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# The impact of supplementing lambs with algae on growth, meat traits and oxidative status

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ABSTRACT

The current study examined the effect of supplementing lambs with algae. Forty, three month old lambs were allocated to receive a control ration based on oats and lupins (n = 20) or the control ration with DHA-Gold™ algae (~2% of the ration, 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). Lamb performance, carcase weight and GR fat content were not affected by treatment diet (control vs algae) or DN (silage vs OSCM). Health claimable omega-3 fatty acids (EPA + DHA) were significantly greater 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 provides a means of improving an aspect of the health status of lamb meat.



## 1. Introduction

The concentrations and sources of variation 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 (Ponnampalam, Butler, Jacob, et al., 2014; Ponnampalam, Butler, Pearce, et al., 2014a,b). These lambs were finished on a range of feed sources including 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, and DHA were significantly affected by site and time of slaughter (Ponnanpalam et al., 2014a). In general, EPA + DHA was greater when lambs were mostly fed quality green pasture and lower when pellets, grain or dry pasture had become a large portion of the diets prior to slaughter (Ponnampalam, Butler, Jacob, et al., 2014). As a result, lamb from several sites would not meet the requirements to be claimed as a 'source' of long-chain omega-3 where a level of 30 mg of EPA + DHA per standard serving size of 135 g is required (FSANZ, 2012).

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 cattle (Nuernberg et al., 2005; Wachira et al., 2002) and sheep (Aurousseau, Bauchart, Calichon, Micol, & Priolo, 2004; Aurousseau et al., 2007) by increasing long-chain omega-3 or reducing long-chain omega-6 fatty acid concentration. A greater omega-3 concentration and lower ratio of omega-6:omega-3 are considered beneficial for human health (Palmquist, 2009). Inclusion of oat grain at 175–245 g per day in the diet of lambs fed supplements significantly reduced total long-chain omega-3 in meat compared with lambs grazing perennial pasture (Ponnampalam, Burnett, Norng, Warner, & Jacobs, 2012), with the results from the IN lambs being consistent with these observations.

The results from recent studies indicate that concentrations of EPA and DHA in *longissimus* muscle (Scerra et al., 2011) and red blood cells (Clayton, Gulliver, Meyer, Piltz, & Friend, 2011) 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 (Scerra et al., 2011) 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-3s. The concentration of health claimable omega-3 in lamb muscle was

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significantly increased following the addition of algae high in long-chain omega-3 as a supplement to low quality ryegrass based roughage diet (Ponnampalam, Burnett, Ji, Dunshea, & Jacobs, 2012). The effect of the addition of algae to the diet on carcase composition and other aspects of meat quality is, however, largely unknown. Therefore, the aim of the current study was to examine the impact of an algae supplement on the growth, carcase and meat quality of lambs fed a grain-based diet.

## 2. Materials and methods

### 2.1. Animal background, feeding and slaughter

The Poll Dorset × Border Leicester × Merino wether lambs (n = 40) used in the current study were produced as part of a study to examine the effect of ewe nutrition at joining on the sex ratio of progeny. The dams of the lambs were fed a ration based on either silage (high in omega-3) or oats and cottonseed meal (OCSM: high in omega-6 fatty acids) for six weeks prior to and, three weeks following, joining, using similar methods to those described previously (Gulliver, Friend, King, Wilkins, & Clayton, 2013). Of the lambs used in the current study, 12 were single born and 28 were a twin, but each lamb came from a separate dam. At the commencement of an introductory feeding period to the base ration, the lambs weighed on average 34.8 ± 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 lambs were grouped into replicates of 5 lambs and then the four replicates per treatment group were allocated at random to 8 pens (1 replicate per pen) (Table 1), with water provided by troughs and shade cloth provided for sun protection. Lambs were fed one of two treatment rations (n = 20 per treatment group) consisting of a basal (control) ration or the basal ration with DHA-Gold™ algae (Martek Biosciences Corporation, Maryland, USA) included at 1.92% dry matter (DM) (algae, Table 2). 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 the treatment rations was analysed using methods described previously (McMurray & Blanchflower, 1979) and 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/hd/day and an energy: protein intake ratio of 0.65. The lambs were weighed at regular intervals and a blood sample was collected at the conclusion of the feeding period. Blood was collected from the jugular vein into a 5 ml Vacutainer™ containing lithium heparin. Following centrifugation at 1500 ×*g* for 10 min (Centra 7R Refrigerated Centrifuge, IEC, USA), plasma was decanted. To 1.5 ml of plasma samples, 15 μl of 20 μM butylated hydroxytoluene was added and the samples held at −80 °C. Total $F_2$-isoprostane (form 8) concentration was analysed in plasma using an enzyme immunoassay (EIA) kit (Caymen Chemical Company, USA) following the manufacturer's instructions. Prior to analysis plasma samples were hydrolysed by addition of 25 μl 2 M NaOH to each 100 μl plasma sample. The samples were incubated at 45 °C for 2 h. Following this, 25 μl 10 M HCl acid was added and the samples were centrifuged for 5 min at 12,000 *g*. The supernatant was removed and used for the determination of total 8-isoprostane using the EIA kit. This assay is based on the competition between 8-isoprostane and an 8-isoprostane acetylcholinesterase (AChE) conjugate for a limited number of 8-isoprostane-specific rabbit anti-serum binding sites. Values were expressed as pg/ml of plasma.

Throughout the feeding period one lamb exhibited slow growth and at the end of the feeding period was subjected to a post-mortem examination by a veterinarian, which revealed pneumonia. The management of the lambs in this study was under the animal ethics approval ORA 12/15/013 issued by the Orange Animal Ethics Committee of NSW Department of Primary Industries.

The lambs were weighed the day before slaughter and 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. All carcases were electrically stimulated pre-dressing with a mid-voltage unit (2000 mA with variable voltage to maintain a constant current, for 25 s at 15 pulses/s, 500 microsecond pulse width, unipolar waveform) (Toohey, Hopkins, Stanley, & Nielsen, 2008). Carcasses were chilled at a mean temperature of 3 °C over a 24 h period. All carcasses were trimmed according to AUS-MEAT specifications (Anonymous, 2005), weighed hot (hot carcase weight, HCW) and the depth of tissue at the GR site (the depth of muscle and fat tissue from the surface of the carcase to the lateral surface of the twelfth rib 110-mm from the midline) was measured using a GR knife (GR).

### 2.2. Measurements

After the commencement of chilling, pH and temperature were measured in the left-hand m. *longissimus thoracis et lumborum* (LL) at the caudal end over the lumbar-sacral junction and then subsequently three times to ensure that the range in temperature from ~35 °C to less than 18 °C was covered. A section of subcutaneous fat and the m. *gluteus medius* were cut away to expose the LL and after measurement the area was resealed with the overlaying tissue. pH was measured using meters with temperature compensation (WP-80, TPS Pty Ltd, Brisbane, Australia) and a polypropylene spear-type gel electrode (Ionode IJ 44), calibrated at ambient temperature. pH of the LL at 24 h post-mortem ($LL_{24}$pH) was measured at the 12th/13th rib site after calibrating the meter at chiller temperatures.

At 24 h post-mortem the LL muscle was removed (Product identification number HAM 4910; Anonymous, 2005) from the left side loin, between the 6th lumbar vertebrae and the 12th rib. Measures of subcutaneous fat depth (Fat C) and muscle depth and width (EMD and EMW; LL) were taken at the 12th rib by experienced personnel using a metal ruler and these values were multiplied by 0.008 to give a cross sectional area estimate (EMA) as applied previously (Hopkins, Gilbert, Pirlot, & Roberts, 1992). The cut surface of the LL was exposed to the air at ambient temperature for 30–40 min and the meat colour was measured using a Minolta Chroma meter (Model CR-400) set on the $L^*$, $a^*$, $b^*$ system (where $L^*$ measures relative lightness, $a^*$ relative redness and $b^*$ relative yellowness), with D65 illuminant and 2° standard observer. The chromameter was calibrated with a white tile (Y = 92.8, x = 0.3160, y = 03323). Three replicate measurements were taken at different positions and an average value was used for analysis. The boned LL section was transported (at 4 °C) in a portable chiller to the Centre for Red Meat and Sheep Development.

A 3 cm length of muscle was cut from the cranial end of the LL, vacuum packed and held at 4 °C for 4 days. Prior to measurement of colour

**Table 1**
Allocation of lambs based on the nutrition of their dam (OCSM or silage) and the feeding treatment in the feedlot (algae or no algae).

| Pen | Dam nutrition + treatment | | | |
|---|---|---|---|---|
| | OCSM + algae | OCSM + control | Silage + algae | Silage + control |
| 1 | 4 | | 1 | |
| 2 | | 2 | | 3 |
| 3 | | 1 | | 4 |
| 4 | 4 | | 1 | |
| 5 | 1 | | 4 | |
| 6 | | 4 | | 1 |
| 7 | 2 | | 3 | |
| 8 | | 4 | | 1 |