# ISOPROSTANES: POTENTIAL BIOMARKERS OF OXIDATIVE STRESS IN MUSCLE

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**Abstract – This study investigated the potential use of blood isoprostane as a biomarker in prime lambs to predict the oxidative status of meat. Eighty-four cross-bred lambs (10 weeks of age) were randomly allocated to four finishing diets in paddocks. The treatment groups were: lucerne pasture (Lucerne); annual ryegrass pasture (Ryegrass); feedlot pellets (Feedlot); combination of annual ryegrass pasture and feedlot pellets (RyeFeedlot). Before adaptation to diets (week 0) and 4, 6 and 8 weeks (prior to slaughter), blood samples were collected from all lambs for isoprostane measurements. Finishing diets affected ($P < 0.01$ and $P < 0.05$) isoprostane concentration in blood collected after 4 and 8 weeks of feeding. Lipid oxidation was lowest for ryegrass, highest for feedlot and modest for the other two treatments ($P < 0.001$). Blood isoprostane collected at 8 weeks of feeding was positively ($P < 0.01$) related to lipid oxidation of meat after 96 h of retail display. Isoprostane concentration in blood can be a useful marker for detecting oxidative stress in farm animals and the quality of meat at the retail point of sale. This is the first report to identify a link between circulating oxidative stress in meat producing animals and a biochemical component in meat that can affect its eating quality.**

## I. INTRODUCTION

Producing lambs extensively year round is challenging due to many constraints, which affects the cost of production and quality of meat through supply chains. Oxidative stress in humans or animals is characterised by an imbalance between increased exposure to free radicals, which can be generated endogenously (eg, acute or chronic diseases) or from exogenous sources (eg, environmental effect) [1], and inadequate antioxidant defence systems.

Farm animals can undergo several challenges, such as a poor immune status, imbalance in feed nutritive values, and heat/cold stress, and these can all influence the oxidative status of individual animals to various degrees. Any variation in the oxidative status of the animal can induce the formation of free radicals, which can lead to further damage to DNA, lipids, and protein [2]. It is logical to think that the oxidative processes in meat across the supply chain can be related to the oxidative stress status of animals on-farm which is mostly linked to changes in lipids or other chemical components in tissues. A major consequence of oxidative stress is lipid oxidation, which is enhanced by the presence of polyunsaturated fatty acids (PUFA) and absence of antioxidants [3, 4].

Consumer choice when purchasing lamb can be influenced directly by the appearance at point of retail sale or indirectly from the perception of the texture and taste/flavour of meat when consumed. Oxidation of meat occurs under post-mortem storage conditions and is inevitable. This oxidation includes biochemical changes in meat leading to changes in lipids mainly PUFA. Consequently undesirable flavours and rancidity develop in meat thereby impacting on consumer satisfaction and perception of meat quality [5]. There have been several methods used to identify the oxidative stress status of humans and laboratory animals, but to-date none are available to assess the oxidative stress of farm animals and relating this to oxidative processes

of muscle and lipid oxidation in meat. A recent review [6] indicated that isoprostanes have the potential to be used as biomarkers in farm animals to assess the oxidative status *in vivo* and relate this to biochemical components in muscle tissue post-mortem and, therefore, offer potential for the prediction of meat quality pre-slaughter. The objective of this study was to investigate the potential use of blood isoprostanes as a biomarker in prime lambs to predict the oxidative status of meat at the retail level.

## II. MATERIALS AND METHODS

Eighty-four second cross lambs (Poll Dorset X Border Leicester Merino) raised on ryegrass pasture with their mothers until weaning (10 weeks of age) were used in this study. The lambs were randomly allocated to four finishing diets (*ad libitum*) in paddocks with two weeks of adaptation to the diets. The treatment groups were: 1) Lambs finished on lucerne pasture (n = 24, **Lucerne**); 2) Lambs finished on annual ryegrass with sub clover pasture (n = 18, **Ryegrass**); Lambs finished on standard commercial feedlot pellets (n = 24, **Feedlot**); Lambs finished on a combination of annual ryegrass based pasture (as in treatment 2) and feedlot pellets (first 2 weeks at 300 g/day/head and the remaining 4 weeks at 500 g/day/head, n = 18, **RyeFeedlot**).

Before adaptation to diets (week 0), and then at 4, 6 and 8 weeks (prior to slaughter), blood samples [10 mL] were collected from all lambs via jugular venapuncture using lithium heparin vacutainer tubes and kept on ice at 4°C for 2 h until centrifugation. Following centrifugation at 3000 x *g* for 10 minutes, plasma was separated into 3 aliquots and stored at -80°C for analysis of $F_2$-isoprostanes (8-isoprostaglandin $F_{2\alpha}$) using a commercially available EIA assay (Sapphire Bioscience, Waterloo, Australia), following the manufacturers protocol. After 8- weeks of feeding that included 2 weeks of adaptation, lambs were transported to a commercial abattoir and slaughtered after 18 h of fasting. At 24 h post-mortem the *longissimus lumborum* (LL, loin) muscle was collected for retail colour (shelf life) assessment over 4 days of display under simulated retail conditions. At the end of retail colour measurement (96 h), all samples were collected and stored at -20°C for determination of lipid oxidation. The lipid oxidation in meat was assessed by the thiobarbituric acid reactive substances (TBARS) procedure and the results were expressed in mg malondialdehyde (MDA)/kg of meat.

The treatment means for isoprostane concentration at different bleed times and MDA concentrations in meat collected at 96 h retail display were analysed by restricted maximum likelihood (REML) mixed model analyses that included a priori random effect for paddocks within treatment. To examine the relationship between lipid oxidation in meat at 96 h of display and the measurements of isoprostanes at different bleeding times, a series of similar REML mixed models were used that included a priori random effect for paddocks within treatment. In these REML analyses, the experimental unit was measurement taken from individual lambs. For each analysis, a residuals versus fitted values plot was examined to determine any extreme outliers.

## III. RESULTS AND DISCUSSION

There were no treatment differences on isoprostane concentration for blood collected at the commencement of study (week 0), and at 6 weeks of feeding the experimental diets. Finishing diets significantly affected ($P < 0.01$ and $P < 0.05$) isoprostane concentration in blood collected after 4 and 8 weeks of feeding the experimental diets (Figure 1). Lambs on the ryefeedlot treatment had the highest concentration and lambs on the feedlot diet had the lowest concentration of isoprostane in their blood after 4 weeks of feeding, but after 8 weeks of feeding, lambs fed the feedlot diet had the highest concentration of isoprostane while lambs fed the lucerne pasture had the lowest concentration and ryegrass and ryefeedlot treatments had intermediate values.

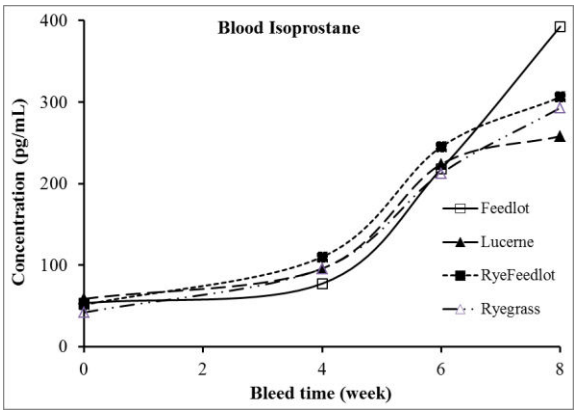


**Figure 1**. $F_2$-isoprostanes (8-isoprostaglandin $F_{2\alpha}$) concentration in blood from lambs fed lucerne, ryegrass, ryegrass with pellets (RyeFeedlot) and a feedlot ration. Blood samples were collected at commencement (before adaptation to diets (Time = 0)), after 4, 6 and 8 weeks of feeding the experimental diets.

There were significant treatment differences in lipid oxidation of the lamb samples at 96 h of simulated retail display ($P < 0.001$; SED = 0.20) (Figure 2). Lipid oxidation was lowest for ryegrass, highest for feedlot and modest for the other two treatments (Figure 1).

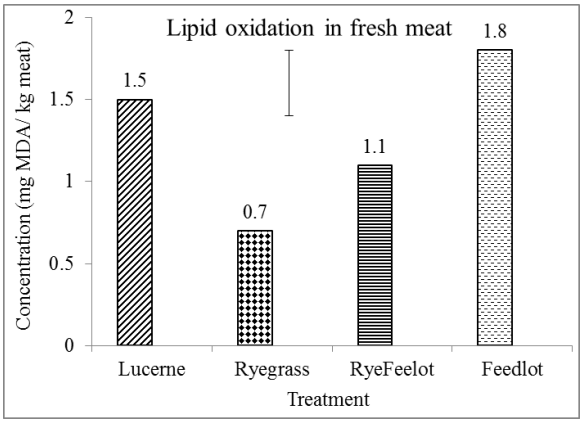


**Figure 2**: Lipid oxidation in fresh meat (5 day post-mortem LL) from lambs fed lucerne, ryegrass, ryegrass with pellets (RyeFeedlot) and a feedlot ration. Muscle LL samples were measured at 96 h of simulated retail display at 3-4°C.

There were no relationships observed between isoprostane and lipid oxidation for samples collected at commencement (Time = 0), 4 and 6 weeks of feeding. Lipid oxidation of meat in the simulated retail display was linearly related ($P < 0.004$) with isoprostane concentration in blood collected after 8 weeks of feeding (i.e., blood samples collected in the paddock 2 days prior to slaughter) (Figure 3).

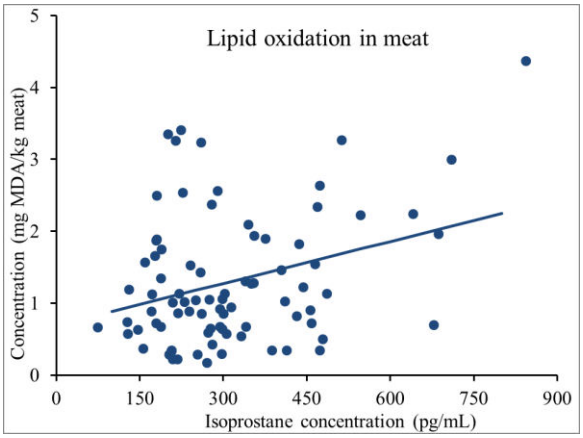


**Figure 3**. Relationship between $F_2$-isoprostanes (8-isoprostaglandin $F_{2\alpha}$) concentration in blood from lambs fed lucerne, ryegrass, ryegrass with pellet (RyeFeedlot) and a feedlot ration. Data from blood samples collected after 8 weeks of feeding the experimental diets.

There is no estimated value for losses due to meat discolouration and undesirable flavour for the Australian meat industry, but these two factors in addition to tenderness have universally been associated with decision making and the purchase of meat. The stability of colour and the flavour of lamb meat are dictated by the rate of oxidative processes post-slaughter, which are regulated by the antioxidant capacity and the reducing systems of muscle tissues at slaughter. The latter can be influenced by nutritional background, genetics, chiller management and packaging of which diet has the major effect. Several studies have indicated lipid oxidation of meat at retail display or during storage was influenced by PUFA and affected flavour due to the formation of rancidity or secondary compounds in meat [5] while others reported lipid soluble antioxidants incorporated (eg, vitamin E) in muscle membranes are the major factor that slows down or avoids oxidation by quenching free radicals produced by post-mortem processing and storage of meat [4].

The results show that finishing diets affected the oxidative stress of lambs as assessed by isoprostane concentration in blood and lipid oxidation of meat during retail display. These latter effects could be due to levels of PUFA and/or vitamin E concentration in the muscles, which need further investigation.

The interesting and main outcome of the present study was the relationship between oxidative stress and lipid oxidation in meat after retail display. This implies that flavour/aroma deterioration due to the lipid oxidation process in meat post farm gate can be detected by measuring the isoprostane concentrations in live animals on farm. This provides valuable information for devising on-farm dietary interventions that would avoid the production of meat with inferior flavour and aroma due to lipid oxidation at the retail level. This is the first investigation reported in farm animals and needs further investigation with larger numbers of animals covering different species, genetics and seasons.

## IV. CONCLUSION

Finishing diets significantly affected isoprostane concentration of blood in prime lambs at 4 and 8 weeks of feeding, but not at 2 or 6 weeks. After 8 weeks, isoprostane concentration was lowest in lambs fed lucerne, highest in lambs fed a feedlot diet and modest in animals fed ryegrass and ryefeedlot treatments. Lambs fed the feedlot diet had the greatest lipid oxidation and ryegrass had lowest and others had intermediate levels. This could be due to variation in antioxidant (vitamin) E concentration or PUFA concentration, which needs further investigation. Blood isoprostane measured in lambs on-farm was significantly related to muscle lipid oxidation. This is the first report to identify a link between oxidative stress in meat producing animals and biochemical component in meat that can affect its eating quality. Isoprostane concentration in blood can be a useful marker for detecting oxidative stress in farm animals and quality of meat at point of retail sale. The findings from this study provide valuable information for devising on-farm dietary interventions that would avoid flavour and aroma deterioration of meat due to lipid oxidation at point of retail sale.

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