

# THE IMPACT OF SUPPLEMENTING LAMBS WITH ALGAE ON MEAT TRAITS AND OXIDATIVE STATUS

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**Abstract – The current study examined the effect of supplementing lambs with algae. Forty lambs, three month old, were allocated to receive a control ration based on oats and lupins (n = 20) or the control ration supplemented with DHA-Gold™ algae (n = 20). These lambs came from dams previously fed a ration based on either silage (high in omega-3) or oats and cottonseed meal (OCSM: high in omega-6) at joining (dam nutrition, DN). The concentration of health claimable omega-3 fatty acids (EPA + DHA) was significantly higher in the LL of lambs fed algae (125 ± 6 mg/100 g meat) compared to those not fed algae (43 ± 6 mg/100 g meat) and this effect was mediated by DN. Supplementing with algae high in DHA improves the health status of lamb meat.**

**Key Words –lamb, muscle, algae, fatty acids.**

## I. INTRODUCTION

The concentrations of health claimable long chain omega-3 fatty acid content in Australian lamb meat have been measured in Sheep CRC Information Nucleus (IN) lambs produced at 8 sites across Australia [1]. These lambs were finished on a range of feed sources of either pasture, supplements with pasture or full concentrate diets. Levels of health claimable omega-3 polyunsaturated fatty acids (n-3 PUFA) including eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) were significantly affected by site and time of slaughter [1]. In general, EPA+DHA was higher when, prior to slaughter, lambs were mostly fed quality green pasture and lower when pellets, grain or dry pasture had become a large portion of the diets [1]. As a result, lamb from several sites could not be claimed as a 'source' of long-chain omega-3 since a level of 30 mg of EPA+DHA per standard serving size of 135 g is required [2].

Replacing grain based silage or concentrate (pellets) diets rich in linoleic acid (18:2n-6) with pasture rich in alpha-linolenic acid (18:3n-3) improves the fat composition of muscle in sheep [3] by increasing long-chain omega-3 or reducing long-chain omega-6 fatty acid concentration. A higher omega-3 concentration and lower ratio of omega-6:omega-3 is considered beneficial for human health [4]. Thus, the provision of grain supplements or concentrates in the diets of sheep can elevate the level of omega-6, the ratio of omega-6:omega-3 and the saturated: polyunsaturated ratio which is detrimental to the nutritional value of meat.

The results from recent studies indicate that concentrations of EPA and DHA in *longissimus* muscle [5] are significantly lower when sheep are fed concentrates compared with pasture. EPA and DHA may be significantly depleted and the ratio of omega-6:omega-3 can be significantly increased with as little as 14 days of concentrate feeding [5] indicating that depletion rates can be rapid. As a consequence, producers require the ability to supplement lambs so as to ensure adequate levels of health claimable omega-3's. The concentration of health claimable omega-3 in lamb muscle was significantly increased following the addition of algae high in long-chain omega-3 as a supplement to low quality ryegrass based roughage diet [6]. The effect of adding algae to the diet on other aspects of meat quality are, however, largely unknown. Therefore, the aim of the current study was to examine the impact of an algae supplement on the meat quality of lambs fed a grain-based diet.

## II. MATERIALS AND METHODS

The Poll Dorset x Border Leicester x Merino wether lambs (n = 40) used in the current study were bred as part of a study to examine the effect of ewe nutrition at joining on progeny sex ratio. The dams of the lambs were fed one of two levels of dam nutrition (DN) for six weeks prior to and three weeks following joining, using similar methods to those described previously [7]. The two levels of DN were silage (high in omega-3) and oats with cottonseed meal (OCSM: high in omega-6 fatty acids). At the commencement of an introductory feeding period to the base ration, the lambs weighed on average 34.8 (sd = 2.5) kg and were 3 months of age. The introductory period lasted for 14 days over which time the proportion of grain was gradually increased until the proportions of the components, oat grain, lupin grain, chopped lucerne, salt and lime were 62.8, 15.7, 19.6, 1.0 and 1.0% respectively of the fed amount. The lambs were then allocated to two treatment groups of 20 based on the nutrition of their mothers at joining (Dam nutrition) and their liveweight. Within each treatment group the 20 lambs were grouped into four replicates of 5 lambs and then the eight replicates were allocated at random to 8 pens, with water provided by troughs and sun protection by shade cloth. Lambs in the first treatment group (4 pens) were fed a basal (control) ration and those in the second treatment group (4 pens) the basal ration with DHA-Gold™ algae (Martek Biosciences Corporation, Maryland, USA) included at 1.92% DM. The metabolisable energy (ME, 11.5 versus 11.6 MJ/kg DM) and crude protein (CP%, 18.0 versus 17.9 %DM) did not differ between the control and algae treatment rations, respectively. The concentration of Vitamin E in both treatment rations was 6.82 mg/kg DM. The lambs were fed daily and refusals weighed each day before feeding. The level of feeding was increased over the 6 weeks of the experiment, based on a target growth rate of 200 g/day and an energy:protein intake ratio of 0.65.

The lambs were transported to a commercial abattoir (200 km), where they were held in lairage overnight and slaughtered the following day. The lambs were slaughtered after head only stunning. Carcasses were chilled at a mean temperature of 3°C over a 24 h period. At 24 h post-mortem the

*m. longissimus thoracis et lumborum* (LL) was removed and the pH measured (pH24).

A 3 cm length of muscle was cut from the cranial end of the LL, vacuum sealed in gas impermeable plastic bags and held at 4°C for 4 days. Prior to measurement of colour a fresh surface was cut and the samples were placed on black styrofoam trays (one per tray) and overwrapped with 15µ polyvinyl chloride (PVC) film. Samples on trays were allowed to bloom for 30-40 minutes at a temperature of 3-4°C before making initial colour measurements. Samples were then displayed for 3 days under fluorescent lights set at ~1000 Lux. A Hunter Lab Mini Scan(tm) XE Plus (Cat. No. 6352, model No. 45/0-L, reading head diameter of 37 mm) was used to measure light reflectance. The light source was set at "D65" with the 10° standard observer. The instrument was calibrated on a black glass followed by a white enamel tile according to the manufacturer's specifications. At each reading the measurement was replicated after rotating the spectrophotometer 90° in the horizontal plane. The percentage of light reflectance at wavelength 630 nm, by the percentage of light reflectance at wavelength 580 nm was calculated.

A 25 g sample of LL was also taken at day 1 and kept frozen at -20°C for later measurement of Vitamin E content as previously described [8]. A 40 g sample of diced LL was collected for the determination of the fatty acid profile. Fatty acid analysis was conducted using the one-step procedure [9] with slight modifications as described previously [10]. At the conclusion of the period of retail display a 25 g sample of LL was also taken and kept frozen at -20°C for later measurement of lipid oxidation. 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. A 1g sample of fresh meat was ground and suspended in 5ml normal saline before analysis using the OXItek TBARS assay kit. The supernatant of standards and samples were read on a spectrophotometer at 540 nm and these then used to linearly calibrate sample concentrations of MDA.

Of the colour traits for meat under display, only the R630/R580 ratio, and redness ( $a^*$ ) values were analysed as these have been related to consumer acceptance [11]. The repeated measures data for R630/R580 ratio and  $a^*$  were each analysed using a Linear Mixed Model (LMM) analysis. The fixed effects included linear regression on day of display, possibly differing across the four DN  $\times$  treatment combinations. Possible deviations from linearity over the four days were also included as fixed effects, with these deviations also allowed to differ across the four DN  $\times$  treatment combinations if required. The covariate pH24 was included, with its effect allowed to possibly differ across the four days. Random effects included pen effects; random regressions on day for carcasses; and finally random error. Stepwise regression was used to remove non-significant ( $P > 0.05$ ) terms in models. The model (DN, treatment, DN  $\times$  treatment, as fixed effects, with pen as random effect) was used for Vitamin E and fatty acid levels in the muscle, and the TBARS data.

### III. RESULTS AND DISCUSSION

The R630/R580 ratio and  $a^*$  during display were not significantly affected by DN or lamb dietary treatment, however, both colour measures significantly ( $P < 0.001$ ) declined with time on display. Both R630/R580 ratio and  $a^*$  on a given day declined with increasing pH24 ( $P < 0.05$ ) with the rates of decline  $0.7 \pm 0.2$  and  $1.5 \pm 0.4$  units, respectively, for each 0.1 unit increase in pH24. The decline in the R630/R580 ratio and redness values was consistent with previous studies [for example 11].

The concentration of vitamin E in the LL was lower ( $P < 0.001$ ) when lambs were offered the algae ration ( $1.27 \pm 0.05$  mg/kg) compared with the Control ration ( $1.64 \pm 0.05$  mg/kg). The concentration of the omega-3 fatty acids EPA and DHA was significantly higher when lambs received the algae treatment ration compared with the control ration (Table 1). The concentration of DHA was highest when the dams of the lambs had previously been fed silage compared with OCSM at the time of joining (Table 1). As a result, the level of omega-3 polyunsaturated fatty acids (PUFA n-3) was significantly higher than the level of omega-6 PUFA,

and there was a significant ( $P < 0.001$ ) effect on the n-6:n-3 ratio. The concentration of TBARS was significantly ( $P < 0.05$ ) higher when lambs received the algae ration ( $5.2 \pm 0.5$  mg MDA/kg meat) compared with the control ration ( $1.5 \pm 0.5$  mg MDA/kg meat).

The elevation of the n-3 PUFA EPA and DHA in the LL of the algae fed lambs was expected given previous research [6]. Enrichment of PUFA levels in meat may lead to negative flavour/aroma development due to lipid oxidation [12] however, lipid oxidation in aged muscle by higher PUFA is not generally considered a problem if vitamin E concentrations are above a critical level of 2.95 mg/kg meat [13]. In the current study, there was a detrimental effect of the increased n-3 PUFA on lipid oxidation with the MDA concentration significantly higher when lambs were fed the algae ration and the level of Vitamin E was not high enough to prevent this oxidation. The higher concentration of n-3 PUFA and the lower concentration of Vitamin E in the muscle of lambs fed algae in the current study may have lead to an increased lipid oxidation. To gain the maximum benefit from algae supplementation, therefore, diets need to contain sufficient Vitamin E.

### IV. CONCLUSION

The concentration of DHA in the LL was significantly higher when lambs received a ration containing DHA rich algae at ~2% of the dry matter. The amount of EPA + DHA in the meat following supplementation would mean that the lamb could be considered a “good source” of omega-3. The concentration of vitamin E was reduced and TBARS was increased following supplementation of algae indicating increased lipid peroxidation, however, the colour shelf life of displayed meat was not adversely affected. Supplementing lambs with vitamin E to increase concentrations above the critical threshold of 2.95 mg/kg meat is recommended in order to prevent this lipid peroxidation.

### ACKNOWLEDGEMENTS

Staff and resources for this work were provided by NSW Department of Primary Industries. The staff of the cooperating abattoir are thanked for their assistance during sample collection.

Table 1 Predicted means and standard error (s.e.) for fatty acid composition of the intramuscular fat (mg/100g muscle) in the *longissimus lumborum* of lambs according to treatment diet and previous dam nutrition.

Fatty acids	Dam Fed Silage		Dam Fed OCSM	
	Control	Algae	Control	Algae
C18:3 n-3	39 (3)	39 (3)	41 (3)	37 (3)
C20:5 n-3 (EPA)	30 (3)b	48 (3)a	29 (3)b	44 (3)a
C22:5 n-3 (DPA)	38 (2)	37 (2)	37 (2)	34 (2)
C22:6 n-3 (DHA)	13 (7)c	92 (8)a	12 (7)c	71 (7)b
EPA + DHA	44 (9)c	140 (10)a	42 (9)c	115 (8)b
n-6 PUFA	433 (23)	467 (24)	425 (22)	449 (21)
n-3 PUFA	124 (12)a	220 (13)b	123 (11)a	190 (11)b
n-6:n-3 Ratio	3.5 (0.1)a	2.1 (0.1)b	3.5 (0.1)a	2.4 (0.1)b

Means for treatment combinations within a trait not having a trailing letter in common are significantly different at  $P = 0.05$ .

PUFA = polyunsaturated fatty acids, n-6:n-3 Ratio = ratio of n-6 PUFA to n-3 PUFA.

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