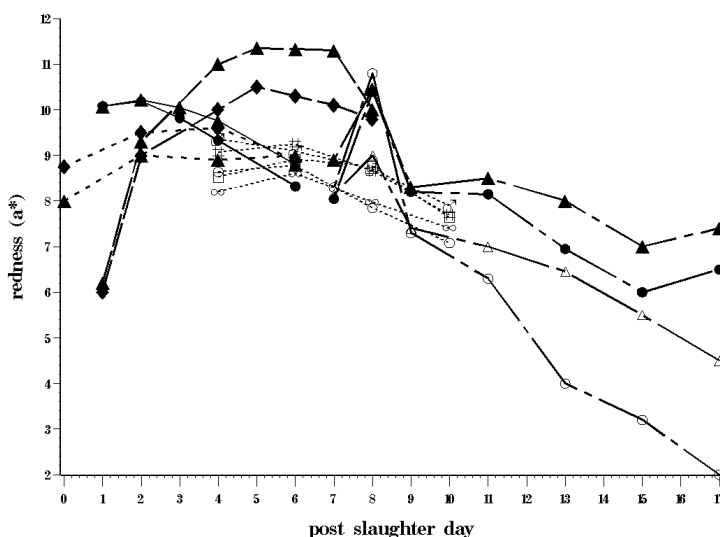


Figure 1

Changes in redness (a^*) in *M. longissimus dorsi* with storage time for various supplementary vitamin E doses from 5 different studies. Vitamin E dose levels: 0 IU (\circ/\bullet), 50 IU (\diamond), 100 IU (\square), 150 IU (\triangle), 200 IU (Δ/\blacktriangle), 300 IU ($\#$), 600 IU (∞). Open and closed symbols refer to storage conditions in which samples were exposed to illumination ('light'), or kept in darkness ('no light'), respectively.

Veränderungen der Fleisch-Röte (a^*) im *M. longissimus dorsi* in Abhängigkeit von der Lagerungszeit für verschiedene Dosierungen von Vitamin E als Futterzusatz, basierend auf 5 verschiedenen Studien. Vitamin E Dosierungen: 0 IU (\circ/\bullet), 50 IU (\diamond), 100 IU (\square), 150 IU (\triangle), 200 IU (Δ/\blacktriangle), 300 IU ($\#$), 600 IU (∞). Gefüllte Symbole ($\bullet, \blacklozenge, \blacktriangle$) beziehen sich auf Schätzwerte für Fleischproben, die im Dunkeln gelagert wurden, während sich helle Symbole ($\circ, \square, \triangle, \#, \infty$) auf Lagerung im Hellen beziehen.

Figure 2 shows the least square (LS) mean values for redness (a^*) estimated from model (1) for different vitamin E doses, storage conditions and storage times.

Confidence intervals of the individual predicted a^* values ranged from 0.34 to 0.92. Distribution of the residuals was uniform over storage time.

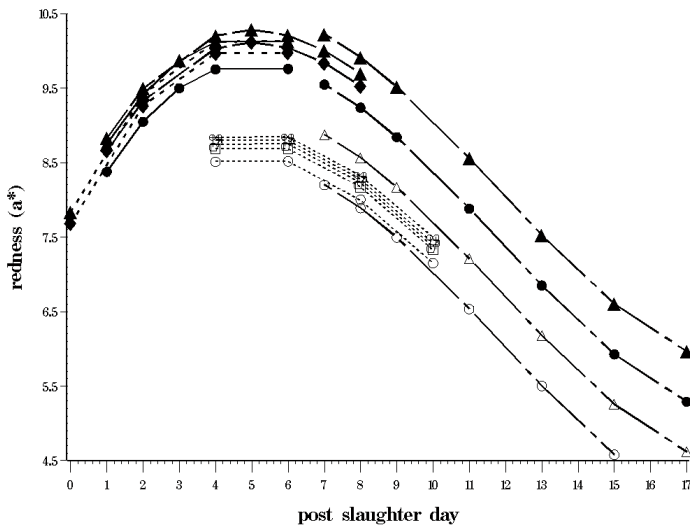


Figure 2

Model (1) predicted redness (a^*) in *M. longissimus dorsi* for different supplementary vitamin E doses and storage light conditions. Vitamin E dose levels: 0 IU (\circ/\bullet), 50 IU (\diamond), 100 IU (\square), 150 IU (\triangleleft), 200 IU (Δ/\blacktriangle), 300 IU ($\#$); 600 IU (∞). The predicted values are presented according to whether pork samples were stored in dark ($\bullet, \blacktriangle, \infty$) or exposed to illumination ($\circ, \square, \triangleleft, \#, \infty$). Schätzwerte von Modell (1) für Fleischröte-Werte (a^*) im *M. longissimus dorsi* für verschiedene Vitamin E Dosierungen und Lagerungslicht-Bedingungen. Vitamin E Dosierungen: 0 IU (\circ/\bullet), 50 IU (\diamond), 100 IU (\square), 150 IU (\triangleleft), 200 IU (Δ/\blacktriangle), 300 IU ($\#$); 600 IU (∞). Gefüllte Symbole ($\bullet, \blacktriangle, \infty$) beziehen sich auf Schätzwerte für Fleischproben, die im Dunkeln gelagert wurden, während sich helle Symbole ($\circ, \square, \triangleleft, \#, \infty$) auf Lagerung im Hellen beziehen.

In model (1) storage time was considered as a continuous variable. This provided a quantitative description for the change in redness of pork with time, but did not allow statistical comparisons of predicted redness between different storage time points. Therefore an alternative model was applied to the data where storage time was considered as a fixed effect. The resulting best fit model (AIC: 238.7, R: 0.91, RSD: 0.95, CD: 0.99) was

$$a^*_{mnpq} = (TOC_cont * StoreTime)_{mnp} + StoreTime_p + StoreLight_q + b_m + e_{mnpq} \quad (4)$$

where a^*_{mnpq} is the a^* value of m -th experiment of n -th dose level at the p -th storage time point under q -th type storage light condition; $TOC_cont * StoreTime_{mnp}$ is the interaction between α -tocopherol concentration of *M. longissimus dorsi* of the m -th experiment of n -th dose level and p -th storage time point; $StoreTime_p$ is the effect of time at p -th storage time point; $StoreLight_q$ is the effect of storage light ($q=0$ refers to 'no light' and $q=1$ refers to 'light'); b_m is the random effect, as random intercept of the m -th experiment and e_{mnpq} denotes the residuals. Detailed analysis of the $TOC_cont * StoreTime_{mnp}$ interaction at individual storage

times showed that the interaction was significant at 1, 6 and the last 3 days post slaughter storage times and their LS means were -0.70 (0.25), 0.39 (0.19), 0.30 (0.12), 0.28 (0.12), 0.28 (0.12), respectively, implying that α -tocopherol concentrations at slaughter affect the change in redness at rather later stages of the oxidation process. This coincides with the period in which redness changes most as LS means of a^* values estimated from model (2) increased from 7.58 (0.54) to 9.30 (0.63) over the first 3 days post slaughter, after which values remained approximately stable until day 8 after which they gradually decreased to 5.05 (0.37). Multiple comparisons of LS means of redness between the different storage days generally showed no significant differences between successive storage days, but significant differences were found between estimates corresponding to short (i.e. four days or less) and long (above a week) storage. As in the case of model (1) a significant difference of 1.43 (0.19) ($P < 0.0001$) was found for predicted redness with lower a^* values when illumination was present during storage. Confidence intervals of the individual predicted redness varied between 0.47 and 1.47 . Distribution of the residuals was uniform over storage time.

In the derivation of the statistical models vitamin E dose as a multi-level fixed effect as well as its accumulation in the form of α -tocopherol were introduced into the models. However, α -tocopherol concentration was found to be the better predictor for redness, and the effect of vitamin E was not significant at the 95 % confidence limit and hence not included in these models. In order to answer the question whether supplementation of vitamin E has a direct effect on the change of redness of pork, a further model was tested in which vitamin E dose was attributed to one of two dose categories corresponding to doses either equal to or less than 50 IU/kg feed (control) or greater than or equal to 100 IU/kg feed (supplementation), respectively. The statistical model applied to the data contained the two simplified dose categories, storage time and storage light as fixed effects. Although the fit of the model did not improve (AIC: 232.7 , R: 0.88 , RSD: 0.97 , CD: 0.99), the model was:

$$a^*_{rstv} = \text{simpDose}_{rs} + \text{StoreTime}_t + \text{StoreLight}_v + b_r + e_{rstv} \quad (5)$$

where a^*_{rstv} is the a^* value of r -th experiment of s -th simplified dose level at the t -th storage time point under v -th type storage light condition; simpDose_{rs} effect of s -th level of simplified vitamin E dose of r -th experiment; StoreTime_t is the effect of time at t -th storage time point; StoreLight_v is the effect of storage light ($v=0$ refers to 'no light' and $v=1$ refers to 'light'); b_r is the random effect, as random intercept of the r -th experiment and e_{rstv} denotes the residuals. All fixed effects were found to be significant, and interactions between fixed effects were found to be non-significant at the 95 % confidence limit. Model (3) gave similar estimation for changes of LS means of redness over time as model (2) e.g. estimated a^* values increased from 7.64 (0.60) to 9.20 (0.60) on the first 3 days post slaughter, after which values remained approximately stable until day 8 after which they gradually decreased to 5.10 (0.42). Furthermore multiple comparisons of estimated redness between the different storage days showed the same patterns as in the case of model (2) and a significant difference of 1.47 (0.22) ($P < 0.0001$) with the same magnitude of model (1) & (2) was found between light and dark storage conditions. In particular a significant difference of -0.48 (0.19) ($P < 0.0162$) was found between predicted redness at lower (≤ 50 IU/kg feed) and higher vitamin E doses (≥ 100 IU/kg feed). Confidence intervals of the individual predicted a^* values varied between 0.55 and 1.20 . Distribution of the residuals was uniform over storage time.

Random intercepts were found to be not significant in either model, implying that experimental conditions of the individual studies did not contribute to the modelled values.

Weighting did not improve the fitting of the models, nor did it change general tendencies in results and only marginally modified predicted values. Hence, weighting factors were excluded from the final models.

The present meta-analysis was based on the period between days 0 and 17 post slaughter. However, data between days 11 and 17 originated from a single reference (LANARI *et al.* 1995). To test for potential bias in the statistical results caused by this single reference for later storage times, the meta-analysis was also carried out using only data from the period between days 0 and 11 post slaughter. Removal of the data referring to the later storage times was however found to have only negligible influence on the statistical model results.

Discussion

In this study a meta-analysis was performed to determine the effects of dietary vitamin E as an antioxidant on the changes of redness of pork during storage.

The meta-analysis combined results from five independent studies to establish quantitative relationships between the changes in pork redness with time and influencing factors of significant importance. To the best of the authors' knowledge this study is the first to quantitatively describe the time dependence of the discolouration process of pork when related to dietary vitamin E and storage in different light conditions.

Model (1) revealed a significant influence of accumulated α -tocopherol concentration in *M. longissimus dorsi*, as well as storage time and storage light on redness and its change over time. The relationship between redness and α -tocopherol concentration was linear, whereas the change of redness with storage time was best described by a third order polynomial. The moderate but positive slope value associated with α -tocopherol concentration in model (1) suggested that an increase of 1 μg of α -tocopherol in the muscle as a result of dietary vitamin E led to an expected increase of 0.11 in redness across all storage times.

A second model was derived using storage time as a fixed effect. Model (2) found an interaction between α -tocopherol concentration of *M. longissimus dorsi* and storage time, indicating that muscle α -tocopherol concentration (and hence vitamin E supplementation) affects the evolution of redness over time. This influence was more frequent after 6 days of storage. Model (2) further shows that redness decreases only slowly over time, and that differences become significant only after about a week of storage.

A third model was developed to establish quantitative relationship between supplemented vitamin E dose, storage conditions and changes in redness. In model (3) definition of dose categories was required to quantify the effect of dose. This simplification may have been due to the relatively low number of data points (80) from 5 references compared to the available number of vitamin E dose levels (7), however this model predicted the changes of redness over time as model (2).

Considering redness changes over time the initial increase in redness during storage is coherent with findings of earlier studies unrelated to the effects of vitamin E (LINDAHL *et al.* 2006, LI *et al.* 2009). While these two studies found that after the initial increase, redness remained stable, other studies found a decrease in redness over several days of storage

(JUNCHER *et al.* 2001, TIKK *et al.* 2008), which is in accordance with the results of this meta-analysis.

To correctly predict redness, all models needed to take into account whether samples were exposed to illumination. This result is a good agreement with the existing literature (FAUSTMAN and CASSENS 1990, HONIKEL 1998) which shows that redness of pork and pork products decrease during exposure to light (CALKINS *et al.* 1986), (ANDERSEN *et al.* 1988). Storage light was found to have a significant influence on redness of pork with a difference of about 1.4 between light and dark storage conditions and this effect was found constant over all storage time by this meta-analysis.

Several other factors that may influence the relationship between vitamin E and colour changes could not be assessed in the present analysis. The length of time supplementary vitamin E was fed was identified as a non-significant factor in the models. Earlier studies established that blood α -tocopherol concentration of pigs stabilise after more than 2 weeks of vitamin E supplementation and a linear relationship was found between blood- and tissue α -tocopherol concentration at slaughter (HOPPE *et al.* 1993). The duration of vitamin E supplementation was considerably longer than 14 days pre slaughter in all references used for the present meta-analysis (Table 1). Thus tissue α -tocopherol concentrations may have reached equilibrium with vitamin E supply, which might explain why supplementation times did not significantly contribute to the models. Furthermore, although studies included in the meta-analysis gave information on the breeds used, mainly modern crossbreds (Table 1), any breed effect could not be assessed. The reason is that pigment (myoglobin) content of muscle fibres is primarily responsible for meat colour (HEDRICK *et al.* 1994, FAUSTMAN and CASSENS 1990) and no marked difference in muscle fibre numbers and size of the *longissimus* muscle were apparent between modern meat-type pig breeds and crosses (PAS 2004). Similarly, the present meta-analysis concerns only redness of a specific muscle, *M. longissimus dorsi*. Among the references used in this analysis only two (HOVING-BOLINK *et al.* 1998, PHILLIPS *et al.* 2001) published changes of a^* values of *M. psoas major* over time. This dataset was too small to carry out a meta-analysis for this muscle. Finally among the references, which satisfied our selection criteria, only 2 references (DIRINCK *et al.* 1996, VAN HEUGTEN *et al.* 2002) contained information on time dependent changes in lightness (L^*) and only one (VAN HEUGTEN *et al.* 2002) on time dependent changes in yellowness (b^*) following the different amounts of vitamin E administration. In summary the present study used meta-analysis based on a small number of carefully selected experiments to establish quantitative relationships for the effect of dietary vitamin E supplementation on change of redness of pork under different storage conditions.

In conclusion, the three statistical models together suggest that vitamin E supplementation affects redness of pork, but only when supplementation of dose exceeds 100 IU/kg and after 6 days post slaughter.

The number of experiments included in this meta-analysis was small, as the aim was to produce statistically robust estimates rather than less reliable estimates based on a larger number of studies but inconsistent experimental conditions.

Further studies would be necessary to confirm the results, to establish and validate the found relationships for other muscles and colour attributes.

Acknowledgements

Financial support for this research was received from EU Sixth Framework Programme Priority Food-2005-T5.4.1.1, ›Improving the quality of pork and pork products for the consumer‹ and Scottish Agricultural College.

The views expressed in this publication are the sole responsibility of the author(s) and do not necessarily reflect the views of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use, which might be made of the information.

References

- Andersen HJ, Bertelsen G, Boegh-Soerensen L, Shek CK, Skibsted LH (1988) Effect of light and packaging conditions on the colour stability of sliced ham. *Meat Sci* 22, 283-92
- Asghar A, Gray JI, Booren AM, Gomaa EA, Abouzied MM, Miller ER (1991) Effects of supranutritional dietary vitamin E levels on subcellular deposition of alpha-tocopherol in the muscle and pork quality. *J Sci Food Agric* 57, 31-41
- Buckley DJ, Morissey PA, Grey JI (1995) Influence of dietary vitamin E on oxidative stability and quality of pig meat. *J Anim Sci* 73, 3122-30
- Bünger L, Herrendörfer G (1994) Analysis of a long-term selection experiment with an exponential model. *J Anim Breed Genet* 111, 1-13
- Calkins CR, Goll SJ, Mandigo RW (1986) Retail display lighting type and fresh pork color. *J Food Sci* 51, 1141-5
- Cannon JE, Morgan JB, Schmidt GR, Tatum JD, Sofos JN, Smith GC, Delmore RJ, Williams SN (1996) Growth and fresh meat quality characteristics of pigs supplemented with Vitamin E. *J Anim Sci* 74, 98-105
- CIE (1976). CIE (Comission International de l'Eclairage) 18th Session. CIE Publication 36, London, England
- Dikeman ME (2007) Effects of metabolic modifiers on carcass traits and meat quality. *Meat Sci* 77, 121-35
- Dirinck P, De Winne A, Casteels M, Frigg M (1996) Studies on Vitamin E and Meat Quality. 1. Effect of feeding high vitamin E levels on time-related pork quality. *J Agric Food Chem* 44, 65-8
- Dunsha FR, D'Souza DN, Pethick DW, Harper GS, Warner RD (2005) Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. *Meat Sci* 71, 38
- Faustman C, Cassens RG (1990) The biochemical basis for discoloration in fresh meat: A Review. *J Muscle Foods* 1, 217-43
- Gray JI, Gomaa EA, Buckley DJ (1996) Oxidative quality and shelf life of meats. *Meat Sci* 43, 111-23
- Hedrick BH, Forrest CJ, Judge DM, Merkel AR (1994) Principles of Meat Science. 3rd edition, Kendal/Hunt Publishing Company, Dubuque, USA
- Honikel KO (1998) Reference methods for the assessment of physical characteristics of meat. *Meat Sci* 49, 447-57
- Hoppe PP, Schöner FJ, Wiesche H, Stahler-Geyer A, Kammer J, Hochadel H (1993) Effect of graded dietary alpha-tocopherol supplementation on concentrations in plasma and selected tissues of pigs from weaning to slaughter. *J Vet Med A* 40, 219-28
- Hoving-Bolink AH, Eikelenboom G, van Diepen JT, Jongbloed AW, Houben JH (1998) Effect of dietary vitamin E supplementation on pork quality. *Meat Sci* 49, 205-12
- HunterLab (2008) Hunter L, a, b Color Scale. http://www.hunterlab.com/appnotes/an08_96a.pdf [last accessed 28.09.2010]
- Jensen C, Guidera J, Skovgaard IM, Staun H, Skibsted LH, Jensen SK, Moller AJ, Buckley J, Bertelsen G (1997) Effects of dietary alpha-tocopheryl acetate supplementation on alpha-tocopherol deposition in porcine m. psoas major and m. longissimus dorsi and on drip loss, colour stability and oxidative stability of pork Meat. *Meat Sci* 45, 491-500

- Juncher D, Ronn B, Mortensen ET, Henckel P, Karlsson A, Skibsted LH, Bertelen G (2001) Effect of pre-slaughter physiological conditions on the oxidative stability of colour and lipid during chill storage of pork. *Meat Sci* 58, 347-57
- Krska P, Lahucky R, Küchenmeister U, Nürnberg K, Palanska O, Bahelka I, Kuhn G, Ender K (2001) Effects of dietary organic selenium and vitamin E supplementation on post mortem oxidative deterioration in muscles of pigs. *Arch Tierz* 44, 193-201
- Lahucky R, Krska P, Küchenmeister U, Nürnberg K, Bahelka I, Demo P, Ender K (2000) Effect of vitamin E on changes in phosphorus compounds assessed by ³¹P NMR spectroscopy and ATPase from post mortem muscle samples and meat quality of pigs. *Arch Tierz* 43, 487-97
- Lahucky R, Kuechenmeister U, Bahelka I, Novotna K, Vasickova K, Ender K (2005) Effects of vitamin E by dietary supplementation and of calcium ascorbate by post mortem injection in muscle on the antioxidative status and meat quality of pigs. *Arch Tierz* 48, 592-600
- Lanari MC, Schaefer DM, Scheller KK (1995) Dietary Vitamin E supplementation and discoloration of pork bone and muscle following modified atmosphere packaging. *Meat Sci* 41, 237-50
- Lauridsen C, Engel H, Craig AM, Traber MG (2002) Relative bioactivity of dietary RRR- and all-rac-alpha-tocopheryl acetates in swine assessed with deuterium-labeled vitamin E. *J Anim Sci* 80, 702-7
- Li C, Wu J, Zhang N, Zhang S, Liu J, Li J, Li H, Feng X, Han Y, Zhu Z, Xu X, Zhou G (2009) Effects of boning method and postmortem aging on meat quality characteristics of pork loin. *Anim Sci J* 80, 591-6
- Lindahl G, Karlsson AH, Lundström K, Andersen HJ (2006) Significance of storage time on degree of blooming and colour stability of pork loin from different crossbreeds. *Meat Sci* 72, 603-12
- Monahan FJ, Gray JI, Asghar A, Booren MA, Miller RE, Buckley DJ, Morissey PA, Gomaa AE (1992) Influence of dietary treatment on lipid and cholesterol oxidation in pork. *J Agric Food Chem* 40, 1310-5
- Oldfield JE (2003) Some recollection of early swine research with selenium and vitamin E. *J Anim Sci* 81 (E. suppl. 2), E145-8
- te Pas MFW (2004). *Muscle development of livestock animals (Physiology, Genetics and Meat Quality)*. 1st edition, CABI Publishing, Wallingford, UK
- Pettigrew JE, Esnaola MA (2001) Swine nutrition and pork quality: A review. *J Anim Sci* 79, 316-42
- Phillips AL, Fustman C, Lynch MP, Govoni KE, Hoagland TA, Zinn SA (2001) Effect of dietary alpha-tocopherol supplementation on color and lipid stability in pork. *Meat Sci* 58, 389-93
- Phillips CJC (2005) Meta-analysis - a systematic and quantitative review of animal experiments to maximise the information derived. *Anim Welf* 14, 333-8
- Rosenvold K, Andersen HJ (2003) Factors of significance for pork quality-a review. *Meat Sci* 64, 219-37
- SAS (2000) *SAS/STAT User's Guide (Release 8)*. SAS Institute Inc, Cary, NC, USA
- Sauvant D, Schmidely P, Daudin JJ, St Pierre NR (2008) Meta-analyses of experimental data in animal nutrition. *Animal* 2, 1203-14
- St-Pierre NR (2001) Invited Review: Integrating quantitative findings from multiple studies using mixed model methodology. *J Dairy Sci* 84, 741-55
- Tikk K, Lindahl G, Karlsson AH, Andersen HJ (2008) The significance of diet, slaughter weight and aging time on pork colour and colour stability. *Meat Sci* 79, 806-16
- Trefan L, Bünger L, Bloom-Hansen J, Rooke JA, Salmi B, Larzul C, Terlouw C, Doeschl-Wilson A (2010) Meta-analysis of the effects of dietary vitamin E supplementation on alpha-tocopherol concentration and lipid oxidation in pork. *Meat Science* (submitted)
- van Heugten E, Hasty JL, See MT, Larick DK (2002) Storage stability of pork from berkshire and hampshire sired pigs following dietary supplementation with vitamin E. *J Muscle Foods* 14, 67-80
- Verbeke G, Molenberghs G (1997) *Linear mixed models in practice: A SAS-Oriented Approach*. Springer-Verlag, New York, USA

Vernet J, Ortigues-Marty I (2005) Conception and development of bibliographic database of blood nutrient fluxes across organs and tissues in ruminants: data gathering management prior to meta-analysis. *Reprod Nutr Dev* 5, 527-46

Whitehead A (2002) *A Meta-Analysis of controlled clinical trials*. John Wiley and Sons, UK, 215-40

Received 21 April 2010, accepted 20 July 2010.

Corresponding author:

LASZLO TREFAN

email: laszlo.trefan@sac.ac.uk

Sustainable Livestock Systems, Scottish Agricultural College, Penicuik, EH26 0PH, United Kingdom
