

Increasing the proportion of female lambs by supplementary feeding oats high in omega-6 fatty acids at joining

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Abstract: At the time of joining, sheep commonly graze pasture which is high in omega-3 fatty acids. If pasture supply is limited, supplements such as grain that are high in omega-6 fatty acids may be fed. Therefore, the aim of the current paper was to review dietary sources of fatty acids in the diet of sheep in south-east Australia and the contribution of these fatty acids to reproduction and, specifically, the sex of lambs. In a series of studies, Merino x Border Leicester or Merino ewes were allocated to one of two dietary treatments, 100% silage (low in omega-6 and high in omega-3) or 70% oat grain and 8% cottonseed meal (CSM, high in omega-6). In study 1, ewes consumed the diets for 44 days prior to the assessment of the prostaglandin (PGF2 α) response to an oxytocin challenge. In studies 2–4, ewes consumed the diets for approximately six weeks prior to and 17 days following joining to assess the effect of diet on the sex ratio of lambs. Plasma omega-6 was higher ($P < 0.001$), PGF2 α response to oxytocin was greater ($P < 0.05$), the time to behavioural oestrus was shorter ($P = 0.006$) and the proportion of female lambs was increased (58.2 versus 43.5%, $P = 0.010$) when ewes were fed the oat grain/CSM compared with the silage diet. Targeted feeding of oats at joining may provide a practical way for producers to manipulate the sex ratio of their flock in favour of females.

Key words: omega-3, oestrus, sex ratio

Introduction

Omega-3 fatty acids have a number of positive effects on human and animal health. In particular, the ratio of omega-6 to omega-3 may play an important role in several aspects of animal production and reproduction (Abayasekara and Wathes 1999). A number of studies have examined the effects of fatty acids on peripheral markers of reproductive success in sheep and cattle such as hormones, oocyte quality or inflammatory markers (Gulliver *et al.* 2012), however, few studies have examined measurable outcomes of reproductive success. The aim of the current paper was to review the sources of omega-3 and omega-6 fatty acids in sheep and cattle diets in Australia and examine their metabolism and effects on reproduction, specifically effects on inflammation and sex ratio of lambs.

Omega-3 and omega-6 fatty acids

The primary fatty acids of interest in studies examining reproduction in animals

are the long-chain omega-3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the long-chain omega-6 fatty acid arachidonic acid (AA). The first double bond in omega-3 fatty acids occurs three bonds from the methyl end of the fatty acid chain, whereas, the first double bond in omega-6 fatty acids occurs six bonds from the methyl end (Figure 1). These long-chain fatty acids are synthesised in the body from the short-chain omega-3 α -linolenic acid (ALA) and omega-6 linoleic acid (LA, Figure 2). The

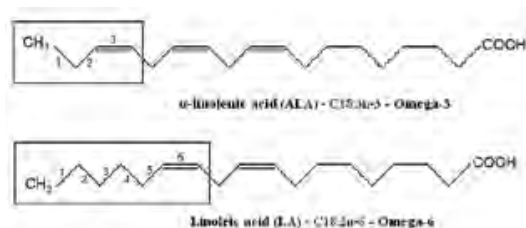


Figure 1. Short-chain omega-3 (α -linolenic acid, ALA) and omega-6 (linoleic acid, LA) fatty acids important in reproduction in sheep and cattle showing the position of the first double bond.

short-chain fatty acids ALA and LA cannot be synthesised by animals (Lands 1992) and, therefore, must be consumed in the diet.

Sheep commonly graze pasture at the time of joining in south-eastern Australia (King *et al.* 2010) and pasture is high in omega-3 fatty acids (Clayton *et al.* 2010). If pasture supply is limited, however, sheep producers may feed supplements, such as grain, that are low in omega-3 and high in omega-6 fatty acids. While the effects of feeding diets high in fatty acids to sheep and cattle on improved reproduction are well established (Gulliver *et al.* 2012), the specific effects of omega-3 and omega-6 fatty acids on reproduction and, specifically, altered sex ratios in sheep, have not previously been reported.

Prostaglandins (PG), in particular $\text{PGF}_{2\alpha}$ and $\text{PGF}_{3\alpha}$, play an important role in several aspects of reproduction, including ovulation, oestrus, embryo survival and parturition (for review, see Gulliver *et al.* 2012). The series-3 PG are less inflammatory, while the series-2 PG are more inflammatory (Lands 1992). The long-chain omega-3 and omega-6 fatty acids EPA and AA are the precursors for these PGs. In the metabolism of these fatty acids to PG, the removal of two double bonds from AA leaves two double bonds and leads to the formation of series-2 PG ($\text{PGF}_{2\alpha}$). Conversely, the removal of two double bonds from EPA leads to the formation of series-3 PG ($\text{PGF}_{3\alpha}$, Figure 2b). Therefore, the ratio of omega-6 to omega-3 in ruminant diets is particularly important

in determining the relative availability of the precursors for PG formation.

Omega-6 fatty acids and prostaglandins

Diets high in omega-6 are associated with increased $\text{PGF}_{2\alpha}$ synthesis, however, few studies have reported the specific effects of omega-6 on the potential PG response to an oxytocin challenge in sheep. The aim of study 1 was to determine whether oxytocin stimulated $\text{PGF}_{2\alpha}$ was significantly increased when ewes were fed a diet high in omega-6 compared with a diet low in omega-6 fatty acids.

Merino x Border Leicester ewes ($n = 30$) were allocated to one of two dietary treatments, either low in omega-6 (100% cereal/legume silage) or high in omega-6 (70% oat grain, 22% silage and 8% cottonseed meal, CSM). Ewes consumed the diets for 44 days prior to two consecutive oxytocin challenges to stimulate PG release.

Plasma omega-6 and $\text{PGF}_{2\alpha}$ metabolite (PGFM) concentrations following oxytocin challenge were greater ($P < 0.05$) when ewes were fed the oat grain/CSM diet high in omega-6 (Figure 3). The time to the onset of behavioural oestrus was also numerically, but not significantly ($P = 0.06$), shorter when ewes were fed the oats/CSM diet. A shorter time to oestrus in ewes fed the high omega-6 may be related to increased *in vivo* synthesis of $\text{PGF}_{2\alpha}$, resulting in a faster initiation of the hormonal sequences leading to oestrus and

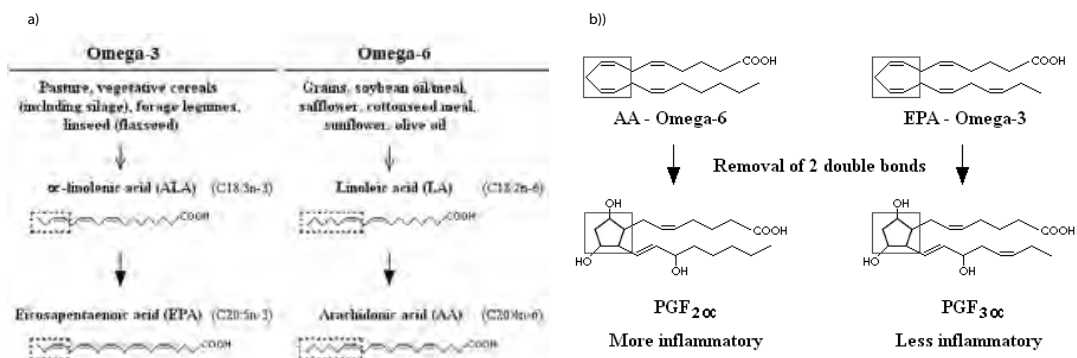


Figure 2. (a) Sources and metabolism of short-chain omega-3 or omega-6 fatty acids from plant material or oilseeds to long-chain fatty acids including arachidonic acid (AA) and eicosapentaenoic acid (EPA) and (b) metabolism to prostaglandin. Sources: (for review, see Gulliver *et al.* 2012).

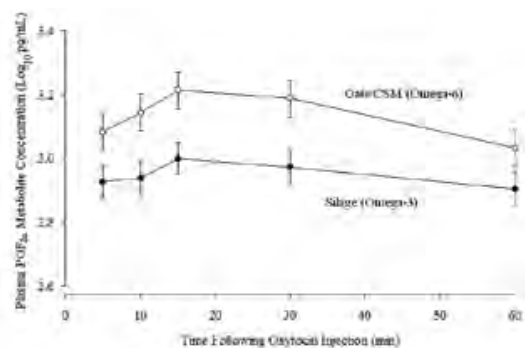


Figure 3. Change in plasma $\text{PGF}_{2\alpha}$ metabolite (PGFM) concentration over time following an oxytocin challenge in ewes fed silage high in omega-3 (●) or oats/cottonseed meal high in omega-6 (◊). Baseline PGFM concentrations for the high omega-3 and high omega-6 diets were 637.1 and 550.2 pg/mL, respectively (re-transformed means) and were included in the statistical analysis as a co-variate. Significant difference between treatment diets, $P = 0.002$.

ovulation, however, further research is required to determine the exact mechanisms involved

Omega-6 fatty acids and sex ratio

Sheep operations would benefit from the ability to skew the sex ratio of offspring towards their preferred gender. For example, male prime lambs grow approximately 20% faster than females and have increased muscle accumulation, thereby reaching a higher market weight over a set time period. First cross enterprises, however, prefer breeding females, which may achieve a higher sale price at weaning.

Maternal nutrition may significantly affect the sex ratio of offspring (for reviews, see Cameron 2004). Maternal body condition, reflecting a high plane of nutrition (Cameron and Linklater 2007; Mathews *et al.* 2008), as well as a number of specific nutritional factors, such as glucose (Kimura *et al.* 2005), total fat (Rosenfeld *et al.* 2003) and polyunsaturated fatty acid (PUFA, Green *et al.* 2008) content of the diet, have been implicated in altering sex ratios. Feeding a diet specifically high in omega-6 fatty acids was also associated with a higher proportion of female offspring in mice (Fountain *et al.* 2008), however, no previous studies have examined the effects of diets high in omega-6 or omega-3 fatty acids on the sex ratio of lambs.

Methods

Animals and dietary treatments

A series of three studies (2–4) with Merino x Border Leicester and Merino ewes were conducted in 2010 and 2011. In study 2 (2010), 296 Merino x Border Leicester (X-Bred) ewes (12 months of age) were allocated to one of two treatment groups. Treatments consisted of pea silage ($n = 148$) high in omega-3 fatty acids or oat grain/cottonseed meal high in omega-6 fatty acids ($n = 148$). Details of animals and feeding have been presented previously (Gulliver *et al.* 2010). In study 3 (2011), 304 X-Bred ewes (12 and 24 months of age) were fed either barley silage or oats/CSM. In study 4 (2011), 320 Merino ewes were fed either ryegrass silage or oats/CSM (Table 1).

In all three studies, animals were fed the treatment diets for approximately six weeks prior to and 17 days following joining. Liveweight and fat score of all ewes was recorded prior to feeding and during pen feeding. Feed samples were collected daily during pen feeding and bulked across weeks of feeding for proximate analysis as described previously (Packer *et al.* 2011). Blood samples were collected from a randomly selected sub-set of ewes prior to the introduction of experimental rations and again following the consumption of treatment diets. Total plasma fatty acids were analysed as described previously (Clayton *et al.* 2012).

Oestrous synchronisation, mating and detection of oestrus

The oestrous cycles of all ewes were synchronised using a controlled internal release device (CIDR, Eazibreed®, Pfizer, Australia) inserted intravaginally for 14 days (King *et al.* 2010). Dorset rams (for X-Bred ewes) or Border Leicester rams (for Merino ewes) were randomly allocated to pen within age (two rams per pen) with a total ram proportion of approximately one ram to 25 ewes as used previously (Robertson *et al.* 2011). Rams were introduced to pens at the estimated time of the first natural oestrus and ram pairs were rotated daily through each pen. Each ram was fitted with a crayon harness and ewes were inspected daily for crayon marks to estimate the time of commencement of behavioural oestrus

from time of ram introduction. Ewes were mated over two consecutive oestrous cycles and were fed for a further 17 days after oestrus detection.

Statistical analyses

Differences in measures between treatment groups were examined using the Mixed Model procedure in SAS with treatment as a fixed effect and individual animal, litter size and pen as random effects (SAS Institute Inc. 1997). Statistical analyses have not been completed for

studies 3 and 4. An alpha of 0.05 was used for all statistical tests.

Results

Plasma omega-6 was higher and omega-3 was lower when ewes were fed the oats/CSM diet high in omega-6 compared with silage diet high in omega-3 (Figure 4). The time to showing behavioural oestrus from ram introduction was shorter (Figure 5) and the proportion of female

Table 1. Components and proximate analysis of diets fed to X-Bred or Merino ewes for approximately six weeks prior to and 17 days following joining in three studies examining the sex ratio of lambs.

Ingredients ^a	Study 2 (X-Bred)		Study 3 (X-Bred)		Study 4 (Merino)	
	Silage	Oats	Silage	Oats	Silage	Oats
Inclusion (% DM)						
Silage	88.3	19.5	88.7	21.8	98.2	21.7
Oat grain	0.0	69.9	0.0	70.1	0.0	68.3
Cottonseed meal	0.0	7.8	0.0	5.5	0.0	7.6
Molasses	9.8	0.0	9.9	0.0	0.0	0.0
Urea	0.0	0.48	0.0	0.44	0.0	0.0
Mineral Premixb	1.84	2.40	1.42	2.22	1.78	2.38
Proximate analysis (% DM)						
Neutral detergent fibre	35.4	37.4	41.8	33.4	50.6	28.3
Acid detergent fibre	26.1	17.9	23.4	19.0	29.1	18.9
Crude protein	12.8	14.8	10.6	16.8	11.2	16.4
ME (MJ/kg DM)	9.6	11.2	9.7	11.2	10.7	11.6
Total lipid	1.15	3.78	2.15	6.83	2.69	4.7
Omega-6:Omega-3 ratio	0.93	13.03	0.41	32.17	0.32	30.00

^a DM = dry matter; ME = Metabolisable energy.

^b Mineral premix (AusFarm Nutrition Products) containing (DM basis) 36.5% NaCl, 21.9% Ca, 2.1% P, 0.10% K, 2.1% S, 3.1% Mg, 52.1 mg/kg Co and 1.04 mg/kg Cu fed at recommended rate of 20 g/hd per day.

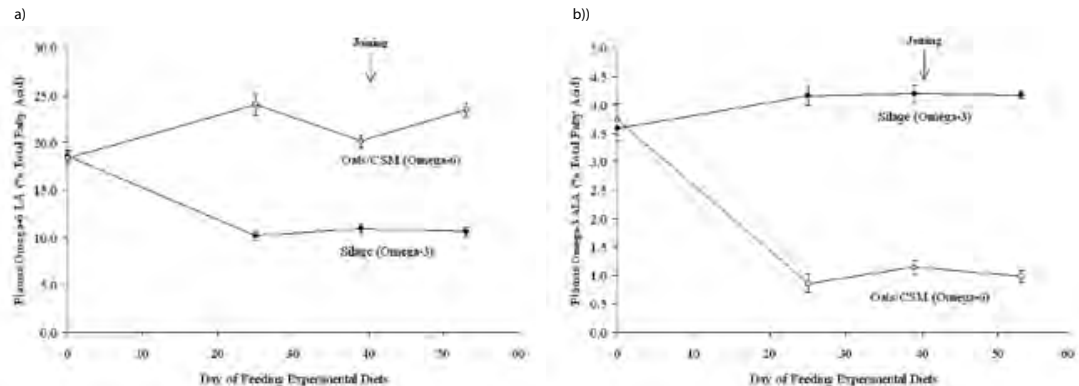


Figure 4. Change in plasma (a) omega-6 and (b) omega-3 in ewes fed silage high in omega-3 (●) or oats/cottonseed meal high in omega-6 (○) for approximately six weeks prior to joining and 17 days following joining.

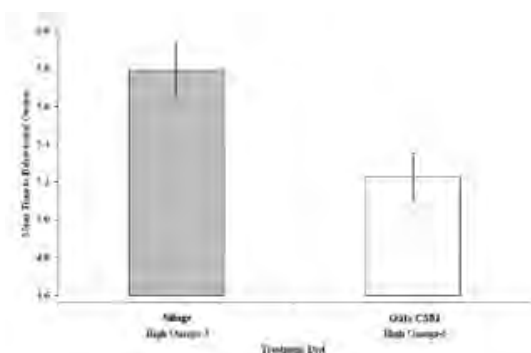


Figure 5. Mean time to behavioural oestrus following synchronisation in first cross ewes fed silage high in omega-3 or oats/cottonseed meal high in omega-6 for approximately six weeks prior to joining.

offspring was higher (58.2 versus 43.5%, $P = 0.010$, Figure 6) when ewes were fed the oats/CSM diet high in omega-6. Although results of studies 3 and 4 have not been statistically analysed, the proportion of female lambs appeared to be consistently higher when ewes were fed the oats/CSM diet in all three studies (Figure 6).

Discussion

The current studies are the first to show an increased $\text{PGF}_{2\alpha}$ response to oxytocin and a consistently higher proportion of female lambs when ewes are fed oats/CSM diets high in omega-6 compared with silage diets low in omega-6 at joining. The exact mechanisms linking the diets with the observed effects are currently not known, however, an increased $\text{PGF}_{2\alpha}$ response and shorter time to oestrus when ewes consumed the oats/CSM diets may affect the timing of conception. The fertilisation of younger ova compared with more mature ova *in vitro*, was associated with a higher proportion of females (Gutierrez-Adan *et al.* 2001; Gutierrez-Adan *et al.* 1999) and the proportion of females was higher in dairy cattle (Pursley *et al.* 1998) and sheep (Gutierrez-Adan *et al.* 1999) when the timing of artificial insemination was closer to ovulation

Selective loss of male embryos post-conception due to increased $\text{PGF}_{2\alpha}$ and increased *in utero* inflammation would also skew the sex ratio in favour of females (Rosenfeld and Roberts 2004).

The higher proportion of female offspring in mice observed previously (Fountain *et al.* 2008) appeared to be due to loss of male embryos (Rosenfeld 2012). In the current studies, however, the total numbers of lambs born were not significantly different when ewes were fed the high omega-6 diets compared with the low omega-6 diets (data not shown), suggesting post-conception loss of male embryos did not occur.

The major limitation of the current studies was that the oats/CSM diets were higher in saturated fatty acids and total fat than the silage-based diets, thereby representing substantial differences apart from omega-3 and omega-6 content. Despite the limitation of differences in diets, the significantly higher proportion of female lambs observed when ewes were fed the high omega-6 diet based on oats/CSM compared with the low omega-6 diet is of considerable practical significance. These changes certainly warrant further investigation in order to determine the mechanisms leading to the observed effects, regardless of whether the effects were related to altered fatty acid status. Feeding a targeted diet for approximately six weeks prior to joining in synchronised ewes may provide a practical mechanism by which to increase the proportion of female lambs.

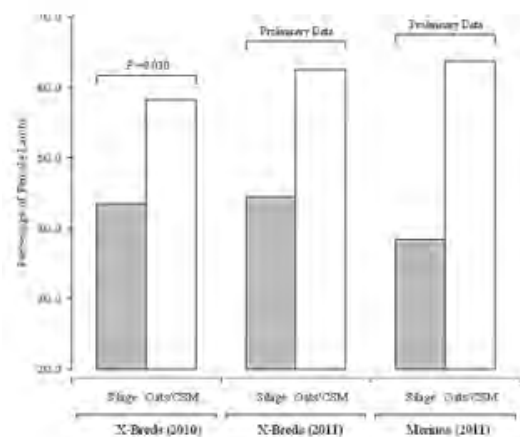


Figure 6. Proportion of female lambs in X-Bred or Merino ewes fed silage high in omega-3 (shaded bars) or oats/cottonseed meal high in omega-6 (unshaded bars) for approximately six weeks prior to joining and 17 days following joining.

A number of on-farm studies are also currently in progress examining whether the feeding regimes can be practically implemented and whether the effects are consistent in unsynchronised ewes.

Conclusions

Feeding diets based on oats/CSM high in omega-6 fatty acids for six weeks prior to joining and 17 days post-joining were associated with an increased PGF_{2α} response to oxytocin, a shorter time to oestrus and a higher proportion of female lambs. Further research is required to determine the mechanisms linking the effects observed in the current study, in particular, whether the effects of omega-6 act pre- or post-conception. If these mechanisms can be identified and there are no detrimental effects to overall lamb survival and subsequent production, practical guidelines may be developed to allow producers to alter female proportions in order to specifically target individual production systems.

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ON FARM R&D



An update on AWI R&D progress to date in the environment, climate change and Carbon area.

ON FARM R&D

Strategy 1: Sheep health, welfare and productivity

Strategy 2: Wool harvesting and clip quality

Strategy 3: The environment, climate change and carbon

Strategy 4: Education and extension

Feedbase & Production Systems

AWI is investing in the following projects to increase pasture resilience and production and overall farm profitability:

- a) Commercialising lotus cultivars: following on from earlier research, AWI is contributing to a Future Farm Industries CRC project which aims to commercialise four lotus cultivars. These new cultivars are suitable for use in acidic, water logged areas as well as low rainfall extensive areas. It is expected these new varieties will be released within the next four years.
- b) EverGraze: AWI, along with MLA and other project partners, continue to support the Future Farm Industries CRC EverGraze project. EverGraze is developing and testing new farming systems in different environments across the high rainfall zone of southern Australia. The target is to increase profits of sheep and cattle enterprises by up to 50% and at the same time improve water management, use of perennials, biodiversity and soil health.
- c) BioControl of Paterson's Curse: AWI is continuing to work with NSW DPI, to establish a self-sustaining network of weed personnel across southern Australia trained in Paterson's curse BioControl with access to BioControl agents.
- d) Grazing management for efficient and sustainable production: AWI, in collaboration with NSW DPI, is funding a research project to develop optimal grazing stocking rates defined as per-head performance. Building on earlier research the project will assess number of paddocks, rotation speed for both native and introduced pastures to develop optimal sustainable stocking rates that increase per head production and achieve NRM outcomes.

Carbon Market Opportunities & Wool Eco-credentials

AWI realises the need for wool producers to be more aware of the carbon market opportunities available as well as making the case for wool.

- a) Wool producers planning for carbon markets on-farm: this pilot project running on the Eyre Peninsula (SA) will work with farm businesses to increase producer capacity in understanding wool grower opportunities in a low carbon economy, from the use of perennial pastures to feed efficiency.
- b) Wool Life Cycle Assessment: a Life Cycle Assessment (LCA) is a review of the environmental impacts associated with all stages of a product's life. AWI is funding a LCA to assess the environmental benefits of wool production from paddock to farm gate to help AWI promote the benefits of wool to urban consumers.
- c) Carbon Credentials: AWI is working with CSIRO to conduct a comprehensive assessment of the contribution wool production systems make in terms of carbon balance in the landscape. The purpose of the project is to help AWI demonstrate to consumers the benefits of wool from an environmental perspective.

For more information call Gus Manatsa – Program Manager, Environment, Climate Change & Carbon on 02 8295 3100.