Supplementation of Merino ewes with Serradella Pod does not increase ovulation or reproductive rate

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Declaration

This thesis has been composed by myself and has not been accepted in any previous application for a degree. The work, of which this is a record, has been done by myself and all sources of information have been cited.

……. Date……

Sydney Fowler

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Literature Review

1. INTRODUCTION

The on-farm value of lambs in Australia exceeds AU$2 billion per annum and reproductive performance is a key profit driver for farms (Young *et al*, 2014). Improving reproductive performance is a research priority for the sheep industry as recently; demand for lamb exceeds supply (Curtis, 2009). Reproductive performance on-farm is determined by three key measures; reproductive rate, marking rate and weaning rate. This study is focused on reproductive rate which is determined by counting the number of foetuses scanned via ultrasound at approximately day 50 of pregnancy per 100 ewes mated. Ovulation rate is the key determinant of reproductive rate, which is determined by the number of eggs released per 100 ewes mated; however this is less practical and economical to measure in an on-farm environment. Increasing ovulation rate can improve reproductive rates, which will improve on-farm profits and assist in supplying extra lamb to meet market demands.

There is evidence that feeding lupins (*Lupinus angustifolius*) for 6-10 days prior to oestrus can increase ovulation rate in Merino ewes (Lightfoot *et al* 1976; Oldham and Lindsay 1984; Stewart and Oldham 1986). Lupins are high in crude protein (CP=32%) and metabolisable energy (ME=13MJ/kg DM) compared to barley (CP=11% & ME=11.9 MJ/kg DM). Recent work has shown that other feeds of similar nutritive value can be used as a short-term supplement to increase ovulation rate in ewes (Wilkins 1997; King *et al* 2010). Tagasaste (C*hamaecytisus palmensis*) is a perennial shrub that is similar in crude protein and metabolisable energy to lupin grain and has been reported to increase ovulation rate by 20 extra eggs released per 100 ewes (Wilkins 1997). Lucerne (*Medicago sativa*) and chicory (*Chicorum intybus*) have been shown to increase ovulation rate in comparison to phalaris (*Phalaris aquatic*) when fed prior to mating in summer and autumn (King *et al* 2010; Thomas *et al* 2010). Lupins, tagasaste, lucerne and chicory have a limited range of environments to which they are adapted therefore a suitable alternative to increase ovulation rate would be beneficial to farmers.

Farmers have been shifting away from traditional pasture legumes such as subterranean clover and annual medics and adopting newer legume species which combat or solve some of the current issues with these older species. Increasing costs of inorganic nitrogen, the need to combat herbicide-resistant weeds and increasing livestock prices will contribute to continued uptake of these new species (Nichols *et al* 2006). Serradella clover is one such legume species

that is currently being adopted by farmers, particularly in Western Australia (Nichols *et al* 2006). Many cultivars of serradella have been developed to suit a range of soils and rainfall zones. Serradella pods are high in crude protein (31.5%) and metabolisable energy (13.6 MJ/kg DM) and are similar in nutritional value to lupins, tagasaste, lucerne and chicory. Therefore serradella could be a suitable alternative to increase ovulation rate when fed to ewes pre-mating.

This review will describe the physiological mechanisms behind the control of ovulation rate and how increasing short-term nutrition prior to ovulation leads to the increased reproductive rate of ewes. In this study, we hypothesise that ewes supplemented with Serradella (pods) will have a higher ovulation rate and consequent reproductive rate than ewes that are not supplemented, similar to the effects observed from short-term supplementation with lupins.

2. PHYSIOLOGICAL MECHANISMS BEHIND CONTROL OF OVULATION RATE

Ovulation rate, as with any reproductive factor is limited by environmental factors such as nutrition. The phenotypic expression of ovulation rate in a ewe has a predetermined range of ovulations that can be affected by a physiological mechanism that incorporates environmental inputs (Scaramuzzi *et al*, 1990). The physiological mechanism behind oestrus and ovulation is the hormonal control by the hypothalamus, anterior pituitary and ovaries.

2.1 OESTRUS, OESTRUS CYCLE & OVULATION

The reproductive physiology of the ewe is particularly complex but essentially revolves around hormonal control of oestrus and ovulation. Oestrus, commonly known as heat, is defined as the period when the ewe is receptive to sexual activity. An oestrus cycle delineates periodic development of follicles (folliculogenesis) which results in release of ova or eggs that have the possibility of being fertilised. Oestrus, in ewes, is displayed for 24–48 hours and the oestrus cycle is approximately 17 days in length. Oestrus and the oestrus cycle are important in our study as we are manipulating these to produce responses to nutritional supplementation.

Ovulation is controlled by the anterior-pituitary and ovarian hormones that also regulate the maturation of follicles (Moyes,*et al.* 2008). A typical ovulation is depicted in Figure 1.1 showing the development of follicles through to ovulation whilst the hormones related to development of follicles are shown in Figure 1.2. The hypothalamus releases Gonadotropin-releasing Hormone (GnRH) triggering the anterior pituitary to secrete pulses of gonadotropins: Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH). This in

turn causes the ovaries to produce ovarian steroid hormones: progesterone and oestrogen. It is these hormones: LH, FSH, progesterone and oestrogen, which control ovulation but to manipulate ovulation we must first understand the mechanisms behind these hormones.

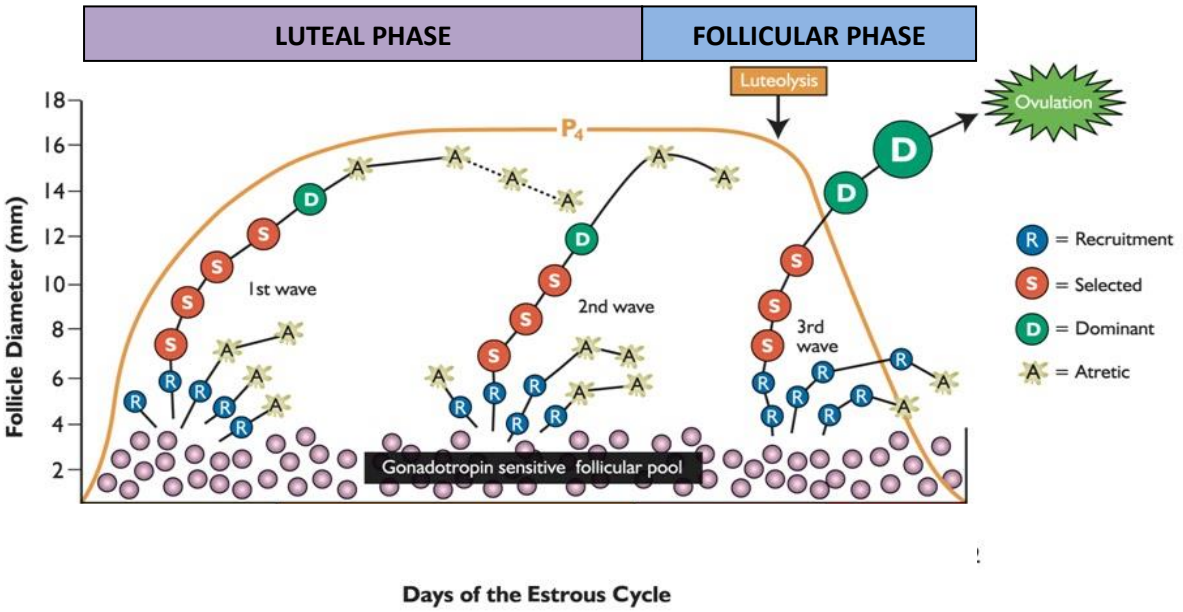

Figure 1.1 Several follicular waves occur during the oestrus cycle. The small filled circles represent gonadotropin-sensitive follicles. During each wave some follicles are recruited (R), some are selected (S) and some become dominant (D). Eventually most follicles undergo atresia. Only follicles recruited after the third wave of after luteolysis of the corpus luteum (CL) produced in the previous cycle will become eligible for ovulation. [Source: Adapted from Moyes and Schulte, 2008]

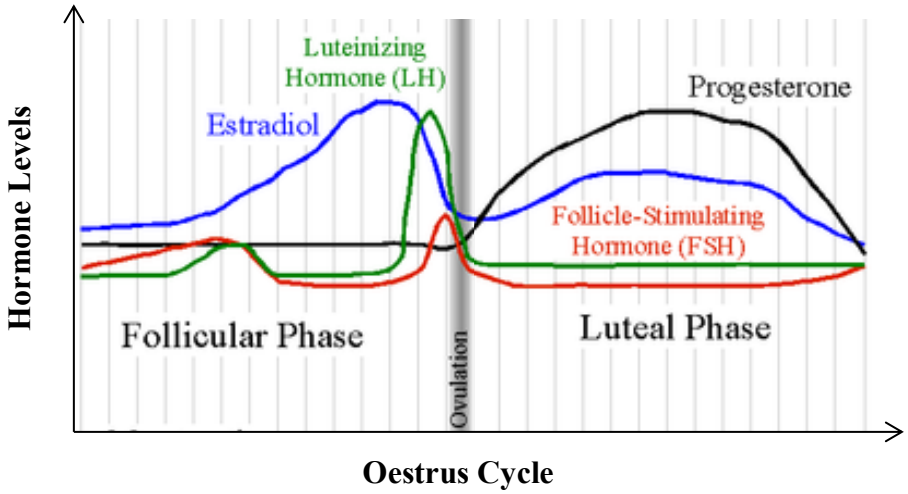

Figure 1.2. Relative changes in secretion of major reproductive hormones during ovine oestrus cycle. During the follicular phase, oestrogen increases due to secretion from recruited and selected follicles. This promotes an increase in FSH with further stimulation of oestrogen secretion and finally the dramatic LH surge that leads to ovulation.

During the late luteal phase, blood concentrations of both oestrogen and progesterone are declining and once they fall below a critical level hypothalamic GnRH is secreted causing a

rapid secretion of FSH accompanied by a slower secretion of LH. During the early follicular phase a subset of follicles from the gonadotropin sensitive follicular pool are recruited and begin to mature. The average number of recruited follicles present at any one time is about equal to the average ovulation rate (Webb & Gauld, 1985). Recent advances have since contradicted these findings (review by Scaramuzzi *et* al. 2011) but the insinuation has remained that ovulation rate is affected by the supply of recruited follicles available for selection and therefore present for ovulation.

Oestrogen secretion increases directly proportion to the proportion of maturing follicles. Increasing oestrogen exerts negative feedback blocking GnRH release and production of LH and FSH, in effect decreasing levels of these three hormones. Decreased FSH levels will cause many of the follicles to undergo atresia but a subset, termed the 'dominant follicles', mature to the point where they can sustain maturation despite falling FSH levels. Atresia refers to the degeneration or regression of an ovarian follicle.

The hypothalamic-pituitary axis reorganises its signalling pathways during the late follicular phase as to reverse the effects of oestrogen. Oestrogen no longer impairs GNRH but instead promotes GnRH release. The dominant follicle/s during late follicular maturation continues to produce oestrogen increases LH levels via a positive feedback mechanism this dramatic increase is termed the LH surge. The LH surge causes granulosa cells to secrete several factors that support development of the ovum. Enzymes are secreted to digest the extracellular matrix between the follicle cells, weakening the follicle and causing it to rupture releasing the ovum.

Just prior to ovulation, the follicle cells increase production of progesterone, marking beginning of ovulation. After ovulation, the remnants of the follicle continue to play an important role in hormone synthesis. Driven by the LH surge, the follicle undergoes a change in structure, increasing in size and complexity as capillaries and fibroblasts penetrate the structure. The remnants of the rupture follicle appear as a dense yellow body in the ovary known as the corpus luteum. The CL secretes high levels of progesterone and lesser amounts of oestrogen sustaining steroid hormone secretion for a time but level begin to decline. If fertilisation occurs the CL develops further but if fertilisation does not occur, progesterone levels continue to decline and the next ovulatory cycle begins.

The hormonal control mechanism of ovulation is essential to our studies as these hormones not only regulate the cyclical maturation of follicles and control ovulation but are also involved in whole-body nutritional homeostasis.

2.2 PHYSIOLOGICAL EFFECTS OF NUTRITON ON OVULATION

The relationship between nutrition and ovulation rate, as a determinant of reproductive performance, is associated with energy balance. Ensuring nutrient intake is adequate for the nutrient requirements of a ewe during joining to maintain the ewe in a condition score of 3.0 or greater and a minimum pasture target of 1000kg DM/ha dry FOO (Fergurson *et al*. 2008). If net nutrient intake was less than net nutrient requirements this would produce a deficit, or negative energy balance, meaning the ewe would have to use energy stores to meet requirements. Whilst if net nutrient intake was more than net nutrient requirements the excess nutrients would be stored and the animal would be in a state of positive energy balance. These metabolic states are regulated by a series of complex interactions of metabolic hormones many of which are also involved in the regulation of the reproductive system. As a result there are many well-defined associations between metabolic state and reproduction as seen in Table 1 (reviewed by Scaramuzzi *et al*, 2006).

A negative energy balance causes hypoinsulinemia, hypoglycaemia, suppressed plasma IGF-1 and elevated plasma GH which effects ovulation mainly through low FSH concentrations leading to inhibition of folliculogenesis, anovulation and anoestrus. Although evidence suggests that negative energy balance does not have any direct ovarian effects in the ewe independent of its effects on the hypothalamus-pituitary axis (Lozana *et al*. 2003; Kiyma *et al*. 2004).

A positive energy balance causes increased levels of insulin and leptin concentrations and increased glucose uptake affecting ovulation by increased FSH concentration leading to enhanced folliculogenesis, ovulation and oestrus. Increasing nutrition, thereby creating a positive energy balance, leads to increased FSH concentrations. There is then a cascade effect where increased FSH concentrations leads to a decrease in atresia, a decrease in atresia leads to an increased proportion of follicles and therefore an increased ovulation rate.

Table 1. Some known associations between energy balance and reproduction.

Metabolic State	Metabolic Consequences	Effects of Reproduction
Negative energy Balance	Weight loss Fat stores depleted Muscle wasting Hypoinsulinemia Hypoglycaemia Elevated βOH butyrates and NEFA Elevated GH & Low Leptin Reduced metabolic heat Suppressed IGF system Elevated urea	Inhibition of GnRH secretion by the hypothalamus Absence of LH pulses Low FSH concentrations Inhibition of folliculogenesis High negative feedback sensitivity Anovulation Anoestrus Delayed puberty
Energy Balance	Weight maintained Fate stores maintained Normal insulin Normoglycaemia Low NEFA and βOH butyrate Normal GH & Leptin Normal IGF system Normal urea	Normal of GnRH secretion by the hypothalamus Normal LH pulsatility Normal FSH concentrations Normal folliculogenesis Normal oestradiol and inhibin Normal negative feedback Ovulation & Oestrus Ovulation rate below natural maximum
Positive energy Balance	Long-term weight gain Fate stores increased Hyperinsulinemia Hyperglycaemia Low NEFA and βOH butyrate Low GH Elevated Leptin Increased metabolic heat Stimulated IGF system Urea normal but can be high if dietary nitrogen is high	Normal of GnRH secretion by the hypothalamus Normal LH pulsatility Increased FSH concentrations Enhanced folliculogenesis Reduced oestradiol Reduced negative feedback Ovulation & Oestrus Maximum natural ovulation rate Advanced puberty

A previous study (Vinoles *et al*. 2005) suggested the need for a standardised model to study the effect of nutrition on follicle growth. The 'first-wave model' was developed as it allows the commencement of feeding the supplement at the expected time of wave emergence so that the peak concentrations of the metabolic hormones coincide with the time of maximum requirement for FSH in the growing follicles. Nutritional metabolic hormones insulin and leptin increased as a result of supplementation which allows more follicle/s to be selected into the ovulatory wave.

3. NUTRITION PRIOR TO OVULATION

Nutrition has been extensively studied particularly in regards to the effect it has on ewe reproduction (McInnes *et al*, 1966; Fletcher, 1971; Lindsay *et al*, 1993). The effects of nutrition prior to ovulation were initially developed in regards to the concept of flushing where a three week period of high-level nutrition increased body mass of the ewe and was used to increase litter size (Clark, 1934). Coop (1966) outlined the 'static' and 'dynamic' effects of nutrition which followed on from Clark's concept of flushing but analysed the mechanisms further. Whilst static effects refer to liveweight and condition score at joining – dynamic effects are associated with the liveweight change during a 6-week flushing period prior to joining (Coop, 1966). The descriptive analysis of the relationship between nutrition and liveweight has led to a classification of nutritional effects on ovulation rate which can be seen in Figure 2. The static effect associated liveweight as a whole, the dynamic effect associated with increasing body weight and the acute effects associated with increase in ovulation rate without a change in body weight. This third phenomenon noted as the 'acute' effect is also referred to as the 'immediate' effect outlined by Smith & Stewart (1990).

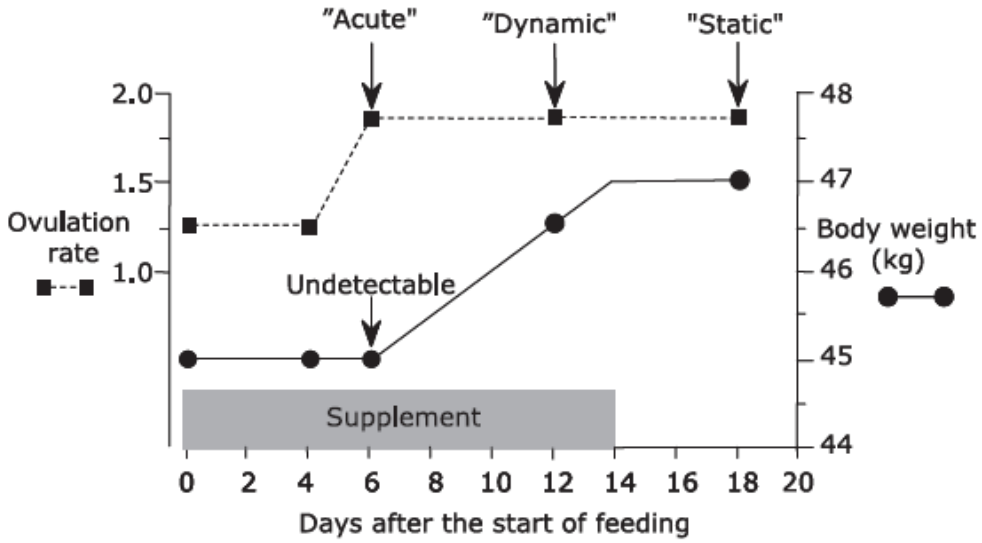

Figure 2. The 'acute', 'dynamic', and 'static' influences of nutrition on ovulation rate in sheep. Acute refers to the effect seen in the absence of a detectable change in body weight. The dynamic effect is associated with increasing body weight. The static effect is associated with elevated body weight overall.

These three patterns and their effects on ovulation rate appear to increase due to surges in the number of gonadotropin-dependent follicles maturing (Rhing & McNeilly, 1986; Xu *et al*. 1989; Smith & Stewart, 1990; Vinoles *et al*. 2002). Stimulation of the hormonal mechanisms that respond to nutrition such as the glucose-insulin system will increase the proportion of gonadotropin-dependent follicles due to physiological mechanisms mentioned previously in this report.

Further studies have shown that feeding lupins (*Lupinus angustifolius*) for 6-10 days prior to oestrus can increase ovulation rate in Merino ewes (Lightfoot *et al*, 1976; Oldham *et al*, 1984; Stewart *et al*, 1986). The response discovered in these studies seem to be independent of the 'static' and 'dynamic' effects mainly due to the significant change in live weight during the period of supplementation (Lindsay, 1976). The practice of 'flushing' – short-term nutritional supplementation of a feed high in protein and energy – has come about from such studies mentioned previously and due to this phenomenon reported by Lindsay (1976). The exact mechanism by which flushing influences ovulation rate is not known but we do know that the desired effects are produced with lupins – a feed that is high in crude protein (32%) and high in metabolisable energy (13 MJ/kg DM). Due to high costs associated with lupin feeding, other feeds that produce similar results would be beneficial to farmers.

4. OTHER FEED OPTIONS FOR INCREASING OVULATION RATE

Ensuring ewes have optimal nutrition during critical periods such as joining is crucial. There is a pasture deficit experienced across Australia during late summer when traditional annual pastures, such as Phalaris (*Phalaris aquatic*) senesce. Producers typically supplement feed with lupins during late summer due to their ability to boost ovulation rates (Smith et al., 1990), but there is increasing evidence that suggests the effects are driven by dietary energy (Vinoles et al., 2005). Recent work has shown that other feeds of similar nutritive value can be used as a short-term supplement to increase ovulation rate in ewes (Wilkins 1997; King *et al* 2010). Lupins are expensive and an alternative that is cost effective with similar results on ovulation rate would be readily adopted.

Tagasaste (C*hamaecytisus palmensis*) is a perennial shrub that is similar in crude protein and metabolisable energy to lupin grain and has been reported to increase ovulation rate by 20 extra eggs released per 100 ewes (Wilkins 1997). Tagasaste is also commonly known as tree lucerne due to its nutritional similarities with perennial pasture lucerne (*Medicago sativa*). Tagasaste was found to be an effective feed supplement to boost ovulation rates but practical issues regarding target feeding and ensuring adequate feed intake make it unsuitable.

Perennial pastures, such as lucerne and chicory, provide similar good quality nutrition (metabolisable energy equals 10-12MJ/kg DM) during summer and autumn (Holst *et al*., 1998). Lucerne and chicory (*Chicorum intybus*) have been shown to increase ovulation rate in comparison to phalaris when fed prior to mating in summer and autumn (King *et al* 2010; Thomas *et al* 2010). Lucerne is suited to medium to high rainfall areas whilst chicory is more tolerant of acidic soils providing an alternative to lucerne. Lupins, tagasaste, lucerne and chicory have a limited range of environments to which they are adapted therefore a suitable alternative to increase ovulation rate would be beneficial to farmers.

Serradella clover is becoming popular with farmers, particularly in Western Australia due to its suitability on a broader range of soil types and environments (Nichols *et al* 2006). There are many cultivars of serradella such as: Yelbini, Charano and Santorini yellow serradellas (low-medium rain fall, allows direct heading, persistence), King yellow serradella (high productivity and persistence in northern New South Wales), Cadiz French serradella (cheap alternative for deep, infertile acid soils), Margurita and Erica French serradellas (higher persistence). Being a leguminous pasture it has the ability to fix nitrogen in the soil leading to more flexibility with mixed farming properties. Serradella pods are high in crude protein

(31.5%) and metabolisable energy (13.6 MJ/kg DM) and are similar in nutritional value to all previously studied feeds. Compared to lupins, tagasaste, lucerne and chicory, serradella could easily be a suitable alternative to increase ovulation rate when fed to ewes pre-mating.

5. CONCLUSION

This paper presents Serradella pods as a potential feed that could be used as an alternative to lupins to boost ovulation rates in Merino ewes. The results from selected experiments lend support to this concept. The use of Serradella pods has a sound base due to nutritional similarities to well researched supplements such as lupins, tagasaste, lucerne and chicory, combined with the added benefits of flexibility as a pasture for farmers. Serradella could potentially increase lamb production by an increase in ovulation rate leading to an increase in reproductive rate. This is of great benefit not only to farmers on an economical level but also to animal production systems as a whole as market demands can be better met. A more complete understanding how short-term supplementary feeding of Serradella (pod) and in the future perhaps Serradella pasture or conserved fodder can only facilitate the economical and effective production of lamb.

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Scientific Paper

Supplementation of Merino ewes with Serradella does not increase ovulation or reproductive rate

Abstract

It is well documented that short-term supplementation of a feedstuff high in energy and protein prior to joining increases reproductive rate and ovulation rate of ewes. In this study, the effect of short-term supplementation with serradella pods on ovulation and reproductive rate was investigated and compared to supplementation with lupins. Nine hundred Merino ewes (4.5 years) sourced from two farms, Site 1 and Site 2 (n=450 per site), had oestrus synchronised with the use of Controlled Internal Drug Release (CIDR) devices. Ewes at each site were randomised based on liveweight and condition score into one of three treatment groups: control (no supplement), lupin and serradella pod. Ewes were fed either: no supplement (control), 700g/hd/day of lupins (ME=13 MJ/kg DM, CP=32%) or 700g/hd/day of serradella pod (ME=12 MJ/kg DM, CP= 24%) for six days prior to joining. In addition to being weighed and condition scored throughout the study, the ewes were scanned for ovulation rate and pregnancy status at nine days and forty days post joining, respectively. At joining there were site by treatment interactions evident for condition score ($P<0.001$). The condition score of ewes at Site 1 was higher for control than both lupins and serradella (3.50, 3.33 and 3.22 respectively) whilst at Site 2 the condition score of ewes fed serradella was highest compared to lupin and control (3.36, 3.16 and 3.04 respectively). Liveweight, ovulation rate and reproductive rate did not differ significantly between treatments ($P>0.05$) and there was no site by treatment interactions. It is concluded that short-term supplementation with serradella pods is not effective for increasing ovulation and reproductive rates.

Key words: Serradella, short-term supplementation, ovulation rate, reproductive rate, ewes

1. INTRODUCTION

The on-farm value of lambs in Australia exceeds AU$2 billion per annum and reproductive performance is a key profit driver for farms (Young *et al*, 2014). As current demand for lamb exceeds supply, farmers need to improve reproductive performance to keep up with market demands whilst ensuring continuation of Australia's sheep flock (Curtis, 2009). Improving reproductive performance is, now more than ever, a research priority for the sheep industry. Reproductive performance on-farm is determined by three key measures; reproductive rate, marking rate and weaning rate. This experiment focusses on how nutrition can influence reproductive rate in Merino ewes. Ovulation rate is the key determinant of reproductive rate; however this is less practical and economical to measure in an on-farm environment. Increasing ovulation rate can improve reproductive rates, which will improve on-farm profits and assist in supplying extra lamb to meet market demands.

There is evidence that feeding lupins (*Lupinus angustifolius*) for 6-10 days prior to oestrus can increase ovulation rate in Merino ewes (Lightfoot *et al* 1976; Oldham and Lindsay 1984; Stewart and Oldham 1986). Lupins are high in crude protein (CP=32%) and metabolisable energy (ME=13MJ/kg DM) and recent work has shown that other feeds of similar nutritive value can be used as a short-term supplement to increase ovulation rate in ewes (Wilkins 1997; King *et al* 2010). Tagasaste (C*hamaecytisus palmensis*) is a perennial shrub that is similar in crude protein and metabolisable energy to lupin grain and has been reported to increase ovulation rate by 20 extra eggs released per 100 ewes (Wilkins 1997). Lucerne (*Medicago sativa*) and chicory (*Chicorum intybus*) have been shown to increase ovulation rate in comparison to phalaris (*Phalaris aquatic*) when fed prior to mating in summer and autumn (King *et al* 2010; Thomas *et al* 2010). Lupins, tagasaste, lucerne and chicory have a limited range of environments to which they are adapted therefore a suitable alternative to increase ovulation rate would be beneficial to farmers.

Farmers have been shifting away from traditional pasture legumes such as subterranean clover and annual medics and adopting newer legume species which combat or solve some of the current issues with these older species. Increasing costs of inorganic nitrogen, the need to combat herbicide-resistant weeds and increasing livestock prices will contribute to continued uptake of these new species (Nichols *et al* 2006). Serradella is one such legume species that is currently being adopted by farmers, particularly in Western Australia (Nichols *et al* 2006). Many cultivars of serradella have been developed to suit a range of soils and rainfall zones.

Serradella is high in crude protein (31.5%) and metabolisable energy (13.6 MJ/kg DM) and are similar in nutritional value to lupins, tagasaste, lucerne and chicory. Therefore serradella could be a suitable alternative to increase ovulation rate when fed to ewes pre-mating. In this paper we hypothesised that short-term supplementation with serradella would increase ovulation rate and reproductive rate compared to ewes that were not supplemented, and the response would be comparable to supplementation with lupins.

2. MATERIAL AND METHODS

All procedures reported in this paper were approved by Murdoch University's Animal Ethics Committee in accordance with the guidelines of the Australian Code of Practice for the Use of Animals for Scientific purposes.

Research sites and experimental design

The research was conducted at two properties located in Wagin (Site 1; 33.3088°S, 117.3439°E) and Katanning (Site 2; 33.6908°S, 117.5553°E), in the southwest of Western Australia. The experiment commenced in early January 2015 and concluded in March 2015. Nine hundred Merino ewes aged 4.5 years were sourced from site 1 and 2 (n=450 per farm). Ewes were allocated into one of three treatment groups; control (no supplement), lupins or serradella, such that the mean liveweight and condition score of each group was similar. Treatments were administered for six days leading up to joining.

Animal management, treatments and animal measurements

All ewes were weighed and condition scored in early January 2015 and grazed on a common paddock at each site. Fifteen days prior to joining all ewes were weighed, condition scored (Russel *et al.* 1969), and had a Controlled Internal Drug Release (CIDR) inserted to synchronise oestrus. CIDRs were inserted on 15-Jan at Site 1 and on 20-Jan at Site 2. Six days prior to joining all ewes were weighed and condition scored again and then separated into treatment groups. Artificial fences and water troughs were set-up within each paddock at each site to separate the three treatment groups for the supplementation period. Ewes were then fed either no supplement (control) or fed a supplement of approximately 700g/hd/day of either lupins (ME=13MJ/kg DM, CP=32%) or serradella (ME=12MJ/kg DM, CP=24%). After six days of supplementation the artificial fences were removed, CIDRs were removed and all

ewes were weighed and condition score again and then joined together with a minimum of 18 rams for one week (4% rams). Nine days after the CIDRs were removed, a sub sample of approximately 50 ewes as close in liveweight to the mean, from each treatment were assessed for ovulation rate via trans-rectal ultrasonography (Vinoles *et al.* 2010). Forty days after the CIDRs were removed all ewes were scanned for pregnancy via trans-abdominal ultrasound.

At Site 1 when the ewes were scanned for ovulation rate nine days after CIDRS were removed 19% of ewes were detected as being >50 days pregnant from exposure to a ram prior to the commencement of the study and consequently removed. Additional ewes were scanned from each treatment to record approximately 50 ovulation rates for analysis.

Nutrition

Ewes were managed to achieve maintenance in liveweight throughout the synchronisation and joining period at each site. Site 1 ewes grazed a dry barley stubble (44.9% DMD, 6.1 MJ/kg DM, 1.2% CP) and Site 2 ewes grazed a senesced annual pasture (47.7% DMD, 6.6 MJ/kg DM, 5.0% CP). Maintenance of liveweight at site 2 was achieved by supplementary feeding 200g/hd/day of oats (feed quality not tested). The daily treatment rations of lupins or serradella pod at both sites were calculated based on the mean liveweight of ewes six days prior to joining, so that the ration would be providing approximately 1 x maintenance requirements. This treatment ration was then provided daily, in addition to the base diet of stubble /senesced pasture & oats at each site.

Statistical Analysis

All statistical analyses were performed using GENSTAT (GENSTAT committee 2008). Ovulation rate and reproductive rate data were analysed using ANOVA. Site, treatment, liveweight and condition score at joining, were fitted as fixed effects including all significant interactions. Ewe identification was fitted as a random effect. The model predictions presented as back-transformed means with 95% confidence intervals. Statistical significance was accepted at $P<0.05$.

3. RESULTS

Liveweight and condition score

The Site 2 ewes were significantly heavier than Site 1 ewes throughout the trial ($P<0.05$; Table 1). During the course of the trial, liveweights between treatments were not significantly different and there were not site by treatment interactions evident ($P>0.05$).

At joining and pregnancy scanning (Days 0 & 40) ewes at Site 1 were in significantly better condition score than Site 2 ewes ($P<0.001$; Table 2). However, there was no difference in condition score between sites at CIDRs In (Day-15; $P>0.05$). Throughout the trial, condition score of ewes did not differ between treatments ($P>0.05$).

There were site by treatment interactions for condition score seen at joining ($P<0.001$) but not at Days -15 and 40 ($P>0.05$). At Site 1, ewes in the control treatment were in better condition score than both lupin and serradella treatments ($P<0.001$) however the lupin and control treatments were not significantly different ($P>0.05$). At Site 2, the serradella treatment ewes were in better condition score than the lupin and control treatments ($P<0.001$) which did not significantly differ from each other ($P>0.05$).

Ovulation rate and reproductive rate

Ovulation rates were not significantly different between sites or between treatments and there was no evidence of site by treatment interactions ($P>0.05$; Table 3).

Ewes from Site 1 and Site 2 did not differ significantly in reproductive rate ($P>0.05$; Table 3). Between treatments, reproductive rate was not significantly different. Site by treatment interactions were non-existent for reproductive rate ($P>0.05$).

Table 1: The mean liveweight (kg) of ewes by site, by treatment and site by treatment that received no supplement (control), or received lupins or serradella for six days pre-joining at two different sites [Least significant differences between treatments are presented with 95% confidence intervals, p-values presented (P<0.05), treatments with different superscripts are considered significantly different at P<0.05]

Treatment		CIDRs In (Day -15)	Joining (Day 0)	Ovulation Scanning (Day 9)	Pregnancy Scanning (Day 40)
Site	**1**	58.7[a]	60.0 [a]	60.7 [a]	61.8 [a]
	2	63.0[b]	62.7 [b]	61.5 [b]	64.4 [b]
	LSD (5%)	0.98	0.96	0.99	0.92
	P-value	<0.001	<0.001	0.075	<0.001
Treatment	**Control**	61.4 [a]	61.5 [a]	60.8 [a]	62.7 [a]
	Lupin	61.4 [a]	62.1 [a]	61.7 [a]	63.7 [a]
	Serradella	61.4 [a]	62.0 [a]	61.1 [a]	63.9 [a]
	LSD (5%)	1.17	1.15	1.18	1.11
	P-value	n.s.	n.s.	n.s.	n.s.
Site by Treatment					
Site 1	**Control**	59.0 [a]	60.1 [a]	60.4 [a]	61.1 [a]
	Lupin	58.6 [a]	60.5 [a]	60.9 [a]	62.1 [a]
	Serradella	58.6 [a]	61.1 [a]	60.8 [a]	62.1 [a]
Site 2	**Control**	62.8 [a]	62.3 [a]	61.0 [a]	63.6 [a]
	Lupin	63.1 [a]	63.1 [a]	62.2 [a]	64.7 [a]
	Serradella	63.0 [a]	62.6 [a]	61.4 [a]	64.9 [a]
	LSD (5%)	1.68	1.65	1.69	1.58
	P-value	n.s.	n.s.	n.s.	n.s.

Table 2: The mean condition score of ewes by site, by treatment and site by treatment that received no supplement (control), or received approximately 700 g/hd/day of lupins or serradella for six days pre-joining at two different sites [Least significant differences between treatments are presented with 95% confidence intervals, p-values presented (P<0.05), treatments with different superscripts are considered significantly different at P<0.05]

Treatment		CIDRs In (Day -15)	Joining (Day 0)	Pregnancy Scanning (Day 40)
Site	**1**	3.04 [a]	3.35 [a]	3.44 [a]
	2	2.98 [a]	3.18 [b]	3.29 [b]
	LSD (5%)	0.80	0.79	0.068
	P-value	n.s.	<0.001	<0.001
Treatment	**Control**	3.07 [a]	3.21 [a]	3.32 [a]
	Lupin	3.00 [a]	3.22 [a]	3.37 [a]
	Serradella	2.98 [a]	3.31 [a]	3.36 [a]
	LSD (5%)	0.096	0.094	0.082
	P-value	n.s.	n.s.	n.s.
Site by Treatment				
Site 1	**Control**	3.04 [a]	3.50 [a]	3.41 [a]
	Lupin	2.96 [a]	3.33 [b]	3.48 [a]
	Serradella	2.94 [a]	3.22 [b]	3.43 [a]
Site 2	**Control**	3.09 [a]	3.04 [a]	3.26 [a]
	Lupin	3.02 [a]	3.16 [a]	3.31 [a]
	Serradella	3.00 [a]	3.36 [b]	3.31 [a]
	LSD (5%)	0.137	0.137	0.117
	P-value	n.s.	<0.001	n.s.

Table 3: The mean ovulation rate and mean reproductive rate of ewes that received no supplement (control), or received lupins or serradella for six days pre-joining at two different sites [Least significant differences between treatments are presented with 95% confidence intervals, p-values presented (P<0.1), treatments with different superscripts are considered significantly different at P<0.05].

Treatment		Ovulation Rate	Reproductive Rate
Site	**1**	1.58 [a]	0.93 [a]
	2	1.61 [a]	1.03 [a]
	LSD (5%)	0.131	0.126
	P-value	n.s.	n.s
Treatment	**Control**	1.54 [a]	0.92 [a]
	Lupin	1.59 [a]	1.07 [a]
	Serradella	1.64 [a]	0.99 [a]
	LSD (5%)	0.158	0.151
	P-value	n.s.	n.s.
Site by Treatment			
Site 1	**Control**	1.60 [a]	0.90 [a]
	Lupin	1.46 [a]	0.98 [a]
	Serradella	1.67 [a]	0.90 [a]
Site 2	**Control**	1.50 [a]	0.92 [a]
	Lupin	1.71 [a]	1.13 [a]
	Serradella	1.61 [a]	1.04 [a]
	LSD (5%)	0.224	0.216
	P-value	0.063	n.s.

4. DISCUSSION

Supplementation with serradella pods for 6 days prior to joining did not significantly increase ovulation rate or reproductive rate in Merino ewes compared to supplementation with lupins or no supplementation. Therefore the hypothesis is rejected. Reproductive rates of control and lupin treatment were almost different (difference of 0.150 & LSD of 0.151). A similar occurrence was seen at Site 2 whereby control and lupin treatments differed by 0.210 whilst the least significant difference was 0.216. This suggests a positive trend however it cannot be statistically supported. The 15% and 21% increase in reproductive rate is consistent with literature whereby reproductive rate typically increases by 10% but can range from -10% to 25% (Croker *et al* 1985; Young *et al* 1990; Kelley and Croker 1990). The response to feeding serradella pods was not comparable to lupins and based on this research alone is not suggested as a suitable alternative for nutritionally flushing ewes. This study was the first to attempt to quantify any effect of serradella on ovulation and reproductive rate in sheep. Consequently, further investigation into the effects of serradella is essential to determine the efficacy and suitability as an alternative to lupins.

The 5% difference in ovulation rate between lupin and control treatments was considerably lower than previously reported – 13-24% by Knight *et al* (1975), 24-38% by Lightfoot and Marshall (1974) and 29-57% by Nottle *et al* (1997). There was conflicting interactions of site and treatment for ovulation rate within this experiment. At Site 1 the ovulation rate of the control treatment was higher than the lupin treatment (difference of 14%) contrasting the results seen at Site 2 where lupin was higher than control (difference of 21%). The conflicting site results may be due to one of three reasons: 1) the out of season introduction of a ram at Site 1 in December 2014, prior to commencement of the experiment which may have affected subsequent oestrus cycles, 2) some studies have shown that theories based on controlled experiments did not hold up in an on-farm situation (Croker *et al* 1985; King and Fisher 1990), and 3) the sample size for ovulation rate scanning was not sufficient to display the response to treatments. Results at Site 2 were more in line with the literature whereby lupin supplemented ewes induced a higher ovulation rate than non-supplemented ewes however we cannot be conclusive as a third replicate is essential to give confidence to the results.

Other supplements have been investigated as alternatives to lupins to increase ovulation rate such as tagasaste (C*hamaecytisus palmensis*), lucerne (*Medicago sativa*) and chicory (*Chicorum intybus*) (Wilkins 1997; King *et al* 2010). Grazing tagasaste has been reported to

increase ovulation rate by 20% compared to control, however was not as effective as supplementation with lupins which increased ovulation rate by 66% (Wilkins 1997). Lucerne and chicory were shown to be more effective than lupins in increasing ovulation rate (13%, 11% and 7% respectively) compared to senesced phalaris pasture (King *et al* 2010). In this study, serradella had the highest ovulation rate and lupin was intermediate to control (164%, 159% and 154%) but these results were not significantly different. However, there were site discrepancies that need to be taken into account. At Site 1, ovulation rate of lupin (146%) was lower than that of the control or serradella (160%, 167%) contrary to previous reports where lupin increased ovulation rate compared to control (Knight *et al* 1975; Oldham and Lindsay 1984). As mentioned previously, the unscheduled exposure of experimental ewes to a ram and the concept that on-farm experiments can have a multitude of unknown interfering factors compared to controlled experiments explains this discrepancy at Site 1. At Site 2, ovulation rate of lupins (171%) was higher than control (150%) and serradella (161%) was intermediate similar to results reported by Wilkins (1997) where tagasaste increased ovulation rate compared to control however was not as effective as lupins. The results at Site 2 are closer to what was expected and the discrepancies, mentioned previously, regarding Site 1 further confirms the need to repeat the experiment with the inclusion of a third replicate which should aid in minimising the unknown surrounding site by treatment interactions.

Producers typically supplement feed with lupins during late summer due to their ability to boost ovulation rates (Smith and Stewart 1990), but there is increasing evidence that suggests the effects are driven by dietary energy (Vinoles *et al* 2005). The energy and protein content of feedstuffs used for flushing varies ranging in metabolisable energy from 8-13MJ/kg DM and crude protein from 20-32%. Serradella falls within this range (ME=12 MJ/kg DM, CP=24%) such that a result would have been expected. As no other studies have been conducted using serradella pods, the optimum feeding rate may have been inconsistent with necessary amount to produce results and as such may need to be fed at a different rate. The absence of a response for ovulation and reproductive rate could also be due to some unforeseen effect of serradella on metabolic or reproductive hormones.

Previous studies have described a positive relationship between liveweight and ovulation rate and condition score and ovulation rate (Gunn and Doney 1975; Morley *et al* 1978). In this study there did not appear to be a trend between liveweight and ovulation rate or condition score and ovulation rate. Despite, site differences and site by treatment interactions evident in liveweight and condition score results. Site differences in liveweight throughout trial were due

to genetic variation between strains of sheep. At Site 1, during ovulation scanning, 19% of ewes were removed due to being greater than 50 days pregnant. Liveweights of the additional ewes scanned to replace the pregnant ewes were further from the mean than those originally selected. Therefore the absence of these pregnant ewes would have affected the liveweight at ovulation scanning. At the commencement of the trial, Day -15, there was no difference in condition score between sites despite an obvious difference throughout the remainder of the trial accounted for by the subjective nature of condition scoring. Site by treatment interactions were evident for condition score data such that at Site 1 lupin and serradella were in significantly lower condition score than control at joining ($P<0.001$) and at Site 2 serradella was in better condition score than control and lupins at joining ($P<0.001$). Site 1 results were unexpected as the lupin and serradella treatment both received one times maintenance above what the non-supplemented control treatment received. This could be due to a myriad of reasons such as genetic variation between individuals or the variation in amount of supplement received by each ewe. Despite minimising every factor that could cause bias or cause variation, the nature of an on-farm experiment remains that there are many interfering factors which can affect the outcome (Croker *et al*, 1985).

Site 2 results were such that serradella was in better condition score than control and lupins at joining and control and control and lupin did not differ significantly. This was what we had expected as far as condition scores however without a third replicate we cannot be conclusive that this difference is a direct result of the serradella treatment.

5. CONCLUSION

This study found that short-term supplementary feeding with serradella did not significantly increase ovulation or reproductive rates in Merino ewes. The response to feeding serradella pods was not comparable to lupins and as such is not suggested as a suitable alternative to nutritionally flush ewes prior to joining based on this research alone. The use of Serradella had a sound base due to nutritional similarities to well researched supplements such as lupins, tagasaste, lucerne and chicory, combined with the added benefits of flexibility as a pasture for farmers. This study was the first to attempt to quantify any effect of serradella pod on ovulation and reproductive rate in sheep hence the need for further investigation. A more complete understanding of how serradella pod affects the reproductive physiology of Merino ewes is essential to determine its suitability as an alternative short-term supplementation to increase ovulation and reproductive rates on farm.

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