IS NUTRITIONAL ENRICHMENT OF OMEGA-3 FATTY ACID IN MEAT A CONCERN FOR QUALITY DETERIORATION BY LIPID OXIDATION?

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Abstract – This study investigated whether polyunsaturated fatty acids (PUFAs), mainly omega-3 (n-3) fatty acids, is a cause of quality deterioration in meat due to lipid oxidation. Lipid oxidation was evaluated in fresh and aged meat (vacuum packaged) (*M. longissimus lumborum)* displayed under refrigerated conditions for 4 days. The aged meat was stored at 3°C for 4 weeks. The effect of muscle PUFAs (n-3 and omega-6 [n-6]), heme iron and vitamin E concentrations on lipid oxidation was examined using regression analysis and shingle plots. Shingle plots were used to investigate the relationship between thiobarbituric acid reactive substances and other variables by conditioning on vitamin E or vice versa. The results showed a strong positive relationship between n-3 or n-6 fatty acids or heme iron and lipid oxidation when vitamin E was below 2.95 mg/kg muscle. The data demonstrate that vitamin E concentration in muscle has a major effect on controlling lipid oxidation in muscle tissues, but n-3 fatty acids play a minor role unless the vitamin E concentration falls below 2.95 mg/kg muscle.

Key Words – antioxidant status, biochemical components, meat.

I. INTRODUCTION

Studies conducted in humans and rodents have shown that n-3 fatty acids can reduce free radical formation and development of secondary metabolites within the circulatory system and body tissues by lowering low density lipoprotein (LDL) production, formation of inflammatory substances and oxidative stress [1,2,3].

Polyunsaturated fatty acids (PUFA), particularly omega-3 (n-3) fatty acids, have been implicated as the primary cause of lipid oxidation in meat [e.g. 4]. Understanding the interrelationship between muscle biochemical components (vitamin E, n-3 PUFA, heme iron) and their interactions with respect to lipid oxidation in muscle tissues is crucial to understanding the involvement of PUFA in lipid oxidation. This will in turn enable the tailoring of the functions of these chemical components in biological systems through nutritional management so that the integrity, nutritional value and freshness of meat are optimized.

The objective of this study was to investigate the combined effect of antioxidants (vitamin E), iron (heme, nonheme) and PUFA (n-3, omega-6 (n-6) fatty acids) on lipid oxidation of muscle tissues in lambs, as a model for quality deterioration of muscle foods in domestic animals. We hypothesise that n-3 fatty acids in muscle are not the major cause for quality deterioration of muscle foods due to lipid oxidation.

II. MATERIALS AND METHODS

Fifty four second cross lambs (Poll Dorset × [Border Leicester × Merino]) were assigned to 18 groups based on initial liveweight (range 28.8 – 39.6 kg) and gender, and then randomly allocated to four dietary treatments. The experiment was an asymmetric split-plot design comprising four treatments: perennial pasture (PP); annual pasture plus supplement of hay (lucerne [alfalfa] and annual ryegrass) and oat grain (AP); AP and whole bruised flaxseed (APFS); AP and flaxmeal (APFM). The supplements for lambs on AP, APFS and APFM were offered as 500 g of pellets daily for the first two weeks and 700 g of pellets daily for the remaining five weeks.

At 24 h post-slaughter, a subset of 36 (9 per treatment) carcases were randomly sampled across treatments from the *M. longissimus lumborum;* (LL). LL muscle was stored at -20°C for the subsequent determination of vitamin E content (α-tocopherol), forms of iron (heme, nonheme) and PUFA (n-6, n-3 fatty acids) composition as described by others (5). Another two sets of samples were collected from the same LL section, one set was used for display as fresh meat and the other set was used for display after ageing in vacuum packaging. Aged meat was stored at 3°C for 4 weeks. Muscle samples (2.5 cm thickness) from fresh and aged meat were displayed under refrigerated conditions (~3-4°C) for four days with fluorescent lights set at 1500 Lux. Fresh and aged meat samples collected after 4 days retail display were used for the determination of lipid oxidative substances. The lipid oxidation in meat was assessed by the thiobarbituric acid reactive substances (TBARS) procedure, expressed in mg of malondialdehyde (MDA) per kg of muscle [6].

Preliminary analyses using summary statistics and scatterplot matrices were carried out to check for data integrity and examine possible relationships between the biochemical variables in muscle. This was followed by more formal statistical methods, namely, restricted maximum likelihood (REML) regression and a more advanced graphical technique known as shingle plots. In the regression analysis, the fixed effects in the full model represented nine terms including all the explanatory variables (vitamin E, heme iron, nonheme iron, total n-3 fatty acids, total n-6 fatty acids and total fatty acid), the covariate terms (initial liveweight and sex) and the treatment term (diets). Shingle plots were used to investigate the relationship between TBARS and other variables by conditioning on vitamin E. The opposite was also done by plotting TBARS against vitamin E and conditioning on other variables. All variables were conditioned by creating 4 shingles or classes of equal count, with 10% overlap between adjacent intervals.

III. RESULTS AND DISCUSSION

The TBARS from fresh meat collected after 4 days of display fell within a narrow range (0.13-0.82 mg MDA/kg meat) and showed no relationship with the variables tested, but TBARS from aged meat showed a wide range of values and had a significant relationship with other biochemical variables. From the scatterplot matrix, lipid oxidation of aged meat appeared to be negatively related to vitamin E and positively related to total n-6 fatty acids, but no relationship was found with total n-3 fatty acids or other variables. When regression analysis was performed, of the variables tested, only vitamin E ($P<0.002$), heme iron ($P<0.05$), total n-6 fats ($P<0.01$) and initial liveweight ($P<0.05$) were significant, indicating that lipid oxidation (TBARS) was not affected by total n-3 fatty acid level.

A simplified model, limited to the significant terms, was fitted to the data. The results showed that the closest competing model was the model that included vitamin E, total n-6 fats and liveweight, which explained 57% of the variation as opposed to 63% by the parsimonious model (model not shown). Of the models that explained greater than 41% of the total variation, all had vitamin E as a term in the model with vitamin E explaining itself 41.2% of variation.

Regression analysis clearly demonstrated that vitamin E in meat explained most of the effect of diet on lipid oxidation of meat. When total n-6 fats and heme iron were included in the model with vitamin E (51.5%), this only explained an extra 10% of the variability as compared with fitting a model containing only vitamin E.

To further examine the effect of vitamin E on lipid oxidation, shingle plots were used to investigate the relationship between TBARS and other variables by conditioning on vitamin E. The shingle plot shown in Figure 1 exhibited a strong positive relationship between heme iron and lipid oxidation when vitamin E was low (bottom left hand panel), that is when vitamin E was below 2.95 mg/ kg muscle. However when vitamin E was above 2.95 mg/ kg muscle, the positive relationship was not evident and became a random scatter implying that vitamin E influences the interaction between lipid oxidation (TBARS) and heme iron.

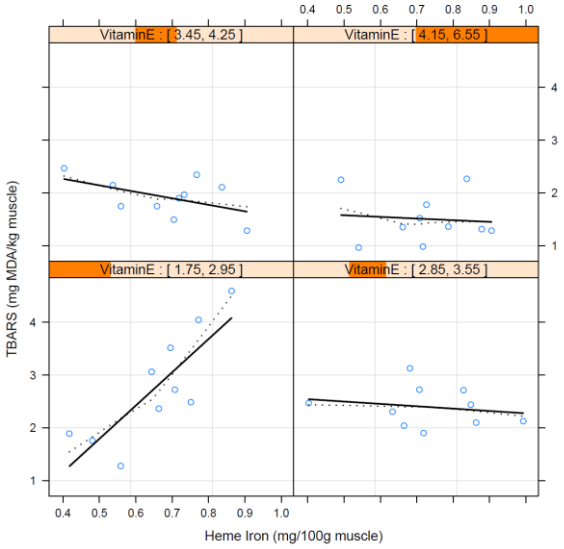

Figure 1. Lipid oxidation (TBARS) (mg MDA/kg muscle) versus heme iron (mg/kg muscle) conditioned on vitamin E.

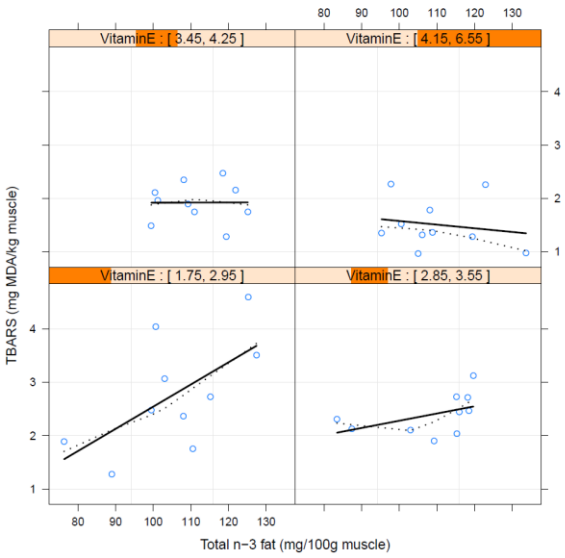

Figure 2. Lipid oxidation (TBARS) (mg MDA/kg muscle) versus total n-3 fatty acids (mg/100 g muscle) conditioned on vitamin E.

This effect was also evident for total n-6 fatty acids and total n-3 fatty acids, which showed a strong positive relationship between total n-6 fatty acids and lipid oxidation (not shown) or total n-3 fatty acids and lipid oxidation (Figure 2) when vitamin E was low (bottom left hand shingle plot), that is when vitamin E was below 2.95 mg/ kg muscle. However, when muscle vitamin E concentration was above 2.95 mg/ kg muscle, the positive relationship between total n-3 fatty acids and lipid oxidation (Figure 2) or total n-6 fatty acids and lipid oxidation (not shown) disappeared and became random scatter.

In many studies, muscle PUFA (n-3 or n-6 fatty acids) concentrations were claimed to be the major cause of lipid oxidation, while vitamin E concentration was not considered or factored in the analysis [e.g. 7]. In this context, we state that if muscle vitamin E content was lower than 2.95 mg/ kg muscle, one would extrapolate that muscle PUFA was the reason for lipid oxidation in muscles, as was found in the present study.

From the results of this study it is evident that the relationship between lipid oxidation and PUFA was substantially reduced when the muscle vitamin E concentration was above 2.95 mg/ kg muscle. It is worth noting that when the vitamin E concentration was between 2.95-3.55 mg/ kg muscle, lipid oxidation (assessed by TBARS) was reduced to below 3 mg MDA/kg muscle while at vitamin E concentrations above 4.15 mg/ kg muscle, the oxidation was further reduced to below 2 mg MDA/kg muscle irrespective of PUFA concentrations. Therefore, dietary management to elevate muscle vitamin E concentration is beneficial to minimise quality deterioration through lipid oxidation. It also clearly shows that vitamin E must be considered as a factor in lipid oxidation when the effect of muscle PUFA concentration is related.

From the shingle plots, there is ample evidence that vitamin E is the main driver of lipid oxidation in meat. Neither n-3 fatty acids nor other variables tested had a positive relationship with lipid oxidation. Rather, they all had a negative relationship with lipid oxidation when vitamin E was included in the analysis. Lipid oxidation appeared to be negatively related to vitamin E, that is, as the level of vitamin E increased, the level of lipid oxidation (TBARS) decreased and this appeared to be true for all

levels of heme iron or n-3 or n-6 fatty acids. Careful interpretation must be undertaken when the interaction between lipid oxidation and n-3 fatty acids in relation to muscle quality deterioration or off flavour development is under examination in the absence of vitamin E and/or heme iron in the model.

IV. CONCLUSION

The results clearly demonstrate that vitamin E is the major muscle component affecting lipid oxidation in meat. When muscle vitamin E concentration was lower than 2.95 mg/ kg muscle, other variables such as n-6 fatty acids or heme iron can override the control mechanism of vitamin E on lipid oxidation. Data show that n-3 fatty acids did not cause muscle quality deterioration of lipid oxidation in aged meat stored for four weeks under refrigerated conditions, provided vitamin E levels are above the 2.95 mg/ kg muscle.

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REFERENCES

1. Simopoulos A. P. (2002). Review: Omega-3 fatty acids in inflammation and autoimmune diseases. Journal of the American College of Nutrition 21: 495-505.
2. Sampath, H. & Ntambi, J. M. (2005). Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annual Review of Nutrition 25: 317-340.
3. Fang, Y., Yang, S. & Wu, G. (2002). Free Radicals, Antioxidants, and Nutrition. Nutrition 18: 872–879.
4. Morrissey, P. A., Sheehy, P. J. A., Galvin, K., Kerry, J. P. & Buckley, D. J. (1998). Lipid stability in meat and meat products. Meat Science 49: 73–86.
5. Ponnampalam, E. N., Butler, K. L., McDonagh M. B., Jacobs J. L. & Hopkins, D. L. (2012). Relationship between muscle antioxidant status, forms of iron, polyunsaturated fatty acids and functionality (retail colour) of meat in lambs. Meat Science 90: 297–303.
6. Witte, V. C., Krause, G. F. & Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. Journal of Food Science 35: 582–585.
7. Díaz, M.T., Cañeque, V., Sánchez, C. I., Lauzurica, S., Pérez, C., Fernández, C., Álvarez, I. & Fuente, J. D. (2011). Nutritional and sensory aspects of light lamb meat enriched in *n*−3 fatty acids during refrigerated storage. Food Chemistry 124: 147–155.