Retail colour (oxidation) of meat is affected by antioxidant status and heme iron but not polyunsaturated fatty acids of muscle in lambs

Ponnampalam E.N.[1,2,*], Butler K.L.[2], McDonagh M.B.[1,2], Jacobs J.L.[2], Dunshea F.R.[3] and Hopkins D.L.[1,4]

[1]CRC for Sheep Industry Innovation, Homestead Building, UNE, Armidale NSW 2350, Australia
[2]AgriFood Production Systems, Department of Primary Industries, VIC 3030, Australia
[3]Melbourne School of Land and Environment, The University of Melbourne, Parkville, Vic. 3010, Australia
[4]NSW Department of Primary Industries, Centre for Red Meat and Sheep Development, PO Box 129, Cowra, NSW 2794, Australia

[*]Corresponding author: Eric N. Ponnampalam. Email: eric.ponnampalam@dpi.vic.gov.au

Abstract: **The relationship between vitamin E, forms of iron, polyunsaturated fatty acids (PUFA) and the redness of meat (retail display) at 72 to 96 h post slaughter from lambs offered 2 different diets was examined in the m. *longissimus lumborum*. Meat redness was positively related to vitamin E and heme iron and negatively related to total n-3 and total n-6 content. However, after adjusting for the effects of vitamin E and heme iron content, there was no indication of any residual relationship between redness at 72 to 96 h of retail display and total n-3 or total n-6. This indicates that the relationship between PUFA and redness in meat is mediated through the effects of heme iron and vitamin E in the muscle. It appears that the level of highly oxidisable PUFAs (n-3 and n-6) in muscle tissues do not play a major role in maintenance of redness when measured at 72 to 96 h of display, but the level of antioxidants (vitamin E) and heme iron content are important.**

Keywords- **Muscle biochemical components, retail colour, oxidation of meat.**

I. INTRODUCTION

There has been a mixed view among researchers on the relationship of muscle vitamin E, heme iron, polyunsaturated fatty acids (PUFA) and colour stability of meat post-farm gate in terms of the oxidation process in muscles. It was reported that an increase in heme pigments can result in an increase in heme iron and therefore the redness (a*-value) of meat, as iron is complexed in the heme moiety of the pigments (1). Myoglobin is a globular heme protein found in the muscle tissues and the concentration can be affected by genetic and dietary background. Others (2,3) have shown vitamin E content in muscle tissue systems is an important factor to protect lipid and myoglobin from oxidation, thus delaying the onset of discolouration in fresh and frozen meat. The oxidation of lipids, especially the highly oxidisable n-6 and n-3 PUFAs, is accentuated immediately after death, leading to deterioration in colour, texture, nutritional value and flavour in meat (4). However, no studies have investigated the joint relationship of vitamin E, heme iron and PUFA on the stability of meat colour. This study investigates the joint relationship of vitamin E, heme iron, n-3 and n-6 PUFAs to a* values 72-96 h post slaughter in a flock of lambs with a wide range in levels of heme and non heme iron, PUFA and vitamin E.

II. MATERIALS AND METHODS

A. Sample collection and analytical procedures

Twenty six, 7 month old crossbred (Poll Dorset x Border Leicester x Merino) lambs fed under grazing conditions for five weeks were used in this study. They were divided into two groups with one group offered low quality pasture only (dry matter, crude protein and metabolisable energy content were 58.9%, 5.1% and 6.9 MJ/kg, respectively) and the other offered hay supplemented with grain (800 g/day air-dry basis). The low quality pasture contained 90% ryegrass (*Lolium perenne*) and 10% barley grass (*Hordeum leporinum*) while the grain diet was a mix of barley grain (80%) and lentils (20%). For grain fed lambs, hay based on capeweed (*Arctotheca calendula*) was available at all times. At the end of a 5 week feeding period lambs were transported 80 km to a commercial abattoir and slaughtered after 12 h off feed, with access to water. At 24 h post-slaughter, muscle samples collected from the m. *longissimus lumborum* (LL), trimmed of all external fat and connective tissue, were used for the determination of vitamin E, PUFA and iron content.

At 24 h postmortem, a section of LL was sliced into three 2.5-cm chops, packed on a black foam tray and over wrapped with a PVC food film (15 µm thickness) as retail packs. Trays were maintained at a refrigerated temperature of 3-4°C for the evaluation of colour stability of meat under fluorescent light (1000 lux). Redness (a*-value) of meat at 24, 48, 72 & 96 h was measured in duplicate on each sample using a Hunter Laboratory Mini

Scan XE Plus meter with a 5-mm aperture, light source set to D65/10 (model 45/0-S, Hunter Associates Laboratory Inc., Reston VA, USA). Values at 72 and 96 h retail display were averaged to produce a combined mean (2 × 3 × 13 at each point), so as to increase the precision of statistical analyses by reducing sampling variation. Details of sample preparation, extraction procedure and quantification for n-3 and n-6 fatty acids, forms of iron and vitamin E in muscle are as described previously (5).

B. Statistical Analysis

Differences between diets were compared using two-sided exact Mann Witney U tests. Then, the redness of the meat at 72 and 96 h retail display was individually related to heme iron, non heme iron, total n-3, total n-6 and vitamin E contents of the muscle for each of the two diet groups using parallel single variable regression lines. A general model was then constructed to jointly relate the redness of meat to these measurements and the two diet groups. The model was evaluated using F ratio tests, residual standard deviation and the percentage of variance accounted for.

III. RESULTS

Heme iron (P = 0 .072) and vitamin E (P < 0.001) were generally less in the grain fed lambs than the pasture fed lambs, while the reverse was true for total n-6 (P = 0.002) and total PUFA (P = 0.014). On an individual variable basis, redness was positively related to vitamin E and negatively related to total n-3 and total n-6 PUFA. None of the 6 measurements (heme iron, non heme iron, total n-3, total n-6, total PUFA or vitamin E) were individually able to explain the effect of diet on the redness of meat at 72-96 h of retail display.

The parsimonious model that best explained redness at 72-96 h of retail display was a multiple linear regression model based on the two independent variables vitamin E and heme iron. Once these two variables were included in the model, there was no indication of any effect of diet (Fig. 1 and Fig. 2, respectively). This model accounted for more of the variation than other competing models (Table 1). In the final model, redness is positively related to both vitamin E (Fig. 1) and heme iron (Fig. 2) content. Once these two variables are taken into account there was no indication of any relationship of redness at 72 and 96 h of retail display with total n-3 (P = 0.60), total n-6 (P = 0.16) or non heme iron (P = 0.22). There was a negative relationship between vitamin E and either total n-3 (Fig. 3) or total n-6 (Fig. 4) PUFA. There was little relationship between heme iron and either total n-3 or total n-6 PUFA (data not shown).

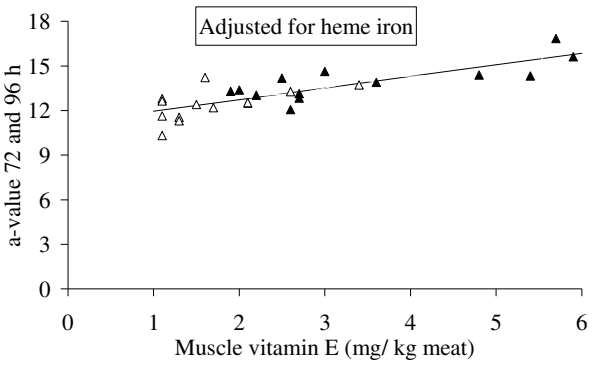

Fig 1: Relationship between redness of meat at 72 and 96 h of retail display and muscle vitamin E ($P = 8.9 \times 10^{-7}$), after the redness has been adjusted for heme iron for lambs offered pasture only (▲) or offered hay supplemented with grain (Δ).

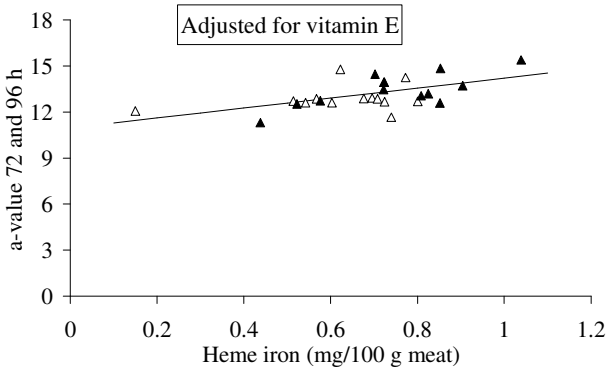

Fig. 2: Relationship between redness of meat at 72 and 96 h of retail display and heme iron (P = 0.0031), after the redness has been adjusted for vitamin E for lambs offered pasture only (▲) or offered hay supplemented with grain (Δ).

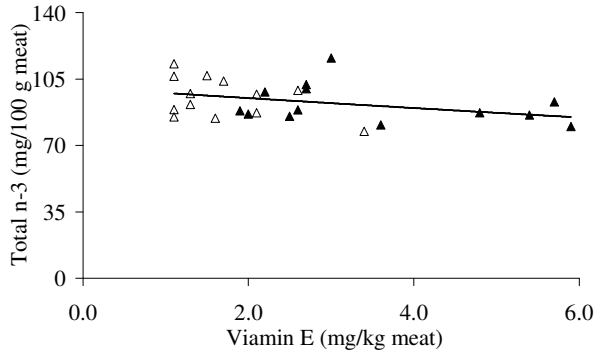

Fig 3: Relationship between vitamin E and total n-3 PUFA (6% of variance accounted for, P = 0.19) in LL muscle for lambs offered pasture only (▲) or offered hay supplemented with grain (Δ). Curve is quadratic best fit.

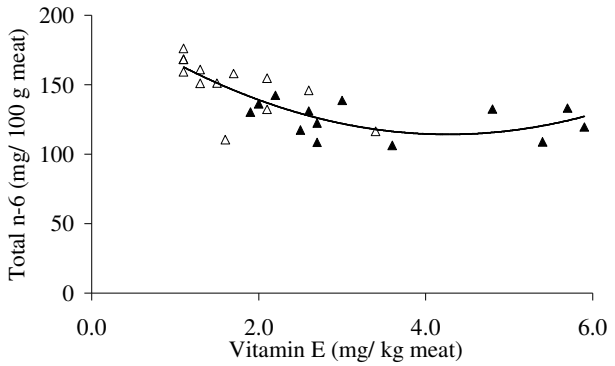

Fig 4: Relationship between vitamin E and total n-6 PUFA (57% of variance accounted for, P = 0.000024) in LL muscle for lambs offered pasture only (▲) or offered hay supplemented with grain (Δ). Curve is quadratic best fit.

Table 1. Residual standard deviation and percentage variance accounted for some competing models to explain redness of meat (a*-value) at 72 and 96 h of retail display.

Terms in Model	Residual standard deviation	Percentage variance accounted for
None	1.55	-
Diet	1.07	39.3
n-3	1.26	16.2
n-6	1.04	42.7
Total PUFA	1.04	42.4
Vitamin E	0.99	48.2
Heme iron	1.21	21.9
Heme iron and diet	1.01	45.7
Heme iron and n-3	1.13	32.3
Heme iron and n-6	0.86	61.3
Heme iron and total PUFA	0.89	58.2
Vitamin E and n-3	1.01	57.5
Vitamin E and n-6	0.99	59.1
Vitamin E and total PUFA	0.99	59.4
Vitamin E and diet	0.94	53.7
Vitamin E and heme iron	0.84	70.2

IV. Discussion

This study shows a negative relationship between redness of muscle and total n-3 or total n-6 PUFA. However, when we also took into account the amount of heme pigment present (through measurement of heme iron content in muscle) together with the amount of vitamin E in the muscle, there was no remaining relationship between total n-3 or total n-6 PUFA and the redness (a*-value) of muscle. This indicates that, despite observing a relationship between components of n-3 and n-6 PUFA and colour stability, this relationship is not direct. Rather, this relationship is mediated through the effects of heme iron and vitamin E in the muscle tissue.

Results from this experiment question the paradigm that antioxidants in muscle tissue, such as vitamin E, reduce the oxidation of pigments such as myoglobin, and thus the colour stability of meat, via the mechanism of reducing oxidation of PUFA in meat. If this paradigm was true, then a direct effect of n-3 or n-6 PUFA on colour stability (as indicated by redness) would be evident, and not mediated through vitamin E and heme iron levels. Thus, some other mechanism must be operative. A likely possibility is that antioxidants, such as vitamin E, reduce the oxidation of pigments directly without the chemical reaction of n-6 or n-3 PUFA (6). In such a situation it would be expected that the redness of meat is jointly related to the heme pigment (i.e., heme iron) present and the level of antioxidants, such as vitamin E, present in the tissue system. Such a conclusion agrees with the findings of this experiment.

A review (7) discussed an interrelationship between myoglobin and lipid oxidation in muscles, (i.e., myoglobin as an initiator of lipid oxidation and vice versa), that in some circumstances, could explain a lack of any direct effect of PUFA on colour stability. They suggested that muscles with greater colour stability were characterised by less oxygen consumption and less lipid oxidation. These authors also suggested that lipid oxidation is tightly related to the level of oxygen present, and oxymyoglobin redox stability is enhanced in high oxygen availability. Thus very high or very low levels of oxygen provide conditions in which the oxidative interaction between myoglobin and lipids is not tightly linked. However, this is not likely to be the reason we found no non-mediated effect of PUFA on colour stability in the present experiment. We found simple effects (i.e., effects with no adjustment for vitamin E and heme iron) of n-3 and n-6 PUFA on colour retention. If their explanation was the reason, then no simple effect of n-3 and n-6 PUFA on colour retention would be expected.

It appears that the apparent relationship between n-3 or n-6 PUFA and muscle redness is actually an artefact of the negative relationship between PUFA and vitamin E. It does not appear to be an artefact of a relationship between n-3 or n-6 PUFA and heme iron (heme pigment) or the total iron, because no such relationship was observed. An explanation of the observed negative association between PUFA (n-3 and n-6 fats) and vitamin E contents in the meat is that PUFA and vitamin E levels are negatively associated in the muscle of the live animal. Vitamin E can be maintained through dietary supplementation or pasture feeding, which in turn leads to improved stability of colour, lipid oxidation and nutritional value of meat post farm gate.

V. CONCLUSIONS

This study indicates that the level of highly oxidisable PUFAs (n-3 and n-6) in muscle tissue systems does not play a major role in the functionality of meat (retention of red colour over 72-96 h of display) post-farm gate. It is the level of antioxidants (vitamin E) and heme iron content that is important. Results demonstrate the interpretation of a relationship between n-3 or n-6 PUFA and retail colour stability (colour deterioration via myoglobin oxidation) without accounting for vitamin E and heme iron content in the tissue system may provide misleading outcomes.

ACKNOWLEDGEMENT

The muscle samples for this study were collected from a project jointly funded by the Australian Sheep Industry CRC and the Department of Primary Industries, Victoria, Australia. The financial support for the measurement of colour in meat, analysis of vitamin E (α-tochopherol) and forms of iron content in meat was provided by the Department of Primary Industries, Victoria (Project – Novel High Value Lamb). We thank Matthew Kerr and Athula Natharampatta for the support with muscle sample collection at slaughter and colour measurement, and Tim Plozza and George Croatto for the assistance in laboratory analysis.

REFERENCES

1. Dunne PG, O'Mara FP, Monahan FJ, Moloney AP (2006) Changes in colour characteristics and pigmentation of subcutaneous adipose tissue and M. *longissimus dorsi* of heifers fed grass, grass silage or concentrate-based diets. Meat Sci 74: 231-241
2. Arnold RN, Arp SC, Scheller KK, Williams SN, Schaefer DM (1993) Tissue equilibration and subcellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. J Anim Sci 71:105-118
3. Faustman C, Chan WKM, Schaefer DM, Havens A (1998) Beef colour update. The role of vitamin E. J Anim Sci 76:1019-1026
4. Morrissey PA, Sheehy PJA, Galvin K, Kerry JP, Buckley DJ (1998) Lipid stability in meat and meat products. Meat Sci 49:73-86
5. Ponnampalam EN, Butler KL, McDonagh, MB, Jacobs JL, Hopkins DL (2011) Relationship between muscle antioxidant status, forms of iron, polyunsaturated fatty acids and functionality (retail colour) of meat in lambs. Meat Sci (in press).
6. Cheah KS, Cheah, AM, Krausgrill DI (1995) Effect of dietary supplementation of vitamin E on pig meat quality. Meat Sci 39: 255–264
7. Faustman C, Sun Q, Mancini R, Suman S (2010) Myoglobin and lipid oxidation interactions: Mechanistic bases and control. Meat Sci 86:86-94