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Health beneficial long chain omega-3 fatty acid levels in Australian lamb managed under extensive finishing systems

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ABSTRACT

The variation in levels of the health claimable long chain omega-3 fatty acids, eicosapentaenoic acid (EPA, 20:5n-3) plus docosahexaenoic acid (DHA, 22:6n-3) across production regions of Australia was studied in 5726 lambs over 3 years completed in 87 slaughter groups. The median level of EPA plus DHA differed dramatically between locations and sometimes between slaughters from the same location. The ratio of EPA plus DHA from lambs with high values (97.5% quantile) to lambs with low values (2.5% quantile) also differed dramatically between locations, and between slaughters from the same location. Consistency between years, at a location, was less for the high to low value ratio of EPA plus DHA than for the median value of EPA plus DHA. To consistently obtain high levels of omega-3 fatty acids in Australian lamb, there must be a focus on lamb finishing diets which are likely to need a supply of α-linolenic acid (18:3n-3), the precursor for EPA and DHA.
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1. Introduction

The value of meat to the purchaser is mostly dependent on eating quality, keeping quality and nutritional value. All these can be influenced by nutrition and the genetic background (Warner, Greenwood, Pethick, & Ferguson, 2010) and the former two also by chilling and processing methods (Hopkins, 2010). Fatty acids in meat, along with minerals including trace elements and vitamins, are vital components that contribute to the nutrient value of meat in terms of physiological and biochemical functions. These components can be influenced by genetics and nutrition. Among fatty acid major groups, polyunsaturated fatty acids such as omega-3 (n-3) and omega-6 (n-6) in foods have been highlighted due to their anti- and pro-effects on inflammatory and autoimmune diseases, respectively (McAfee et al., 2010; Palmquist, 2009; Simopoulos, 2002).

According to Australian standards, to claim meat as a source of omega-3, it needs to have 30 mg of long chain omega-3 fatty acids per 100 g of meat in the form of eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). By contrast, the European standard for a source of omega-3 is 40 mg per 100 g (Commission Regulation (EU), 2010). Lamb production in Australia and some other countries is primarily based on year round extensive finishing systems. Under these finishing systems, animals are often finished on widely differing diets. These diets include both irrigated and dryland pasture, green and senesced pasture as well as feed supplements including hay, grain legumes, cereal grains, oil seed by-products or crop residues (e.g. Dixon & Stockdale, 1999; Hopkins, Beattie, & Pirlot, 1995; Hopkins, Holst, & Hall, 1995). It is known that diet has a major effect on polyunsaturated fats in meat (Daley, Abbott, Doyle, Nader, & Larson, 2010; Simopoulos, 1999). Pannier et al. (2010) reported that extensive lamb production sometimes produces meat with high levels of long chain omega-3 fatty acids, but not always. This is not surprising because of the wide range of finishing diets used in extensive grazing systems.

Ponnampalam et al. (2014) showed that, under production systems that finish lambs at similar live weights, the largest systematic sources of variation in EPA plus DHA concentrations were 1) variation between location and slaughter time within location and 2) differences between slaughter times in the amount of between animal variation. Other

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sources of systematic variation such as genetics (sire, dam and breed effects), rearing type (singles versus multiples) and gender were much smaller. We would suggest that, from a practical point of view, these latter sources of variation of EPA plus DHA can be ignored when finishing lambs in extensive grazing systems.

The present paper documents 1) variation between location and slaughter times within location and 2) differences between slaughter times in the amount of between animal variation for EPA plus DHA. It covers 87 slaughter times from 3 matings (across 3 years) of 8 commercial-like lamb production locations in the wheat-sheep and high rainfall grazing zones (ABARE, 2003) of Australia. At each slaughter time the lambs were slaughtered at a similar target carcass weight (about 21–22 kg). The present study discusses the characteristics of those locations that were able to maintain higher levels of EPA plus DHA over a 3 year production period (2007–2010).

2. Materials and methods

2.1. Experimental design and sample collection and measurement

This large research study was approved by 5 respective Animal Experimentation Committees across 4 states of Australia. van der Werf, Kinghorn, and Banks (2010) describe fully the design of the experiment from which the lambs were sourced for the current work, including the procedure used to select the sires for AI mating with the flocks' base ewes. Sires were selected from a range of breeds used in the Australian sheep industry (Merino, maternal and terminal meat breeds). The base ewes, depending on the research site usually consisted of approximately 80% Merino ewes and 20% Border Leicester × Merino ewes. Lambs were generally maintained under extensive pasture conditions at 8 lamb production sites, but were fed grain, hay or feedlot pellets when the supply was limited (Table 1). In Table 1 early post-weaning is the feed availability to lambs during the first half of the period since lambs were separated from dams before slaughter and late post-weaning is the feed availability to lambs during the second half of the period before slaughter, respectively. Lambs were slaughtered in three consecutive years, with between 28 and 30 kills in each year. The slaughter procedure has been reported elsewhere (Pannier, Pethick, Geesink, Ball, Jacob, & Gardner, 2014). In brief, during the years 2008–2010, *longissimus lumborum* (LL) muscle samples from approximately 5726 lambs were collected at 24 h post-mortem. There were several slaughter occasions (kills) at each location in each year (Fig. 1).

These LL samples (~20 g) were dissected without any visible external fat (subcutaneous), freeze dried and ground using a FOSS Knifetech™ 1095 sample mill (FOSS Pacific, Unit 2, 112–118 Talavera Road, North Ryde, NSW 2113). The same grinding equipment was used across all laboratories. A homogeneous 0.5 g ground sample was used for fatty acid extraction, methylation and quantification by gas chromatography as described by Ponnampalam et al. (2014), a rapid modified procedure developed from the method reported by O'Fallon, Busboom, Nelson, and Gaskins (2007). One hundred μL of nonadecanoic acid methyl ester (C19:0, Sigma Aldrich Pty Ltd, Castle Hill, NSW 2154, Australia) was added to muscle samples as an internal standard dissolved in chloroform (10 mg C19:0/mL $CHCl_3$). The contents were hydrolysed using 0.7 mL of 10 N KOH in water and 5.3 mL of methanol to form free fatty acids. After mixing well with a vortex, the contents were incubated at 55 °C for 1.5 h, with vigorous mixing at 20 min intervals and then cooled to room temperature using tap water. Upon cooling, the contents were mixed with 0.6 mL of 24 N sulphuric acid in water and the mixing, incubation and cooling process occurred as above. After cooling the tubes to room temperature, the fatty acid methyl ester (FAME) was separated with 1 mL of hexane solvent by mixing for 5 min and centrifuging at 2000 rpm for 10 min. Two hundred μL of hexane containing FAME was collected into a Gas Chromatograph (GC) vial and fatty acid fractions were quantified by capillary GC (HP INNOWAX 60 m × 0.25 mm, 0.5 μm, Agilent J & W Scientific, Santa Clara, CA, USA).

Samples collected from the 8 sites were systematically allocated in order of sample to three laboratories for sample processing and fatty acid determination. For 2008 born progeny, muscle samples were distributed to three laboratories (Department of Primary Industries, Victoria; Food & Agriculture, Western Australia; and Commonwealth Scientific International Research Organisation, Western Australia) for fatty acid determination. For 2009 and 2010, samples were analysed at the latter two laboratories. Each laboratory followed the same procedures, columns and temperature setup. Calibration was achieved by testing the same pool sample 10 times each year. A variation of less than 5% between laboratories was maintained in the current study. All fatty acid peaks were identified using a reference standard (Supelco C4–C24 mix, Sigma Aldrich Pty Ltd, NSW 2154, Australia), which was run in each batch. Fatty acid levels in the muscles are reported in mg/100 g meat. Before reporting, fatty acid traces from each year were tabulated, tested for outliers, and analysed for predicted means of EPA (20:5n-3) and DHA (22:6n-3). The total amount of EPA and DHA (EPA plus DHA) was calculated as the sum from the total fatty acid profiles of GC quantification.

2.2. Statistical analysis

Our approach was to firstly develop a statistical model for EPA plus DHA that took into account the variances and covariances that are induced by the effects of sires, dams and the between lamb variation. It was important to allow the between lamb variation to differ with each slaughter time within each location (Ponnampalam et al., 2014). Using this statistical model, the median value of EPA plus DHA at each location and slaughter time was calculated; this represents the value of EPA plus DHA for a typical lamb. The model was further used to calculate, for each site and location, the ratio of a large EPA plus DHA lamb (with only 2.5% of lambs having a greater value) compared to a small EPA plus DHA lamb (with only 2.5% of lambs having a lower value). For instance, if lambs with greater EPA plus DHA concentrations had twice the level of EPA plus DHA than lambs with lower EPA plus DHA concentrations then the ratio will be 2. Finally the ratio for each location and slaughter time was plotted against the median for each location and slaughter time, which allows identification of site and slaughter time combinations for which a *typical* lamb has EPA plus DHA > 23 mg/100 g (which is equivalent to >30 mg per 135 g serving) and site and slaughter time combinations for which *most* lambs have EPA plus DHA > 23 mg/100 g. Details of these analyses are given in the next two paragraphs.

The natural logarithm of EPA plus DHA was analysed as a restricted maximum likelihood (REML) model with a fixed effect for each location by slaughter time combination, random effects for sire identity and dam identity and a separate residual variance for each location by slaughter time combination. A logarithmic transformation was chosen so that the between sire variance was similar at kills with both low and high values of EPA plus DHA. Chi square change in deviance tests were used to examine the necessity of including various effects in the model. Only one of 5726 lambs with necessary measurements was excluded as a statistical outlier.

The median EPA plus DHA concentration for each location by slaughter time combination was obtained from the predicted mean value on the logarithmic scale and back-transforming. Asymptotic normal confidence intervals were calculated on the logarithmic scale and then back transformed to the original scale. The residual variation for each location by slaughter time combination is summarised as the ratio of EPA plus DHA from lambs with high values (97.5% quantile of EPA plus DHA) to lambs with low values (2.5% quantile of EPA plus DHA). The ratio is calculated as $\exp(2 \times 1.96 \times \sigma)$, where exp denotes the exponential function and σ is the estimated residual standard deviation obtained for a location by slaughter time combination using the REML analysis. Confidence

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