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Ruminant finishing diet affects nutritional value and preservative aspects of meat

Use of lucerne hay in ruminant feeds to improve animal productivity, meat nutritional value and meat preservation under a more variable climate

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Abstract

This study investigated the effect of low energy (LE) and high energy (HE) diets fed to Crossbred and Merino sheep on carcass weight, meat nutritional value (essential fatty acids) and preservative (shelf life) aspects. Animals were slaughtered after seven weeks of feeding and the *longissimus lumborum* (LL) and *rectus femoris* (RF) muscles collected for measurement of fatty acid concentration and retail colour. Liveweight and carcass weight at slaughter were not affected by dietary treatments. Adding lucerne hay as an ingredient to LE diet increased ($P < 0.001$) omega-3 fatty acids concentrations and lowered ($P < 0.001$) the omega-6: omega-3 ratio in meat. The redness of meat for LL and RF muscles at simulated retail display was higher ($P < 0.05$) for lambs fed LE than lambs fed HE diet. Results indicate that adding lucerne hay as an ingredient in ruminant diets has potential for maintaining liveweight (wellbeing) of animals as well as nutritional value and preservative aspects of meat.

Keywords: Ruminant animal production; drought; pasture availability; supplementary feeding; meat integrity.

1. Introduction

Animal production systems in Australia can be classified as extensive, intensive or semi-intensive based on the resource availability, production purpose and climatic conditions. Extensive grazing systems predominate in Australian ruminant meat and dairy production systems and such systems are believed to be more economically viable and animal welfare friendly than the other production systems. Increased environmental temperatures and low rainfall from late spring to early autumn reduces the density, yield and nutrient value of pasture, which in turn reduces the performance of lambs grazing such pastures (Ponnampalam, Linden, Mitchell, Hopkins, & Jacobs, 2017a). When pasture and pasture hay forages are insufficient for ruminant animal production, supplementation of diets with cereal grains, oilseed meals or concentrates (feedlot) enables faster animal growth rates and a quicker turnaround to market, but such supplementation strategies are also costly. Supplementation also helps in maintaining the health and wellness of ruminants during periods of insufficient pasture availability. The application of concentrate diets high in cereal grains or feedlot diets can alter carcass composition and meat preservative (shelf-life) aspects compared with traditional grazing systems (Ponnampalam, Butler, McDonagh, Jacobs, & Hopkins, 2012a; Ponnampalam et al., 2017b). Integration of pasture-, fodder- or tree-legumes into crop and animal production systems has many advantages but recent climate variability also impacts upon the survival and persistence of some legume cultivars across many regions of Australia. Lucerne is one potential legume, capable of not only withstanding climate variability but also fulfilling the nutrient needs during drought or insufficient feed availability in many parts of southern Australia.

Recent climate variability in Australia and other parts of the world has impacted pasture and crop production, which in turn has resulted in reduced production of meat, milk and wool from sheep, cattle and other ruminants (Campbell et al., 2016). One example is that the recent (2018-2019) drought in eastern Australia covering the states of NSW, Queensland, Victoria and ACT considerably reduced the persistence, nutritional value, yield and availability of pasture and fodder crops, which in turn heavily affected livestock productivity and the supply of meat from sheep and cattle for national and international markets during 2018 and 2019 (ABARES, 2018; MLA, 2019). Under these

67 circumstances, farmers need to consider whether to continuously feed their animals with supplementary
68 feeds purchased at higher prices from other locations, or to sell their stock. Such decisions are generally
69 based on previous experiences and current information available on climate outlooks.

70 In previous studies (Ponnampalam, Burnett, Norng, Warner, & Jacobs, 2012b; Ponnampalam et al.,
71 2017b, Burnett, Seymour, Norng, Jacobs, & Ponnampalam, 2012) it was reported that sheep grazing
72 lucerne pasture had higher carcass weight, greater muscle essential omega-3 fatty acids and vitamin E
73 concentrations than those grazing annual pasture with concentrate offered or fed feedlot diet. The
74 potential of lucerne hay as a feed ingredient in a mixed ration, as opposed to cereal grain ingredients,
75 has not been examined. This study investigated the effects of including a high proportion (~54%) of
76 lucerne hay as opposed to the use of a high proportion of barley-oat cereal grains (~54%) into a mixed
77 ration on the nutritional value (essential polyunsaturated fatty acids, PUFA) and preservative aspects
78 (retail colour stability) of meat from pure Merino and Crossbred (Poll Dorset × Border Leicester ×
79 Merino) wether lambs. The lucerne hay supplemented ration was classified as a low energy (LE) diet
80 while the barley-oat grains supplemented ration was classified as a high energy (HE) diet in the present
81 experiment. We hypothesised that using lucerne hay as an ingredient in the LE diet will not affect
82 liveweight or carcass weight at slaughter but would improve nutritional value and preservative attributes
83 of meat. Crossbred and Merino animals were used in this study as they are used for lamb (young sheep)
84 and mutton (yearling sheep) production, respectively in Australia.

85

86 **2. Materials and methods**

87 ***2.1. Experimental design and animal feeding***

88 This experiment was approved by the Animal Ethics Committee (AEC # 2694) Department of
89 Economic Development, Jobs, Transport and Resources, Victoria. All procedures were conducted in
90 accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes
91 (National Health and Medical Research Council, 2013).

92 A companion paper covering the effect of diet and genetic variation on muscle glycogen concentration
93 and eating quality of meat has been reported by Chauhan, Ponnampalam, Dunshea, & Warner (2019),
94 and reports the experimental design, animal type, dietary background and slaughter procedure in detail.
95 In brief, the ingredients used in the low energy (LE) diet were lucerne chaff (53.24%), oaten straw
96 (44.45%), oil (0.21%), salt (1%), limestone (1%) and mineral-vitamin mix (0.1%). The ingredients used
97 for high energy (HE) diet were barley grain (42.19%), oat grain (12.81%), oaten straw (27.97%), lupins
98 (11.83%), oil (3.0%), salt (1%), limestone (1%), chromium (0.1%) and mineral-vitamin mix (0.1%).
99 All ingredients (grain and straw) used for both dietary treatments were smashed using a hammer mill
100 (a 5 mm screen used to control particle size) and pellets were made for feeding. The dietary ingredients
101 were chosen to manage the crude protein (CP) concentration of the diets to be similar, but metabolisable
102 energy (MJ/kg DM, ME) differed with values of 7.8 and 11.8 MJ/ kg dry matter (DM), respectively.
103 The CP concentration of both diets was maintained at 12%.

104 Six-month old pure Merino (*n*=24) and Crossbred (Poll Dorset × Border Leicester × Merino; *n*=24;)
105 wether lambs were allocated to two dietary treatments (LE versus HE) by stratified randomisation using
106 liveweight. Animals were housed in individual pens at Animal Facilities, Werribee, Victoria and fed
107 daily at *ad libitum* amounts for the first 4 weeks and then on restricted intakes for the following 3 weeks
108 to maintain a similar growth rate of animals consuming the same diet between breed. The four
109 treatments applied were high energy diet fed to Crossbred (HEC); high energy diet fed to Merino
110 (HEM); low energy diet fed to Crossbred (LEC) and low energy diet fed to Merino (LEM). After seven
111 weeks of feeding, animals were slaughtered at the research facilities, Department of Primary Industries,
112 Werribee 3030, Victoria using standard commercial stunning and slaughter procedures (see Chauhan et
113 al., 2019 for details on pre-slaughter management and post-slaughter muscle sampling). At 1 h *post-*
114 *mortem*, carcass weight and GR fat depth (GR = total muscle and adipose tissue depth at $11^{th}/12^{th}$
115 intersection, 110 mm away from midline) were recorded. Carcasses were chilled overnight at 3-4 ºC
116 and the *longissimus lumborum* (LL) and *rectus femoris* (RF) muscles (100 g) were collected for retail
117 colour assessment. Another set of muscle LL (20 g) was taken for determination of fatty acid

concentrations. The external subcutaneous fat and connective tissue were removed from the muscle LL and RF samples at the time of preparation for colour assessment and fatty acid extraction.

2.2. Measurement of meat colour and fatty acid concentration

Samples of LL and RF collected for colour evaluation were subjected to simulated retail display and measurements of colour recorded at day 0 (1 h display), 3 and 6 of display. The details of sample preparation, packaging and colour measurement procedures have been previously reported in Chauhan et al. (2019). In brief, the selected portion of muscle LL and RF were sliced to create a fresh surface (30 mm thickness), placed on a black styrofoam tray (12 X 12 cm) and over wrapped with oxygen-permeable PVC film (15 µm). The packaged trays were maintained at 3–4 °C under fluorescent light (1000 lux) and placed on a display cabinet having 3 shelves with appropriate ventilation and lighting. Colour measurements (redness of meat; a*-value) were made fresh (after a 30 min bloom at 4°C, day 1) and then on days 3 and 6 of display, using a Hunter lab Miniscan™ XE Plus 45/10 (Reston, VA, USA). The light source was set at illuminant D65 with the 10° standard observer. A homogeneous 0.5 g (freeze dried) ground sample was used for fatty acid extraction, methylation and quantification by gas chromatography as described by Ponnampalam et al. (2017b) using a rapid modified procedure developed from the method reported by O'Fallon, Busboom, Nelson, & Gaskins (2007). One hundred µL of nonadecanoic acid methyl ester (C19:0, Sigma Aldrich Pty Ltd., Castle Hill, NSW2154, Australia) was added to muscle samples as an internal standard dissolved in chloroform (10 mg C19:0/mL $CHCl_3$). The fatty acid methyl esters (FAMEs) were separated with 1 mL of hexane solvent by mixing for five minutes 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-micron, Agilent J & W Scientific, Santa Clara, CA, USA). 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 concentrations in muscle are reported in mg/100 g meat. The amounts of major fatty acid groups such as eicosapentaenoic acid (EPA) + docosapentaenoic acid (DPA) + docosahexaenoic acid (DHA), total omega-3 (n−3), total omega-6 (n−6), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA),

PUFA, n−6/n−3 ratio and PUFA/SFA ratio were calculated as the sum of fatty acid profiles of the GC quantification. Total muscle fat content was calculated in mg/100 g of muscle as the sum of SFA, MUFA and PUFA.

2.3. Statistical analyses

A 2 by 2 factorial design (two diets and two genetic background) enable the implementation of a four treatment fully randomised design. Results from 48 animals maintained in single pens were used in the analyses. Each experimental unit was a pen, having 12 lambs per treatment. Data were analysed using an ANOVA procedure in GenStat (GenStat Release 18 Edition, VSN International, 2015). Diet and genetic background were used as main effects and pen was used as a block in order to obtain treatment means for variables of carcass traits, muscle individual fatty acids and the major fatty acid groups. Initial liveweight of the animal was used as a covariate for the analyses of carcass weight and GR fat depth. Meat retail colour data were analysed using ANOVA with "display time" points as repeated measurements in order to obtain the means of interactions between diet × genetic background × display time, diet × genetic background, diet × display time and genetic background × display time. F-tests were used to determine the overall significant difference among the predicted means.

3. Results

3.1. Carcass traits

Crossbred lambs were heavier (38.1 vs 33.4, SED [standard error difference of means] = 0.44; $P < 0.001$) than Merino lambs at the commencement of experiment but the weight were similar for both diets within each breed. The average weights for HEC, LEC, HEM and LEM were 38.0, 38.2, 33.5 and 33.3 kg, respectively (SED for breed = 0.44, SED for diet 0.15). This resulted in a heavier ($P < 0.001$) liveweight for Crossbred animals at slaughter compared with the Merino counterparts (41.4 vs 36.6 kg, SED = 0.67) but no diet (weights for HE and LE were 39.2 vs 38.8 kg, SED = 0.44) or diet by genotype interaction were observed ($P > 0.05$ for both). Similarly, carcass weights at slaughter were greater ($P < 0.002$) for Crossbred than Merino lambs (18.7 vs 16.2, SED = 0.72) but there were no effects of diet

(17.7 vs 17.3, SED = 0.27) or diet by genotype interaction ($P > 0.05$) for both. Merinos had lower ($P < 0.02$) GR than Crossbred lambs (5.27 vs 10.6 mm, SED = 2.0) while LE fed lambs tended to have lower ($P < 0.06$) GR than their HE counterparts (7.28 vs 8.60 mm, SED = 0.67).

3.2. Muscle longissimus lumborum fatty acid concentration

There were no diet by genotype interactions ($P > 0.05$) except for linoleic acid (C18:2n6-*cis*) and EPA (C20:5n3) concentrations. The HE diet increased ($P < 0.002$) total muscle fat content when compared with the LE diet. This in turn resulted in higher values for most of the individual fatty acids and major group fatty acids for lambs fed the HE diet (Table 1). In contrast, the concentration of health enhancing *n*-3 fatty acid of alpha-linolenic acid (ALA, C18:3n-3), EPA, DPA (C22:5n-3) and DHA (C22:6n-3) were higher ($P < 0.01$) in meat from LE fed lambs. This resulted in higher ($P < 0.001$) muscle EPA+DHA, EPA+DPA+DHA, total *n*-3 concentrations and lower ($P < 0.001$) n-6/n-3 for the LE group than the HE groups (Table 2), which is noted as health beneficial (Decker, Akoh, & Wilkes, 2012; Russo, 2009). There was no significant breed effect ($P > 0.05$) observed for muscle intramuscular fat (Table 1). Merino lambs had a higher concentration of arachidonic acid (AA, C20:4n-6; $P < 0.03$) and adrenic acid (AdA, C22:4n-6 = docosatetraenoic acid; $P < 0.001$), both of which belong to the *n*-6 fatty acid family, compared with Crossbred lambs even though the total muscle fat concentration was lower with Merinos, which is also considered unhealthy (Simopoulos, 2006; Russo, 2009).

Insert Table 1 and Table 2 here

3.3. Meat retail colour

When redness of meat (a*-value) for muscle LL and RF was evaluated from day 0 to day 6 of simulated retail display, there was no ($P > 0.05$) diet × breed × display time interaction or diet × breed interaction or main breed effects. However, there was a diet × display time effect in that the redness of meat, with the a* values for lambs fed LE diet found to be greater in both LL (Fig. 1a; $P < 0.04$) and RF (Fig. 1b; $P < 0.10$) muscles than for lambs fed the HE diet, which can be clearly seen on day 6 of simulated retail display.

Insert Figure 1 here

197

198 **4. Discussion**

199 This study shows a promising outcome for the use of lucerne hay as a supplement in forage-based diets
200 to improve productivity (liveweight and carcass weight), meat nutritional value (*n*-3 PUFA) and
201 preservative (redness of meat) aspects during times of insufficient pasture availability. This finding
202 suggests that other sources of legumes such as tree-legumes [leucaena (*Leucaena leucocephala*), honey
203 locust (*Gleditsia triacanthos*), gliricidia (*Gliricidia sepium*), erythrina forage (*Erythrina species*)] and
204 other fodder-legumes, may have potential to be integrated into the pasture or crop production systems
205 to extend the nutritional value and feeding length of ruminants during the dry season or drought. The
206 tree-legumes and fodder-legumes can establish rapidly and provide an excellent source of high-protein
207 for ruminants as fodder feeds. It is feasible that the high protein legume leaves, foliage, pods and grains
208 will complement high fibrous, low-nutritive and low-digestible cereal stubbles, hay or pasture hay in
209 elevating nutrient supply to animals. Tree-legumes have additional advantages of providing shade to
210 livestock to avoid heat stress during hot days. Based on the observations under temperate climate
211 conditions in Australia (Victoria), the annual and perennial clover, medic or vetch species are suitable
212 for late winter to mid spring for grazing as pasture or senesced hay along with other annual and perennial
213 grass species. They are not suitable for summer or post-summer dry seasons as these pastures dry off,
214 decompose (decay) and lose their nutrient value as the season become drier due to high temperature and
215 low rainfall. However, silage or hay prepared from perennial clover, when nutritive characteristics are
216 high, may be utilised during summer as a small portion of ration to improve liveweight gain. Therefore,
217 we realise that the senesced hay or silage from clover, medic or vetch cannot be used as major portion
218 of ruminant diets during summer or dry seasons to improve animal performance, meat nutritional value
219 and meat preservation.

220 Results indicate that the productivity of lambs fed the LE diet, containing a higher proportion of lucerne
221 chaff (~54%), was equivalent to the HE diet having a higher proportion of cereal grain (~54%) based
222 on liveweight and carcass weight at slaughter. This was similar to the finding from a recent study
223 conducted in lambs under extensive grazing conditions, where weaned lambs rotationally grazing

lucerne pasture for six weeks produced premium quality carcasses, equivalent to lambs fed a feedlot concentrate diet (Ponnampalam et al., 2017a). We have also previously reported that sheep grazing perennial pasture that contained mainly senesced lucerne, had greater liveweight gain and carcass weight than those grazing senesced annual ryegrass pasture supplemented with cereal grains (Burnett et al., 2012). The application of cereal grain or legume grain-based supplements to sheep (Dixon & Stockdale, 1999; Dixon & Egan, 2000) and cattle (Beever & Thorp, 1997; Doreau, Bauchart, & Chilliard, 2011) have been recommended to maintain or improve the growth performance and productivity during times of insufficient feed availability. It is noted that an appropriate supplementary feeding strategy within the grazing system is essential, otherwise consumption of cereal or legume grains at a greater level by dominant animals can cause acidosis or rumen bloat, respectively. Such cases can be costly and affect average flock performance and wellness of animals.

Increasing the health enhancing *n*-3 fatty acid concentration in meat and milk from livestock is a challenging process but research clearly shows that this is achievable (Decker, Akoh, & Wilkes, 2012; Ponnampalam, Hopkins, & Jacobs, 2018). Another advantage observed in this study was the LE diet containing lucerne chaff significantly increased the health enhancing PUFAs precursor (ALA) and product (EPA, DPA and DHA) fatty acids in muscle LL of both Merino and Crossbred sheep. Previous studies conducted in Australia with lambs grazing lucerne pasture have also been shown to increase ALA, longer chain *n*-3 fatty acids and vitamin E concentrations in meat from diverse sheep genetic backgrounds (Ponnampalam 2012b; Ponnampalam et al., 2014; Ponnampalam 2017b). Vitamin E is fat soluble, and it is likely that vitamin E in the lucerne offers protection against oxidation of ALA through digestion, enterocyte absorption and tissue deposition (muscle or liver). Alternatively, it is also possible that secondary metabolites, such as tannins and flavonoids present in lucerne can protect dietary PUFA from the hydrolysis and biohydrogenation in the rumen. Hence, increased ALA is available for absorption across enterocytes and therefore increased deposition within the muscle tissue and meat.

Seed, meal and forage supplementation of brassica species (canola, camelina, flax, rape) to sheep, goats and cattle have also been reported to significantly increase essential *n*-3 PUFA concentrations in meat (Kronberg, Barceló-Coblijn, Shin, Lee, & Murphy, 2006; Corazzin, Bovolenta, Sepulcri, & Piasentier,

2012; Najafi et al., 2012; Karami, Ponnampalam, & Hopkins, 2013) and milk (Mierlita, 2015). In this study, diet × genotype interactions for LA and EPA concentrations were identified and not for the other individual fatty acids. ALA is the precursor n-3 PUFA for the production of longer chain n-3 EPA, DPA and DHA, whilst LA is the precursor n-6 PUFA for the production of AA in animal tissue. It has been reported that the presence of any family of PUFAs in excess will interfere with the metabolism of others, reducing incorporation into tissues, subsequently altering PUFA deposition in tissues and the overall biological effects (Palmquist, 2009; Ruxton, Reed, Simpson & Millington, 2004; Ponnampalam, Hopkins, & Jacobs, 2018). In the present study, when animals were fed LE diet, the crossbred lambs produced higher ALA in the tissues and had 20 mg higher ALA/100 g tissue than the Merino lambs. This pattern was not seen in animals fed HE diet. The highest ALA concentration in muscle tissues from crossbred lambs fed the LE diet might have suppressed the LA deposition whilst enhancing the elongation process of ALA to its longer chain EPA. This supports our previous findings that the production of EPA plus DHA, through enzymatic conversion of ALA, appears to be inhibited by the levels of LA present in muscle tissues, particularly at concentrations above 150 mg/100 g of muscle (Ponnampalam, Hopkins, Butler, & Pethick, 2013). Research from the authors' group has also showed that not only do ALA and LA compete for the elongation process but both ALA and DHA can compete for absorption at the level of gut enterocyte or peripheral tissue (Ponnampalam et al., 2015).

The higher ratios of *n*-6 to *n*-3 in the diet of humans in recent years, as forewarned by health professionals has been due to a higher dietary consumption of oilseeds and grains high in *n*-6 while decreasing *n*-3 intake during the last few decades (Simopoulos, 2006). The actual *n*-6/*n*-3 in the western diet is believed to be between 15-20:1 while the recommended ratio is closer to 1-4:1 in order to protect against degenerative diseases such as cancer, cardiovascular disease, inflammation, autoimmune disease and alzheimer's disease (Simopoulos, 2002; Russo, 2009). This study shows that adding lucerne chaff to the LE diet resulted in an *n*-6/*n*-3 in the meat below 4 for both Crossbred and Merino lambs. However, meat from the HE diet, containing greater levels of cereal grain, resulted in an *n*-6/*n*-3 above 4, which may be considered detrimental to human health.

277 An increase in *n*-6/*n*-3 and *n*-6 PUFA consumption in the human diet can change the production of
278 important mediators and regulators of inflammation and immune responses towards a pro-inflammatory
279 manner (Russo, 2009). Arachidonic acid and EPA are precursors for the production of different classes
280 of eicosanoids that are pro-inflammatory and anti-inflammatory, respectively contributing to risks and
281 benefits of consuming different classes of PUFA. One thing to note with the Merino breed in this study
282 was that meat from Merino breed had greater levels of AA and AdA, both considered pro-inflammatory.
283 The actual reason for this outcome is not known, perhaps associated with the stress susceptibility and
284 fatty acid metabolism observed previously in Merino breeds, as they show different basal and hormone
285 stimulated energy metabolism when compared with Crossbred sheep (Ponnampalam, Warner, &
286 Dunshea, 2012c). A recent study also showed that a diet-induced increase in precursor ALA resulted in
287 altered activities and mRNA expression of antioxidant enzymes in muscle tissues, which, in turn, can
288 provide the animals with a higher antioxidant potential to alleviate oxidative stress and immune
289 suppression, and protective mechanisms from adverse environmental conditions (Ponnampalam et al.,
290 2019).

291 Another beneficial outcome observed was the meat colour during simulated display from lambs fed the
292 LE diet had better retail colour (high in redness) at day 6 of display than the lambs fed HE diet. This
293 finding was observed in both LL and RF muscles and occurred despite the increased concentrations of
294 *n*-3 fatty acid which are more susceptible to oxidation than shorter, more SFA. The latter observation
295 might be due to an increased vitamin E concentration in meat from lucerne hay fed lambs as was found
296 in a recent study where lambs grazing lucerne pasture had higher muscle vitamin E concentration as
297 opposed to lambs grazing a feedlot diet (Ponnampalam et al., 2017b). A previous study (Ponnampalam
298 2012b) also supports this, when a senesced perennial lucerne-dominant pasture fed to lambs
299 significantly increased muscle vitamin E concentration compared to senesced annual ryegrass pasture
300 supplemented with oat grain.

301

302 **5. Conclusions**

303 Feeding LE diet containing greater proportion of lucerne hay did not affect liveweight or carcass weight
304 at slaughter compared with a HE diet containing greater proportion of cereal grain. Although the
305 animals fed LE diet had lower total muscle fat content than the HE diet, the concentration of parent and
306 precursor *n*-3 fatty acids were higher in meat from sheep fed the LE diet. The redness of meat from
307 lambs fed LE diet was greater for both LL and RF muscles than those fed the HE diet. The findings of
308 this study show that the addition of lucerne hay at ~54% to the LE diet resulted in increased health
309 enhancing *n*-3 fatty acids and retail colour of meat compared with HE diet containing ~54% cereal
310 grains (barley and oat), without affecting liveweight or carcass weight of animals. Lucerne or potentially
311 other legume feeds as dietary ingredients provide opportunity to improve animal wellbeing as well as
312 nutritional and preservative attributes of meat during times of insufficient feed availability.

313

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316 Agriculture Victoria, Department of Jobs, Precincts and Regions) and Meat and Livestock Australia.

317

318 **Conflict of interest**

319 The authors declare no conflict of interest.

320

321 **References**

322 ABARES Insight (2018). Analysis of 2018 drought. Department of Agriculture and Water Resources,
323 Australian Government. http://www.agriculture.gov.au/abares/Documents/abares-insights-analysis-
324 2018-drought.pdf (accessed on 10 - 08 - 2019).

Beever, D. E., & Thorp, C.L. (1997). Supplementation of forage diets. Page 419 in Milk Composition, Production and Biotechnology. R.A.S. Welch, D.J.W. Burns, S.R. Davis, A.I. Popay, and C.G. Prosser, eds. CAB International, Oxon, UK.

Burnett, V. F., Seymour, G. R., Norng, S., Jacobs, J. L., & Ponnampalam, E.N. (2012). Lamb growth performance and carcass weight from rotationally grazed perennial pasture systems compared with annual pasture systems with supplements. *Animal Production Science, 52,* 248–254.

Campbell, B. M., Vermeulen, S. J., Aggarwal, P. K., Corner-Dolloff, C., Girvetz, E., Loboguerrero, A., M., Ramirez-Villegas, J., Rosenstock, T., Sebastian, L., Thornton, P. & Wollenberg, E. (2016). Reducing risks to food security from climate change. *Global Food Security, 11*, 34-43.

Chauhan, S. S., Ponnampalam, E. N., Dunshea, F. R., & Warner, R. D. (2019). Breed and nutrition effects on meat quality and retail color after lamb pre-slaughter stress. *Meat and Muscle Biology, 3*, 147-157.

Corazzin, M., Bovolenta, S., Sepulcri, A., & Piasentier, E. (2012). Effect of whole linseed addition on meat production and quality of Italian Simmental and Holstein young bulls. *Meat Science, 90,* 99-105.

Decker, E. A., Akoh, C.C., & Wilkes, R.S. (2012). Incorporation of (n-3) fatty acids in foods: challenges and opportunities. *Journal of Nutrition, 142,* 610S–613S.

Dixon, R. M., & Egan, A. R. (2000). Response of lambs fed low quality roughage to supplements based on urea, cereal grain, or protein meals. *Australian Journal of Agriculture Research, 51,* 811–821.

Dixon, R. M., & Stockdale, C.R. (1999). Associative effects between forages and grains: Consequences for feed utilisation. *Australian Journal of Agriculture Research, 50,* 757–774.

Doreau, M., Bauchart, D., & Chilliard, Y., (2011). Enhancing fatty acid composition of milk and meat through animal feeding. *Animal Production Science, 51,* 19–29.

347 Karami, M., Ponnampalam, E. N., & Hopkins, D. L. (2013). The effect of palm oil or canola oil on
348 feedlot performance, plasma and tissue fatty acid profile and meat quality in goats. *Meat Science, 94,*
349 165–169.

350 Kronberg, S. L., Barceló-Coblijn, G., Shin, J., Lee, K., & Murphy, E. J. (2006). Bovine muscle n-3 fatty
351 acid content is increased with flaxseed feeding. *Lipids, 41,* 1059-68.

352 Mierlita, D. (2015). Conjugated linoleic acid and ω-3 fatty acid in sheep milk was increased by part-
353 time grazing and camelina seed. *Journal of Food, Agriculture & Environment, 13,* 76-81.

354 Meat and Livestock Australia Industry: Industry Projection 2019.
355 https://www.mla.com.au/globalassets/mla-corporate/prices--markets/documents/trends--
356 analysis/sheep-projections/mla-june-2019-update-australian-sheep-industry-projections.pdf (accessed
357 29-08-2019).

358 Najafi, M. H., Zeinoaldini, S., Ganjkhanlou, M., Mohammadi, H., Hopkins, D. L. & Ponnampalam, E.
359 N. (2012). Performance, carcass traits, muscle fatty acid composition and meat sensory properties of
360 male Mahabadi goat kids fed palm oil, soybean oil or fish oil. *Meat Science, 92,* 848–854.

361 National Health and Medical Research Council, (2013). Australian Code of Practice for the Care and
362 Use of Animals for Scientific Purposes, 8th ed. Australian Government, Canberra.

363 O'Fallon, J. V., Busboom, J. R., Nelson, M. L. & Gaskins, C. T. (2007). A Direct Method for Fatty
364 Acid Methyl Ester Synthesis: Application to Wet Meat Tissues, Oils, and Feedstuffs. *Journal of Animal*
365 *Science, 85,* 1511-1521.

366 Palmquist D.L. (2009). Omega-3 fatty acids in metabolism, health, and nutrition and for modified
367 animal product foods. *Professional Animal Scientist 25*, 207–249.

368 Ponnampalam, E. N., Butler, K. L., McDonagh, M. B., Jacobs, J. L., & Hopkins, D. L. (2012a).
369 Relationship between muscle antioxidant status, forms of iron, polyunsaturated fatty acids and
370 functionality (retail colour) of meat in lambs. *Meat Science, 90,* 297–303.

371 Ponnampalam, E. N., Burnett, V. F., Norng, S., Warner, R. D., & Jacobs, J. L. (2012b). Vitamin E and
372 fatty acid content of lamb meat from perennial or annual pasture systems with supplements. *Animal*
373 *Production Science, 52*, 255–262.

374 Ponnampalam, E. N., Warner, R. D., & Dunshea, F. R. (2012c). Basal and hormone-stimulated
375 metabolism in lambs varies with breed and diet quality. *Domestic Animal Endocrinology, 42,* 94-102.

376 Ponnampalam, E. N., Hopkins, D. L., Butler, K. L., & Pethick, D. W. (2013). The relationship between
377 product (EPA & DHA) and precursor (ALA) omega-3 fatty acids in lamb. *59th International Congress*
378 *of Meat Science and Technology,* 18-23rd August 2013, Izmir, Turkey.

379 Ponnampalam, E. N., Butler, K. L., Jacob, R. H., Pethick, D. W., Ball, A. J., Hocking Edwards, J. E.,
380 Geesink, G., & Hopkins, D. L. (2014). Health beneficial long chain omega-3 fatty acids in Australian
381 lambs managed under extensive finishing systems. *Meat Science, 96*, 1104-1110.

382 Ponnampalam E.N., Lewandowski P.A., Fahri F.T., Burnett V.F., Dunshea F.R., Tim Plozza T. &
383 Jacobs J.L. (2015). Forms of n-3 (ALA, C18:3n-3 or DHA, C22:6n-3) fatty acids affect carcass yield,
384 blood lipids, muscle n-3 fatty acids and liver gene expression in lambs. *Lipids, 50,* 1133–1143.

385 Ponnampalam, E. N., Linden, N. P., Mitchell, M. L., Hopkins, D. L., & Jacobs, J. L. (2017a). Production
386 systems to deliver premium grade lambs to the growing international and Australian markets. *Small*
387 *Ruminant Research, 157,* 32-39.

388 Ponnampalam, E.N., Plozza, T., Kerr, M.G., Linden, N., Mitchell, M., Bekhit, A. E., Jacobs, J.L., &
389 Hopkins, D.L. (2017b). Interaction of diet and long ageing period on lipid oxidation and colour stability
390 of lamb meat. *Meat Science, 129,* 43-49.

391 Ponnampalam, E. N., Hopkins, D. L., & Jacobs, J. L. (2018). Increasing omega-3 levels in meat from
392 ruminants under pasture-based systems: An invited review. *Scientific and Technical Review of the*
393 *Office International des Epizooties, 37,* 57–70.

394 Ponnampalam, E. N., Vahedi, V., Giri, K., Lewandowski, P., Joe L. Jacobs, J. L., & Dunshea, F. R.
395 (2019). Muscle Antioxidant Enzymes Activity and Gene Expression Are Altered by Diet-Induced

Increase in Muscle Essential Fatty Acid (α-linolenic acid) Concentration in Sheep Used as a Model. *Nutrients, 11,* 723.

Russo, G. L. (2009). Dietary n−6 and n−3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. *Biochemical Pharmacology, 77,* 937-946.

Ruxton C. H. S., Reed S. C., Simpson M. J. A. & Millington K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: A review of the evidence. *Journal of Human Nutrition and Dietetics, 17*, 449–459.

Simopoulos, A. P. (2002). Review: Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition, 21*, 495–505.

Simopoulos, A. P. (2006). Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. *Biomedicine & Pharmacotherapy, 60,* 502–507.

VSN International (2015). GenStat for Windows 18th Edition. VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk

410 **Table 1.** Effect of diet (high energy vs low energy) and genetic variation (Crossbred vs Merino) on

411 individual and total fatty acids in mg per 100 grams of meat

	High energy		Low energy		SED	*P*-value		
	Crossbred	Merino	Crossbred	Merino		Diet (D)	Genotype (G)	D x G
C12:0	3.74	2.92	3.29	2.01	0.97	0.09	0.35	0.55
C14:0	76.0	56.6	60.6	40.9	14.9	0.01	0.28	0.98
C14:1	5.48	5.40	5.45	5.07	0.72	0.46	0.79	0.56
C16:0	783	549	607	426	129	0.003	0.21	0.57
C16:1	42.7	31.7	37.1	23.3	8.5	0.05	0.21	0.69
C18:0	622	485	471	393	99	0.003	0.38	0.42
C18:1n-9 *cis, trans*	1401	882	1042	655	219	0.002	0.09	0.44
C18:2n-6 *cis*	168	138	103	95	14.6	0.001	0.28	0.05
C18:2n-6 *trans*	33.6	14.3	32.0	12.1	5.4	0.28	0.01	0.87
C18:3n-3	30.8	15.3	50.6	30.9	5.3	0.001	0.01	0.29
C18:3n-6	1.85	1.78	1.32	1.60	0.41	0.06	0.81	0.35
C18:4n-3	1.29	0.31	1.45	0.32	0.31	0.37	0.02	0.46
C20:0	3.65	3.07	2.86	2.52	0.69	0.02	0.57	0.65
C20:1n-9	7.34	6.03	4.08	3.62	1.28	0.001	0.56	0.39
C20:2n-6	2.29	1.35	1.87	1.15	0.27	0.009	0.02	0.31
C20:4n-6	31.5	42.7	26.0	40.2	4.1	0.006	0.03	0.28
C20:5n-3	12.9	8.1	21.3	13.8	1.9	0.001	0.02	0.03
C22:4n-6	0.53	1.67	0.59	1.78	0.23	0.43	0.001	0.81
C22:5n-3	7.18	7.62	12.65	11.74	1.16	0.001	0.85	0.18
C22:6n-3	3.63	2.75	4.79	3.98	0.54	0.001	0.24	0.85
Total muscle fat (IMF)	3318	2317	2550	1813	490	0.002	0.15	0.48

412 The energy concentration of low energy and high energy diets were 7.8 and 11.8 MJ/ kg DM,

413 respectively. Merino (Merino x Merino) and Crossbred (Poll Dorset × Border Leicester × Merino).

414 IMF = intramuscular fat. SED = standard error difference of means for diet × genetic interaction.

415

Table 2. Effect of diet (high energy vs low energy) and genetic variation (Crossbred vs Merino) on essential polyunsaturated fatty acid groups, ratio of omega-6 to omega-3 fatty acid (n-6/n-3), and the ratio of polyunsaturated to saturated fatty acid (PUFA/SFA) in mg per 100 grams of meat

	High energy		Low energy		SED	*P*-value		
	Crossbred	Merino	Crossbred	Merino		Diet (D)	Genotype (G)	D x G
EPA+DPA	16.26	10.59	25.84	17.99	2.5	0.001	0.06	0.12
EPA+DPA+DHA	23.27	18.01	38.33	29.90	3.6	0.001	0.17	0.16
Total n-3 FA	54.5	33.8	89.4	60.4	7.1	0.001	0.009	0.11
Total n-6 FA	234	197	162	149	19	0.001	0.29	0.09
MUFA	1460	928	1090	689	227	0.002	0.09	0.46
PUFA	289	230	251	210	25	0.003	0.11	0.35
SFA	1562	1152	1201	908	251	0.003	0.26	0.53
n-6/n-3	4.1	6.3	1.6	2.8	0.49	0.001	0.005	0.03
PUFA/SFA	0.20	0.23	0.22	0.24	0.05	0.38	0.58	0.64

The energy concentration of low energy and high energy diets were 7.8 and 11.8 MJ/ kg DM, respectively. Merino (Merino x Merino) and Crossbred (Poll Dorset × Border Leicester × Merino). SED = standard error difference of means for diet × genetic interaction.

EPA (C20:5n-3) = eicosapentaenoic acid; DPA (C22:5n-3) = docosapentaenoic acid; DHA (C22:6n-3) = docosahexaenoic acid.

FA = fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid.

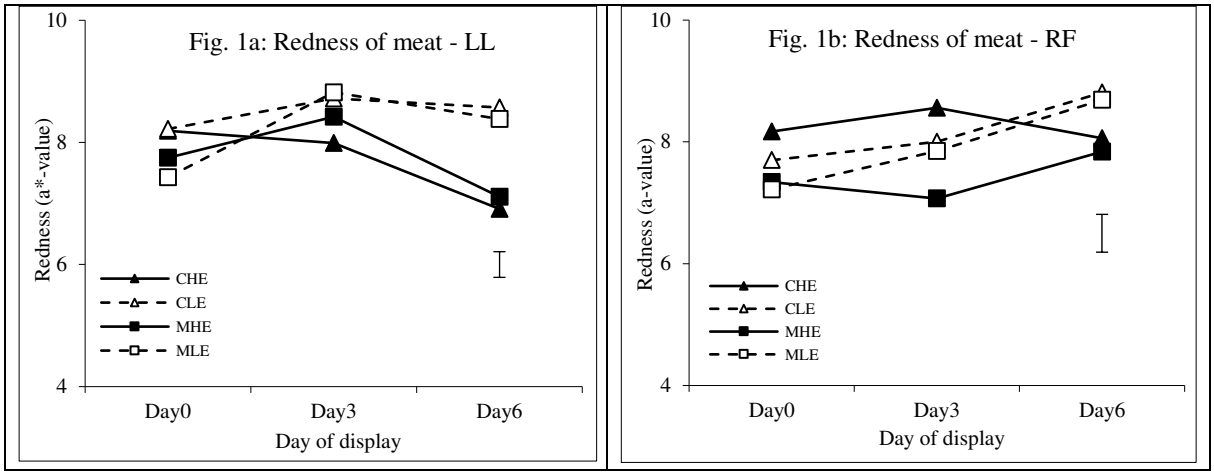

Figure 1. Effect of diet (high energy vs low energy) and genetic variation (Crossbred vs Merino) on meat redness (a*-value) of muscle *longissimus lumborum* (LL, Fig.1a) and muscle *rectus femoris* (RF, Fig.1b) of sheep under day 0 (1h), day 3 and day 6 of simulated retail display.

CHE = Crossbred high energy, CLE = Crossbred low energy, MHE = Merino high energy, MLE = Merino low energy, SED = standard error difference of means for diet × genetic interaction. The pooled SED is displayed on Day 6.

The energy concentration of low energy and high energy diets were 7.8 and 11.8 MJ/ kg DM, respectively. Merino (Merino x Merino) and Crossbred (Poll Dorset × Border Leicester × Merino).