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Increasing male lamb proportion by feeding ewes omega-3 fatty acids

Final report on the effects of omega-3 in Merino and Border Leicester x Merino first cross ewes and intergenerational effects in maidens

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Project Investigators

The studies presented as part of the current project were conducted by researchers at the NSW Department of Primary Industries (Wagga Wagga Agricultural Institute) and Charles Sturt University through the Graham Centre for Agricultural Innovation, which is a strategic alliance between NSW DPI and CSU. The following researchers contributed significantly to the design and conduct of the studies or analysis and interpretation of results.

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Abstract

Objectives
To determine whether the proportion of female lambs was higher when Border Leicester x Merino ewes or Merino ewes were fed a diet high in omega-3 or omega-6 fatty acids and to determine whether the effect was greater when ewes were fed either pre or post-conception and whether these effects carry over into the second generation.

Methods
The project involved a series of intensive pen feeding studies with Border Leicester x Merino or Merino ewes and on-farm demonstration trials. Ewes were fed a diet high in either omega-3 or omega-6 for 6 weeks pre-conception only or 6 weeks pre and 17 days post-conception. Maiden ewes bred from Merino ewes were also fed omega-3 or omega-6 to determine the intergenerational effects of these diets. Plasma fatty acids, the time to oestrus and joining as well as the sex ratio of lambs was determined.

Results
The proportion of female lambs was 14% higher when ewes were fed a diet high in omega-6 fatty acids both pre- and post-conception. The time to oestrus and parturition was shorter when ewes were fed the High omega-6 diet. The concentration of progesterone was lower when BL x Merino ewes, but not Merino ewes, were fed the High omega-6 diet.

Conclusions
Producers requiring high value breeding females may benefit from feeding omega-6 at joining, whereas those requiring males for meat production may benefit from feeding omega-3.

Executive Summary

Background and objectives
Preliminary trials indicated that feeding a diet high in omega-3 (low in omega-6) to ewes reduces potential inflammation (Gulliver et al., 2013a) and was associated with a higher proportion of male lambs (Gulliver et al., 2013b), while a diet low in omega-3 (high in omega-6) was associated with a higher proportion of female lambs. The effect of these diets on altering the sex ratio of different breeds of sheep and whether the effect of the diets were pre- or post-conception was, however, unknown. Therefore, the objective of the current project was to determine whether the proportion of female lambs was higher when Border Leicester (BL) x Merino ewes or Merino ewes were fed a diet high in omega-3 or omega-6 fatty acids at mating and to determine whether the effect of the experimental diets was greater when ewes were fed either pre-conception only or both pre- and post-conception.

Methodology
The project was conducted in four Phases. Phases 1 and 2 involved a series of intensive pen feeding studies examining the effects of the high omega-3 or high omega-6 fatty acid diets on the sex ratio of lambs from BL x Merino first cross ewes (Phase 1) or Merino ewes (Phase 2) and whether the effect of the diets was greater when ewes were fed either pre-conception only or both pre- and post-conception. Phase 3 involved feeding the experimental diets during on-farm demonstration trials and Phase 4 involved feeding the experimental diets to the maiden first cross ewes born to the Merino ewes in the first year of the current study.

A total of 304 BL x Merino ewes and 320 Merino ewes were enrolled in Phase 1 and 2 of the project. Ewes were allocated to 1 of 4 treatment groups based on property source, fat score then liveweight using a stratified randomised block allocation procedure. Ewes were fed a diet high in omega-3 fatty acids based on ryegrass/cereal silage or high in omega-6 fatty acids based on oats and cottonseed meal (CSM) and fed either pre-conception only or both pre- and post-conception in a 2 x 2 factorial design. The oestrous cycle of ewes was synchronised using CIDRs and all ewes naturally mated with Poll Dorset (BL x Merino ewes) or Border Leicester (Merino ewes) rams and the time of oestrus was estimated by monitoring crayon marks from harnessed rams. The time of lambing and sex, birthweight and head circumference of lambs was determined at parturition. The studies were conducted over 2 years, with pen-feeding repeated in year 2 using the same animals as year 1 with a cross-over design for diets. Results were combined across the two years (Total for both years; n = 160 ewes per treatment group for Merinos and n =152 ewes per treatment for BL x Merino ewes).

The maiden BL x Merino ewes bred from the Merino ewes in the first year of the current study (n = 130) were also fed a diet high in omega-3 or omega-6 fatty acids for 6 weeks pre- and 17 days post-conception in a 2 x 2 factorial design with ewes allocated to treatment group according to the diet fed to their dam. The experimental procedures for the maiden ewes were similar to their dams.

Outcomes
The proportion of female lambs was 9% higher when Border Leicester x Merino ewes were fed a diet high in omega-6 fatty acids (53.3% females) compared with omega-3 fatty acids (44.6% females) either pre-conception or pre- and post-conception; and 12% higher when Merino ewes were fed a diet high in omega-6 (58.7% females) compared with omega-3 (46.7% females) fatty acids. The increased proportion of female lambs when BL x Merino ewes were fed the high omega-6 compared with the high omega-3 diet was greatest in single-bearing ewes (59.4% vs 37.8%, 21% more females) compared with twin-bearing

(51.4% vs 47.8%, 3.6% more females) or triplet-bearing ewes (56.1% vs 46.3%, 10% more females) regardless of the length of time of feeding either pre- or post-conception.

In contrast, the increased proportion of female lambs when Merino ewes were fed the high omega-6 diet compared with the igh omega-3 diet was greater when ewes were fed both pre- and post-conception (64.2% vs 43.9%, 20% more females) compared with pre-conception only (53.1% vs 49.5%, 3.6% more females) regardless of whether ewes gave birth to single or twin lambs.

The proportion of female lambs was 13% higher (55.8 vs 43.1%) when maiden BL x Merino ewes born to the Merino ewes in Phase 2 of the project were also fed the High omega-6 diet compared with the High omega-3 diet at mating. The increased proportion of female lambs when ewes were fed the High omega-6 diet appeared to be greater when ewe dams were also previously fed a diet high in omega-6 (21% more females) compared with omega-3 fatty acids at conception, although none of these differences were statistically significant. The differential effect of diets high in omega-3 or omega-6 fatty acids depending on the diet fed to dams at conception appears to warrant further investigation.

The time from synchronisation of the oestrous cycle to the onset of behavioural oestrus was also significantly shorter when ewes were fed the High omega-6 diet prior to mating. The impact this alteration has on the sex ratio of lambs should be investigated. In particular, an altered timing of oestrus may have impacts in sheep operations using artificial insemination. The observed shorter time to oestrus in BL x Merino ewes may have been related to a lower concentration of plasma progesterone leading up to mating, however, these results were not consistent in Merino ewes.

The results of the sex ratio in the on-farm trials were not consistent with the intensive pen-feeding studies. The proportion of female lambs was not higher when ewes were supplementary fed a diet high in omega-6 fatty acids on-farm. There were a number of results of the current on-farm trials, however, that supported results of the intensive pen-feeding studies. The higher concentration of omega-6 fatty acids in plasma of ewes fed the High omega-6 diet in on-farm Trial 1 demonstrated that systemic fatty acid status can be altered during on-farm feeding. The estimated shorter time to conception when ewes were offered the High omega-6 ration in on-farm Trial 1 indicates that there were significant effects of the different diets on some reproduction parameters. Further refinements to the feeding protocol may be necessary in order to maximise the effect of the diet on the sex ratio of lambs in practical feeding situations.

Adoption strategies and benefits to the industry

Sheep operations would benefit from the opportunity to skew the sex ratio of offspring towards their preferred gender. For example, terminal sire enterprises prefer male prime lambs, as they grow approximately 20% faster than females and have increased muscle accumulation, thereby reaching a higher market weight over a set time period. Self-replacing and first cross enterprises however, prefer breeding females, which may lead to a $30 - $80 higher sale price at weaning.

The results of intensive pen-feeding studies indicate there is the potential to alter the sex ratio of lambs through manipulation of ewe nutrition at conception. Although results were not consistent on-farm in the current project, there is an opportunity to make producers aware of the current feeding strategies employed in the project while the practical on-farm feeding protocols are refined in future studies. In particular, the effect of concentrations of omega-3 available in different pasture species on the proportion of male lambs and the effect of different types of grain as a source of omega-6, the quanitity of grain to feed and the length of time to feed need to be determined in order to implement these feeding strategies successfully on-farm.

The aim of the adoption strategy of the current project is to have a 10% adoption from producers by 2018-19. The enterprises most likely to adopt the technology may be early adopters who have a relatively higher proportion of the total terminal sire or self-replacing ewe flocks in Australia. If the feeding protocols on-farm can be improved, the alteration of sex ratio in practical situations may be increased and there may be an opportunity to improve adoption.

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Glossary of Terms

Term	Description
Behavioural oestrus	Presence of crayon marks on the rump of a ewe from harnessed rams. Also termed “standing heat” by some researchers.
Conception	Estimated time of fertilisation of ova after mating/insemination from the ram. Although the time of conception occurs sometime after behavioural oestrus and mating/insemination, the length of time of feeding in intensive pen trials was in relation to the estimated time of conception in synchronised ewes.
Dam diet	Diet previously fed to BL x Merino maiden ewe dams (Merino ewes) in the first year of Phase 2 of the current project in 2011.
Day of feeding	Day of feeding experimental diets from the first day ewes were introduced to the diets in each study. Refers to the length of time of feeding in days rather than the time period of feeding either pre- or post-conception (see below).
Foetal rate	Mean number of foetuses per ewe scanned pregnant or mean number of foetuses per ewe joined.
Gestation length	Time interval from the time of conception to parturition. This interval will be shorter than the oestrus to parturition interval, as conception occurs some time after the onset of oestrus.
Mating	Describes the time period of introduction of rams to the ewes. The time of mating is estimated from the display of oestrus in synchronised ewes and by assessing crayon marks left by harnessed rams. The time of mating does not indicate that the ova was successfully fertilised at that time. The terms “mating” and “joining” can be used interchangeably, but the terms usually describe synchronised or unsynchronised ewes.
Mating to parturition interval	Time interval between the first day rams were introduced to ewes in pens and the time of parturition for each ewe.
Joining	Refers to the introduction of rams to ewes. This term is usually used to describe the time period of mating in unsynchronised ewes in on-farm trials. The time of conception can be estimated from the age of the foetus at scanning in relation to the time of ram introduction at joining.
Oestrus to parturition interval	Time interval between the display of behavioural oestrus as observed from crayon marks left by rams with crayon harnesses and the time of parturition for each ewe.
Pre-conception	Time period of feeding experimental diets prior to the estimated time of conception. The presence of a crayon mark from harnessed rams indicating behavioural oestrus estimates the time of conception. In all studies ewes were fed experimental rations for at least 40 days prior to the introduction of rams and

Term	Description
	mating/insemination (which estimates the time of conception).
Pre and post-conception	Time period of feeding experimental diets prior to and, following, the estimated time of conception in intensive pen studies. In all studies ewes were fed experimental rations for at least 40 days prior to and, 17 days following, mating/insemination (which estimates the time of conception).
Time of feeding	Length of time of feeding experimental diets, either pre-conception only or both pre- and post-conception.
Time to oestrus	Mean time to the display of behavioural oestrus from the first day any ewe showed oestrus. Describes the first natural oestrus after the synchronised oestrus following CIDR removal.
Time to parturition	Mean time to parturition from the first day any ewe lambed. This time interval could be described also from the first day rams were introduced to ewes.

1.0 General introduction and background

1.1 Introduction and industry relevance

Sheep operations would benefit from the opportunity 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 lead to a $30 - $80 higher sale price at weaning.

Preliminary trials have indicated that feeding a diet high in omega-3 fatty acids (low in omega-6) to ewes reduces potential inflammation (Gulliver et al., 2013a) and was associated with a higher proportion of male lambs (Gulliver et al., 2013b), while a diet low in omega-3 (high in omega-6) was associated with a higher proportion of female lambs. Therefore, there may be an opportunity to selectively increase the proportion of male or female lambs depending on sheep enterprise in order to maximise profits.

The following report presents the results of a reseach program designed to further examine the effect of diets high in omega-3 or omega-6 fatty acids on the sex ratio of lambs from Merino or Border Leicester (BL) x Merino first cross ewes and whether the effect of diet is greatest prior to or following conception. A variation to the project to examine the intergenerational effects of these diets in the BL x Merino first cross maiden ewes bred from the Merino ewes in the current proejct was approved in April 2013. An interim final report presented results from the first 3 phases of the project including BL x Merino ewes, Merino ewes and on-farm demonstration trials. The current final report presents results from all phases of the project including BL x Merino maiden ewes.

1.2 Review of literature

The reproductive ability of the ewe is significantly affected by condition score and, therefore, nutrition, leading up to mating. The effect of nutrition on reproduction in ruminants has been reviewed in detail previously (Lindsay et al., 1993; Scaramuzzi et al., 2006). Maternal nutrition has a significant effect on many stages of reproduction, in particular, the effect of an increasing plane of nutrition leading up to mating on increasing the ovulation rate of ewes has been studied extensively (for reviews, see Scaramuzzi et al., 2011; Scaramuzzi et al., 2006).

A number of specific nutritional factors, such as glucose (Kimura et al., 2005), total fat (Rosenfeld et al., 2003) and fatty acids (Fountain et al., 2008; Green et al., 2008) may significantly affect several aspects of ruminant reproduction. In particular, the effect of dietary fatty acids on reproduction in sheep and cattle have been studied previously (for example, see Funston, 2004; Hess et al., 2008; Santos et al., 2008; Staples et al., 1998; Sturmey et al., 2009), however, these studies have primarily focused on the effects of total dietary fat and energy balance, rather than specific fatty acids.

Several fatty acids may have specific effects on many aspects of ruminant reproduction. In particular, the effects of omega-3 and omega-6 fatty acids on health and reproduction have been studied in detail in humans and ruminants. The following section will provide an overview of omega-3 and omega-6 fatty acids and previously identified effects on ewe reproduction.

1.2.1 Omega-3 and omega-6 fatty acids

Omega-3 polyunsaturated fatty acids (omega-3) are associated with several health benefits in humans. There is growing evidence that omega-3 may be beneficial in the prevention of cardiovascular disease (Horrobin and Bennett, 1999; Simopoulos, 1991) and the treatment of several medical conditions such as asthma (Das, 2004), Crohn's disease (Romano et al., 2005) and psychiatric disorders such as Attention Deficit Hyperactivity Disorder (ADHD), depression and bipolar disorder (Clayton et al., 2007; Parker et al., 2006). Omega-3 fatty acids and, in particular, the ratio of omega-6 polyunsaturated fatty acids (omega-6) to omega-3, may also have important effects of several aspects of animal health and reproduction (Abayasekara and Wathes, 1999; Gulliver et al., 2012).

The primary fatty acids of interest in studies examining reproduction in animals are the long-chain omega-3's eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) and the long-chain omega-6 arachidonic acid (ARA, 20:4n-3). These long-chain polyunsaturated fatty acids (PUFA) are synthesised in the body from the short-chain omega-3 α-linolenic acid (ALA, 18:3n-3) and omega-6 linoleic acid (LA, C18:2n-6) through a number of steps involving desaturation and elongation (Figure 1.1). The short-chain ALA and LA cannot be synthesised by animals (Lands, 1992) and, therefore, must be consumed in the diet. The concentration of omega-3 in plasma (Kemp et al., 1998), red blood cells (Clayton et al., 2012; Gulliver et al., 2010), meat (Scollan et al., 2006), milk (Dewhurst et al., 2003) and reproductive tissue (Kim et al., 2001) are influenced by concentrations of ALA and LA in the diet. Details of the nomenclature used to describe fatty acids is provided in Appendix 4.

1.2.2 Omega-3 fatty acids and prostaglandin

Most of the research examining the effects of omega-3 and omega-6 fatty acids in reproduction in ruminants has involved the study of their effects on prostaglandin. The long-chain fatty acids EPA and ARA are the precursors for eicosanoids including prostaglandins (PG), prostacyclins (PGI), thromboxanes (TX) and leukotrienes (LT, Abayasekara and Wathes, 1999; Smith et al., 1991). The removal of two double bonds from ARA (20:4n-6) by prostaglandin H synthase (PGHS, also called cyclooxygenase, COX) leaves two double bonds and leads to the formation of series-2 eicosanoids, while the removal of two double bonds from EPA (20:5n-3) leads to the formation of series-3 eicosanoids (Figure 1.2).

The eicosanoids are signalling molecules associated with a number of functions in the body including inflammation (Peet and Stokes, 2005). The series-1 and series-3 PG are less inflammatory, while the series-2 PG are more inflammatory (Horrobin and Bennett, 1999; Lands, 1992). The PGs, in particular series-2 PGs including $PGF_{2\alpha}$, play an important role in several aspects of reproduction, including ovulation, oestrus, embryo survival and parturition (for review, see Abayasekara and Wathes, 1999; Gulliver et al., 2012).

Omega-3 and omega-6 fatty acids have opposing biological effects (Stoll et al., 1999) and, in general, omega-3 are anti-inflammatory while omega-6 are pro-inflammatory (Horrobin and Bennett, 1999). Omega-3 fatty acids inhibit uterine secretion of the inflammatory prostaglandin $PGF_{2\alpha}$ (Caldari-Torres et al., 2006) and may, therefore, reduce potential *in utero* inflammation (Gulliver et al., 2013a). In contrast, omega-6 fatty acids are associated with increased inflammatory $PGF_{2\alpha}$ production (Bagga et al., 2003).

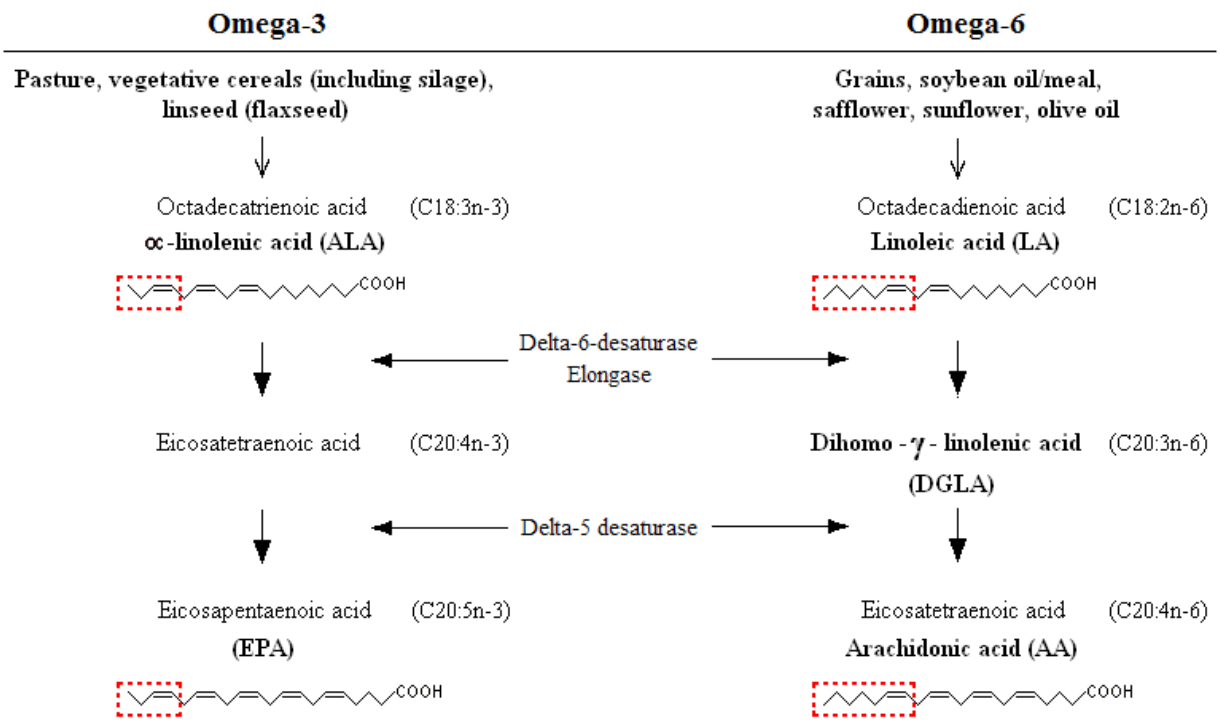


Figure 1.1 Sources and metabolism of short-chain omega-3 or omega-6 fatty acids in ruminant diets to long-chain fatty acids including dihomo-γ-linolenic acid (DGLA), arachidonic acid (ARA) and eicosapentaenoic acid (EPA) important in the production of eicosanoids. Sources: (Clayton et al., 2007; Moore et al., 1991; Parker et al., 2006; Wang and Anderson, 1993).

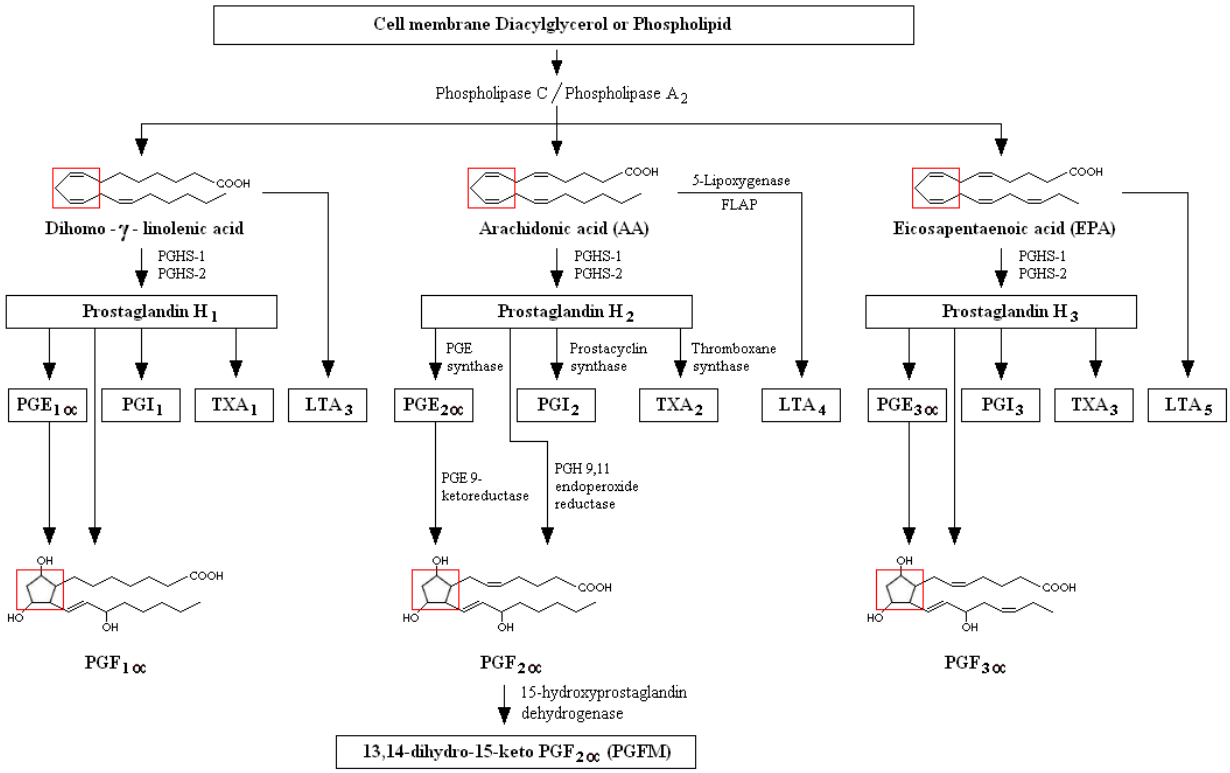


Figure 1.2 Summary of the metabolism of fatty acids to series 1, 2 or series 3 eicosanoids including prostaglandin (Sources, Cheng et al., 2005; Dozier et al., 2008; Fortier et al., 2008; Murphy and Gijon, 2007; Watanabe, 2002; Wathes et al., 2007). PGHS = Prostaglandin H synthase, PGF = Prostaglandin F series, PGI = Prostacyclin, TXA = Thromboxane A, LTA = Leukotriene A, FLAP = 5-lipoxygenase activating protein.

1.2.3 Omega-3 in sheep nutrition

There are several sources of the short-chain omega-3 ALA in ruminant diets, including forages and linseed (Figure 1.1). Long-chain omega-3 (20 carbons or more) purified from sources such as fish oil and fish meal can also be fed to ruminants, however, these supplements are usually expensive and are not often fed on a commercial basis. In particular, the feeding of fishmeal directly to sheep and cattle is banned in Europe and in many countries including Australia. The short-chain omega-6 LA is available from a number of sources, including grains, soybean, safflower and sunflower (Figure 1.1).

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., 2009). In addition, many producers in south east Australia conserve green feed such as cereal crops, lucerne or pasture as silage (Kaiser and Piltz, 2004). The omega-3 content of these silages is similar to the parent material and silages produced from vegetative cereals contain relatively high concentrations of omega-3 and low concentrations of omega-6 fatty acids (Clayton et al., 2010). If pasture supply is limited, however, sheep producers may feed supplements, such as grain (Vinoles et al., 2009), that are high in omega-6 fatty acids (Scollan et al., 2006).

Omega-3 and omega-6 fatty acids may affect several aspects of reproduction (Gulliver et al., 2012). In particular, the ratio of omega-3 and omega-6 fatty acids may affect the sex ratio of offspring. Details of these effects will be described below and form the basis for the conduct of the current project.

1.2.4 Fatty acids and sex ratio

Maternal nutrition may significantly affect several aspects of reproduction, including the sex ratio of offspring (for reviews, see Cameron, 2004; Grant and Chamley, 2010). Maternal body condition, reflecting the plane of nutrition received by the animal (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, Fountain et al., 2008; Green et al., 2008) content of the diet, have been implicated in altering sex ratios.

Specific alterations in the type of fatty acids consumed at the time of conception may also affect the sex ratio of offspring. A diet high in omega-6 fatty acids was associated with an increased proportion of female offspring in mice, which was proposed to be associated with a higher loss of male embryos post-conception (Fountain et al., 2008). The proportion of male offspring was also higher when female opossum were supplemented with sardines high in omega-3 fatty acids prior to conception (Austad and Sunquist, 1986). Omega-3 may have anti-inflammatory effects *in-utero* (Abayasekara and Wathes, 1999) leading to selective fertilisation or reduced loss of male embryos (Hansen et al., 2004; Wamsley et al., 2005).

Preliminary evidence from research conducted at the Wagga Wagga Agricultural Institute (WWAI) indicated that potential prostaglandin synthesis was lower (Gulliver et al., 2013a) and the proportion of male offspring was higher (Gulliver et al., 2013b) when ewes were fed a diet high in omega-3 based on silage compared with a diet high in omega-6 fatty acids based on oats and cottonseed meal (CSM). In addition, ewes fed a high omega-3 diet showed oestrus and lambed later than ewes fed a High omega-6 diet following synchronisation, indicating the time of conception was later when ewes were fed the omega-3 diet. The mechanisms linking these diets high in omega-3 or omega-6 fatty acids with the altered sex ratio are, however, unknown.

2.0 Project aims and objectives

2.1 General project aim and objective

The objective of the current project was to determine whether the sex ratio of lambs was consistently altered when ewes were fed a diet high in omega-3 or omega-6 fatty acids and to determine whether the effects were greater when ewes were fed pre-conception only or both pre- and post-conception. The project was conducted in a number of phases which included pen feeding studies and on-farm demonstration trials.

2.2 Project aims

The aim of the current project was to examine the physiological mechanisms linking diets rich in omega-3 or omega-6 fatty acids with altered sex ratio. Specific aims were;

1. To determine whether the proportion of male lambs was increased when ewes were fed omega-3 either pre- or post-conception.
2. To determine whether the effects of omega-3 and omega-6 were consistent in Merino and Border Leicester x Merino first cross ewes.
3. To determine whether the time to oestrus following synchronisation was consistently longer when ewes were fed omega-3 pre-conception.

4. To determine whether the effects of omega-3 and omega-6 were consistent in on-farm situations.
5. To determine determine the intergenerational effects of diets high in omega-3 or omega-6 and examine whether the effect of omega-3 and omega-6 on the sex ratio of lambs is mediated by diet previously fed to ewe dams at conception.

2.3 Specific project objectives

The specific objectives of the project were;

- By May 2012: Evaluate the impact of feeding diets high in omega-3 to 320 Merino and 288 Border Leicester x Merino first cross ewes on the proportion of male and female lambs.
- By May 2013: Evaluate the impact of feeding diets high in omega-3 to a further 320 Merino and 288 Border Leicester x Merino first cross ewes on the proportion of male and female lambs.
- By May 2013: Complete evaluation of feed and blood samples examining potential mechanisms linking omega-3 and omega-6 with altered sex ratio of lambs.
- By May 2013: Evaluate the impact of feeding diets high in omega-3 or omega-6 to ewes on the proportion of male and female lambs at 3 demonstration trial sites on-farm.
- By December 2013: Evaluate the impact of feeding diets high in omega-3 to Merino x Border Leicester first cross maiden ewes on the proportion of male and female lambs and Determine whether there are intergenerational effects of the diet previously fed to the first cross ewe dams at joining.

2.4 Project outcomes/deliverables

The proposed outcomes and deliverables of the project were to;

1. Increase the proportion of male first cross and second cross lambs by 14% after feeding ewes a diet high in omega-3.
2. Determine the effect of feeding ewes a diet high in omega-3 on the time to oestrus following synchronisation.
3. Examine the relationship between blood fatty acids and sex hormones with the sex ratio of lambs.
4. Publish results on the relationship between dietary omega-6 and omega-3 fatty acids and sex ratio in a peer-reviewed journal.

3.0 General methodology

3.1 Methodological approach

The current project was conducted in four phases; Phase 1 involved Border Leicester x Merino first cross ewes, Phase 2 involved Merino ewes, Phase 3 involved the conduct on-farm demonstration trials and Phase 4 involved the BL x Merino maiden ewes born to the Merino ewes in Phase 2 of the project. Phases 1, 2 and 4 involved a series of pen studies conducted at the Wagga Wagga Agricultural Institue (WWAI) and Phase 3 involved 3 on-farm demonstration trials. A total of 4 pen experiments in Phase 1 and 2 and a single

experiment in Phase 4 were conducted at the WWAI and common methodology for these pen experiments will be presented in detail in the current section. Variations from common methodology for each pen feeding study will be presented in the section detailing each phase of the project (Sections 4, 5 and 7) and the methodology employed for the on-farm demonstration trials will be reported separately in Section 6.

The pen experiments in Phase 1 and 2 were conducted over 2 years in 2011 and 2012 with experiments in year 2 involving the same animals as in year 1 with a cross-over for diets. The study design was a randomised comparative trial with ewes fed a diet high in omega-3 or high in omega-6 fatty acids either pre-conception only or both pre and post-conception in a 2 x 2 factorial design. The primary outcome measure of the studies was the sex ratio of lambs at birth. Secondary outcome measures included the concentration of fatty acids and hormones in plasma and the timing of oestrus or parturition.

3.2 Power analysis

The number of sheep required was determined using a retrospective analysis of outcome measures in a previous study (Gulliver et al., 2013b) and a power analysis program (Faul et al., 2007). For the comparison of sex ratio with the main effect of treatment using a chi-square analysis, compromise analysis, a medium effect size (sex ratio deviation from preliminary data 43:57 to 57:43 - 14% deviation, effect size (w) = 0.282), alpha of 0.05 and a sample size of 110 ewes per treatment, the power was calculated to be 0.90. Considering previous experience with differences in conception rates between Merino and BL x Merino ewes, a total of 160 Merino and 144 BL x Merino first cross ewes per treatment were enrolled in the study.

3.3 Ethics and experimental guidelines

Experimental procedures for the pen studies were approved by the Charles Sturt University Animal Care and Ethics Committee (Phase 1 and 2 Approval No: 11/023, Phase 4 Approval No: 13/017) with co-approval by the NSW Department of Primary Industries (Orange) Animal Care and Ethics Committee. The research was compliant with the *Animal Research Act* 1985 (as amended) in accordance with the ethical principles that have their origins in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2004) and the Australian Model Code of Practice for the Welfare of Animals - Sheep (PISC, 2004). Experimental procedures for the on-farm demonstration trials were approved separately by the Charles Sturt University Animal Care and Ethics Committee (Approval No: 12/001).

All studies were conducted with cognisance of the principles outlined in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary medicinal Products ‘Good Clinical Practice’ (VICH, GL9 - GCP) guidelines. The studies were not monitored by an external monitor but the Animal Nutrition unit at the WWAI was accredited within the ISO9001:2008 quality assurance system.

3.4 Blinding

The study was conducted in a single-blind fashion, whereby personnel conducting laboratory analyses were blinded to treatment.

3.5 Animals

All ewes and rams were purchased for the study by the NSW Department of Primary Industries. The income from all progeny or ewe sales was returned to the NSW DPI. The source of animals for each Phase of the study is shown below.

3.5.1 Phase 1 - Border Leicester x Merino first cross ewes

A total of 304 Border Leicester x Merino ewes were enrolled into the study on the 08/05/2011, including 204 ewes (24 months old) that were part of the current WWAI commercial flock and were involved in a previous sex ratio study (Gulliver et al., 2013b) and 100 maiden ewes purchased for the current study. The details of the ewes are shown below (Table 3.1). Ewes were shorn on the 05/05/2011 prior to study commencement.

Table 3.1 Details of Border Leicester x Merino first cross ewes enrolled into the project in 2011.

Date of Birth (Age)	Source	Property of Origin	Date Purchased	Number
April 2010 (Maidens)	Purchased	"Delco", Ardlethan	19/04/2011	100
April 2009 (24 months)	WWAI	"Wywury", Beckom	23/02/2010	140
April 2009 (24 months)	WWAI	"Fairview", Barellan	23/02/2010	64
			Total	304

A total of 16 Poll Dorset rams were used in the study each year. Rams were from 3 different age groups and 2 properties (Table 3.2). Rams were randomly allocated to pen within age (2 rams from different age groups per pen) with a total ram proportion of approximately 1 ram to 25 ewes as used previously (Robertson et al., 2011).

Table 3.2 Details of Poll Dorset rams used in the project in 2011 and 2012.

Date of Birth	Property of Origin	Date Purchased	Number Used
2007	NI540126	2009	4
2008	NJ552495	March 2010	6
2009	NJ552495	March 2011	6
		Total	16

3.5.2 Phase 2 - Merino ewes

A total of 320 Merino ewes were enrolled in the study on the 21/09/2011. The details of the ewes are shown below (Table 3.3). All ewes were purchased from "Wyoming Station" via Hay on the 30/08/2011. The ewes were from a number of sources and were introduced to Wyoming Station on 07/11/2010. Ewes were shorn on the 29/08/2011 prior to purchase and study enrolment.

Table 3.3 Details of Merino ewes enrolled into the project in 2011.

Age	Property Tag	Number
22-28 months (4-6T)	A5R	151
32 months (6T)	6TS	100
22-28 months (4-6T)	T5L	58
28-32 months (4-6T)	Mixed	11
	Total	320

A total of 16 Border Leicester rams were used in the study each year. Rams were purchased from "Cadell" stud (Ardlethan) at 12 months of age in 2011 (Table 3.4). In the second year of the study (2012), a further 5 rams were purchased from "Gleneith" stud via Ganmain (2011 drop, 12 months of age).

Table 3.4 Details of Border Leicester rams used in the project in 2011 and 2012.

Date of Birth	Property of Origin	Date Purchased	Number Used 2011	Number Used 2012
August 2010	"Cadell"	August 2011	16	11
August 2011	"Gleneith"	September 2012	0	5
		Total	16	16

3.6 Animal housing

All pen studies were conducted at the WWAI Animal Nutrition Unit outdoor pens in Agriculture Avenue (35°02'55"S 147°19'34"E) off Pine Gully Road Wagga Wagga NSW 2650. Ewes were housed in one of 8 outdoor pens (Figure 3.1) for feeding (four 12 m x 7 m pens per treatment, n = 38 ewes per pen for BL x Merino ewes and n = 40 ewes/pen for Merino ewes). Each pen contained adequate trough space for silage or oat grain fed separately (Plate 3.1) and all animals had free access to fresh water. Climatic data were obtained from the Bureau of Meteorology (Wagga Wagga Airport) as an indication of climate at the experimental site (Appendix 5).

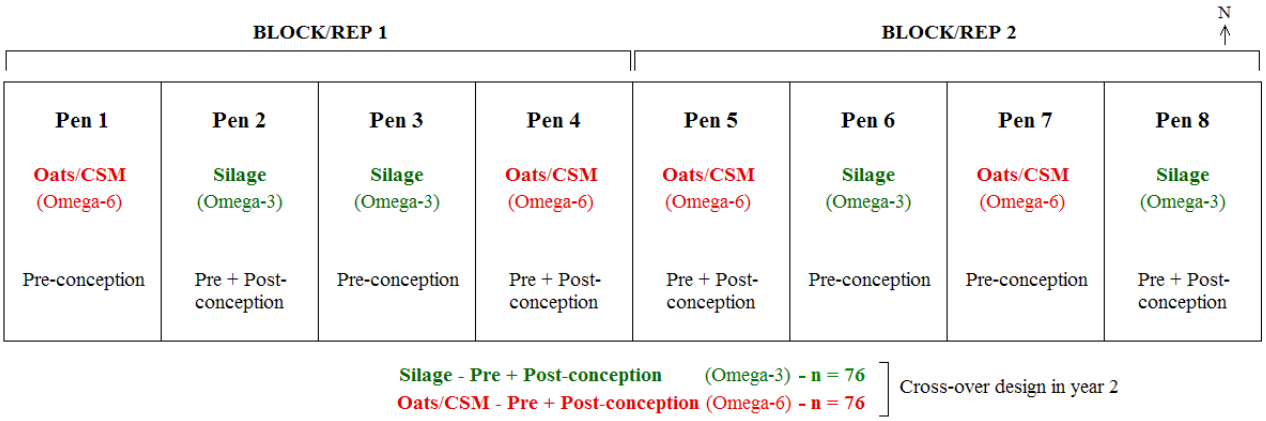


Figure 3.1 Pen layout for feeding studies conducted in 2011 and 2012.

Plate 3.1 Pens used for feeding studies in 2011 and 2012 showing BL x Merino ewes and separate troughs for feeding silage or grain.

3.7 Treatment group allocation

Ewes were randomly allocated to treatment groups using a stratified block randomisation procedure based on age, ewe property source, fat score and then liveweight. Animal weight and fat score was assessed prior to treatment and data was entered into an Excel spreadsheet. Animals were ranked according to age, ewe property source, increasing fat score then increasing live weight. Animals were split into 76 blocks of 4 animals per block (for BL x Merino ewes) or 80 blocks of 4 animals per block (for Merino ewes) and a random number (generated using the "=rand ()" function in Excel) added to each animal. Each block was then sorted by random number and treatment groups assigned to each animal in sequential order from 1 to 4. Animals were sorted by group (n = 76 or 80) and mean fat score and liveweight was calculated to check group balance. Ewes were further allocated to Rep 1 or Rep 2 within treatment group. Treatment groups within reps were then randomly allocated to pen.

3.8 Dietary treatments

Ewes in each pen feeding study were allocated to receive 1 of 2 dietary treatments fed either pre-conception or pre- and post-conception in a 2 x 2 factorial design. Dietary treatments consisted of either a diet high in omega-3 (High omega-3) based on silage, similar to the diet expected when ewes graze fresh pasture (Clayton et al., 2010), or a diet high in omega-6 (High omega-6) based on oat grain and cottonseed meal (CSM, Table 3.5). Diets were formulated to be similar in protein concentration. Groups were fed at levels that provide an approximately equivalent energy intake per day. A commercial vitamin/mineral premix was added to both diets and details of diets fed in each individual pen feeding study are presented in Sections 4 and 5 below.

Table 3.5 Details of the dietary treatments and feeding regime used in each study (2011 or 2012).

Group	Dietary Treatment	Diet[1]	Feeding Regime	Number of Ewes	
				BL x M[2]	Merino
1	High omega-3 (Low omega-6)	Silage (~90%)	Pre-conception	38	40
2	High omega-3 (Low omega-6)	Silage (~90%)	Pre and post-conception	38	40
3	High omega-6 (Low omega-3)	Oat grain (~70%) Silage (~20%) CSM (~8%)	Pre-conception	38	40
4	High omega-6 (Low omega-3)	Oat grain (~70%) Silage (~20%) CSM (~8%)	Pre and post-conception	38	40
			Totals	304	320

[1]Description of silages fed to ewes in individual studies will be presented in Sections 4 and 5.
[2]BL x M = Border Leicester x Merino ewe.

Each pen feeding study was conducted twice over 2 years with the same experimental protocol each year. The experiment was a cross-over design whereby ewes fed the High omega-3 diet in Year 1 were fed the High omega-6 diet in Year 2.

3.9 Animal feeding and ewe management

3.9.1 Feeding

Prior to the experiment, ewes were selected from the NSW Department of Primary Industries (NSW DPI) Wagga Wagga Agricultural Institute (WWAI) commercial sheep flock or purchased from commercial operators. Ewes grazed a mixture of native and improved pastures with the WWAI commercial flock until they were moved into outdoor pens for feeding. Prior to entering each pen feeding study ewes were vaccinated with a 6 in 1 clostridal vaccine and drenched with an effective anthelmintic (Triton, Merial or HatTrick, Ancare).

Ewes were introduced to the experimental rations over a period of 10 days following pen introduction. Details of the introduction of oat grain to all ewes are shown below (Table 3.6). All animals were fed at 1.2 times maintenance (SCA, 1990) with total energy and protein intake per head approximately equivalent between treatment groups. Details of individual rations fed during each pen feeding study will be provided in detail in Sections 4 and 5 below.

Table 3.6 Introduction of oat grain to ewes during pen feeding studies in 2011 and 2012

Day of Feeding	Proportion of Ration (% DM)
1	0
2-4	20
5-7	40
8-10	60
11+	70

Ewes were fed treatment diets for 42 days prior to mating only (pre-conception) or 42 days prior to and 17 days following mating (pre and post-conception) depending on treatment group allocation (Figure 3.3). At the end of treatment feeding (at conception or 17 days post-conception), ewes were removed from the outdoor pens and managed as one group in grazing paddocks with the WWAI commercial flock until lambing. Pregnancy scanning of all ewes was performed approximately 45-65 days after mating.

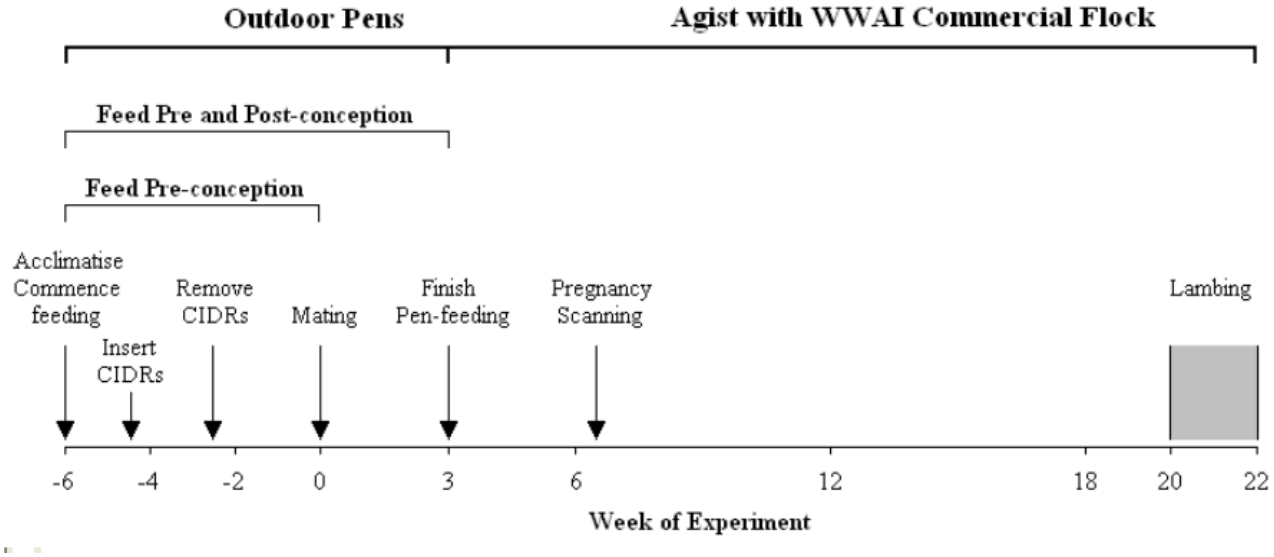


Figure 3.2 Timeline for pen feeding and lambing for studies conducted in 2011 and 2012.

3.9.2 Oestrus synchronisation and mating

The oestrous cycles of all ewes were synchronised using a controlled internal drug release dispenser (CIDR, Eazibreed®, Pfizer, Australia) containing 0.3 g of progesterone. CIDRs were inserted intra-vaginally for 14 days. Ewes were expected to ovulate approximately 54 hr following CIDR removal (King et al., 2010) and were naturally mated with rams on the first natural oestrus after the synchronised oestrus. All ewes were mated with rams at a ratio of approximately 20:1 (ewes:rams, 2 rams per pen) as used previously (Robertson et al., 2011). Merino ewes were mated with Border Leicester rams and BL x Merino ewes were mated with Poll Dorset rams. Rams were introduced to pens 39 days following the introduction of experimental rations (14 days following CIDR removal, Figure 3.3). Rams remained in the pens for 27 days and ram pairs were rotated daily through each pen. Each ram was fitted with a crayon harness (Mating Mark, Rurtec Ltd, Hamilton, New Zealand) with each ram in a pair receiving a different coloured crayon.

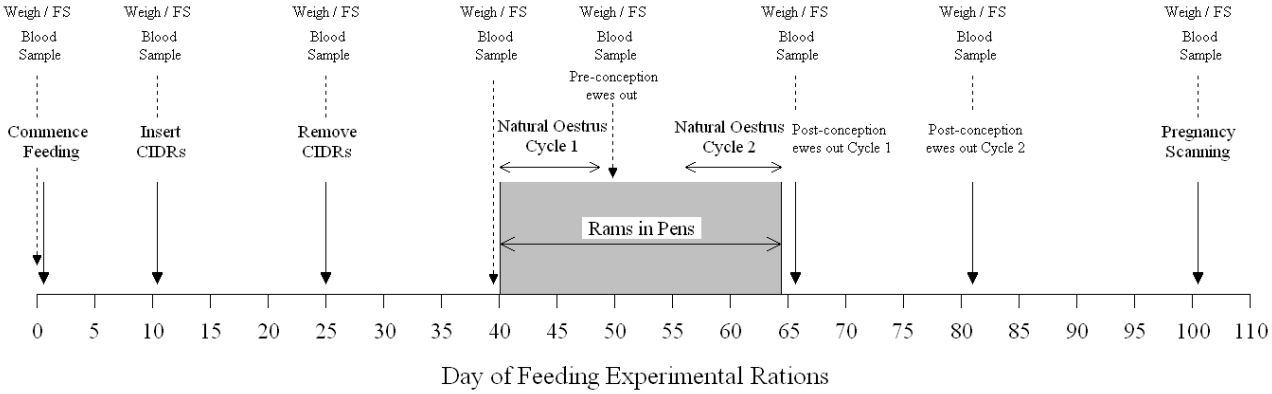


Figure 3.3 Detailed timeline for pen feeding and joining for studies conducted in 2011 and 2012.

Ewes were inspected daily for crayon marks to estimate the time of commencement of behavioural oestrus from time of ram introduction. Ewes were allowed to mate over two consecutive natural oestrous cycles (Cycle 1 or Cycle 2) subsequent to the first synchronised oestrus following CIDR removal. Ewes allocated to receive treatment diets pre-conception only were removed from pens after all ewes had shown oestrus in each oestrous cycle. Ewes allocated to receive treatment diets pre and post-conception remained in pens for a further 17 days after oestrus detection (Figure 3.3).

3.9.3 Ewe management during pregnancy and parturition

Following pen feeding and mating, ewes were managed as a single group and grazed a mixture of native and improved pastures with greater than 1000 kg DM/ha feed on offer (Bell, 2007) throughout pregnancy. Details of ewe management during parturition will be described in detail for each study in Sections 4 and 5.

3.9.4 Concomitant treatments

All ewes were treated as necessary for internal or external parasite control according to standard commercial practices. All treatments were recorded and are available if required. Ewes were shorn yearly in March for BL x Merino ewes and in August for Merino ewes.

3.9.5 Disposal of study animals

At the completion of each study all animals were managed with the commercial flock at the WWAI as per standard commercial practice. At the completion of the live phase of the project all ewes were returned to the WWAI.

3.10 Data collection

3.10.1 Ewe weight and fat score

Ewes were weighed using electronic scales (Ruddweigh 300) and scales were checked for accuracy prior to use on each occasion using a calibrated check-weight. Ewe fat scores were assessed according to the NSW Department of Primary Industries Primefact 'Fat scoring sheep and lambs' (White and Holst, 2006). The weight and fat score of ewes was assessed in each study prior to commcencement of pen feeding, at the time of CIDR

insertion and removal, at the completion of pen feeding, at pregnancy scanning and 3-4 weeks prior to parturition (Figure 3.3)

3.10.2 Feed sample collection

Feed samples were collected daily during pen feeding. Samples were bulked across weeks of pen feeding and a sub-sample collected and frozen at -20^{o}C prior to analysis. For analysis, duplicate samples of each bulked sub-sample were thawed and dried in a fan forced dehydrator for 24 hr at 80^{o}C for proximate analysis. All samples were ground through a 5 mm then a 1 mm screen prior to analysis. A separate sub-sample of each feed for fatty acid analysis was also lyophilised in a freeze-drier at -70^{o}C. Freeze-dried samples were also ground through a 1 mm screen and frozen prior to analysis.

3.10.3 Blood collection

Blood samples were collected from a randomly selected sub-set of 4 ewes per pen (a total of 16 ewes per treatment) prior to the introduction of experimental rations and again at approximately 2 week intervals during pen feeding, at pregnancy scanning (Figure 3.3) and again approximately 4 weeks prior to lambing, for analysis of fatty acid concentrations in plasma and red blood cells. Samples were collected in the morning prior to feeding. Blood was collected from the jugular vein into a 4 mL evacuated tube containing lithium heparin (Vacutainer^{TM}, Interleuvenlaan 40, Terumo Corporation, Belgium).

Blood samples were centrifuged as soon as practicable following collection (at least within 2.5 hr) for the separation of plasma and red blood cells (RBC). Samples were centrifuged at 1500 x g for 10 min and plasma decanted using a disposable plastic pipette into a 1.5 mL plastic eppendorf tube. RBC were then washed with isotonic saline (0.9% NaCl) and centrifuged at 1500 x g for 10 min according to methods outlined previously (Clayton et al., 2008). After decanting saline, RBC were collected and stored separately from plasma.

3.10.4 Pregnancy scanning

The pregnancy status of ewes, litter size and age of the foetus was determined by ultrasonography approximately 40 - 75 days post mating. In 2011 (Year 1) scanning was conducted with an Aloka SSD500 ultrasound scanner (Aloka Co., Tokyo, Japan) with an external 3.5 MHz linear transducer (Wilkins, 1997) at 38 or 54 days post mating (BL x Merino ewes) and 51 or 66 days post mating (Merino ewes) according to whether ewes were mated in Cycle 2 or Cycle 1, respectively. In 2012 (Year 2) scanning was conducted with an Ovi-Scan 6 ultrasound scanner (BCF Technology Ltd, Livington, Scotland) with an external 3.5 MHz sector transducer at 60 or 76 days post mating (BL x Merino ewes) and 46 or 62 days post mating (Merino ewes). The time of conception was estimated from the age of the foetus.

3.10.5 Parturition

Ewes were moved to lambing paddocks at least 3 weeks prior to parturition. Prior to lambing, ewes were acclimatised to the presence of humans in close proximity within their paddocks for at least two weeks. Ewes were observed twice daily for the duration of lambing. All lambs born (dead or alive) were ear-tagged and their dam identified. Sex, litter size (single, twin or triplet), birth weight, head circumference in the coronal plane (Jamison et al., 1961), ewe and lamb behaviour (see details in Appendix 6) and survival to 24 hr was recorded. The time of lambing for each ewe, in relation to the time of birth of the first lamb from any ewe in each study, was defined as the “time to parturition”. Ewes were also examined at lamb marking to determine those that gave birth to a lamb (Dun, 1963).

3.11 Laboratory analyses

3.11.1 Proximate analysis

Proximate analyses including fibre and digestibility (% DM) were determined using near infra-red reflectance spectroscopy (NIR) with a Bruker multi-purpose analyser (MPA, Bruker Optik GmbH, Ettlingen, Germany) and OPUS software (version 5.1) with calibrations developed by the NSW DPI Feed Quality Service (FQS) as described previously (Packer et al., 2011). Calibrations were based on the following methods including; neutral detergent fibre (NDF) and acid detergent fibre (ADF) analysed sequentially (Van Soest et al., 1991) using the filter bag method (Ankom® 200/220 fibre analyser, ANKOM technology, Macedon, NY, USA), ash by heating a sample in a muffle furnace at 550^{o}C for 6 hr (AOAC, 1990a) and digestible organic matter in the dry matter (DOMD) using the pepsin cellulase digestibility assay (Clarke et al., 1982).

In addition, the concentration of crude protein (CP) and total fat in feed was determined by wet chemistry. Concentrations of nitrogen (N) were determined using using the Dumas combustion method with a Leco CNS 2000® analyser (Leco, St. Joseph, MI, USA) (AOAC, 1990b). Total lipid concentration (ether extract, EE) was determined by solvent extraction using the Randall method (Randall, 1974) with hexane, which has been validated for use with animal feed (Thiex et al., 2003) using a FOSS solvent extraction system (Soxtec™ 2050 Avanti Extraction Unit, FOSS, Hoganas, Sweden) according to the FOSS Application Note (FOSS, 2003).

Crude Protein (CP) was estimated from N (CP (%DM) = N (%DM) x 6.25). Metabolisable energy (ME) for was estimated from DOMD using the following equations (AFIA, 2006; Kaiser et al., 2005):

Pasture - ME (MJ/kg DM) = 0.203 x DOMD (% DM) - 3.001
Silage - ME (MJ/kg DM) = 0.16 x DOMD (% DM)
Grain - ME (MJ/kg DM) =0.138 x DOMD (% DM) + 0.272 x EE + 0.858

3.11.2 Analysis of fatty acid methyl esters (FAME) in plasma

Reagents and standards
Methanol, toluene, acetyl chloride and potassium carbonate (K_2CO_3) were all purchased from Sigma. FAME standards were purchased from Nu Chek Prep (GLC68C, GLC463 and C22:5n-6, Elysian, MN USA), Supelco (37 Component FAME Mix, Cat No. 47885-U and C18:1n-7, Cat No. 4-6904, Supelco, Bellefonte, PA) and Sigma (C18:3n-6, Cat No. L6503-100MG, C20:5n-3, Cat No. E2012-5MG and C22:2n-6, Cat No. D4034-25MG, St Louis, MO). The tridecanoic acid (C13:0, Cat No. T0627-1G) and nonadecanoic acid (C19:0, Cat No. N5377-1G) methyl ester internal standards were also purchased from Sigma (St Louis, MO).

Fatty acid extraction and methylation
Total plasma lipids were extracted and fatty acids were methylated using the one-step procedure of Lepage and Roy as described previously (Clayton et al., 2012). In brief, 100 μL of plasma was added to an 8.0 mL glass culture tube and exactly 2.0 mL of methanol:toluene (4:1 v/v) containing C19:0 (0.02 mg/mL) internal standard was added and vortexed vigorously. Fatty acids were methylated by adding 200 μL acetyl chloride drop-wise while vortexing, followed by heating to 100^{o}C for 1 hr. After cooling, the reaction was stopped by slowly adding 5 mL of 6% K_2CO_3 while mixing by vortex. The sample was centrifuged at 1500 x g for 10 min to facilitate the separation of layers. The upper toluene layer containing

the FAME was transferred to a 2 mL glass vial containing a 400 μL glass insert and sealed with a teflon lined screw-cap for subsequent analysis by gas chromatography.

Gas chromatography

Individual fatty acid methyl esters (FAME) were identified using either an Agilent 6890N gas chromatograph (GC) with a single flame ionisation detector (FID) for studies conducted in 2011 or an Agilent 7890A GC with dual FIDs for studies conducted in 2012. In all analyses, FAME were separated using a fused carbon-silica column, coated with cyanopropylphenyl (BPX70, 30 m x 0.25 mm i.d. and 0.25 um film thickness, SGE International, Ringwood, Victoria, Australia, P/N 054622). The carrier gas was helium with a total flow rate of 12.4 mL min^{-1}, with a split ratio of 10:1 and a column flow of 0.9 mL min^{-1}. The inlet temperature was 250^{o}C, inlet pressure was 107.8 kPa and injection volume was 3 μL into a focus inlet liner (4 mm i.d. SGE Analytical Science, P/N 092018) that was changed at least every 150 injections.

The oven temperature was set at 150^{o}C and held for 0.5 min, increased 10^{o}C min^{-1} to 180^{o}C, increased 1.5^{o}C min^{-1} to 220^{o}C and then increased 30^{o}C min^{-1} up to 260^{o}C and maintained for 5 min to give a total run time of 36.5 min. The FID temperature was set at 280^{o}C with the following gas flow rates; hydrogen = 35 mL min^{-1}, instrument air = 350 mL min^{-1} and nitrogen make-up gas = 30 mL min^{-1}.

Identification and quantification of FAME

Sample FAME peaks were identified by comparing their retention times with those of a standard mixture of genuine FAME (Nu Chek Prep, Elysian, MN USA; Supelco Bellefonte, PA and Sigma, St Louis, MO as indicated above) and quantified using Agilent Chemstations Version B.01.03 or Version C.01.03 and Microsoft Excel using a four point standard curve for each FAME using nonadecanoic acid methyl ester (C19:0) as the internal standard. In addition, some peaks for branched-chain fatty acids, for which standard FAME were not available, were identified by comparison with published data as described by (Or-Rashid et al., 2010). A number of fatty acids were not identified in Study 1 (2011, see Table 4.7), but were subsequently identified in all other studies. All results were calculated as μg/mL plasma. A detailed summary of the fatty acid nomenclature used in the current report is included in Appendix 4.

The lower limit of detection (LoD) was calculated from three times the standard deviation of the estimated peak area:internal standard peak area ratio for a concentration equal to 0 interpolated from the four standards (NATA, 2009). The LoD was less than 0.01 μg/mL for all FAME analysed. The co-efficient of variation (CV) for the analyses ranged between 1.1-2.2% for fatty acids comprising greater than 1% of total identified FAME.

Markers of fatty acid metabolism

In addition to the concentration of fatty acids in plasma, a number of indices were calculated. These indices provide further information on fatty acid metabolism.

DHA Deficiency Index (DHADI)

The DHA Deficiency Index (DHADI) is considered a marker of DHA deficiency (van den Ham et al., 2001). A deficit in DHA is accompanied by an increased conversion of C22:4n-6 to C22:5n-6 resulting in a higher DHADI (Neuringer et al., 1986). The DHADI is calculated using the following formula (Neuringer et al., 1986);

DHA Deficiency Index (DHADI) = 22:5n-6 (DPA n-6, Osbond acid) / C22:4n-6 (Adrenic acid)

DHA Sufficiency Index (DHASI)
The DHA Sufficiency Index (DHASI) is also used as an indicator of DHA status (van den Ham et al., 2001). A deficiency of DHA stimulates the synthesis of the most comparable omega-6 fatty acid, which is C22:5n-6 (DPA n-6, or Osbond acid), therefore, representing the ratio of the end-products of omega-3 and omega-6 metabolism (Hoffman and Uauy, 1992; Hornstra and De Vriese, 2003). Factors that reduce DHA status lower DHASI (van den Ham et al., 2001). The DHASI is calculated using the following formula (Hoffman and Uauy, 1992);

DHA Sufficiency Index (DHASI) = 22:6n-3 (DHA) / 22:5n-6 (DPA n-6, Osbond acid)

Essential Fatty Acid Status Index (EFI)
The Essential Fatty Acid Status Index (EFI) represents the ratio of polyunsaturated to monounsaturated fatty acids. A higher EFI is considered healthier and is calculated using the following formula (de Groot et al., 2004);

EFI = (Omega-3 PUFA + Omega-6 PUFA) / (n-7 MUFA + n-9 MUFA)

Polyunsaturated Fatty Acid to Saturated Fatty Acid) Ratio (P:S Ratio)
The polyunsaturated fatty acid (PUFA) to saturated fatty acid (SFA) ratio (P:S Ratio) is also used as a marker of healthy fatty acid status. A higher P:S ratio indicates that the diet is higher in PUFA compared with SFA and, in humans, is thought to represent a lower risk of inflammatory diseases and cardiovascular disease (Hoffman and Uauy, 1992). The P:S ratio is calculated using the following formula (Singer et al., 1986);

P:S Ratio = Sum of PUFA / Sum of SFA

3.11.3 Analysis of fatty acid methyl esters (FAME) in feed

Total fatty acids were extracted and methylated directly from a freeze-dried sample using the one-step procedure of Lepage and Roy (1986) as modified by Outen et al. (1976). The method was similar to that employed for plasma as described above. For pasture and silage, a 0.02 g sample was methylated with the addition of C19:0 internal standard, while for grain a 0.01 g sample was methylated with the addition of C13:0 internal standard.

3.11.4 Analysis of oestradiol and progesterone in plasma

Oestradiol-17β (E_2)
The concentration of oestradiol-17β (E_2) in plasma was determined in duplicate by RIA using an Adaltis MAIA E_2 kit from Diagnostic Technology (Belrose, NSW 2085, Australia) by the University of Western Australia. The kit was modified to obtain a lower limit of detection of 0.2 pg/mL. In brief, the number 6 E_2 standard in the kit was diluted to give a standard range from 0.2 - 50 pg/mL and the volume of each standard and plasma sample used was increased to 250 µL. Each sample was extracted with 2.0 mL of high purity diethyl ether (Cat No: 31685, Sigma Aldrich) prior to analysis. The extracted samples were left to dry overnight and then reconstituted in 250 µL of 0.01 M phosphate buffered saline containing 0.1% gelatin (PBSG). The 1st antibody was diluted 1 in 6 in PBSG and 100 µL was added to each sample. After incubating for an hour at room temperature, 50 µL of tracer (diluted to 10,000 cpm/50 µL) was added and samples incubated for 3 hr at room temperature. A separation reagent (250 µL) was added and samples were incubated for a further 30 min prior to centrifugation at 2500 x g for 40 min. The supernatant was then used in the analysis of E_2. The lower limit of detection of the assay was 0.2 pg/mL and the intra-assay coefficient of variation (CV) was 2.2% for E_2 concentrations up to 4.5 pg/ml and 0.6% for E_2 concentrations up to 17.2 pg/ml.

Progesterone (P_4)
The concentration of progesterone in plasma was analysed in duplicate using an Immunotech RIA Progesterone kit (Beckman Coulter Gladesville, NSW 2111, Australia). The lower limit of detection was 0.1 ng/ml. The lower limit of detection of the assay was 0.1 ng/mL and the intra-assay coefficient of variation (CV) was 1.4% for low (1.22 ng/ml) and 3.3% for high (5.6 ng/ml) P_4 concentrations and the inter-assay CV was 3.9 and 7.7% respectively.

3.12 Data manipulation and statistical analyses

3.12.1 Data manipulation

Prior to analysis, data were assessed for linearity and homoscedasticity assumptions using the Scatterplot option and for normality assumptions using the Explore option of the Statistical Package for Social Sciences (SPSS) version 19.0 for Windows (Coakes and Steed, 2001). Where appropriate, data were transformed prior to analysis.

Ewes that were allocated to receive treatment diets both pre- and post-conception but were mated in Cycle 2 and were removed from pens immediately following mating, were classed as having been fed pre-conception only. If ewes were allocated to receive treatment diets pre-conception only and they were removed from pens after showing oestrus in the first cycle but subsequently were found to have conceived in the second cycle, data from these ewes was not included in the statistical analysis of sex ratio as the ewes were not fed up until the time of conception.

3.12.2 Statistical analyses

Changes in live weight, fat score, plasma fatty acids and hormones over time between treatment groups (high omega-3 vs high omega-6 and pre-conception vs pre- and post-conception) were analysed by repeated measures analysis using the Mixed Model procedure in the SAS statistical program (SAS Institute Inc., 1997). The restricted maximum likelihood (REML) estimation used “ewe x year” as the individual experimental unit and “ewe within treatment”, “ewe x year” or “ewe x pen” as random effects (Clayton et al., 2008; Littell et al., 1998). The most appropriate covariance structure for each analysis was determined by reference to the Schwarz’s Bayesian Information Criterion (BIC, Wang and Goonewardene, 2004). The analysis determined the fixed effects of dietary treatment, time of feeding (pre-conception versus pre- and post-conception) and day of feeding (day of feeding experimental diets) as well as the interaction between fixed effects. Where appropriate, baseline values were analysed as covariates and where significant, were included in the final model.
Differences in the time to showing behavioural oestrus (from the first day any ewe showed oestrus) or the time to parturition (from the day the first lamb was born) between treatment groups were determined using Cox’s Proportional Hazards Regression Analysis (Cox, 1972) in SAS. The use of Hazards Regression analysis to calculate the Relative Risk (RR) of an event, particularly the time of conception or lambing, has been reviewed previously (Cunningham et al., 1981; Holst et al., 1986).

An estimated oestrus to parturition interval was calculated from the time to showing behavioural oestrus and the day of parturition for each ewe. The mean time to showing behavioural oestrus, mean time to lambing, oestrus to parturition interval and lamb birthweight and head circumference were also analysed using the Mixed Model procedure in SAS with treatment diet or time of feeding as fixed effects and “pen” or “lambing cycle” as random effects. Sub-group analyses also determined the effect of litter size on reproduction data.

Reproduction data, including the proportion of ewes pregnant or lambed were analysed by Chi-square analysis using the Frequency procedure in SAS. Differences in the proportion of male and female lambs between treatment groups were analysed using the Mixed Model procedure in SAS with treatment diet, time of feeding and litter size as fixed effects and "individual ewe" or "pen" as random effects. The relationship between the concentration of fatty acids in feed, the concentration of fatty acids in plasma and reproduction data were analysed by Pearson correlation using SAS.

Data from one BL x Merino ewe fed the high omega-3 diet in Study 1 was excluded from the gestation length and lamb birth weight analyses as she gave birth to premature twin lambs (estimated day 117 of gestation). An alpha of 0.05 was used for all statistical tests.

4.0 Phase 1: Border Leicester x merino first cross ewes

4.1 Introduction

In our previous study, the proportion of female lambs was significantly higher when Border Leicester (BL) x Merino first cross ewes were fed a diet high in omega-6 (low in omega-3 fatty acids) for 6 weeks prior to and, 3 weeks following, mating (Gulliver et al., 2013b). Although the study involved 290 first cross ewes, in order to confirm the effects of diets either high or low in omega-3 fatty acids on the sex ratio of lambs, it was necessary to repeat the experiment with larger numbers. In addition, it was not possible to determine from our previous study whether the effect of omega-3 or omega-6 fatty acids was greater when ewes were fed experimental rations pre or post-conception. Therefore, the aim of the current Phase of the study was to determine whether the proportion of female of lambs was consistently higher when BL x Merino ewes were fed a diet high in omega-6 compared with omega-3 fatty acids and whether the effect was greater when ewes were fed either pre-conception only or both pre- and post-conception.

As indicated above, the current project involved 3 Phases. Phase 1 involved 2 pen studies with BL x Merino ewes conducted in 2011 and 2012 with a cross-over for diets in the second year. The current section presents details of these 2 pen feeding studies. Details of methodology specific to these studies will be presented, including details of specific diets used and animal management. Results for the effect of diet on ewe weight, fat score and plasma concentrations of fatty acids will be presented for each study. Data for the primary reproduction outcomes including the timing of oestrus and parturition, reproduction hormones and the sex ratio of lambs were combined across years and analysed together.

4.2 Methods

4.2.1 Study 1

The first pen feeding study commenced in May 2011 (WWAI Study ID: 11-07). Details of experimental procedures for Study 1 are outlined below.

Animals
A total of 304 BL x Merino ewes (12 or 24 months of age, see Table 3.1) were vaccinated against clostridial diseases and caseous lymphadenitis (CLA, Glanvac 6, Pfizer Animal Health, West Ryde, Sydney) and treated with a combination anthelmintic drench (Triton, Merial, NSW) prior to enrolment in the study. The study was conducted at the WWAI with pen feeding commencing in May (Autumn) and lambing occurring in November 2011.

Experimental diets

Ewes were randomly allocated to 1 of 4 treatment groups (as indicated in Section 3.8) according to property source, fat score (FS, mean = 3.32 ± 0.03) then live weight (mean = 64.8 ± 0.55 kg). Treatments consisted of either a diet based on barley silage (n = 152) high in omega-3 or a diet based on oat grain and CSM high in omega-6 (n = 152) fatty acids (Table 4.1).

Table 4.1 Components and proximate analysis of diets offered to BL x Merino ewes for 6 weeks prior to mating or 6 weeks prior to and 17 days following mating in Study 1 conducted in 2011.

Ingredients	Treatment Diet	
	High Omega-3 (Silage)	High Omega-6 (Oats/CSM)
Inclusion	**(%DM)**	
Silage	89.36	22.05
Oat grain	0.0	70.87
Cottonseed Meal	0.0	5.51
Molasses	9.92	0.0
Urea	0.0	0.45
Mineral Premix[1]	0.72	1.12
Proximate Analysis	**(%DM)**	
Neutral Detergent Fibre	42.14	33.98
Acid Detergent Fibre	23.60	19.06
Crude Protein	12.51	16.20
Total Lipid	2.17	7.18
ME (MJ/kg DM)	9.75	11.43
Fatty Acid Composition	**g/kg DM (% Total Fatty Acids)**	
C14:0	0.40 (2.17%)	0.26 (0.37%)
C16:0	3.23 (17.5%)	10.90 (15.2%)
C18:0	0.51 (2.75%)	1.25 (1.74%)
C18:1n-9	0.65 (3.51%)	24.41 (34.0%)
C18:1n-7	0.14 (0.76%)	0.47 (0.66%)
C18:2n-6	2.36 (12.8%)	22.74 (31.7%)
C18:3n-3	5.80 (31.4%)	2.12 (2.95%)
Ratio of n-6:n-3[2]	0.41	10.73
Feed Offered[3]	**(per head)**	
DM (kg/day)	1.40	0.89
ME (MJ/day)	13.62	10.16
CP (g/day)	174.75	144.03

[1]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 10 g/head per day.
[2]Ratio of n-6:n-3 = ratio of omega-6 to omega-3 fatty acids.
[3]DM = dry matter, ME = metabolisable energy, CP = crude protein.

Feeding and ewe management

Ewes were housed in 1 of 8 pens (38 ewes/pen). The majority of ewes in both groups allocated to receive treatment diets pre-conception only showed oestrus in Cycle 1 (High omega-3 = 88.2%, Low omega-3 = 98.6%). Pen feeding was, therefore, ceased for all ewes in these pens after the last ewe showed oestrus in Cycle 1 and ewes were moved to paddocks. Ewes allocated to receive treatment diets pre- and post-conception remained in pens and received treatment diets for a further 17 days before being moved to paddocks with the ewes that were fed pre-conception only. The timing of insemination (Cycle 1 or 2) was confirmed for each ewe at pregnancy scanning.

Animal withdrawals during the study

Two ewes (fed the High omega-6 diet) were stolen prior to mating. One ewe also fed the High omega-6 diet died between pen feeding and pregnancy scanning. Details of animal withdrawals can be found in Appendix 3.

Data collection

Ewe weight and fat score was assessed during pen feeding as described previously (Section 3.10). Blood samples were also collected from a sub-set of ewes in each pen for fatty acid and hormone analysis as described previously. Pregnancy scanning was conducted 54 or 38 days after the last ewe showed oestrus in Cycle 1 or Cycle 2, respectively.

Ewe management during parturition

Ewes lambed in 1 of 3 paddocks depending on whether ewes were scanned as conceiving in Cycle 1 or 2 and carrying single or twin foetuses (Table 4.2). A mixture of ewes from both dietary treatment groups were run together in each paddock. Ewes were branded with their individual ear tag number on each side for identification during lambing (Plate 4.1).

Table 4.2 Details of lambing paddocks used for BL x Merino ewes in Study 1 in 2011.

Paddock	Pasture	Cycle	Scanned	Dietary Treatment	Number
1	Lucerne	1	Single	High n-3 + High n-6	127
2	Mixed	1	Multiple	High n-3 + High n-6	133
3	Mixed	2	Dry/Single/Multiple	High n-3 + High n-6	43
Total					303

Plate 4.1 Identification of Border Leicester x Merino ewes at lambing in Study 1 in 2011

4.2.2 Study 2

The second pen feeding study commenced in April 2012 (WWAI Study ID: 12-09). Details of experimental procedures for Study 2 are outlined below.

Animals
Due to the loss of a number of ewes between lamb marking in 2011 and the commencement of pen feeding in 2012, only 291 BL x Merino ewes (24 or 36 months of age) were available for feeding in Study 2 (Table 4.2). All ewes were vaccinated against clostridial diseases and caseous lymphadenitis (CLA, Glanvac 6, Pfizer Animal Health, West Ryde, Sydney) and treated with a combination anthelmintic drench (HatTrick, Ancare, NSW) prior to the start of the second pen feeding study. Pen feeding commencing in April (Autumn) and lambing occurred in October 2012.

The mean weight of ewes at the commencement of the pen feeding study in 2012 was significantly (p = 0.023) higher when ewes were fed the high omega-6 (Oats/CSM) diet (62.6 ± 0.90) compared with the high omega-3 (silage) diet (60.5 ± 0.89) the previous year in 2011. The mean fat score of ewes at the commencement of the pen feeding study in 2012 was not, however, significantly (p = 0.056) higher when ewes were fed the high omega-6 diet (3.47 ± 0.09) compared with the high omega-3 diet (3.28 ± 0.09) in 2011. More details of the change in weight during the pen feeding studies will be shown in Section 4.3 below.

Table 4.3 Number of BL x Merino ewes from each source enrolled in Study 2 in 2012.

Source	High Omega-3 (Silage)	High Omega-6 (Oats/CSM)	Total
Delco	49	48	97
Wywurry	67	64	131
Fairview	32	31	63
Total	148	143	291

Experimental diets
In Study 2 in 2012, treatment diets were either based on oaten silage (high omega-3) or oat grain and CSM (high omega-6, Table 4.4). The silage diet used in the current study was also used in Study 4. The amount of feed offered to ewes in each treatment group is shown below (Table 4.4). Ewes allocated to receive the High omega-3 diet in Study 1 received the High omega-6 diet in Study 2. Ewes that were allocated to receive treatment diets pre-conception only or both pre- and post-conception received diets for the same length of time in Study 2 as in Study 1.

Feeding and ewe management
Ewes were housed in the same pens as in 2011 with a total of 34-38 ewes/pen depending on the ewes available in each treatment group. The majority of ewes in both groups allocated to receive treatment diets pre-conception only showed oestrus in Cycle 1 (high omega-3 = 95.8%, high omega-6 = 99.3%). Pen feeding was, therefore, ceased for all ewes in these pens after the last ewe showed oestrus in Cycle 1 and ewes were moved to paddocks. Ewes allocated to receive treatment diets pre- and post-conception remained in pens and received treatment diets for a further 17 days before being moved to paddocks with the ewes that were fed pre-conception only. The timing of insemination (Cycle 1 or 2) was confirmed for each ewe at pregnancy scanning.

Table 4.4 Components and proximate analysis offered to BL x Merino ewes for 6 weeks prior to mating or 6 weeks prior to and 17 days following mating in study 2 conducted in 2012.

Ingredients	Treatment Diet	
	High Omega-3 (Silage)	High Omega-6 (Oats/CSM)
Inclusion	**(%DM)**	
Silage	89.38	22.12
Oat grain	0.0	71.19
Cottonseed Meal	0.0	5.57
Molasses	9.91	0.0
Mineral Premix[1]	0.71	1.12
Proximate Analysis	**(%DM)**	
Neutral Detergent Fibre	55.45	35.78
Acid Detergent Fibre	31.78	19.95
Crude Protein	10.23	14.69
Total Lipid	2.51	5.08
ME (MJ/kg DM)	9.45	11.49
Fatty Acid Composition	**g/kg DM (% total fatty acids)**	
C14:0	0.24 (1.26%)	0.15 (0.29%)
C16:0	4.00 (21.2%)	7.90 (15.6%)
C18:0	0.47 (2.51%)	0.98 (1.93%)
C18:1n-9	3.84 (20.3%)	17.34 (34.2%)
C18:1n-7	0.13 (0.67%)	0.26 (0.52%)
C18:2n-6	3.58 (18.9%)	15.64 (30.8%)
C18:3n-3	6.67 (35.2%)	2.10 (4.14%)
Ratio of n-6:n-3[2]	0.54	7.44
Feed Offered[3]	**(per head)**	
DM (kg/day)	1.40	0.89
ME (MJ/day)	13.21	10.27
CP (g/day)	143.07	131.33

[1]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 10 g/head per day.
[2]Ratio of n-6:n-3 = ratio of omega-6 to omega-3 fatty acids.
[3]DM = dry matter, ME = metabolisable energy, CP = crude protein.

Animal withdrawals during the study

One ewe allocated to receive the high omega-3 diet was withdrawn from the study prior to mating due to cancer. Details of animal withdrawals can be found in Appendix 3.

Data collection

Ewe weight and fat score was assessed during pen feeding as described previously (Section 3.10). Blood samples were also collected from the same sub-set of ewes in each pen as in Study 1 for fatty acid and hormone analysis as described previously. Pregnancy scanning was conducted 76 or 60 days after the last ewe showed oestrus in Cycle 1 or Cycle 2, respectively.

Ewe management during parturition

Ewes lambed in 1 of 10 paddocks depending on whether ewes were scanned as conceiving in Cycle 1 or 2 and carrying single or multiple foetuses (Table 4.5). Ewes from each dietary treatment group scanned as having multiple foetuses were allocated to lamb in separate paddocks, with 4 replicates per treatment for multiple bearing ewes. Ewes from both dietary treatment groups scanned as having a single foetus or ewes scanned as being fertilised in Cycle 2 lambed together in 2 paddocks (Table 4.5). Ewes were fitted with neck plates with a

number that was coded to their individual ear tag number for identification during lambing (Plate 4.1).

Table 4.5 Details of lambing paddocks used for BL x Merino ewes in Study 2 in 2012.

Paddock	Pasture	Cycle	Scanned	Dietary Treatment	Number
1	Clover/Ryegrass	1	Multiple	High Omega-6	27
2	Clover/Ryegrass	1	Multiple	High Omega-3	30
3	Clover/Ryegrass	1	Multiple	High Omega-3	27
4	Clover/Ryegrass	1	Multiple	High Omega-6	32
5	Barley forage	1	Multiple	High Omega-3	21
6	Barley forage	1	Multiple	High Omega-6	22
7	Barley forage	1	Multiple	High Omega-6	23
8	Barley forage	1	Multiple	High Omega-3	21
9	Mixed pasture	1	Single	High n-3 + High n-6	55
10	Mixed pasture	2	Dry/Single/Multiple	High n-3 + High n-6	33
Total					291

Plate 4.2 Identification of BL x Merino ewes at lambing in Study 2 in 2012

4.3 Results

The results for weight, fat score and plasma fatty acids will be presented separately for each study in the following sections in order to assess the effects of individual diets. Reproduction data, however, will be combined for both years.

4.3.1 Ewe weight and fat score

Study 1 (2011)
Ewe weight increased significantly ($p < 0.001$) over the time of feeding experimental diets (Figure 4.1A) and was significantly higher at the completion of pen feeding when ewes were fed a diet high in omega-6 fatty acids compared with omega-3 fatty acids both pre- and post-conception ($p = 0.028$) but not pre-conception only ($p = 0.083$). Ewe weight remained significantly higher at preg-scanning ($p = 0.028$) and 4 weeks prior to parturition ($p = 0.014$) when ewes were fed the High omega-6 diet compared with the High omega-3 diet at joining (Figure 4.1A).

Ewe fat score was significantly ($p < 0.001$) higher when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids either pre-conception only or pre- and post-conception (Table 4.6). Ewe fat score was significantly higher at the completion of pen feeding, at preg-scanning and prior to parturition when ewes were fed the High omega-6 diet at joining (Figure 4.1B)

Table 4.6 Mean weight and fat score of BL x Merino ewes following the consumption of a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating showing the main effects of diet and day of feeding.

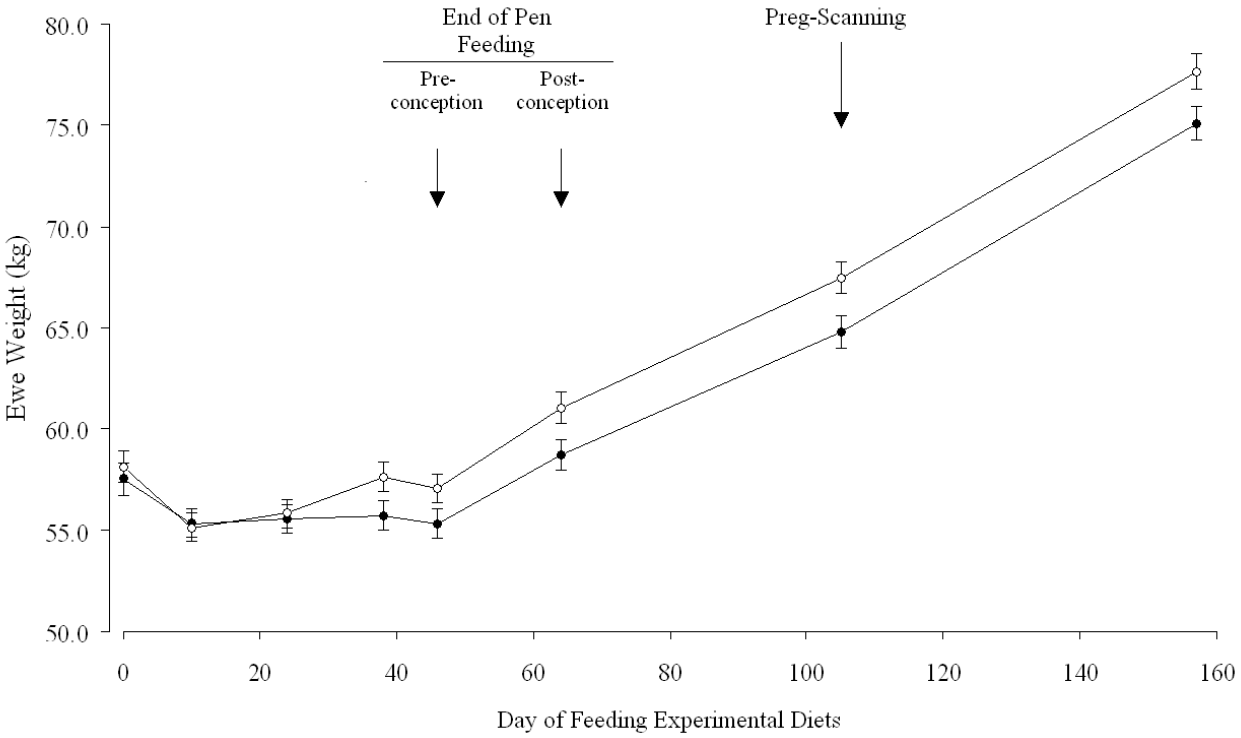
				p-values		
Study	Measure	High Omega-3	High Omega-6	Diet	Day	Diet x Day
Study 1 (2011)	Weight (kg)	59.7 (± 0.72)	61.2 (± 0.72)	0.141	< 0.001	< 0.001
	Fat Score	3.54 (± 0.03)	3.69 (± 0.03)	< 0.001	< 0.001	< 0.001
Study 2 (2012)	Weight (kg)	67.8 (± 0.64)	67.2 (± 0.63)	0.558	< 0.001	< 0.001
	Fat Score	3.34 (± 0.04)	3.46 (± 0.04)	0.049	< 0.001	< 0.001

Study 2 (2012)
Ewe weight was significantly ($p = 0.041$) higher at the commencement of pen feeding in the second year when ewes were fed the high omega-6 diet (66.6 ± 0.69) compared with the high omega-3 diet (64.6 ± 0.68) the previous year (Figure 4.2A). Mean liveweight over the duration of the study was not significantly ($p = 0.558$) different when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids at joining (Table 4.6). The interaction between diet and day of feeding, however, was significant ($p < 0.001$) and ewe weight increased to a greater extent over the period of pen feeding when ewes were fed the High omega-6 diet compared with the High omega-3 diet (Figure 4.2A). Ewe weight was not significantly higher, however, at preg-scanning ($p = 0.135$) or prior to parturition ($p = 0.063$) when ewes were fed the High omega-6 diet.

In contrast to ewe weight, ewe fat score was not significantly ($p = 0.064$) higher at the commencement of pen feeding in the second year when ewes were fed the high omega-6 diet (3.58 ± 0.07) compared with the high omega-3 diet (3.40 ± 0.07) the previous year (Figure 4.2B). Fat score was, however, significantly higher over the duration of the study when ewes were fed the high omega-6 diet compared with the high omega-3 diet (Table 4.6).

Fat score was significantly higher at preg-scannning (p = 0.007) but not prior to parturition (p = 0.178) when ewes were fed the high omega-6 diet.

A



B

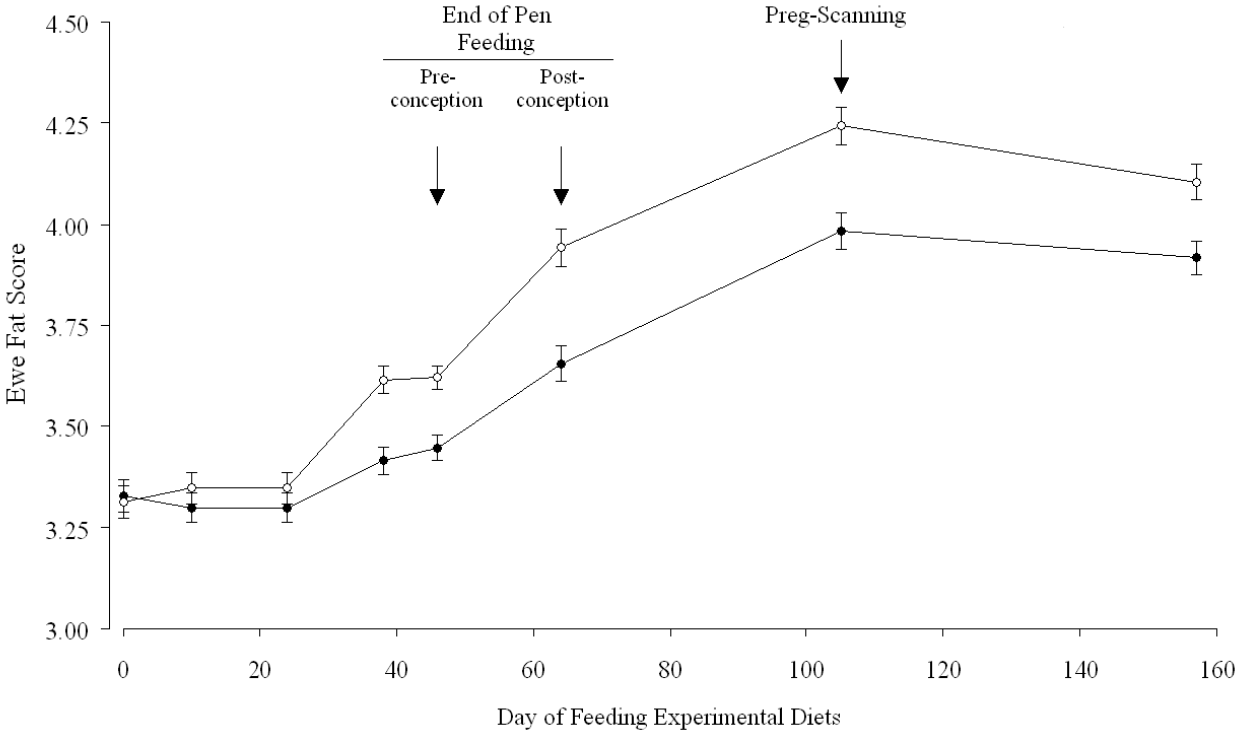


Figure 4.1 Mean weight (A) and fat score (B) for BL x Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating or 42 days prior to and 17 days following mating in Study 1 (2011).

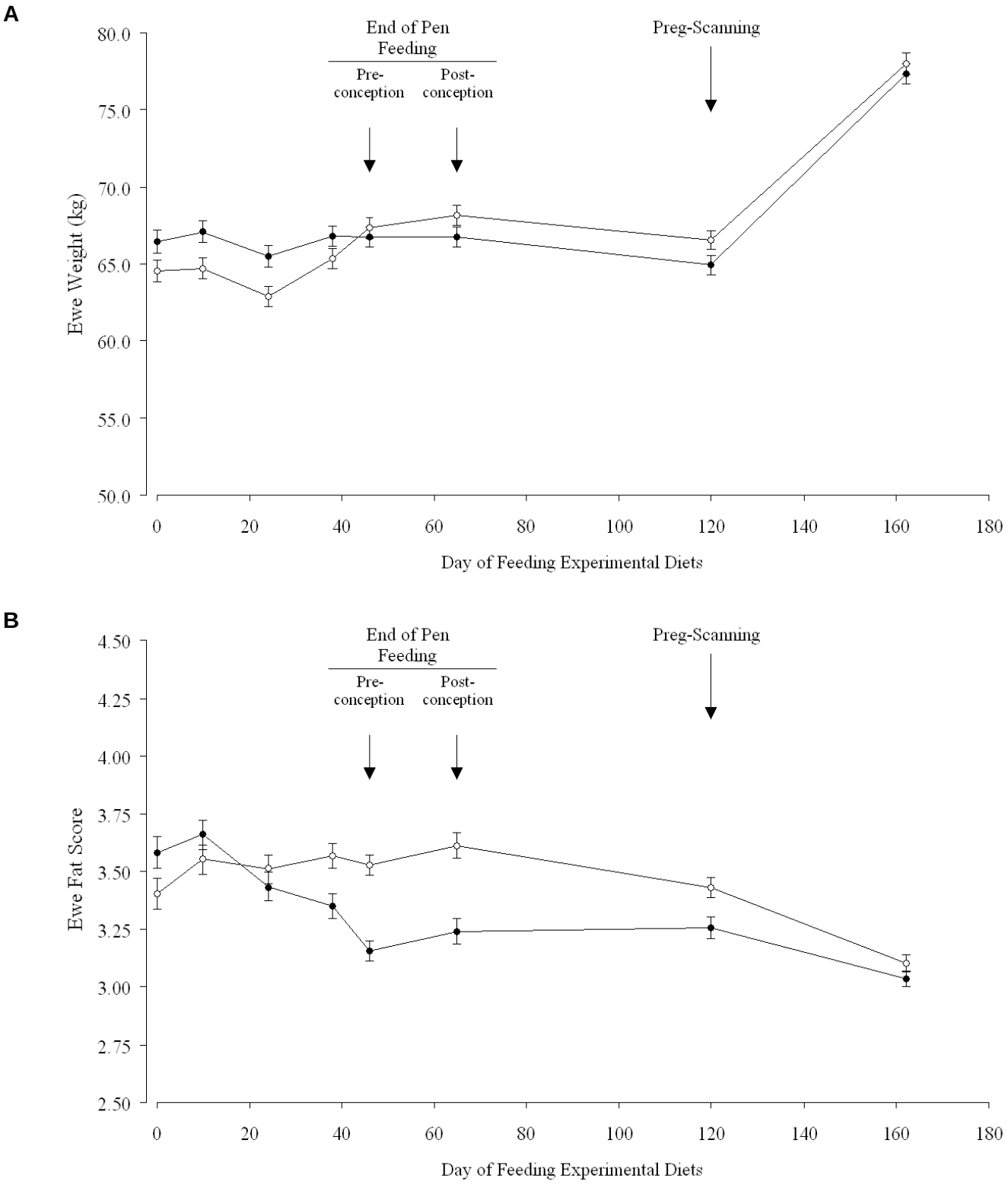


Figure 4.2 Mean weight (A) and fat score (B) for BL x Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating or 42 days prior to and 17 days following mating in Study 2 (2012).

4.3.2 Plasma fatty acid concentration

Plasma fatty acid concentrations were related to the diet fed during each study, particularly, the type and fatty acid profile of the silage used. Fatty acid concentrations will be presented separately for each study with details provided for ewes that were fed pre-conception only or for the longer time period both pre- and post-conception.

Study 1 (2011)
The concentration of omega-3 fatty acids including C18:3n-3, C20:5n-3 (EPA) and C22:5n-3 (DPA) were significantly ($p < 0.001$) higher when ewes were fed a diet high in omega-3 fatty acids compared with omega-6 fatty acids either pre-conception only or pre- and post-conception (Table 4.7). The interaction between diet and length of time of feeding (pre-conception or pre- and post-conception) was significant for the concentration of C18:3n-3 ($p = 0.002$) and C20:5n-3 ($p = 0.005$).

The concentration of ALA and EPA decreased significantly ($p < 0.001$) following the introduction of the high omega-6 diet (Figures 4.3A and B) and remained relatively constant while ewes were fed the experimental rations. The concentration of EPA was significantly lower at preg-scanning ($p = 0.035$) and pre-lambing ($p = 0.003$) when ewes were fed the high omega-6 diet both pre- and post-conception (Figure 4.3B), but not when ewe were fed pre-conception only (preg-scanning, $p = 0.572$; pre-lambing, $p = 0.683$, Figure 4.3A). The concentration of DHA in the plasma of ewes pre-lambing, however, was not significantly ($p = 0.109$) lower when ewes were fed the High omega-6 diet both pre- and post-conception (Figure 5.3B).

The concentration of all omega-6 fatty acids measured, including C18:2n-6, C20:3n-6 and C20:4n-6 (ARA) were significantly ($p < 0.01$) higher when ewes were fed a diet high in omega-6 fatty acids compared with omega-3 fatty acids either pre-conception only or pre- and post-conception (Table 4.7). The concentration of omega-6 fatty acids increased significantly ($p < 0.001$) following the introduction of the high omega-6 diet (Figures 4.4A and B) and remained relatively constant while ewes were fed the experimental rations. The concentration of C18:2n-6 and C20:4n-6 was not significantly different, however, at preg-scanning or pre-lambing when ewes were fed the high omega-3 or high omega-6 diet at mating.

Table 4.7 Mean concentration (μg/mL) of fatty acid methyl esters in the plasma of BL x Merino ewes following the consumption of a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating in Study 1 (2011).

	High Omega-3 (Silage)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception	Pre- and Post-Conception	Diet	Diet x Time
SFA						
C8:0	-	-	-	-	-	-
C9:0	-	-	-	-	-	-
C10:0	4.56 (± 0.26)	5.95 (± 0.26)	4.76 (± 0.29)	4.73 (± 0.27)	0.069	0.022
C11:0	-	-	-	-	-	-
C12:0	1.29 (± 0.06)	1.40 (± 0.05)	1.41 (± 0.06)	1.38 (± 0.06)	0.406	0.496
C14:0	8.14 (± 0.25)	8.71 (± 0.24)	7.81 (± 0.25)	7.05 (± 0.25)	< 0.001	0.046
iC15:0	2.52 (± 0.13)	2.50 (± 0.13)	2.42 (± 0.13)	2.19 (± 0.13)	0.134	0.046
aiC15:0	3.65 (± 0.20)	3.51 (± 0.19)	3.90 (± 0.20)	3.80 (± 0.20)	0.190	0.376
C15:0	6.47 (± 0.27)	6.86 (± 0.26)	6.35 (± 0.27)	5.62 (± 0.27)	0.016	0.005
C16:0	149.5 (± 4.68)	159.2 (± 4.51)	180.9 (± 4.71)	171.5 (± 4.80)	< 0.001	0.127
iC17:0	8.18 (± 0.43)	7.86 (± 0.41)	10.05 (± 0.44)	9.65 (± 0.44)	0.000	0.687
aiC17:0	9.17 (± 0.48)	9.62 (± 0.47)	5.89 (± 0.50)	3.98 (± 0.50)	< 0.001	0.001
C17:0	11.09 (± 0.34)	11.38 (± 0.33)	11.25 (± 0.34)	9.95 (± 0.35)	0.072	0.008
C18:0	154.7 (± 7.45)	159.0 (± 7.35)	211.6 (± 7.76)	208.7 (± 7.87)	< 0.001	0.001
C20:0	1.37 (± 0.08)	1.52 (± 0.08)	1.61 (± 0.08)	1.60 (± 0.08)	0.048	0.920
C21:0	0.66 (± 0.04)	0.63 (± 0.04)	0.63 (± 0.04)	0.60 (± 0.04)	0.409	0.349
C22:0	2.68 (± 0.11)	2.80 (± 0.10)	3.20 (± 0.10)	2.87 (± 0.11)	0.008	0.644
C23:0	4.85 (± 0.18)	4.82 (± 0.17)	5.48 (± 0.17)	4.69 (± 0.18)	0.169	0.795
C24:0	4.14 (± 0.16)	4.20 (± 0.15)	5.05 (± 0.16)	4.30 (± 0.16)	0.003	0.742
Total SFA	380.6 (± 12.92)	400.5 (± 12.69)	470.8 (± 13.34)	451.7 (± 13.56)	< 0.001	0.028
MUFA						
C11:1n-1	-	-	-	-	-	-
C12:1n-7	-	-	-	-	-	-
C13:1n-1	-	-	-	-	-	-
C14:1n-5	0.39 (± 0.02)	0.45 (± 0.02)	0.44 (± 0.02)	0.45 (± 0.02)	0.215	0.864
C15:1n-5	0.52 (± 0.03)	0.58 (± 0.03)	0.63 (± 0.03)	0.63 (± 0.03)	0.015	0.409
C16:1n-7t	-	-	-	-	-	-
C16:1n-7	14.94 (± 0.64)	16.13 (± 0.62)	15.59 (± 0.64)	13.92 (± 0.66)	0.235	0.285
C17:1n-7	1.53 (± 0.09)	1.79 (± 0.09)	1.78 (± 0.09)	1.72 (± 0.09)	0.325	0.603
C18:1n9t	3.96 (± 0.38)	3.51 (± 0.38)	5.02 (± 0.39)	4.56 (± 0.41)	0.011	0.001
C18:1n7t	-	-	-	-	-	-
C18:1n-12	-	-	-	-	-	-
C18:1n-9	176.1 (± 5.86)	188.0 (± 5.54)	221.9 (± 5.76)	205.4 (± 5.90)	< 0.001	< 0.001
C18:1n-7	7.51 (± 0.32)	7.99 (± 0.31)	7.35 (± 0.33)	7.41 (± 0.33)	0.262	0.246
C19:1n-12	-	-	-	-	-	-
C20:1n-15	-	-	-	-	-	-
C20:1n-12	-	-	-	-	-	-
C20:1n-9	0.90 (± 0.06)	1.06 (± 0.06)	1.17 (± 0.06)	1.30 (± 0.06)	< 0.001	0.213

	High Omega-3 (Silage)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception	Pre- and Post-Conception	Diet	Diet x Time
C22:1n-9	0.69 (± 0.05)	0.84 (± 0.05)	0.64 (± 0.05)	0.72 (± 0.05)	0.085	0.683
C24:1n-9	4.51 (± 0.17)	4.89 (± 0.17)	5.52 (± 0.17)	5.39 (± 0.18)	< 0.001	0.098
Total MUFA	222.0 (± 6.73)	234.2 (± 6.38)	268.9 (± 6.64)	251.2 (± 6.79)	< 0.001	0.001
n-3 PUFA						
C18:3n-3	44.01 (± 1.68)	39.9 (± 1.57)	41.2 (± 1.63)	28.67 (± 1.67)	< 0.001	0.002
C18:4n-3	-	-	-	-	-	-
C20:3n-3	0.43 (± 0.02)	0.42 (± 0.02)	0.43 (± 0.02)	0.40 (± 0.02)	0.548	0.155
C20:5n-3	18.62 (± 0.94)	21.28 (± 0.87)	16.21 (± 0.90)	13.68 (± 0.93)	< 0.001	0.005
C22:5n-3	22.40 (± 0.76)	23.76 (± 0.70)	20.19 (± 0.72)	19.22 (± 0.74)	< 0.001	0.071
C22:6n-3	16.11 (± 0.85)	17.90 (± 0.80)	17.07 (± 0.82)	16.03 (± 0.85)	0.586	0.780
Total n-3	102.21 (± 3.19)	104.05 (± 2.99)	96.04 (± 3.11)	78.56 (± 3.18)	< 0.001	0.001
n-6 PUFA						
C18:2n-6t	1.07 (± 0.11)	1.22 (± 0.11)	1.25 (± 0.11)	1.00 (± 0.11)	0.838	0.046
C18:2n-6	126.5 (± 7.84)	123.5 (± 7.59)	215.3 (± 7.98)	205.7 (± 8.10)	< 0.001	< 0.001
C18:3n-6	3.78 (± 0.28)	3.82 (± 0.27)	5.84 (± 0.29)	5.29 (± 0.29)	< 0.001	0.012
C20:2n-6	0.53 (± 0.02)	0.54 (± 0.02)	0.61 (± 0.02)	0.62 (± 0.02)	0.002	0.119
C20:3n-6	2.59 (± 0.11)	2.54 (± 0.10)	3.14 (± 0.11)	3.29 (± 0.11)	< 0.001	< 0.001
C20:4n-6	31.89 (± 1.75)	34.09 (± 1.68)	39.77 (± 1.73)	38.36 (± 1.80)	0.002	0.171
C22:2n-6	-	-	-	-	-	-
C22:4n-6	-	-	-	-	-	-
C22:5n-6	-	-	-	-	-	-
Total n-6	166.9 (± 8.93)	166.4 (± 8.63)	266.5 (± 9.04)	255.3 (± 9.22)	< 0.001	< 0.001
Total ID	872.0 (± 27.95)	904.3 (± 27.16)	1101.9 (± 28.44)	1035.9 (± 28.99)	< 0.001	0.005
n-6:n-3	1.64 (± 0.18)	1.60 (± 0.18)	3.58 (± 0.18)	4.00 (± 0.19)	< 0.001	< 0.001
DHADI	-	-	-	-	-	-
DHASI	-	-	-	-	-	-
EFI	1.31 (± 0.04)	1.25 (± 0.04)	1.47 (± 0.04)	1.43 (± 0.04)	< 0.001	0.351
P:S	0.70 (± 0.01)	0.68 (± 0.01)	0.77 (± 0.01)	0.75 (± 0.01)	< 0.001	0.224

[1]FAME = fatty acid methyl ester, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, Total ID = total concentration of identified fatty acids, n-6:n-3 = ratio of n-6 PUFA : n-3 PUFA, DHADI = DHA Deficiency Index, 22:5n-6 / C22:4n-6 (Neuringer et al., 1986), DHASI = DHA Sufficiency Index, C22:6n-3 / C22:5n-6 (Hoffman and Uauy, 1992), EFI = Essential Fatty Acid Status Index, ratio of (n-3 PUFA + n-6 PUFA) : (n-7 MUFA + n-9 MUFA), P:S = ratio of (n-3 PUFA + n-6 PUFA) : SFA. A number of fatty acids were not identified in the current study but were identified in subsequent studies.

[2]Pre-conception = ewes fed experimental rations for 42 days prior to mating only, Pre- and post-conception = ewes fed experimental rations for 42 days prior to mating and 17 days post-mating.

[3]The *p*-value for Diet x Time represents the interaction between dietary treatment group and time of feeding either pre-conception or both pre- and post-conception.

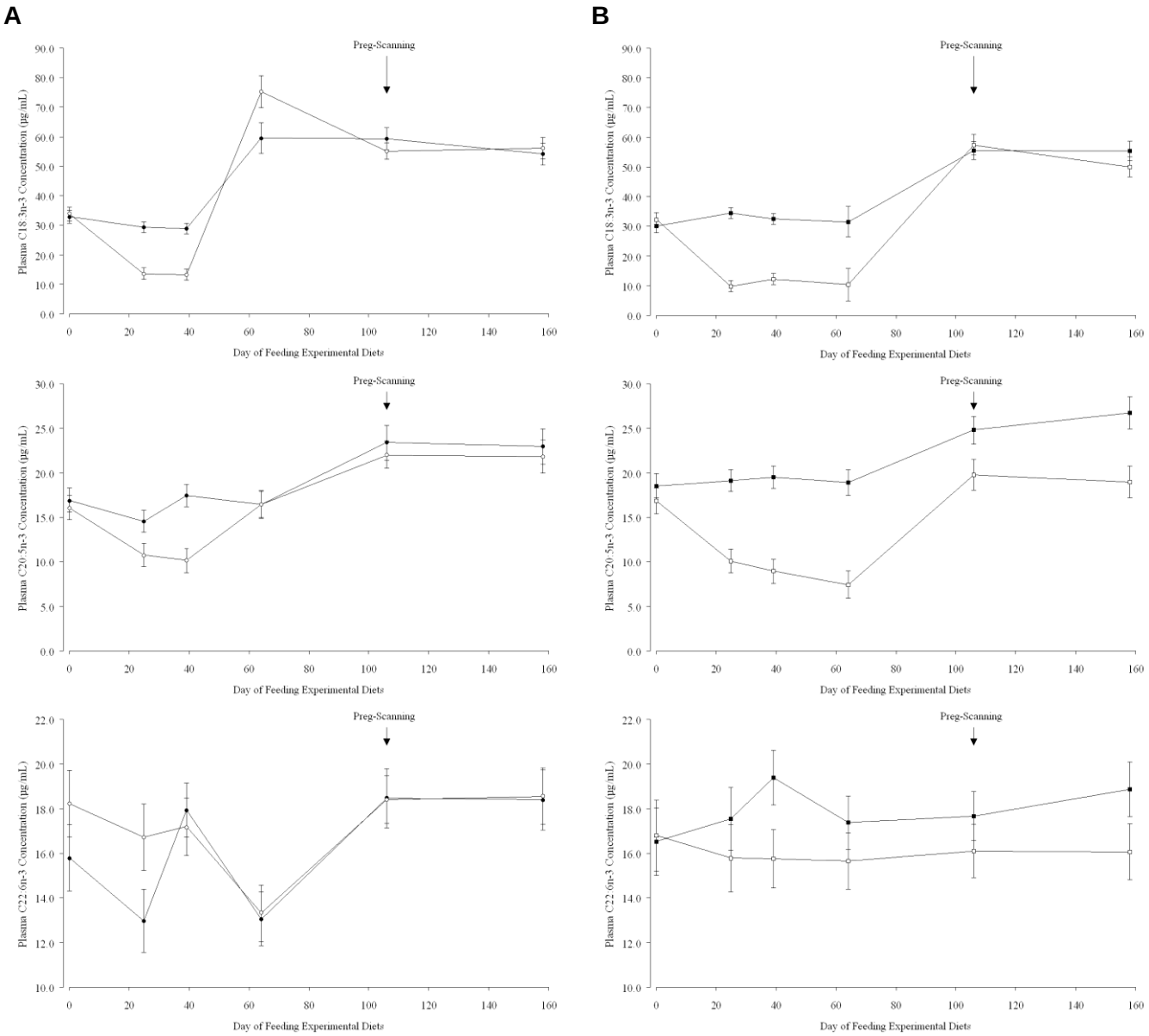


Figure 4.3 Mean concentration of α-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in the plasma of BL x Merino ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for either (A) 42 days prior to mating (pre-conception, ● omega-3, ○omega-6) or (B) 42 days prior to and 17 days following mating (pre and post-conception, ■ omega-3, □ omega-6) in Study 1 (2011).

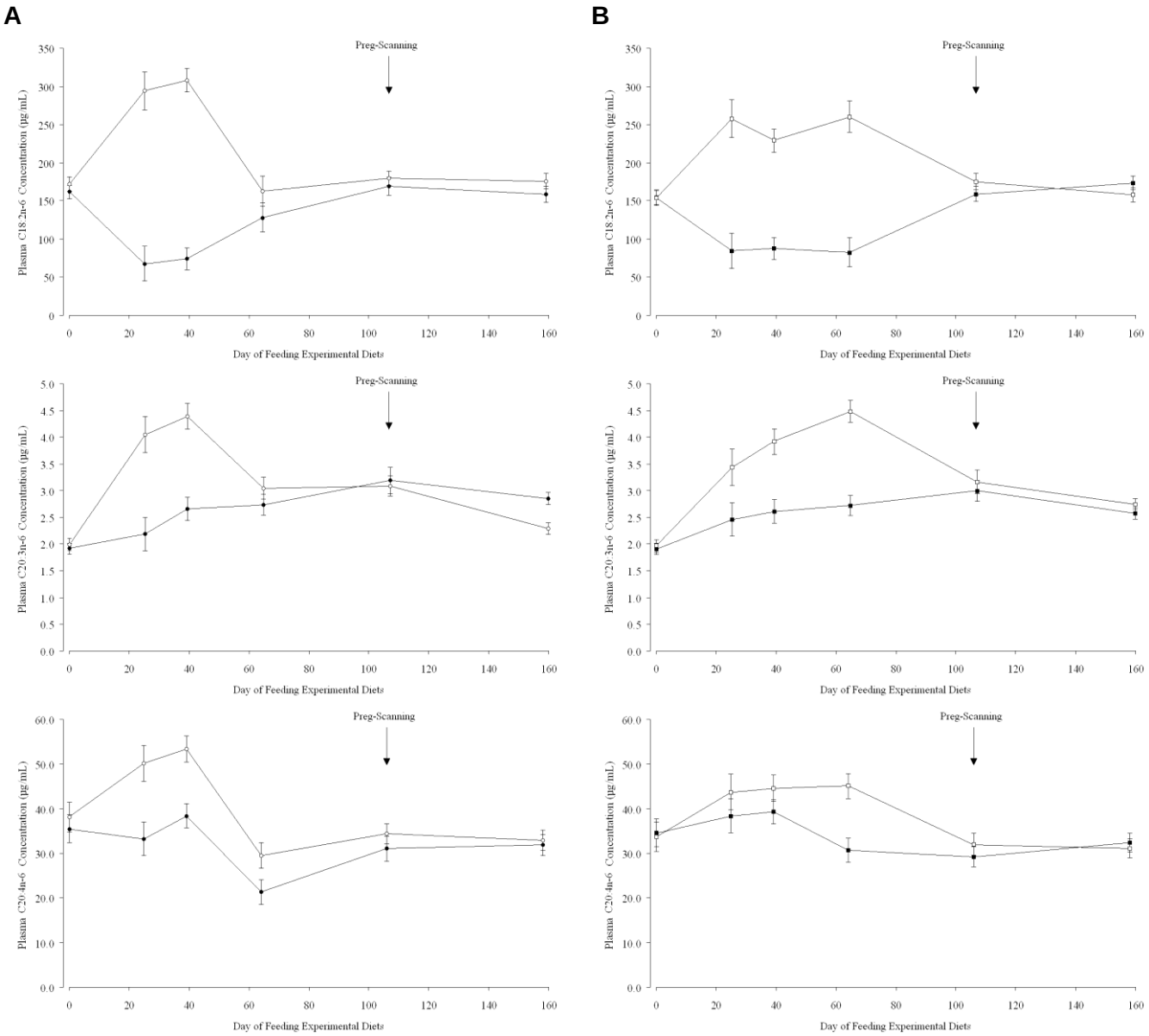


Figure 4.4 Mean concentration of linoleic acid (LA, C18:2n-6), dihomo-γ-linolenic acid (DGLA, C20:3n-6) and arachidonic acid (ARA, C20:4n-3) in the plasma of BL x Merino ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for either (A) 42 days prior to mating (pre-conception, ● omega-3, ○ omega-6) or (B) 42 days prior to and 17 days following mating (pre and post-conception, ■ omega-3, □ omega-6) in Study 1 (2011).

Study 2 (2012)

The concentration of C18:3n-3 ($p < 0.001$) and C20:5n-3 ($p < 0.001$), but not C22:5n-3 ($p = 0.062$) and C22:6n-3 ($p = 0.167$) were significantly higher when ewes were fed a diet high in omega-3 fatty acids compared with omega-6 fatty acids either pre-conception only or pre- and post-conception (Table 4.8). The interaction between diet and length of time of feeding (pre-conception or pre- and post-conception) was significant for the concentration of C18:3n-3 ($p < 0.001$).

The concentration of ALA and EPA decreased significantly ($p < 0.001$) following the introduction of the high omega-6 diet (Figures 4.5A and B) and remained relatively constant while ewes were fed the experimental rations. The concentration of EPA was significantly lower at preg-scanning ($p = 0.022$) when ewes were fed the high omega-6 diet compared with the high omega-3 diet pre-conception only (Figure 4.5A), but not when ewe were fed both pre- and post-conception (Figure 4.5B). The concentration of EPA was not significantly different pre-lambing when ewes were fed either the high omega-3 or high omega-6 diet pre- or post-conception. The concentration of DHA was significantly lower at preg-scanning ($p = 0.033$) and pre-lambing ($p = 0.018$) when ewes were fed the high omega-6 diet compared with the high omega-3 diet pre-conception only.

The concentration of all omega-6 fatty acids measured, including C18:2n-6, C20:3n-6 and C20:4n-6 (ARA) were significantly ($p < 0.01$) higher when ewes were fed a diet high in omega-6 fatty acids compared with omega-3 fatty acids either pre-conception only or pre- and post-conception (Table 4.8). The concentration of omega-6 fatty acids increased significantly ($p < 0.001$) following the introduction of the High omega-6 diet (Figures 4.6A and B). The concentration of C20:4n-6 was significantly higher at preg-scanning ($p = 0.016$) and pre-lambing ($p = 0.036$) when ewes were fed the high omega-6 diet compared with the high omega-3 diet at mating.

Table 4.8 Mean concentration (μg/mL) of fatty acid methyl esters in the plasma of BL x Merino ewes following the consumption of a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating in Study 2 (2012).

	High Omega-3 (Silage)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception	Pre- and Post-Conception	Diet	Diet x Time
SFA						
C8:0	7.40 (± 0.64)	7.34 (± 0.57)	8.80 (± 0.57)	9.28 (± 0.56)	0.008	0.221
C9:0	0.48 (± 0.08)	0.18 (± 0.07)	0.27 (± 0.07)	0.22 (± 0.07)	0.223	0.719
C10:0	3.43 (± 0.14)	3.70 (± 0.12)	3.65 (± 0.12)	3.78 (± 0.12)	0.253	0.801
C11:0	3.43 (± 0.14)	3.56 (± 0.13)	3.61 (± 0.13)	4.00 (± 0.12)	0.023	0.372
C12:0	1.85 (± 0.20)	2.05 (± 0.18)	1.88 (± 0.18)	2.26 (± 0.18)	0.506	0.378
C14:0	13.50 (± 0.95)	12.10 (± 0.87)	9.19 (± 0.87)	9.19 (± 0.87)	< 0.001	0.007
iC15:0	3.71 (± 0.17)	2.86 (± 0.16)	2.86 (± 0.16)	2.74 (± 0.16)	0.007	0.552
aiC15:0	4.84 (± 0.24)	3.87 (± 0.23)	4.40 (± 0.23)	4.28 (± 0.23)	0.940	0.576
C15:0	8.62 (± 0.35)	7.00 (± 0.32)	7.21 (± 0.32)	6.37 (± 0.32)	0.004	0.385
C16:0	188.4 (± 8.50)	167.8 (± 7.66)	184.5 (± 7.64)	200.9 (± 7.64)	0.075	0.142
iC17:0	10.45 (± 0.48)	8.87 (± 0.45)	10.44 (± 0.45)	10.63 (± 0.45)	0.065	0.253
aiC17:0	14.76 (± 1.11)	13.10 (± 1.03)	6.66 (± 1.03)	5.16 (± 1.03)	< 0.001	< 0.001
C17:0	12.94 (± 0.46)	11.04 (± 0.42)	11.25 (± 0.42)	9.99 (± 0.42)	0.004	0.638
C18:0	206.8 (± 10.82)	183.8 (± 9.92)	226.6 (± 9.90)	269.4 (± 9.89)	< 0.001	0.001
C20:0	1.87 (± 0.10)	1.55 (± 0.09)	1.81 (± 0.09)	1.99 (± 0.09)	0.046	0.207
C21:0	2.04 (± 0.07)	1.94 (± 0.07)	2.13 (± 0.07)	1.92 (± 0.07)	0.605	0.838
C22:0	2.99 (± 0.10)	2.51 (± 0.09)	3.27 (± 0.09)	3.18 (± 0.09)	< 0.001	0.004
C23:0	5.52 (± 0.17)	4.50 (± 0.16)	5.63 (± 0.16)	5.04 (± 0.16)	0.055	0.062
C24:0	4.26 (± 0.14)	3.44 (± 0.13)	4.55 (± 0.13)	4.28 (± 0.13)	< 0.001	0.042
Total SFA	489.5 (± 21.20)	433.6 (± 19.31)	490.7 (± 19.28)	546.6 (± 19.26)	0.008	0.082
MUFA						
C11:1n-1	0.82 (± 0.05)	0.86 (± 0.04)	0.89 (± 0.04)	0.96 (± 0.04)	0.061	0.104
C12:1n-7	1.24 (± 0.07)	1.05 (± 0.06)	1.02 (± 0.06)	1.02 (± 0.06)	0.052	0.822
C13:1n-1	0.86 (± 0.07)	0.83 (± 0.06)	0.76 (± 0.06)	0.80 (± 0.06)	0.288	0.663
C14:1n-5	0.26 (± 0.03)	0.25 (± 0.03)	0.22 (± 0.03)	0.26 (± 0.03)	0.679	0.885
C15:1n-5	0.52 (± 0.03)	0.44 (± 0.03)	0.45 (± 0.03)	0.44 (± 0.03)	0.250	0.229
C16:1n-7t	2.61 (± 0.17)	2.58 (± 0.15)	2.12 (± 0.15)	1.87 (± 0.15)	0.001	0.512
C16:1n-7	22.34 (± 1.31)	17.98 (± 1.22)	18.19 (± 1.22)	17.78 (± 1.22)	0.091	0.618
C17:1n-7	2.08 (± 0.09)	1.90 (± 0.08)	2.17 (± 0.08)	2.32 (± 0.08)	0.006	0.016
C18:1n9t	5.46 (± 0.71)	4.61 (± 0.62)	4.63 (± 0.62)	5.97 (± 0.62)	0.690	< 0.001
C18:1n7t	15.57 (± 1.10)	13.47 (± 1.00)	13.34 (± 1.00)	10.86 (± 1.00)	0.026	0.290
C18:1n-12	2.06 (± 0.12)	2.09 (± 0.11)	1.92 (± 0.11)	1.80 (± 0.11)	0.059	0.471
C18:1n-9	252.8 (± 11.58)	226.9 (± 10.77)	254.5 (± 10.74)	287.8 (± 10.73)	0.008	0.022
C18:1n-7	8.84 (± 0.44)	7.59 (± 0.41)	8.19 (± 0.41)	7.73 (± 0.41)	0.545	0.880
C19:1n-12	0.38 (± 0.07)	0.58 (± 0.06)	0.49 (± 0.06)	0.47 (± 0.06)	0.960	0.095
C20:1n-15	0.60 (± 0.06)	0.46 (± 0.05)	0.58 (± 0.05)	0.59 (± 0.05)	0.326	0.365
C20:1n-12	0.48 (± 0.05)	0.39 (± 0.04)	0.41 (± 0.04)	0.43 (± 0.04)	0.773	0.669
C20:1n-9	0.91 (± 0.04)	0.84 (± 0.04)	0.92 (± 0.04)	1.01 (± 0.04)	0.030	0.185

	High Omega-3 (Silage)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception	Pre- and Post-Conception	Diet	Diet x Time
C22:1n-9	0.65 (± 0.04)	0.71 (± 0.04)	0.69 (± 0.04)	0.75 (± 0.04)	0.324	< 0.001
C24:1n-9	4.51 (± 0.16)	4.48 (± 0.15)	4.79 (± 0.15)	5.31 (± 0.15)	0.001	0.115
Total MUFA	321.7 (± 13.36)	287.1 (± 12.38)	315.3 (± 12.35)	347.1 (± 12.34)	0.043	0.081
n-3 PUFA						
C18:3n-3	40.99 (± 1.37)	32.87 (± 1.26)	33.04 (± 1.26)	22.49 (± 1.24)	< 0.001	< 0.001
C18:4n-3	2.50 (± 0.09)	2.06 (± 0.09)	2.02 (± 0.08)	1.76 (± 0.08)	< 0.001	0.822
C20:3n-3	0.53 (± 0.02)	0.51 (± 0.02)	0.55 (± 0.02)	0.52 (± 0.02)	0.453	0.251
C20:5n-3	20.58 (± 0.99)	17.67 (± 0.92)	15.4 (± 0.92)	14.99 (± 0.92)	< 0.001	0.079
C22:5n-3	19.04 (± 0.61)	18.51 (± 0.57)	18.35 (± 0.57)	16.93 (± 0.57)	0.062	0.439
C22:6n-3	18.06 (± 1.04)	16.97 (± 0.97)	15.62 (± 0.97)	16.60 (± 0.97)	0.167	0.601
Total n-3	101.16 (± 2.89)	88.64 (± 2.68)	84.41 (± 2.67)	73.29 (± 2.66)	< 0.001	0.079
n-6 PUFA						
C18:2n-6t	0.75 (± 0.06)	0.76 (± 0.05)	0.58 (± 0.05)	0.55 (± 0.05)	0.001	0.331
C18:2n-6	137.5 (± 11.84)	105.3 (± 10.98)	202.9 (± 10.98)	235.7 (± 10.97)	< 0.001	< 0.001
C18:3n-6	6.00 (± 0.34)	4.25 (± 0.32)	6.43 (± 0.32)	6.94 (± 0.32)	< 0.001	< 0.001
C20:2n-6	0.75 (± 0.05)	0.70 (± 0.05)	0.86 (± 0.05)	0.87 (± 0.05)	0.007	0.057
C20:3n-6	2.93 (± 0.17)	2.53 (± 0.16)	3.38 (± 0.16)	3.55 (± 0.16)	< 0.001	0.001
C20:4n-6	37.11 (± 1.85)	34.02 (± 1.72)	41.12 (± 1.72)	41.90 (± 1.72)	0.002	0.248
C22:2n-6	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	< 0.001	< 0.001
C22:4n-6	1.18 (± 0.13)	1.19 (± 0.12)	1.60 (± 0.12)	1.52 (± 0.12)	0.004	0.223
C22:5n-6	0.93 (± 0.11)	0.75 (± 0.10)	1.04 (± 0.10)	1.14 (± 0.10)	0.026	0.410
Total n-6	163.1 (± 6.09)	143.3 (± 6.46)	256.4 (± 6.24)	271.9 (± 6.04)	< 0.001	< 0.001
Total ID	1098.8 (± 40.53)	958.6 (± 37.32)	1148.4 (± 37.24)	1259.2 (± 37.20)	< 0.001	0.007
n-6:n-3	1.85 (± 0.21)	1.70 (± 0.20)	3.32 (± 0.20)	4.43 (± 0.20)	< 0.001	< 0.001
DHADI	1.40 (± 0.20)	0.73 (± 0.18)	0.76 (± 0.18)	0.94 (± 0.18)	0.249	0.021
DHASI	21.05 (± 2.25)	25.77 (± 2.10)	18.17 (± 2.09)	18.10 (± 2.09)	0.020	0.686
EFI	1.05 (± 0.05)	0.98 (± 0.05)	1.24 (± 0.05)	1.19 (± 0.05)	< 0.001	0.392
P:S	0.61 (± 0.02)	0.57 (± 0.02)	0.70 (± 0.02)	0.68 (± 0.02)	< 0.001	0.011

[1]FAME = fatty acid methyl ester, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, Total ID = total concentration of identified fatty acids, n-6:n-3 = ratio of n-6 PUFA : n-3 PUFA, DHADI = DHA Deficiency Index, 22:5n-6 / C22:4n-6 (Neuringer et al., 1986), DHASI = DHA Sufficiency Index, C22:6n-3 / C22:5n-6 (Hoffman and Uauy, 1992), EFI = Essential Fatty Acid Status Index, ratio of (n-3 PUFA + n-6 PUFA) : (n-7 MUFA + n-9 MUFA), P:S = ratio of (n-3 PUFA + n-6 PUFA) : SFA.
[2]Pre-conception = ewes fed experimental rations for 42 days prior to mating only, Pre- and post-conception = ewes fed experimental rations for 42 days prior to mating and 17 days post-mating.
[3]The *p*-value for Diet x Time represents the interaction between dietary treatment group and time of feeding either pre-conception or both pre- and post-conception.

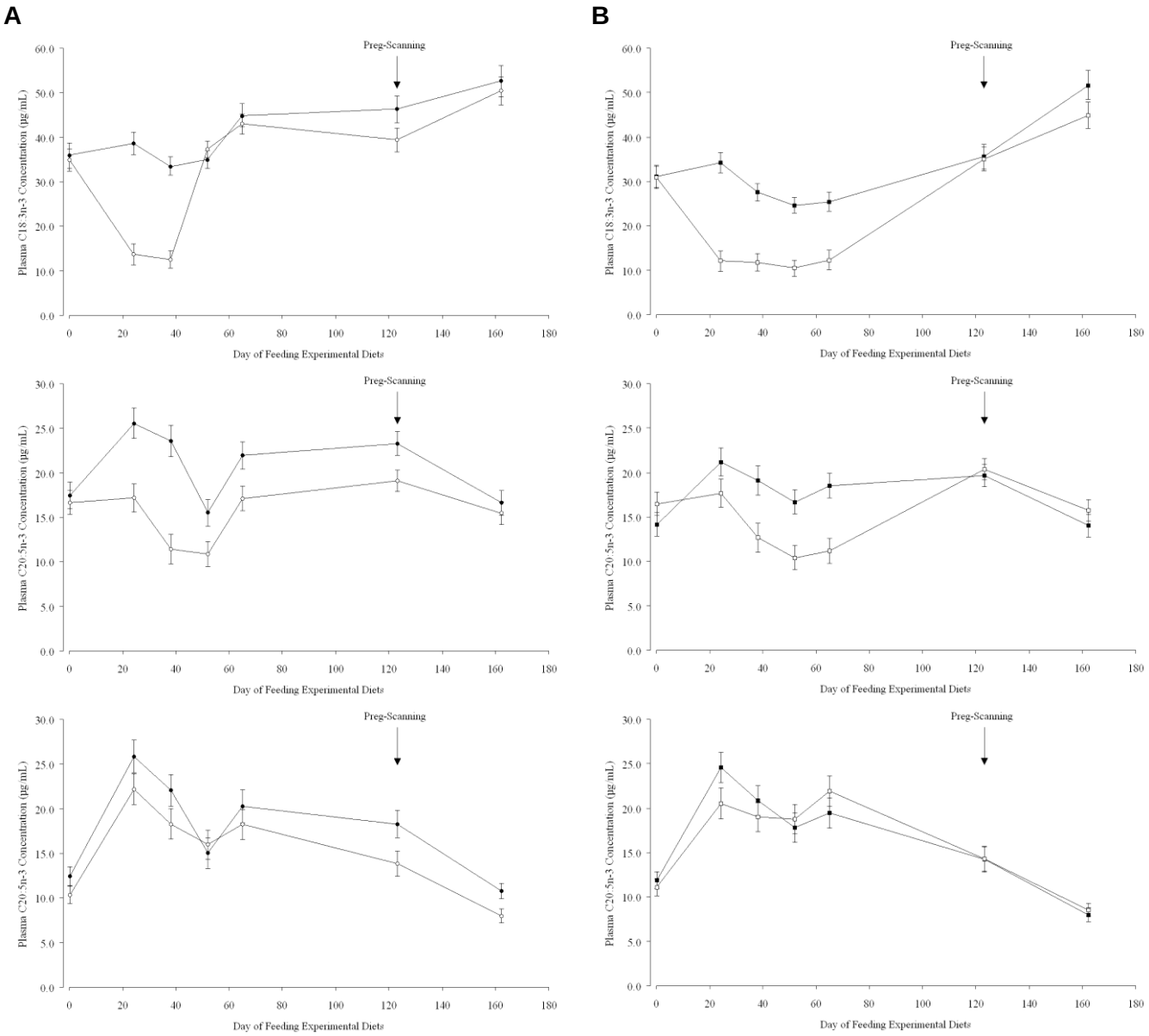


Figure 4.5 Mean concentration of α-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in the plasma of BL x Merino ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for either (A) 42 days prior to mating (pre-conception, ● omega-3, ○omega-6) or (B) 42 days prior to and 17 days following mating (pre and post-conception, ■ omega-3, □ omega-6) in Study 2 (2012).

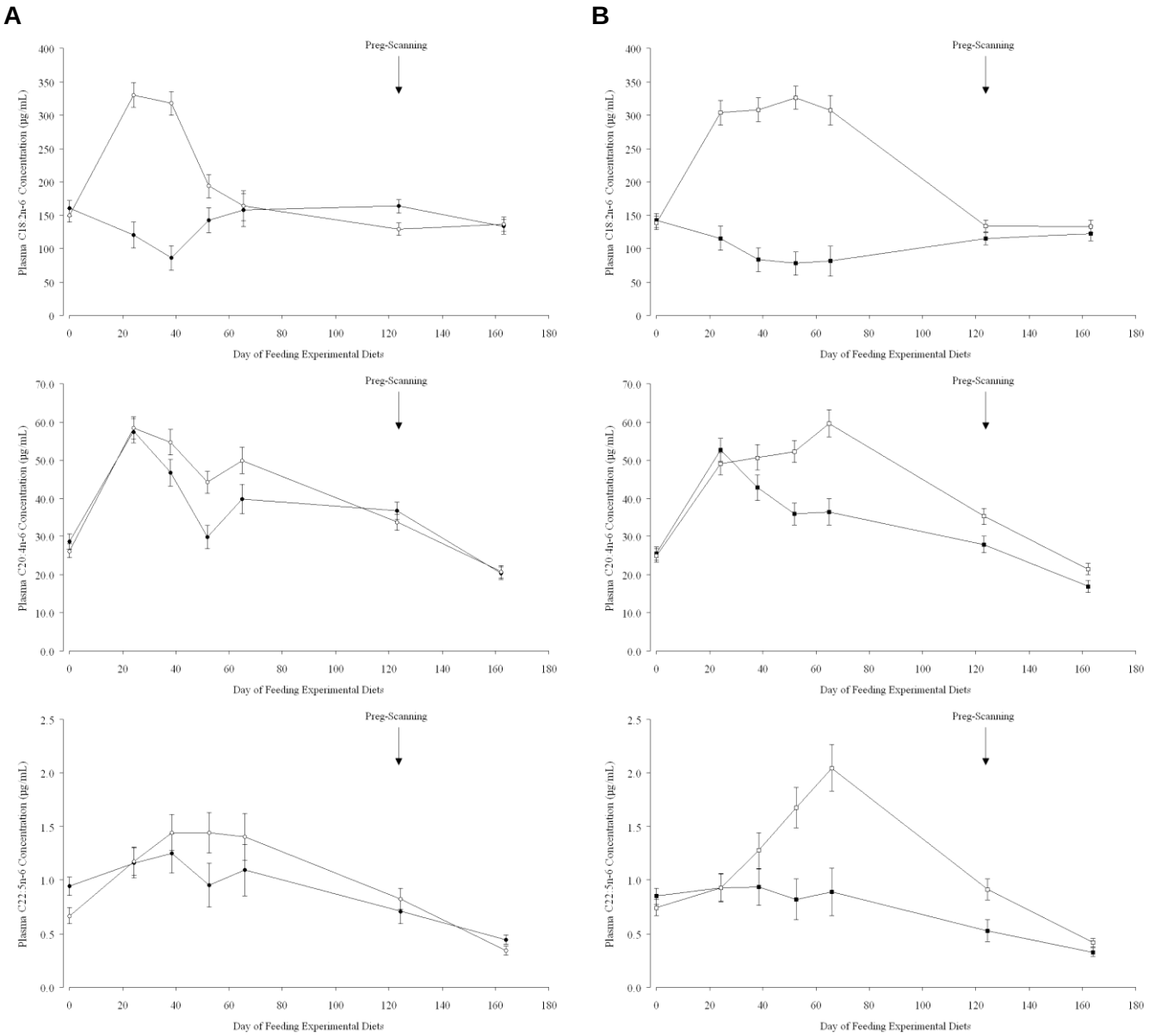


Figure 4.6 Mean concentration of linoleic acid (LA, C18:2n-6), dihomo-γ-linolenic acid (DGLA, C20:3n-6) and arachidonic acid (ARA, C20:4n-3) in the plasma of BL x Merino ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for either (A) 42 days prior to mating (pre-conception, ● omega-3, ○ omega-6) or (B) 42 days prior to and 17 days following mating (pre and post-conception, ■ omega-3, □ omega-6) in Study 2 (2012).

4.3.3 Plasma hormone concentrations

Progesterone (P_4)

The mean concentration of plasma progesterone was significantly (p = 0.030) lower when ewes were fed the high omega-6 diet (7.17 ± 0.21 ng/mL) compared with the high omega-3 diet (6.53 ± 0.20 ng/mL) for 42 days prior to mating (Figure 4.5A). The percentage change in progesterone concentration from baseline was also significantly (p < 0.001) lower when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids prior to mating (Figure 4.5B).

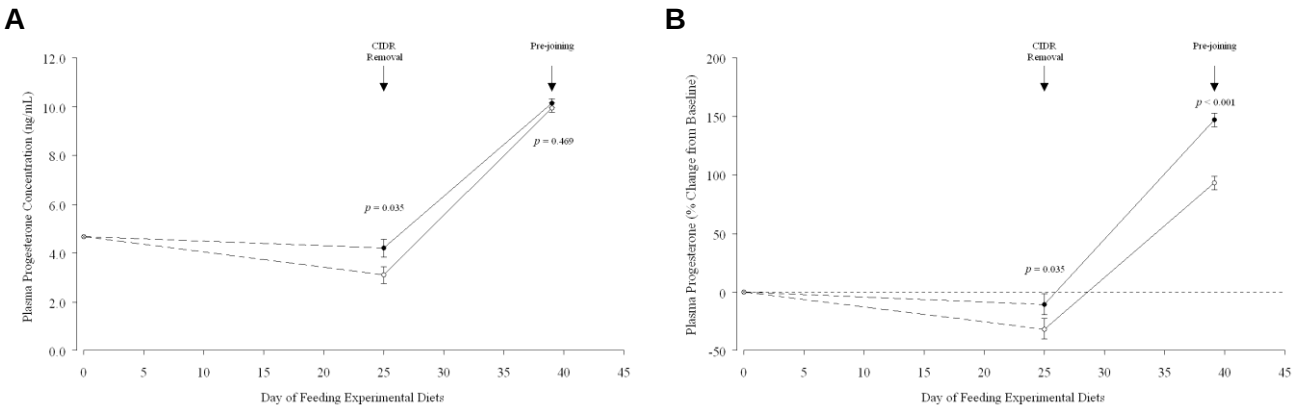


Figure 4.7 Mean (A) concentration of plasma progesterone (ng/mL) and (B) the percentage change in progesterone from baseline for BL x Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating. Baseline progesterone concentrations for the High omega-3 and High omega-6 diets were 3.25 ± 0.70 and 5.92 ± 0.67 ng/mL respectively and were included in the statistical analysis as a co-variate. Significant difference between treatment diets (A) p = 0.030, (B) p < 0.001.

Oestradiol (E_2)

The mean concentration of plasma oestradiol was not significantly (p = 0.058) higher when ewes were fed the high omega-6 diet (3.73 ± 0.59 pg/mL) compared with the high omega-3 diet (2.28 ± 0.60 pg/mL) for 42 days prior to mating (Figure 4.6A). The percentage change in oestradiol concentration from baseline was also not significantly (p = 0.967) different when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids prior to mating (Figure 4.6B).

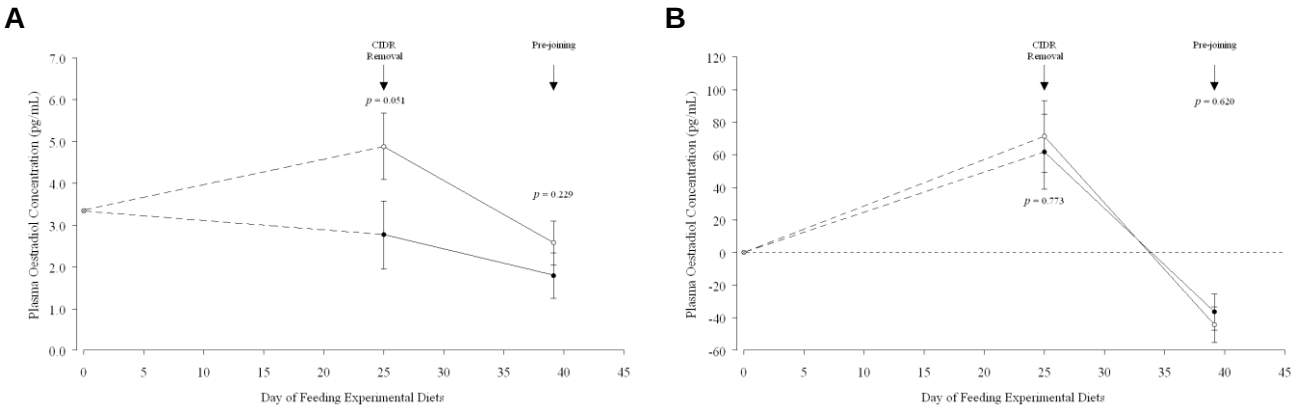


Figure 4.8 Mean (A) concentration of plasma oestradiol (pg/mL) and (B) the percentage change in oestradiol from baseline for BL x Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating. Baseline progesterone concentrations for the High omega-3 and High omega-6 diets were 2.45 ± 0.65 and 3.77 ± 0.62 pg/mL respectively and were included in the statistical analysis as a co-variate. Significant difference between treatment diets (A) $p = 0.058$, (B) $p = 0.967$.

4.3.4 Time of oestrus and parturition

Details of the results for oestrus and parturition will be presented in the following section. Data for the time to oestrus will be presented for each study as well as the combined results for the overall effect of diet. Results for parturition will be presented with the main effects of diet and time of feeding either pre-conception only or both pre- and post-conception.

Oestrus

The proportion of ewes showing oestrus was significantly higher when ewes were fed the high omega-6 diet compared with the high omega-3 diet prior to mating (Table 4.10). The cumulative proportion of ewes showing oestrus over time was signify cantly greater (Relative Risk = 1.37, $p < 0.001$, Figure 4.6) when ewes were fed the high omega-6 diet compared with the high omega-3 diet.

The time from CIDR removal to the first day any ewe showed oestrus was 15 days in Study 1 (2011) and 14 days in Study 2 (2012). The mean time to oestrus from the first day any ewe showed oestrus was also significantly ($p < 0.001$) shorter when ewes were fed the high omega-6 diet compared with the high omega-3 diet (Table 4.10). The time to oestrus was shorter when ewes were fed the diet high in omega-6 in both Study 1 and 2 (Figure 4.7).

Table 4.9 Proportion of BL x Merino ewes showing oestrus or lambed and the time to oestrus or parturition following the consumption of a diet based on either silage (High Omega-3) or oats and cottonseed meal (High Omega-6) for 42 days prior to and, 17 days following, mating.

Outcome[1]	Treatment: High Omega-3 (Silage)	Treatment: High Omega-6 (Oats/CSM[2])	*p*-values
Oestrus			
Proportion of ewes showing oestrus (%)	92.2%	97.3%	0.005
Relative Risk of Oestrus (Cox's PHR[3])	1.00	1.37	< 0.001
Mean time to behavioural oestrus (Days)	5.76 (± 0.09)	5.33 (± 0.08)	< 0.001
Parturition			
Proportion of ewes lambed[4] (%)	94.8%	91.8%	0.155
Relative Risk of lambing (Cox's PHR)	1.00	1.32	0.002
Mean time to parturition (Days)	7.96 (± 0.14)	6.62 (± 0.14)	< 0.001

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means) including all ewes that could be identified with their lamb in each treatment group.
[2]CSM = cottonseed meal.
[3]Cox's PHR = Cox's Proportional Hazards Regression Analysis (Cox, 1972).
[4]Including only those ewes that could be positively identified with their lamb.

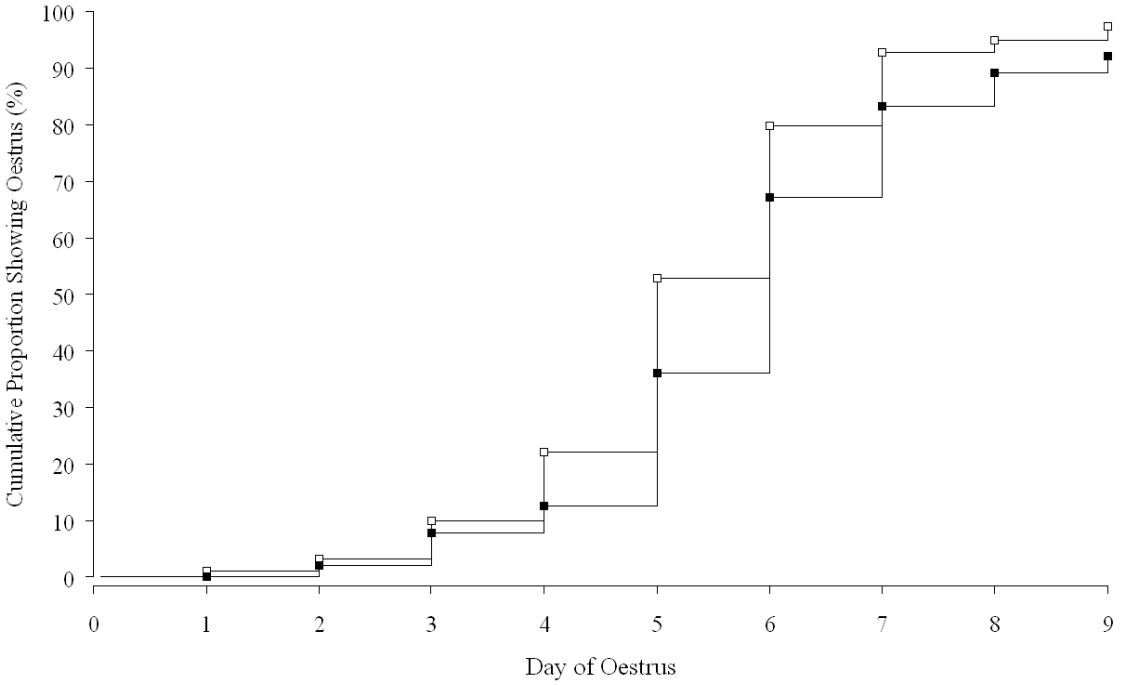


Figure 4.9 Cumulative proportion of of BL x Merino ewes showing behavioural oestrus (from the first day any ewe showed oestrus) following the consumption of a diet high in omega-3 (■) or omega-6 (□) fatty acids for 42 days prior to mating. The time from CIDR removal to the first day any ewe showed oestrus was 14.5 days. Relative Risk (Cox, 1972) of ewes fed the omega-6 diet showing oestrus = 1.37 ($p < 0.001$).

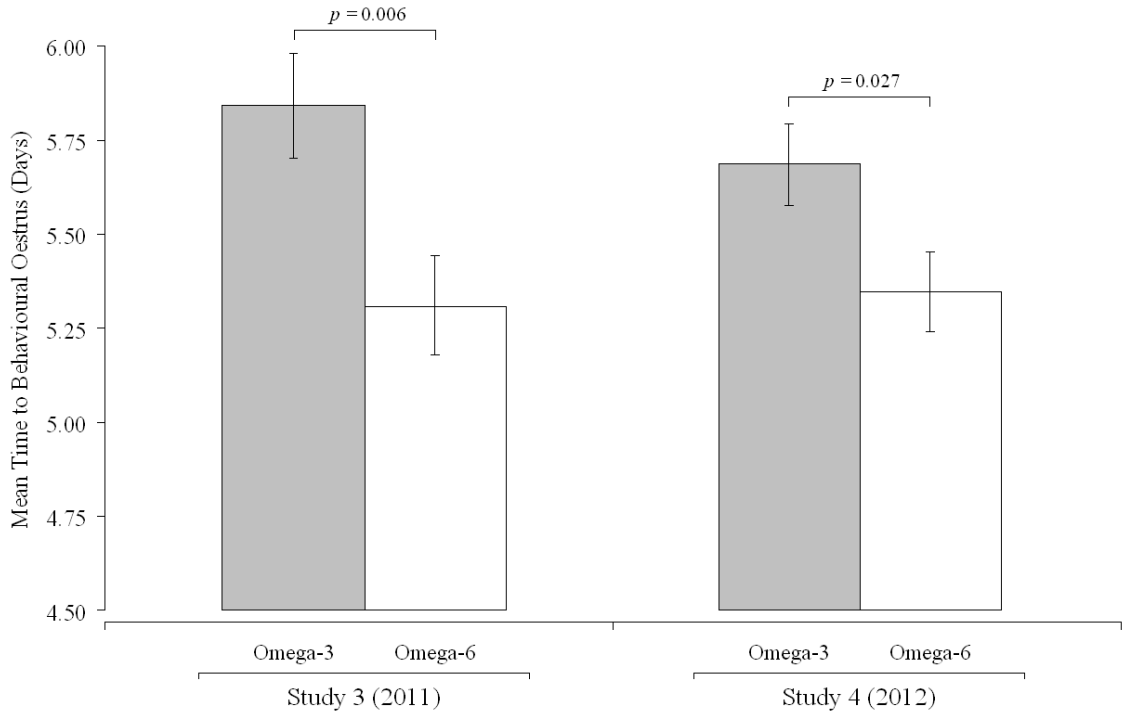


Figure 4.10 Mean time to showing behavioural oestrus (from the first day any ewe showed oestrus) for BL x Merino ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids for 42 days prior to mating. The mean time from CIDR removal to the first day any ewe showed oestrus in Studies 1 and 2 was 14.5 days.

Parturition

The proportion of ewes that lambed was not significantly higher when ewes were fed the high omega-3 diet compared with the high omega-6 diet prior to or following mating (Table 4.9). Further details outlining the effect of feeding experimental diets either pre-conception or pre- and post-conception will be shown in Section 4.3.5. The cumulative proportion of ewes that lambed over time was significantly higher (Relative Risk = 1.30, p = 0.003, Figure 4.8) when ewes were fed the high omega-6 diet compared with the high omega-3 diet.

The first day any ewe lambed was 159 or 161 days after CIDR removal in Study 1 and 2, respectively and, 144 or 147 days after the first ewe showed oestrus in Study 1 and 2, respectively. The mean time to parturition from the first day any ewe lambed was significantly shorter when ewes were fed the high omega-6 diet (6.6 ± 0.14 days) compared with the high omega-3 diet (8.0 ± 0.14 days, Table 4.10). The time to parturition was significantly shorter when ewes were fed the high omega-6 diet compared with the high omega-3 diet either pre-conception only (p < 0.005), or both pre- and post-conception (p < 0.001, Figure 4.9). The oestrus to parturition interval was significantly shorter when were fed the high omega-6 diet compared with the high omega-3 diet both pre- and post-conception (p < 0.001), but not when ewes were fed pre-conception only (p = 0.915, Figure 4.10)

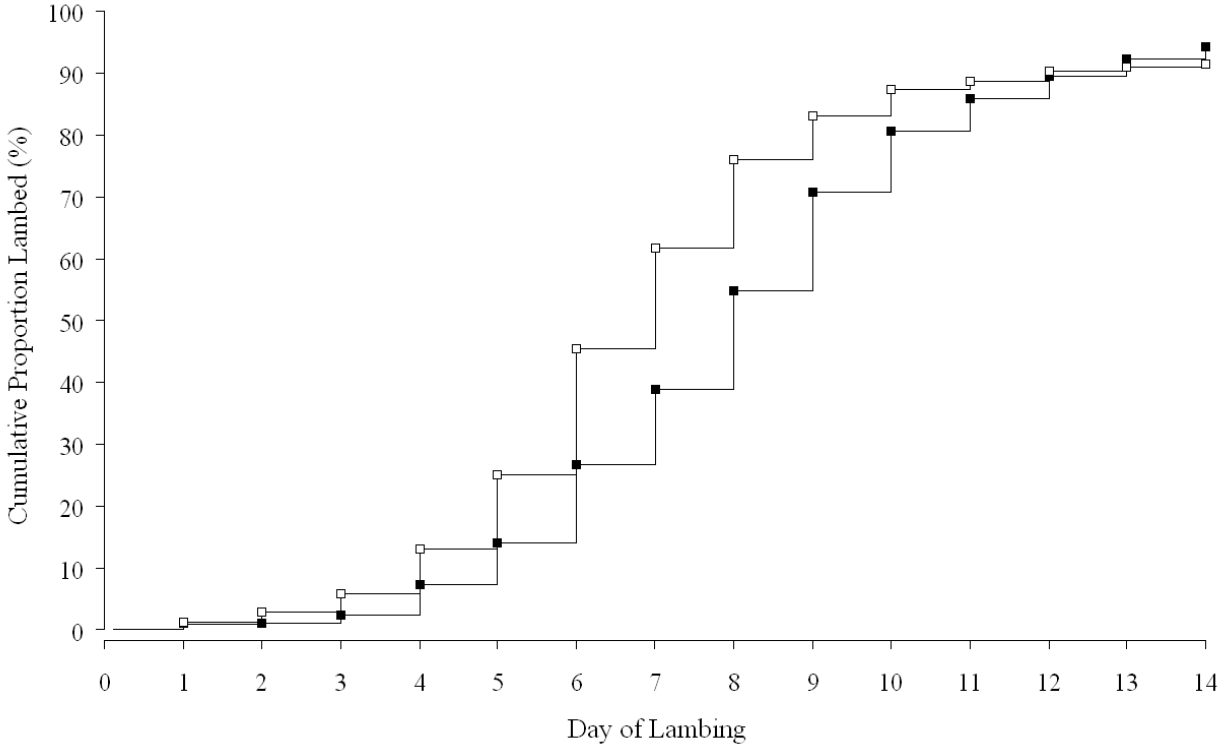


Figure 4.11 Cumulative proportion of BL x Merino ewes that lambed (from the first day any ewe lambed) following the consumption of a diet high in omega-3 (■) or omega-6 (□) fatty acids for 42 days prior to mating. Relative Risk (Cox, 1972) of ewes fed the omega-6 diet lambing = 1.32 (p = 0.002).

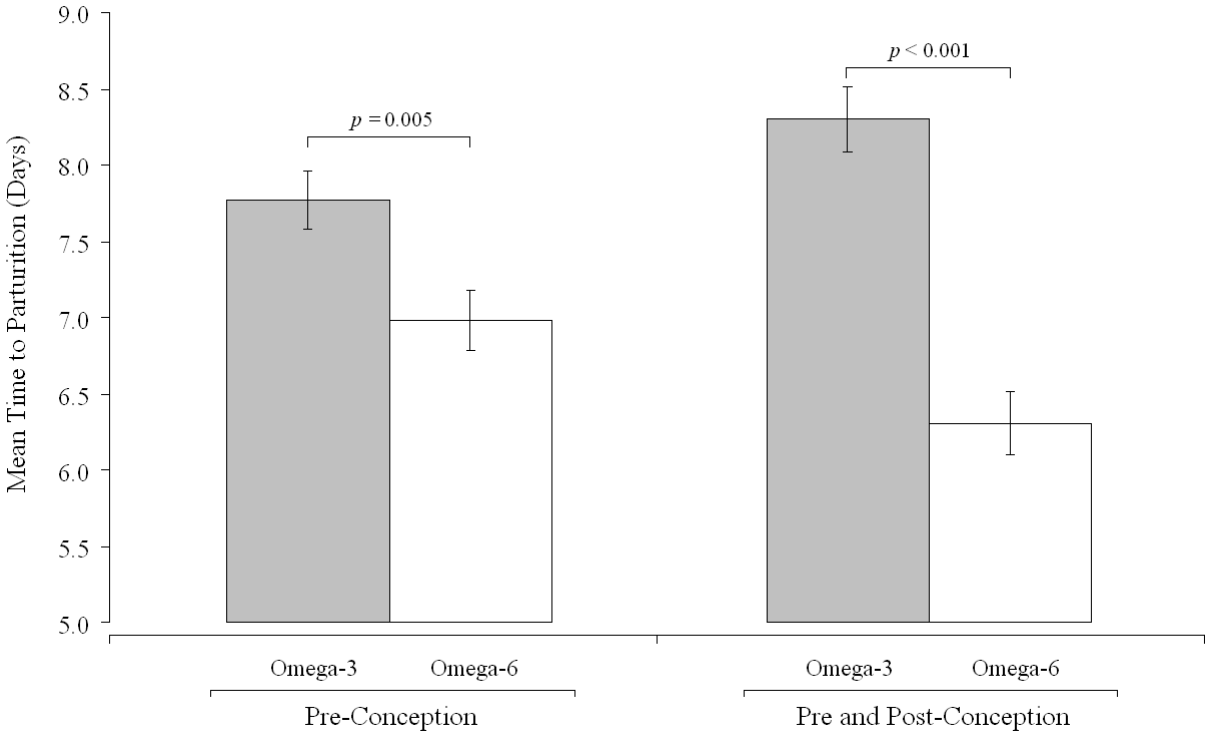


Figure 4.12 Mean time to parturition (from the first day any ewe lambed) for BL x Merino ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids either 42 days prior to mating (Pre-conception), or 42 days prior to and 17 days following mating (Pre and Post-conception).

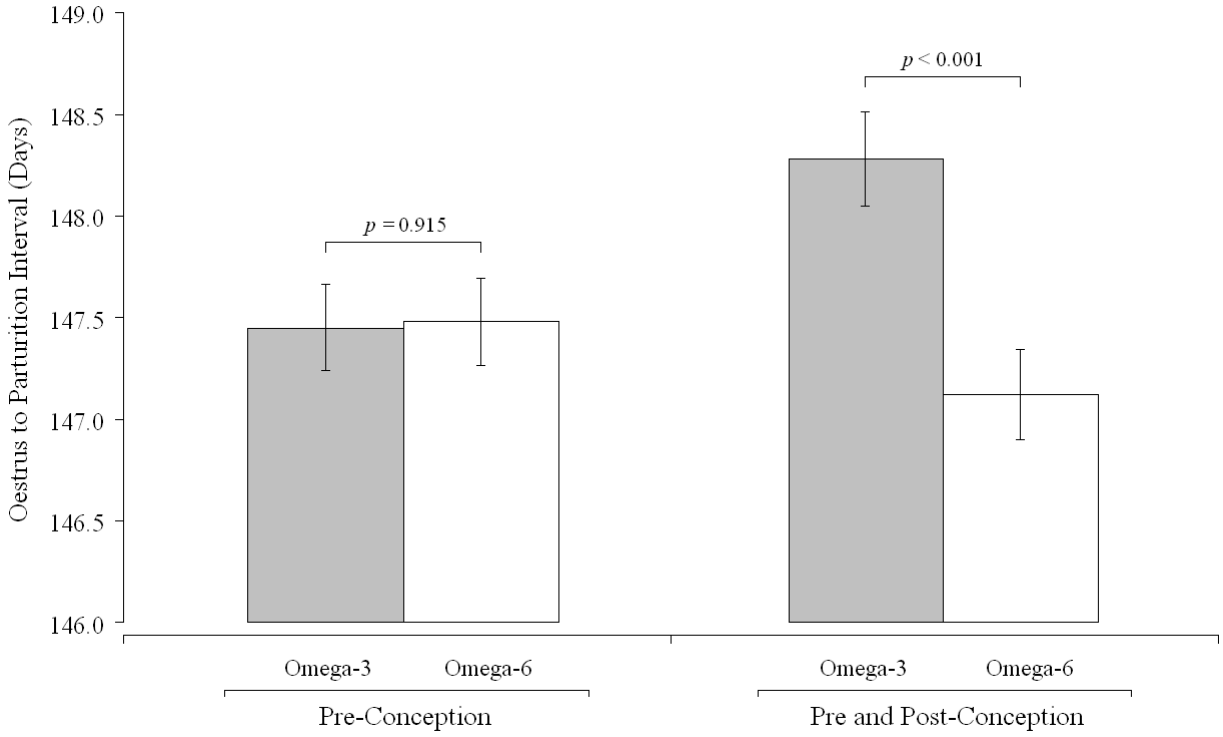


Figure 4.13 Mean oestrus to parturition interval for BL x Merino ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids either 42 days prior to mating (Pre-conception), or 42 days prior to and 17 days following mating (Pre and Post-conception).

4.3.5 Reproduction outcomes

The proportion of ewes pregnant and the proportion of ewes that lambed was not significantly different when ewes were fed the high omega-3 diet compared with the high omega-6 diet prior to or following mating (Table 4.11). Similarly, the mean foetal rate and the mean number of lambs born was not significantly different when ewes were fed the silage diet high in omega-3 compared with the oats/CSM diet high in omega-6 and the interaction between diet and length of time of feeding was also not significant for any reproduction parameters (Table 4.10).

4.3.6 Sex ratio of lambs

A total of 945 lambs were born over the two years. Of those lambs born, a total of 907 lambs (Males = 471, Females = 436) could be identified with their dam and included in the sex ratio analysis. The proportion of female lambs was significantly (p = 0.009) higher when ewes were fed the high omega-6 diet (55.7% female) compared with the high omega-3 diet (43.9% female) either pre-conception or both pre- and post-conception.

The proportion of female lambs was higher when ewes were fed the diet high in omega-6 compared with omega-3 fatty acids both pre- and post-conception (57.2% vs 43.4% respectively) but not when ewes were fed pre-conception only (54.1% vs 44.4% respectively, Figure 4.11). The interaction between diet and time, however, was not significant (p = 0.405). The proportion of female lambs was also higher when single-bearing ewes were fed the high omega-6 diet compared with high omega-3 diet either pre-conception (57.8 vs 38.4%) or pre

and post conception (60.9 vs 37.1%) but was not statistically higher when twin-bearing or triplet-bearing ewes were fed either pre-conception or pre- and post-conception (Figure 4.12).

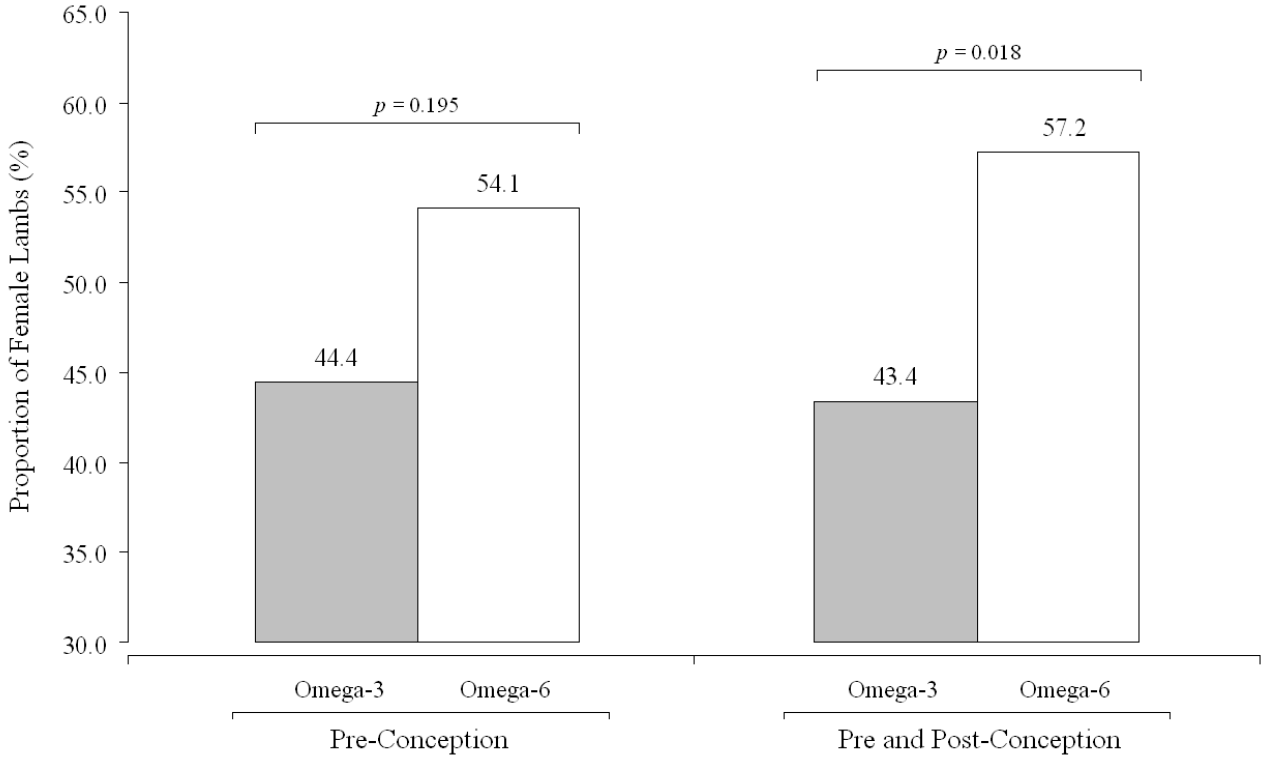


Figure 4.14 Proportion of female lambs when BL x Merino ewes were fed a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids for either 42 days prior to mating (Pre-conception) or 42 days prior to and 17 days following mating (Pre and Post-conception).

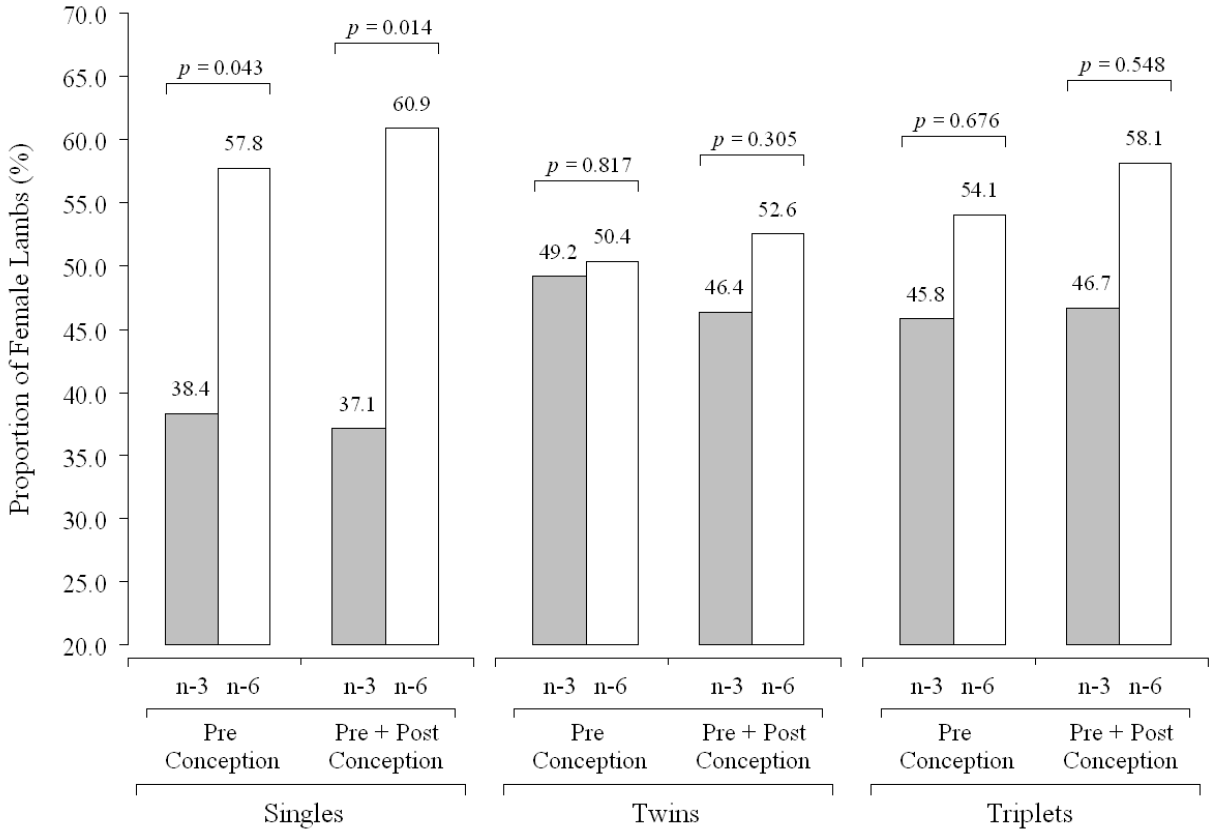


Figure 4.15 Proportion of female lambs when single, twin or triplet-bearing BL x Merino ewes were fed a diet high in omega-3 (n-3, shaded bars) or omega-6 (n-6, unshaded bars) fatty acids either 42 days prior to mating (Pre-conception) or 42 days prior to and 17 days following mating (Pre + Post-conception).

The concentration of progesterone, oestradiol and fatty acids in plasma at the time of mating was not significantly correlated with lamb sex ($p > 0.05$). The concentration of plasma oestradiol pre-conception was, however, significantly positively correlated with the time of oestrus ($r^2 = 0.022$, $p = 0.049$) but not significantly correlated with the time of lambing ($r^2 = 0.058$, $p = 0.241$), indicating that the concentration of E_2 in plasma was higher when the time to oestrus or lambing was longer.

4.3.7 Lamb birthweight, vigour and survival

Lamb birthweight was significantly ($p < 0.001$) higher for male lambs versus female lambs (Table 4.12). Lamb birthweight was not significantly ($p = 0.144$) higher when ewes were fed the high omega-3 diet compared with the high omega-6 diet at mating. Lamb birthweight was significantly positively correlated (r (857) = 0.312, $p < 0.001$) with day of lambing, indicating that birthweight was lower when lambs were born earlier. The birthweight of male (5.70 ± 0.08 vs 5.62 0.07, $p = 0.397$) and female (5.18 ± 0.07 vs 5.16 0.08, $p = 0.849$) lambs was also not significantly different when ewes were fed the high omega-6 compared with the high omega-3 diet at mating when the day of lambing was included in the analysis as a co-variate. Lamb head circumference, vigour and survival were not significantly different when ewes were fed the diet high in omega-3 or omega-6 fatty acids at mating (Table 4.11).

Table 4.10 Proportion of BL x Merino ewes pregnant or lambed and mean pregnancy or lambing rates following the consumption of a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating.

Reproduction Measure[1]	High Omega-3 (Silage)		High Omega-6 (Oats/CSM[2])		*p*-values[3]		
	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception[2]	Pre- and Post-Conception	Diet	Time	Diet x Time
Proportion scanned pregnant (%)	96.27%	94.81%	91.67%	95.74%	0.324	0.482	0.137
Mean foetal rate							
For ewes scanned pregnant	1.65 (± 0.04)	1.59 (± 0.04)	1.70 (± 0.04)	1.63 (± 0.04)	0.325	0.133	0.889
For all ewes joined	1.59 (± 0.05)	1.51 (± 0.05)	1.56 (± 0.05)	1.56 (± 0.05)	0.868	0.450	0.420
Pregnancy loss[4] (%)	2.48%	0.74%	1.92%	0.71%	0.769	0.143	0.793
Proportion lambed							
For ewes scanned pregnant[5] (%)	97.42%	99.24%	97.90%	99.26%	0.812	0.135	0.828
For all ewes joined[5] (%)	93.21%	96.30%	89.17%	94.37%	0.151	0.047	0.612
Mean number of lambs							
For ewes that lambed	1.62 (± 0.05)	1.60 (± 0.05)	1.68 (± 0.05)	1.69 (± 0.05)	0.110	0.904	0.808
For all ewes joined	1.51 (± 0.06)	1.50 (± 0.06)	1.48 (± 0.06)	1.56 (± 0.06)	0.834	0.510	0.483

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means) including all lambs that could be identified with their dam in each treatment group.
[2]CSM = cottonseed meal.
[3]Diet = main effect of dietary treatment (High omega-3 versus High omega-6), Time = main effect of time of feeding (Pre-conception only versus both Pre- and Post-conception).
[4]Proportion of ewes scanned as pregnant that were identified as not having lambed at lamb marking (Dun, 1963).
[5]Including only those lambs that could be positively identified with their dam. Also includes lambs from ewes that could not be identified pregnant at pregnancy scanning that lambed.

Table 4.11 Lamb birthweight, head circumference, vigour and survival and maternal score at parturition when BL x Merino ewes were fed a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating.

Lamb Measure[1]	Male		Female		*p*-values[3]		
	High Omega-3[2]	High Omega-6	High Omega-3[2]	High Omega-6	Diet	Sex	Diet x Sex
Birthweight (kg)	5.67 (± 0.08)	5.57 (± 0.09)	5.16 (± 0.09)	5.05 (± 0.08)	0.144	< 0.001	0.888
Head Circumference[4] (cm)	27.6 (± 0.13)	27.6 (± 0.14)	27.2 (± 0.14)	27.0 (± 0.13)	0.328	< 0.001	0.524
Lamb vigour[5]	2.60 (± 0.06)	2.44 (± 0.06)	2.43 (± 0.06)	2.39 (± 0.06)	0.088	0.068	0.263
Lamb Survival							
Birth	96.3%	98.0%	96.9%	96.1%	0.688	0.547	0.289
24 hr	93.9%	95.3%	95.0%	96.8%	0.409	0.507	0.916

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means) including all lambs that could be identified with their dam in each treatment group.
[2]High omega-3 treatment based on silage, High omega-6 treatment based on oats and cottonseed meal.
[3]Diet = main effect of dietary treatment (High omega-3 versus High omega-6), Time = main effect of time of feeding (Pre-conception only versus both Pre- and Post-conception).
[4]Head circumference in the coronal plane (Jamison et al., 1961).
[5]Lamb vigour measured on a scale of 1 to 5 where 1 = more vigorous and 5 = least vigorous.

4.4 Discussion

The proportion of female lambs was 12% higher (55.7 vs 43.9%) when ewes were fed the high omega-6 diet compared with the high omega-3 diet at mating. The increased proportion of female lambs when ewes were fed the high omega-6 diet was greatest in single-bearing compared with multiple-bearing ewes (21% vs 5% more females) or when ewes were fed both pre- and post-conception compared with pre-conception only (14% vs 10% more females). The greatest difference in the proportion of female lambs occurred when single-bearing ewes were fed the high omega-6 diet both pre- and post-conception (24% more females).

Ewe weight and fat score was higher when ewes received the high omega-6 diet based on oats/CSM compared with the high omega-3 diet based on silage in Study 1 and 2. This change in weight and fat score indicates that the energy intake of ewes fed the high omega-6 diet was consistently higher during pen feeding, despite a similar amount of energy being offered to ewes in both treatment groups. The alteration in sex ratio, however, was not significantly correlated with ewe weight or fat score, or ewe weight and fat score change over the period of pen feeding, indicating that maternal condition at mating was not likely to be responsible for the observed differences in sex ratio between treatment groups.

The time to showing behavioural oestrus and the time to parturition was consistently shorter when ewes were fed the high omega-6 compared with the high omega-3 diet at mating. The difference in the time to parturition between dietary treatment groups was greater when ewes were fed the experimental diets both pre- and post-conception. This shorter time to parturition is consistent with previously observed effects of a high omega-6 diet fed pre- and post-conception (Gulliver et al., 2013b) and may be the result of the observed shorter time to oestrus or a shorter time interval between oestrus and parturition.

The difference in the oestrus to parturition interval between dietary treatment groups was greatest when single-bearing (p = 0.001) and twin-bearing (p = 0.099) ewes were fed treatment diets both pre- and post-conception. The oestrus to parturition interval was not, however, significantly different between ewes fed either diet when single-bearing (p = 0.867) or twin-bearing (p = 0.961) ewes were fed pre-conception only. This shorter time interval between oestrus and parturition when ewes were fed the high omega-6 diet both pre- and post-conception is also consistent with previously observed effects in these BL x Merino ewes (Gulliver et al., 2013b). The difference in the oestrus-parturition interval between length of time of feeding the experimental diets indicates that the may have been some carry over effects of dietary fatty acids post-conception, rather than only affecting the time of mating and conception.

The concentration of EPA in the plasma of ewes was significantly lower at preg-scanning when ewes were fed the high omega-6 compard with the high omega-3 diet both pre- and post-conception in Study 1, but not in Study 2. The reason for the differential effect of dietary treatments on the time taken for plasma fatty acid concentrations to return to similar values for both treatment groups is unclear. Ewes were fed the opposite diet in Study 2 as to Study 1, however, the length of time of feeding the diets was the same for ewes in both years. That is, ewes that were fed the high omega-3 diet both pre- and post-conception in Study 1 were fed the high omega-6 diet both pre- and post-conception in Study 2. The differential response in plasma fatty acid concentrations between Study 1 and Study 2 may, therefore, be the result of some carry over from the previous year, however, it is not possible to tell whether this is the case in the current study.

The lower progesterone in the plasma of BL x Merino ewes indicates that the ewes were closer to the time of ovulation at blood collection. Although the sex ratio of lambs was not

significantly related to the concentration of hormones or fatty acids at the time of conception, the relationship between the time of oestrus and ovulation with the observed differences in sex ratio warrants further investigation.

5.0 Phase 2: Merino ewes

5.1 Introduction

The proportion of female lambs was significantly higher when BL x Merino first cross ewes were fed a diet low in omega-3 (and high in omega-6 fatty acids) for 6 weeks prior to and, 3 weeks following, mating (Gulliver et al., 2013b). The effect of these diets on the sex ratio of lambs born to Merino ewes has not, however, previously been examined. Therefore, the aim of the current Phase of the study was to determine whether the proportion of female lambs was increased when Merino ewes were fed a diet high in omega-6 compared with omega-3 fatty acids and whether the effect was greater when ewes were fed either pre-conception only or both pre- and post-conception.

Phase 2 of the current project involved 2 pen studies with Merino ewes in 2011 and 2012 with a cross-over for diets in the second year. The current section presents details of these 2 pen feeding studies. Details of methodology specific to these studies will be presented, including details of specific diets used and animal management. Results for the effect of diet on ewe weight, fat score and plasma concentrations of fatty acids will be presented for each study. Data for the primary reproduction outcomes including the timing of oestrus and parturition, reproduction hormones and the sex ratio of lambs were combined across years and analysed together.

5.2 Methods

5.2.1 Study 3 (2011)

The third pen feeding study commenced in September 2011 (WWAI Study ID: 11-22). Details of experimental procedures for study 1 are outlined below.

Animals
A total of 320 Merino ewes (24-32 months of age, see Table 3.1) were vaccinated against clostridial diseases and caseous lymphadenitis (CLA, Glanvac 6, Pfizer Animal Health, West Ryde, Sydney) and treated with a combination anthelmintic drench (HatTrick, Ancare, NSW) prior to enrolment in the study. The study was conducted at the WWAI with pen feeding commencing in September (Spring) and lambing occurring in April 2012.

Experimental diets
Ewes were randomly allocated to 1 of 4 treatment groups (as indicated in Section 3.8) according to property source, fat score (FS, mean = 3.44 ± 0.02) then live weight (mean = 58.2 ± 0.34 kg). Treatments consisted of either a diet based on ryegrass silage (n = 160) high in omega-3 or a diet based on oat grain and CSM low in omega-3 (high in omega-6, n = 160) fatty acids (Table 5.1).

Table 5.1 Components and proximate analysis of diets offered to Merino ewes for 6 weeks prior to mating or 6 weeks prior to and 17 days following mating in Study 3 conducted in 2011.

Ingredients	Treatment Diet	
	High Omega-3 (Silage)	High Omega-6 (Oats/CSM)
Inclusion	**(%DM)**	
Silage	99.10	22.00
Oat grain	0.0	69.11
Cottonseed Meal	0.0	7.69
Molasses	0.0	0.0
Urea	0.0	0.0
Mineral Premix[1]	0.90	1.20
Proximate Analysis	**(%DM)**	
Neutral Detergent Fibre	51.06	28.20
Acid Detergent Fibre	29.36	18.85
Crude Protein	10.89	13.96
Total Lipid	2.76	4.91
ME (MJ/kg DM)	10.83	11.79
Fatty Acid Composition	**g/kg DM (% total fatty acids)**	
C14:0	0.16 (0.67%)	0.14 (0.28%)
C16:0	4.07 (17.3%)	8.08 (16.4%)
C18:0	0.52 (2.23%)	1.08 (2.19%)
C18:1n-9	1.03 (4.40%)	14.61 (29.8%)
C18:1n-7	0.09 (0.38%)	0.36 (0.73%)
C18:2n-6	3.59 (15.3%)	15.84 (32.3%)
C18:3n-3	11.55 (49.1%)	3.03 (6.17%)
Ratio of n-6:n-3[2]	0.31	5.23
Feed Offered[3]	**(per head)**	
DM (kg/day)	1.11	0.83
ME (MJ/day)	12.03	9.81
CP (g/day)	121.00	116.15

[1]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 10 g/head per day.
[2]Ratio of n-6:n-3 = ratio of omega-6 to omega-3 fatty acids.
[3]DM = dry matter, ME = metabolisable energy, CP = crude protein.

Feeding and ewe management

Ewes were housed in 1 of 8 pens (40 ewes/pen). Ewes were mated over 2 consecutive oestrous cycles and approximately 40% of the ewes in each treatment group showed oestrus in each cycle (Table 5.2). If ewes were allocated to receive treatment diets pre-conception only and they showed oestrus in Cycle 1, pen feeding was ceased for these ewes and ewes were moved to paddocks when the last ewe showed oestrus in Cycle 1. The remainder of the ewes allocated to receive treatment diets pre-conception only were removed from pens when the last ewe showed oestrus in Cycle 2.

If ewes were allocated to receive treatment diets both pre- and post-conception and they showed oestrus in Cycle 1, pen feeding was ceased for these ewes 17 days after the last ewe showed oestrus in Cycle 1. The remainder of the ewes allocated to receive treatment diets pre- and post-conception were removed from pens 17 days after the last ewe showed oestrus in Cycle 2. The proportion of all ewes that showed oestrus in each cycle is shown in Table 5.2 and the timing of insemination (Cycle 1 or 2) was confirmed for each ewe at pregnancy scanning.

Table 5.2 Proportion of Merino ewes that showed oestrus in Cycle 1 or 2 when ewes were allocated to receive treatment diets either pre-conception only or both pre- and post-conception.

Treatment Diet[1]	Feeding Time	Proportion Showing Oestrus (%)		
		Cycle 1	Cycle 2	Both
High Omega-3 (Silage)	Pre	34.6	51.3	83.8
High Omega-3 (Silage)	Pre + Post	41.0	50.0	82.3
High Omega-6 (Oats/CSM)	Pre	38.0	51.9	85.9
High Omega-6 (Oats/CSM)	Pre + Post	51.3	47.5	88.8

[1]Silage = ryegrass silage, CSM = cottonseed meal.
[2]Pre = ewes allocated to receive treatment diets pre-conception only, Pre + Post = ewes allocated to receive treatment diets both pre- and post-conception.

Animal withdrawals during the study

One ewe (fed the high omega-3 diet) was withdrawn from pen feeding prior to joining as it was pregnant prior to the commencement of the study and one ewe (also fed the high omega-3 diet) was removed from pen feeding prior to joining due to low body weight and fat score. Details of animal withdrawals can be found in Appendix 3.

Data Collection

Ewe weight and fat score was assessed during pen feeding as described previously (Section 3.10). Blood samples were also collected from a sub-set of ewes in each pen for fatty acid and hormone analysis as described previously. Pregnancy scanning was conducted 66 or 51 days after the last ewe showed oestrus in Cycle 1 or Cycle 2, respectively.

Ewe management during parturition

Ewes lambed in 1 of 3 paddocks depending on whether ewes were scanned as conceiving in Cycle 1 or 2 or were scanned as being not pregnant (Table 4.2). A mixture of ewes from both dietary treatment groups and those scanned as having single or multiple foetuses were run together in each paddock.

Table 5.3 Details of lambing paddocks used for Merino ewes in Study 3 in 2011.

Paddock	Pasture	Cycle	Scanned	Number of Ewes		
				High Omega-3	High Omega-6	Total
1	Oats	1	Single/Multiple	64	64	128
2	Oats	2	Single/Multiple	49	51	100
3	Mixed	1 and 2	Dry	47	45	92
Total						320

5.2.2 Study 4 (2012)

The second pen feeding study commenced in October 2012 (WWAI Study ID: 12-27). Details of experimental procedures for Study 4 are outlined below.

Animals

Due to ewe losses between lamb marking and the commencement of pen feeding in 2012, only 317 (out of 320) Merino ewes (24 or 36 months of age) were available for pen feeding in Study 4 (Table 5.4). All ewes were vaccinated against clostridial diseases and caseous lymphadenitis (CLA, Glanvac 6, Pfizer Animal Health, West Ryde, Sydney) and treated with a combination anthelmintic drench (HatTrick, Ancare, NSW) prior to the start of the fourth

pen feeding study. Pen feeding commencing in October (Spring) and lambing occurring in April 2013.

The mean weight of ewes at the commencement of the pen feeding study in 2013 was not significantly ($p > 0.05$) different when ewes were fed the high omega-3 (silage) diet (61.7 ± 0.51) compared with the high omega-6 (Oats/CSM) diet (62.1 ± 0.58) the previous year in 2011. The mean fat score of ewes at the commencement of the pen feeding study in 2012 was also not significantly ($p > 0.05$) different when ewes were fed the high omega-3 diet (3.11 ± 0.05) compared with the high omega-6 diet (3.06 ± 0.05) in 2011. More details of the change in weight during the pen feeding studies will be shown in Section 4.3 below.

Table 5.4 Number of Merino ewes from each source enrolled in Study 2 in 2012.

Source[1]	High Omega-3 (Silage)	High Omega-6 (Oats/CSM[2])	Total
A5R	74	75	149
6TS	50	49	99
T5L	29	29	58
Mixed	5	6	11
Total	158	159	**317**

[1]Source = property of origin identification tag.
[2]CSM = cottonseed meal.

Experimental diets

In Study 4 in 2012, treatment diets were either based based on oaten silage (High omega-3) or oat grain and CSM (High omega-6, Table 4.3). The same silage diet was used in Study 4 as was used in Study 2. The amount of feed offered to ewes in each treatment group is shown below (Table 4.3).

Table 5.5 Components and proximate analysis of diets offered to Merino ewes for 6 weeks prior to mating or 6 weeks prior to and 17 days following mating in Study 4 conducted in 2012.

Ingredients	**Treatment Diet**	
	High Omega-3 (Silage)	High Omega-6 (Oats/CSM)
Inclusion	**(%DM)**	
Silage	89.38	22.12
Oat grain	0.0	71.19
Cottonseed Meal	0.0	5.57
Molasses	9.91	0.0
Mineral Premix[1]	0.71	1.12
Proximate Analysis	**(%DM)**	
Neutral Detergent Fibre	51.06	28.20
Acid Detergent Fibre	29.36	18.85
Crude Protein	10.89	13.96
Total Lipid	2.76	4.91
ME (MJ/kg DM)	10.83	11.79
Fatty Acid Composition	**g/kg DM g/kg DM (% total fatty acids)**	
C14:0	0.24 (1.00%)	0.15 (0.30%)
C16:0	4.24 (18.0%)	7.67 (15.6%)
C18:0	0.59 (2.49%)	0.98 (2.00%)
C18:1n-9	3.41 (14.6%)	16.39 (33.4%)
C18:1n-7	0.13 (0.56%)	0.36 (0.73%)
C18:2n-6	3.73 (15.9%)	15.17 (30.9%)
C18:3n-3	7.82 (33.1%)	2.14 (4.37%)
Ratio of n-6:n-3[2]	0.48	7.07

Feed Offered[3]	(per head)	
DM (kg/day)	1.11	0.83
ME (MJ/day)	12.03	9.81
CP (g/day)	121.00	116.15

[1]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 10 g/head per day.
[2]Ratio of n-6:n-3 = ratio of omega-6 to omega-3 fatty acids.
[3]DM = dry matter, ME = metabolisable energy, CP = crude protein.

Feeding and ewe management

Ewes were housed in the same pens as in 2011 with a total of 34-38 ewes/pen depending on the ewes available in each treatment group. The majority of ewes in both groups allocated to receive treatment diets pre-conception only showed oestrus in Cycle 1 (high omega-3 = 95.8%, high omega-6 = 99.3%). Pen feeding was, therefore, ceased for all ewes in these pens after the last ewe showed oestrus in Cycle 1 and ewes were moved to paddocks. Ewes allocated to receive treatment diets pre- and post-conception remained in pens and received treatment diets for a further 17 days before being moved to paddocks with the ewes that were fed pre-conception only. The timing of insemination (Cycle 1 or 2) was confirmed for each ewe at pregnancy scanning.

Animal withdrawals during the study

One ewe (allocated to the High omega-6 dietary treatment group) was withdrawn prior to the commencement of pen feeding as it was pregnant prior to the commencement of the study and two ewe (fed the high omega-3 diet) were removed from pen feeding prior to joining due to low body weight and fat score. One ewe fed the high omega-6 diet was also withdrawn from the study at pregnancy scanning due to cancer. Details of animal withdrawals can be found in Appendix 3.

Data collection

Ewe weight and fat score was assessed during pen feeding as described previously (Section 3.10). Blood samples were also collected from a sub-set of ewes in each pen for fatty acid and hormone analysis as described previously. Pregnancy scanning was conducted 62 or 46 days after the last ewe showed oestrus in Cycle 1 or Cycle 2, respectively.

Ewe management during parturition

Ewes lambed in 1 of 10 paddocks depending on whether ewes were scanned as conceiving in Cycle 1 or 2 and carrying single or multiple foetuses (Table 5.6). Ewes from each dietary treatment group scanned as having single or multiple foetuses were allocated to lamb in separate paddocks, with 3 replicates per treatment for single bearing ewes. Ewes from both dietary treatment groups scanned as having multiple foetuses and scanned as being pregnant in Cycle 2 lambed together in a separate paddock (Table 5.6). Ewes were branded with a series of 1, 2 or 3 numbers (0 or 1) that was coded to their individual ear tag number for identification during lambing (Plate 5.2).

Table 5.6 Details of lambing paddocks used for Merino ewes in Study 4 in 2012 (Total n = 313).

Paddock	Pasture	Cycle	Scanned	Dietary Treatment	Number
1	Lucerne	1	Single	High Omega-6	30
2	Lucerne	1	Single	High Omega-3	30
3	Lucerne	1	Single	High Omega-3	30
4	Lucerne	1	Single	High Omega-6	29
5	Clover/Ryegrass	1	Multiple	High Omega-3	21
6	Clover/Ryegrass	1	Multiple	High Omega-6	22
7	Clover/Ryegrass	2	Single	High Omega-6	32
8	Clover/Ryegrass	2	Single	High Omega-3	29
9	Mixed	2	Multiple	High n-3 + High n-6	14
10	Mixed	-	Dry	High n-3 + High n-6	76
Total Pregnant					237

Plate 5.2 Identification of Merino ewes at lambing in Study 4 in 2012.

5.3 Results

Similar to the previous section for BL x Merino ewes, the results for weight, fat score and plasma fatty acids will be presented separately for each study in the following sections in order to assess the effects of individual diets. Reproduction data will then be combined for both years.

5.3.1 Ewe weight and Fat score

Study 3 (2011)
Ewe weight initially decreased during pen feeding when ewes were fed the high omega-6 diet based on oats/CSM (Figure 5.1). Mean ewe weight was not significantly different when ewes were fed the high omega-3 compared with the high omega-6 diet, however, ewe weight changed significantly over time for ewes fed the diet high in omega-6 compared with ewes fed the high omega-3 diet ($p < 0.001$, Table 5.6). Ewe weight was not significantly higher at preg-scanning ($p = 0.070$) and 4 weeks prior to parturition ($p = 0.126$) when ewes were fed the high omega-6 diet compared with the high omega-3 diet at the time of mating (Figure 5.1A).

Similar to BL x Merino ewes, ewe fat score was significantly ($p < 0.001$) higher when Merino ewes were fed a diet high in omega-6 compared with omega-3 fatty acids either pre-conception or pre- and post-conception (Table 5.7). Ewe fat score was significantly higher at the completion of pen feeding, at preg-scanning and prior to parturition when ewes were fed the High omega-6 diet (Figure 4.1B).

Table 5.7 Mean weight and fat score of Merino ewes following the consumption of a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating showing the main effects of diet and day of feeding.

				p-values		
Study	Measure	High Omega-3	High Omega-6	Diet	Day	Diet x Day
Study 3 (2011)	Weight (kg)	61.1 (± 0.49)	60.7 (± 0.49)	0.588	< 0.001	< 0.001
	Fat Score	3.44 (± 0.02)	3.60 (± 0.02)	< 0.001	< 0.001	0.001
Study 4 (2012)	Weight (kg)	62.0 (± 0.52)	63.9 (± 0.52)	0.012	< 0.001	< 0.001
	Fat Score	2.98 (± 0.03)	3.20 (± 0.03)	< 0.001	< 0.001	< 0.001

Study 4 (2012)

Ewe weight and fat score (Figures 5.2A and B) were not significantly different between dietary treatment groups at the commencement of pen feeding in the second year. Mean liveweight over the duration of the study was, however, significantly ($p = 0.012$) higher when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids at joining (Table 4.6). Ewe weight was significantly higher at preg-scanning ($p = 0.022$) and prior to parturition ($p = 0.030$) when ewes were fed the high omega-6 diet compared with the high omega-3 diet (Figure 5.2A).

Similar to Study 3, ewe fat score was significantly higher over the duration of Study 4 when ewes were fed the high omega-6 diet compared with the high omega-3 diet (Table 4.6). Fat score was significantly higher at preg-scannning ($p < 0.001$) and prior to parturition ($p = 0.001$) when ewes were fed the high omega-6 diet (Figure 5.2B).

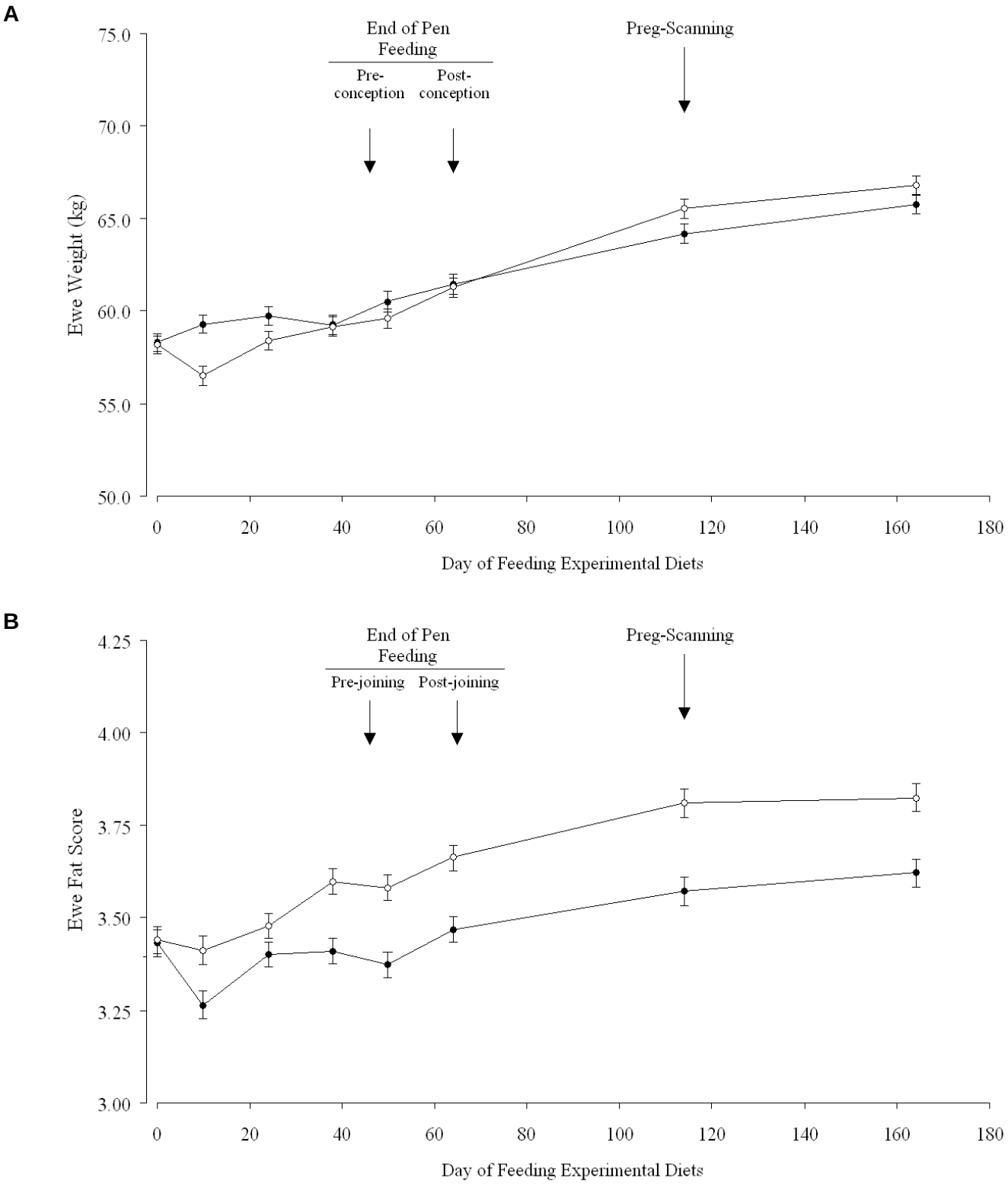


Figure 5.1 Mean weight (A) and fat score (B) for Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating or 42 days prior to and 17 days following mating in Study 3 (2011).

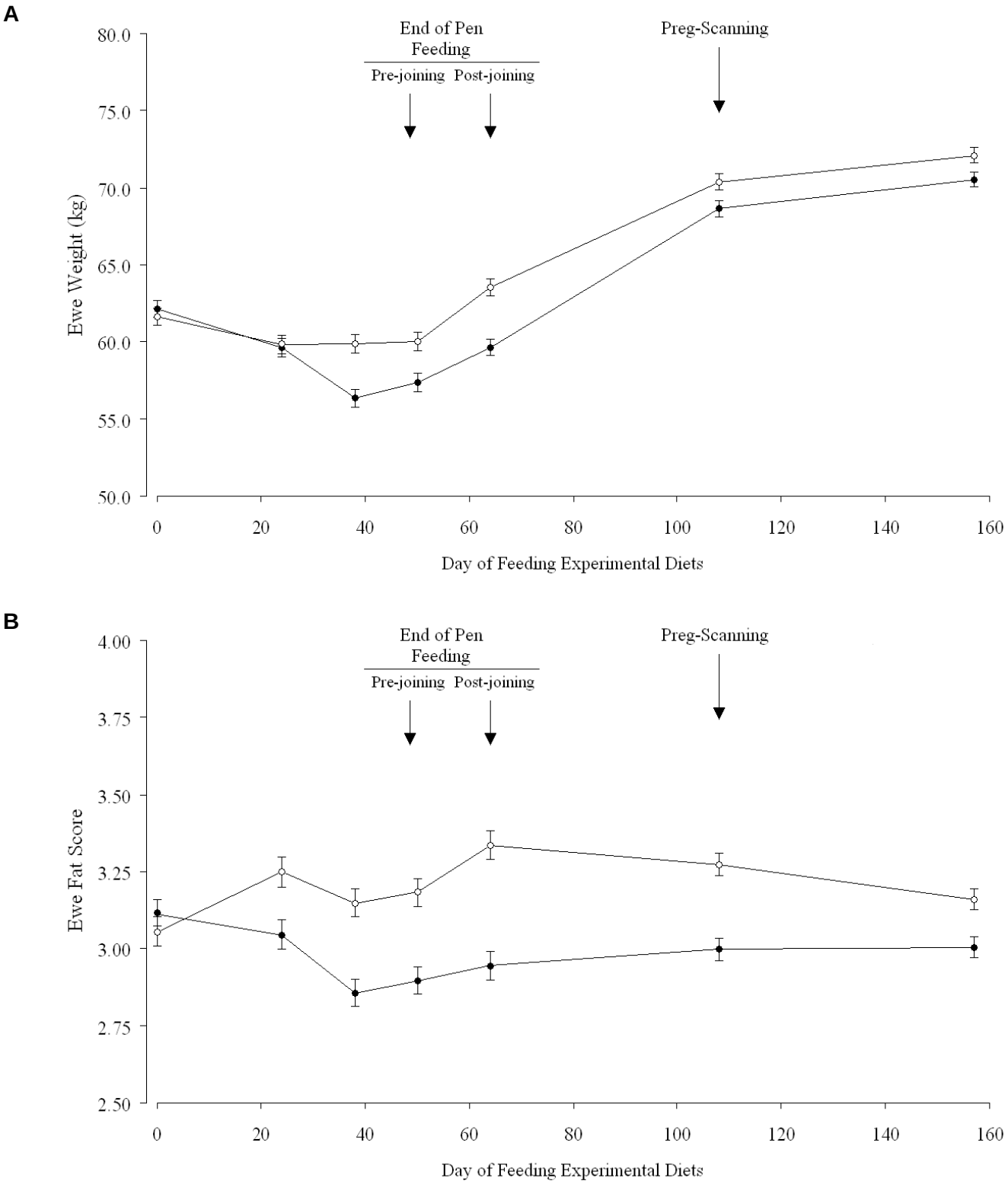


Figure 5.2 Mean weight (A) and fat score (B) for Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating or 42 days prior to and 17 days following mating in Study 4 (2012).

5.3.2 Plasma fatty acid concentration

Plasma fatty acid concentrations were related to the diet fed during each study, particularly, the type and fatty acid profile of the silage used. Fatty acid concentrations will be presented separately for each study with details provided for ewes that were fed pre-conception only or for the longer time period both pre- and post-conception.

Study 3 (2011)
The concentration of all omega-3 fatty acids measured in Study 3, including ALA, EPA and DHA was significantly higher when ewes were fed a diet high in omega-3 fatty acids based on ryegrass silage compared with ewes fed a diet high in omega-6 fatty acids based on oats/CSM (Table 5.8). The interaction between diet and length of time of feeding (pre-conception or pre- and post-conception) was not significant for any omega-3 fatty acids.

The concentration of ALA, EPA and DHA increased significantly ($p < 0.001$) following the introduction of the high omega-3 diet (Figures 5.3A and B) and remained relatively constant while ewes were fed the experimental rations. The concentration of EPA was significantly lower at preg-scanning ($p = 0.039$) and pre-lambing ($p < 0.001$) when ewes were fed the high omega-6 diet both pre- and post-conception (Figure 5.3B), but not when ewes were fed pre-conception only (preg-scanning, $p = 0.194$; pre-lambing, $p = 0.135$, Figure 5.3A). In contrast, the concentration of DHA in the plasma of ewes pre-lambing was not significantly lower when ewes were fed the high omega-6 diet both pre- and post-conception ($p = 0.109$), but was lower when ewes were fed pre-conception only ($p = 0.034$, Figure 5.3A).

The concentration of all major omega-6 fatty acids (including C18:2n-6 and C22:5n-6) except for C20:4n-6 (ARA) were significantly ($p < 0.001$) higher when ewes were fed a diet high in omega-6 fatty acids compared with omega-3 fatty acids either pre-conception only or pre- and post-conception (Table 5.8). The concentration of omega-6 fatty acids increased significantly ($p < 0.001$) following the introduction of the high omega-6 diet (Figures 5.4A and B) and remained higher while ewes were fed the experimental rations. The concentration of C18:2n-6 and C20:4n-6 was not significantly different pre-lambing when ewes were fed the high omega-3 or high omega-6 diet at joining.

Table 5.8 Mean concentration (μg/mL) of fatty acid methyl esters (FAME) in the plasma of Merino ewes following the consumption of a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating in Study 3 (2011).

	High Omega-3 (Silage)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception	Pre- and Post-Conception	Diet	Diet x Time
SFA						
C8:0	5.41 (± 0.71)	7.04 (± 0.72)	8.57 (± 0.75)	6.59 (± 0.73)	0.073	0.008
C9:0	1.63 (± 0.16)	1.83 (± 0.16)	2.01 (± 0.18)	1.84 (± 0.16)	0.246	0.203
C10:0	1.54 (± 0.15)	1.92 (± 0.15)	2.19 (± 0.19)	1.65 (± 0.14)	0.252	0.006
C11:0	2.54 (± 0.20)	2.35 (± 0.20)	2.31 (± 0.21)	2.75 (± 0.20)	0.669	0.227
C12:0	1.47 (± 0.72)	1.71 (± 0.72)	4.29 (± 10)	1.55 (± 0.66)	0.101	0.176
C14:0	11.42 (± 1.39)	14.11 (± 1.38)	10.41 (± 1.92)	7.41 (± 1.30)	0.017	0.268
iC15:0	3.88 (± 0.18)	3.54 (± 0.18)	2.63 (± 0.19)	2.20 (± 0.18)	< 0.001	0.604
aiC15:0	4.75 (± 0.19)	4.62 (± 0.20)	3.81 (± 0.21)	3.34 (± 0.20)	< 0.001	0.535
C15:0	10.72 (± 0.33)	10.05 (± 0.33)	7.50 (± 0.35)	6.73 (± 0.34)	< 0.001	0.360
C16:0	182.0 (± 5.15)	171.3 (± 4.98)	193.0 (± 5.15)	182.3 (± 5.26)	0.040	0.396
iC17:0	9.07 (± 0.52)	8.39 (± 0.50)	8.48 (± 0.53)	8.25 (± 0.52)	0.490	0.363
aiC17:0	24.88 (± 1.62)	27.24 (± 1.57)	9.56 (± 1.71)	7.23 (± 1.63)	< 0.001	0.532
C17:0	14.31 (± 0.40)	12.92 (± 0.38)	10.57 (± 0.40)	10.08 (± 0.40)	< 0.001	0.084
C18:0	193.9 (± 8.37)	189.9 (± 8.12)	219.2 (± 8.37)	221.1 (± 8.59)	0.002	0.081
C20:0	1.62 (± 0.08)	1.56 (± 0.08)	1.73 (± 0.08)	1.69 (± 0.08)	0.161	0.864
C21:0	0.99 (± 0.03)	0.92 (± 0.03)	0.74 (± 0.03)	0.75 (± 0.03)	< 0.001	0.732
C22:0	3.22 (± 0.12)	2.85 (± 0.12)	2.89 (± 0.12)	2.98 (± 0.12)	0.392	0.039
C23:0	6.56 (± 0.22)	5.87 (± 0.21)	5.61 (± 0.21)	5.80 (± 0.21)	0.023	0.100
C24:0	4.95 (± 0.21)	4.18 (± 0.21)	4.53 (± 0.21)	4.50 (± 0.21)	0.825	0.019
Total SFA	479.0 (± 14.45)	465.7 (± 14.11)	493.5 (± 14.76)	474.1 (± 14.83)	0.436	0.326
MUFA						
C11:1n-1	1.41 (± 0.13)	1.44 (± 0.13)	1.22 (± 0.14)	1.20 (± 0.13)	0.104	0.293
C12:1n-7	0.93 (± 0.05)	1.03 (± 0.05)	0.75 (± 0.06)	0.71 (± 0.05)	< 0.001	0.679
C13:1n-1	1.35 (± 1.08)	3.45 (± 1.08)	0.50 (± 1.51)	0.73 (± 1.00)	0.142	0.042
C14:1n-5	0.51 (± 0.07)	0.49 (± 0.06)	0.41 (± 0.07)	0.41 (± 0.06)	0.185	0.038
C15:1n-5	0.44 (± 0.03)	0.40 (± 0.03)	0.40 (± 0.03)	0.40 (± 0.03)	0.651	0.332
C16:1n-7t	2.68 (± 0.83)	4.09 (± 0.83)	2.44 (± 1.17)	2.17 (± 0.77)	0.246	0.159
C16:1n-7	16.69 (± 0.95)	16.83 (± 0.92)	17.50 (± 0.94)	14.77 (± 0.97)	0.514	0.610
C17:1n-7	1.87 (± 0.14)	1.76 (± 0.13)	1.55 (± 0.18)	1.68 (± 0.13)	0.188	0.063
C18:1n9t	2.96 (± 0.22)	3.24 (± 0.22)	4.66 (± 0.24)	3.83 (± 0.23)	< 0.001	0.628
C18:1n7t	11.21 (± 0.76)	11.28 (± 0.71)	11.76 (± 0.76)	10.40 (± 0.76)	0.830	0.037
C18:1n-12	2.19 (± 0.15)	1.75 (± 0.15)	1.09 (± 0.18)	1.69 (± 0.15)	0.001	< 0.001
C18:1n-9	195.7 (± 7.22)	191.4 (± 6.91)	227.9 (± 7.23)	211.5 (± 7.21)	0.001	0.718
C18:1n-7	6.63 (± 0.35)	6.72 (± 0.34)	8.25 (± 0.35)	7.26 (± 0.35)	0.004	0.111
C19:1n-12	1.57 (± 0.11)	1.49 (± 0.11)	0.63 (± 0.12)	0.78 (± 0.12)	< 0.001	0.586
C20:1n-15	0.30 (± 0.05)	0.37 (± 0.04)	0.44 (± 0.05)	0.38 (± 0.05)	0.116	0.096
C20:1n-12	0.37 (± 0.02)	0.37 (± 0.02)	0.44 (± 0.02)	0.42 (± 0.02)	0.012	0.025
C20:1n-9	0.90 (± 0.05)	0.83 (± 0.05)	1.01 (± 0.06)	1.05 (± 0.05)	0.003	0.649
C22:1n-9	0.80 (± 0.07)	0.77 (± 0.07)	0.69 (± 0.07)	0.64 (± 0.06)	0.088	0.998

	High Omega-3 (Silage)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception	Pre- and Post-Conception	Diet	Diet x Time
C24:1n-9	4.92 (± 0.41)	4.32 (± 0.4)	4.89 (± 0.54)	4.75 (± 0.38)	0.654	0.055
Total MUFA	252.3 (± 8.65)	250.8 (± 8.3)	285.9 (± 8.89)	264 (± 8.6)	0.011	0.666
n-3 PUFA						
C18:3n-3	61.88 (± 1.76)	55.07 (± 1.73)	25.27 (± 1.85)	25.41 (± 1.79)	< 0.001	0.146
C18:4n-3	2.33 (± 0.13)	2.56 (± 0.13)	1.64 (± 0.14)	1.40 (± 0.14)	< 0.001	0.340
C20:3n-3	0.40 (± 0.06)	0.47 (± 0.05)	0.25 (± 0.07)	0.30 (± 0.05)	0.012	0.400
C20:5n-3	24.05 (± 1.08)	25.54 (± 1.04)	12.03 (± 1.09)	13.04 (± 1.08)	< 0.001	0.241
C22:5n-3	23.55 (± 0.66)	21.04 (± 0.64)	15.79 (± 0.67)	16.59 (± 0.66)	< 0.001	0.451
C22:6n-3	25.29 (± 1.07)	23.79 (± 1.02)	16.90 (± 1.06)	17.34 (± 1.07)	< 0.001	0.339
Total n-3	137.63 (± 3.81)	128.45 (± 3.70)	71.90 (± 3.89)	74.07 (± 3.86)	< 0.001	0.227
n-6 PUFA						
C18:2n-6t	0.85 (± 0.07)	0.87 (± 0.06)	0.82 (± 0.07)	0.61 (± 0.06)	0.029	0.485
C18:2n-6	134.0 (± 9.18)	123.6 (± 8.73)	206.2 (± 8.90)	207.1 (± 9.23)	< 0.001	0.513
C18:3n-6	3.91 (± 0.48)	4.08 (± 0.46)	7.58 (± 0.47)	5.77 (± 0.48)	< 0.001	0.006
C20:2n-6	0.54 (± 0.03)	0.50 (± 0.03)	0.70 (± 0.03)	0.66 (± 0.03)	< 0.001	0.495
C20:3n-6	2.44 (± 0.23)	2.43 (± 0.22)	3.49 (± 0.22)	3.46 (± 0.23)	< 0.001	0.188
C20:4n-6	37.01 (± 2.03)	31.04 (± 1.93)	36.22 (± 2.00)	36.67 (± 2.03)	0.235	0.298
C22:2n-6	-	-	-	-	-	-
C22:4n-6	1.55 (± 0.14)	1.20 (± 0.14)	2.28 (± 0.14)	1.94 (± 0.15)	< 0.001	0.457
C22:5n-6	0.86 (± 0.11)	0.78 (± 0.10)	1.50 (± 0.11)	1.16 (± 0.11)	< 0.001	0.020
Total n-6	166.9 (± 8.93)	166.4 (± 8.63)	266.5 (± 9.04)	255.3 (± 9.22)	< 0.001	< 0.001
Total ID	1050.9 (± 31.54)	1008.5 (± 30.45)	1111.4 (± 31.73)	1069.9 (± 31.98)	0.062	0.434
n-6:n-3	1.42 (± 0.18)	1.46 (± 0.18)	4.19 (± 0.19)	3.72 (± 0.19)	< 0.001	0.099
DHADI	0.61 (± 0.04)	0.82 (± 0.04)	0.69 (± 0.05)	0.62 (± 0.04)	0.139	0.001
DHASI	33.47 (± 3.61)	37.63 (± 3.46)	16.97 (± 3.73)	19.80 (± 3.54)	< 0.001	0.183
EFI	1.41 (± 0.05)	1.33 (± 0.05)	1.30 (± 0.05)	1.37 (± 0.05)	0.485	0.217
P:S	0.67 (± 0.01)	0.63 (± 0.01)	0.68 (± 0.01)	0.69 (± 0.01)	0.018	0.157

[1]FAME = fatty acid methyl ester, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, Total ID = total concentration of identified fatty acids, n-6:n-3 = ratio of n-6 PUFA : n-3 PUFA, DHADI = DHA Deficiency Index (Ref), DHASI = DHA Sufficiency Index (Ref), EFI = Essential Fatty Acid Status Index, ratio of (n-3 PUFA + n-6 PUFA) : (n-7 MUFA + n-9 MUFA), P:S = ratio of (n-3 PUFA + n-6 PUFA) : SFA.

[2]Pre-conception = ewes fed experimental rations for 42 days prior to mating only, Pre- and post-conception = ewes fed experimental rations for 42 days prior to mating and 17 days post-mating.

[3]The *p*-value for Diet x Time represents the interaction between dietary treatment group and time of feeding either pre-conception or both pre- and post-conception.

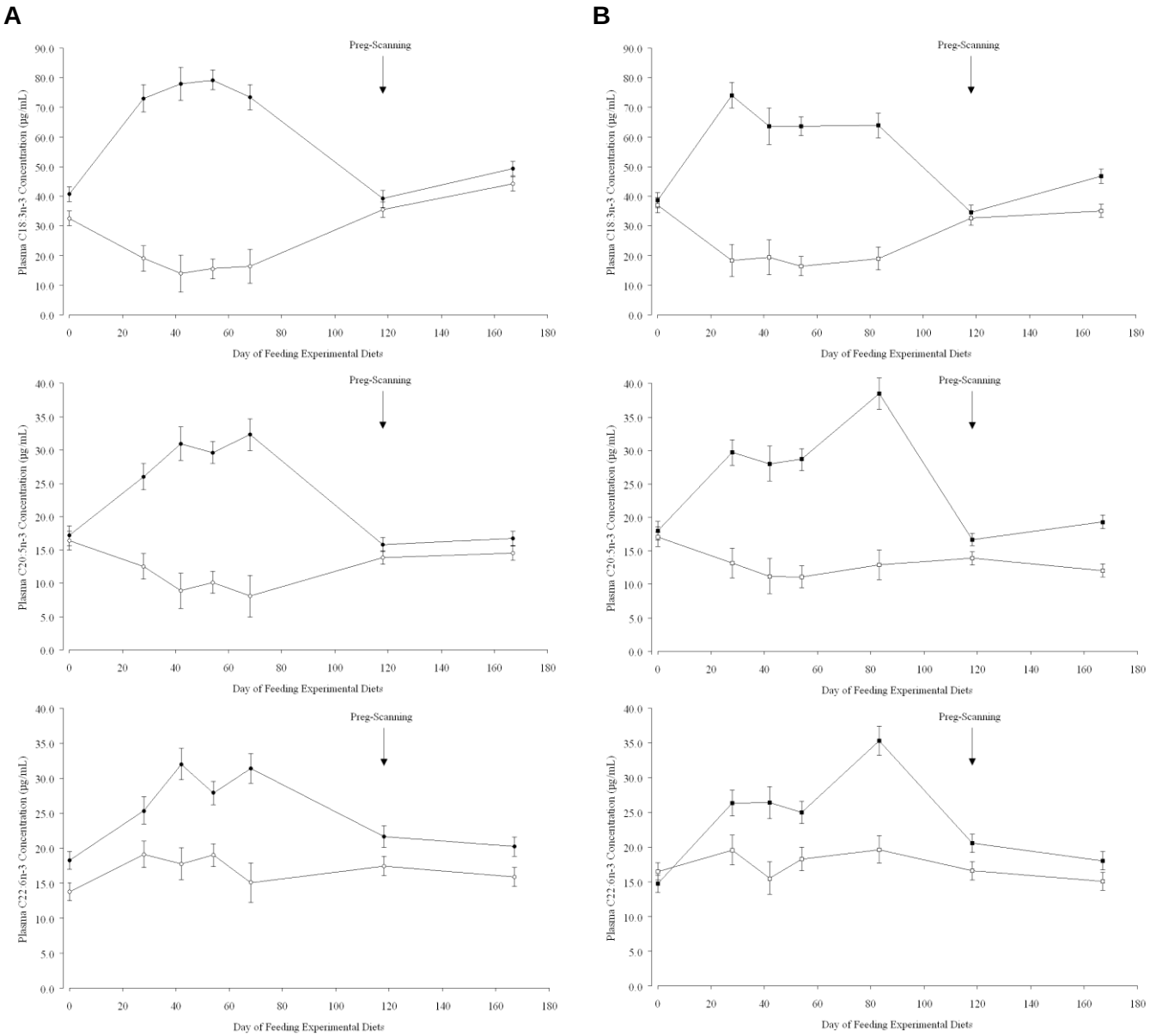


Figure 5.3 Mean concentration of α-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in the plasma of Merino ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for either (A) 42 days prior to mating (pre-conception, ● omega-3, ○ omega-6) or (B) 42 days prior to and 17 days following mating (pre and post-conception, ■ omega-3, □ omega-6) in Study 3 (2011).

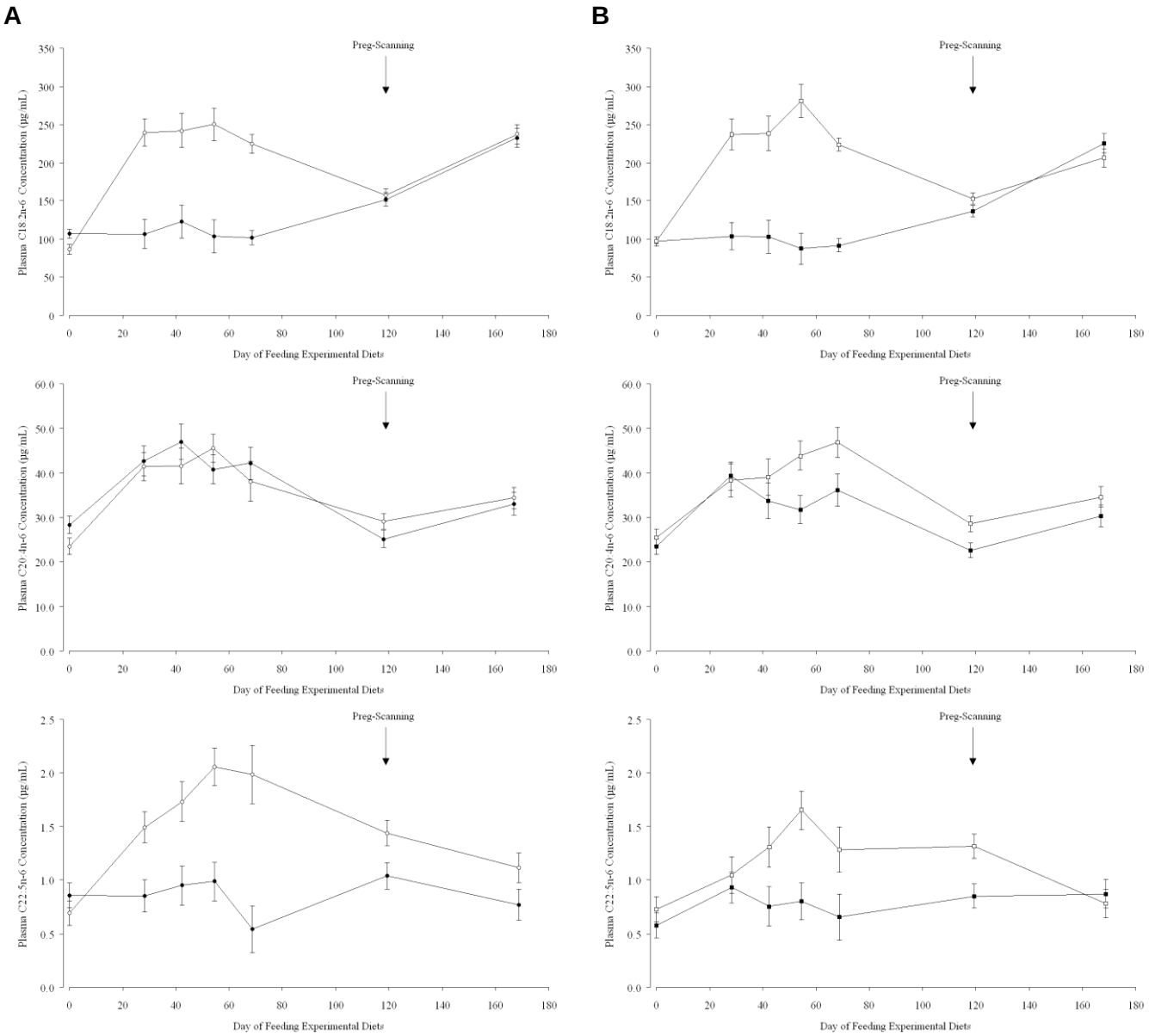


Figure 5.4 Mean concentration of linoleic acid (LA, C18:2n-6), arachidonic acid (ARA, C20:4n-3) and docosapentaenoic acid n-6 (DPA n-6, C22:5n-6) in the plasma of Merino ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for either (A) 42 days prior to mating (pre-conception, ● omega-3, ○ omega-6) or (B) 42 days prior to and 17 days following mating (pre and post-conception, ■ omega-3, □ omega-6) in Study 3 (2011).

Study 4 (2012)

The concentration of ALA and EPA in plasma was significantly lower when ewes were fed the high omega-6 diet either pre-conception only or both pre- and post-conception compared with the high omega-3 diet based on oaten silage (Figures 5.5A and B). The concentration of ALA and EPA remained lower while ewes were fed the high omega-6 diet, but were not significantly different at preg-scanning or pre-lambing when ewes were fed the high omega-3 or high omega-6 diets either pre-conception or pre- and post-conception. In contrast to Study 3, the concentration of DHA in plasma was not significantly different during pen feeding when ewes were fed the experimental rations either pre-conception or pre- and post-conception (Figures 5.5A and B).

The concentration of all major omega-6 fatty acids, including C18:2n-6, C20:4n-6 and C22:5n-6, was significantly ($p < 0.05$) higher when ewes were fed a diet high in omega-6 fatty acids compared with omega-3 fatty acids either pre-conception only or pre- and post-conception (Table 5.9). The concentration of omega-6 fatty acids increased significantly ($p < 0.001$) following the introduction of the high omega-6 diet (Figures 5.6A and B) and remained higher while ewes were fed the experimental rations. The concentration of EPA was significantly lower at preg-scanning ($p < 0.001$) and pre-lambing ($p = 0.002$) when ewes were fed the high omega-6 diet both pre- and post-conception (Figure 5.3B), but not when ewe were fed pre-conception only (preg-scanning, $p = 0.108$; pre-lambing, $p = 0.110$, Figure 5.3A).

Table 5.9 Mean concentration (μg/mL) of fatty acid methyl esters (FAME) in the plasma of Merino ewes following the consumption of a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating in Study 4 (2012).

	High Omega-3 (Silage)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception	Pre- and Post-Conception	Diet	Diet x Time
SFA						
C8:0	10.23 (± 0.44)	9.23 (± 0.47)	8.78 (± 0.53)	10.05 (± 0.43)	0.511	0.025
C9:0	0.27 (± 0.04)	0.19 (± 0.05)	0.27 (± 0.05)	0.25 (± 0.04)	0.427	0.820
C10:0	1.97 (± 0.07)	1.85 (± 0.08)	1.83 (± 0.09)	2.02 (± 0.07)	0.867	0.040
C11:0	1.51 (± 0.06)	1.39 (± 0.06)	1.34 (± 0.06)	1.41 (± 0.06)	0.207	0.001
C12:0	1.80 (± 0.09)	1.63 (± 0.10)	1.62 (± 0.11)	1.63 (± 0.09)	0.382	0.261
C14:0	8.94 (± 0.37)	8.39 (± 0.39)	6.71 (± 0.38)	5.63 (± 0.38)	< 0.001	< 0.001
iC15:0	2.70 (± 0.14)	2.34 (± 0.14)	2.43 (± 0.14)	2.08 (± 0.14)	0.068	0.071
aiC15:0	3.76 (± 0.19)	3.08 (± 0.21)	3.77 (± 0.20)	3.45 (± 0.20)	0.348	0.657
C15:0	7.53 (± 0.26)	6.45 (± 0.27)	6.84 (± 0.28)	5.81 (± 0.26)	0.019	0.024
C16:0	150.8 (± 5.12)	138.0 (± 5.38)	164 (± 5.14)	156.6 (± 5.11)	0.005	0.343
iC17:0	7.96 (± 0.36)	7.19 (± 0.38)	8.20 (± 0.37)	7.90 (± 0.36)	0.205	0.127
aiC17:0	14.28 (± 0.87)	11.73 (± 0.93)	6.24 (± 0.85)	4.91 (± 0.87)	< 0.001	< 0.001
C17:0	10.68 (± 0.32)	9.57 (± 0.34)	9.88 (± 0.33)	8.34 (± 0.32)	0.004	0.297
C18:0	162.1 (± 7.50)	147.6 (± 7.98)	212.9 (± 7.51)	209.8 (± 7.54)	< 0.001	0.480
C20:0	1.57 (± 0.1)	1.36 (± 0.10)	2.02 (± 0.09)	1.84 (± 0.09)	< 0.001	0.423
C21:0	1.65 (± 0.06)	1.53 (± 0.06)	1.59 (± 0.07)	1.50 (± 0.06)	0.458	0.782
C22:0	2.61 (± 0.13)	2.31 (± 0.14)	3.28 (± 0.13)	2.97 (± 0.13)	< 0.001	0.632
C23:0	4.95 (± 0.2)	4.47 (± 0.21)	5.64 (± 0.20)	4.74 (± 0.20)	0.024	0.694
C24:0	3.98 (± 0.16)	3.62 (± 0.16)	4.69 (± 0.16)	4.16 (± 0.16)	< 0.001	0.256
Total SFA	393.6 (± 14.28)	356.8 (± 15.10)	445.5 (± 14.24)	430.1 (± 14.28)	< 0.001	0.664
MUFA						
C11:1n-1	0.81 (± 0.06)	0.79 (± 0.06)	0.80 (± 0.06)	0.87 (± 0.06)	0.555	0.040
C12:1n-7	1.27 (± 0.05)	0.98 (± 0.05)	0.92 (± 0.06)	0.87 (± 0.05)	< 0.001	0.008
C13:1n-1	0.77 (± 0.03)	0.63 (± 0.03)	0.64 (± 0.03)	0.58 (± 0.03)	0.005	< 0.001
C14:1n-5	0.12 (± 0.01)	0.13 (± 0.01)	0.11 (± 0.01)	0.10 (± 0.01)	0.056	0.567
C15:1n-5	0.30 (± 0.02)	0.25 (± 0.02)	0.36 (± 0.02)	0.32 (± 0.02)	0.003	0.668
C16:1n-7t	2.73 (± 0.17)	2.35 (± 0.18)	2.09 (± 0.17)	1.68 (± 0.17)	0.001	0.098
C16:1n-7	16.03 (± 0.96)	14.52 (± 0.99)	12.28 (± 0.94)	12.15 (± 0.96)	0.003	< 0.001
C17:1n-7	1.47 (± 0.08)	1.42 (± 0.09)	1.53 (± 0.08)	1.51 (± 0.08)	0.344	0.559
C18:1n9t	3.78 (± 0.24)	3.28 (± 0.25)	4.16 (± 0.26)	3.93 (± 0.25)	0.046	0.188
C18:1n7t	13.38 (± 0.83)	10.90 (± 0.89)	10.81 (± 0.86)	10.79 (± 0.86)	0.130	0.285
C18:1n-12	4.56 (± 1.44)	1.84 (± 1.44)	2.26 (± 1.54)	1.88 (± 1.54)	0.453	0.364
C18:1n-9	197.6 (± 9.00)	194.0 (± 9.48)	185.4 (± 8.85)	186.8 (± 8.97)	0.295	0.877
C18:1n-7	11.60 (± 1.18)	9.74 (± 1.26)	9.18 (± 1.24)	9.91 (± 1.21)	0.365	0.275
C19:1n-12	0.36 (± 0.06)	0.25 (± 0.06)	0.33 (± 0.06)	0.27 (± 0.06)	0.994	0.696
C20:1n-15	0.46 (± 0.05)	0.38 (± 0.05)	0.45 (± 0.04)	0.40 (± 0.04)	0.958	0.007
C20:1n-12	0.39 (± 0.03)	0.35 (± 0.03)	0.31 (± 0.03)	0.33 (± 0.03)	0.106	0.001
C20:1n-9	0.94 (± 0.04)	0.87 (± 0.05)	1.03 (± 0.05)	1.09 (± 0.04)	0.002	0.785

	High Omega-3 (Silage)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception	Pre- and Post-Conception	Diet	Diet x Time
C22:1n-9	0.55 (± 0.02)	0.47 (± 0.03)	0.61 (± 0.03)	0.51 (± 0.02)	0.040	0.510
C24:1n-9	4.28 (± 0.18)	4.37 (± 0.20)	4.50 (± 0.18)	4.10 (± 0.18)	0.897	0.669
Total MUFA	260 (± 9.95)	246.9 (± 10.54)	236.7 (± 9.89)	236.6 (± 9.91)	0.106	0.853
n-3 PUFA						
C18:3n-3	33.20 (± 1.18)	30.05 (± 1.23)	26.83 (± 1.16)	21.91 (± 1.14)	< 0.001	< 0.001
C18:4n-3	2.26 (± 0.12)	1.87 (± 0.13)	1.86 (± 0.12)	1.71 (± 0.12)	0.029	0.053
C20:3n-3	0.40 (± 0.02)	0.36 (± 0.02)	0.41 (± 0.02)	0.37 (± 0.02)	0.816	0.246
C20:5n-3	15.71 (± 0.76)	15.26 (± 0.80)	14.06 (± 0.74)	12.50 (± 0.75)	0.007	0.256
C22:5n-3	16.24 (± 0.55)	15.84 (± 0.58)	15.57 (± 0.55)	13.20 (± 0.54)	0.006	0.147
C22:6n-3	18.11 (± 0.88)	16.32 (± 0.93)	19.63 (± 0.87)	18.74 (± 0.86)	0.033	0.982
Total n-3	86.42 (± 2.81)	80.30 (± 2.99)	78.73 (± 2.79)	68.55 (± 2.77)	0.002	0.129
n-6 PUFA						
C18:2n-6t	0.80 (± 0.05)	0.66 (± 0.05)	0.68 (± 0.05)	0.61 (± 0.05)	0.083	0.088
C18:2n-6	115.9 (± 4.32)	98.30 (± 4.55)	205.1 (± 4.21)	222.2 (± 4.13)	< 0.001	< 0.001
C18:3n-6	4.43 (± 0.39)	3.79 (± 0.40)	5.58 (± 0.38)	6.40 (± 0.39)	< 0.001	0.004
C20:2n-6	0.61 (± 0.04)	0.54 (± 0.05)	0.74 (± 0.05)	0.78 (± 0.04)	< 0.001	0.824
C20:3n-6	2.66 (± 0.14)	2.56 (± 0.15)	3.19 (± 0.14)	3.32 (± 0.14)	< 0.001	0.538
C20:4n-6	32.53 (± 1.72)	30.78 (± 1.80)	38.18 (± 1.67)	39.69 (± 1.67)	< 0.001	0.352
C22:2n-6	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)	< 0.001	< 0.001
C22:4n-6	1.20 (± 0.08)	1.04 (± 0.09)	1.17 (± 0.08)	1.15 (± 0.08)	0.622	0.269
C22:5n-6	0.95 (± 0.06)	0.66 (± 0.07)	0.90 (± 0.06)	1.01 (± 0.06)	0.033	0.289
Total n-6	163.1 (± 6.09)	143.3 (± 6.46)	256.4 (± 6.24)	271.9 (± 6.04)	< 0.001	< 0.001
Total ID	903.1 (± 29.73)	827.3 (± 31.47)	1018.3 (± 29.64)	1007.5 (± 29.59)	< 0.001	0.341
n-6:n-3	1.92 (± 0.11)	1.82 (± 0.12)	3.39 (± 0.11)	4.34 (± 0.11)	< 0.001	< 0.001
DHADI	0.84 (± 0.06)	0.67 (± 0.06)	0.81 (± 0.06)	0.91 (± 0.06)	0.067	0.358
DHASI	19.73 (± 2.03)	25.24 (± 2.06)	24.02 (± 2.01)	20.48 (± 2.01)	0.911	0.057
EFI	1.34 (± 0.11)	1.12 (± 0.11)	1.62 (± 0.11)	1.60 (± 0.11)	< 0.001	0.088
P:S	0.64 (± 0.02)	0.64 (± 0.02)	0.76 (± 0.02)	0.79 (± 0.02)	< 0.001	0.007

[1]FAME = fatty acid methyl ester, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, Total ID = total concentration of identified fatty acids, n-6:n-3 = ratio of n-6 PUFA : n-3 PUFA, DHADI = DHA Deficiency Index (Ref), DHASI = DHA Sufficiency Index (Ref), EFI = Essential Fatty Acid Status Index, ratio of (n-3 PUFA + n-6 PUFA) : (n-7 MUFA + n-9 MUFA), P:S = ratio of (n-3 PUFA + n-6 PUFA) : SFA.

[2]Pre-conception = ewes fed experimental rations for 42 days prior to mating only, Pre- and post-conception = ewes fed experimental rations for 42 days prior to mating and 17 days post-mating.

[3]The *p*-value for Diet x Time represents the interaction between dietary treatment group and time of feeding either pre-conception or both pre- and post-conception.

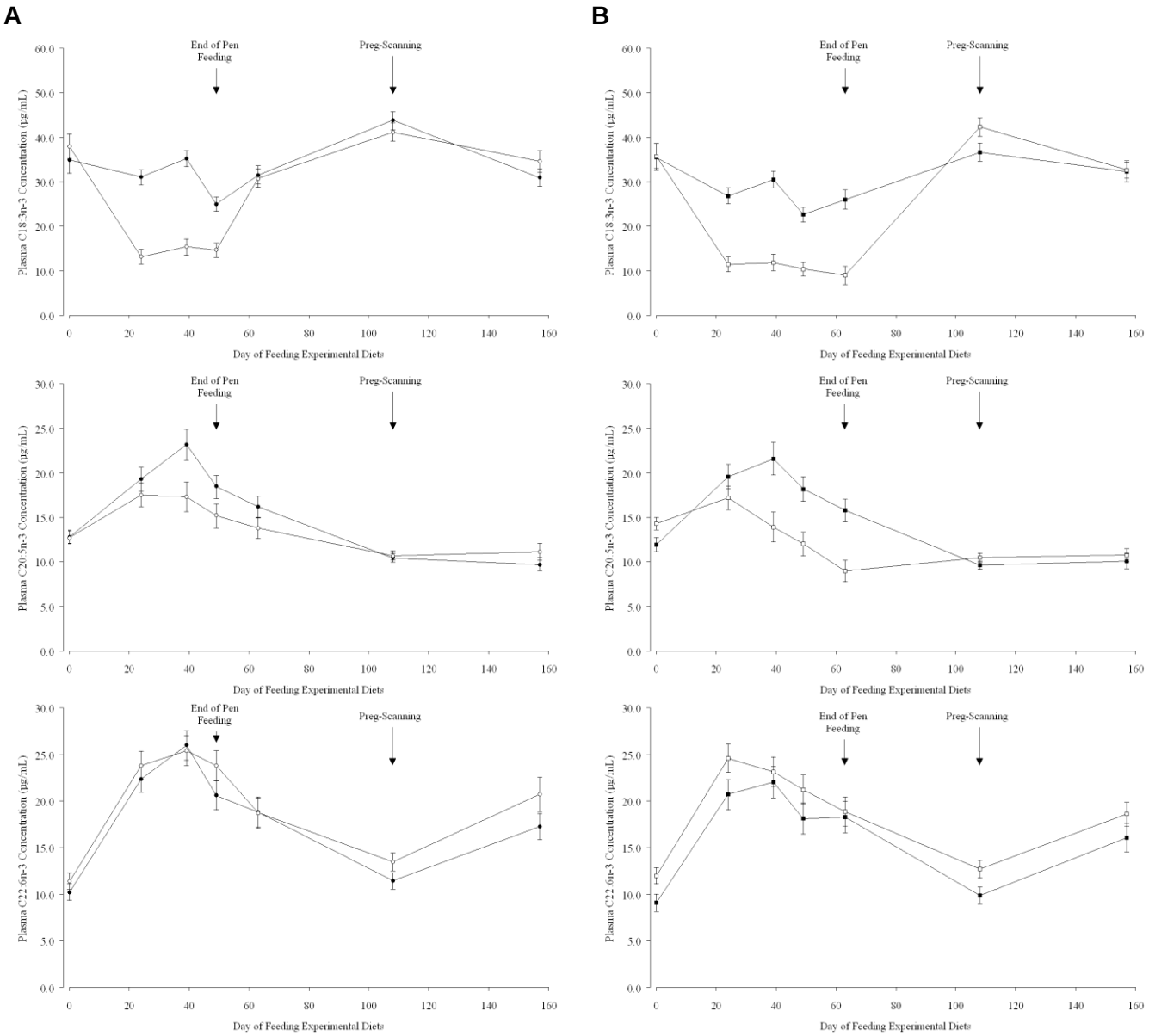


Figure 5.5 Mean concentration of α-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in the plasma of Merino ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for either (A) 42 days prior to mating (pre-conception, ● omega-3, ○ omega-6) or (B) 42 days prior to and 17 days following mating (pre and post-conception, ■ omega-3, □ omega-6) in Study 4 (2012).

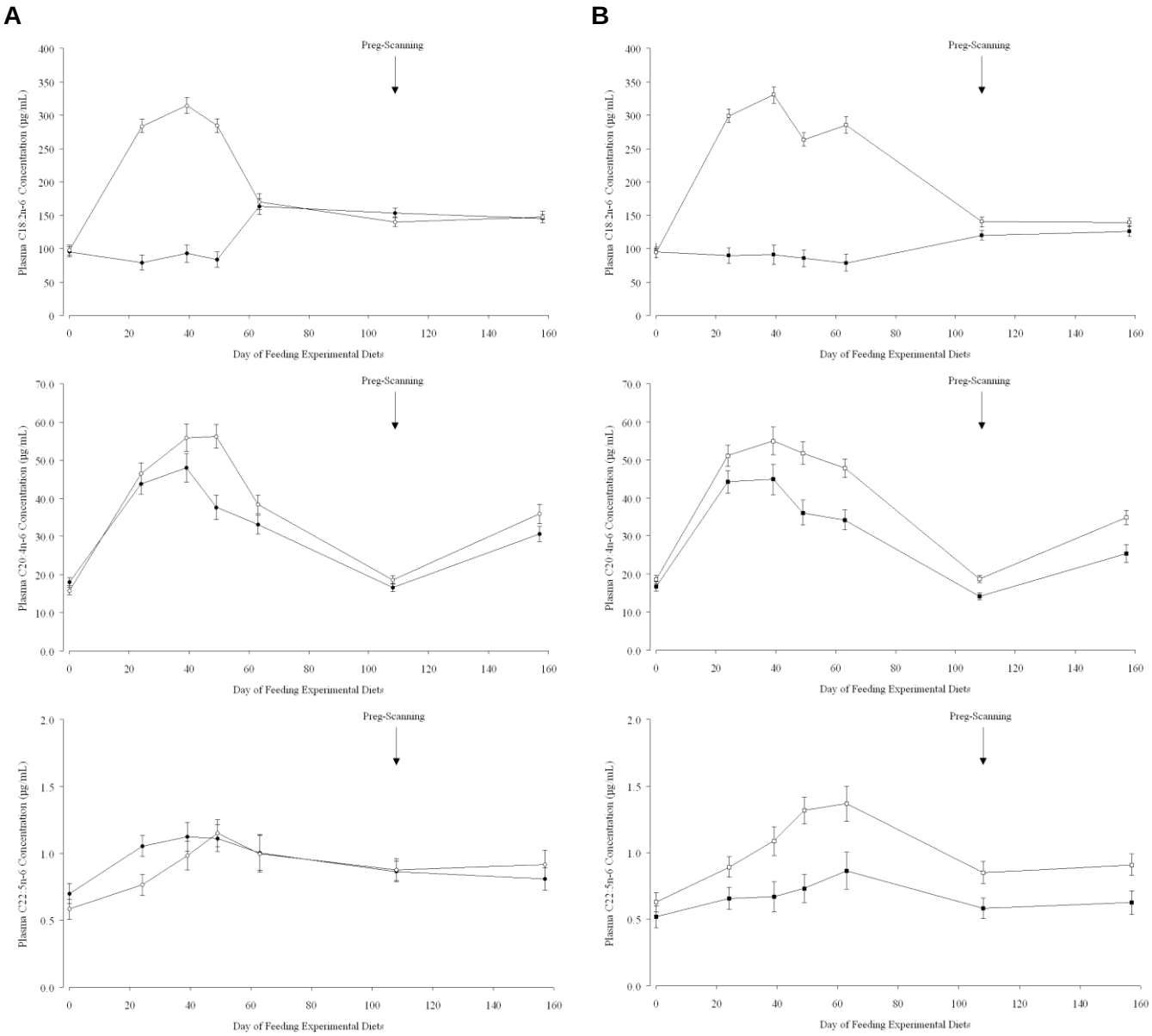


Figure 5.6 Mean concentration of linoleic acid (LA, C18:2n-6), arachidonic acid (ARA, C20:4n-3) and docosapentaenoic acid n-6 (DPA n-6, C22:5n-6) in the plasma of Merino ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for either (A) 42 days prior to mating (pre-conception, ● omega-3, ○ omega-6) or (B) 42 days prior to and 17 days following mating (pre and post-conception, ■ omega-3, □ omega-6) in Study 4 (2012).

5.3.3 Plasma hormone concentrations

Progesterone (P_4)

The mean concentration of plasma progesterone was significantly (p = 0.006) higher when ewes were fed the High omega-6 diet (2.16 ± 0.15 ng/mL) compared with the High omega-3 diet (1.56 ± 0.14 ng/mL) for 42 days prior to mating (Figure 5.7A). The percentage change in progesterone concentration from baseline was also significantly (p < 0.001) higher when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids prior to mating (Figure 5.7B).

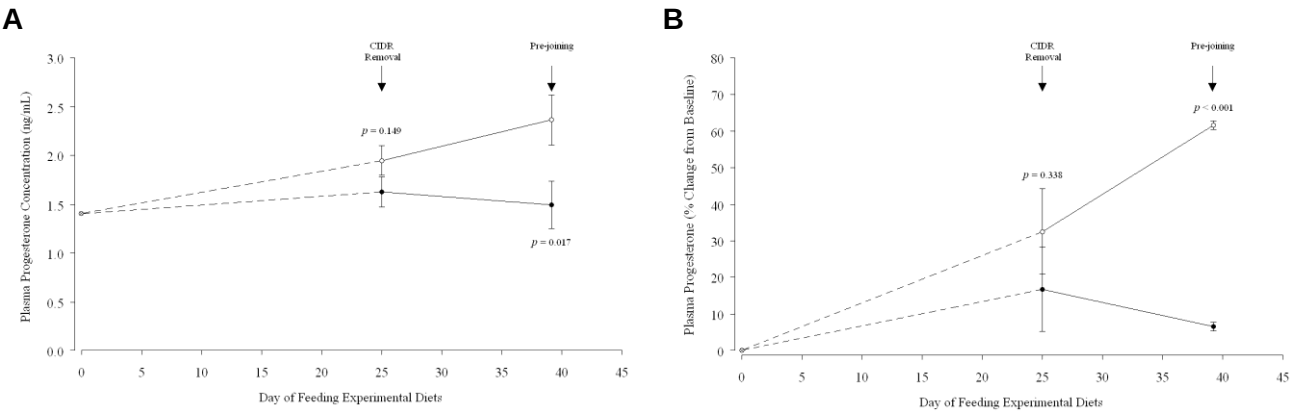


Figure 5.7 Mean (A) concentration of plasma progesterone (ng/mL) and (B) the percentage change in progesterone from baseline for Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating. Baseline progesterone concentrations for the High omega-3 and High omega-6 diets were 1.34 ± 0.28 and 1.44 ± 0.28 ng/mL respectively and were included in the statistical analysis as a co-variate. Significant difference between treatment diets (A) p = 0.006, (B) p < 0.001.

Oestradiol (E_2)

The mean concentration of plasma oestradiol was not significantly (p = 0.318) higher when ewes were fed the high omega-6 diet (1.28 ± 0.20 pg/mL) compared with the Hgh omega-3 diet (0.98 ± 0.20 pg/mL) for 42 days prior to mating (Figure 5.8A). The percentage change in oestradiol concentration from baseline was also not significantly (p = 0.252) different when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids prior to mating (Figure 5.8B).

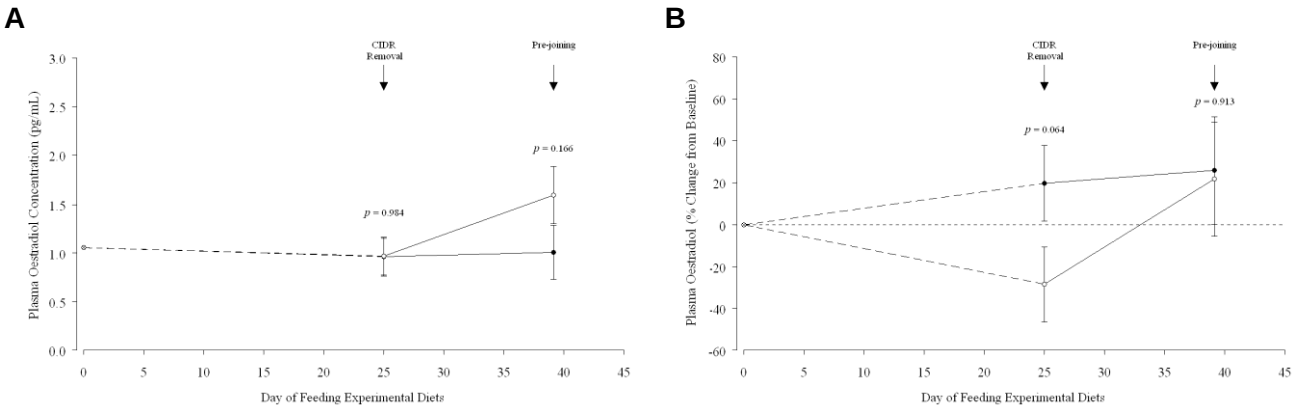


Figure 5.8 Mean (A) concentration of plasma oestradiol (pg/mL) and (B) the percentage change in oestradiol from baseline for BL x Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating. Baseline progesterone concentrations for the High omega-3 and High omega-6 diets were 2.45 ± 0.65 and 3.77 ± 0.62 pg/mL respectively and were included in the statistical analysis as a co-variate. Significant difference between treatment diets (A) p = 0.058, (B) p = 0.967.

5.3.4 Time of oestrus and parturition

Details of the results for oestrus and parturition will be presented in the following section. Data for the time to oestrus will be presented for each study as well as the combined results for the overall effect of diet. Results for parturition will be presented with the main effects of diet and time of feeding either pre-conception only or both pre- and post-conception.

Oestrus

The proportion of ewes showing oestrus was not significantly different when ewes were fed the high omega-6 diet compared with the high omega-3 diet prior to mating (Table 5.10). The cumulative proportion of ewes showing oestrus over time was also not significantly different (Relative Risk = 1.15, p = 0.167, Figure 5.9) when ewes were fed the high omega-6 diet compared with the High omega-3 diet.

The time from CIDR removal to the first day any ewe showed oestrus was 15 days in Study 3 (2011) and 16 days in Study 4 (2012). The mean time to oestrus from the first day any ewe showed oestrus was significantly (p = 0.001) shorter when ewes were fed the High omega-6 diet compared with the High omega-3 diet (Table 5.10). The time to oestrus was shorter when ewes were fed the diet high in omega-6 in both Study 3 and 4 (Figure 5.10).

Table 5.10 Proportion of Merino ewes showing oestrus or lambed and the time to oestrus or parturition following the consumption of a diet based on either silage (High Omega-3) or oats and cottonseed meal (High Omega-6) for 42 days prior to and, 17 days following, mating.

Outcome[1]	Treatment		*p*-values
	High Omega-3 (Silage)	High Omega-6 (Oats/CSM[2])	
Oestrus			
Proportion of ewes showing oestrus (%)	84.1%	85.9%	0.545
Relative Risk of Oestrus (Cox's PHR[3])	1.00	1.15	0.167
Mean time to behavioural oestrus (Days)	5.12 (± 0.10)	4.63 (± 0.10)	0.001
Parturition			
Proportion of ewes lambed[4] (%)	66.1%	67.5%	0.701
Relative risk of lambing (Cox's PHR)	1.00	1.13	0.217
Mean time to parturition (Days)	6.85 (± 0.22)	6.00 (± 0.22)	0.006

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means) including all ewes that could be identified with their lamb in each treatment group.
[2]CSM = cottonseed meal.
[3]Cox's PHR = Cox's Proportional Hazards Regression Analysis (Cox, 1972).
[4]Including only those ewes that could be positively identified with their lamb.

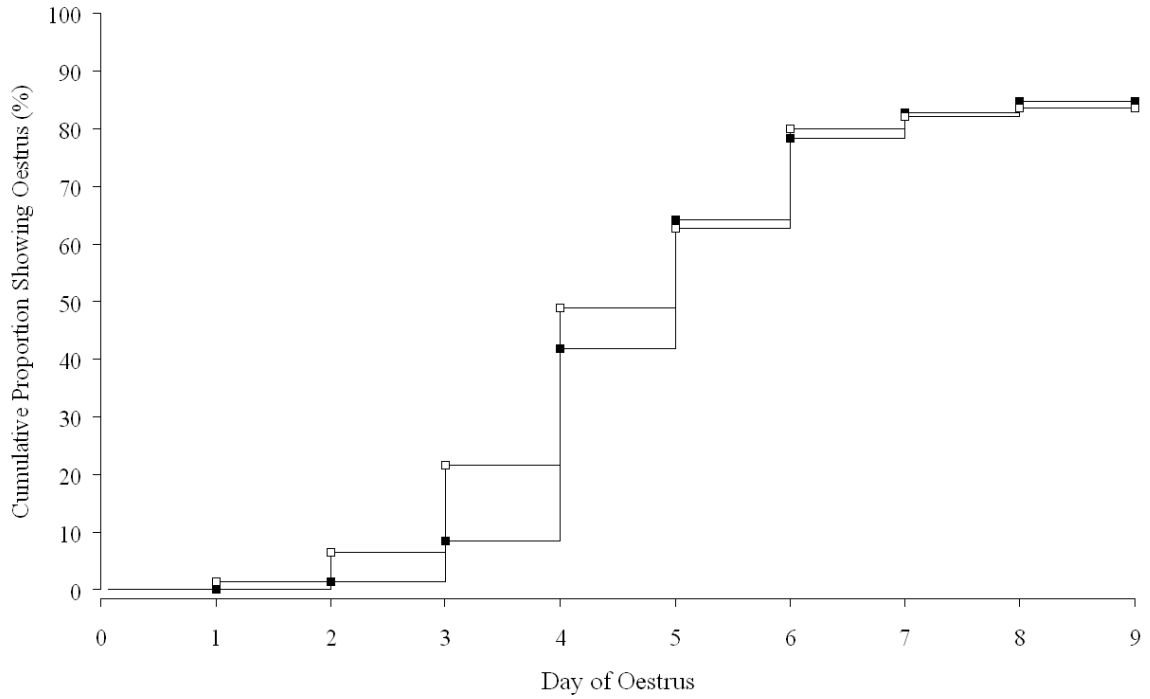


Figure 5.9 Cumulative proportion of of Merino ewes showing behavioural oestrus (from the first day any ewe showed oestrus) following the consumption of a diet high in omega-3 (■) or omega-6 (□) fatty acids for 42 days prior to mating. The time from CIDR removal to the first day any ewe showed oestrus was 14.5 days. Relative Risk (Cox, 1972) of ewes fed the omega-6 diet showing oestrus = 1.37 ($p < 0.001$).

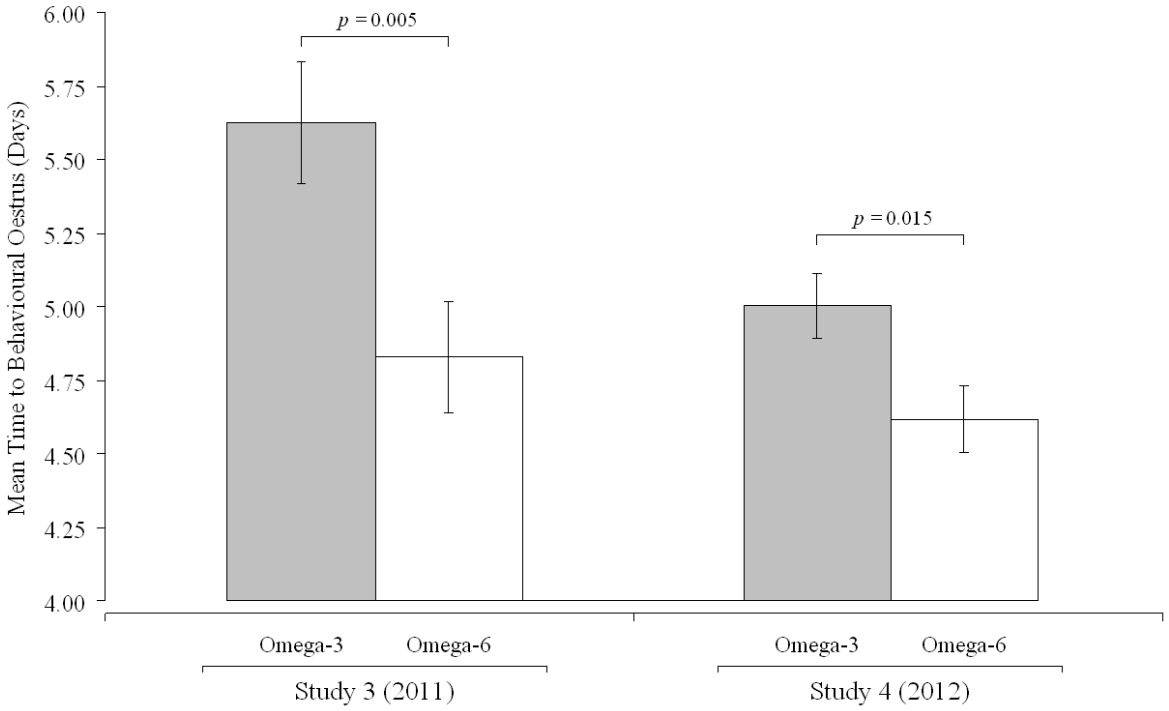


Figure 5.10 Mean time to showing behavioural oestrus (from the first day any ewe showed oestrus) for Merino ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids for 42 days prior to mating. The mean time from CIDR removal to the first day any ewe showed oestrus in Studies 3 and 4 was 14.5 days.

Parturition

The proportion of ewes that lambed was not significantly higher when ewes were fed the high omega-3 diet compared with the high omega-6 diet prior to or following mating (Table 5.10). Further details outlining the effect of feeding experimental diets either pre-conception or pre- and post-conception will be shown in Section 5.3.5. The cumulative proportion of ewes that lambed over time was not significantly higher (Relative Risk = 1.13, p = 0.217, Figure 5.11) when ewes were fed the high omega-6 diet compared with the high omega-3 diet.

The first day any ewe lambed was 162 or 163 days after CIDR removal in Study 3 and 4, respectively and, 147 days after the first ewe showed oestrus in both Study 3 and 4. The mean time to parturition from the first day any ewe lambed was significantly shorter when ewes were fed the high omega-6 diet (6.0 ± 0.22 days) compared with the high omega-3 diet (6.9 ± 0.22 days, Table 5.10). The time to parturition was significantly shorter when ewes were fed the high omega-6 diet compared with the high omega-3 diet pre-conception only (p = 0.010), but not when ewes were fed both pre- and post-conception (p = 0.179, Figure 5.12). The oestrus to parturition interval was not significantly different when ewes were fed the high omega-6 diet compared with the high omega-3 diet either pre-conception (p = 0.987) or pre- and post-conception (p = 0.734, Figure 5.13)

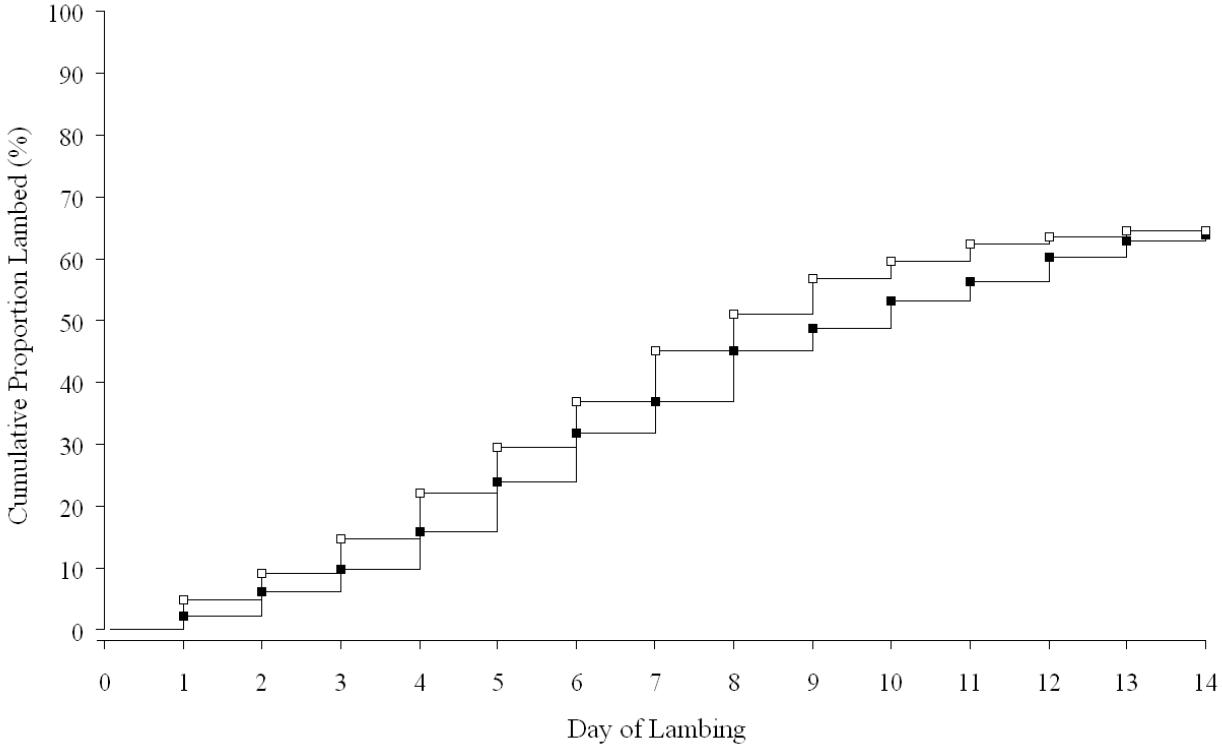


Figure 5.11 Cumulative proportion of Merino ewes that lambed (from the first day any ewe lambed) following the consumption of a diet high in omega-3 (■) or omega-6 (□) fatty acids for 42 days prior to mating. Relative Risk (Cox, 1972) of ewes fed the omega-6 diet lambing = 1.32 (p = 0.002).

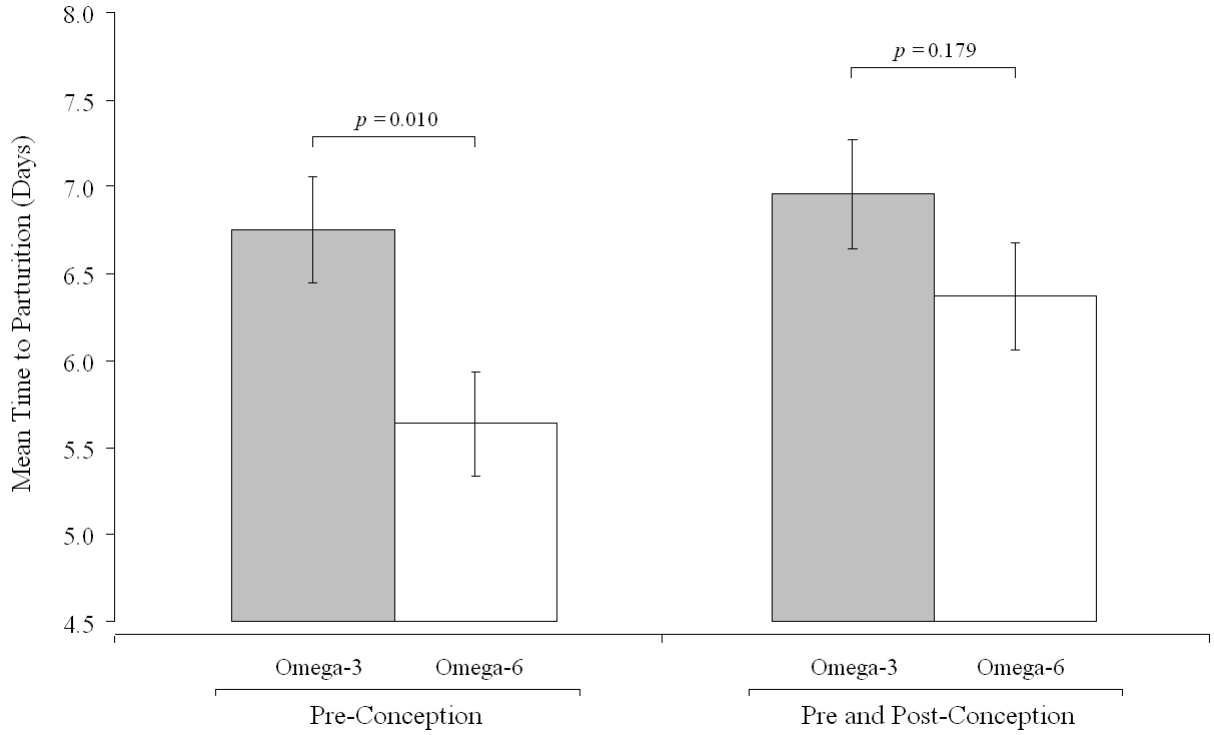


Figure 5.12 Mean time to parturition (from the first day any ewe lambed) for Merino ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids either 42 days prior to mating (Pre-conception), or 42 days prior to and 17 days following mating (Pre and Post-conception).

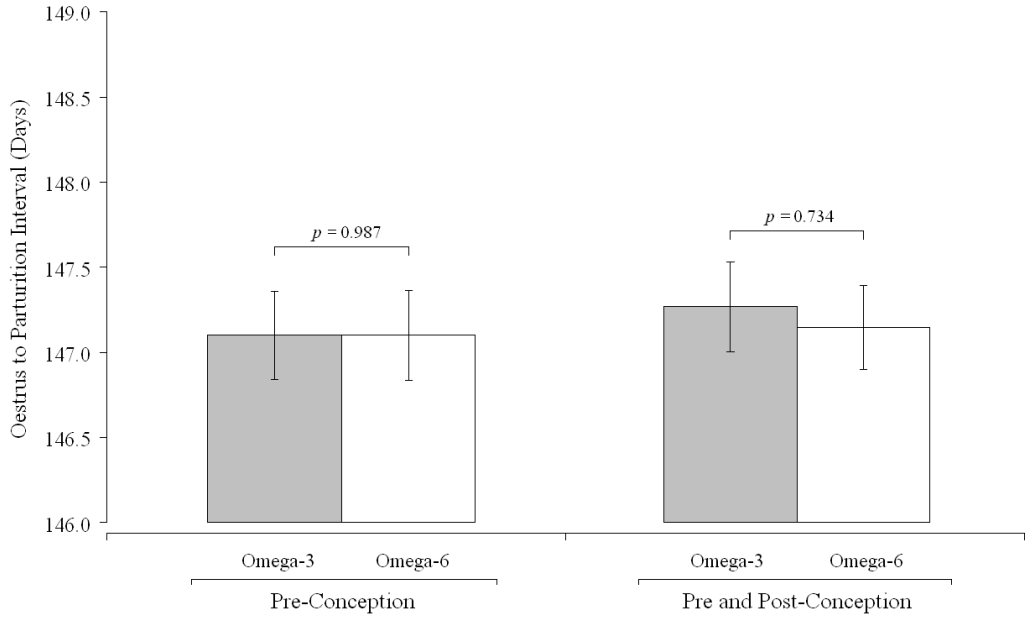


Figure 5.13 Mean oestrus to parturition interval for Merino ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids either 42 days prior to mating (Pre-conception), or 42 days prior to and 17 days following mating (Pre and Post-conception).

5.3.5 Reproduction outcomes

The proportion of ewes pregnant and the proportion of ewes that lambed was not significantly different when ewes were fed the high omega-3 diet compared with the high omega-6 diet prior to or following mating (Table 4.11). Similarly, the mean foetal rate and the mean number of lambs born was not significantly different when ewes were fed the silage diet high in omega-3 compared with the oats/CSM diet high in omega-6 and the interaction between diet and length of time of feeding was also not significant for any reproduction parameters (Table 5.11).

5.3.6 Sex ratio of lambs

A total of 517 lambs were born over the two years. Of those lambs born, a total of 496 lambs (Males = 236, Females = 260) could be identified with their dam and included in the sex ratio analysis. The proportion of female lambs was significantly (p = 0.008) higher when ewes were fed the high omega-6 diet (58.7% female) compared with the high omega-3 diet (46.7% female) either pre-conception or both pre- and post-conception.

The proportion of female lambs was higher when ewes were fed the diet high in omega-6 compared with omega-3 fatty acids both pre- and post-conception (64.2% vs 43.9% respectively) but not when ewes were fed pre-conception only (53.1% vs 49.5% respectively, Figure 5.14). The interaction between diet and time, however, was not significant (p = 0.075). The proportion of female lambs was also significantly (p = 0.012) higher when single-bearing ewes were fed the High omega-6 diet compared with High omega-3 diet both pre- and post-conception (64.0% vs 44.7%), but not pre-conception only (53.7% vs 50.0%, Figure 5.15). The proportion of female lambs was not statistically significantly (p = 0.055) higher when twin-bearing ewes were fed the High omega-6 diet compared with High omega-3 diet both pre- and post-conception (64.0% vs 44.7%, Figure 5.15).

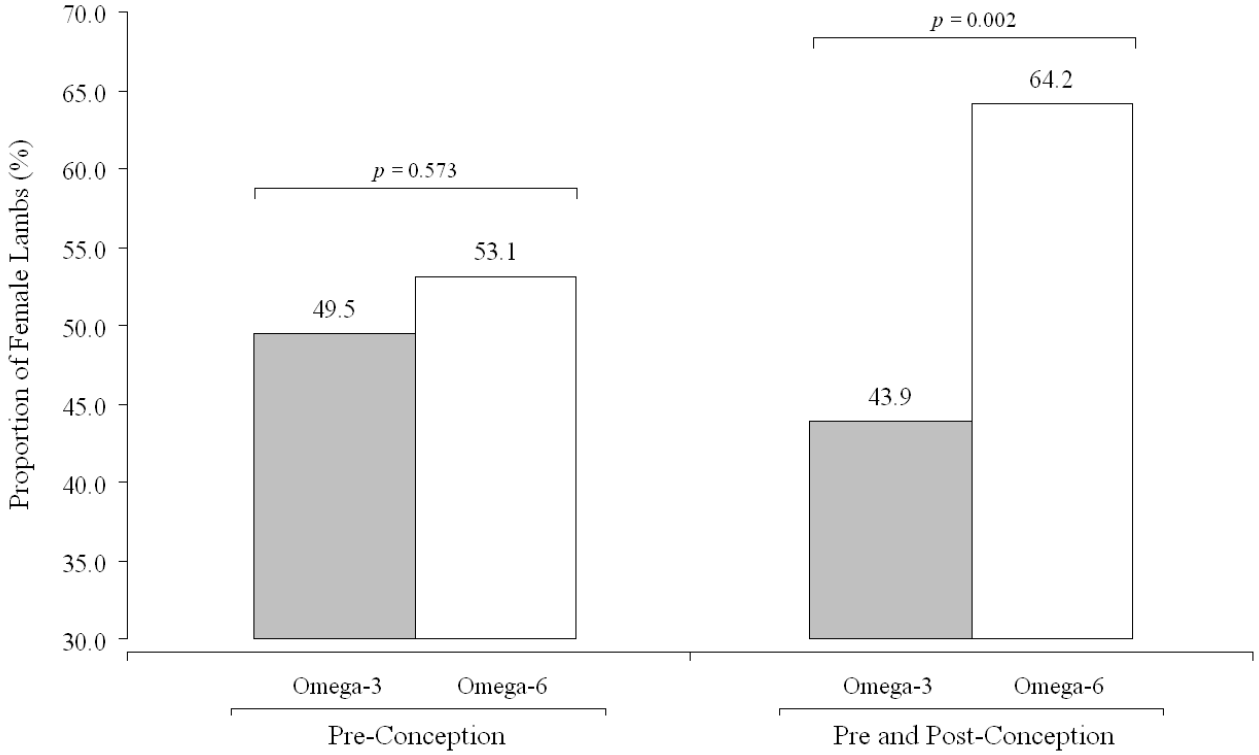


Figure 5.14 Proportion of female lambs when Merino ewes were fed a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids for either 42 days prior to mating (Pre-conception) or 42 days prior to and 17 days following mating (Pre and Post-conception).

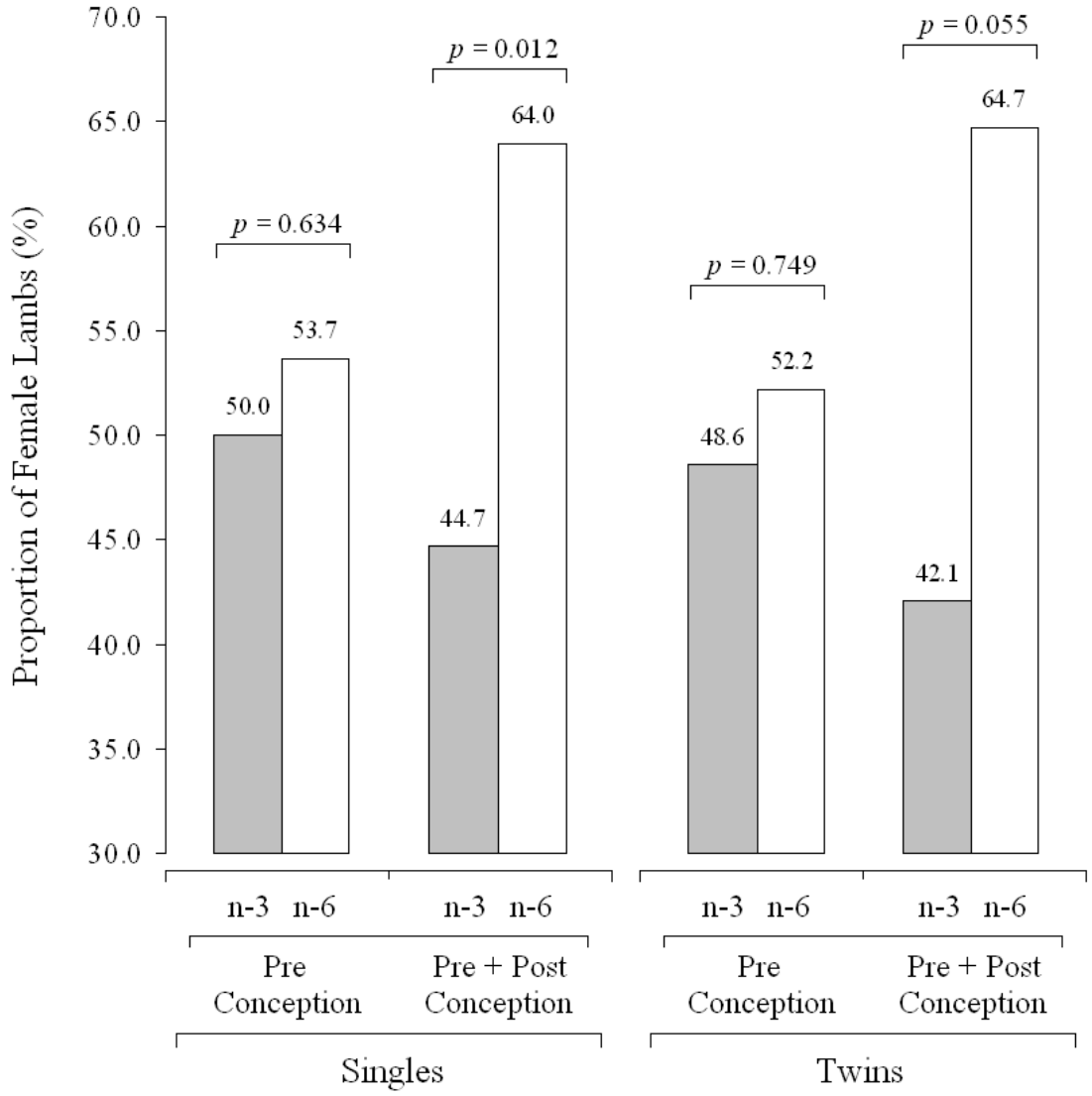


Figure 5.15 Proportion of female lambs when single or twin-bearing Merino ewes were fed a diet high in omega-3 (n-3, shaded bars) or omega-6 (n-6, unshaded bars) fatty acids either 42 days prior to mating (Pre-conception) or 42 days prior to and 17 days following mating (Pre + Post-conception).

5.3.7 Lamb birthweight, vigour and survival

Lamb birthweight was significantly (p = 0.009) higher for male lambs versus female lambs (Table 5.12). Lamb birthweight was not significantly (p = 0.334) higher when ewes were fed the high omega-3 diet compared with the high omega-6 diet at joining. The birthweight of male lambs was not numerically, but not statistically significantly (p = 0.131) higher, when ewes were fed the high omega-3 diet. Lamb head circumference, vigour and survival were not significantly different when ewes were fed the diet high in omega-3 or omega-6 fatty acids at joining (Table 5.12).

Table 5.11 Proportion of Merino ewes pregnant or lambed and mean pregnancy or lambing rates following the consumption of a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating.

Reproduction Measure[1]	High Omega-3 (Silage)		High Omega-6 (Oats/CSM[2])		*p*-values[3]		
	Pre-Conception[2]	Pre- and Post-Conception	Pre-Conception[2]	Pre- and Post-Conception	Diet	Time	Diet x Time
Proportion scanned pregnant (%)	67.68%	77.62%	72.73%	75.68%	0.664	0.071	0.327
Mean foetal rate							
For ewes scanned pregnant	1.21 (± 0.04)	1.16 (± 0.04)	1.22 (± 0.04)	1.18 (± 0.04)	0.750	0.286	0.943
For all ewes joined	0.82 (± 0.05)	0.90 (± 0.05)	0.88 (± 0.05)	0.89 (± 0.05)	0.527	0.404	0.486
Pregnancy loss[4] (%)	5.49%	9.09%	5.46%	6.76%	0.555	0.221	0.566
Proportion lambed							
For ewes scanned pregnant[5] (%)	89.20%	89.39%	87.50%	90.18%	0.855	0.884	0.456
For all ewes joined[5] (%)	62.20%	69.93%	65.45%	69.59%	0.701	0.119	0.636
Mean number of lambs							
For ewes that lambed	1.19 (± 0.04)	1.18 (± 0.04)	1.23 (± 0.04)	1.17 (± 0.04)	0.609	0.420	0.519
For all ewes joined	0.74 (± 0.05)	0.83 (± 0.05)	0.81 (± 0.05)	0.82 (± 0.05)	0.562	0.345	0.469

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means) including all lambs that could be identified with their dam in each treatment group.
[2]CSM = cottonseed meal.
[3]Diet = main effect of dietary treatment (High omega-3 versus High omega-6), Time = main effect of time of feeding (Pre-conception only versus both Pre- and Post-conception).
[4]Proportion of ewes scanned as pregnant that were identified as not having lambed at lamb marking (Dun, 1963).
[5]Including only those lambs that could be positively identified with their dam. Also includes lambs from ewes that could not be identified pregnant at pregnancy scanning that lambed.

Table 5.12 Lamb birthweight, head circumference, vigour and survival and maternal score at parturition when Merino ewes were fed a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating.

Lamb Measure[1]	Male		Female		*p*-values[3]		
	High Omega-3[2]	High Omega-6	High Omega-3[2]	High Omega-6	Diet	Sex	Diet x Sex
Birthweight (kg)	4.82 (± 0.18)	4.57 (± 0.18)	4.38 (± 0.18)	4.41 (± 0.17)	0.334	0.009	0.219
Head Circumference[4] (cm)	27.2 (± 0.26)	27.0 (± 0.27)	26.7 (± 0.27)	26.8 (± 0.26)	0.910	0.051	0.451
Lamb vigour[5]	2.15 (± 0.08)	2.16 (± 0.09)	2.13 (± 0.08)	2.14 (± 0.07)	0.842	0.807	0.996
Lamb Survival							
Birth	96.2%	96.2%	99.1%	99.3%	0.942	0.023	0.947
24 hr	94.7%	95.2%	97.4%	98.6%	0.576	0.064	0.837

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means) including all lambs that could be identified with their dam in each treatment group.
[2]High omega-3 treatment based on silage, High omega-6 treatment based on oats and cottonseed meal.
[3]Diet = main effect of dietary treatment (High omega-3 versus High omega-6), Time = main effect of time of feeding (Pre-conception only versus both Pre- and Post-conception).
[4]Head circumference in the coronal plane (Jamison et al., 1961).
[5]Lamb vigour measured on a scale of 1 to 5 where 1 = more vigorous and 5 = least vigorous.

5.4 Discussion

The proportion of female lambs was 12% higher (58.7 vs 46.7%) when ewes were fed the high omega-6 diet compared with the high omega-3 diet at mating. The difference in the proportion of female lambs between treatment groups was greatest when ewes were fed both pre- and post-conception compared with pre-conception only (14% and 4% more females, respectively when ewes were fed the high omega-6 diet). The most significant difference in the proportion of female lambs occurred when single-bearing ewes were fed theh high omega-6 diet both pre- and post-conception (approximately 20% more females, p = 0.012).

The proportion of ewes pregnant, the proportion lambed and the number of lambs born per ewe joined was not significantly different when ewes were fed either treatment diet. The pregnancy and lambing rates were lower than anticipated, but were similar to rates seen on farm. The lower than expected lambing rate was partly due to low numbers of ewes conceiving over the 2 oestrous cycles during pen feeding and a higher than expected loss (5-9%) between pregnancy scanning and lambing. Although the difference in the proportion of ewes scanned pregnant (69.8 vs 63.8%) and the proportion that lambed (0.82 vs 0.79 lambs per ewe joined) between ewes fed either pre-conception only or both pre- and post-conception was not statistically significant, these changes represent a potentially significant production benefit on-farm.

It is unclear why the lambing rate was lower when ewes were fed pre-conception only, however, it is possible that a specific nutritional deficiency occurred due to low quality pasture on offer during late spring following the conclusion of pen feeding which lead to a reduction in the ability of the ewe to maintain pregnancy. This greater than expected pregnancy loss, particularly in Study 4, may have led to a lower than expected number of male lambs born and the higher proportion of females observed when ewes were fed experimental diets pre-conception only in Study 4 compared with Study 3 (see Milestone Report 6).

The lower survival of male lambs 24 hr after birth needs to be monitored in future studies. The lower 24 hr survival for male lambs did not seem to be due to problems associated with dystocia at birth. The birthweight of lambs was lower in female compared with male lambs, as expected, but was not significantly different between treatment groups.

Ewe weight and fat score was higher when ewes received the high omega-6 diet based on oats/CSM compared with the high omega-3 diet based on silage in Study 3 and 4, similar to the results seen with BL x Merino ewes. The carry over effect of the diet from year 1 to year 2 was not, however, as great as that observed in BL x Merino ewes. Similar to results observed with BL x Merino ewes, the alteration in sex ratio was not significantly correlated with ewe weight or fat score, or ewe weight and fat score change over the period of pen feeding, indicating that maternal condition at joining was not likely to be responsible for the observed differences in sex ratio between treatment groups.

The time to showing behavioural oestrus and the time to parturition was consistently shorter when ewes were fed the igh omega-6 compared with the high omega-3 diet at mating. In contrast to results observed with BL x Merino ewes, the difference in the time to parturition between dietary treatment groups was greater when ewes were fed the experimental diets pre-conception only, rather than both pre- and post-conception. The shorter time to parturition overall was consistent with previously observed effects of a high omega-6 diet fed pre- and post-conception (Gulliver et al., 2013b) and may be the result of the observed shorter time to oestrus.

The oestrus to parturition interval was not different between treatment groups when ewes were fed treatment diets either pre-conception only or both pre- and post-conception, which is in contrast to those results observed with BL x Merino ewes in the current study and results observed previously (Gulliver et al., 2013b). The oestrus-parturition interval was not significantly related to the sex of lambs, so the practical significance of this finding remains unclear.

The concentration of EPA in the plasma of ewes was significantly lower at preg-scanning when ewes were fed the high omega-6 compared with the high omega-3 diet both pre- and post-conception in Study 3, but not in Study 4, which is similar to the results observed with BL x Merino ewes. The length of time of feeding experimental diets was the same for ewes in both years, however, the silage fed to ewes in Study 4 (Merino ewes) was the same silage as fed to ewes in Study 2 (BL x Merino ewes). It is possible, therefore, that the level of omega-3 in the silage was not high enough to lead to a sustained difference in fatty acid concentrations in plasma after the completion of pen feeding. The relationship between the concentration of omega-3 in feed and plasma will be discussed in more detail in the General Discussion (Section 7.0).

The concentration of progesterone in the plasma of ewes pre-lambing was significantly higher when ewes were fed the high omega-6 diet compared with the high omega-3 diet, which is in contrast to results observed for BL x Merino ewes. The reason for the differential response to P_4 with feeding the experimental rations is unclear. It is possible, therefore, that the shorter time to oestrus observed in the Merino ewes was not related to an altered concentration of P_4 leading up to mating. The relationship between plasma fatty acids and hormones and potential mechanisms linking the experimental diets with altered sex ratio will be discussed further in the General Discussion in Section 7.

6.0 Phase 3: On-farm demonstration trials

6.1 Introduction

Previous studies examining the effect of diets high in omega-3 or omega-6 fatty acids on the sex ratio of lambs were conducted in a controlled pen-feeding environment. In order to determine the feasibility of implementing these feeding strategies in practical situations, it is necessary to conduct on-farm trials. Therefore, the aim of the Phase 3 of the current project was to determine whether the proportion of female lambs was increased when ewes were fed a diet high in omega-3 or omega-6 fatty acids in on-farm situations.

Phase 3 of the current project involved 3 on-farm demonstration trials and the current section presents details of these trials. Details of methodology and results for each study will be presented, including details of specific diets used, animal management and reproduction outcomes.

6.2 On-Farm Trial 1 - Euchareena

The first on-farm demonstration trial commenced in January 2012 (Trial ID: 12-02). Details of experimental procedures for on-farm Trial 1 are outlined below.

6.2.1 Trial location and animal details

The trial was conducted at the property 'Maroombah' via Euchareena, NSW (32°58'15"S, 149°03'23"E). A total of 559 primiparous Merino ewes ('Egelabra' bloodline) were enrolled in the trial. Ewes were born on-farm in July 2009 and had their first lamb in July-August 2011. The lambs were weaned in December 2011 and ewes grazed as one mob until the commencement of the current study (January 2012). All ewes were treated with a fenbendazole (50 g/L) + levamisole hydrochloride (80 g/L) combination drench (Combimax, 6 mL/hd Bayer) in September 2011. Feeding of ewes commenced in January (Summer), joining commenced in February and lambing occurrred in July-August 2012.

6.2.2 Allocation to treatment group and experimental diets

Ewes (mean weight = 50.3 $\pm$ 0.20 kg, fat score 2.73 $\pm$ 0.01) were randomly allocated to one of two treatment groups by drafting alternate groups of 10 animals through a drafting race. Ewes were allocated to receive one of two treatment diets; either pasture only (high omega-3 diet) or oat grain in addition to pasture (high omega-6 diet, Table 6.1).

Table 6.1 Experimental diets either high in omega-3 or omega-6 fatty acids offered to Merino ewes for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 1.

Treatment	No. of Ewes	Dietary Treatment	Feed Offered	Feeding Period
1	279	High omega-3	Pasture *ad libitum*	Throughout trial
2	280	High omega-6 (Low omega-3)	Pasture *ad libitum* + 585 g/hd per day of oats	4 weeks pre-joining + during joining

The pasture available was a mixture of native and improved pasture including *Danthonia sp.* (Wallaby Grass), *Themeda australis* (Kangaroo Grass), *Bothriacloa macra* (Red Grass), *Poa sp.* (Tussock Poa), *Dactylis glomerata* (Cocksfoot), *Phalaris aquatica* (Phalaris) and *Hordeum leporinum* (Barley Grass). The proximate analysis of the diets can be seen in Table 6.2.

Table 6.2 Components and proximate analysis of pasture and oats offered to Merino ewes for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 1.

	Treatment Diet		
	High Omega-3	High Omega-6	
	Pasture	Pasture	Oats
Dry Matter (%)	58.91	50.31	93.46
Proximate Analysis[1]	**(%DM)**		
Neutral Detergent Fibre	73.08	70.45	33.89
Acid Detergent Fibre	45.27	43.81	19.50
Crude Protein	6.38	7.36	8.76
Total Lipid	1.42	1.99	5.84
ME (MJ/kg DM)	6.49	7.18	11.86
Fatty Acid Composition	**% Total Fatty Acids**		
C14:0	1.72	1.55	0.25
C16:0	18.43	18.81	17.58
C18:0	2.85	2.83	1.98
C18:1n-9	6.98	6.59	41.46
C18:1n-7	0.77	0.76	0.81
C18:2n-6	17.99	19.65	34.10
C18:3n-3	28.82	29.39	1.08
n-6:n-3 Ratio	0.62	0.67	31.61

[1]DM = dry matter, ME = metabolisable energy, CP = crude protein, n-6 = omega-6 polyunsaturated fatty acid, n-3 = omega-3 polyunsaturated fatty acid, n-6:n-3 Ratio = ratio of C18:2n-6 to C18:3n-3.

Oat grain was introduced to the ewes over a period of approximately 2 weeks (Table 6.3) and ewes were offered oats for four weeks prior to joining and during the six weeks of joining. The amount of oats offered to ewes was reduced during day 10 to 17 of feeding due to wet weather. Ewes were fed the oats by trailing the grain onto the ground (Plate 6.1) and ewes received the oats in addition to *ad libitum* pasture.

Table 6.3 Details of the introduction of oat grain to Merino ewes for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 1.

Day	Feeding Frequency	Oats Offered (g/hd per day)
1 to 3	Daily	250
4 to 6	Daily	360
7 to 9	Daily	490
10 to 17	Every 2nd Day	340
18 to 73	Every 3rd Day	580

Plate 6.1 Supplementary feeding of oats to Merino ewes during on-farm Trial 1

6.2.3 Ewe management and joining

Joining occurred as per normal farm practice. Four rams were allocated to each treatment group (3 x 3 year old rams and 1 x 2 year old ram per group). Rams remained with ewes for six weeks (Figure 6.1). All ewes remained in separate paddocks for the duration of the study. Ewes lambed in 1 of 2 paddocks with similar pasture to that present at joining. A number of ewes and lambs died during lambing due to a *Haemoncus contortus* (Barber's pole) internal parasite infection. The number of dead lambs was assessed in the paddock, however, predation by foxes prohibited the identification of the sex of dead lambs. The number of ewes joined and the number that died during the study is outlined in Figure 6.2.

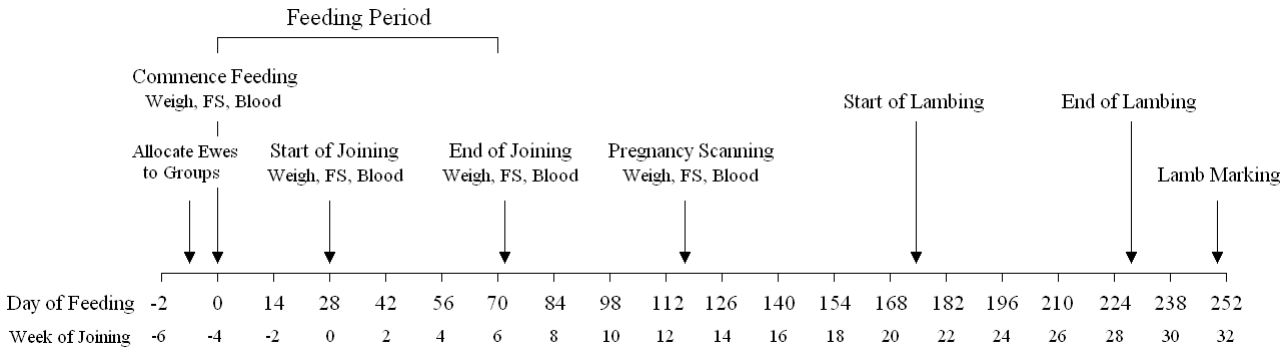


Figure 6.1 Timeline for the timing of feeding and Merino ewe management during the conduct of on-farm Trial 1 in 2012.

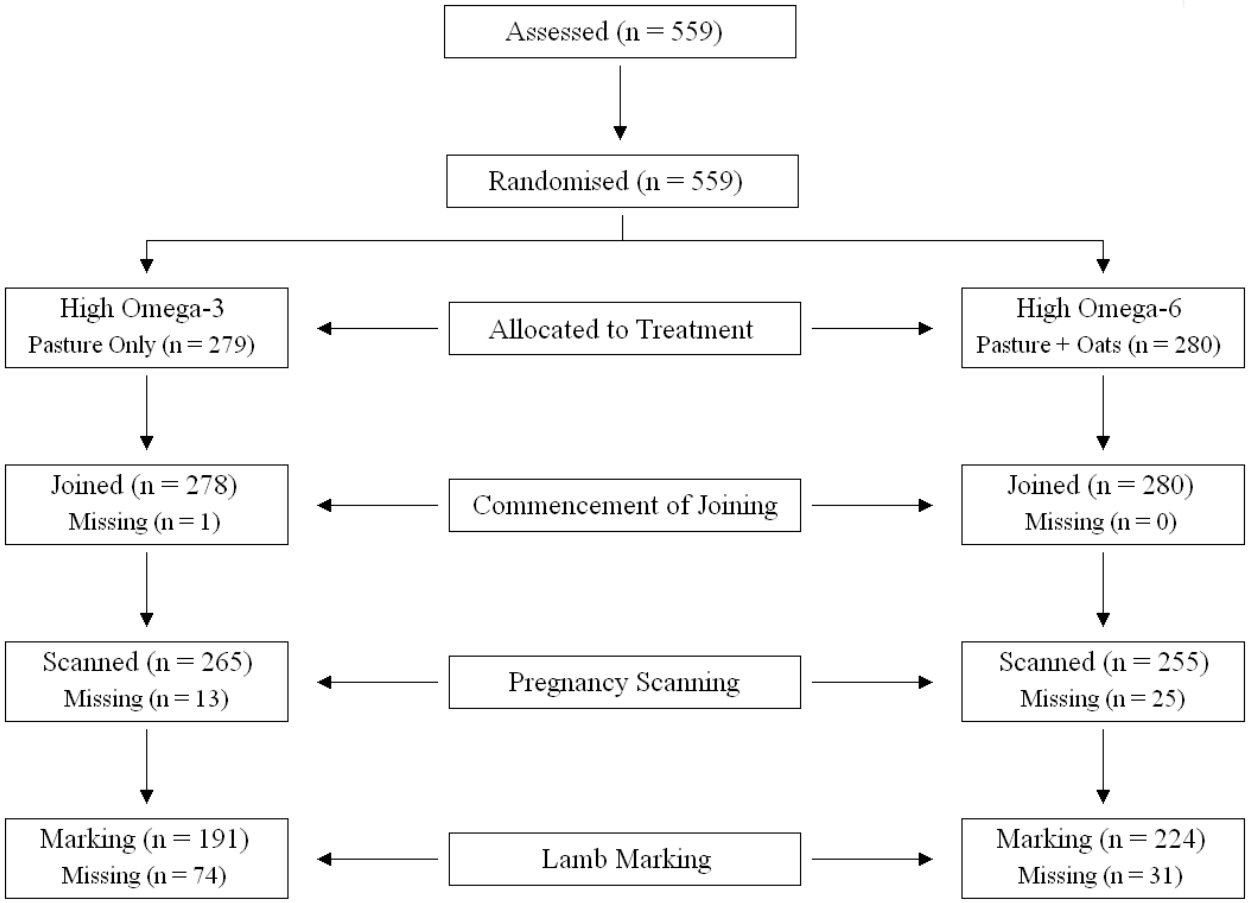


Figure 6.2 Number of ewes allocated to treatments and details of the number of ewes alive at pregnancy scanning or lamb marking during the conduct of on-farm Trial 1 in 2012.

6.2.4 Weight, fat score and blood collection

The weight and fat score of ewes was assessed prior to commencement of feeding of experimental rations, 4 weeks following the commencement of feeding at the time of commencement of joining, following 6 weeks of joining and again at pregnancy scanning according to the methods described in Section 3.10.1. Blood samples were collected from a randomly selected subset of fifteen ewes per treatment group for analysis of fatty acid concentrations in plasma. The samples were collected from the same animals at each collection time and were collected at the same time as the assessment of weight and fat score. Blood samples were collected according to the methods described in Section 3.10.3.

6.2.5 Pregnancy scanning

The pregnancy status of the ewes, litter size and age of the foetus was determined by ultrasonography with a SonoScape A6V ultrasound (SonoScape, Shenzhen, China) using a 3.5 MHz curve array probe (C543) 84 and 33 days after the start and end of joining, respectively. Foetal rate was calculated as the number of foetuses per ewe scanned or the number of foetuses per ewe scanned as pregnant. The time of conception was estimated from the age of the foetus in comparison with the commencement of joining.

6.2.6 Laboratory analyses

Pasture and grain samples were collected at regular intervals during the study. Pasture samples were collected using the median quadrat technique (Bell, 2007). Proximate analysis of feed samples was determined as described previously (Section 3.11.1). The concentration of fatty acids in feed and plasma were also determined as described previously in Section 3.11.

6.2.7 Sex ratio of lambs

The sex ratio of lambs was determined at lamb marking (242 days after the commencement of feeding experimental diets). The percentage of lambs alive was determined as a proportion of ewes joined or ewes alive at lamb marking. The proportion of female lambs in 7 mobs of non-trial Merino ewes joined to either Merino or Border Leicester rams was also assessed at lamb marking.

6.2.8 Statistical analysis

Statistical analyses were determined using the procedures outlined previously (Section 3.12). Ewe weight and fat score as well as plasma fatty acid concentrations were analysed using repeated measures analysis and the difference in sex ratio between treatment groups was determined by chi-squared or Mixed Model analysis.

6.2.9 Results

Ewe weight and fat score
Mean ewe live weight and fat score was not significantly different between treatment groups prior to feeding the experimental diets (Figures 6.3A and B). Mean ewe live weight (51.4 $\pm$ 0.27 versus 49.2 $\pm$ 0.27 kg) and fat score (2.83 $\pm$ 0.014 versus 2.62 $\pm$ 0.014), was significantly ($p < 0.001$) higher when ewes grazed pasture and were supplementary fed oats compared with ewes that grazed pasture only (Figure 6.3A and B). Mean ewe live weight did not change significantly over the experimental period when ewes were fed pasture, however, mean ewe live weight and fat score increased significantly over the period of supplementary feeding oats (Figure 6.3A).

Plasma fatty acid concentrations
Plasma fatty acid concentrations did not differ between the two groups prior to the commencement of feeding experimental rations (data not shown). Total identified fatty acids ($p = 0.997$) and the total concentration of SFA ($p = 0.913$) and MUFA ($p = 0.415$) were not significantly different between ewes that grazed pasture only compared with ewes that grazed pasture and were supplementary fed oats. The concentration of major omega-6 fatty acids in plasma including C18:2n-6 and C20:4n-6 increased significantly over time when ewes were fed the high omega-6 diet compared with the high omega-3 diet (Table 6.4). The concentration of all omega-3 fatty acids analysed, except for C22:6n-3 (DHA), was significantly higher when ewes were fed the high omega-3 diet compared with the high omega-6 diet (Table 6.4). The interaction between diet and time was significant for DHA, however, with the concentration of DHA being significantly lower at the start of joining ($p = 0.037$) but not at the completion of joining ($p = 0.099$) when ewes were fed the High omega-6 diet compared with the High omega-3 diet (Figure 6.4A).

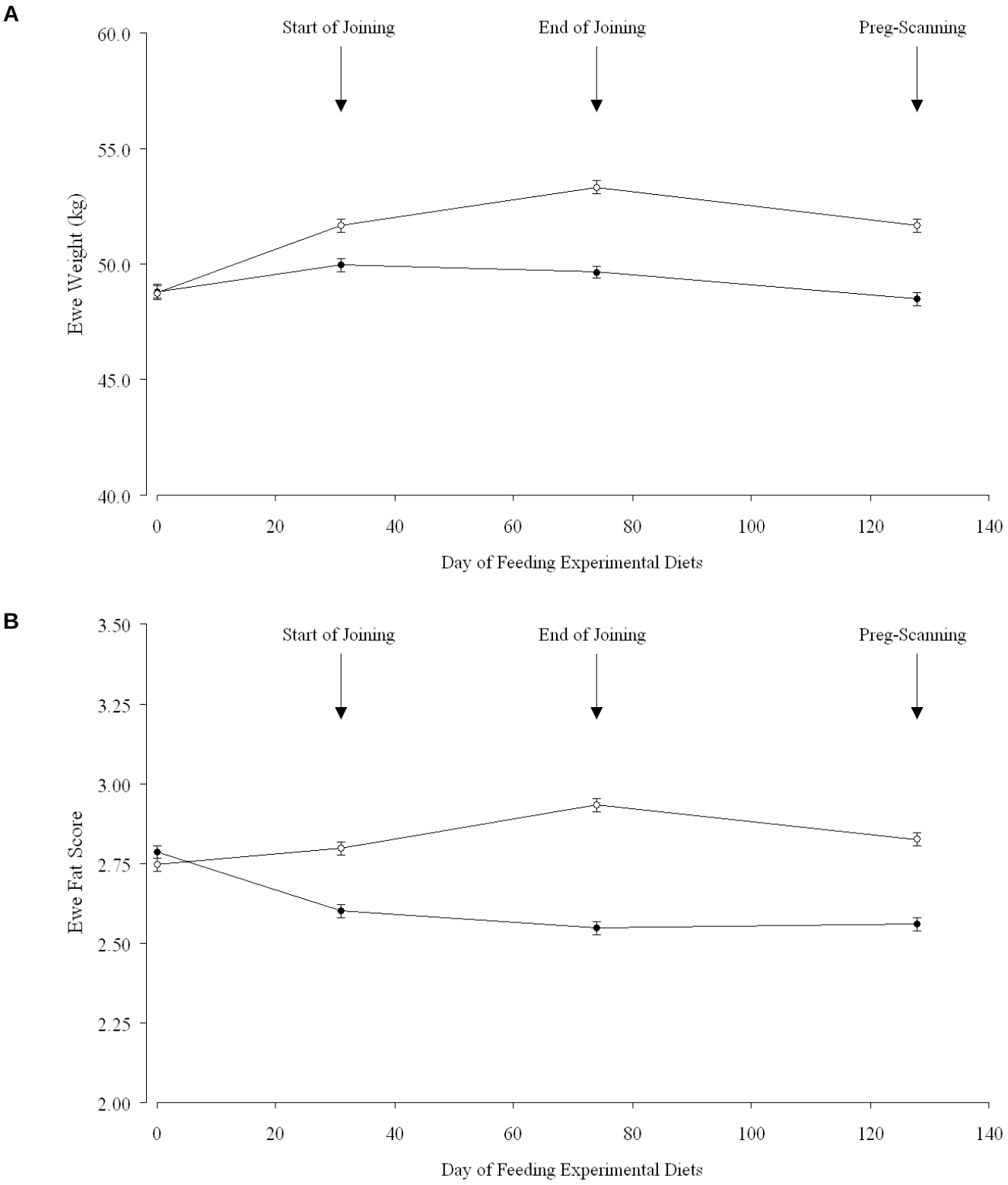


Figure 6.3 Mean weight (A) and fat score (B) for Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 1 (2012).

Table 6.4 Mean concentration (µg/mL) of fatty acids in the plasma of Merino ewes prior to and during the consumption of a diet consisting of pasture only (High omega-3) or a diet of pasture plus oats (High omega-6) for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 1.

FAME[1]	High Omega-3 (Pasture)	High Omega-6 (Pasture + Oats)	*p* - values[2]		
			Diet	Time	Diet x Time
SFA					
C8:0	11.85 (± 0.74)	9.98 (± 0.76)	0.087	0.580	< 0.001
C10:0	1.06 (± 0.06)	0.98 (± 0.07)	0.403	0.005	0.002
C11:0	1.64 (± 0.14)	1.66 (± 0.14)	0.942	0.004	0.037
C12:0	1.63 (± 0.13)	1.33 (± 0.13)	0.113	0.345	0.070
C14:0	10.80 (± 0.33)	9.76 (± 0.33)	0.034	< 0.001	0.005
iC15:0	4.37 (± 0.14)	3.60 (± 0.14)	0.001	< 0.001	< 0.001
aiC15:0	5.24 (± 0.19)	4.20 (± 0.19)	< 0.001	< 0.001	< 0.001
C15:0	8.28 (± 0.27)	7.07 (± 0.27)	0.004	< 0.001	< 0.001
C16:0	144.9 (± 3.24)	150.3 (± 3.28)	0.246	< 0.001	0.007
iC17:0	9.36 (± 0.35)	8.19 (± 0.36)	0.025	< 0.001	0.016
aiC17:0	13.56 (± 0.57)	12.66 (± 0.58)	0.277	< 0.001	0.031
C17:0	10.69 (± 0.34)	10.06 (± 0.34)	0.197	< 0.001	< 0.001
C18:0	166.9 (± 4.54)	170.6 (± 4.58)	0.571	< 0.001	0.020
C20:0	1.29 (± 0.05)	1.22 (± 0.05)	0.261	< 0.001	0.001
C21:0	2.20 (± 0.05)	2.06 (± 0.05)	0.061	< 0.001	0.009
C22:0	2.73 (± 0.08)	2.60 (± 0.08)	0.235	< 0.001	0.177
C23:0	6.41 (± 0.22	6.84 (± 0.22)	0.169	0.338	0.141
C24:0	4.37 (± 0.11)	4.33 (± 0.12)	0.788	< 0.001	0.409
Total SFA	405.8 (± 9.51)	407.3 (± 9.60)	0.913	< 0.001	0.001
MUFA					
C11:1n-1	0.86 (± 0.05)	0.87 (± 0.06)	0.960	0.009	0.021
C12:1n-7	2.33 (± 0.11)	1.21 (± 0.12)	< 0.001	< 0.001	< 0.001
C13:1n-1	0.70 (± 0.04)	0.49 (± 0.04)	0.001	0.030	0.006
C14:1n-5	0.19 (± 0.02)	0.18 (± 0.02)	0.866	0.012	0.394
C15:1n-5	0.40 (± 0.02)	0.32 (± 0.02)	0.022	0.001	0.812
C16:1n-7t	4.44 (± 0.17)	3.25 (± 0.17)	< 0.001	< 0.001	< 0.001
C16:1n-7	17.12 (± 0.64)	15.33 (± 0.64)	0.058	< 0.001	0.018
C17:1n-7	1.68 (± 0.06)	1.53 (± 0.06)	0.114	< 0.001	0.017
C18:1n-9t	4.84 (± 0.15)	5.11 (± 0.15)	0.191	< 0.001	0.008
C18:1n-9	205.7 (± 5.97)	208.3 (± 6.01)	0.754	< 0.001	0.091
C18:1n-7	7.31 (± 0.23)	6.68 (± 0.24)	0.069	< 0.001	0.015
C19:1n-12	0.86 (± 0.09)	0.55 (± 0.10)	0.033	< 0.001	0.184
C20:1n-15	0.64 (± 0.05)	0.49 (± 0.05)	0.028	0.102	0.007
C20:1n-12	2.57 (± 0.11)	2.19 (± 0.11)	0.024	< 0.001	< 0.001
C20:1n-9	0.75 (± 0.03)	0.74 (± 0.03)	0.799	0.042	0.395
C22:1n-9	0.89 (± 0.05)	0.60 (± 0.05)	0.001	< 0.001	0.187
C24:ln-9	3.38 (± 0.12)	3.59 (± 0.12)	0.224	< 0.001	0.091
Total MUFA	259.7 (± 1.08)	251.5 (± 7.10)	0.415	< 0.001	0.036

FAME[1]	High Omega-3 (Pasture)	High Omega-6 (Pasture + Oats)	*p* - values[2]		
			Diet	Time	Diet x Time
n-6 PUFA					
C18:2n-6t	0.30 (± 0.02)	0.31 (± 0.02)	0.869	< 0.001	0.559
C18:2n-6	108.5 (± 3.76)	123.87 (± 3.79)	0.007	< 0.001	< 0.001
C18:3n-6	5.23 (± 0.24)	5.67 (± 0.24)	0.201	< 0.001	0.067
C20:2n-6	0.67 (± 0.02)	0.59 (± 0.02)	0.008	< 0.001	0.362
C20:3n-6	3.10 (± 0.14)	3.22 (± 0.14)	0.543	0.301	0.687
C20:4n-6	22.72 (± 0.94)	22.79 (± 0.95)	0.954	< 0.001	< 0.001
C22:4n-6	0.82 (± 0.04)	0.95 (± 0.04)	0.032	0.173	< 0.001
C22:5n-6	0.74 (± 0.04)	0.89 (± 0.04)	0.017	0.012	0.429
Total n-6	142.2 (± 4.58)	158.3 (± 4.61)	0.019	< 0.001	< 0.001
n-3 PUFA					
C18:3n-3	38.37 (± 1.38)	30.09 (± 1.39)	< 0.001	< 0.001	< 0.001
C18:4n-3	0.59 (± 0.08)	0.27 (± 0.09)	0.012	< 0.001	< 0.001
C20:3n-3	0.38 (± 0.02)	0.31 (± 0.02)	0.038	0.146	0.053
C20:5n-3	19.57 (± 0.86)	15.53 (± 0.87)	0.002	< 0.001	< 0.001
C22:5n-3	16.80 (± 0.50)	14.85 (± 0.50)	0.009	< 0.001	< 0.001
C22:6n-3	14.31 (± 0.80)	14.20 (± 0.80)	0.924	< 0.001	< 0.001
Total n-3	90.02 (± 2.95)	75.25 (± 2.97)	0.001	< 0.001	< 0.001
Total ID	972.9 (± 24.32)	973.0 (± 24.50)	0.997	< 0.001	< 0.001
n-6:n-3	1.59 (± 0.05)	2.25 (± 0.05)	< 0.001	< 0.001	< 0.001
DHADI	0.93 (± 0.05)	0.98 (± 0.05)	0.546	0.007	0.262
DHASI	19.81 (± 0.88)	16.73 (± 0.88)	0.019	< 0.001	0.013
EFI	0.98 (± 0.02)	0.98 (± 0.02)	0.863	< 0.001	0.016
P:S	0.57 (± 0.01)	0.57 (± 0.01)	0.767	0.032	0.002

[1]FAME = fatty acid methyl ester, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, Total ID = total concentration of identified fatty acids, n-6:n-3 = ratio of n-6 PUFA : n-3 PUFA, DHADI = DHA Deficiency Index, 22:5n-6 / C22:4n-6 (Neuringer et al., 1986), DHASI = DHA Sufficiency Index, C22:6n-3 / C22:5n-6 (Hoffman and Uauy, 1992), EFI = Essential Fatty Acid Status Index, ratio of (n-3 PUFA + n-6 PUFA) : (n-7 MUFA + n-9 MUFA), P:S = ratio of (n-3 PUFA + n-6 PUFA) : SFA.
[2]The *p*-value for Diet x Time represents the interaction between dietary treatment group and length of time of feeding.

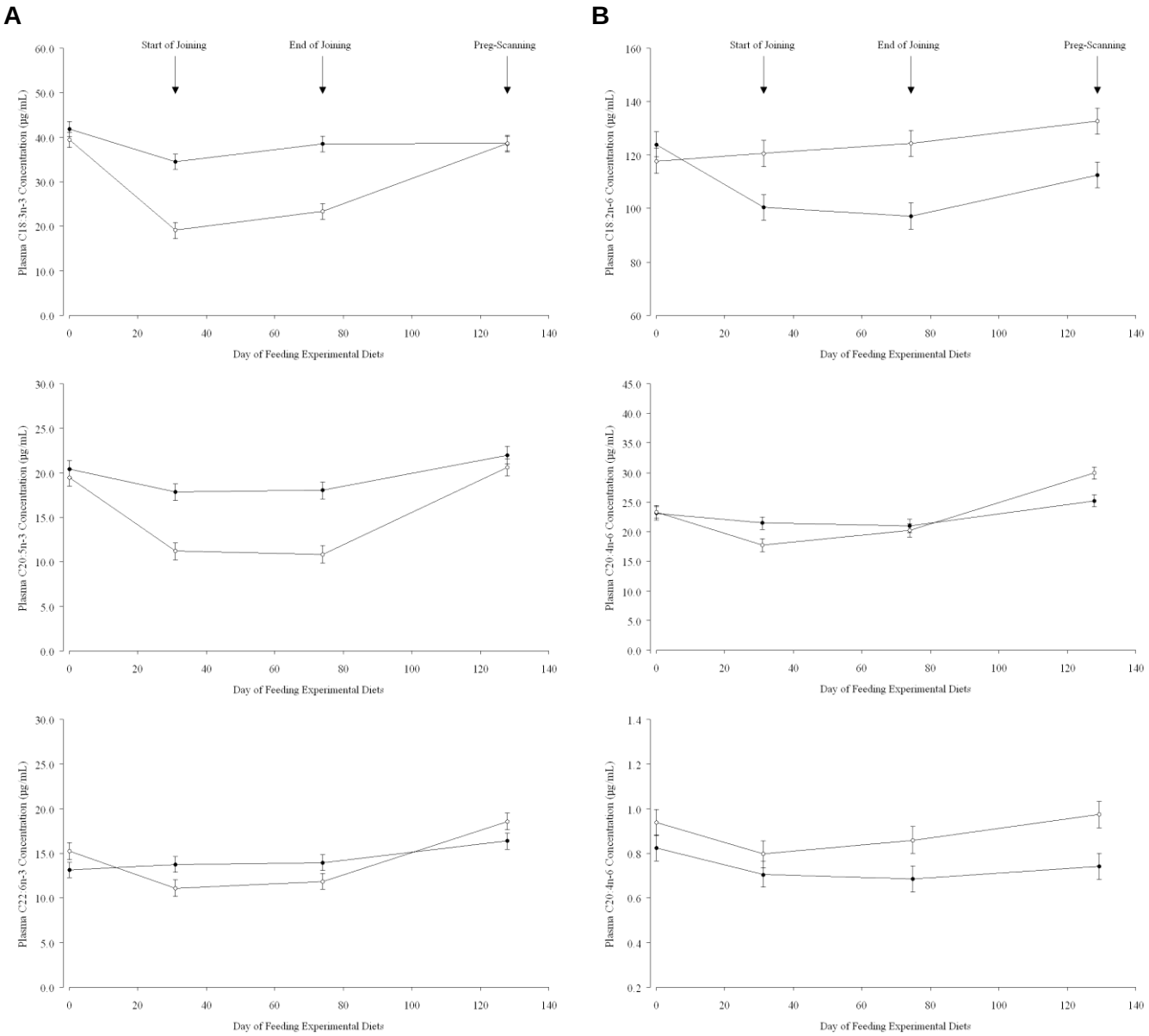


Figure 6.4 Mean concentration of (A) omega-3 and (B) omega-6 fatty acids in the plasma of Merino ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 1 (2012).

Time of joining and reproduction outcomes

The estimated time of conception was significantly ($p < 0.001$) shorter when ewes were fed the high omega-6 diet compared with high omega-3 diet (Figure 6.5). The proportion of ewes pregnant was not significantly different when ewes were fed the diet high in omega-3 or omega-6 fatty acids (Table 6.5). The foetal rate was, however, significantly higher when ewes were fed the high omega-6 diet supplemented with oats compared with the high omega-3 diet of pasture only.

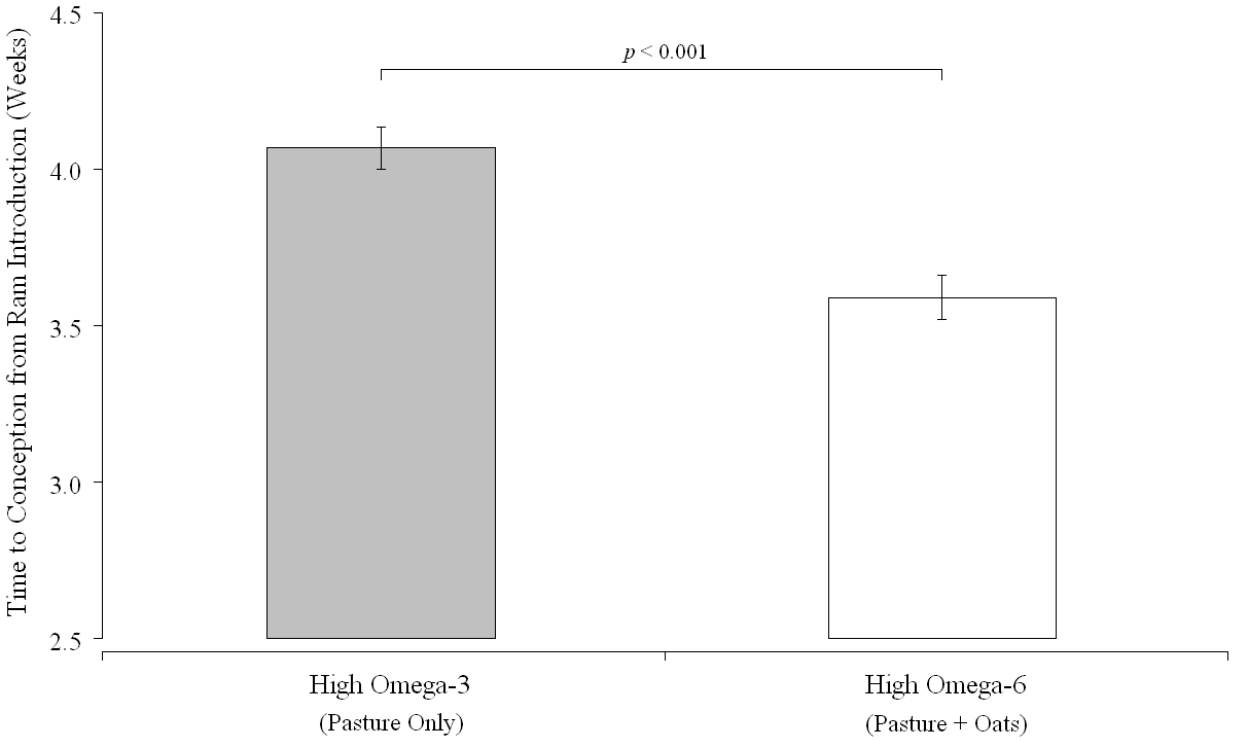


Figure 6.5 Time to conception from ram introduction when Merino ewes were fed a diet consisting of pasture only (High omega-3, shaded bar) or a diet of pasture plus oats (High omega-6, unshaded bar) for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 1. Values are least squares means ± the standard error of least squares means.

The lamb marking rate was significantly higher when ewes were fed the high omega-6 diet compared with high omega-3 diet (Table 6.5). The lamb marking rate was higher when lambs were assessed as a proportion of ewes pregnant or ewes joined.

Table 6.5 Proportion of ewes pregnant, foetal rate and lamb marking rate when Merino ewes were fed a diet consisting of pasture only (High omega-3) or a diet of pasture plus oats (High omega-6) for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 1.

Reprouction Measure[1]	High Omega-3 (Pasture)	High Omega-6 (Pasture + Oats)	*p* - value
Proportion pregnant (%)	89.0	87.0	0.479
Foetal rate			
Ewes pregnant	1.06 (± 0.02)	1.22 (± 0.02)	< 0.001
Ewes Scanned	0.95 (± 0.03)	1.06 (± 0.03)	0.008
Lamb marking rate			
Ewes at marking	0.31 (± 0.03)	0.52 (± 0.03)	< 0.001
Ewes pregnant	0.25 (± 0.03)	0.52 (± 0.03)	< 0.001
Ewes joined	0.22 (± 0.02)	0.41 (± 0.03)	< 0.001

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means).

Sex ratio of lambs

The proportion of female lambs was not significantly different from 50% when ewes were fed the high omega-6 diet at joining (p = 0.878), however, the proportion of female lambs was significantly higher than 50% when ewes were fed the igh omega-3 diet (p = 0.030). The proportion of female lambs was significantly (p = 0.032) higher when ewes were fed the pasture only diet high in omega-3 compared with the oats supplemented diet high in omega-6 (Figure 6.6). The proportion of female lambs was not, however, significantly (p > 0.180) different when ewes were supplementary fed the high omega-6 diet compared with any other unsupplemented non-trial sheep joined to Merino rams (Table 6.6).

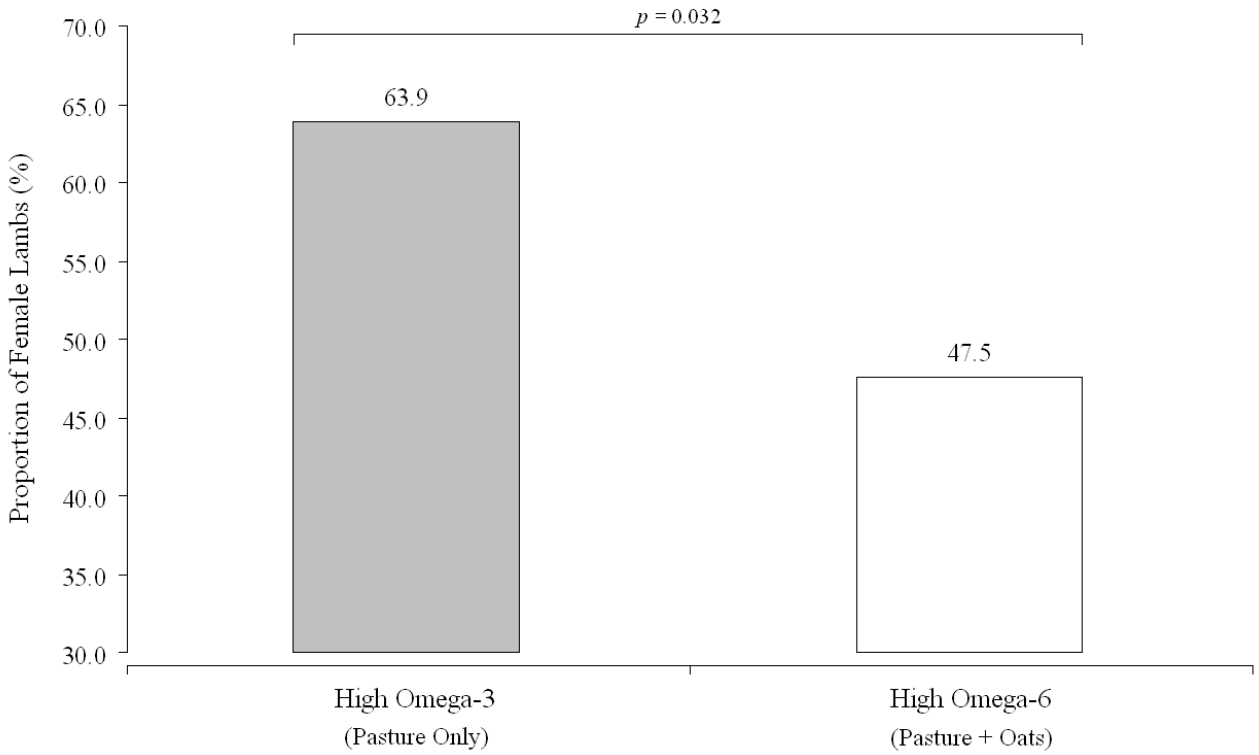


Figure 6.6 Proportion of female lambs at lamb marking when when Merino ewes were fed a diet consisting of pasture only (High omega-3) or a diet of pasture plus oats (High omega-6) for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 1.

Table 6.6 Number of lambs at lamb marking and the proportion of female lambs for non-trial Merino ewes joined to either Merino or Border Leicester rams on the property where on-farm Trial 1 was conducted.

Mob	Rams	Ewes Joined	Lamb Marking Rate (%)		Number of Lambs			Proportion of Females	
			Ewes at Marking	Ewes Joined	Male	Female	Total	(%)	*p*-value (50:50)
Pdk 1	Merino	215	92.5	86.5	83	103	186	55.4	0.143
Pdk 2	Merino	280	82.8	77.1	105	111	216	51.4	0.683
Pdk 3	Merino	367	93.3	90.5	160	172	332	51.8	0.510
Pdk 4	Merino	394	74.1	69.5	141	133	274	48.5	0.629
Pdk 5	Border Leicester	253	79.9	75.5	93	98	191	51.3	0.718
Pdk 6	Border Leicester	290	78.0	63.4	96	88	184	47.8	0.555
Pdk 7	Border Leicester	406	100.8	87.9	165	192	357	53.8	0.153
	Merino	1256	84.8	80.3	489	519	1008	51.5	ns
	Border Leicester	949	88.3	77.1	354	378	732	51.6	ns
	Overall	2205	86.3	78.9	843	897	1740	51.6	ns

Values are proportions (percentages) of lambs at lamb marking. ns = not significantly different from a ratio of 50:50.

6.3 On-Farm Trial 2 - Bookham

The second on-farm demonstration trial commenced in February 2012 (Trial ID: 12-05). Details of experimental procedures for on-farm Trial 2 are outlined below.

6.3.1 Trial location and animal details

The trial was conducted at the property 'Kia-Ora' via Bookham, NSW (34°48'38"S, 148°35'25"E). A total of 822 primiparous Merino ewes ('Bogo' bloodline) were enrolled in the trial. Ewes were born on-farm and had previously had 2 lambs. All ewes grazed as one mob from weaning the previous year until the commencement of the current study (February 2012). Feeding of ewes commenced in February (Summer), joining commenced in March and lambing occurred in August-September 2012.

6.3.2 Allocation to treatment group and experimental diets

Ewes were randomly allocated to one of two treatment groups by drafting alternate groups of 10 animals through a drafting race. Ewes were allocated to receive one of two treatment diets; either pasture only (High omega-3 diet) or barley grain in addition to pasture (High omega-6 diet, Table 6.7). The proximate analysis of the barley can be seen in Table 6.8.

Table 6.7 Experimental diets either high in omega-3 or omega-6 fatty acids offered to Merino ewes for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 2.

Treatment	No. of Ewes	Dietary Treatment	Feed Offered	Feeding Period
1	575	High omega-3	Pasture *ad libitum*	Throughout trial
2	250	High omega-6 (Low omega-3)	Pasture *ad libitum* + 600 g/hd per day of barley	4 weeks pre-joining + during joining

Barley was introduced to the ewes over a period of approximately 2 weeks (Table 6.9) and ewes were offered barley for four weeks prior to joining and during the six weeks of joining. Ewes were fed the barley by trailing the grain onto the ground and ewes received the barley in addition to *ad libitum* pasture.

6.3.3 Ewe management and joining

Joining and animal husbandry, including drenching and flystrike prevention occurred as per normal farm practice. All ewes remained in separate paddocks for the duration of the study.

6.3.4 Pregnancy scanning

The pregnancy status of the ewes, litter size and age of the foetus was determined by ultrasonography with an Ovi-Scan 6 ultrasound scanner (BCF Technology Ltd, Livington, Scotland) with an external 3.5 MHz sector transducer 91 and 48 days after the start and end of joining, respectively. Foetal rate was calculated as the number of foetuses per ewe joined or the number of foetuses per ewe scanned as pregnant. The time of conception was estimated from the age of the foetus in comparison with the commencement of joining. Single-bearing and twin-bearing ewes from each treatment group were kept in separate paddocks until lambing.

Table 6.8 Components and proximate analysis of barley offered to Merino ewes for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 2.

	Barley
Dry Matter (%)	92.9
Proximate Analysis[1]	**(%DM)**
Neutral Detergent Fibre	10.01
Acid Detergent Fibre	6.11
Crude Protein	12.97
Total Lipid	0.54
ME (MJ/kg DM)	12.66
Fatty Acid Composition	**% Total Fatty Acids**
C14:0	0.62
C16:0	22.07
C18:0	3.27
C18:1n-9	13.58
C18:1n-7	0.70
C18:2n-6	47.63
C18:3n-3	4.32
n-6:n-3 Ratio	11.02

[1]DM = dry matter, ME = metabolisable energy, CP = crude protein, n-6 = omega-6 polyunsaturated fatty acid, n-3 = omega-3 polyunsaturated fatty acid, n-6:n-3 Ratio = ratio of C18:2n-6 to C18:3n-3.

Table 6.9 Details of the introduction of barley grain to Merino ewes for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 2.

Day	Feeding Frequency	Oats Offered (g/hd per day)
1 to 3	Daily	200
4 to 6	Daily	350
7 to 9	Daily	450
10 to 12	Daily	500
13 to 14	Daily	600
15 to 18	Every 2nd Day	600
19 to 64	Every 3rd Day	600

6.3.5 Sex ratio of lambs

The sex ratio of lambs was determined at lamb marking. The percentage of lambs alive was determined as a proportion of ewes joined or ewes alive at lamb marking. The sex ratio of single and twin lambs in the high omega-3 group were assessed together at marking.

6.3.6 Statistical analysis

Statistical analyses were determined using the procedures outlined previously (Section 3.12).

6.3.7 Results

Time of joining and reproduction outcomes

The estimated time of conception was significantly shorter when twin-bearing (p = 0.031), but not single-bearing (p = 0.289) ewes were fed the high omega-6 diet compared with high omega-3 diet (Figure 6.7). The proportion of ewes pregnant and the foetal rate was not significantly different when ewes were fed the diet high in omega-3 or omega-6 fatty acids (Table 6.10).

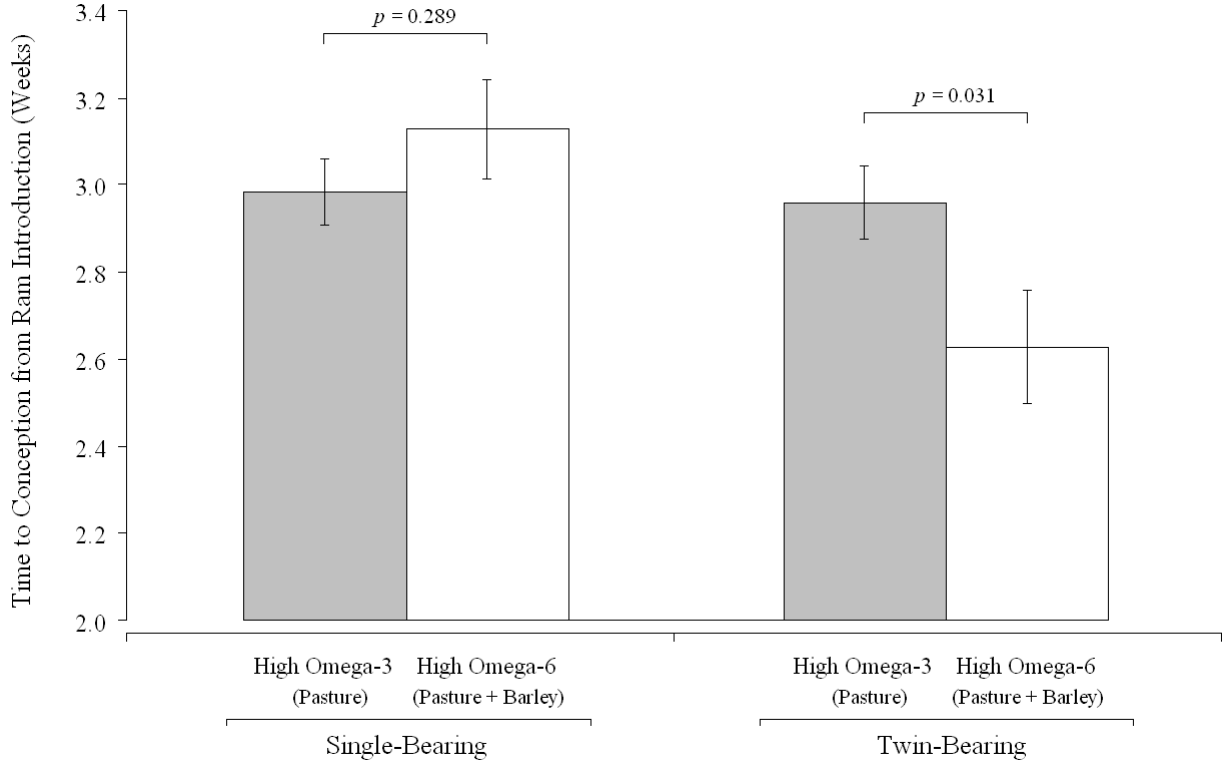


Figure 6.7 Time to conception from ram introduction when Merino ewes were fed a diet consisting of pasture only (High omega-3, shaded bar) or a diet of pasture plus oats (High omega-6, unshaded bar) for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 2. Values are least squares means ± the standard error of least squares means.

The lamb marking rate was not significantly different when ewes were fed the high omega-6 diet compared with high omega-3 diet (Table 6.10). The lamb marking rate was also not significantly different when lambs were assessed as a proportion of ewes joined.

Table 6.10 Proportion of ewes pregnant, foetal rate and lamb marking rate when Merino ewes were fed a diet consisting of pasture only (High omega-3) or a diet of pasture plus barley (High omega-6) for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 2

Reprouction Measure[1]	High Omega-3 (Pasture)	High Omega-6 (Pasture + Barley)	p - value
Proportion pregnant (%)	95.5	95.1	0.833
Foetal rate			
Ewes pregnant	1.45 (± 0.02)	1.43 (± 0.03)	0.649
Ewes joined	1.39 (± 0.02)	1.35 (± 0.04)	0.385
Lamb marking rate			
Ewes at marking	1.02 (± 0.03)	1.03 (± 0.05)	0.907
Ewes joined	0.99 (± 0.03)	0.96 (± 0.05)	0.562

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means).

Sex ratio of lambs

The proportion of female lambs was not significantly different from 50% when ewes were fed the high omega-3 diet (p = 0.496), or when single-bearing (p = 0.074) or twin bearing (p = 0.657) ewes were fed the high omega-6 diet at joining. The proportion of female lambs was not significantly (p = 0.997) different when ewes were fed the pasture only diet high in omega-3 (47.1%) compared with the diet supplemented with barley high in omega-6 (47.1%). The proportion of female lambs was also not significantly different when single-bearing (p = 0.385) or twin-bearing (p = 0.429) ewes were fed the high omega-6 diet compared with all ewes fed the high omega-3 diet (Figure 6.8)

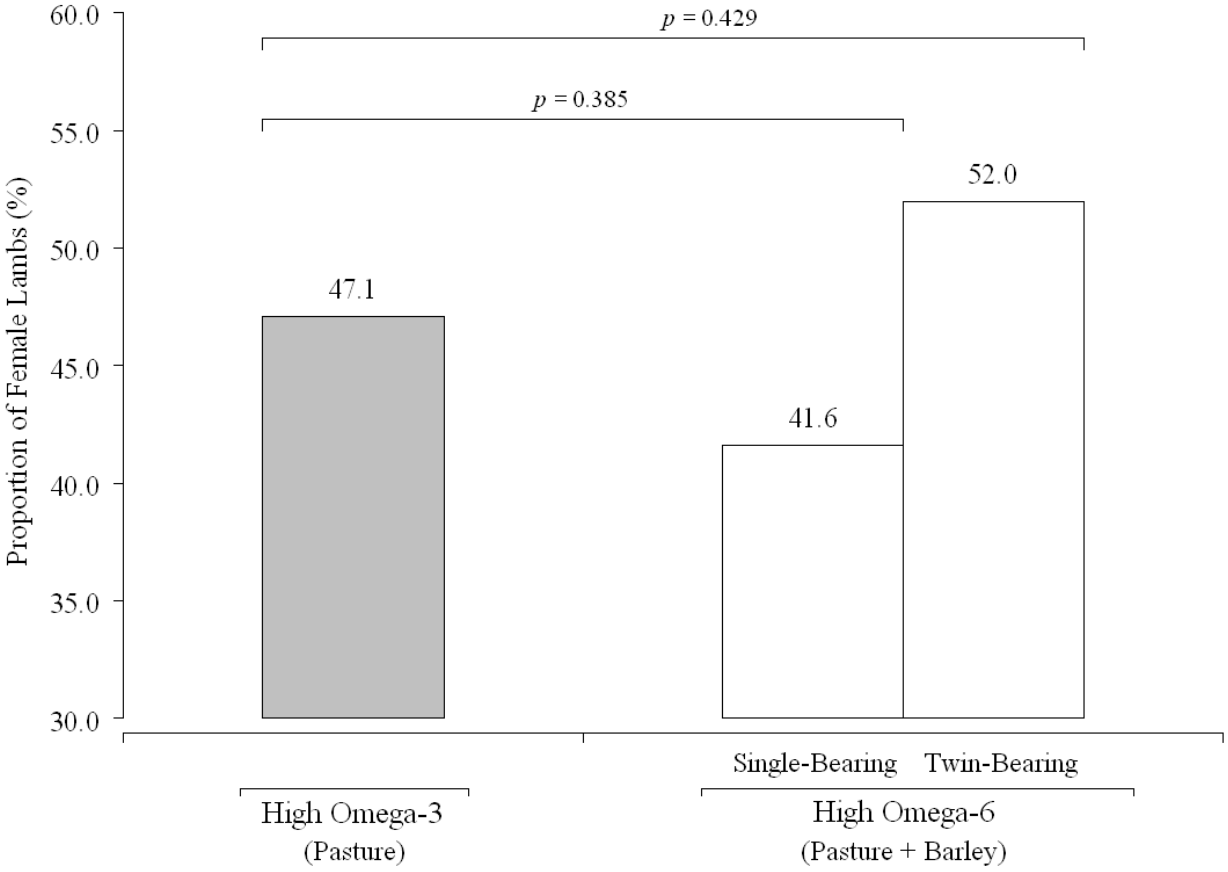


Figure 6.8 Proportion of female lambs at lamb marking when when Merino ewes were fed a diet consisting of pasture only (High omega-3) or a diet of pasture plus barley (High omega-6) for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 2. *p*-values indicate significant differences between all lambs from ewes fed the High Omega-3 diet compared with lambs from either single-bearing or twin-bearing ewes fed the High Omega-6 diet.

6.4 On-Farm Trial 3 - Wagga

The third on-farm demonstration trial commenced in May 2013 (Trial ID: 13-02). Details of experimental procedures for on-farm Trial 3 are outlined below.

6.4.1 Trial location and animal details

The trial was conducted at the property 'Richlands Park' via Wagga Wagga, NSW (35°01'58"S 147°19'11"E). A total of 254 primiparous Border Leicester x Merino ewes were

enrolled in the trial. Ewes were originally purchased from commercial properties and had previously had 3 lambs. All ewes grazed as one mob from weaning (February 2013) until the commencement of the current study (May 2013). Feeding of ewes commenced in May (Autumn), joining commenced in June and lambing occurred in November-December 2013.

6.4.2 Allocation to treatment group and experimental diets

Ewes were randomly allocated to one of two treatment groups by drafting alternate groups of 15 animals through a drafting race. Ewes were allocated to receive one of two treatment diets; either pasture only (high omega-3 diet) or a mixed grain ration (wheat, barley, lupins and peas, approximate proportions = 40:30:20:10) in addition to pasture (high omega-6 diet, Table 6.11). The proximate analysis of the mixed grain ration is shown in Table 6.12.

Table 6.11 Experimental diets either high in omega-3 or omega-6 fatty acids offered to BL x Merino ewes for 4 weeks prior to joining and 5 weeks during joining in on-farm Trial 3.

Treatment	No. of Ewes	Dietary Treatment	Feed Offered	Feeding Period
1	127	High omega-3	Pasture *ad libitum*	Throughout trial
2	127	High omega-6 (Low omega-3)	Pasture *ad libitum* + 600 g/hd per day of mixed grain	4 weeks pre-joining + during joining

The mixed grain was introduced to the ewes over a period of approximately 2 weeks (Table 6.13) and ewes were offered the grain for four weeks prior to joining and during five weeks of joining. Ewes were fed the the mixed grain by trailing the feed onto the ground and ewes received the grain in addition to *ad libitum* pasture.

6.4.3 Ewe management and joining

Joining and animal husbandry, including drenching and flystrike prevention occurred as per normal farm practice. All ewes grazed the same paddock following joining up until 3 weeks prior to parturition.

6.4.4 Pregnancy scanning and parturition

The pregnancy status of the ewes, litter size and age of the foetus was determined by ultrasonography with an Ovi-Scan 6 ultrasound scanner (BCF Technology Ltd, Livington, Scotland) with an external 3.5 MHz sector transducer 73 and 38 days after the start and end of joining, respectively. Foetal rate was calculated as the number of foetuses per ewe joined or the number of foetuses per ewe scanned as pregnant. The time of conception was estimated from the age of the foetus in comparison with the commencement of joining.

Ewes were monitored closely during lambing and the total number of lambs born was monitored daily. The sex ratio of lambs was determined at birth and will be monitored at lamb marking and weaning.

Table 6.12 Components and proximate analysis of mixed grain offered to BL x Merino ewes for 4 weeks prior to joining and 5 weeks during joining in on-farm Trial 3.

	Mixed Grain
Dry Matter (%)	92.9
Proximate Analysis[1]	**(%DM)**
Neutral Detergent Fibre	18.06
Acid Detergent Fibre	7.45
Crude Protein	11.85
Total Lipid	1.90
ME (MJ/kg DM)	12.62
Fatty Acid Composition	**% Total Fatty Acids**
C14:0	0.55
C16:0	22.96
C18:0	2.56
C18:1n-9	17.57
C18:1n-7	0.79
C18:2n-6	56.75
C18:3n-3	4.75
n-6:n-3 Ratio	11.95

[1]DM = dry matter, ME = metabolisable energy, CP = crude protein, n-6 = omega-6 polyunsaturated fatty acid, n-3 = omega-3 polyunsaturated fatty acid, n-6:n-3 Ratio = ratio of C18:2n-6 to C18:3n-3.

Table 6.13 Details of the introduction of mixed grain to BL x Merino ewes for 4 weeks prior to joining and 5 weeks during joining in on-farm Trial 3.

Day	Feeding Frequency	Oats Offered (g/hd per day)
1 to 3	Daily	200
4 to 6	Daily	350
7 to 9	Daily	450
10 to 12	Daily	500
13 to 14	Daily	600
15 to 18	Every 2nd Day	600
19 to 76	Every 3rd Day	600

6.4.5 Results

Time of joining and reproduction outcomes

The estimated time of conception was not significantly (p = 0.763) shorter when ewes were fed the high omega-3 diet compared with high omega-6 diet (Figure 6.9). The proportion of ewes pregnant and the foetal rate was not significantly different when ewes were fed the diet high in omega-3 or omega-6 fatty acids (Table 6.14). The lambing rate (lambs born or lambs marked) was also not significantly different when ewes were fed the high omega-6 diet compared with high omega-3 diet (Table 6.14).

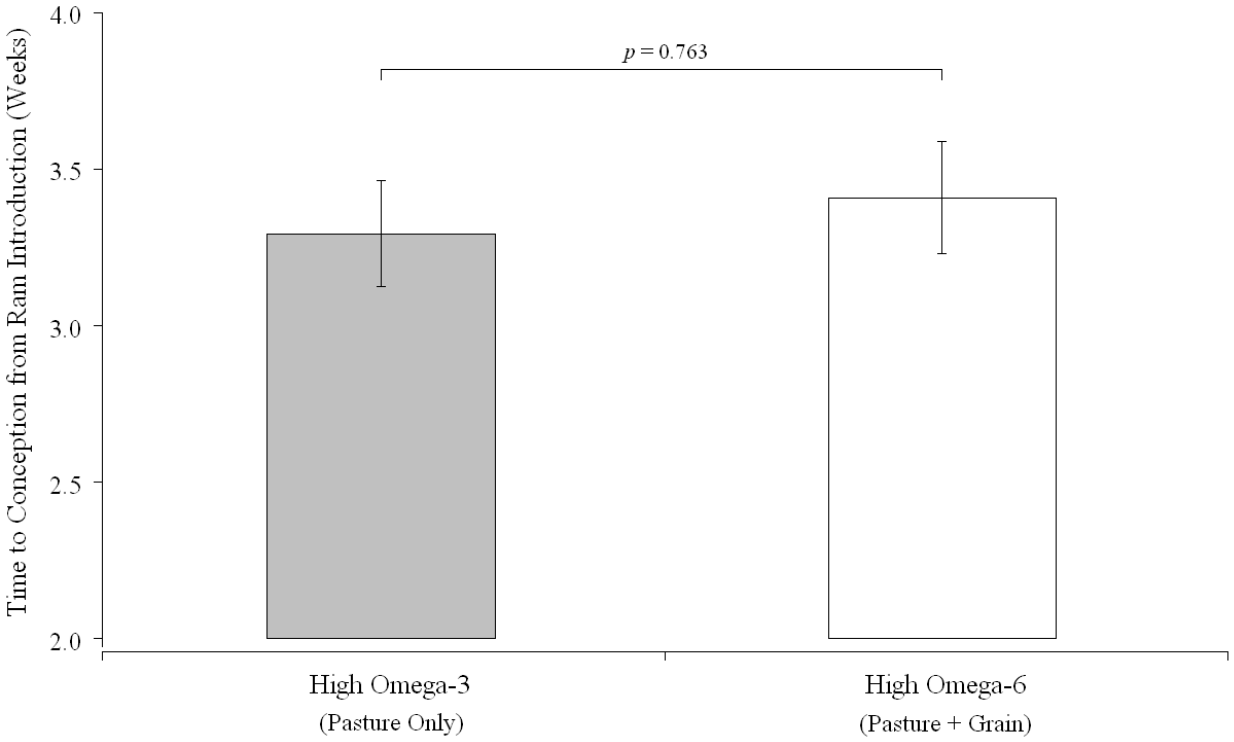


Figure 6.9 Time to conception from ram introduction when BL x Merino ewes were fed a diet consisting of pasture only (High omega-3, shaded bar) or a diet of pasture plus mixed grain (High omega-6, unshaded bar) for 4 weeks prior to joining and 6 weeks during joining in on-farm Trial 3. Values are least squares means ± the standard error of least squares means.

Table 6.14 Proportion of ewes pregnant, foetal rate and lamb marking rate when BL x Merino ewes were fed a diet consisting of pasture only (High omega-3) or a diet of pasture plus mixed grain (High omega-6) for 4 weeks prior to joining and 5 weeks during joining in on-farm Trial 3

Reprouction Measure[1]	High Omega-3 (Pasture)	High Omega-6 (Pasture + Grain)	*p* - value
Proportion pregnant (%)	96.8	96.8	0.991
Foetal rate			
Ewes pregnant	1.76 (± 0.04)	1.76 (± 0.04)	0.972
Ewes joined	1.70 (± 0.05)	1.70 (± 0.05)	0.972
Mean number of lambs (*ewes joined*)			
Lambs born	1.66 (± 0.06)	1.69 (± 0.07)	0.711
Lambs marked	1.32 (± 0.08)	1.37 (± 0.08)	0.654

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means).

Sex ratio of lambs

The proportion of female lambs was not significantly different when single-bearing (p = 0.425), twin-bearing (p = 0.927) or triplet-bearing (p = 0.053) ewes were fed the high omega-6 diet compared with all ewes fed the high omega-3 diet (Figure 6.10). The proportion of female lambs was higher when ewes received the high omega-3 diet and gave birth to triplet lambs compared with single or twin lambs, however, these differences were not statistically significant (p > 0.10).

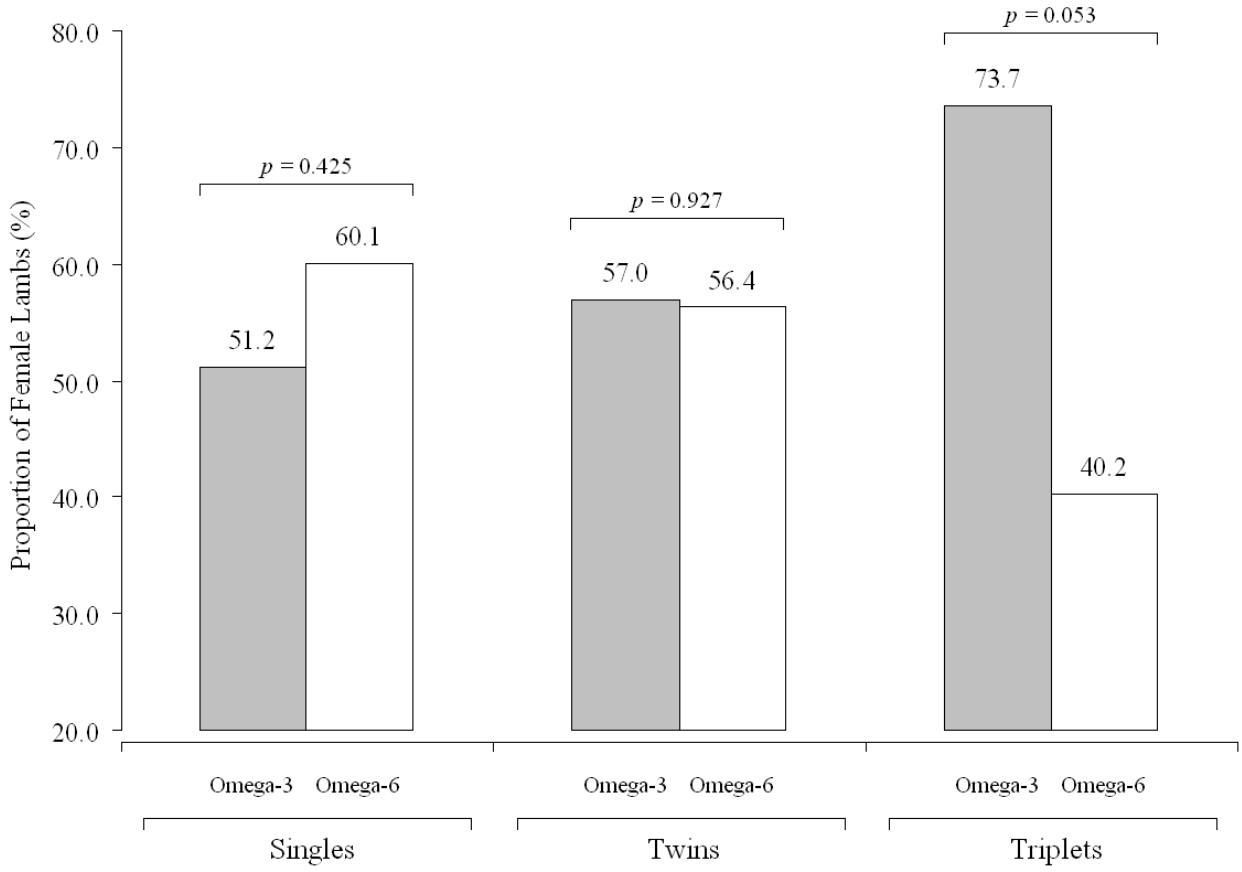


Figure 6.10 Proportion of female lambs when when BL x Merino ewes were fed a diet consisting of pasture only (High omega-3) or a diet of pasture plus mixed grain (High omega-6) for 4 weeks prior to joining and 5 weeks during joining in on-farm Trial 3 and ewes gave birth to single, twin or triplet lambs.

6.5 Discussion

The results of the current on-farm demonstration trials do not indicate that the proportion of female lambs is consistently increased when ewes are offered a diet high in omega-6 fatty acids at joining. The significant loss of lambs between pregnancy scanning and lamb marking during on-farm Trial 1 does not, however, allow firm conclusions to be drawn. It is not possible to determine whether the proportion of female lambs was higher at birth when ewes were fed the high omega-3 (pasture) diet or whether male lamb mortality was significantly higher than female lamb mortality between birth and marking. The field trials will have to be conducted with a larger numbers of ewes or the number of live and dead lambs at birth will need to be assessed more closely in order to more accurately determine the effect of the diets on the sex ratio of lambs on-farm.

There were a number of results of the current on-farm trials that confirm results of the intensive pen-feeding studies. The higher concentration of omega-6 fatty acids in plasma of ewes fed the high omega-6 diet in on-farm Trial 1 demonstrate that systemic fatty acid status can be altered during on-farm feeding. The estimated shorter time to conception when ewes were offered the high omega-6 ration in on-farm Trial 1 indicates that there were significant effects of the different diets on some reproduction parameters. Results from pregnancy scanning during on-farm Trial 2 indicate that the estimated time to conception for twin-bearing ewes was shorter when ewes were offered the high omega-6 diet compared with the high omega-3 diet at joining. The proportion of female lambs was highest when the estimated time to conception was shortest in these twin-bearing ewes fed the high omega-6 diet. The relationship between the time of conception and the proportion of male and female lambs warrants further investigation during future on-farm feeding trials.

The pregnancy scanning and lamb marking percentages were significantly higher when ewes were supplementary fed oats at joining in on-farm Trial 1. The proportion of ewes pregnant, the scanning and lamb marking rates were not significantly altered when ewes were offered barley at joining in Field Trial 2. These results indicate that there appear to be few negative effects of offering ewes a high omega-6 diet at joining. The increased scanning and marking percentage in on-farm Trial 1 is consistent with a “flushing” effect of an increasing plane of nutrition leading up to joining (Vinoles et al., 2009). If these results could be repeated on a larger scale, there is the potential to increase the reproductive efficiency of ewes through an additive effect of increasing fertility and the number of lambs weaned per ewe joined as well as alter the sex of lambs towards a preferred gender.

7.0 Phase 4: Border Leicester x merino first cross maiden ewes

7.1 Introduction

In our previous study, the proportion of female lambs was significantly higher when Border Leicester (BL) x Merino first cross ewes were fed a diet high in omega-6 (low in omega-3 fatty acids) for 6 weeks prior to and, 3 weeks following, mating (Gulliver et al., 2013b). Preliminary evidence also indicated that the concentration of omega-3 (EPA and DHA) in the plasma of lambs was significantly lower when their dams were fed omega-6 compared with omega-3 at joining (unpublished observations). The effect of diet previously fed to dams on the subsequent sex ratio in the second generation of lambs is unknown. Therefore, the aim of this Phase of the study was to determine the intergenerational effects of diets high in omega-3 and omega-6 fatty acids and, specifically, to determine whether the proportion of female of lambs was higher when BL x Merino maiden ewes were fed a diet high in omega-6 compared with omega-3 fatty acids and whether the effect was mediated by diet previously fed to ewe dams

As indicated above, this project involved 4 Phases. Phase 4 involved a pen study with the BL x Merino first cross maiden ewes bred from the Merino ewes in Phase 2 in 2011. The current section presents details of this pen feeding study. Details of methodology specific to the study will be presented, including details of the diet used and animal management as well as results for all physical and reproduction outcomes.

7.2 Methods

7.2.1 Experimental design

The study was a 2 x 2 factorial randomised controlled design and commenced in May 2013 (WWAI Study ID: 13-01). Maiden ewes were allocated to treatment group based on the diet previously fed to their dam at joining. Details of experimental procedures are outlined below.

7.2.2 Animals

A total of 130 BL x Merino maiden ewes (12 months of age, see Table 7.1) bred as part of Phase 2 of the current project in 2011 were vaccinated against clostridial diseases and caseous lymphadenitis (CLA, Glanvac 6, Pfizer Animal Health, West Ryde, Sydney) and treated with a combination anthelmintic drench (HatTrick, Ancare, NSW) prior to enrolment in the study. The study was conducted at the WWAI with pen feeding commencing in May (Autumn) and lambing occurring in October 2013.

7.2.3 Experimental diets

Ewes were randomly allocated to 1 of 2 treatment groups (as indicated in Section 3.8) according to diet previously fed to the ewe dam, fat score (FS, mean = 3.69 ± 0.05) then live weight (mean = 53.7 ± 0.46 kg). Treatments consisted of either a diet based on lucerne chaff (n = 65) high in omega-3 or a diet based on oat grain and CSM high in omega-6 (n = 65) fatty acids (Table 7.2).

Table 7.1 Number of BL x Merino maiden ewes enrolled into the study.

Source	High Omega-3 (Lucerne)	High Omega-6 (Oats/CSM)	Total
Dam Omega-3	25	28	53
Dam Omega-6	34	31	65
Dam Unknown[1]	6	6	12
Total	65	65	**130**

[1]A number of maiden ewes lost ear tags prior to the commencement of the study and could not be positively identified with their dam.

Table 7.2 Components and proximate analysis of diets offered to BL x Merino maiden ewes for 6 weeks prior to and 17 days following mating.

Ingredients	Treatment Diet	
	High Omega-3 (Lucerne)	High Omega-6 (Oats/CSM)
Inclusion	**(%DM)**	
Lucerne	87.01	21.00
Oat grain	0.00	70.34
Cottonseed Meal	0.00	6.68
Molasses	12.01	0.00
Urea	0.00	0.99
Mineral Premix[1]	0.97	0.99
Proximate Analysis	**(%DM)**	
Neutral Detergent Fibre	43.05	43.42
Acid Detergent Fibre	29.69	23.58
Crude Protein	19.73	13.39
Total Lipid	1.90	4.02
ME (MJ/kg DM)	10.39	10.95
Fatty Acid Composition	**g/kg DM (% Total Fatty Acids)**	
C14:0	0.13 (1.54)	0.10 (0.21)
C16:0	2.70 (32.8)	6.11 (12.7)
C18:0	0.49 (5.88)	0.71 (1.47)
C18:1n-9	0.34 (4.12)	13.15 (27.3)
C18:1n-7	0.11 (1.31)	0.19 (0.40)
C18:2n-6	1.85 (22.4)	11.12 (23.1)
C18:3n-3	2.40 (29.1)	0.93 (1.94)
Ratio of n-6:n-3[2]	0.77	11.90
Feed Offered[3]	**(per head)**	
DM (kg/day)	0.92	0.81
ME (MJ/day)	9.60	8.85
CP (g/day)	182.31	108.19

[1]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 10 g/head per day.
[2]Ratio of n-6:n-3 = ratio of omega-6 to omega-3 fatty acids.
[3]DM = dry matter, ME = metabolisable energy, CP = crude protein.

7.2.4 Feeding and ewe management

Ewes were housed in 1 of 8 pens (15-17 ewes/pen). The majority of ewes in both treatment groups showed oestrus in Cycle 1 (high omega-3 = 88.2%, high omega-6 = 98.6%). Pen feeding was, therefore, ceased for all ewes 17 days after the last ewe showed oestrus in Cycle 1. The timing of insemination (Cycle 1 or 2) was confirmed for each ewe at pregnancy scanning and parturition.

7.2.5 Animal withdrawals during the study

One ewe (fed the High omega-3 diet) was removed from pen feeding prior to joining due to a lung infection. Details of animal withdrawals can be found in Appendix 3.

7.2.6 Data collection

Ewe weight and fat score was assessed during pen feeding as described previously (Section 3.10). Blood samples were also collected from a sub-set of ewes in each pen for fatty acid and hormone analysis as described previously. Pregnancy scanning was conducted 63 or 46 days after the last ewe showed oestrus in Cycle 1 or Cycle 2, respectively.

7.2.7 Ewe management during parturition

Ewes lambed in 1 of 6 paddocks depending on whether ewes were scanned as conceiving in Cycle 1 or 2 and carrying single or twin foetuses (Table 7.3). Ewes from each dietary treatment group scanned as having single or multiple foetuses were allocated to lamb in separate paddocks, with 3 replicates per dietary treatment (Table 7.3). Ewes were branded with a series of 1, 2 or 3 numbers (0 or 1) that was coded to their individual ear tag number for identification during lambing (Plate 7.1).

Table 7.3 Details of lambing paddocks used for BL x Merino maiden ewes.

Paddock	Pasture	Cycle	Scanned	Dietary Treatment	Number
1	Clover/Ryegrass	1	Multiple	High Omega-6	21
2	Clover/Ryegrass	1	Single	High Omega-3	19
3	Clover/Ryegrass	1 and 2	Multiple	High Omega-3	22
4	Clover/Ryegrass	1	Single/Multiple	High Omega-6	20
5	Mixed pasture	1 and 2	Single	High Omega-3	27
6	Mixed pasture	1 and 2	Single/Multiple	High Omega-6	20
Total					122

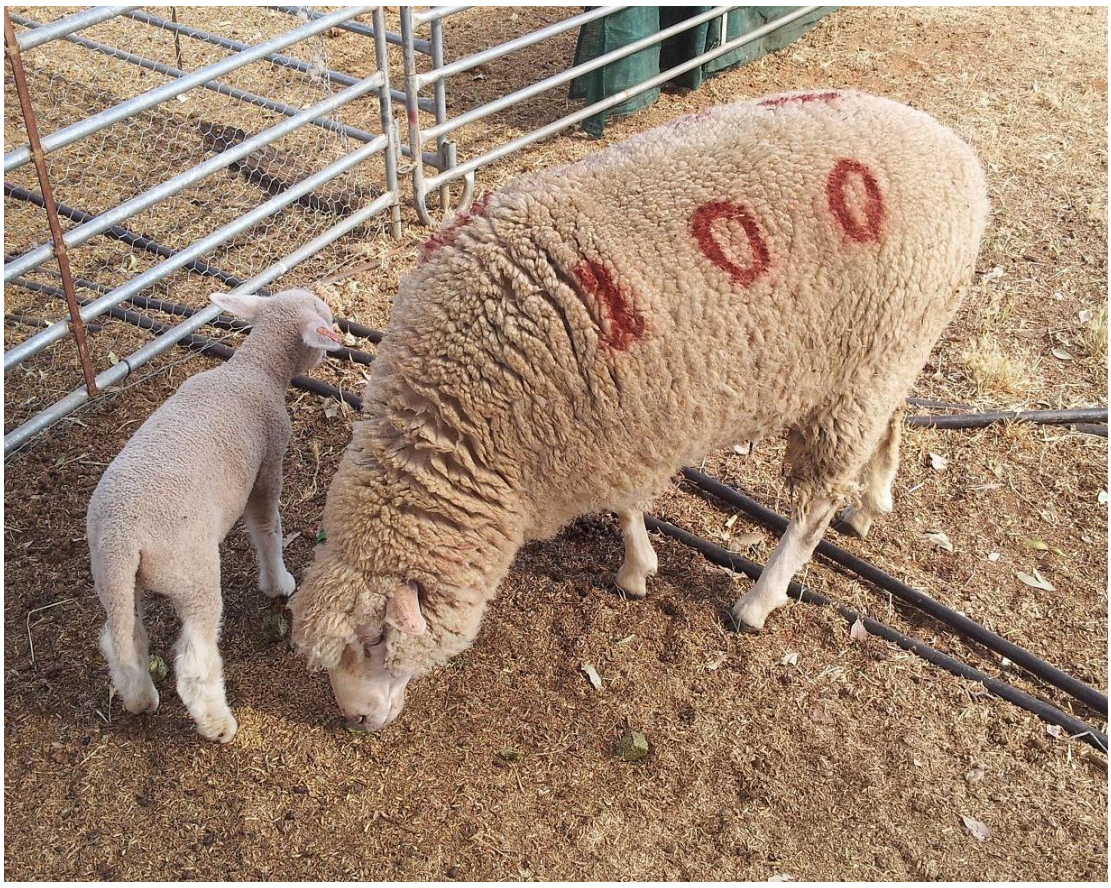


Plate 7.1 Identification of Border Leicester x Merino maiden ewes at lambing.

7.3 Results

7.3.1 Ewe weight and fat score

Ewe weight did not increase significantly ($p > 0.05$) over the time of feeding experimental diets and was not significantly ($p = 0.074$) higher at the completion of pen feeding when all ewes were fed a diet high in omega-6 fatty acids compared with omega-3 fatty acids. When ewe dams had previously been fed omega-6 at joining, however, weight at the completion of pen feeding ($p = 0.030$) and preg scanning ($p = 0.040$) was significantly higher when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids (Figure 7.1B). Ewe weight increased significantly over the entire experimental period between pen feeding and pre-lambing (Table 7.4).

Ewe fat score was not significantly ($p = 0.380$) higher when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids (Table 7.3). Ewe fat score was also not significantly higher at the completion of pen feeding ($p = 0.208$), at preg-scanning ($p = 0.147$) and prior to parturition ($p = 0.616$) when ewes were fed the high omega-6 compared with high omega-3 diet at joining and their dams had also previously been fed omega-6 (Figure 7.2B) at joining. The interaction between diet, dam diet and time of feeding was also not significant ($p = 0.669$) indicating that there was not a differential change in fat score over time depending on the diet fed to the maiden dams (Figures 7.2A and B).

Table 7.4 Mean weight and fat score of BL x Merino maiden ewes following the consumption of a High omega-3 diet based on silage or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating showing the main effects of diet and day of feeding.

			p-values		
Measure	High Omega-3	High Omega-6	Diet	Day	Diet x Day
Weight (kg)	58.4 (± 0.78)	59.2 (± 0.77)	0.503	< 0.001	< 0.001
Fat Score	3.70 (± 0.05)	3.76 (± 0.05)	0.380	< 0.001	0.822

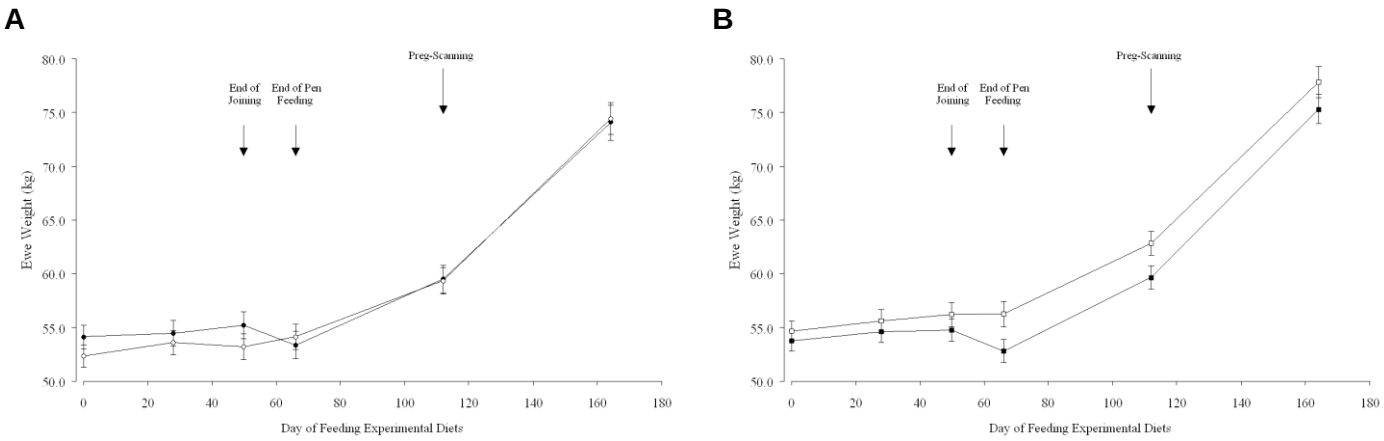


Figure 7.1 Mean weight of BL x Merino maiden ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for 42 days prior to and 17 days following mating when their dams were previously fed (A) a diet high in omega-3 (● maiden omega-3, ○ maiden omega-6) or (B) omega-6 (■ maiden omega-3, □ maiden omega-6).

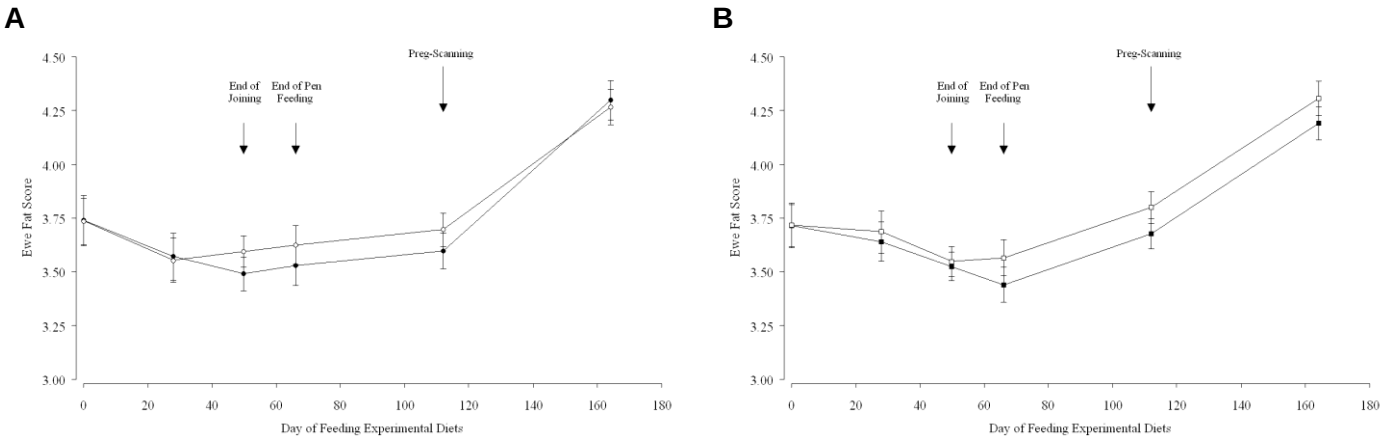


Figure 7.2 Mean fat score of BL x Merino maiden ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for 42 days prior to and 17 days following mating when their dams were previously fed (A) a diet high in omega-3 (● maiden omega-3, ○ maiden omega-6) or (B) omega-6 (■ maiden omega-3, □ maiden omega-6).

7.3.2 Plasma fatty acid proportions

The proportion of all omega-3 fatty acids including C18:3n-3, C20:5n-3 (EPA) and C22:5n-3 (DPA) were significantly ($p < 0.001$) higher when ewes were fed a diet high in omega-3 fatty acids compared with omega-6 fatty acids and ewe dams were previously fed either a High n-3 or High n-6 diet at mating (Table 7.5). The interaction between diet and Dam diet (Dam n-3 or Dam n-6) was not significant for the proportion of C18:3n-3 ($p = 0.192$), C20:5n-3 ($p = 0.750$) or C22:6n-3 ($p = 0.201$). The proportion of ALA and EPA decreased significantly ($p < 0.001$) following the introduction of the high omega-6 diet (Figures 7.3A and B) and remained relatively constant while ewes were fed the experimental rations.

The proportion of the omega-6 fatty acids C18:2n-6, C18:3n-6 and C20:3n-6 were significantly ($p < 0.001$) higher when ewes were fed a diet high in omega-6 fatty acids compared with omega-3 fatty acids and ewe dams were previously fed either a High n-3 or High n-6 diet at mating (Table 7.5). The interaction between diet and Dam diet (Dam n-3 or Dam n-6) was not significant for the proportion of any omega-6 fatty acids measured. The proportion of C18:2n-6 increased significantly ($p < 0.001$) following the introduction of the high omega-6 diet (Figures 7.4A and B) and remained relatively constant while ewes were fed the experimental rations.

Table 7.5 Mean proportion (% total identified FAME) of fatty acid methyl esters in the plasma of BL x Merino maiden ewes following the consumption of a High omega-3 diet based on lucerne or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating.

	High Omega-3 (Lucerne)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Dam Omega-3[2]	Dam Omega-6	Dam Omega-3	Dam Omega-6	Diet	Dam Diet
SFA						
C8:0	1.40 (± 0.11)	1.21 (± 0.11)	1.08 (± 0.11)	1.04 (± 0.11)	0.041	0.308
C9:0	-	-	-	-	-	-
C10:0	0.75 (± 0.05)	0.84 (± 0.05)	0.55 (± 0.05)	0.55 (± 0.05)	< 0.001	0.355
C11:0	0.61 (± 0.04)	0.65 (± 0.04)	0.44 (± 0.04)	0.42 (± 0.04)	< 0.001	0.795
C12:0	0.27 (± 0.03)	0.20 (± 0.03)	0.19 (± 0.03)	0.19 (± 0.03)	0.089	0.119
C14:0	0.81 (± 0.03)	0.80 (± 0.03)	0.64 (± 0.03)	0.65 (± 0.03)	< 0.001	0.936
iC15:0	0.21 (± 0.01)	0.20 (± 0.01)	0.15 (± 0.01)	0.15 (± 0.01)	< 0.001	0.454
aiC15:0	0.24 (± 0.01)	0.25 (± 0.01)	0.22 (± 0.01)	0.21 (± 0.01)	0.018	0.947
C15:0	0.62 (± 0.02)	0.64 (± 0.02)	0.46 (± 0.02)	0.43 (± 0.02)	< 0.001	0.743
C16:0	16.6 (± 0.14)	16.7 (± 0.14)	16.2 (± 0.14)	16.2 (± 0.14)	0.008	0.615
iC17:0	0.72 (± 0.02)	0.75 (± 0.02)	0.62 (± 0.02)	0.58 (± 0.02)	< 0.001	0.915
aiC17:0	0.48 (± 0.03)	0.48 (± 0.03)	0.31 (± 0.03)	0.32 (± 0.03)	< 0.001	0.895
C17:0	1.37 (± 0.02)	1.36 (± 0.02)	0.83 (± 0.02)	0.83 (± 0.02)	< 0.001	0.756
C18:0	18.6 (± 0.27)	18.3 (± 0.27)	19.8 (± 0.27)	19.9 (± 0.27)	< 0.001	0.592
C20:0	0.14 (± 0.01)	0.14 (± 0.01)	0.16 (± 0.01)	0.15 (± 0.01)	0.202	0.409
C21:0	0.09 (± 0.01)	0.09 (± 0.01)	0.07 (± 0.01)	0.07 (± 0.01)	0.028	0.966
C22:0	0.30 (± 0.01)	0.29 (± 0.01)	0.30 (± 0.01)	0.30 (± 0.01)	0.669	0.775
C23:0	0.66 (± 0.02)	0.66 (± 0.02)	0.53 (± 0.02)	0.57 (± 0.02)	< 0.001	0.215
C24:0	0.40 (± 0.02)	0.37 (± 0.02)	0.40 (± 0.02)	0.38 (± 0.02)	0.685	0.334
Total SFA	44.1 (± 0.34)	43.9 (± 0.33)	42.9 (± 0.33)	42.9 (± 0.33)	0.003	0.671
MUFA						
C11:1n-1	0.15 (± 0.01)	0.07 (± 0.01)	0.09 (± 0.01)	0.07 (± 0.01)	0.015	0.001
C12:1n-7	0.19 (± 0.02)	0.15 (± 0.02)	0.15 (± 0.02)	0.12 (± 0.02)	0.057	0.055
C13:1n-1	0.11 (± 0.01)	0.06 (± 0.01)	0.07 (± 0.01)	0.06 (± 0.01)	0.016	0.002
C14:1n-5	0.023 (± 0.00)	0.023 (± 0.00)	0.015 (± 0.00)	0.018 (± 0.00)	< 0.001	0.431
C15:1n-5	0.03 (± 0.00)	0.03 (± 0.00)	0.02 (± 0.00)	0.03 (± 0.00)	0.070	0.985
C16:1n-7t	0.26 (± 0.02)	0.28 (± 0.02)	0.24 (± 0.02)	0.26 (± 0.02)	0.227	0.202
C16:1n-7	1.21 (± 0.04)	1.3 (± 0.04)	1.24 (± 0.04)	1.17 (± 0.04)	0.302	0.809
C17:1n-7	0.17 (± 0.01)	0.17 (± 0.01)	0.1 (± 0.01)	0.11 (± 0.01)	< 0.001	0.894
C18:1n9t	0.23 (± 0.02)	0.22 (± 0.02)	0.42 (± 0.02)	0.37 (± 0.02)	< 0.001	0.149
C18:1n7t	1.25 (± 0.09)	1.25 (± 0.09)	1.03 (± 0.09)	1.14 (± 0.09)	0.067	0.565
C18:1n-12	0.21 (± 0.01)	0.19 (± 0.01)	0.26 (± 0.01)	0.27 (± 0.01)	< 0.001	0.488
C18:1n-9	18.6 (± 0.44)	19.3 (± 0.43)	19.9 (± 0.43)	19.8 (± 0.43)	0.048	0.436
C18:1n-7	0.70 (± 0.03)	0.75 (± 0.03)	0.66 (± 0.03)	0.66 (± 0.03)	0.044	0.401
C19:1n-12	0.04 (± 0.01)	0.04 (± 0.01)	0.02 (± 0.01)	0.02 (± 0.01)	0.002	0.876
C20:1n-15	0.02 (± 0.00)	0.02 (±0.00)	0.02 (±0.00)	0.03 (±0.00)	0.166	0.562
C20:1n-12	0.04 (±0.00)	0.04 (±0.00)	0.04 (±0.00)	0.04 (±0.00)	0.490	0.791
C20:1n-9	0.08 (± 0.01)	0.08 (± 0.01)	0.10 (± 0.01)	0.10 (± 0.01)	0.001	0.506

	High Omega-3 (Lucerne)		High Omega-6 (Oats/CSM)		*p*-values[3]	
FAME[1]	Dam Omega-3[2]	Dam Omega-6	Dam Omega-3	Dam Omega-6	Diet	Dam Diet
C22:1n-9	0.08 (± 0.01)	0.06 (± 0.01)	0.06 (± 0.01)	0.06 (± 0.01)	0.049	0.192
C24:1n-9	0.34 (± 0.01)	0.33 (± 0.01)	0.41 (± 0.01)	0.39 (± 0.01)	< 0.001	0.317
Total MUFA	23.7 (± 0.5)	24.4 (± 0.49)	24.8 (± 0.49)	24.8 (± 0.49)	0.159	0.515
n-3 PUFA						
C18:3n-3	4.62 (± 0.15)	4.42 (± 0.15)	2.58 (± 0.15)	2.78 (± 0.15)	< 0.001	0.982
C18:4n-3	0.14 (± 0.01)	0.14 (± 0.01)	0.10 (± 0.01)	0.10 (± 0.01)	< 0.001	0.962
C20:3n-3	0.05 (± 0.00)	0.05 (±0.00)	0.03 (±0.00)	0.04 (±0.00)	< 0.001	0.972
C20:5n-3	1.88 (± 0.07)	1.91 (± 0.07)	1.48 (± 0.07)	1.56 (± 0.07)	< 0.001	0.431
C22:5n-3	2.32 (± 0.06)	2.40 (± 0.06)	1.65 (± 0.06)	1.77 (± 0.06)	< 0.001	0.143
C22:6n-3	2.76 (± 0.11)	2.65 (± 0.11)	1.81 (± 0.11)	1.97 (± 0.11)	< 0.001	0.784
Total n-3	11.68 (± 0.26)	11.47 (± 0.26)	7.60 (± 0.26)	8.16 (± 0.26)	< 0.001	0.500
n-6 PUFA						
C18:2n-6t	0.11 (± 0.01)	0.10 (± 0.01)	0.05 (± 0.01)	0.06 (± 0.01)	< 0.001	0.967
C18:2n-6	15.6 (± 0.46)	15.1 (± 0.46)	19.6 (± 0.46)	18.9 (± 0.46)	< 0.001	0.197
C18:3n-6	0.31 (± 0.02)	0.34 (± 0.02)	0.47 (± 0.02)	0.42 (± 0.02)	< 0.001	0.700
C20:2n-6	0.09 (± 0.01)	0.08 (± 0.01)	0.06 (± 0.01)	0.07 (± 0.01)	0.126	0.985
C20:3n-6	0.24 (± 0.01)	0.23 (± 0.01)	0.29 (± 0.01)	0.27 (± 0.01)	< 0.001	0.093
C20:4n-6	3.96 (± 0.14)	4.19 (± 0.14)	3.98 (± 0.14)	4.26 (± 0.14)	0.748	0.075
C22:2n-6	-	-	-	-	-	-
C22:4n-6	0.11 (± 0.01)	0.15 (± 0.01)	0.14 (± 0.01)	0.13 (± 0.01)	0.730	0.226
C22:5n-6	0.07 (± 0.01)	0.07 (± 0.01)	0.08 (± 0.01)	0.07 (± 0.01)	0.808	0.335
Total n-6	20.5 (± 0.53)	20.2 (± 0.52)	24.7 (± 0.52)	24.2 (± 0.52)	< 0.001	0.484
Total ID	750.5 (± 28.88)	781.6 (± 28.65)	1038.5 (± 28.7)	1079.2 (± 28.7)	< 0.001	0.221
n-6:n-3	1.76 (± 0.20)	1.76 (± 0.20)	3.82 (± 0.20)	3.44 (± 0.20)	< 0.001	0.362
DHADI	0.78 (± 0.23)	0.70 (± 0.24)	1.29 (± 0.23)	0.74 (± 0.23)	0.239	0.179
DHASI	48.21 (± 5.71)	43.54 (± 5.7)	29.64 (± 5.7)	37.56 (± 5.7)	0.040	0.777
EFI	1.55 (± 0.05)	1.47 (± 0.05)	1.47 (± 0.05)	1.48 (± 0.05)	0.489	0.496
P:S	0.73 (± 0.02)	0.73 (± 0.02)	0.76 (± 0.02)	0.76 (± 0.02)	0.058	0.948

[1]FAME = fatty acid methyl ester, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, n-3 PUFA = omega-3 polyunsaturated fatty acids, n-6 PUFA = omega-6 polyunsaturated fatty acids, Total ID = total concentration of identified fatty acids (μg/mL), n-6:n-3 = ratio of n-6 PUFA : n-3 PUFA, DHADI = DHA Deficiency Index, 22:5n-6 / C22:4n-6 (Neuringer et al., 1986), DHASI = DHA Sufficiency Index, C22:6n-3 / C22:5n-6 (Hoffman and Uauy, 1992), EFI = Essential Fatty Acid Status Index, ratio of (n-3 PUFA + n-6 PUFA) : (n-7 MUFA + n-9 MUFA), P:S = ratio of (n-3 PUFA + n-6 PUFA) : SFA.

[2]Dam omega-3 = High omega-3 diet previously fed to ewe dams at joining, Dam omega-6 = High omega-6 diet previously fed to ewe dams at joining. Ewes in the current study were fed experimental rations for 42 days prior to mating and 17 days post-mating.

[3]The *p*-value for Diet x Dam Diet represents the interaction between dietary treatment group and diet previously fed to ewe dams (High omega-3 or High omega-6).

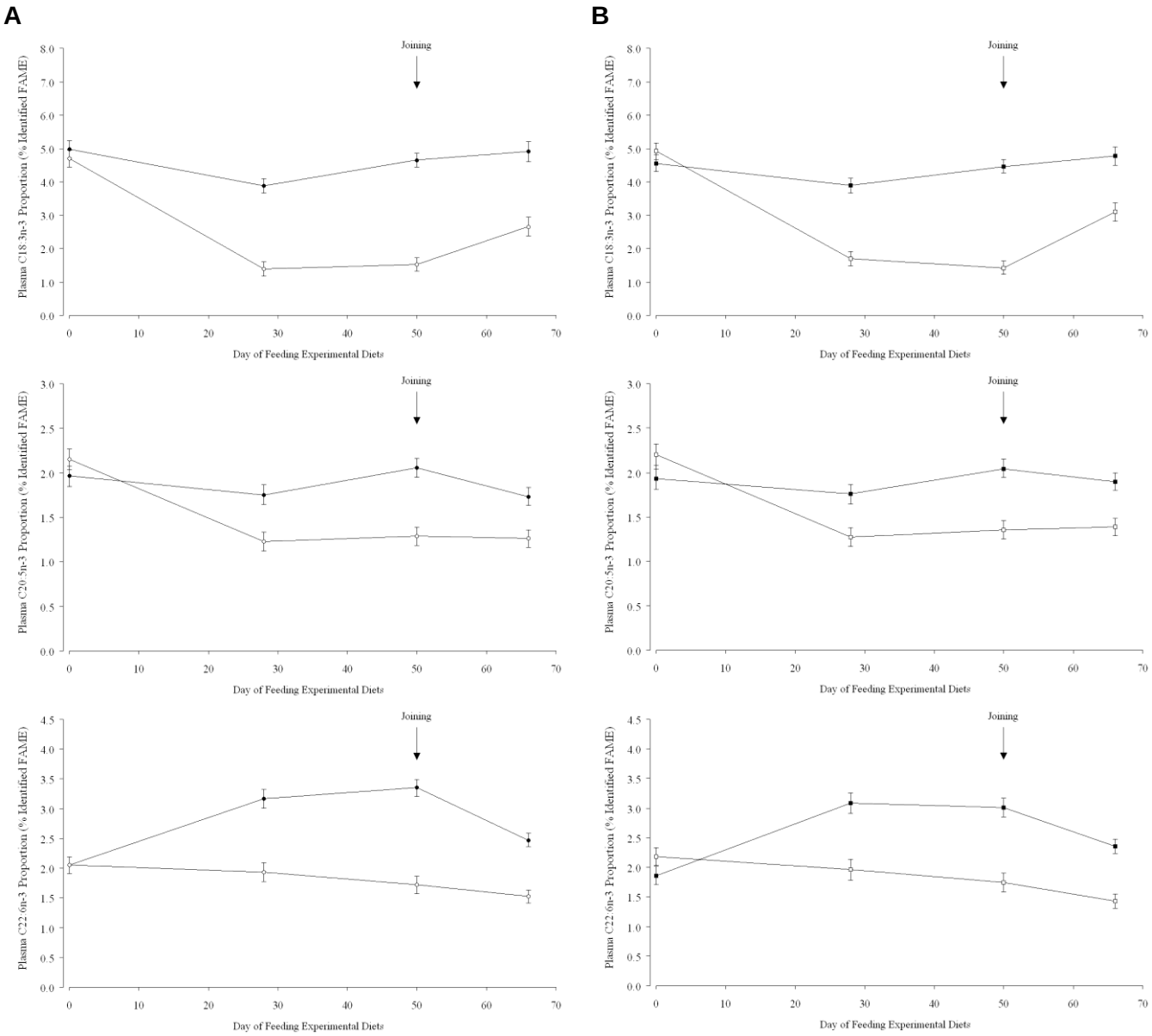


Figure 7.3 Mean concentration of α-linolenic acid (ALA, C18:3n-3), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in the plasma of BL x Merino maiden ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for 42 days prior to and 17 days following mating when their dams were previously fed (A) a diet high in omega-3 (● maiden omega-3, ○ maiden omega-6) or (B) high in omega-6 (■ maiden omega-3, □ maiden omega-6).

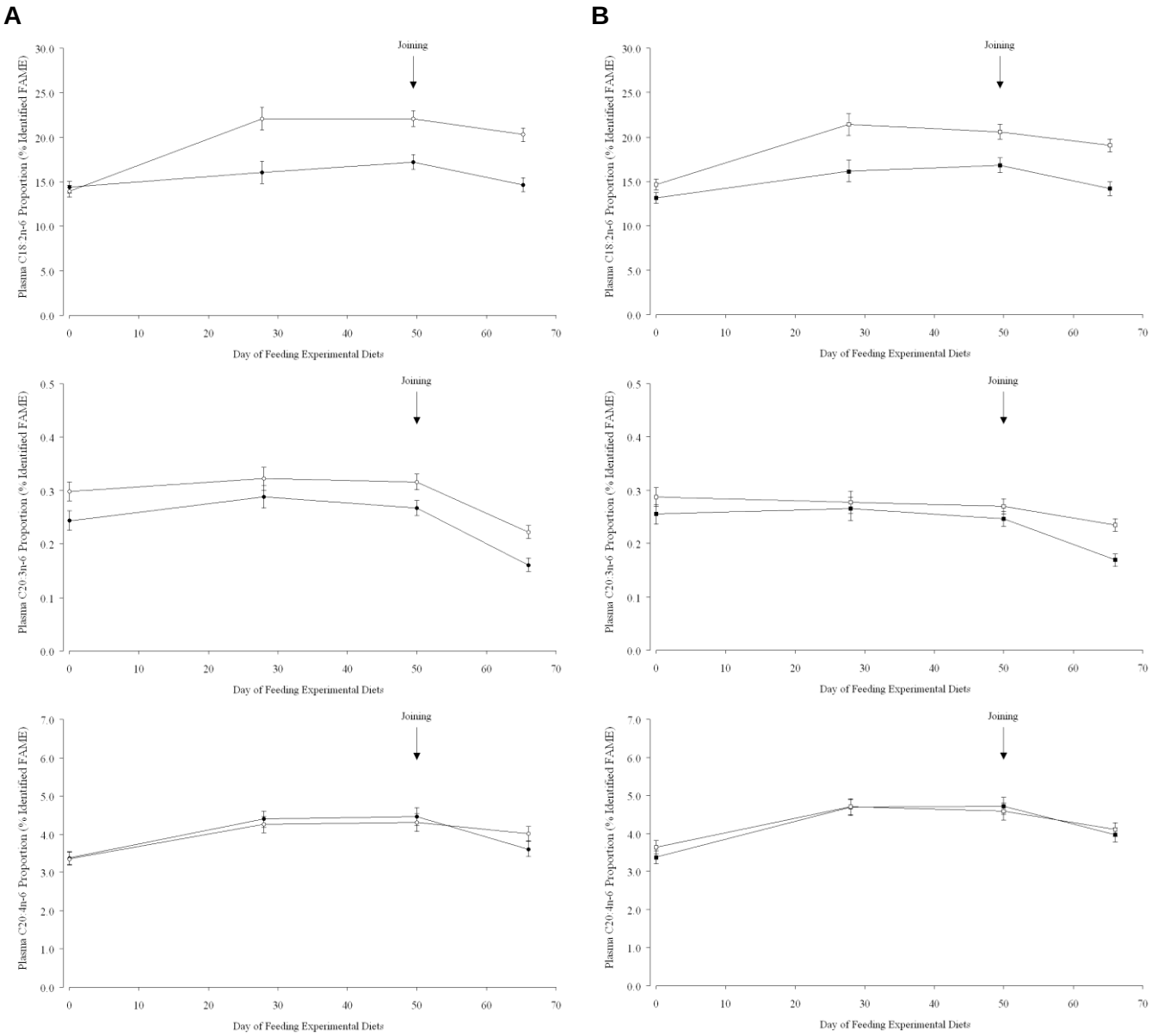


Figure 7.4 Mean concentration of linoleic acid (LA, C18:2n-6), dihomo-γ-linolenic acid (DGLA, C20:3n-6) and arachidonic acid (ARA, C20:4n-3) in the plasma of BL x Merino maiden ewes following the consumption of a diet high in omega-3 or omega-6 fatty acids for 42 days prior to and 17 days following mating when their dams were previously fed (A) a diet high in omega-3 (● maiden omega-3, ○ maiden omega-6) or (B) high in omega-6 (■ maiden omega-3, □ maiden omega-6).

7.3.3 Plasma hormone concentrations

Progesterone (P_4)

The mean concentration of plasma progesterone was not significantly (p = 0.614) different when ewes were fed the high omega-6 diet (2.95 ± 0.22 ng/mL) compared with the high omega-3 diet (2.79 ± 0.22 ng/mL) for 42 days prior to mating (Figure 7.5A). The percentage change in progesterone concentration from baseline was also not significantly (p = 0.086) different when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids prior to mating (Figure 7.5B).

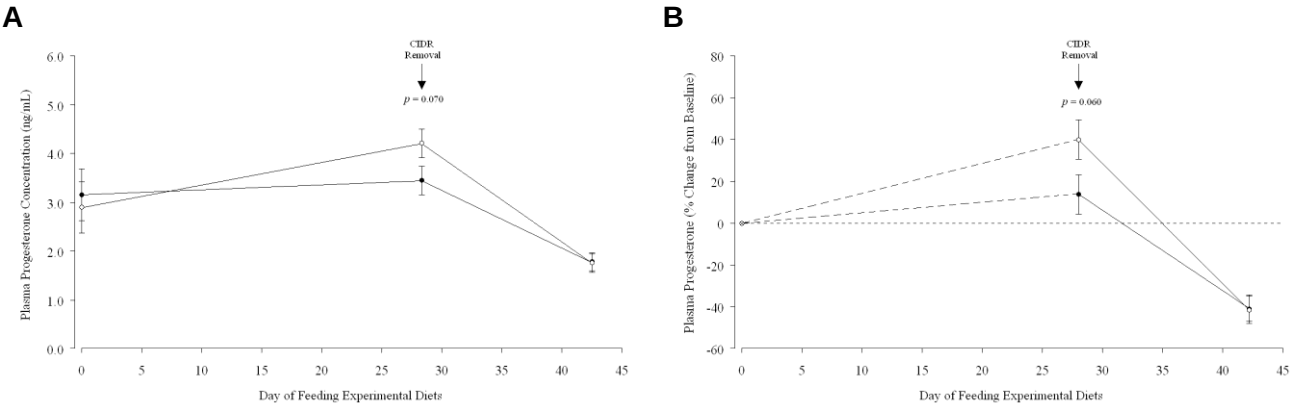


Figure 7.5 Mean (A) concentration of plasma progesterone (ng/mL) and (B) the percentage change in progesterone from baseline for BL x Merino maiden ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating. Statistical difference between treatment diets (A) p = 0.614, (B) p = 0.086.

Oestradiol (E_2)

The mean concentration of plasma oestradiol was not significantly (p = 0.388) higher when ewes were fed the high omega-6 diet (0.93 ± 0.08 pg/mL) compared with the high omega-3 diet (0.83 ± 0.08 pg/mL) for 42 days prior to mating (Figure 76A). The percentage change in oestradiol concentration from baseline was also not significantly (p = 0.089) different when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids prior to mating (Figure 7.6B).

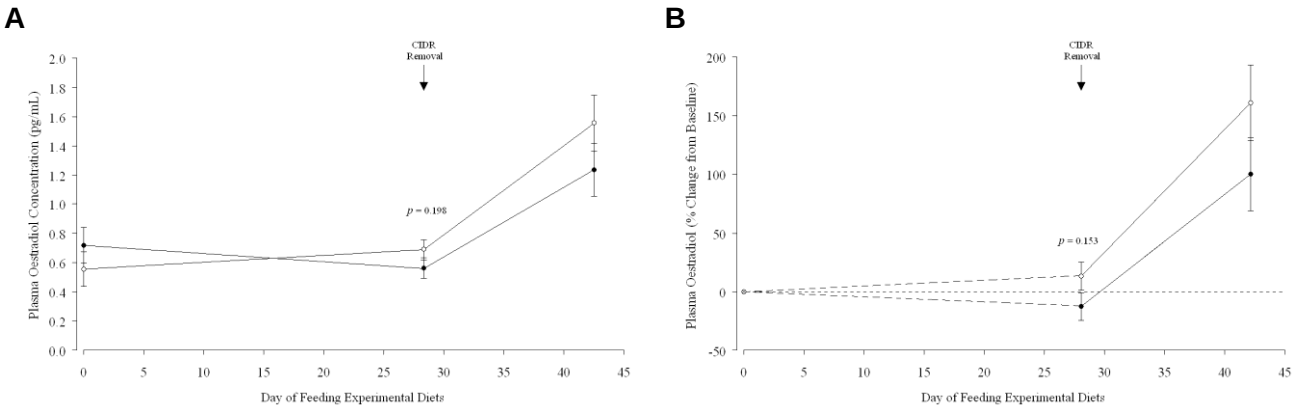


Figure 7.6 Mean (A) concentration of plasma oestradiol (pg/mL) and (B) the percentage change in oestradiol from baseline for BL x Merino maiden ewes following the consumption of a diet high in omega-3 (●) or omega-6 (○) fatty acids for 42 days prior to mating. Baseline progesterone concentrations for the High omega-3 and High omega-6 diets were 2.45 ± 0.65 and 3.77 ± 0.62 pg/mL respectively and were included in the statistical analysis as a co-variate. Significant difference between treatment diets (A) p = 0.058, (B) p = 0.967.

7.3.4 Time of oestrus and parturition

Details of the results for oestrus and parturition will be presented in the following section. Results for parturition will be presented with the main effects of diet fed to the maidens and whether their dams were also fed omega-3 or omega-6 at joining.

Oestrus

The proportion of ewes showing oestrus was not significantly higher when ewes were fed the high omega-3 diet compared with the high omega-6 diet prior to mating (Table 7.6). The cumulative proportion of ewes showing oestrus over time was also not significantly greater (Relative Risk = 1.02, p = 0.914, Figure 7.7) when ewes were fed the high omega-3 diet compared with the high omega-6 diet.

The time from CIDR removal to the first day any ewe showed oestrus was 16 days. The mean time to oestrus from the first day any ewe showed oestrus was significantly (p = 0.018) shorter when ewes were fed the high omega-6 diet compared with the high omega-3 diet (Table 7.6). The effect of the high omega-6 diet on decreasing the time to oestrus appeared to be greatest when ewe dams were also previously fed a high omega-6 diet at joining (Figure 7.8).

Table 7.6 Proportion of BL x Merino maiden ewes showing oestrus or lambed and the time to oestrus or parturition following the consumption of a diet based on either lucerne (High Omega-3) or oats and cottonseed meal (High Omega-6) for 42 days prior to and, 17 days following, mating.

Outcome[1]	Treatment		*p*-values
	High Omega-3 (Lucerne)	High Omega-6 (Oats/CSM[2])	
Oestrus			
Proportion of ewes showing oestrus (%)	96.5%	86.6%	0.059
Relative Risk of Oestrus (Cox's PHR[3])	1.02	1.00	0.914
Mean time to behavioural oestrus (Days)	4.31 (± 0.15)	3.79 (± 0.16)	0.018
Parturition			
Proportion of ewes lambed[4] (%)	96.4%	93.2%	0.448
Relative Risk of lambing (Cox's PHR)	1.00	1.05	0.788
Mean time to parturition (Days)	6.30 (± 0.32)	5.54 (± 0.31)	0.095

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means) including all ewes that could be identified with their lamb in each treatment group.
[2]CSM = cottonseed meal.
[3]Cox's PHR = Cox's Proportional Hazards Regression Analysis (Cox, 1972).
[4]Proportion of all ewes joined.

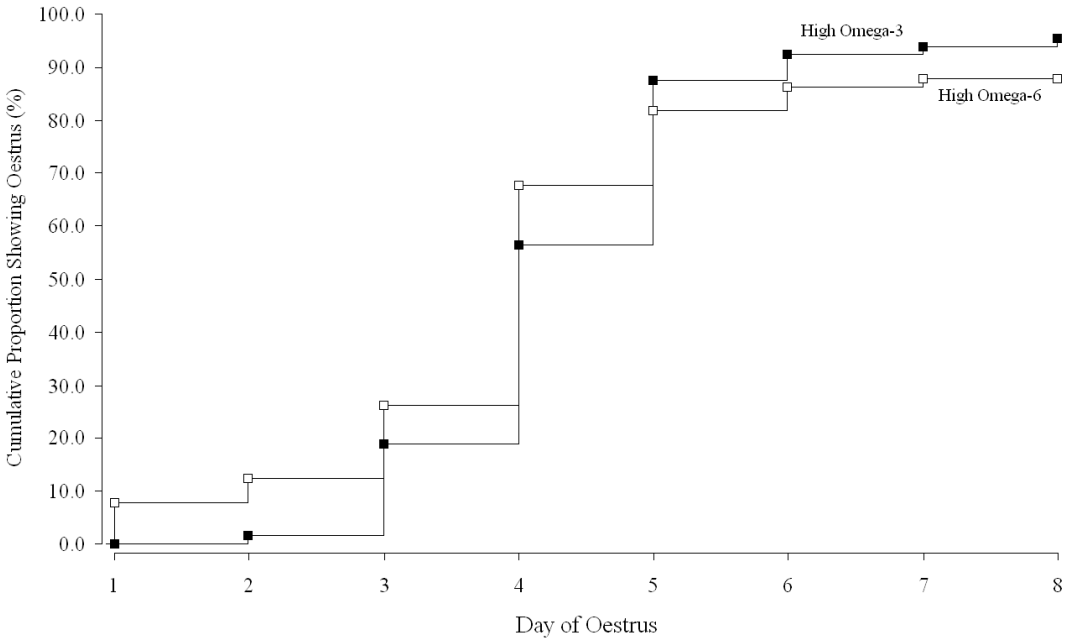


Figure 7.7 Cumulative proportion of of BL x Merino maiden ewes showing behavioural oestrus (from the first day any ewe showed oestrus) following the consumption of a diet high in omega-3 (■) or omega-6 (□) fatty acids for 42 days prior to mating. The time from CIDR removal to the first day any ewe showed oestrus was 16 days. Relative Risk (Cox, 1972) of ewes fed the omega-3 diet showing oestrus = 1.02 (p = 0.914).

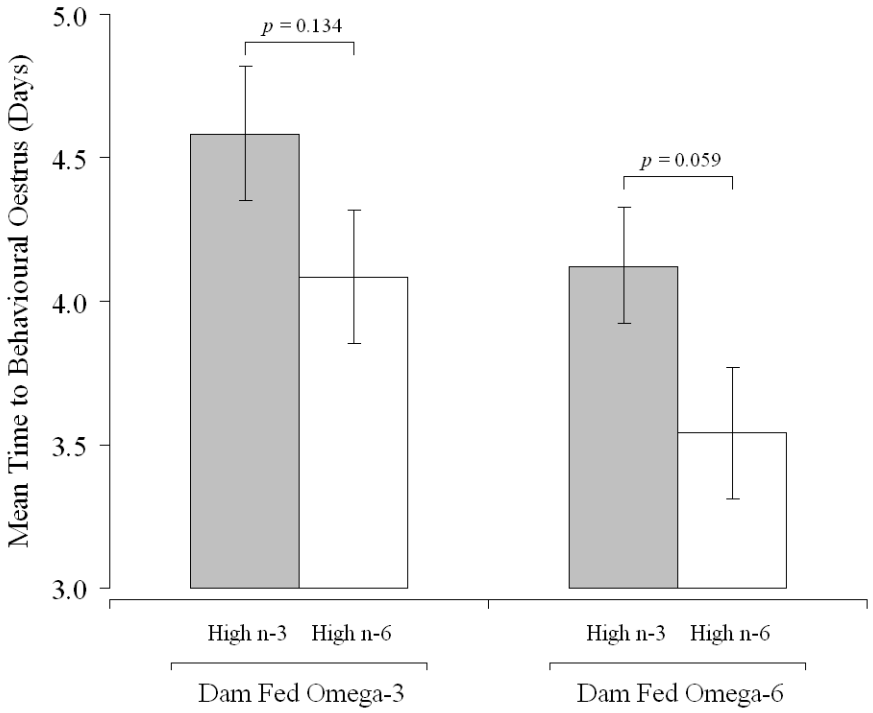


Figure 7.8 Mean time to showing behavioural oestrus (from the first day any ewe showed oestrus) for BL x Merino maiden ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids for 42 days prior to mating when their dams were also previously fed a diet high in omega-3 or omega-6 at mating. The time from CIDR removal to the first day any ewe showed oestrus was 16 days.

Parturition

The proportion of ewes that lambed was not significantly higher (p = 0.448) when ewes were fed the high omega-3 diet compared with the high omega-6 diet prior to or following mating (Table 7.6). Further details outlining the effect of diet previously fed to ewe dams will be shown in Section 7.3.5. The cumulative proportion of ewes that lambed over time was not significantly higher (Relative Risk = 1.05, p = 0.788) when ewes were fed the high omega-6 diet compared with the High omega-3 diet (Figure 7.9).

The first day any ewe lambed was 161 days after CIDR removal and 145 days after the first ewe showed oestrus. The mean time to parturition from the first day any ewe lambed was not significantly shorter when ewes were fed the high omega-6 diet (5.5 ± 0.31 days) compared with the high omega-3 diet (6.3 ± 0.32 days, Table 7.6). The time to parturition was also not significantly shorter when ewes were fed the high omega-6 diet compared with the high omega-3 diet and ewe dams were previously fed a high omega-3 (p = 0.324) or high omega-6 diet (p = 0.788, Figure 7.10). The oestrus to parturition interval was not significantly (p = 0.843) different when ewes were fed the high omega-6 diet compared with the high omega-3 diet (Figure 7.11)

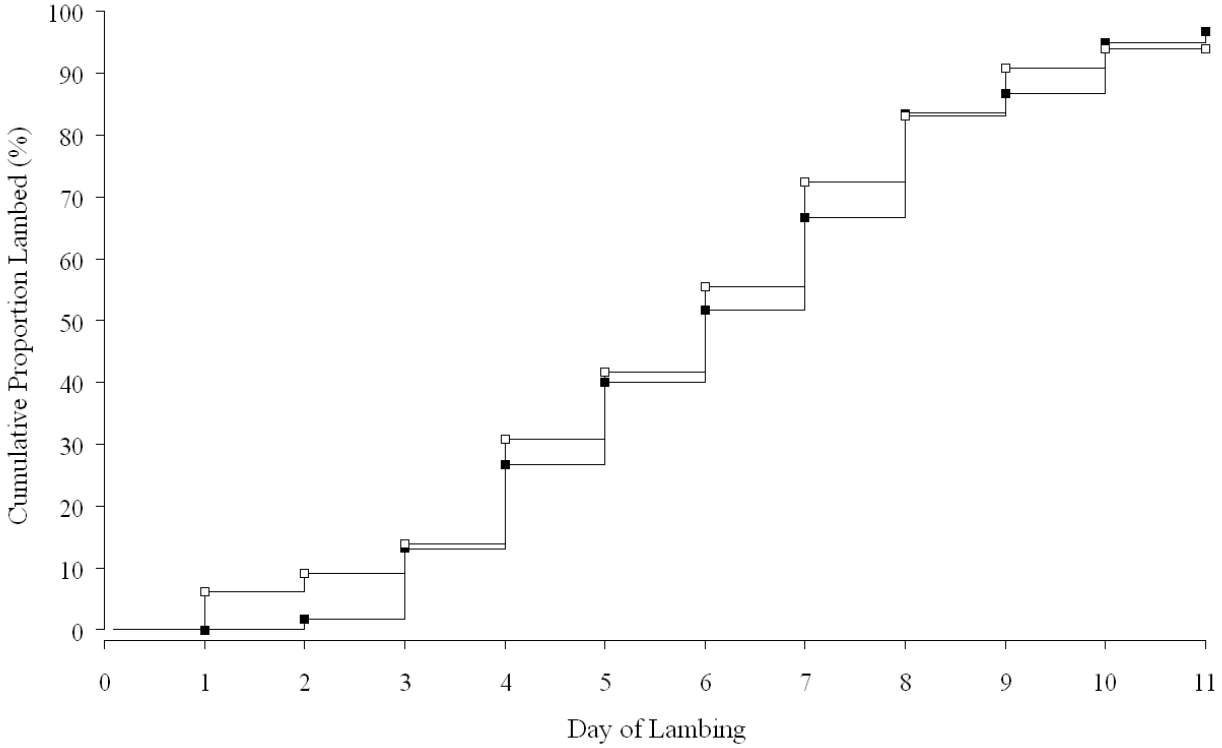


Figure 7.9 Cumulative proportion of BL x Merino maiden ewes that lambed (from the first day any ewe lambed) following the consumption of a diet high in omega-3 (■) or omega-6 (□) fatty acids for 42 days prior to and 17 days following mating. Relative Risk (Cox, 1972) of ewes fed the omega-6 diet lambing = 1.05 (p = 0.788).

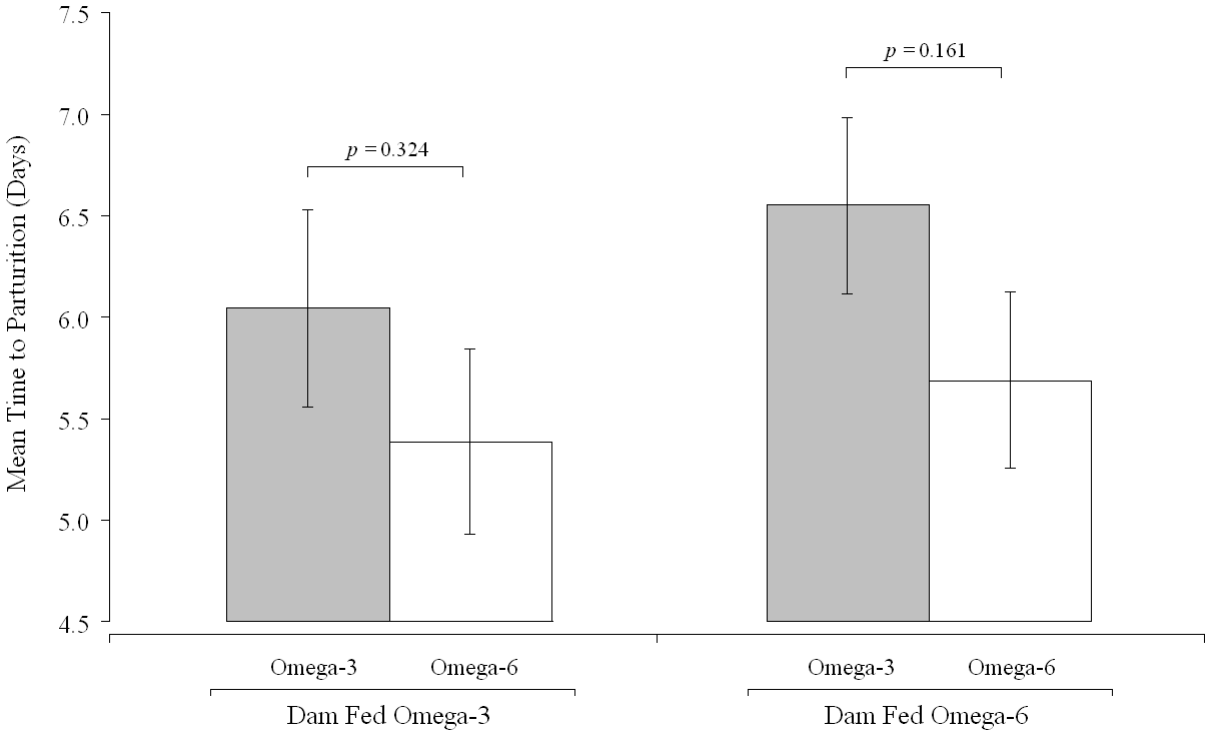


Figure 7.10 Mean time to parturition (from the first day any ewe lambed) for BL x Merino maiden ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids for 42 days prior to and 17 days following mating when their dams were also previously fed a diet high in omega-3 or omega-6 at mating.

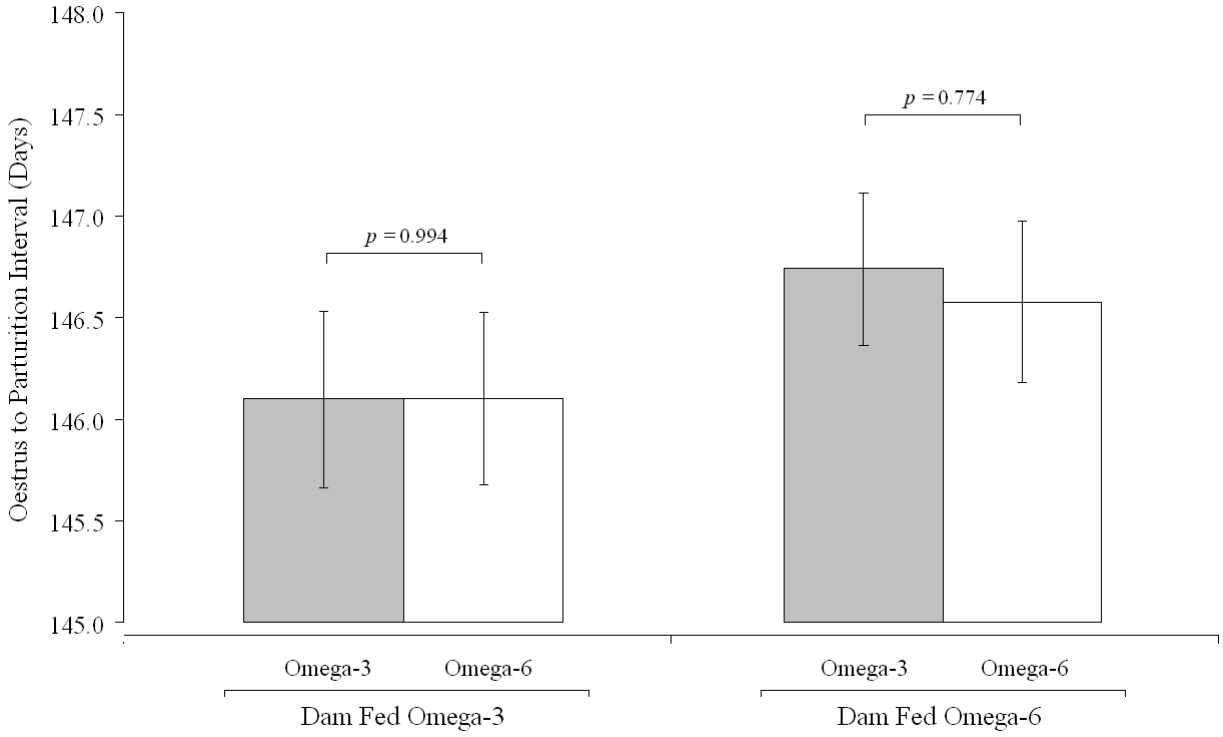


Figure 7.11 Mean oestrus to parturition interval for BL x Merino maiden ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids for 42 days prior to and 17 days following mating when their dams were also previously fed a diet high in omega-3 or omega-6 at mating.

7.3.5 Reproduction outcomes

The proportion of ewes pregnant and the proportion of ewes that lambed was not significantly different when ewes were fed the high omega-3 diet compared with the high omega-6 diet prior to and following mating (Table 7.7). The mean foetal rate and the mean number of lambs born was not significantly different when ewes were fed the diet high in omega-3 compared with the diet high in omega-6 and the interaction between ewe diet and diet previously fed to ewe dams was also not significant for any reproduction parameters (Table 7.7). The mean foetal rate for ewes joined (p = 0.002, Figure 7.12) and the mean number of lambs born per ewe that lambed (p = 0.033) was, however, significantly higher when ewe dams were previously fed a high omega-6 compared with a high omega-3 diet at joining. Although ewe weight at joining was significantly (r^2 = 0.25, p = 0.006) positively correlated with foetal rate, the mean foetal rate for ewes joined was still significantly (p = 0.002) higher when ewe dams were previously fed a high omega-6 (1.58 ± 0.06) compared with a high omega-3 (1.29 ± 0.07) diet at joining after controlling for ewe weight at joining as a co-variate.

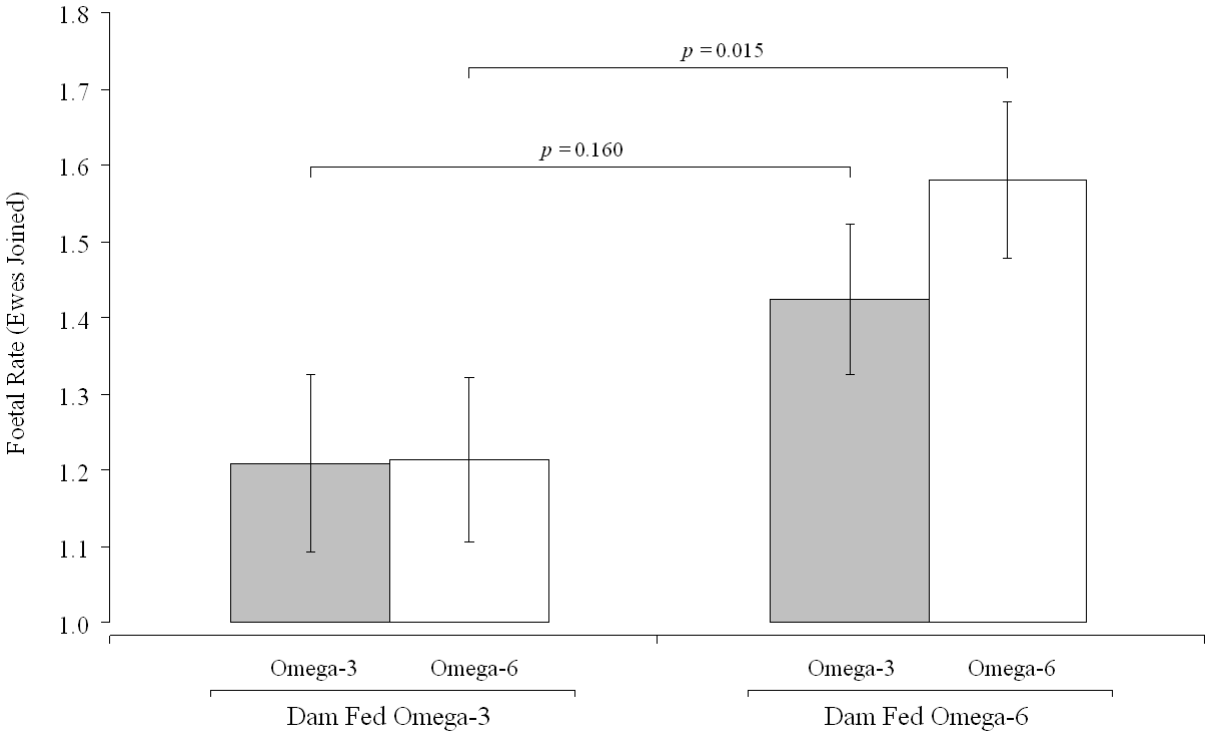


Figure 7.12 Mean foetal rate (foetuses per ewe joined) for BL x Merino maiden ewes following the consumption of a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids for 42 days prior to and 17 days following mating when their dams were also previously fed a diet high in omega-3 or omega-6 at mating.

7.3.6 Sex ratio of lambs

A total of 166 lambs were born and all of those (Males = 81, Females = 85) could be identified with their dam and included in the sex ratio analysis. The proportion of female lambs was not significantly (p = 0.243) higher when ewes were fed the high omega-6 diet (55.8% female) compared with the High omega-3 diet (43.1% female). The proportion of female lambs was also not significantly (p = 0.193) higher when ewes were fed the diet high in omega-6 compared with omega-3 fatty acids and ewe dams had previously been fed a high omega-6 diet at mating (Figure 7.13).

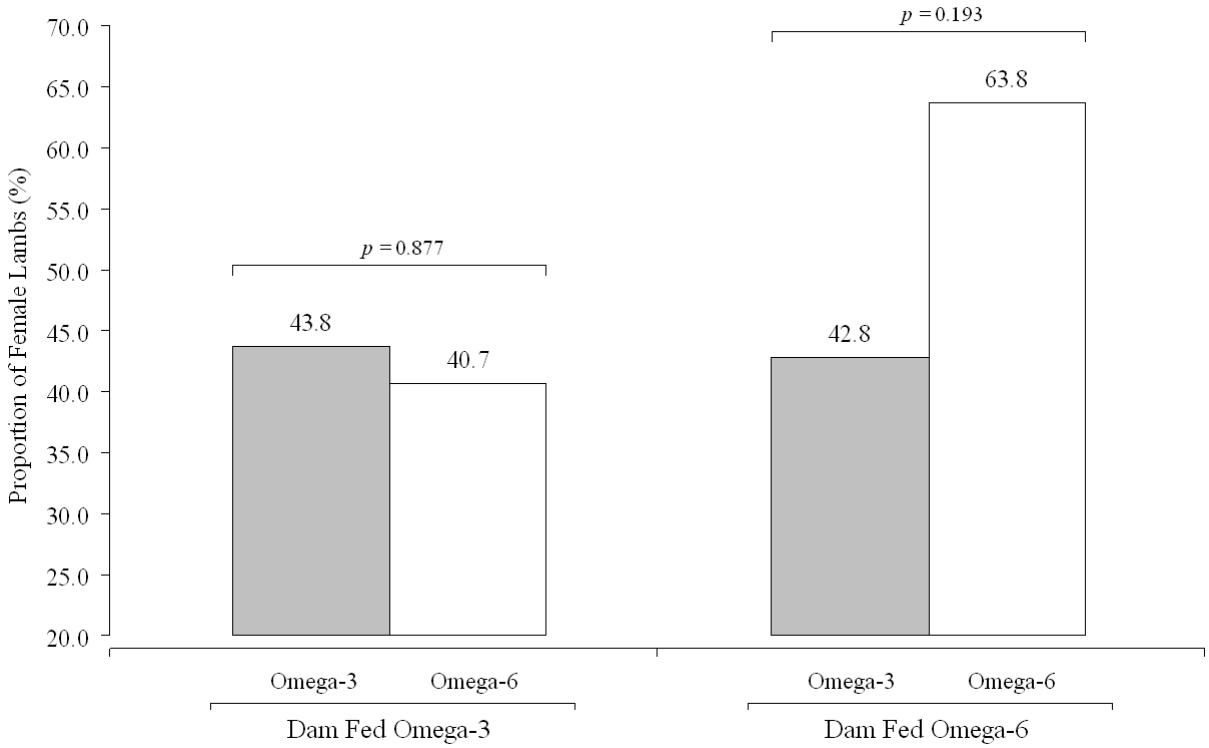


Figure 7.13 Proportion of female lambs when BL x Merino maiden ewes were fed a diet high in omega-3 (shaded bars) or omega-6 (unshaded bars) fatty acids for 42 days prior to and 17 days following mating when their dams were also previously fed a diet high in omega-3 or omega-6 at mating.

A small number of lambs were born in the second cycle (high omega-3 n = 14, high omega-6 n = 11). The proportion of female lambs was not significantly (p = 0.083) higher when ewes were fed the high omega-6 diet (63.6% female) compared with the high omega-3 diet (28.6% female) and conceived in the second natural oestrous cycle (Cycle 2).

The concentration of progesterone, oestradiol and fatty acids in plasma at the time of mating was not significantly correlated with lamb sex ($p > 0.05$). The concentration of plasma oestradiol and progesterone prior to mating was also not significantly correlated with the time to oestrus or lambing.

7.3.7 Lamb birthweight, vigour and survival

Lamb birthweight was not significantly ($p = 0.144$) higher for male versus female lambs (Table 7.8). Lamb birthweight was not significantly ($p = 0.253$) higher when ewes were fed the high omega-3 diet compared with the high omega-6 diet at mating. Lamb birthweight was significantly positively correlated ($r^2 = 0.08$, $p = 0.002$) with day of lambing, indicating that birthweight was lower when lambs were born earlier. The birthweight of male (5.26 ± 0.15 vs 5.49 ± 0.18 kg, $p = 0.330$) and female (5.17 ± 0.16 vs 5.28 ± 0.15 kg, $p = 0.614$) lambs was not significantly different when ewes were fed the high omega-6 compared with the high omega-3 diet at mating when the day of lambing was included in the analysis as a co-variate.

Lamb birthweight was significantly ($p = 0.043$) lower when ewe dams were previously fed a diet high in omega-6 (5.15 ± 0.11 kg) compared with omega-3 (5.50 ± 0.13 kg) fatty acids at joining. Birthweight was, however, significantly ($p < 0.001$) lower for lambs born in twin (4.81

± 0.09 kg) compared with single (5.85 ± 0.10 kg) litters. Lamb birthweight was not significantly (p = 0.784) different when ewe dams were previously fed a diet high in omega-6 (5.27 ± 0.09 kg) compared with omega-3 (5.31 ± 0.12 kg) fatty acids at joining when litter size were included in the analysis as a co-variate, indicating that the lower birthweight when ewe dams were fed the high omega-6 compared with the high omega-3 diets was mediated by the higher proportion of twin lambs when ewe dams were previously fed omega-6 at joining.

Lamb survival at birth was significantly (p = 0.011) lower when ewes were fed the high omega-3 diet (89.9%) compared with the high omega-6 diet (98.8%) at mating (Table 7.8). Lamb survival at birth was also significantly lower when ewes were fed the high omega-3 compared with the high omega-6 diet when ewe dams were previously fed the diet high in omega-6 (87.0 vs 97.7%, p = 0.031) but not omega-3 (92.6 vs 100.0%, p = 0.221) fatty acids at joining. The lower lamb survival at birth was associated with a higher incidence of dystocia when ewes were fed the high omega-3 compared with omega-6 diet and lamb survival was significantly (r (122) = -0.23, p = 0.011) negatively correated to birth weight. Lamb surval at birth was not significantly (p = 0.284) lower when ewes were fed the high omega-3 diet (94.7%) compared with the high omega-6 diet (98.6%) at mating when birthweight was included in the analysis as a co-variate. Lamb survival to 24 hr and lamb marking was not significantly different when ewes were fed the high omega-3 compared with the high omega-6 diet at mating (survival to 24 hr = 89.8 vs 95.4%, respectively, p = 0.174 and survival to marking = 83.2 vs 82.6%, respectively, p = 0.909). Lamb survival to marking was not significantly (p = 0.107) lower when ewe dams were previously fed the high omega-6 diet (78.7%) compared with the high omega-3 diet (88.9%) at mating.

Lamb vigour was not significantly different when ewes were fed the high omega-3 compared with the high omega-6 diet (Table 7.8). Vigour was, however, significantly (p < 0.001) higher (lower score) for female (1.84 ± 0.17) compared with male (2.40 ± 0.17) lambs as a lower score indicates a greater level of vigour.

Table 7.7 Proportion of BL x Merino maiden ewes pregnant or lambed and mean pregnancy or lambing rates following the consumption of a High omega-3 diet based on lucerne or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating.

	High Omega-3 (Lucerne)		High Omega-6 (Oats/CSM[2])		*p*-values[3]		
Reproduction Measure[1]	Dam Omega-3	Dam Omega-6	Dam Omega-3	Dam Omega-6	Diet	Dam Diet	Diet x Dam Diet
Proportion scanned pregnant (%)	95.83%	96.97%	92.86%	93.55%	0.448	0.828	0.958
Mean foetal rate							
For ewes scanned pregnant	1.26 (± 0.10)	1.47 (± 0.08)	1.31 (± 0.09)	1.69 (± 0.09)	0.147	0.002	0.345
For all ewes joined	1.21 (± 0.12)	1.42 (± 0.10)	1.21 (± 0.11)	1.58 (± 0.10)	0.447	0.007	0.481
Pregnancy loss[4] (%)	3.45%	4.23%	0.00%	10.20%	0.725	0.122	0.187
Proportion lambed							
For ewes scanned pregnant[5] (%)	100.00%	100.00%	100.00%	100.00%	1.000	1.000	1.000
For all ewes joined[5] (%)	95.83%	96.97%	92.86%	93.55%	0.448	0.828	0.958
Mean number of lambs							
For ewes that lambed	1.22 (± 0.10)	1.41 (± 0.08)	1.31 (± 0.09)	1.52 (± 0.09)	0.278	0.033	0.911
For all ewes joined	1.17 (± 0.11)	1.36 (± 0.10)	1.21 (± 0.11)	1.42 (± 0.10)	0.623	0.058	0.969

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means) including all lambs that could be identified with their dam in each treatment group.
[2]CSM = cottonseed meal.
[3]Diet = main effect of dietary treatment (High omega-3 versus High omega-6), Dam Diet = main effect of diet previously fed to maiden dams (High omega-3 versus High omega-6).
[4]Proportion of foetuses identified at scanning that were not identified at lambing or ewes identified as not having lambed at lamb marking (Dun, 1963).
[5]Including only those lambs that could be positively identified with their dam.

Table 7.8 Lamb birthweight, head circumference, vigour and survival and maternal score at parturition when BL x Merino maiden ewes were fed a High omega-3 diet based on lucerne or a High omega-6 diet based on oats and CSM for 42 days prior to and, 17 days following, mating.

Lamb Measure[1]	Male		Female		*p*-values[3]		
	High Omega-3[2]	High Omega-6	High Omega-3[2]	High Omega-6	Diet	Sex	Diet x Sex
Birthweight (kg)	5.56 (± 0.18)	5.27 (± 0.15)	5.21 (± 0.15)	5.13 (± 0.16)	0.253	0.144	0.531
Head Circumference[4] (cm)	27.5 (± 0.25)	27.4 (± 0.20)	27.5 (± 0.20)	27.8 (± 0.21)	0.615	0.364	0.364
Lamb vigour[5]	2.19 (± 0.22)	2.61 (± 0.18)	1.98 (± 0.20)	1.70 (± 0.20)	0.662	< 0.001	0.029
Lamb Survival							
Birth	86.8%[b]	100.0%[a]	92.9%[ab]	97.7%[a]	0.011	0.596	0.232
24 hr	86.8%	93.0%	92.9%	97.7%	0.174	0.188	0.866

[1]Values are proportions (percentages) or least squares means (± standard errors of the least squares means) including all lambs that could be identified with their dam in each treatment group.
[2]High omega-3 treatment based on lucerne, High omega-6 treatment based on oats and cottonseed meal.
[3]Diet = main effect of dietary treatment (High omega-3 versus High omega-6), Sex = main effect of lamb sex (Male versus Female).
[4]Head circumference in the coronal plane (Jamison et al., 1961).
[5]Lamb vigour measured on a scale of 1 to 5 where 1 = more vigorous and 5 = least vigorous.

7.4 Discussion

The proportion of female lambs was 13% higher (55.8 vs 43.1%) when ewes were fed the high omega-6 diet compared with the high omega-3 diet at mating. Although the higher proportion of female lambs was not statistically different due to the small number of lambs in the current study, the effect was similar to that observed in first cross ewes in Phase 1 of the current project. The increased proportion of female lambs when ewes were fed the high omega-6 diet appeared to be greater when ewe dams were also previously fed a diet high in omega-6 (21% more females) compared with omega-3 fatty acids at conception, although none of these differences were statistically significant.

The foetal rate at pregnancy scanning and the lambing rate for ewes that lambed was higher when ewe dams were previously fed a diet high in omega-6 compared with omega-3 fatty acids at joining, regardless of the diet fed during the current study. Although ewe liveweight at the time of conception was higher when ewe dams were previously fed the high omega-6 diet, the effect of diet previously fed to ewe dams appeared to be independent of the effect on liveweight during pen feeding. This carry over effect of the previous diet high in omega-6 could have significant implications for lifetime productivity of ewes. If Merino ewes are fed a high omega-6 diet at joining and a higher proportion of female lambs are born, the increased productivity of these ewe lambs could be significantly greater. These results need to be interpreted with caution, however, due to the small number of ewes in the current study. The maiden ewes born to Merino ewes in the second year of Phase 2 of the current project should be joined in 2014 to further examine the intergenerational effects of the High omega-3 and omega-6 diets on ewe productivity.

Similar to previous results in Phase 1 and 2 of the current project, the alteration in sex ratio in maiden ewes was not significantly correlated with ewe weight or fat score, or ewe weight and fat score change over the period of pen feeding. These results indicate that maternal condition at mating was not likely to be responsible for the observed differences in sex ratio between treatment groups, although, the higher weight of ewes from dams previously fed omega-6 at joining may have mediated the higher foetal rate observed.

The time to showing behavioural oestrus but not the time to parturition was shorter when ewes were fed the high omega-6 compared with the high omega-3 diet at mating. The difference in the time to oestrus between dietary treatment groups was greater when ewe dams were previously fed a diet high in omega-6 at joining. The non significant difference in the time to joining may have been a result of the small number of ewes in the current study. The oestrus to parturition interval was not different between dietary treatment groups which is not consistent with results observed in previous studies. The lack of difference in the oestrus-parturition interval may indicate that the carry over effects of dietary fatty acids post-conception may not have been as great as effects observed previously.

The proportion of EPA in the plasma of ewes was significantly lower and the proportion of linoleic acid (C18:2n-6) but nor ARA (C20:4n-6) was significantly higher after pen-feeding when ewes were fed the high omega-6 compard with the high omega-3 diet. The interaction between diet and Dam diet was not significant, indicating that the change in fatty acids following dietary treatment in the current study did not appear to be mediated by previous diet fed to ewe dams.

The higher oestradiol in the plasma of BL x Merino maiden ewes indicates that the ewes were closer to the time of ovulation at blood collection. Although the sex ratio of lambs was not significantly related to the concentration of hormones or fatty acids at the time of conception, the relationship between the time of oestrus and ovulation with the observed differences in sex ratio warrants further investigation.

8.0 General discussion

The proportion of female lambs was consistently higher when Border Leicester x Merino ewes (53% vs 44% females, difference = 9%) or Merino ewes (56% vs 44% females, difference = 12%) were fed a diet high in omega-6 compared with omega-3 fatty acids at the time of mating in pen-feeding studies. The effect of the high omega-6 diet on increasing the proportion of female lambs was greatest in single-bearing compared with twin-bearing BL x Merino ewes, regardless of time of feeding, however, the effect was greatest when Merino ewes were fed both pre- and post-conception compared with pre-conception only, regardless of whether ewes were single or twin-bearing.

The increase in the proportion of female lambs when ewes were fed the high omega-6 diet also appeared to be greatest when maiden BL x Merino ewe dams were also fed a high omega-6 diet at conception. This result should be interpreted with caution due to the low number of ewes and lambs in the current study, however, the effect of previous diet at conception on life-time productivity and the sex of lambs in the second generation appears to warrant further investigation.

The concentration of fatty acids in plasma was consistently related to dietary treatment across all studies in the current project, indicating the success of being able to alter systemic fatty acid status through dietary manipulation. The relationship between dietary and plasma fatty acids with the altered sex ratio will be described below in Section 8.1.2. The concentration of oestradiol and progesterone, however, was not consistently altered by dietary treatment. The concentration of progesterone in the plasma of BL x Merino ewes was significantly lower and the concentration of oestradiol was numerically higher when ewes were fed the high omega-6 compared with the high omega-3 diet, which is consistent with the significantly shorter time to oestrus observed. The concentration of progesterone was higher, however, when Merino ewes were fed the high omega-6 diet. The reason for the differential response in Progesterone observed between studies is unclear. The analysis of progesterone at more frequent intervals prior to the onset of oestrus and ovulation may indicate more accurately the pattern of change on hormone concentrations between breeds.

It is possible that there was a carry over effect of fatty acids from feeding diets pre-conception only. There is evidence that plasma fatty acids increase rapidly following the commencement of feeding of the high omega-6 diet (Gulliver et al., 2013a), however, the time taken for the concentration of omega-6 to decrease to pre-feeding values is not well known. Future studies should feed the experimental rations post-conception only, or monitor more closely the changes in fatty acids post-feeding in order to assess the length of time of changes.

The mechanism linking the diets varying in omega-3 and omega-6 concentration with the observed alteration of sex ratio in the current project remains unclear. The results of the current study indicate that a post-conception mechanism may have a greater or additive effect on the proportion of female lambs, particularly in Merino ewes. The proportion of ewes lambed and the lambing rate was not significantly different between treatment groups. Therefore, it seems unlikely that the increased proportion of females when ewes were fed the high omega-6 diet was due to loss of male embryos post-conception. It is possible, however, that the proportion of ewes ovulating and the number of embryos was greater immediately after conception when ewes were fed the high omega-6 diet, however, there was a preferential loss of males and the total number of foetuses (and lambs) left alive was similar between groups.

The birthweight of lambs was numerically (p = 0.144) lower (100g for male and 110g for female lambs) when BL x Merino ewes were fed the high omega-6 diet compared with the

high omega-3 diet. The birthweight of male (250g lower), but not female lambs, was also lower (p = 0.131) when Merino ewes were fed the high omega-6 diet. This lower birthweight was related to the shorter time to parturition when ewes were fed the high omega-6 diet and the difference in birthweight between treatment groups was not statistically different when the day of lambing was included in the analysis as a co-variate. The effect this earlier time of lambing and lower birthweight has on lamb survival is not clear and should be monitored closely in future studies. Vigour was numerically lower when lambs were born to BL x Merino ewes fed the high omega-6 diet, but this did not seem to be related to lamb survival.

8.1.1 Combined results for sex ratio across studies

The results for the sex ratio of all lambs born in Phases 1 and 2 of the current project were combined in order to assess the overall effect of the experimental diets. When results for all 4 pen feeding studies were combined, the sex ratio of lambs was significantly different from an expected 50:50 when ewes were fed either the High omega-3 diet (p = 0.007) or the High omega-6 diet (p = 0.024) pre-conception or pre- and post-conception. The proportion of female lambs was 10% higher (54.6% vs 44.7% females, p < 0.001) when ewes were fed a High omega-6 diet compard with a High omega-3 diet at mating. The proportion of female lambs was significantly higher when ewes were fed the High omega-6 diet both pre- and post-conception (57% vs 43% females, difference = 14%, p < 0.001) but not when ewes were fed pre-conception only (52% vs 47% females, difference = 5%, p = 0.160).

The results for the sex ratio of all lambs born in the current project were also combined with results obtained in our previous study conducted at the WWAI with the same BL x Merino ewes in 2010 (Gulliver et al., 2013b). In all studies, the experimental diets were fed to a total of 1524 ewes with a total of 1814 lambs assessed for sex ratio. When results were combined for all studies, the proportion of female lambs was 10% higher when ewes were fed the High omega-6 diet compared with the High omega-3 diet (55% vs 45% females, difference = 10%, p < 0.001). The interaction between diet and time was not significant (p = 0.080), however, the effect of the High omega-6 diet was greatest when ewes were fed both pre- and post-conception (57% vs 43% females, difference = 14%, p < 0.001), but was not statistically significantly higher when ewes were fed pre-conception only (52% vs 47% females, difference = 5%, p = 0.160). Similar to results obtained in the current project, the largest difference in sex ratio between dietary treatment groups was greatest (21.2%) when experimental rations were fed to single-bearing ewes both pre- and post-conception (High omega-6 = 62.5% females, High omega-3 = 41.3% females).

8.1.2 Relationship between fatty acids in feed and plasma and the sex ratio of lambs

The concentration of omega-3 in silage varied across studies due to the quality of the forage ensiled each year. When results for the sex ratio of lambs in the current project were combined with results obtained in our previous study (Gulliver et al., 2013b), the concentration of C18:3n-3 and C20:5n-3 in plasma prior to joining was significantly positively correlated with the concentration of C18:3n-3 in silage when ewes fed the High omega-3 diets (Figure 7.1). This indicates that the concentration of omega-3 in plasma can be increased to a greater extent when the concentration of omega-3 in feed consumed is higher.

The conversion of C18:3n-3 to long-chain omega-3 fatty acids including C20:5n-3 is rate limited in a number of species (Emken et al., 1994; Pawlosky et al., 2001), however, the change in C20:5n-3 in the current project when ewes were fed silage with increasing concentrations of C18:3n-3 indicates significant conversion occurs. This finding agrees with previous research indicating that significant metabolism of C18:3n-3 to C20:5n-3 occurs in ruminants and the concentration of omega-3 in plasma (Kemp et al., 1998), red blood cells (Gulliver et al., 2010), meat (Scollan et al., 2006), milk (Dewhurst et al., 2003) and

reproductive tissue (Kim et al., 2001) is influenced by the concentration of C18:3n-3 in the diet.

Combined results from the current project and our previous study were also assessed for the relationship between the concentration of fatty acids in plasma and the proportion of female lambs born. When results were included from ewes that were fed experimental diets both pre- and post-conception and when BL x Merino ewes gave birth to single lambs, the proportion of female lambs was negatively correlated with the concentration of C18:3n-3 (p = 0.048) but not C20:5n-3 (p = 0.070) in plasma immediately prior to mating (Figure 7.2). That is, the sex ratio was skewed in favour of males to a greater extent when the plasma omega-3 concentration was higher and skewed in favour of females when the plasma omega-3 concentration was lower.

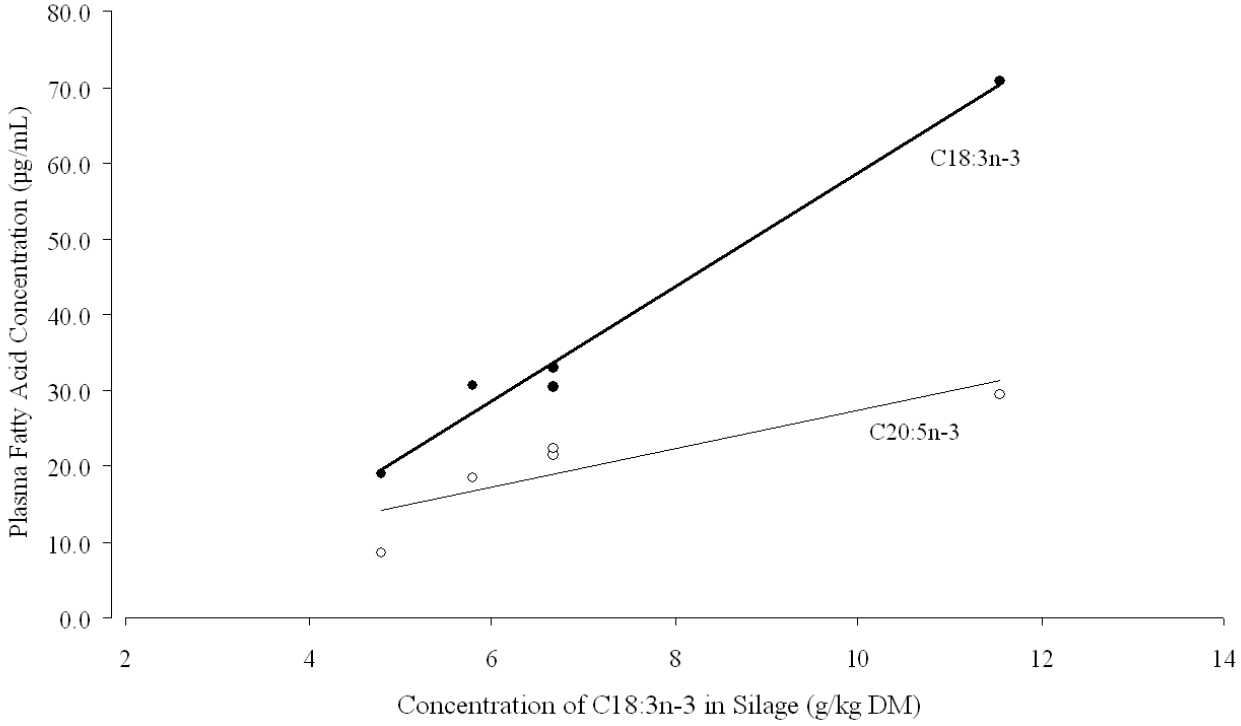


Figure 8.1 Correlation between the concentration of C18:3n-3 in silage and the concentration of C18:3n-3 (●) or C20:5n-3 (○) in the plasma of Border Leicester x Merino ewes or Merino ewes 6 weeks after the commencement of feeding experimental rations in sex ratio studies conducted in 2010, 2011 or 2012. Plasma fatty acid concentration (µg/mL) = 7.55 x C18:3n-3 concentration in feed (g/kg DM) – 16.80, r^2 = 0.98, p < 0.001 (for plasma C18:3n-3) or 2.54 x C18:3n-3 concentration in feed (g/kg DM) + 1.98, r^2 = 0.75, p = 0.056 (for plasma C20:5n-3).

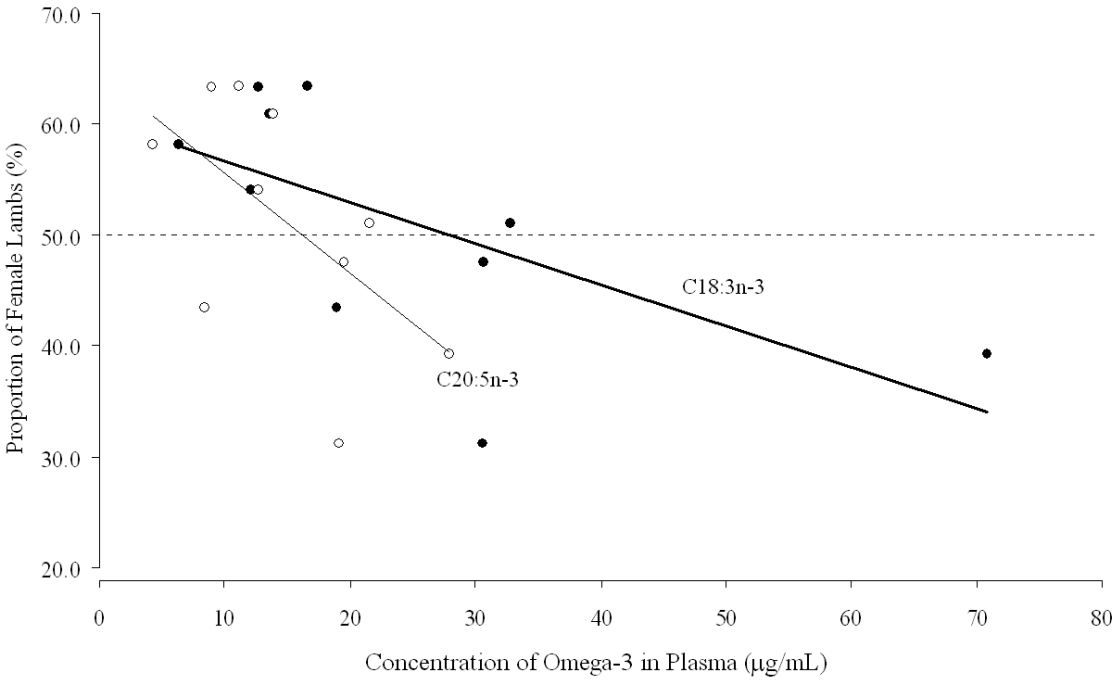


Figure 8.2 Correlation between the concentration of ALA (C18:3n-3, ●) or EPA (C20:5n-3, ○) in the plasma Border Leicester x Merino ewes or Merino ewes prior to joining and the proportion of female lambs in sex ratio studies conducted in 2010, 2011 or 2012. Values are for all ewes fed experimental rations both pre- and post-conception including single-bearing BL x Merino ewes. Proportion of female lambs (%) = -0.37 x plama C18:3n-3 concentration (μg/mL) + 60.36, r^2 = 0.41, p = 0.048 or -0.90 x plama C20:5n-3 concentration (μg/mL) + 64.56, r^2 = 0.35, p = 0.070.

8.1.3 Time to oestrus and parturition

The greatest effect of the experimental diets, apart from the altered sex ratio, was the change in the time to oestrus and parturition. The reduced length of time to showing behavioural oestrus and the shorter time to parturition when ewes were fed the High omega-6 diet is consistent with previous research at the WWAI (Gulliver et al., 2013b). The time to oestrus was shorter when sheep were infused with omega-6 fatty acids (Burke et al., 1996) and when non oestrous-synchronised (Jones et al., 2008) and synchronised (Zachut et al., 2010) dairy cattle received omega-6 fatty acid supplements. The time to parturition (gestation length) was also shorter when sheep (Baguma-Nibasheka et al., 1999; Capper et al., 2006) and goats (Titi and Awad, 2007) received diets higher in omega-6 fatty acids during gestation. Altered concentrations of progesterone may have been responsible for the alterations in gestation length observed in sheep (Baguma-Nibasheka et al., 1999), which agree with the lower plasma progesterone observed pre-mating in BL x Merino ewes in the current project.

The time of insemination in relation to oestrus and ovulation may be a potential mechanism linking the diets in the current study with an altered sex ratio of lambs (Gulliver et al., 2013b). The oestrus to parturition interval was shorter when BL x Merino ewes (but not Merino ewes) were fed the High omega-6 compared with the High omega-3 diet in the current study, which agrees with previous findings (Gulliver et al., 2013b). Fertilisation of younger compared with older ova was associated with a higher proportion of females (Gutierrez-Adan et al., 2001; Gutierrez-Adan et al., 1999). The proportion of females was also higher in dairy cattle (Pursley et al., 1998), sheep (Gutierrez-Adan et al., 1999) and deer (Rorie, 1999) when the timing of artificial insemination was closer to ovulation. The effect of the time of insemination

in relation to ovulation on altering the sex ratio of offspring has also been studied in white-tailed deer (Verme and Ozoga, 1981), Barbary macaques (Paul and Kuester, 1987) and rats (Hedricks and Mcclintock, 1990). A Ph.D study is currently underway at the WWAI examining the preliminary relationship between the timing of oestrus and ovulation when ewes are fed diets high in omega-3 or omega-6 fatty acids.

8.1.4 Practical applications

The results of the sex ratio in the on-farm trials were not consistent with the intensive pen-feeding studies, as the proportion of female lambs was not higher when ewes were supplementary fed a diet high in omega-6 fatty acids on-farm. These results should be interpreted with caution, however, as there were several limitations of the current on-farm trials. For example, the higher than expected lamb mortality during on-farm Trial 1 precluded an accurate assessment of the sex of lambs born compared with lambs marked.

There are a number of other dietary factors that need to be considered before the feeding strategies on-farm can be assessed in detail. In particular, the concentration of omega-3 available in pastures in Australia is largely unknown. It may be possible to alter the amount of omega-3 consumed by ewes at joining on-farm by selecting pasture species high in omega-3 or by manipulating pastures through fertiliser or grazing management to maximise the concentration of omega-3 available at different times of the year. These alterations may provide simple and cost-effective methods for producers to implement feeding strategies to alter the sex ratio in large numbers of lambs.

In addition to assessing the effect of altering the availability of omega-3 from pasture on the sex ratio of lambs, the effect of omega-6 from different sources of grain on altering systemic fatty acid status in ewes and subsequent effects on sex ratio are currently unknown. For example, the ratio of omega-6:omega-3 in some grain types is similar, however, the ratio in barley is lower than oats (11.01 versus 25.9) and the total concentration of lipid and fatty acids varies between grain types as well as varieties (Boschin et al., 2008). The quantity of grain to feed and, the length of time to feed grain, in order to alter fatty acid status to an extent sufficient to alter the sex ratio of lambs is also unknown.

The change in concentration of omega-3 fatty acids when ewes received a diet high in omega-6 fatty acids in on-farm Trial 1 was similar to those changes observed in pen feeding studies. The change in concentration of omega-6 fatty acids, however, was not as great as those observed under intensive pen-feeding situations. Further refinements in the feeding protocol may be necessary in order to maximise the effect of the omega-6 diet. One difference between the diets offered to ewes in the intensive pen feeding studies and on-farm Trial 1, in particular, was that the pen feeding diets also contained cottonseed meal which has a significantly higher concentration of omega-6 than grain alone. The addition of CSM in the diet offered to ewes on-farm may increase the potential effect of omega-6. Other sources of omega-6 fatty acids, including sunflower or safflower meal, may provide other alternatives to increase the amount of omega-6 fatty acids that can be included in on-farm diets. The cost of these supplements would need to be assessed carefully in order to determine the overall benefit in on-farm production systems.

8.1.5 Identification of future projects

Thre are a number of potential future projects that could be conducted after the assessment of results from the current project. These potential projects include;

1. Characterising the concentration of omega-3 and omega-6 fatty acids in pastures available on-farms in south-eastern Australia. This may allow an assessment of the

ability of the basal diet available to ewes to influence the sex ratio of lambs across Australia.
2. To determine the ability of different grain types to increase the concentration of omega-6 in ewe blood. To also determine the rate of increase in omega-6 in blood following the commencement of feeding and the length of time that blood omega-6 concentration stays elevated after the cessation of grain feeding.
3. Examining the effect of diets high in omega-3 or omega-6 fatty acids on the sex ratio of lambs born to ewes in the second generation (examine results in larger numbers).
4. Examine the intergenerational effects of diets high in omega-3 or omega-6 fatty acids fed to dams on other production parameters from male and female offspring.
5. Determining the effect of diets fed to ewes at joining on the fatty acid concentration in the blood of lambs at birth and on subsequent fatty acid metabolism in these lambs.
6. Examining the effect of diets high in omega-3 or omega-6 fatty acids on the sex ratio of lambs in unsynchronised ewes.
7. Determine the mechanisms linking the diets examined in the current project on the sex ratio of lambs. There is a Ph.D project currently underway through the Graham Centre, but this examination will require more research.
8. Examining the effect of diets high in omega-3 or omega-6 fatty acids on the sex ratio of calves.
9. Practical feeding strategies, including the type of grain to feed, the amount of grain required per head and the length of time of feeding required to get the observed effect. In particular, the effect of the experimental diets post-conception only would be required to determine the contribution of post-conception mechanisms only to the alteration in sex ratio.

8.2 Success in achieving project objectives

8.2.1 Statement of outcomes

1. The current project determined the effect of feeding diets high in omega-3 or omega-6 fatty acids to 595 BL x Merino ewes and 633 Merino ewes on the proportion of male and female lambs. The proportion of female lambs was 10% higher (55% vs 45% females, $p < 0.001$) when ewes were fed a High omega-6 diet compard with a High omega-3 diet.

2. The current project determined that the proportion of female lambs was consistently higher when Border Leicester x Merino ewes (53% vs 44% females, difference = 9%, $p = 0.009$) or Merino ewes (56% vs 44% females, difference = 12%, $p = 0.009$) were fed a diet high in omega-6 compared with omega-3 fatty acids.

3. The current project determined that the effect of the High omega-6 diet on increasing the proportion of female lambs was greatest in single-bearing compared with twin-bearing BL x Merino ewes, regardless of time of feeding, however, the effect was greatest when Merino ewes were fed both pre- and post-conception compared with pre-conception only, regardless of whether ewes were single or twin-bearing.

4. The current project determined that the effect of the High omega-6 diet on increasing the proportion of female lambs was greatest when ewes were fed both pre- and post-conception (57% vs 43% females, difference = 14%, $p < 0.001$) compared with pre-conception only (52% vs 47% females, difference = 5%, $p = 0.160$).

5. The current project determined that the effect of the High omega-6 diet on increasing the proportion of female lambs did not appear to be mediated by diet previously fed to ewe dams at conception, however, lifetime ewe reproduction may be affected by previous diet.

6. The time to oestrus and the time to parturition was consistently shorter when ewes were fed the High omega-6 diet compard with the High omega-3 diet pre-conception.

7. The effect of feeding the High omega-3 or High omega-6 diets was completed in 3 on-farm demonstration trials. The effect of omega-3 and omega-6 fatty acids were not consistent in on-farm feeding trials, however, preliminary results indicated that the time to joining was shorter and the concentration of omega-6 in plasma was higher when ewes were fed the High omega-6 diet, indicating that there is potential for the diet to affect the sex ratio of lambs. Several potential future collaborators have also been identified during the current project that have expressed interest in conducting further on-farm demonstration trials

8. The concentration of progesterone in plasma was significantly lower when ewes were fed the High omega-6 compared with the High omega-3 diet prior to joining. This change may be a potential mechanism whereby the diet high in omega-6 fatty acids lead to the shorter time to oestrus. More work is needed to determine the exact mechanisms linking the experimental diets with the observed alteration in sex ratio.

8.2.2 Objectives of the current project that were not met

The objective of the current study that results would be published in a peer-review journal has also not been completed at this stage. Preliminary results of the research have been presented at the Grasslands Society of NSW Annual Conference (see Appendix 1) and it is anticipated that a refereed journal article will be prepared by the middle of 2014.

8.3 Significant obstacles overcome during the course of the project

There was a higher than expected loss of BL x Merino ewes between Year 1 and 2 of the pen-feeding studies. This meant that the power to detect a significant difference in the sex ratio of lambs between dietary treatment groups was slightly reduced. Despite this loss of ewes, a statistical difference in the sex ratio of lambs could still be detected. Future studies will more closely monitor ewes while they are being managed by the WWAI commercial operation.

The large number of twin-bearing BL x Merino ewes in Year 1 meant that lambing was challenging and matching individual ewes and lambs was difficult. In Year 2, ewes lambed in smaller paddocks with only 15 ewes/Ha. This stocking rate was the optimum rate for matching ewes and lambs and reducing the risk of mis-mothering.

There were a number of different types of silage used as a source of omega-3 in the different pen-feeding studies. This variability could have meant that the observed sex ratios differences were also too variable in order to detect statistically significant results. The variation observed, however, added valuable information to the effect of omega-3 on altering the proportion of males (see Figure 7.2).

It was difficult to control sheep management in the conduct of the on-farm demonstration trials. The Barber's pole internal parasite infection during on-farm Trial 1 led to a reduced ability to detect an accurate effect of experimental diets. As mentioned previously, the field trials will have to be conducted with a larger numbers of ewes or the number of live and dead lambs at birth would need to be assessed more closely in order to more accurately determine the effect of the diets on the sex ratio of lambs on-farm.

8.4 Impact on the sheep industry now and in five years time

There is the potential to alter the sex ratio of lambs on-farm by up to 14% through dietary manipulation around the time of conception. If ewes are fed a diet high in omega-3 fatty acids based on fresh pasture or forage, the proportion of female lambs may be as low as 40%. Alternatively, if ewes are fed a diet high in omega-6 fatty acids for 6 weeks prior to mating and 17 days following mating, the proportion of female lambs could be increased by approximately 14% compared with a pasture-based diet or increased to be as high as 60%.

Sheep operations would benefit from the opportunity 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 lead to a $30-80 higher sale price at weaning. Therefore, terminal sire enterprises producing lambs for slaughter would prefer males, while self-replacing enterprises and producers of stud ewe or first cross ewes to supply terminal sire enterprises would prefer breeding females.

Terminal sired lambs account for approximately one third of the approximately 21 million prime lambs slaughtered each year. The aim will be to target 10% of these producers by 2018-19. An increase in male lamb proportion of approximately 10% from an expected ratio of 50:50 could potentially increase the profitability of these industries. The most likely enterprises to adopt the technology are likely to be early adopters who are likely to have a relatively higher proportion of the total terminal sire flock. There are approximatley 42 million head of Merino ewes in Australia and a large proportion of these are in self-replacing flocks. The aim will be to target 10% of these enterprises by 2018-19. An increase in female lamb proportion of approximately 10% could significantly improve profitability by reducing costs of buying replacement ewes and having to sell wethers of lower value.

It is anticipated that there will be a 10% adoption of the technology to influence the sex ratio of lambs in 5 years (by 2018-19). The awareness, participation and adoption objectives are shown in Table 7.1 below.

Table 8.1 Awareness, participation and adoption objectives for altering the sex ratio of lambs on-farm.

Year	Awareness	Participation	Adoption
Year 1 (2014)	5%	0%	0%
Year 2 (2015)	7.5%	5%	0%
Year 3 (2016)	10%	7.5%	5.0%
Year 4 (2017)	20%	10%	7.5%
Year 5 (2018)	50%	15%	10%

8.5 Industry adoption

The awareness of the current technology across the sheep industry will be achieved using a number of approaches. These include the development of scientific publications and extension packages. The objectives of the extension of the technology is shown below;

Dec 2013 – Conference presentations and preparation of refereed scientific publications from the study
Jul 2014 – Refereed scientific publications submitted
Oct 2014 – Refereed scientific publications available
Dec 2014 – MLA publications, information brochures and web-site tools available
Oct 2014 – Graham Centre for Agricultural Innovation producer field days

We will also work with the NSW Department of Primary Industries Research and Development Officers to develop and deliver extension packages to be provided to several groups, including government agricultural advisors (for example Local Land Services and other state Department of Primary Industries), private advisors, pasture agronomists and producer groups. Several producers groups may be involved, including the Holbrook Landcare, Monaro Farming Systems and Victorian Best Wool-Best Lamb producer groups. We aim to contact group facilitators and provide information packages and presentations to the group through regular meetings and the development of further on-farm demonstration sites.

9.0 Conclusions and recommendations

The sex ratio of lambs was significantly altered in pen-feeding studies when ewes were fed diets high in either omega-3 or omega-6 fatty acids. The proportion of female lambs was 9% higher in Border Leicester x Merino ewes and 12% higher in Merino ewes when ewes were fed a diet high in omega-6 compared with omega-3 fatty acids at the time of mating. The effect of the High omega-6 diet on increasing the proportion of female lambs was greatest in single-bearing compared with twin-bearing BL x Merino ewes, regardless of time of feeding, however, the effect was greatest when Merino ewes were fed both pre- and post-conception compared with pre-conception only, regardless of whether ewes were single or twin-bearing. The proportion of male or female lambs may be related to the concentration of omega-3 in feed and the ability of the diet to alter fatty acid concentrations in the ewe. The sex ratio of lambs was related to plasma fatty acid concentration in single-bearing BL x Merino ewes and Merino ewes that were fed experimental diets both pre- and post-conception.

The shorter time to oestrus and parturition observed may be related to altered progesterone or oestradiol concentrations at the time of mating, although the results were not consistent between BL x Merino ewes and Merino ewes in the current project. The relationship between fatty acid status, hormone concentration and the timing of oestrus and ovulation should be examined in detail in future studies.

The results of the sex ratio in the on-farm trials were not consistent with the intensive pen-feeding studies, as the proportion of female lambs was not higher when ewes were supplementary fed a diet high in omega-6 fatty acids on-farm. These results should be interpreted with caution, however, as there were several limitations of the current on-farm trials. The higher concentration of omega-6 fatty acids in plasma and the estimated shorter time to conception when ewes were offered the High omega-6 ration, also indicates that there were significant effects of the different diets on some reproduction parameters on-farm. Further refinements to the feeding protocol are necessary in order to alter the sex ratio of lambs in practical on-farm feeding situations.

A number of potential projects were identified that could examine several aspects of omega-3 and omega-6 metabolism and effects on sex ratio. In particular, the effect of diets high in omega-3 and omega-6 fatty acids on the sex ratio of calves and the intergenerational effects of these diets in ewes may be most beneficial to producers.

The results of the current project indicate that the sex ratio of lambs can be altered by feeding diets differing in omega-3 and omega-6 concentration in some feeding situations. The feeding strategies employed in the current project may be of immediate benefit to producers who can intensively feed ewes, in particular, stud breeding operations that could significantly increase profitability by altering the sex ratio of lambs. Further research is required though to refine the feeding protocols in order to maximise the effect in more extensive on-farm situations.

10.0 References

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12.0 Appendices

12.1 Appendix 1 - Publications arising from work completed during this project

12.1.1 Refereed Conference Proceedings

Clayton EH, Gulliver CE, Wilkins JF, King BJ, Meyer RJ and Friend MF (2012). Increasing the proportion of female lambs by supplementary feeding oats high in omega-6 fatty acids at joining. *In* "Proceedings of the 27th Annual Conference of the Grassland Society of NSW Inc." Wagga Wagga NSW, 24-26 July 2012, pp. 107-113.

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 ($PGF_{2\alpha}$) response to an oxytocin challenge. In studies 2-4 ewes consumed the diets for approximately 6 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), $PGF_{2\alpha}$ 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 vs 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

Clayton EH, Wilkins JF, King BJ, Meyer RJ and Friend MF (2013). Can we increase the proportion of female lambs by feeding oats at joining? *In* "Proceedings of the 54th Annual Conference of the Grassland Society of Southern Australia Inc.", Albury NSW, 17-18 July 2013, pp. 97-102.

Can we increase the proportion of female lambs by feeding oats at joining?

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[C]Graham Centre for Agricultural Innovation (NSW DPI and Charles Sturt University), Wagga Wagga, NSW 2678.

Abstract: The proportion of female lambs was previously found to be higher when ewes were fed a diet based on oat grain and cottonseed meal (CSM) high in omega-6 fatty acids at joining. These results have now been confirmed in a number of studies, both with Border Leicester x Merino ewes and Merino ewes. The current paper presents results from the latest experiments conducted at the Wagga Wagga Agricultural Institute and further details of possible mechanisms underlying the observed effects. The time to lambing was significantly shorter when ewes were fed a diet high in omega-6 compared with omega-3 at joining. The shorter time to lambing appeared to be greatest when ewes were fed the high omega-6 diet both pre- and post-conception. In addition, the difference in the sex ratio between ewes fed a diet high in omega-3 or omega-6 fatty acids also appeared to be greatest when ewes were fed pre and post-conception. Additional research is required in order to determine the optimal amount of omega-3 or omega-6 to feed to ewes at joining in order to maximise the effect of maternal nutrition on the sex ratio of lambs.

Key words: omega-3, omega-6, prostaglandin, sex ratio

Ed,

I am inviting you to speak on Thursday July 18, at the Grasslands Society of Southern Australia Conference in Albury. We'd ask you to speak on Increasing the proportion of female lambs by supplementary feeding oats high in omega-6 fatty acids at joining, including any recent findings (or other species work in this area). Please see attached draft program to see where your talk fits into the schedule.

Please let me know if you can make it.

Kind regards,

Marcus Richardson

12.1.2 Magazines

MLA Feedback Magazine (2012)

Feeding regimes

What did they eat?

The two rations were (on a dry matter (DM) basis):

Group 1: 90% cereal/legume silage, 10% molasses

Group 2: 70% whole oat grain, 8% cottonseed meal (CSM), 22% cereal/legume silage

Both groups were fed approximately 1.07 kg/day total DM for the silage group and 0.81 kg/day total DM for the oats/CSM group.

Each group also received 20g/hd/day of a commercial mineral mix.

Oats for girls, silage for boys

Different feeding regimes for ewes at conception may influence the sex ratio of their offspring, according to preliminary findings of MLA-funded research, with grain high in omega 6 leading to more female lambs while omega 3-rich silage resulting in more males.

62–64%
female lambs from Merino and crossbred ewes fed grain-based ration

40–45%
female lambs from Merino and crossbred ewes fed silage-based ration

Eighteen months of the research (on top of earlier research funded by the EH Graham Centre) at Wagga in southern NSW has found the supplementary feed type, fed to both first cross and Merino ewes for six weeks prior to joining and for 17 days following, had an impact on the sex ratio of the flock's offspring and the time it takes to reach oestrus.

Project Leader Dr Edward Clayton, a ruminant nutrition specialist with the NSW Department of Primary Industries (DPI), said the 2011 MLA-funded research, conducted on 600 ewes, found 64% of the Merino offspring were female when ewes were fed an oat grain/cottonseed meal (CSM) based ration, while the crossbreds produced 62% females. Ewes fed a silage based ration produced, in both Merinos and crossbreds, between 40 and 45% female offspring.

"Why would producers want to change the sex ratio of their lambs? If you are running a terminal sire flock for prime lamb production more males could be useful or if you are building up your flock, more females could help you get their faster," Ed said.

The research also found ewes fed the oats/CSM reached oestrus approximately half a day earlier than those fed silage. While Ed said this may not seem hugely significant, it could be beneficial when seeking greater synchronisation of ewes for a tighter joining period, for AI or for using fewer rams and warranted further investigation.

It could be in the timing

The research is continuing with trials also examining whether the impacts of the timing of supplementary feeding on the sex ratio is from the feeding prior to or following joining. This year the ewes will be fed the opposite diet to the one received in the first year of the project.

Blood samples have also been taken from all offspring and, this year, the lambs will be measured after slaughter to assess if the diet impacted on the omega 3 or omega 6 levels and other eating quality traits in their meat.

Dr Edward Clayton
E: edward.clayton@dpi.nsw.gov.au

12.1.3 Newspaper Articles

THE RURAL
Thursday July 11, 2013

Find out how diet may affect the sex of lambs

June 10, 2013, 12:30 a.m.

Edward Clayton.

Sheep breeding enterprises would undoubtedly benefit from the ability to skew the sex ratio of offspring towards their preferred gender. NSW Department of Primary Industries (NSW DPI) research leader, Dr Ed Clayton, will give attendees at the Grassland Society of Southern Australia.

The conference is being held on July 16 to 18 at the SS&A Club. Dr Clayton said the research, which started in 2010, has involved more than 1500 ewes, examines the ratio of omega-3 and omega-6 fatty acids in the diet of ewes and the effect it has on the sex ratio of lambs.

The work being carried out by the NSW DPI in conjunction with the Graham Centre, (an alliance between Charles Sturt University and NSW DPI) is being funded by Meat and Livestock Australia (MLA). Dr Clayton said recent trials have examined whether the skewing effect was pre or post-conception and if the effect was similar in first-cross ewes and Merinos.

"We think at this stage that pre-conception has the most important effect on the sex of the lamb. However, there may be some additional changes with feeding post conception, but we are still doing work on that," he said."We are keeping the female progeny and have set up a new trial, with additional funding from MLA, to see if there are any carry over effects of our feed regimes into the next generation that can affect the sex of lambs.
"The original research conducted at the Wagga Wagga Agricultural Institute involved 300 first-cross ewes split into two groups."The first group was fed omega-6 sourced from oat

grain and the second group was fed omega-3 sourced from a diet of pea silage," Dr Clayton said.

"Ewes were fed the two diets for six weeks prior to joining to Dorset rams and three weeks after joining. "After lambing, the lambs were tagged, and we identified a very surprising 15 per cent increase in the number of female lambs from sheep fed high omega-6 (grain) compared with those fed high omega-3 (pea silage) diets.

"For a self-replacing ewe flock or for first-cross ewe breeders, to increase the number of female lambs relative to the number of male lambs, a diet high in omega-6 could be fed at joining. "Alternatively, if producers wanted more male lambs (wethers develop more quickly and have more muscle) for prime lamb production systems, they might consider feeding a diet high in omega-3 at joining. To register phone 1300 137 550

Source: http://www.therural.com.au/story/1561454/find-out-how-diet-may-affect-the-sex-of-lambs/?cs=1280

Accessed: 10/07/2013

SOUTHERN WEEKLY

Diet may have an affect on sheep genetics

June 14, 2013, 2:48 p.m.

New research by the NSW Department of Primary Industries (DPI) is helping sheep breeders to skew the sex ratio of offspring towards their preferred gender.

Research developed by NSW DPI research leader Dr Ed Clayton points to evidence that diet may affect the sex of lambs.

Dr Clayton said the research, which started in 2010 and has involved more than 1500 ewes, examines the ratio of omega-3 and omega-6 fatty acids in the diet of ewes and the effect it has on the sex ratio of lambs.

Recent trials have examined whether the skewing effect was pre or post-conception and if the effect was similar in both first-cross ewes and Merinos.

“We think at this stage that pre-conception has the most important effect on the sex of the lamb. However, there may be some additional changes with feeding post conception, but we are still doing work on that,” Dr Clayton said.

“We are keeping the female progeny and have set up a new trial, with additional funding from MLA, to see if there are any carry over effects of our feed regimes into the next generation that can affect the sex of lambs.”

The original research, which was conducted at the Wagga Wagga Agricultural Institute, involved 300 first-cross ewes split into two groups.

“The first group was fed omega-6 sourced from oat grain and the second group was fed omega-3 sourced from a diet of pea silage,” Dr Clayton said.

“Ewes were fed the two diets for six weeks prior to joining to Dorset rams and three weeks after joining.

“After lambing, the lambs were tagged, and we identified a very surprising 15 per cent increase in the number of female lambs from sheep fed high omega-6 (grain) compared with those fed high omega-3 (pea silage) diets.

“For a self-replacing ewe flock or for first-cross ewe breeders, to increase the number of female lambs relative to the number of male lambs, a diet high in omega-6 could be fed at joining.

“Alternatively, if producers wanted more male lambs (wethers develop more quickly and have more muscle) for prime lamb production systems, they might consider feeding a diet high in omega-3 at joining.”

The research has been funded by Meat and Livestock Australia and comes from a joint alliance between Charles Sturt University and the NSW DPI at the Graham Centre.

Dr Clayton is presenting his most recent findings this week in Albury at the Grassland Society of Southern Australia’s annual conference.

Source: http://www.southernweekly.com.au/story/1573300/diet-may-have-an-affect-on-sheep-genetics/

Accessed: 10/07/2013

STOCK & LAND

Diet may affect sex of lambs

17 Jun, 2013 04:00 AM

NSW DPI research leader Ed Clayton will speak at the Grassland Society of Southern Australia's annual conference in Albury about ground breaking research that shows diet may affect the sex of lambs.

SHEEP breeding enterprises would undoubtedly benefit from the ability to skew the sex ratio of offspring towards their preferred gender.

NSW Department of Primary Industries research leader, Dr Ed Clayton, will give attendees at the Grassland Society of Southern Australia's annual conference at Albury an update on ground breaking research that shows diet may well affect the sex of lambs.

The conference is being held on July 16 to 18 at the SS&A Club.

Dr Clayton said the research, which started in 2010 and has involved more than 1500 ewes, examines the ratio of omega-3 and omega-6 fatty acids in the diet of ewes and the effect it has on the sex ratio of lambs.

The work being carried out by the NSW DPI in conjunction with the Graham Centre, (an alliance between Charles Sturt University and NSW DPI) is being funded by Meat and Livestock Australia (MLA).

Dr Clayton said recent trials have examined whether the skewing effect was pre or post-conception and if the effect was similar in first-cross ewes and Merinos.

"We think at this stage that pre-conception has the most important effect on the sex of the lamb. However, there may be some additional changes with feeding post conception, but we are still doing work on that," he said.

“We are keeping the female progeny and have set up a new trial, with additional funding from MLA, to see if there are any carry over effects of our feed regimes into the next generation that can affect the sex of lambs.”

The original research conducted at the Wagga Wagga Agricultural Institute involved 300 first-cross ewes split into two groups.

The first group was fed omega-6 sourced from oat grain and the second group was fed omega-3 sourced from a diet of pea silage.

Ewes were fed the two diets for six weeks prior to joining to Dorset rams and three weeks after joining.

After lambing, the lambs were tagged, which Dr Clayton said identified a surprising 15 per cent increase in the number of female lambs from sheep fed high omega-6 (grain) compared with those fed high omega-3 (pea silage) diets.

“For a self-replacing ewe flock or for first-cross ewe breeders, to increase the number of female lambs relative to the number of male lambs, a diet high in omega-6 could be fed at joining," he said.

“Alternatively, if producers wanted more male lambs (wethers develop more quickly and have more muscle) for prime lamb production systems, they might consider feeding a diet high in omega-3 at joining.”

Source: http://www.stockandland.com.au/news/agriculture/livestock/sheep-general/diet-may-affect-sex-of-lambs/2659723.aspx

Accessed: 10/07/2013

Mildura Weekly

Diet could prove key in determining sex of sheep offspring

Posted on June 11, 2013

IT'S a commonly shared theory among sheep breeders that they would be better off knowing the sex ratio of their offspring.

The idea is that breeders would be able to meet certain market needs if they could predict the sex of their lamb, with the theory shared by New South Wales Department of Primary Industries research leader, Dr Ed Clayton.

Dr Clayton will present research that demonstrates how a sheep's diet may affect the sex of lambs at the upcoming Grassland Society of Southern Australia's annual conference, which will be held at Albury's SS&A Club between July 16 and 18.

Dr Clayton said the research, which started in 2010 and has involved more than 1500 ewes, examined the ratio of omega-3 and omega-6 fatty acids in the diet of ewes, and its effect on the sex ratio of lambs.

"Recent trials have examined whether the skewing effect was pre or post-conception, and if it was similar in first-cross ewes and Merinos," he said.

"We think at this stage that pre-conception has the most important effect on the sex of the lamb.

"However, there may be some additional changes with feeding post conception, but we are still doing work on that.

"We are keeping the female progeny and have set up a new trial to see if there are any carry over effects of our feed regimes in the next generation that can affect the sex of lambs."

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“After lambing, the lambs were tagged, and we identified a very surprising 15 percent increase in the number of female lambs from sheep fed high omega-6 (grain) compared with those fed high omega-3 (pea silage) diets.

“For a self-replacing ewe flock or for first-cross ewe breeders, to increase the number of female lambs relative to the number of male lambs, a diet high in omega-6 could be fed at joining.

“Alternatively, if producers wanted more male lambs for prime lamb production systems, they might consider feeding a diet high in omega-3 at joining.”

More information about the upcoming Grasslands Conference is available by visiting www.grasslands.org.au (with those interested in attending also able to register online), or by contacting 1300 137 550.

Source: http://www.milduraweekly.com.au/2013/06/11/diet-could-prove-key-in-determining-sex-of-sheep-offspring/

Accessed: 10/07/2013

12.1.4 National Radio Interviews

ABC Victoria - Rural Report - Friday 7th June, 2013
774 ABC Melbourne - Breakfast show with Red Symons - Friday 14th June, 2013
2GB Radio, Sydney - National News - Thursday 15th June, 2013
Southern Cross Radio News network - Monday 18th June, 2013

12.1.5 Television Interviews

Prime Television – Monday 10th June 2013

Food the key to a lamb's sex

ABC Rural
Libby Price

Updated Tue Jun 25, 2013 6:06pm AEST

Photo: What you feed your ewes will have an effect on the sex of its lambs. (Laurissa Smith)

Wouldn't it be nice if you could somehow have more ewe lambs than ram lambs?
After all, you can never have enough females, especially for stud breeders.

While they don't know why, researchers at the Department of Primary Industries at Wagga Wagga have found a way to increase the percentages of females being born.

Dr Edward Clayton is a Livestock Research Officer specialising in ruminant nutrition, which is a bit of a clue.

It's all in what the sheep eat. Dr Clayton said the research, which started in 2010 and has involved more than 1500 ewes, examines the ratio of omega-3 and omega-6 fatty acids in the diet of ewes and the effect it has on the sex ratio of lambs.

Dr Clayton said recent trials have examined whether the skewing effect was pre or post-conception and if the effect was similar in first-cross ewes and Merinos.

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"Alternatively, if producers wanted more male lambs (wethers develop more quickly and have more muscle) for prime lamb production systems, they might consider feeding a diet high in omega-3 at joining."

Source: http://www.abc.net.au/news/2013-06-12/feeding-key-to-more-ewe-lambs/4749264

Accessed: 10/07/2013

12.2 Appendix 2 - Presentations Given to Producer Groups

2013 Australian White Suffolk Association Annual Conference, Cowra - 11/02/2013

NSW Department of Primary Industries 'TopCrop' Meeting, Junee Reefs - 22/02/2013

From: Philip Bowden/DII/NSW
Date: 18/02/2013 10:22 AM
Subject: Junee Reefs Topcrop 8th March ALL Welcome

Greetings, We'll kick off the season with a Topcrop meeting...all welcome...open discussion about all the issues... BBQ after...please pass this on to any neighbours or colleagues. cheers Phil

Phil Bowden
District Agronomist
Department of Primary Industries
Cootamundra, NSW 2590

FarmLink – Mixed Farming Forum, Temora Ag Innovation Centre - 18/07/2013

Hi Ed,
I met you at Junee Reefs Top crop group meeting early this year. We are planning a Mixed farming forum for July 31 at the Temora Ag Innovation centre. Your research into omega 3 & 6 impacts on male lamb percentages would be a relevant and well received topic. Would you be available then to come along and present your findings to farmers & advisors?

Paul Breust
Research & Extension Co-ordinator
FarmLink Research Limited
17 Denison Street (PO Box 240)
Junee NSW 2663
0407 009750
02 69244633
Fax 02 69244677
Email paul@farmlink.com.au

12.3 Appendix 3 - Details of ewes withdrawn from pen feeding studies

Table A6.1 Details of BL x Merino ewes withdrawn from Study 1 (2011) or 2 (2012) or missing prior to the commencement of the cross-over study in 2012.

Study	Ewe ID	Dietary Treatment	Date of Removal	Reason for Removal
Study 1 (11-07)	#086	High n-6	28/05/2011	Stolen
	#361	High n-6	28/05/2011	Stolen
	#663	High n-3	Aug 2011	Missing between pen feeding and preg scanning
	204	High n-6	Nov 2011	During lambing 2011?
Prior to Study 2				**Allocated to receive the treatment diet shown**
	27	High n-3		Missing between lamb marking and weaning
	323	High n-6		Missing between lamb marking and weaning
	329	High n-6		Missing between lamb marking and weaning
	372	High n-3		Missing between lamb marking and weaning
	377	High n-3		Missing between lamb marking and weaning
	484	High n-3		Missing between lamb marking and weaning
	842	High n-6		Missing between lamb marking and weaning
	346	High n-3		Missing between weaning and pre-trial (March)
	667	High n-6		Missing between weaning and pre-trial (March)
	360	High n-3		Missing pre-lambing
Study 2 (12-09)	#016	High n-3		Withdrawn, cancer

Table A6.2 Details of Merino ewes withdrawn from Study 3 (2011) or 4 (2012) or missing prior to the commencement of the cross-over study in 2012.

Study	Ewe ID	Dietary Treatment	Date of Removal	Reason for Removal
Study 3 (11-22)	#364	High n-3		Pregnant prior to trial commencement
	#149	High n-3		Withdrawn, low weight and fat score
Prior to Study 4				**Allocated to receive the treatment diet shown**
	#414	High n-3		Missing between lambing and lamb marking
	#424	High n-6		Missing between lambing and lamb marking
	#150	High n-3		Missing between weaning and pre-trial (March)
Study 4 (12-27)	#324	High n-6	01/10/2013	Pregnant prior to trial commencement
	#105	High n-3	09/11/2012	Withdrawn, low weight and fat score
	#441	High n-3	09/11/2012	Withdrawn, low weight and fat score
	#328	High n-6	18/01/2013	Withdrawn, cancer udder

Table A6.3 Details of BL x Merino maiden ewes withdrawn from Study 5 (2013).

Study	Ewe ID	Dietary Treatment	Date of Removal	Reason for Removal
Study 5 (13-01)	#630	High n-3	27/05/2013	Withdrawn, low weight and fat score

12.4 Appendix 4 - Nomenclature for fatty acids identified during the current project

Fatty Acid	Scientific Name	Common Name
SFA		
C8:0	Octanoic acid	Caprylic
C9:0	Nonanoic acid	Pelargonic
C10:0	Decanoic acid	Capric
C11:0	Undecanoic acid	Undecanic
C12:0	Dodecanoic acid	Lauric acid
C14:0	Tetradecanoic acid	Myristic acid
iC15:0	*iso*-Pentadecanoic acid	-
aiC15:0	*anteiso*-Pentadecanoic acid	-
C15:0	Pentadecanoic acid	Pentadecylic acid
C16:0	Hexadecanoic acid	Palmitic acid
iC17:0	*iso*-Heptadecanoic acid	-
aiC17:0	*anteiso*-Heptadecanoic acid	-
C17:0	Heptadecanoic acid	Margaric (daturic)
C18:0	Octadecanoic acid	Stearic acid
C20:0	Eicosanoic acid	Arachidic
C21:0	Heneicosanoic acid	Heneicosylic acid
C22:0	Docosanoic acd	Behenic
C23:0	Tricosanoic acid	Tricosylic acid
C24:0	Tetracosanoic acid	Lignoceric acid
MUFA		
C11:1n-1	10-undecanoic	Undecylenic
C12:1n-7	5-Lauroleic acid	Lauroleic acid
C13:1n-1	12-Tridecenoic acid	-
C14:1n-5	9-Tetradecaenoic acid	Myristoleic acid
C15:1n-5	cis-10-Pentadecenoic acid	Pentadecanoic
C16:1n-7t	9-Hexadecaenoic acid (*trans*)	Palmitelaidic acid
C16:1n-7	9-Hexadecaenoic acid (*cis*)	Palmitoleic acid
C17:1n-7	cis-10-Heptadecenoic acid	-
C18:1n-9t	9-Octadecanoic acid (*trans*)	Elaidic acid
C18:1n-7t	11-Octadecanoic acid (*trans*)	Trans Vaccenic acid
C18:1n-12	cis-6-octadecenoic acid	Petroselenic acid
C18:1n-9	9-Octadecanoic acid (*cis*)	Oleic acid
C18:1n-7	11-Octadecanoic acid (*cis*)	Vaccenic acid
C19:1n-12	7-Nonadecanoic acid	-
C20:1n-15	5-Eicosenoic acid (*cis*)	Eicosenoic acid
C20:1n-12	8-Eicosenoic acid (*cis*)	Eicosenoic acid
C20:1n-9	11-Eicosenoic acid (*cis*)	Gondoic acid
C22:1n-9	13-Docosaenoic acid (*cis*)	Erucic acid
C24:1n-9	15-Tetracosaenoic acid (*cis*)	Nervonic acid

Fatty Acid	Scientific Name	Common Name
n-3 PUFA		
C18:3n-3	9,12,15-Octadecatrienoic acid (*cis*)	α-Linolenic acid **(ALA)**
C18:4n-3	6,9,12,15-Octadecatetraenoic acid	Stearidonic acid **(SDA)**
C20:3n-3	8,11,14-Eicosatrienoic acid (*cis*)	Eicosatrienoic acid **(ETA)**
C20:5n-3	5,8,11,14,17-Eicosapentaenoic acid (*cis*)	Timnodonic acid **(EPA)**
C22:5n-3	7,10,13,16,19-Docosapentaenoic acid (*cis*)	Clupanodonic acid **(DPA n-3)**
C22:6n-3	4,7,10,13,16,19-Docosahexaenoic acid (*cis*)	Docosahexaenoic acid **(DHA)**
n-6 PUFA		
C18:2n-6t	9,12-Octadecadienoic acid (*trans*)	Linolelaidic acid
C18:2n-6	9,12-Octadecadienoic acid (*cis*)	Linoleic acid **(LA)**
C18:3n-6	6,9,12-Octadecatrienoic acid (*cis*)	γ-Linolenic **(GLA)**
C20:2n-6	11,14-Eicosadienoic acid	Eicosadienoic acid
C20:3n-6	8,11,14-Eicosatrienoic acid (*cis*)	Dihomo-γ-linolenic acid
C20:4n-6	5,8,11,14-Eicosatetraenoic acid (*cis*)	Arachidonic acid **(ARA)**
C22:4n-6	7,10,13,16-docosatetraenoic acid	Adrenic acid
C22:5n-6	4,7,10,13,16-Docosapentaenoic acid (*cis*)	**DPA n-6**

12.5 Appendix 5 - Meteorological Observations During Pen Feeding Studies at the WWAI

Study 1 - BL x Merino ewes (2011)

	April 2011			May 2011			June 2011		
	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall
Day	Min	Max	(mm)	Min	Max	(mm)	Min	Max	(mm)
1	10.6	24.8	0.0	8.2	22.0	0.0	3.6	19.7	0.0
2	9.3	22.7	0.0	12.1	21.3	0.0	4.4	18.9	0.0
3	5.3	23.5	0.0	8.2	21.1	8.0	3.5	18.1	0.0
4	5.9	23.0	0.0	6.3	17.8	0.8	8.1	12.6	1.6
5	7.1	24.3	0.0	3.1	18.8	0.0	5.0	10.3	4.2
6	9.3	23.9	0.0	2.3	18.3	0.0	5.8	11.8	1.4
7	9.9	23.7	0.0	2.3	18.3	0.0	-1.9	11.4	0.0
8	12.2	24.5	0.0	-0.4	16.3	0.0	0.1	9.3	3.0
9	9.5	27.0	0.0	0.2	18.1	0.0	2.8	14.0	0.0
10	12.1	17.1	12.4	-0.4	15.9	0.0	1.4	15.2	0.0
11	4.1	14.6	0.6	1.8	11.5	2.2	-0.5	15.2	0.0
12	9.0	14.8	3.6	3.6	11.6	0.6	-1.8	16.3	0.0
13	10.2	16.9	2.6	5.9	13.6	5.6	0.8	16.6	0.0
14	7.9	20.3	0.4	3.9	13.6	0.4	-0.3	17.1	0.0
15	5.7	21.6	0.0	-2.9	14.3	0.0	-0.5	18.0	0.0
16	9.7	21.7	0.0	-2.6	11.2	0.0	0.4	17.3	0.0
17	5.1	21.9	0.0	-1.7	15.0	0.0	3.0	11.3	1.0
18	6.5	22.6	0.0	-1.0	18.2	0.0	2.9	12.5	0.2
19	6.9	23.7	0.0	3.4	20.8	0.0	6.1	13.3	0.0
20	10.6	17.4	0.0	3.4	17.9	0.0	8.0	14.3	0.8
21	10.5	21.2	2.0	4.7	22.0	0.0	6.2	10.9	6.8
22	8.5	17.9	0.0	8.2	23.3	0.0	5.1	10.8	2.4
23	2.4	19.4	0.0	12.3	13.9	5.6	5.6	14.0	1.8
24	2.4	21.1	0.0	9.9	13.6	7.8	2.2	15.0	0.2
25	7.2	23.4	0.0	2.3	15.3	0.0	1.8	15.4	0.0
26	8.4	23.4	0.0	-0.6	14.8	0.0	-1.6	15.8	0.0
27	4.6	20.9	0.0	6.4	13.4	0.0	1.2	16.6	0.0
28	4.4	22.2	0.0	-0.4	14.8	0.0	-0.4	16.0	0.0
29	5.0	22.0	0.0	-0.6	15.7	0.0	2.9	17.0	0.0
30	6.2	23.4	0.0	3.2	17.7	0.0	3.8	16.9	0.0
31				2.2	20.5	0.0			

Study 2 - BL x Merino ewes (2012)

	April 2012			May 2012			June 2012		
	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall
Day	Min	Max	(mm)	Min	Max	(mm)	Min	Max	(mm)
1	14.0	22.9	0.0	9.1	22.4	0.0	4.0	17.8	0.0
2	13.3	25.9	1.6	10.1	18.5	11.4	4.1	14.8	0.0
3	11.3	30.5	0.0	2.8	14.7	8.6	8.2	15.1	16.4
4	11.1	28.3	0.0	1.4	16.4	0.0	5.7	12.8	0.2
5	13.0	28.5	0.0	0.4	16.2	0.0	6.3	15.4	3.2
6	16.7	29.8	0.0	2.0	13.9	0.2	0.4	16.5	0.0
7	9.4	20.8	0.0	4.4	15.3	0.0	2.7	14.6	0.0
8	4.6	20.7	0.0	5.4	22.3	0.0	-1.6	13.5	0.0
9	5.1	19.4	0.0	8.4	24.4	0.0	-0.4	14.6	0.0
10	1.3	17.4	0.0	7.5	24.0	0.0	0.7	16.0	0.0
11	0.9	19.8	0.0	9.5	21.5	0.2	0.1	16.5	0.0
12	3.6	22.1	0.0	3.0	15.0	0.0	0.2	17.2	0.0
13	4.7	23.1	0.0	3.4	15.6	0.0	1.6	17.2	0.0
14	7.4	20.8	0.0	-0.4	14.5	0.0	4.7	17.3	0.0
15	8.6	25.5	0.0	-0.4	17.7	0.0	10.1	17.2	0.0
16	7.7	26.2	0.0	1.8	18.4	0.0	3.8	11.4	0.0
17	7.9	25.1	0.0	2.1	17.6	0.0	5.1	13.5	1.6
18	13.9	23.5	0.6	1.4	18.3	0.0	2.3	13.4	0.0
19	16.6	22.4	2.2	1.6	18.7	0.0	5.8	13.9	1.0
20	15.2	23.0	0.6	0.3	17.7	0.0	-2.6	12.0	0.0
21	9.9	25.8	0.0	0.7	16.2	0.0	2.3	16.1	0.0
22	12.3	23.0	0.4	-1.6	17.6	0.0	7.9	10.1	2.2
23	11.5	23.4	1.4	2.7	21.1	0.0	-0.3	10.3	1.2
24	9.9	15.4	0.2	7.2	13.3	0.2	1.8	13.8	0.0
25	6.2	16.0	0.8	6.4	9.3	26.0	1.7	13.1	0.4
26	4.4	15.4	0.0	5.3	11.8	2.8	2.3	14.4	0.6
27	2.3	19.5	0.0	6.5	15.6	0.4	4.5	15.2	0.0
28	1.2	21.2	0.0	-0.3	15.5	0.0	3.2	14.6	0.0
29	2.2	20.5	0.0	2.0	17.5	0.0	7.6	17.1	0.6
30	4.5	20.9	0.0	1.5	17.6	0.0	1.5	13.1	2.2
31				2.5	17.4	0.0			

Study 3 - Merino ewes (2011)

	October 2011			November 2011			December 2011		
	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall
Day	Min	Max	(mm)	Min	Max	(mm)	Min	Max	(mm)
1	8.2	11.6	1.4	6.3	26.6	0.0	7.7	23.1	1.8
2	2.9	19.2	6.6	10.7	25.0	0.0	9.1	24.0	0.0
3	3.0	18.4	2.8	9.1	24.5	0.0	11.0	26.8	0.0
4	2.9	20.5	0.0	5.5	27.7	0.0	12.5	25.2	0.0
5	5.4	19.8	0.0	9.6	32.2	0.0	6.9	23.5	0.0
6	11.7	14.0	0.8	16.4	33.0	0.0	8.7	23.4	0.0
7	12.3	19.8	0.2	18.3	29.8	4.0	11.2	25.7	0.0
8	8.4	22.4	0.0	19.2	30.3	0.0	16.1	27.7	0.0
9	9.1	19.0	0.0	17.0	32.7	0.2	15.0	28.6	0.0
10	4.7	16.6	0.2	13.6	23.7	22.8	18.7	26.0	0.0
11	2.6	17.6	0.0	7.4	26.2	0.0	16.5	27.3	10.4
12	0.9	19.7	0.0	12.2	28.5	0.0	12.3	27.1	1.4
13	3.4	23.0	0.0	13.6	30.5	0.0	9.9	26.0	0.0
14	13.6	24.0	0.0	18.9	31.0	0.0	10.6	25.9	0.0
15	12.9	27.1	0.0	10.2	31.8	0.0	13.6	27.7	0.0
16	9.2	19.3	0.0	14.0	24.6	0.0	15.3	28.9	0.0
17	1.3	21.8	0.0	13.8	29.1	0.0	15.4	29.3	0.0
18	6.3	24.4	0.0	16.6	33.5	0.0	17.2	25.3	0.0
19	5.7	27.0	0.0	16.3	35.8	0.0	17.8	27.2	12.8
20	8.4	28.6	0.0	18.3	22.2	6.8	12.7	28.2	0.0
21	9.7	30.0	0.0	8.6	24.5	7.4	16.2	28.6	0.0
22	11.7	30.2	0.0	10.9	20.2	0.0	17.1	28.5	0.0
23	12.6	32.9	0.4	9.2	27.1	0.0	15.7	30.2	20.6
24	15.8	33.0	0.0	10.8	24.4	0.0	15.9	32.7	0.0
25	14.4	20.4	4.6	12.9	20.0	18.8	17.2	31.6	3.4
26	8.1	22.7	0.0	14.0	24.8	34.2	17.1	27.7	16.2
27	9.0	24.9	0.0	15.2	27.3	0.4	14.3	27.6	3.6
28	12.6	29.2	0.0	12.1	31.8	0.0	14.8	28.0	0.0
29	17.4	25.2	1.8	18.1	34.4	0.0	13.5	29.3	0.0
30	9.0	21.6	0.0	17.9	25.4	57.8	13.3	28.9	0.0
31	5.3	24.7	0.0				15.6	30.3	0.0

Study 4 - Merino ewes (2012)

	October 2012			November 2012			December 2012		
	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall
Day	Min	Max	(mm)	Min	Max	(mm)	Min	Max	(mm)
1	-1.6	20.3	0.0	15.3	22.6	0.0	23.5	29.0	0.2
2	2.6	22.6	0.0	3.9	23.0	0.0	16.9	28.0	0.6
3	2.7	25.5	0.0	8.6	23.8	0.0	9.7	28.0	0.0
4	7.8	29.8	0.0	12.7	29.8	0.0	12.0	22.4	0.0
5	6.9	28.3	0.0	15.7	32.6	0.0	7.0	21.8	0.0
6	12.1	18.4	0.6	21.5	31.8	0.0	6.0	26.5	0.0
7	2.9	17.2	3.4	18.2	20.2	19.6	13.2	29.4	0.0
8	2.1	17.9	0.0	15.4	26.8	22.2	14.1	34.2	0.0
9	2.6	19.3	0.0	10.9	23.0	4.6	14.2	31.5	0.0
10	1.7	19.4	0.0	5.9	22.9	0.0	12.2	27.9	0.0
11	8.2	11.6	0.8	8.3	24.7	0.0	13.8	29.4	0.0
12	5.8	18.1	25.6	13.0	31.0	0.0	16.3	31.9	0.0
13	4.5	18.7	0.0	10.6	26.5	0.0	17.5	34.1	0.0
14	2.6	21.8	0.0	7.8	25.8	0.0	19.3	33.3	0.0
15	7.2	27.5	0.0	10.0	28.5	0.0	19.7	26.0	0.8
16	10.9	24.8	0.0	13.3	26.4	0.0	15.5	28.2	6.8
17	5.0	20.3	0.2	7.9	26.2	0.0	9.7	27.9	0.0
18	3.5	25.2	0.0	7.2	23.1	0.0	9.7	30.2	0.0
19	8.9	30.4	0.0	7.1	26.4	0.0	14.4	36.5	0.0
20	8.4	26.6	0.0	11.2	26.7	0.0	15.7	28.0	4.0
21	6.9	23.6	0.0	14.7	32.4	0.0	12.2	31.6	0.0
22	5.4	20.6	0.0	8.6	28.6	0.0	17.3	35.3	0.0
23	1.6	23.2	0.0	10.2	29.5	0.0	21.7	39.1	0.0
24	7.7	25.5	0.0	13.9	32.1	0.0	20.8	38.6	10.2
25	13.5	27.9	0.0	15.5	37.0	0.0	14.5	30.4	0.2
26	6.8	20.6	0.0	16.4	34.6	0.0	12.6	28.0	0.0
27	5.2	21.8	0.0	17.7	30.7	0.2	13.1	30.5	0.0
28	5.0	25.1	0.0	18.4	32.8	13.6	14.8	30.3	0.0
29	10.3	28.0	0.0	19.5	38.6	0.0	12.0	32.3	0.0
30	13.3	30.4	0.0	25.2	35.7	0.0	13.1	32.7	0.0
31	13.5	33.1	0.0				17.6	34.8	0.0

Study 5 - BL x Merino maiden ewes (2013)

	April 2013			May 2013			June 2013		
	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall	Temperature (°C)		Rainfall
Day	Min	Max	(mm)	Min	Max	(mm)	Min	Max	(mm)
1	8.2	23.4	0.0	8.2	21.3	0.0	13.1	14.6	24.4
2	7.5	23.8	0.0	3.4	19.3	0.0	12.3	16.4	42.0
3	9.9	24.6	0.0	3.5	21.6	0.0	0.8	13.7	0.0
4	11.9	24.6	0.0	7.3	20.7	0.0	2.3	14.8	0.0
5	13.5	25.3	0.0	2.3	18.9	0.0	4.7	14.6	0.0
6	13.6	25.4	0.0	3.8	19.5	0.0	7.4	16.7	0.0
7	11.1	26.1	0.0	9.4	20.7	0.0	10.7	15.7	0.0
8	10.5	27.1	0.0	12.6	21.7	0.8	1.7	14.5	0.0
9	11.4	26.8	0.0	5.8	23.3	0.0	2.3	16.7	0.0
10	10.8	27.2	0.0	7.9	25.3	0.0	7.3	16.9	0.0
11	10.2	27.7	0.0	8.1	24.5	0.0	2.7	14.7	0.0
12	16.0	27.9	0.0	8.1	24.8	0.0	7.8	14.0	7.6
13	11.3	28.2	0.0	12.9	14.9	13.8	9.8	13.0	10.2
14	13.8	29.8	0.0	3.1	12.6	13.8	9.3	14.0	2.6
15	16.8	25.6	0.0	7.6	13.7	12.2	3.2	14.8	0.0
16	8.5	24.2	0.0	9.2	15.4	0.8	0.8	14.8	0.0
17	9.1	22.6	0.0	4.3	13.0	1.4	0.1	13.0	0.0
18	6.5	23.2	0.0	3.5	13.5	0.0	2.6	11.5	0.4
19	7.5	19.7	0.0	4.2	14.6	0.0	3.8	13.9	1.6
20	3.1	22.5	0.0	2.8	15.0	0.8	1.7	13.2	0.0
21	5.4	21.5	0.0	7.4	17.0	1.2	-0.1	14.3	0.0
22	11.4	18.9	5.0	4.6	12.5	0.0	0.2	13.9	0.0
23	8.1	20.7	0.0	7.3	18.4	1.4	0.0	14.2	0.0
24	3.0	18.3	0.0	2.4	17.3	0.0	4.3	11.8	0.4
25	1.6	21.2	0.0	-0.7	16.3	0.0	4.1	17.6	8.2
26	4.0	22.3	0.0	1.3	16.5	0.0	1.3	17.6	0.0
27	4.2	23.4	0.0	0.7	18.0	0.0	2.3	16.0	0.0
28	7.5	28.1	0.0	6.2	19.7	0.0	6.7	15.7	0.0
29	9.5	22.4	0.0	7.2	21.0	0.0	7.2	15.1	0.0
30	11.8	21.8	2.2	10.4	15.5	0.2	1.9	15.4	0.0
31				12.4	17.4	8.8			

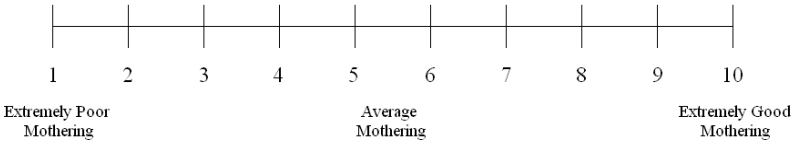
12.6 Appendix 6 - Details of Observations Carried out on Lambs at Birth

Table A6.1 Details of behavioural observations carried out on ewes and lambs at birth.

Maternal score (wait for 30 seconds after lamb release)

Score	Description
0	Lamb dead – invalid record
1	Ewe stays close to lamb/s while tagging occurs
2	Ewe retreats but stays within 10 m and returns immediately shepherd leaves
3	Ewe circles at a distance and continues to show interest in lamb (>10m)
4	Ewe retreats >20 m but cautiously comes back to lamb as shepherd leaves
5	Ewe flees at approach of shepherd, shows no interest in lamb and does not return

Mothering (Subjective assessment by person tagging lamb)



Lamb vigour (record within 30 sec of release)

Score	Description
0	Lamb still wet – new born – invalid record
1	Constant struggle – bleat in response to ewe – on release reaches ewe quickly and follows
2	Regular struggle while held – moves to the ewe on release – bleating common
3	Some struggle – walking in direction of ewe bleats but no contact – may bleat
4	Some struggle – attempts to walk but aimless – no apparent response to ewe bleats
5	Little movement when held – lies on release

Lamb age (record before sampling)
0 = Newborn; 1 = Wet; 2 = Dry