THE RELATIONSHIP BETWEEN PRODUCT (EPA & DHA) AND PRECURSOR (ALA) OMEGA-3 FATTY ACIDS IN LAMB

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Abstract – This study investigated the relationship between precursor (alpha linolenic acid, ALA) and products (eicosapentaenoic, EPA and docosahexaenoic, DHA acids) omega-3 fatty acid in muscle, for lamb flocks from different production sites in Australia. *Longissimus lumborum* (LL) muscle samples from approximately 1950 lambs were collected at 24 h post-mortem, freeze dried and a homogenous sample of 0.5 mg was used for the determination of fatty acid concentration. Fatty acid concentrations of ALA, linoleic acid (LA), EPA and DHA were used to understand the relationship between parent ALA and product EPA plus DHA with different levels of LA present. EPA plus DHA concentration increased in the muscles as ALA concentration increased. EPA plus DHA showed a response to ALA levels as mediated through desaturase enzymatic conversion up to a level of around 80 mg/ 100 g muscle. The results also indicate that the conversion of EPA plus DHA from ALA in the muscle can be inhibited by the level of LA present in the muscles, especially with LA at high levels (> 150 mg/ 100 g muscle).

Key Words – Competition between LA and ALA, Enzymatic desaturation and Elongation, Long chain omega-3 fatty acid in meat

I. INTRODUCTION

In nature there are two forms of omega-3 fatty acids: short-chain omega-3 fatty acids (SCOFA) and long chain omega-3 fatty acids (LCOFA). Alpha linolenic acid is a SCOFA while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are LCOFA. The SCOFA such as ALA are very common in nature, found in plants, nuts and oilseeds while LCOFA are found in marine based products [1]. Among omega-3 fatty acids, ALA is considered as an essential fatty acid because this fatty acid cannot be synthesized by human or animal bodies and therefore must be supplied by dietary sources. ALA is converted to EPA and DHA in the body [2] but the efficiency of conversion varies due to factors such as dietary type, sex and genetics. One of the influencing factors from the diet that affects the conversion of ALA to EPA and DHA is another polyunsaturated fatty acid called linoleic acid (LA), since there is competition for an enzyme that converts both ALA to EPA & DHA and LA to arachidonic acid (AA) [3]. LA is an omega-6 fatty acid, which cannot be synthesized by human or animal bodies, and thus must also be supplied by the diet. While there is strong evidence that EPA and DHA have important roles in brain and visual function, human health, and welfare, ALA does not have the same benefits as its LCOFA counterparts [4]. This paper investigates the product (EPA and DHA) and precursor (ALA) relationship in lamb muscle, and how this relationship is affected by the LA in the muscle, for lamb flocks from different production sites in Australia.

II. MATERIALS AND METHODS

Within the Australian sheep industry CRC meat program approximately 10,000 lambs produced in the major production sites of Australia have been slaughtered over five years. The design of the Information Nucleus, including the procedure used to select the sires for AI mating with the flocks' base ewes has been described [5]. 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 x Merino ewes. Lambs were generally maintained under extensive pasture conditions at the 8 lamb production sites, but were fed grain, hay or feedlot pellets when the pasture supply was limited. Lambs were slaughtered at 28-30 kills in each year. The slaughter procedure has been reported elsewhere [6]. In particular, during the years 2008-2009, *longissimus lumborum* (LL) muscle samples from approximately 1950 lambs were collected at 24 h post-mortem.

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 [7]. Samples collected from the 8 sites were systematically allocated in order of sample to two laboratories for sample processing and fatty acid determination. 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. The total amount of EPA and DHA (EPA plus DHA) was calculated as the sum from the total fatty acid profiles of GC quantification.

We have previously presented a REML mixed model that jointly related the logarithm of EPA plus DHA in the LL muscle of slaughtered lambs to lifetime factors of those lambs, production site and slaughter within production site [7]. Our approach was to modify this model to develop a parsimonious model that also includes effects related to ALA and LA in the LL muscle. Terms were excluded or included in a model using chi-squared change in deviance tests for random effects and Wald F tests for fixed effects. The final parsimonious model for the logarithm of EPA plus DHA was of the same form as the model given previously [6] except that, in addition, there was a separate linear response to the logarithm of ALA for each slaughter of each site, there was a separate linear response to the logarithm of LA for each slaughter of each site, there was a response to the product of the logarithm of ALA and the logarithm of LA, and there was a response to age of lamb at slaughter (Table 1). In addition the gender effect was excluded as it was no longer statistically significant ($P = 0.89$) when terms for ALA and LA were included. The responses of EPA plus DHA to ALA and LA are presented for the first slaughter at each site. These responses are shown for lambs that were typical for the slaughters presented. Thus results are presented for Poll Dorset sires and Merino dams (cross bred dams at Rutherglen because Merino dams were not present at the site), for single lambs at Trangie, Hamilton, Struan and Turretfield, multiple lambs at Cowra, Trangie, Rutherglen and Katanning, and for the median age of lambs at a slaughter. Confidence intervals were calculated on the log scale for EPA plus DHA, and back transformed.

III. RESULTS AND DISCUSSION

The relationship between the precursor (ALA) and the product (EPA plus DHA) is presented for lambs slaughtered in the first kill, at each site (Fig 1 to 8). Although only the first slaughter at each site is presented due to space restrictions, the responses at the first slaughter are typical of responses at other slaughters. Generally as ALA increases in the muscle, the elongation products, EPA plus DHA, considerably increase in the muscle up to around 80 mg/ 100 g muscle. At greater levels of ALA, there was minimal response of EPA plus DHA to ALA. Similar to other reports [1,2], the level of LA present in meat appeared to have an influence on the conversion of ALA in the production of its longer chain omega-3 metabolites EPA and DHA. At a number of sites and slaughters, and especially when LA was more than 150 mg/100 g of muscle, the production of EPA plus DHA from ALA was significantly reduced with greater LA. The influence of LA might be due to the competition between ALA and LA for the same enzymes delta-6 desaturaze and delta-5 desaturaze. The results from the analyses in this study were also found in similar analysis

examining the product-precursor relationship in LL muscle from animals slaughtered in the first year (2007-2008) of the Information Nucleus study (data not shown).

Table 1 Tests for terms included in final parsimonious model. All terms are adjusted for other terms in the model.

Terms	Degrees of Freedom	P-Value
Terms Included		
Sire identity	1	3.2×10^{-15}
Dam identity	1	0.0044
Residual variance differs between slaughters at the same site	21	8.0×10^{-9}
The difference between single and multiple reared lambs is different for each site	7, 526.5	0.0016
Sire breed effect differs with site	39, 784.6	0.00024
Interaction of sire breed with dam breed	5, 333.5	0.0015
Age at slaughter	1, 941.9	0.015
Log(ALA) response differs between slaughter at the same site	21, 474.8	1.0×10^{-7}
Log(LA) response differs between slaughter at the same site	21, 450.5	0.0078
Product of log(ALA) and log(LA)	1, 1314.7	0.0015

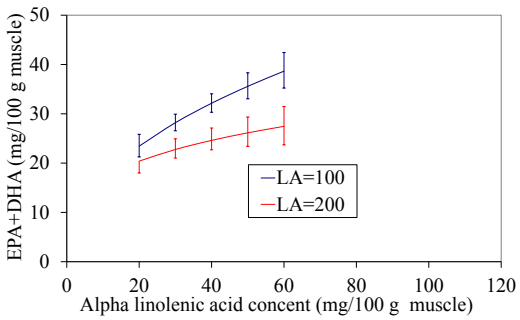

Figure 1: Kirby – New South Wales

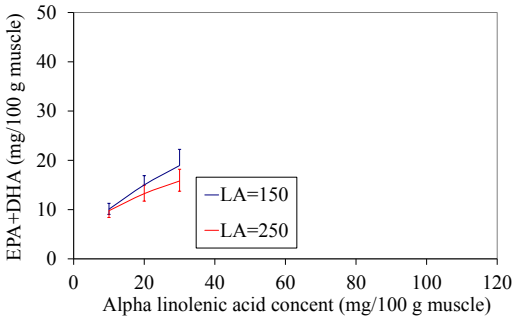

Figure 2: Katanning – Western Australia

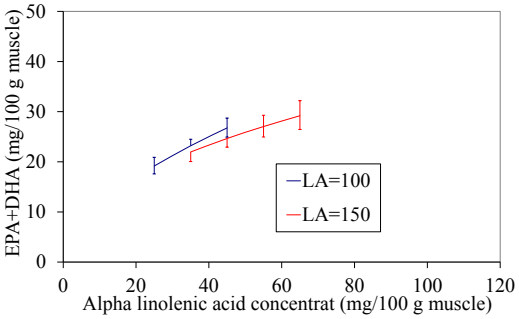

Figure 3: Trangie – New South Wales

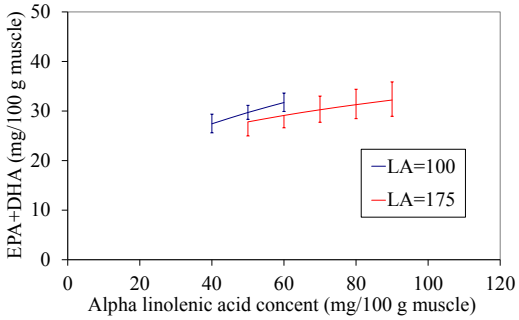

Figure 4: Cowra – New South Wales

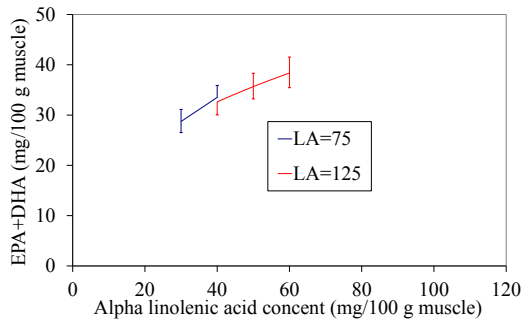

Figure 5: Rutherglen – Victoria

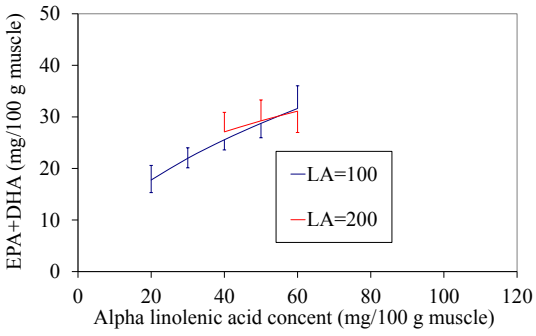

Figure 6: Hamilton – Victoria

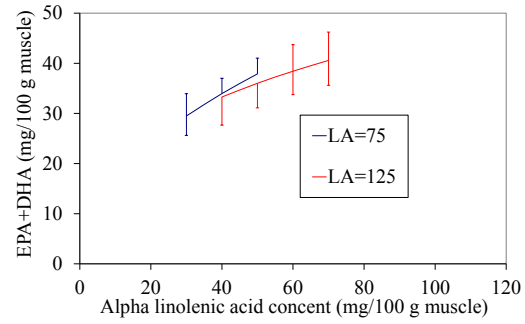

Figure 7: Struan –South Australia

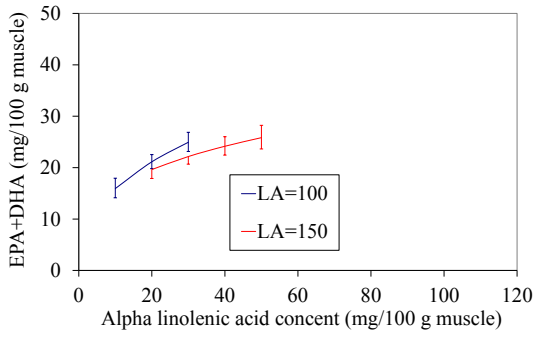

Figure 8: Turretfield –South Australia

IV. CONCLUSION

The production of EPA plus DHA is related to the level of ALA in the muscle tissues. Increasing the ALA content in muscle through feeding systems up to around 80 mg/100 g muscle is associated with considerable improvement in the EPA plus DHA. There is minimal response to ALA above this level. The production of EPA plus DHA through enzymatic conversion of ALA appears to be inhibited by the levels of LA in muscle tissues, particularly at concentrations above 150 mg LA/100 g of muscle.

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