CHAPTER ONE

GENERAL INTRODUCTION

Ruminant productivity in industrialised countries is supported with high quality feeds including highly digestible forages such as silage, hay or grazing or on grains combined with high protein oilseed meals. Leng (1985) has stressed that in reality most ruminant production systems in Asia are based on low quality crop residues, and thus improved production will depend on strategic supplementation of diets with high quality protein forage or other sources. In developing countries such as Indonesia, that rely on the agricultural by products for ruminant productivity, maximisation of the utilisation of agricultural by-products is the major alternative to increase animal production. Basal diets in a traditional farming system vary considerably and include both forage based diets alone or together with a range of agricultural by-products such as rice bran, cassava tubers, soybean curd waste, banana shoots and pineapple waste.

Industrialisation, together with an increasing population density, results in a reduction in availability for feed production for ruminants in many developing countries and there is an increasing dependency on agricultural by-product residues. Feeding strategies in this situation, therefore, must focus on: (1) the optimisation of rumen

fermentation, to obtain more nutrients available from forages and straw as basal diets, and (2) supplementation with nutrients that escape unchanged in the lower digestive tract and "by-passing" rumen fermentation. This can produce an optimum balance of nutrients and hence efficient use of the nutrients absorbed (Preston and Leng, 1987).

Increasing animal productivity is not often accomplished by enhancing animal reproduction rate, where this is low. High rates of reproduction are strongly associated with an appropriate balance of nutrients for the animal. Therefore, providing feeds rich in by-pass protein increases the efficiency of utilisation of nutrients absorbed from forage based diets in the rumen, and thus the opportunity for increasing animal production that utilises agricultural by-products.

From recent studies, reproduction rate can be increased by feeding ruminant animals with high quality feed that provide essential rumen microbial nutrients as well as nutrients to the host. Experiments this thesis are aimed at finding ways: (1) to which nutritional regime may effect reproduction rate of small ruminants; (2) to increase nutrients availability (ie. protein) of small ruminants by a strategy of supplementation involving rumen manipulation; (3) to increase in microbial balance and feeding high protein meals, which in turn increase their reproductive performance. Such an approach of supplementation that involves rumen manipulation is highly applicable in tropical countries such as Indonesia. Therefore, in the final experiment (Chapter 5) of the series, urea multi-nutrient molasses block (UMMB) as a means to provide nutrients to the rumen microbial ecosystem (Hendratno *et al.*, 1990) of goats fed crop residue in Indonesia was investigated, to assess the potential to increase reproductive performance at the level of the traditional farming system. This UMMB supplementation has become an applicable and a practical way to supplement animals under small farmer management in Indonesia. The major objective of this experiment is to increase animal productivity and in turn increase farmers' daily income and standard of living.

CHAPTER TWO

LITERATURE REVIEW

Interaction of Nutrition and Reproduction

2.1 INTRODUCTION

Nutrition and reproduction are two components that are closely interrelated. Reproduction is a complex multi-function physiological process and involves oestrus and ovulation, mating, fertilisation, embryo development, pregnancy, parturition, milk production and weaning. These physiological events are interrelated with particular levels and composition of nutritional status (Egan, 1984). Nutrition in this matter plays its role in determining the success of individual reproductive traits. However, the requirements of nutrition in reproduction fluctuate widely throughout the year, and between time of year and the age of the animal (ie. small ruminants). Other factors besides nutrition are also important such as environmental factors, and there must also be taken into account (Thwaites, 1967).

In this chapter the manipulation of reproductive hormones and feeding strategy to improve the reproductive efficiency of small ruminants, particularly the level of absorbed amino acids will be discussed. The interaction between reproductive status and level of nutrition, mostly in small ruminants, which affect reproductive traits such as the onset of oestrus, ovulation rate, pregnancy and *post partum* ovarian activity will also be reviewed.

2.2 THE MANIPULATION OF REPRODUCTIVE HORMONES

The control of oestrus and ovulation in small ruminants has been sought for many years. Methods for the control of the time of oestrus and ovulation in cyclic sheep with progesterone administration (Robinson *et al.*, 1967), and in anoestrous sheep with pregnant mare serum gonadotrophin, PMSG (Moore and Holst, 1967; Robinson and Smith, 1967), have been evaluated as being simple and effective.

Control of the normal reproductive endocrine system in the female generally involves the hypothalamus-pituitary and ovarian axis (Peters and McNatty, 1980), and also the pineal gland (Lindsay, 1988). The management of all reproductive endocrine systems seems to be mediated via the pituitary (Peters and McNatty, 1980). The neuro-hormonal messages from the hypothalamus or hormonal feed-back to the pituitary are processed and then transferred and secreted into the blood stream as a signal to the ovary in the form of pulses of hormones (Peters and McNatty, 1980; Lindsay, 1988). Because of this, a review of the normal system and some manipulations of ovulation in oestrous and seasonally and lactationally anoestrous sheep is appropriate before further discussion of the interaction between nutrition and reproduction.

2.2.1 Oestrus Cycle

Serum LH and FSH, which are collectively called gonadotrophins, are two hormones that play a major role in the oestrous cycle and ovulation. These hormones are synthesised and stored in the pituitary and control the functions of the ovary. There is a third gonadotrophin secreted from the anterior pituitary gland, named prolactin. The role of prolactin in ovarian function is uncertain and complex. There is evidence that under certain circumstances, prolactin may suppress follicular growth, probably by inhibiting the conversion of androgens to oestrogens (Baird, 1975).

The release of the LH and FSH is controlled primarily by the pulse secretions of the releasing factors (GnRH) from the hypothalamus (Peters and McNatty, 1980). Therefore, the release of LH and FSH can be induced by the injection of synthetic GnRH in lactationally anoestrous ewes (Reeves *et al.*, 1972; Haresign *et al.*, 1983). A schematic diagram of the changes in these hormone concentrations during normal oestrous cycle is shown in Figure 2.1. This figure illustrates the pre-ovulatory surge of LH and FSH prior to ovulation. The pre-ovulatory surge of LH is initiated by an increase in peripheral oestrogen, that has positive feedback effect to the hypothalamic-pituitary axis, which in turn induces the release of LH and FSH surge (Haresign *et al.*, 1983). Haresign *et al.* (1983) further stated that the final phase of follicular development is dependent on the pattern of LH surge secretion.

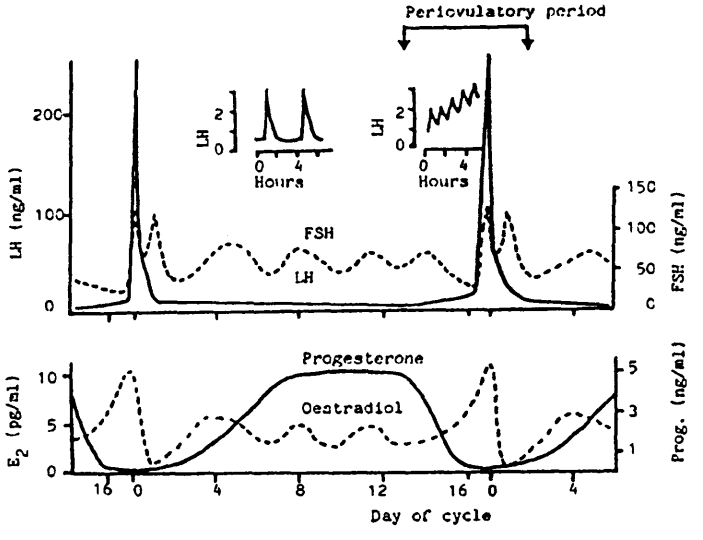


Figure 2.1 The changes of peripheral plasma reproductive hormone concentrations during the oestrus cycle of ewe (from Haresign *et al*, 1985).

Oestrogen and progesterone are hormones produced by the ovary during the oestrous cycle, and both are also involved in the cyclicity of oestrus. Progesterone is of corpus luteum origin whilst oestrogen is a steroid hormone produced by the granulosa cells of the hollow lining cells of the follicles (Lindsay, 1988). The high concentration of progesterone during the luteal phase inhibits positive feedback and enhances its negative feedback by dampening the endocrine activity of the ewe, slowing down the rate of production of GnRH from the hypothalamus, and therefore the LH release (Peters and McNatty, 1980). Thus progesterone can be used to synchronise oestrus and ovulation in the ewe.

There is evidence that by administration of synthetic progesterone, the oestrous cycle and ovulation can be controlled, with considerable commercial impact in the ruminants industry. This synthetic progesterone has a similar action to endogenous progesterone during the luteal phase and suppresses the release of GnRH surge. Supplementation of progesterone could be oral through feeding (Baker *et al.*, 1964). However, this has been evaluated to be ineffective compared with daily injections of progesterone (Lindsay *et al.*, 1967). A more recent finding indicated that insertion of intravaginal progesterone pessaries or sponges was more effective than previous methods, and the progesterone more consistently available (Haresign, 1978). According to Polge (1973), the effect of progesterone administration could be abruptly terminated by ceasing feeding or injections, or by withdrawing the subcutaneous or vaginal pessaries to stimulate normal luteolysis. A side effect of progesterone exposure is the increase in the sensitivity of the animal to oestrogen (Lynch *et al.*, 1992). At this moment the central nervous system is 'primed' to be very sensitive to the presence of progesterone (Lindsay, 1988; Lynch *et al.*, 1992). This priming increases the intensity of oestrous behaviour.

Follicle stimulating hormone stimulates the growth of ovarian follicles which secrete oestrogens that are associated with the onset of oestrus of the ewe (Lynch *et al.*, 1992). During the follicular phase, the level of oestrogen exerts positive feedback to the

hypothalamus to stimulate the secretion of LH pulses which in turn signals the ovary to ovulate (Lindsay, 1988).

Others possibilities of potential sites to increase ovulation rate and higher pregnancy rates by pharmacological methods have been proposed by Scaramuzzi and Martin (1984) as in the endocrine reproductive pathways (Figure 2.2). The sites in which reproductive control may be taken are: the olfactory system, the pineal gland, the anterior pituitary and the ovary.

Olfactory system

In terms of inducing ovulation in anovulatory ewes, Knight (1983) suggested that pheromones may play an important roles in stimulating onset of oestrus and ovulation through the olfactory system. This pheromone is produced from glands over most of the ram's body but not in its urine (Knight and Lynch, 1980a, 1980b; Walkden-Brown *et al.*, 1993a; 1993b). Evidence showed that male animals have this pheromone, but it tends to be specific to animal species. Knight *et al.* (1983b) has been able to show that boar's pheromone is not as effective as compared to ram's and buck's; but buck's pheromone is still less effective compared to ram's. Therefore, the technique of re-introducing a teaser ram could be used to control the onset of oestrus in anovular ewes, and advance the time of breeding season (Lindsay, 1988).

The ram-stimulated ovulation is the result of an apparently normal secretion of gonadotrophin (LH) pulses from the pituitary which occurs within 10 minutes of the introduction of the ram (Martin *et al.*, 1980), but there is no interaction with the level of nutrition fed to the animal (Knight *et al.*, 1983b).

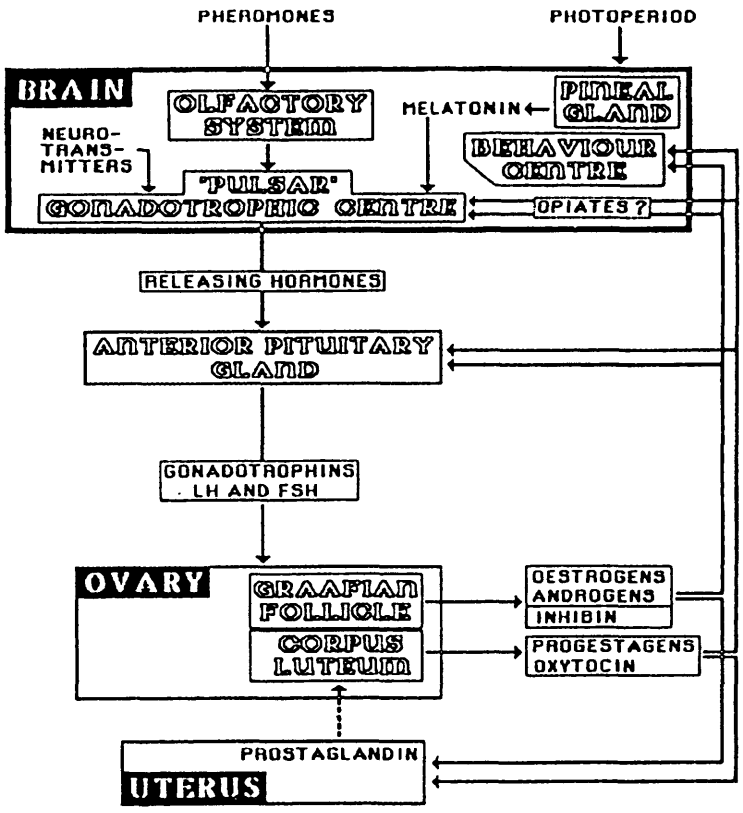


Figure 2.2 A schematic illustration of the reproductive endocrine controls showing several sites that may be manipulated in terms of controlling the reproductive system in ewes (from Scaramuzzi and Martin, 1984).

Previous work indicated that isolation periods of ewes from ram of 17 and 34 days induced 47 and 44% of those ewes, respectively, to ovulated as compare to only 3% of ewes that were not isolated from the ram (Oldham, 1980). Injection of progesterone is suggested, when rams are introduced, with the response of a high degree of oestrous synchronisation 19 to 21 days later, and this injection eliminates the biphasic onset of first oestrus due to premature regression of corpus luteum (CL) (Knight, 1983).

On the evidence of recent studies, ovulation can be induced earlier in the breeding season by introducing rams or bucks into the flocks of ewes (Al-Mauly *et al.*, 1991;

Cushwa *et al.*, 1992; Sweeney and O'Calaghan, 1993), and of goats (Walkden-Brown *et al.*, 1993a; 1993b). These experiments suggest that introducing male sheep or bucks can become one of the strategies for reproductive development and can be used to stimulate the onset of oestrus in seasonally anovulatory animals.

Pineal gland

Many techniques pertaining to manipulation of the pineal gland involve anoestrous animals in the non-breeding season. Photoperiod, which controls the breeding season, involves the pineal gland in addition to the hypothalamus-pituitary-ovarian axis. Light is received by the light receptors in the eye and transmitted to the medio basal part of hypothalamus or surrounding tissue (Malpaux *et al.*, 1993) and modified in the pineal gland, according to the length of exposure of the light, and depending on the reproductive and nutritional stage of the animal (Lindsay, 1988). The pineal gland converts the neural signal received into a hormonal signal, by releasing melatonin as illustrated in Figure 2.3 (see Karsch, 1984). This indicates that the physiological response to photoperiod is mediated by the pineal gland through its circadian pattern of melatonin release, and therefore this becomes a potential site for control of the oestrous cycle and ovulation.

Artificial-light exposure for a definite period during the non-breeding season does not consistency to induce oestrus (McDonald and Hopkins, 1984), which results in low fertility in sheep (Lindsay, 1988), and perhaps causes refractoriness on short days (Karsch *et al.*, 1986; Robinson and Karsch, 1984; Robinson *et al.*, 1985); additional exogenous melatonin hormone can be applied to overcome these problems. Moreover, a light proof building would not be a feasible investment (see Chemineau *et al.*, 1992).

Exogenous melatonin can override the photoperiod effect by mimicking the signal gives by short days (ie. simulatory photoperiod) and present the hypothalamus with false information concerning the season. Several recent studies indicated that the onset of

oestrus and the breeding season can be advanced by injecting or feeding melatonin (Kennaway *et al.*, 1982; Nett and Niswender, 1982).

Kennaway *et al.* (1982) and Arendt *et al.* (1983) in their earlier findings, have shown that ovulation and oestrus can be induced under the influence of an inhibitory photoperiod. The finding of Arendt *et al.* (1983) showed that melatonin fed to anoestrous Suffolk-cross ewes in the absence of rams, induced changes in plasma progesterone indicative of oestrus 2 to 8 weeks ahead of untreated animals.

The success of melatonin administration (eg. injection or feeding) in inducing oestrus and ovulation has been discussed by Staples *et al.* (1992), who found that the response to melatonin is characterised by a greater proportion of ewes ovulating and a higher ovulation rate as compared to untreated ewes. In addition, cyclic and ovulatory activities continue into spring for goats treated with melatonin (Chemineau, *et al.*, 1992). Staples *et al.* (1992) indicated that treatment with melatonin implants is effective in increasing reproduction rate in Merino, Corriedale and Romney Marsh ewes. This has also been confirmed with administration of melatonin in continuous release implants, which induced onset of oestrus in anoestrous ewes (Williams and Helliwell, 1993).

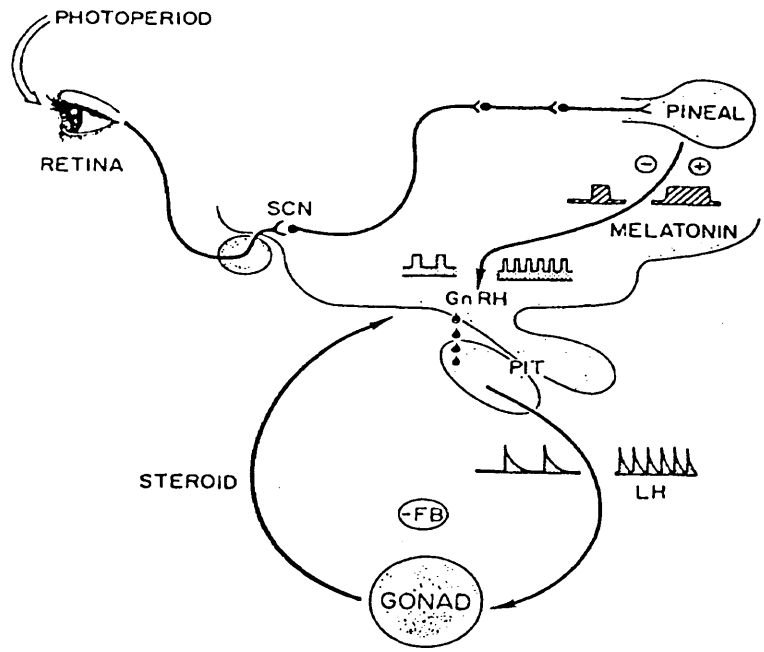


Figure 2.3 The illustration of photoneuroendocrine pathway to the LH pulse generator of the ewe (from Karsch, 1984). Photoperiodic message is received by photoreceptors and then transmitted via superior cervical ganglia (SCN) to the pineal gland. The pineal converts this into a circadian rhythm of melatonin secretion which is directly proportional to the length of day-light and interpreted as *inductive* or *suppressive*. Inductive melatonin signals stimulate the pulse generator of GnRH to the anterior pituitary (PIT) and become resistant to the oestradiol (steroids).

Anterior Pituitary and Ovary

As discussed earlier LH and FSH are gonadotrophins that have a major role in controlling oestrous cycle in the female. The release of these gonadotrophins are dependent on the surge level of GnRH. Therefore, additional exogenous GnRH, artificial positive or negative feedback to the hypothalamic-pituitary axis (McNeilly and Wallace, 1987; Miller and Martin 1993), and increased sensitivity of the pituitary to the releasing hormones are possible ways for manipulating pituitary involvement in reproductive activities.

The techniques of controlling ovulation rate by administration of exogenous GnRH are usually related to superovulation by using pregnant mare serum gonadotrophin (PMSG) (Ryan *et al.*, 1992), where it is used for special purposes, such as artificial insemination (AI) and embryo transfer. Administration of PMSG to the ewe during the luteal phase results in greater recruitment of follicles destined to ovulate (Hay and Moor, 1978). They explained that PMSG administration does not, however, change the number of follicles in the ovary, but increases the proportion of follicles of the size of >3.0 mm compared to those in the size of <3.0 mm in diameter. Walker *et al.* (1989) has shown that GnRH injected into ewes could be used to synchronise the ovulation time at doses as low as 6.25 μg per ewe.

McNeilly and Wallace (1987) and Miller and Martin (1993) found that ovulation rate of small ruminants can be increased using bovine follicular fluid (bFF). This follicular fluid from a variety of species has been shown to suppress FSH when administered (Lindsay, 1988). This effect is due to the presence of a non-steroidal substance (inhibin), which operates at the pituitary level suppressing FSH secretion when inhibin in no longer circulating (de Jong and Robertson, 1985). Increases in ovulation rate after cessation of bFF treatment appear because of the large rebound release of FSH from the pituitary (McNeilly and Wallace, 1987). This has been demonstrated by a number of earlier studies

(McNatty *et al.*, 1985; McNeilly, 1985; Wright *et al.*, 1981), which indicate that administration of exogenous FSH to ewes during luteal regression and through the follicular phase, increased ovulation rate. However, a later experiment showed that administration with an inhibin-like fraction of bFF does not consistently elevate FSH, but it is true that inhibin is part of the feedback control mechanism of FSH secretion at certain times of the oestrous cycle (Al-Obaidi *et al.*, 1987).

Progesterone and oestradiol are two hormones synthesised by the ovary. Progesterone is a steroid produced by the corpora lutea during the luteal phase, while oestrogen is pre-dominant during the follicular phase. Oestrogen stimulates the release of LH which in turn signals the ovary to ovulate (Lindsay, 1988). The ovary is the 'target' organ of these gonadotrophins; therefore a manipulation of this site to control the oestrous cycle and ovulation might be possible.

It has already been mentioned that administration of exogenous synthetic progesterone (ie. orally or by injection, subcutaneous implants or vaginal pessaries) could result in oestrous synchronisation, which in turn leads to synchronisation of ovulation. A further alternative is the administration of prostaglandin (PGF2α). Prostaglandins are produced by the uterus during the luteal phase and results in luteolysis if there is no indication of pregnancy. The corpus luteum has been demonstrated to be sensitive to prostaglandin between day 4 and day 14 of the oestrous cycle (Acritopoulou *et al.*, 1977; Douglas and Ginther, 1973). More recent findings indicated that administration of a combination of GnRH and PGF2α induced *post partum* fertility in cows (Al-Raheem and Al-Ani, 1991). However, Lindsay (1988) argued that although techniques based on prostaglandin have become available as a commercial system, they still result in a lower fertility and cannot be used for out-of-season breeding.

2.2.2 Anoestrus

Lindsay (1988) suggested that there are several anoestrus periods; anoestrus due to prepuberty, lactational anoestrus and anoestrus in non-breeding season. No ovarian activity is found during this period of anoestrus. In anoestrous ewes due to prepuberty, increased frequency of pulsatile LH secretion, presumably driven by the hypothalamic GnRH, together with sufficient concentration of FSH, might be able to stimulate oestrogen production by the maturition of preovulatory follicles. Due to higher LH pulse frequency, the circulating oestrogen produces a high surge of pituitary gonadotrophins, which in turn induces ovulation (see Yellon *et al.*, 1992). However, these authors argued that the sensitivity of the GnRH pulse generator to oestrogen declines after puberty. In this stage (ie. puberty), sensitivity to GnRH in the feedback mechanism becomes more responsive to peripheral progesterone concentration produced by the corpus luteum (Karsch *et al.*, 1984).

With respect to anoestrus in the non-breeding season, a possible manipulation to induce the onset of oestrus and ovulation is via the hypothalamic-pituitary-ovarian axis and the pineal gland. Manipulation of photoperiod exposure, physically or chemically (eg. administration of exogenous melatonin), has been discussed in the previous section of this chapter.

2.3 FEEDING STRATEGY FOR IMPROVING THE REPRODUCTIVE EFFICIENCY OF SMALL RUMINANTS

Goats and sheep are ruminant animals that have a multi-compartmental stomach which distinguishes them from simple stomach non-ruminants such as pigs and poultry. The mouth and teeth of ruminants fragment the coarse plant feed material before it enters the rumen and later re-gurgitation, allows the animals to re-masticate their feed. Ruminant animals are adapted to high fibre diets (eg. grass and other forages) that require microbial degradation for digestion, and so are generally not considered to be competing with humans for food.

The major proportion of feed ingested by ruminants is held whilst being digested in the fore-stomach (the reticulo-rumen). The main microorganisms present in the rumen are bacteria, protozoa and fungi. The microbial ecosystem in the rumen is complex and is highly dependent on the quality and quantity of feed consumed (Hungate, 1966). Rumen microbes in turn are a valuable source of protein for the ruminant animals; therefore, it is important to provide sufficient nitrogen and protein for rumen microbial protein synthesis which in turn provides for the host animal (Leng, 1970).

Ruminants reproduction rate, in developed countries, is often improved by using high quality feed for specific purposes (as will be discussed in the next section), such as increasing ovulation rate prior to joining, shortening the acyclic period in association with early weaning and supplementation of the basal feed for lactating females. Most ruminants in Asia, especially in Indonesia depend on low quality crop residues for their nutrition. Therefore, improved animal production depends on supplementation of diets with green forage with high protein (Leng, 1985). ACIAR (1992) identified a total of 136 species of forage plants as used by village farmers in Indonesia and found that many of these species were of high quality in terms of proximate composition. However, Leng (1993) mentioned that forage from tropical pastures or crop residues are often deficient in

essential nutrients, particularly trace elements and nitrogen needed by the rumen microbes for efficient growth.

Maximising the efficiency of utilisation of fibrous diets in the rumen, by ensuring that nutrient requirements of rumen microbes are met, is important. This review will briefly discuss manipulation of rumen function to give rise to greater quantities of amino acids for absorption, and possibly the mechanism for bypassing rumen fermentation so as to provide more protein for the host animal, which in turn results in an increase in its reproductive efficiency.

2.3.1 Modification of rumen fermentation

Preston and Leng (1987) classified the requirements for growth of microbial cells in the rumen as: (1) fermentable carbohydrate, as the source of energy, (2) nitrogen (N), as the source for ammonia and some amino acids, and (3) minerals and vitamins. These authors, furthermore, stated that adenosine tri-phosphate (ATP) generated from carbohydrate fermentation is the energy used for the microbes to meet their requirement. This may increase the energy availability from fermented carbohydrate by 11 to 30 % more energy to the animal if digested post-ruminally than when fermented in the rumen; and a similar pattern also occurs in the availability of amino acids protein (Leng, 1981). Thus there is potential for increasing the availability of protein and energy to both the microbes and the animal by processing feeds to decrease energy fermentation and amino acids degradation, by bypassing rumen fermentation (Leng *et al.*, 1977; Leng 1981). This microbial growth efficiency is expressed in terms of Y_{ATP}, which is the weight (g) of dry cells that is produced per mole ATP available (Preston and Leng, 1987).

Previous studies of Nolan (1975) and Nolan *et al.* (1976) showed that about 30-80% of the microbial N of mixed bacteria is apparently derived from dietary peptides and amino acids. If amino acids are fermented in the rumen, they are converted to volatile

fatty acids (VFA) and ammonia. Ammonia, in this matter, can become the sole source of N for protein and other nitrogenous compounds syntheses in the majority of rumen microbes (Leng *et al.*, 1977). It has been shown in the study of Nolan and Leng (1972) that 60-70 % of nitrogen in rumen microbes is derived from ammonia. This indicates that other non-protein nitrogen sources may possibly be used to fulfil rumen microbial N requirement.

A working assumption is that if a ruminant obtains most of its amino acids from microbial proteins produced in the rumen from virtually any source of dietary nitrogen, an extensive use of non-protein nitrogen (NPN), such as urea, can become a source of dietary nitrogen for ruminants producing meat and milk fed low-protein high-carbohydrate feeds (Leng *et al.*, 1977). This concept has already been demonstrated as a successful approach in increasing production in animals fed high fibrous agricultural residues, by decreasing the cost of the feed preparations in Asia (Wanapat, 1986; Cheva-Isarakul and Kanjanapruthipong, 1986).

Preston and Leng (1987) introduced the term of protein to energy (P:E) ratio, that is, the yield to the intestines of microbial cells per mole dietary organic matter (OM) digested in the rumen. This P:E ratio is not always exactly the same as in the theoretical approach. Leng (1981) explained that this might be because of utilisation of energy yielding from carbohydrate fermentation (ie. ATP) by the microbes for: (1) maintaining microbial cellular homeostasis but not for microbial protein synthesis, (2) dissociation of catabolism from anabolism that may cause energy-spilling due to nutrients deficiency, and (3) utilisation of debris material from death microbes, due to bacteriophages, by other microbes. The potential for manipulating the rumen system to increase P:E ratio for absorption, therefore, can be realised by optimising ATP use for protein and cell synthesis and reducing ATP energy dissipation in maintenance, energy-spilling and/or extracellular recycling process (Preston and Leng, 1987). To overcome these problems, supplementation of critical nutrients for rumen microbial growth often increases the

microbial cell yield per unit of digested substrate (organic matter truly digested in the rumen - OMTDR), and therefore increases Y_{ATP} (Leng, 1986; Preston and Leng, 1987). However, increase in Y_{ATP} results in a greater fraction of the digested organic matter used to provide microbial cells polymer synthesis; and as a consequence less is fermented to VFA and also methane in association with heat production, and this increases the protein/VFA output from the rumen and thus the P:E ratio (Preston and Leng, 1987). This has been confirmed by the recent study of Bird (1991) on optimising the use of poor quality forage-feed resources for ruminant production; with not only pre-treated forage and dietary supplements but also by elimination of protozoa from the rumen. Figure 2.4 illustrates the relationship of microbial growth efficiency (Y_{ATP}) to microbial cell dry matter and the VFA production.

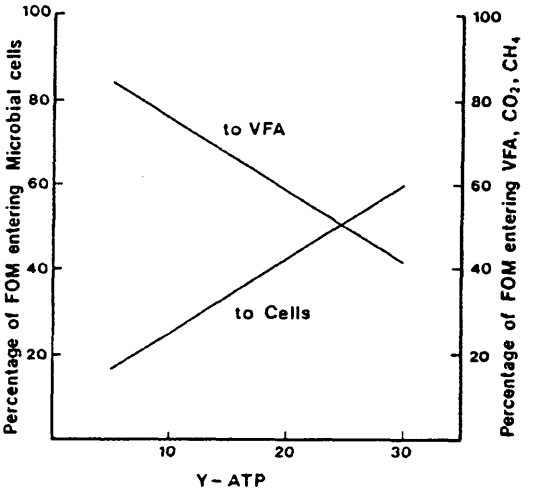


Figure 2.4 Relationship of microbial growth efficiency (Y_{ATP}) and the proportion of the fermented organic matter that is partitioned into VFA and CH_4 and CO_2, and that entering into microbial cells (from Leng 1981; Preston and Leng, 1987).

There are two main methods, described by Preston and Leng (1987) and Leng (1990), to alter P:E ratio in the nutrients absorbed, which are: (1) manipulation in the rumen by providing a protein source and VFA as the energy source for microbial growth, and (2) feeding a ration of protein that escapes rumen microbial degradation. Point (1) leads to a suggestion that supplying nutrients required for the rumen microbes, such as ammonia, or some amino acids and/or other source of nitrogen (eg. urea), increase the efficiency of microbial growth and hence automatically improves the P:E ratio of the nutrients available to the animals. In regard to the latter point, dietary bypass protein that is given to increase the P:E ratio of the nutrients absorbed, the ratio of microbial protein to VFA produced to provide an optimal environment for the rumen microbial growth becomes less important.

The use of urea and molasses as a source of NPN and carbohydrate has been widely used to supply nutrients for rumen microbial growth, especially in the areas that mostly rely on agricultural byproducts. Even urea is not the only alternative source of NPN (Leng *et al.*, 1973), it can replace other nitrogenous sources in the rumen and be transformed into ammonia that is required for the growth of rumen micro-organisms, whilst molasses is all fermented to VFAs which release energy, in the fermentation reactions, and can be utilised by the rumen microbes (Leng, 1986). Increase in ruminant production in animals given low quality basal diets (sugar cane and local cut-and-carry grass) supplemented with urea and molasses has been shown in the recent studies of Soetanto *et al.* (1987), Hendratno (1985) and Hendratno *et al.* (1990) in Indonesia. Nolan and Leng (1970) have demonstrated that the greater requirement of protein of ewes in late pregnancy, might be fulfilled by urea supplementation as it was shown by higher degradation rate of urea in the digestive tract of pregnant as compared to non-pregnant sheep. This indicates that urea and molasses are applicable to increase the utilisation of low quality basal diets and increase animal production.

2.3.2 Effects of rumen manipulation on reproductive performance of ruminants

Instead of supplementing rumen bypass energy and protein, utilisation of agricultural by-products that supply critical nutrients and mineral needs for the microbes, supplements in the form of urea-multinutrients molasses block (UMMB; Preston and Leng 1987; Kunju, 1986, 1990; Hendratno *et al.*, 1990, 1991), might be more applicable to increase ruminant productivity at the level of village farmers, particularly in developing countries. Improved reproductive performance in small ruminants supplemented with UMMB has been demonstrated in previous findings of Tjiptosumirat *et al.* (1990; 1991; 1993). This author has been able to demonstrate an increased in kid birth weight from does supplemented with UMMB during the last trimester of pregnancy and increase in does' milk production, by 15% and 27%, respectively, in Indonesia, as shown in Table 2.1. Basal diets used in those experiments were a mixture of low quality cut-and-carry grass or forages, with protein contents about 9 % crude protein (CP) of dry matter. There was a difference of 40 % more nitrogen intake in UMMB supplemented goats compared to control goats, indicating that the supplemented goats had a balance of nutrients that promote microbial growth in the rumen.

Other work that shows improved reproductive performance due to UMMB supplementation, at the level of village farmers in Indonesia, has been reported by Hendratno *et al.* (1991) in Friesian-Holstein dairy cattle given cut-and-carry green forage. Their results indicated that milk production could be increased by up to 28 % by using a UMMB supplement compared to those cows that were not supplemented. These studies have indicated that reproductive rate in ruminants has been strongly affected by supplementation to balance nutrition of ruminants fed low quality forage diets.

Table 2.1 Effects of UMMB supplements on reproductive performance of Ettawa Crossbred goats fed with mix cut-and-carry grass or forages in Sumberrejo Kendal (Tjiptosumirat *et al.*, 1990).

Parameters	-UMMB	+UMMB	% improvement
Does:			
Milk production (l/d):			
- maximum yield (l)	1.1	1.4	27.3
- average in 14 weeks (l/h/d)	0.8	1.0	20.3
Gestation length (days)	152	144	5.3
Kidding interval (days)	201	192	4.5
Kid:			
Mean birthweight (kg)	3.3	3.8	15.2
Average daily gain (g/d)	91.6	105.2	15.0

Lindsay *et al.* (1982) and Stephenson *et al.* (1981) have shown that offspring birth weights were affected by feeding nutrients to balance the diet and thus promote microbial growth in the rumen of cattle and sheep. However, many investigations which has examined nutritional effect on reproductive performance of small ruminants, have utilised high quality basal diets and degradable supplements, as will be discussed in the following section.

2.4 THE ROLE OF NUTRITION IN REPRODUCTIVE PERFORMANCE

There is no doubt that efficiency of reproductive performance of female animals is related to nutritional status that is to some degree reflected in the changes of live weight or body metabolic status, known as the "dynamic" effect, and live weight or body condition; the "static" live weight effect (see Allen and Lamming, 1961; Coop, 1966; Ducker and Boyd, 1977; Adalsteinsson, 1979). These effects are thought to be associated with the level of protein and/or energy body reserves (Egan, 1984). The dynamic effect is characterised by a "flushing" effect prior to ovulation or joining (Killeen, 1967; Haresign, 1981; Killeen, 1982), while the static effect is more associated with absolute live weight and body condition (Knight, 1980; Knight and Hockey, 1982; Zhengzhong *et al.*, 1989).

Haresign (1981) suggested that at one period of the oestrous cycle flushing may be effective in increasing ovulation rate by preventing large follicles (>3 mm in diameter) from becoming atretic. Higher ovulation rates in heavier ewes is associated with the pattern of ovarian follicular development in which there is more recruitment in the early follicular phase for maturation and lower numbers of atretic follicles in the late follicular phase (Zhengzhong *et al.*, 1989). Moreover, the study of Morley *et al.* (1978) suggested that ovulation rate in most strains of ewes (Merinos, Romney Marsh, Border Leicester, Perendales and Scottish Blackfaces) is strongly related to live weight. However, feeding a high nutrient concentration feed to ewes that are already in a good condition appears to result in no differences in ovulation rate (Gunn *et al.*, 1972). The discussion in this section, therefore, will emphasise the effects of nutrition on ovulation and onset of oestrus in cycling animals, *pre* and *post partum* (including birth weight, milk production and lamb survival rate) and on the onset of first oestrus in anoestrous ewes.

2.4.1 Effect of nutrition on oestrous animals

The timing and period of protein and/or energy supplementation may affect ovulatory responses in ruminants. Lindsay (1976) obtained responses to lupin feeding over a 6 day period prior to expected ovulation; whereas Haresign (1981) suggested that at least one oestrous cycle was required in order for flushing to increase ovulation rate. Later findings showed that to increase ovulation rate, animals had to be offered protein or energy supplementation in the critical few days (4-6 days) before expected ovulation (Stewart and Oldham, 1986; Nottle *et al.*, 1990), which confirmed the earlier finding of Lindsay (1976).

The onset of oestrus in the ewe is usually associated with the expression of ovulation. This is because the reproductive behaviour of ewes is strongly determined by hormonal effects (Lynch *et al.*, 1992). These authors explained that the follicle stimulating hormone (FSH) stimulates the development of ovarian follicles, which produce oestrogen, and the secretion of luteinising hormone (LH), which in turn influences the release of ovum/ova from the ovary. FSH and LH are the two main gonadotrophic hormones secreted by the pituitary gland and are important in the control of the oestrous cycle. Many earlier findings have highlighted the effect of nutrition in altering ovulation rate via the release of these gonadotrophins; for examples, the effect of plane of nutrition (Haresign, 1981; Davis *et al.*, 1981; Zhengzhong, 1989), dietary energy (for review see Schillo, 1992) and dietary protein (Davis *et al.*, 1981; Nottle *et al.*, 1987; Cruickshank, *et al.*, 1990; Jabbour *et al.*, 1991). These findings suggested that the effect of nutrition, may be mediated through an increased secretion of FSH and/or LH. However, Radford *et al.* (1980) and Ritar and Adams (1988) suggested that it may be due to an increased sensitivity of the ovary to circulating gonadotrophins. There has been much discussion as to whether the ovary is the site of the nutritional effects (Haresign, 1981; Davis *et al.*,

1981; Zhengzhong, 1989), and the question of how nutrition alters ovulation remains to be answered.

The onset of oestrus and of ovulation in ruminants can be changed by altering the plane of nutrition. This has been highlighted in section 2.4. Most recent studies have used sheep supplemented with lupin grain (protein contents 30%) at the rate of 500 - 750 g/head/day (Gherardi and Lindsay, 1982; Oldham and Lindsay, 1984; Stewart and Oldham, 1986; Nottle *et al.*, 1987), with basal feeds as wheaten hay or pasture, result in an increase in ovulation rate of up to 20% in supplemented as compared to unsupplemented ewes. Cruickshank *et al.* (1988) stated that although many positive effects on ovulation rate were found in the ewes that were supplemented with protein, there is a possibility that this effect is also due to energy, as was shown previously by Davis *et al.* (1981). Supplementation studies on the effect of dietary energy as the nutrient responsible for the increase in ovulation rate have been conducted (Cruickshank *et al.* 1988; Teleni *et al.*, 1984, 1989b). Teleni *et al.* (1989b) reported that an increase in ovulation rate of 25.0, 26.5 and 24.2% were found in sheep fed lupin grain, infused intravenously with glucose, and infused with glucose + acetate, compared to the corresponding control. They suggested that this effect may be mediated via biological pathways associated with the synthesis of glucose and/or the utilisation of glucose and/or acetate. Therefore, it seems that both protein and energy content of animal feed can influence ovulation rate in ewes and may increase the ovulation rate via independent physiological mechanisms (Smith, 1985; 1988).

The nutrients, ie. dietary constituents or in particular essential amino acids (eAA) for protein and/or the energy content of feed, that are responsible for increases in ovulation rate remain unclear. Several recent studies (Waghorn, 1986; Downing *et al.*, 1990; Smith, *unpublished* **in** Smith, 1991) found that the branched chain amino acids (BCAA: valine, leucine and isoleucine) are amino acids that may be responsible for the variation in ovulation rate in ewes. Waghorn (1986) found a strong relationship between

ovulation rate and BCAA plasma concentration. More recently Downing *et al.* (1990) by infusing BCAA into the jugular vein of ewes fed low quality basal diets (ie. low energy content), showed that infusion of BCAA significantly ($P<0.02$) stimulated ovulation rate by up to 50 % but did not change the level of FSH and LH compared to those ewes infused with saline. This study also indicated an increase in insulin levels in association with BCAA infusions, and hence they postulated that insulin could also alter ovarian sensitivity to gonadotrophins (Downing *et al.*, 1990), as was previously suggested by Hinch and Raelofs (1986). Waghorn and Smith (1990) found that a change in plasma BCAA concentration, due to protein supplementation during the follicular development, may affect follicular activity and ovulation rate.

From these findings, BCAA may be one of the nutrients that contribute to the variation in ovulation rate. Branched chain amino acids are eAA precursors and are necessary for the production traits such as growth (in pigs, rats and chicken) and milk production (McDonald, 1989). Furthermore, McDonald (1989) stated that BCAA are additional long chain fatty acid end-products from metabolism of carbohydrate by rumen micro organisms and later will be deaminated into isobutyric acids and will contribute as glucogenic precursors together with valeric (Kempton and Leng, 1977) and other eAA (Preston and Leng, 1987). Preston and Leng (1987), further, argued that there is a possibility that some of these amino acids can be deaminated and thus become glucogenic precursors, but the proportion is low due to competition for amino acids for protein synthesis. However, as the BCAA are included in the group of essential amino acids precursors that are not synthesised by animal tissue and hence must be obtained from digested feed or microbial protein, the availability of these amino acids can be manipulated through feeding supplements that escape rumen degradation. All of these studies suggest the existence of an integral relationship between reproductive performance, reproductive hormone secretion and/or synthesis and ruminal energy (VFA) and protein production.

2.4.2 Effect of nutrition on pregnancy

Lindsay (1988) indicated that pregnant ewes are very sensitive to nutritional status, especially multiple bearing ewes. He further suggested that the timing of feeding and/or supplementation of good quality feed is of major importance.

Recent studies have shown that periods of supplementation during pregnancy of small ruminants (eg. additional protein and/or energy) can be categorised into three stages; early, 1-35 days gestation (Parr *et al.*, 1986; 1987; Parr, 1992; Robinson, 1986; Robertson and Hinch, 1990); mid, 40-102 days pregnancy (Kleemann *et al.*, 1990; McCrabb *et al.*, 1991), and late pregnancy, 100 days of pregnancy to parturition (Chandler *et al.*, 1985; Hinch and Thwaites, 1990; Knights and Pritchard, 1990; McCrabb *et al.*, 1990).

Day 15 to day 30 after conception, is the period of the implantation phase of the embryo that can be affected by excessive nutrition (see Brien, 1987 and Robinson, 1986). Extreme over-nutrition during the joining period should be avoided and ewes kept at a maintenance level of feeding in the first month of pregnancy (Robinson, 1990). A possibility reason for this is embryo loss resulting from an increase in the clearance rate of peripheral progesterone, which thus lowers lambing rate, as the consequence of a reduction in pregnancy rate in ewes supplemented with high nutrients and high feed intakes immediately after joining (Parr *et al.*, 1987). This finding was confirmed with the more recent findings of Robertson and Hinch (1990). Parr (1992) showed more evidence that ewes fed twice the maintenance level of feeding in the post mating period had a pregnancy rate of 48% compared to ewes fed in a similar manner but receiving exogenous progesterone, which had a pregnancy rate of 76%, and ewes that were fed a maintenance level without additional exogenous progesterone had a pregnancy rate of 64%. This result led to the suggestion that a strategic use of supplementation could be applied emphasising each stage of pregnancy (ie. foetal development and/or ewe body reserves for milk production *post partum*).

In recent studies the effect of nutrients supplementation commenced in mid or late pregnancy (ie. ± 50-100 days of pregnancy and ± 100 days onward to term, respectively) has been examined.

The studies carried out by Kleemann *et al.* (1990) using lupins and Lynch *et al.* (1990) using cottonseed pellets (75% cottonseed and 31.5% protein), reported that the impact of mid-pregnancy supplementation may be carried over to the period post treatment, increasing lamb survival and birthweight for litter sizes 1, 2 and 3. McCrabb *et al.* (1991) reported that maternal under-nutrition from days 30 to 96 of pregnancy increased placental weight, but had no significant effect on foetal growth to days 96 post conception compared to corresponding control ewes which were well-fed. Chandler *et al.* (1985) reported that even when maternal live weight decreased by 14%, there was no significant change in foetal and placental weight due to under-nutrition in late pregnancy. This result led to the suggestion that mid pregnancy under-nutrition will influence placental size which will in turn influence foetal nutrient supply (ie. glucose as the major metabolic substrate required by foetus), but not necessarily limit foetal growth in late pregnancy. The conclusion of those studies is that there is a possibility that feeding a high plane of nutrition during mid pregnancy may give residual positive effects to both dams and their offspring.

The effect of under-nutrition in late pregnancy can be seen after parturition with lower birthweight and lower survival of lambs (Robinson, 1983). Robinson argued that it is reasonable to expect a higher lamb birthweight if the ewe is fed a higher metabolisable energy (ME) level. This statement was confirmed by Kelly and Newnham (1990) who showed that by nutritional management of pregnant ewes during the period of late pregnancy lamb birthweight and survival rate can be improved. Evidence of this has also been presented by Hinch and Thwaites (1990), who showed that supplementation with 250 g/day/head of lupin between days 100 and 145 of pregnancy has a positive effect on milk production and subsequent lamb growth, especially in twin lambs. Ginting *et al.*

(1992), in Indonesia, has also successfully demonstrated that protein and energy supplementation with agricultural by-products (ie. rice bran, molasses, cassava meal, fish meal, urea and lime stone) and rubberseed at 2 weeks *pre partum* continuing until 6 weeks *post partum*, increased the performance of offspring compared with offspring of goats fed with local forages and rubberseed.

The foetus grows at a rapid rate during the last trimester of pregnancy, and is dependent on an increasing nutrient supply during this time. Robinson (1985) further explained that by assuming that if live weight of ewes remained constant in the last trimester of pregnancy, the nutrient supply for the foetus to grow required both extra protein and energy from the feed. However, with sub-maintenance nutrient supply, mobilisation of body reserves of the dam will occur. If the rate of mobilisation is high, ketosis or *pregnancy toxaemia* can occur and may cause death (Egan, 1984; Lindsay, 1988). Figure 2.5 illustrates a scheme of the development of pregnancy toxaemia.

From the above discussion, nutrition in the period of late pregnancy is important, as the ewe needs to prepare itself for body tissue storage that in the later stages might be transformed in order to meet the needs for foetus growth, maintenance of the placenta and for milk production. Most of the sources are stored as body fat reserves and will be broken down into amino acids and glucose that are required for the synthesis of milk protein and milk lactose, glycerol of triglycerides and for oxidation to provide the co-factors of milk synthesis (Preston and Leng, 1987).

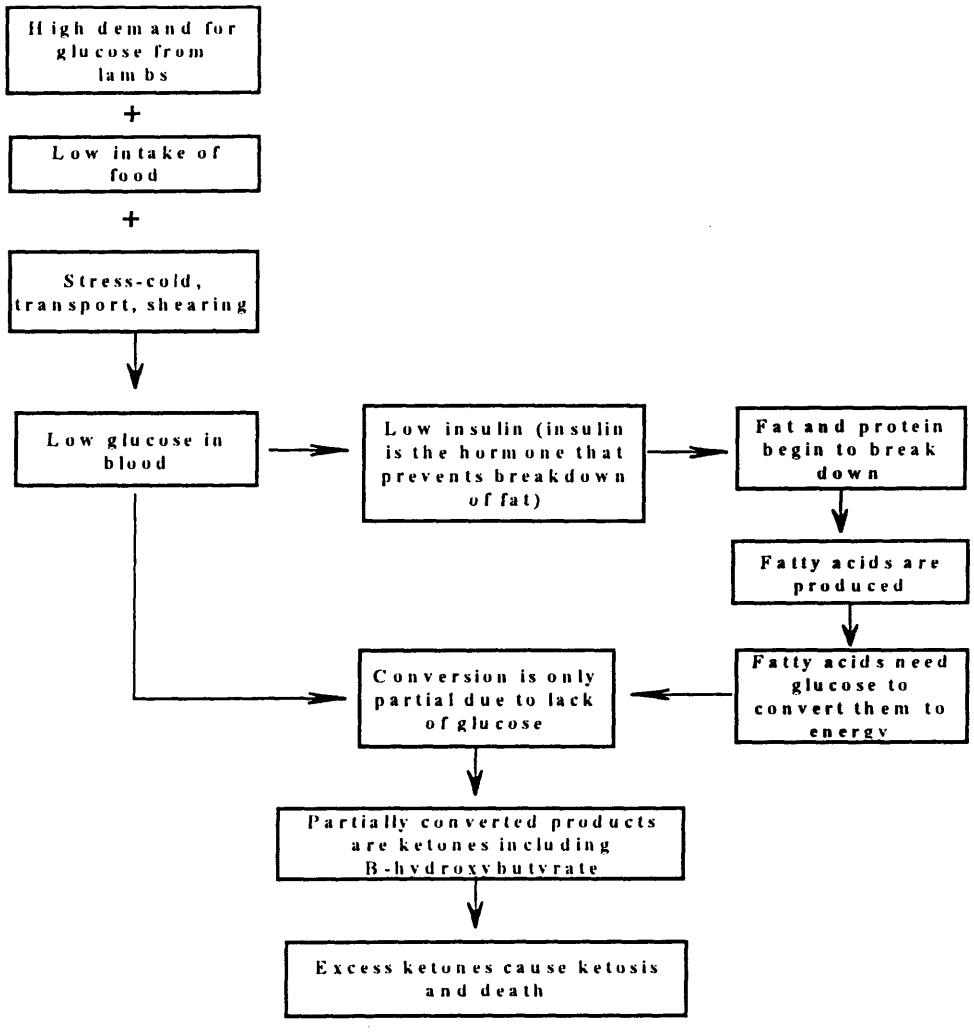


Figure 2.5 Development of ketosis or pregnancy toxaemia in late-pregnant ewes (from Lindsay, 1988).

2.4.3 Effect of nutrition on anoestrous animals

The hypothalamus-pituitary-ovarian axis and also pineal gland are all involved in determining the onset of oestrus. The ovaries of a ewe that is anoestrus generally remain dormant, and thus there is no ovulation and no behavioural oestrus. Anoestrus is found when ovarian activity is not present during the period of pre-puberty or in the non-breeding season or lactating period. In the period of pre-puberty, plane of nutrition may

play its role by advancing the onset of oestrus. Mukasa-Mugerwa *et al.*, (1991) showed that the plane of nutrition and dietary nutrients could alter or trigger puberty through increasing daily live weight gain in ewes, and perhaps together by perturbing the mechanism controlling gonadotrophin releasing hormones (GnRH) neurons that become sensitive to changes in the nutritional status of the animal (Robinson, 1990).

The surge of LH from the pituitary of pre-puberal heifers tends to be more sensitive to GnRH in animals which have been infused with propionate abomasally (Rutter *et al.* 1983). In another study reported by Mukasa-Mugerwa *et al.* (1991) using a mixture of a concentrate of 10.5 MJ/kg DM ME and 15.2 g/kg DM digestible protein, the onset of puberty in Menz ewe lambs was advanced by 153 and 155 days for medium and high plane of nutrition groups, respectively, as compared with the control sheep on a poor plane of nutrition. Both of these studies suggest that changing ruminal VFA production as the source of energy may be one possibility for modulating the responsiveness of pituitary to GnRH.

Lactational anoestrus may differ from seasonal anoestrus because of the impact of the continuous suckling stimulus and the drain of nutrients through lactation. There is also the possibility that *post partum* reproductive rate of different breeds of ewe varies with season and management of the flocks (Pope *et al.*, 1989).

William (1989) showed that ovarian activity of lactating beef cows can be induced within 50 days *post partum* by increasing the plasma level of lipoproteins (lipid metabolites; triglycerides and cholesterol). This also tends to induce longer luteal activity (± 15 days) compared to control animals (± 7 days) that received no lipoproteins. This finding also showed that animals that consumed high lipid diets exhibited higher plasma progesterone levels, indicating ovulation and corpus luteum formation 3 days after GnRH administration.

The interval between parturition and the onset of first oestrus and ovulation *post partum* may be altered by plane of nutrition. Rutter and Randel (1984), using a mixture of feedstuff of coastal bermuda grass hay, corn and cottonseed meal, showed significant shortening of *post partum* interval to onset of oestrus of 57.5, 40.3 and 34.7 days, for low, maintenance and high feeding level of protein and energy respectively; however, no significant difference was found in the plasma LH level. Furthermore, Rutter and Randel (1984) suggested that maintained body condition of the dam *post partum*, due to supplied nutrients requirement, stimulates pituitary function and enhances reproductive potential. Sasser *et al.* (1988), by using a mix of blue grass straw, soybean meal, corn, potato starch and molasses, confirmed this with a significant proportion of 89% of animals fed with adequate or maintenance level of protein (960 g CP/day), showing oestrus at the period of 45-110 days *post partum*, compared with 63% from those fed at sub-maintenance level of 360 g CP/day. A more recent study carried out by Ghosh *et al.* (1993) using UMMB supplementation on low quality base diets (rice straw and cut-and-carry grass), showed that ovarian activity can be stimulated by such supplementation with cycling levels of plasma progesterone observed between days 14 and 44 (25.5±3.4 days) *post partum* compared with days 46 to 81 (67.6±5.5 days) *post partum* for the control. These experiments have shown in general the importance of supplementation and adequate nutrient supply, which maintains the condition of the animal *post partum* during lactational period, may improve the reproductive performance.

According to Richards *et al.* (1989) the period of anoestrus is associated with low body condition in beef cows and a lower pulse frequency of LH; an increase in nutrient intake after a period of severe restriction in the anoestrus period might result in resumption of oestrous cycles and normal pregnancy rates. A recent study examined the impact of protein supplementation with oaten chaff basal diets on the onset of oestrus and ovarian activity in seasonally anoestrous small ruminants has been conducted by Pearse *et al.* (1991). These workers were unable to show any significant effect of lupin

supplementation on ovarian function in seasonal anoestrous ewes stimulated with exogenous gonadotrophins or GnRH. The authors argued that a possible mechanism by which lupin supplementation alters ovulation rate is neural rather than via a direct effect to on either the pituitary or ovaries.

2.5 CONCLUSIONS

The increase in reproductive performance of small ruminants may be improved either by manipulation through reproductive hormone mechanisms and/or by increasing the nutrient availability, through rumen manipulation of microbial growth, that in turn leads to improved nutritional status of the animals. The latter can be achieved by feeding supplements rich in protein and/or energy that will be reflected in the changes of body reserves status and body condition of the animals. Manipulation of reproductive hormone mechanisms may be useful and necessary to support the feeding system.

With respect to high protein and/or energy supplementation feed, manipulation of rumen functions is one solution to increase the availability of nutrient supply for the animal, especially when animal are fed with low quality basal diets. The use of UMMB supplementation, as a rumen microbe stimulator, is one of the alternatives to manipulate rumen function and increase microbial protein synthesis, which in turn increase the availability of nutrients for the host.

Both strategies, efficiency of feeding system and manipulation of reproductive hormone mechanisms as a mean to increase reproductive rate in small ruminants, have to be congruent. However, which nutrient (ie. energy and/or protein) that perturb reproductive performance is still need to be answered. Therefore, studies emphasising on the importance of protein and energy, or the interaction between both by such a feeding strategy on rumen function manipulation, to reproductive performance have to be elucidated.

CHAPTER THREE

EXPERIMENT 1

The effect of Protein Nutrition on Reproductive Parameters in Cycling Ewes

3.1 INTRODUCTION

Nutrition plays an important role in influencing the reproductive function of small ruminants. This can occur in both the male and the female and in their offspring pre and post weaning. However, most of the nutritional treatments used flushing with high qualities feed over a short period prior to expected ovulation or joining, for example, supplementation of lupin grain, with protein contents of 30%, at rate of 500 - 750 g/day, and for a period between 6 and 14 days prior to expected ovulation or joining (Knight *et al.*, 1975; Gherardi and Lindsay, 1982; Oldham and Lindsay, 1984; Nottle *et al.*, 1987, 1988, 1990). Most of basal diets that used in these supplementation experiments were of low quality, such as oaten straw (Downing *et al.*, 1990), wheaten hay (Nottle *et al.*, 1987, 1988, 1990) or pasture (Gherardi and Lindsay, 1982; Oldham and Lindsay, 1984).

It was mentioned, in the literature review, that ovulation rate is influenced by the balance of nutrients available to sheep; and so the objective of the experiment reported here was to evaluate the impact of long-term (ie. 53 days) supplementation with cottonseed pellets (CSP; a high quality source of bypass protein) on oestrus and ovarian activity during and after the cessation of supplementation.

3.2 MATERIALS AND METHODS

3.2.1 Location

The investigations were undertaken at the CSIRO research station, Chiswick (17 km south of Armidale) commencing in May 1992. Animals were maintained in small paddocks (± 1.0 ha) with an average pasture availability of each paddock at the commencement of the feeding trial of 1446 ± 35 kg DM/ha, as measured with a pasture probe TM MK-III (Product of Mosaic System Ltd.; Anonymous, 1991). The animals and were regularly rotated through 6 paddocks. The dominant pasture species were phalaris and white clover, but all feed was apparently of low quality due to frosting.

3.2.2 Animals

Two strains of ewes, Booroola Merinos (*F+*), heterozygous carriers for the fecundity gene (see Turner, 1982; Bindon *et al.*, 1985), and Merinos were used with 48 ewes in each group, the groups ranging in age from 2 to 3 years and with an average body weight of 38.8 ± 0.05 kg.

3.2.3 Diets and feeding

Cottonseed pellets (32% CP and 2% urea, 92% DM; Millmaster Feeds) were used as a source of supplemental rumen by-pass protein. As the sheep had never been previously exposed to "pellet" supplementation, a period of three weeks before the experiment commenced was used to train the sheep to eat the pellets.

Animals were offered an average of 70 g/h/day CSP during the training period and were held for a short time each day in a corner of the paddock where the pellets were

spread on the ground. The day before the commencement of the experiment, and when the animals had had time to become accustomed to the pellets, lithium-cottonseed pellets (Li-CSP) were offered to record the distribution of pellet intake within the flock. The measurement of pellet intake was made twice during the supplementation period; the day prior the commencement of trial and after three weeks of supplementation. The aim for the second estimation of CSP intake was to determine whether ingestion of the supplement by the experimental ewes remained consistent throughout the period.

Each strain of ewes was allocated into three groups (n = 33) for CSP supplementation, using stratified randomisation on body weight and initial CSP (Li-CSP) intake. All groups of ewes had similar access to nature and dry phalaris/clover pasture. Group 1 had access to pasture only (G1; control), while groups 2 and 3 were offered an additional supplement of CSP of 100 g/h/d and 200 g/h/d, respectively for a period of 53 days. Water was available *ad libitum* during the experiment period. On the day following the second laparoscopy all experimental ewes were restricted to access to pasture only.

3.2.4 Experimental Procedure

At the commencement of the experiment on 15 May 1992, all the ewes were oestrous synchronised using two 500 μg Prostaglandin-F2α (Estrumate, Intervet) intramuscular injections 10 days apart (ie. 15 May and 25 May 1992). The assessment of ovulation rate was based on the number of corpus luteum (CL) observed after a synchronised oestrus and introduction of a nutritional regimen. Ovulation rate was recorded twice: first, at 7 days after the expected day of second ovulation (25 days after the second PGF2α injection; 19 June 1992) and the second at 43 days post synchronisation (ie. 7 July 1992). A laparoscopy technique (Killeen and Caffery, 1982) was used to record these ovarian characteristics. Ovarian structures were categorised as: large follicles (≥6 mm in diameter; ∅), corpus luteum (CL) and regressing CL. Eighteen

days after the feeding trial ended (71 days post synchronisation; 25 July 1992), a third laparoscopy was conducted. Live weight of all experimental animals were recorded for each period prior to laparoscopy.

During the period of supplementation, 3 raddle-harnessed wethers were exposed to each treatment group and were used to monitor oestrus throughout the 71 days of the experiment. The wethers were introduced to the flocks the day after the second injection of PGF2α. These wethers had previously been injected with 500 μg/head testosterone (Duratestone, Intervet) to maintained their libido. The number of ewes that showed oestrus, as indicated by those being marked by the wethers, was recorded prior to each laparoscopy, and the colour of the raddles was changed at each laparoscopy. Oestrus detection were carried intensively for 8 days prior to each laparoscopy.

3.2.5 Lithium-CSP measurement

The method of using lithium as a tracer to measure feed intake was developed by Suharyono *et al.* (1991) and Suharyono (1992). The preparation for this measurement began with labelling 10 kg of a commercial CSP by placing them into a rotating feed mixer. A solution of a weighed amount of lithium chloride (100 g LiCl/500 ml H_20) was then sprayed over the mixing pellets. Five minutes after the spraying with LiCl, the pellets were spread and dried at room temperature of 25^o - 30^o C for about 72 h. Pellets were turned daily to increase drying efficiency and to prevent pellet aggregation.

The 10 kg of Li-CSP were offered to all animals in the paddock. To reduce competition labelled-pellets were spread in a trail so that each animal had access to approximately 0.3 m of the trail, which contained on average of 70 g Li-CSP. Eighteen h following the presentation of the Li-CSP, a blood sample (8 ml) was taken from all animals using sodium heparin vacutainer tubes. Blood samples were also collected from

10 sheep randomly chosen from the flock prior to Li-CSP ingestion. Blood sample was immediately stored in iced.

After the completion of sampling, blood tubes were centrifuged at 3000 rpm for 10 minutes, and the plasma then removed and frozen (-20^{o} C). The concentration of plasma lithium was determined on diluted samples (50 fold with Corning 460/405 diluent concentrate) using a flame photometer (Corning, 405). A commercial standard of LiCl was used which included sodium, potassium and calcium at physiological concentrations and which was also used to calibrate the photometer.

The assumptions suggested by Suharyono (1992) in the estimation of pellet intake is that the effective volume of distribution of lithium is proportional to body weight. Therefore, the concentration of plasma lithium ([*Li*]; mmol/L) was multiplied by individual body weights (*W*; kg) to obtain a constant value of lithium-live weight (*F*), shown in the following equation.

$$F_i = [Li]_i \times W_i \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.2.4.1)$$

This F_i value for each animal in the group is then summed to give a flock lithium-live weight value (ΣF_i), which was then used to determined the individual proportion of lithium ingested with pellets ($\frac{Fi}{\sum Fi}$). This value, is multiplied by the weight of Li-CSP (*S*; gram) offered to all animals gives an estimate of individual pellet intake. Therefore, the estimation of CSP intake of the i^{th} ewe is :

$$\frac{Fi}{\sum Fi} \times S_i \quad = \text{gram CSP} \quad \ldots\ldots\ldots\ldots \quad (3.2.4.2)$$

3.2.6 Laparoscopy Procedure

All animals were premedicated with 2 ml of a board spectrum of long acting antibiotic (Terramycine L.A.; Intervet) 24-28 h before surgery and were fasted (including water) overnight preceding laparoscopy. Each of the ewe was sedated with single injection (*i.m.*) of 250 µl A.C.E. Promazine (Delta Veterinary Laboratories PTY. Ltd.) then restrained on a laparotomy cradle. The belly wool cranial to the udder was clipped close to the skin and this area was prepared aseptically with hibitane detergent solution and swabbed with alcohol iodine.

Two sites, one on each side of the cranial aspect of the inguinal area (approximately 4 cm from the mid-line and 6 cm from the anterior margin of the udder), was injected (*i.m.*) with 2 ml of 2 % lignocaine (Intervet). Five to 10 min. later, a puncture wound into the abdominal cavity was made at each site, using a sterile trochar and cannula (5 mm in diameter). An endoscope was then inserted through the cannula and the abdominal cavity inflated. By viewing through the eye-piece of the endoscope, the state of the ovary could be recorded. At the completion of observations (30-60 seconds), both cannulas were withdrawn and a topical antibiotic spray was applied.

3.3 DATA ANALYSIS

Data were analysed using analysis of variance and covariance on the computer statistical software package BMDP-2V (Jennrich, 1981). A repeated measures design was used to analyse number of CLs, follicles and total stimulation with two grouping factors (3 treatments and 2 breeds) and one covariate (live weight). The data relates to the onset of oestrus were analysed as binomial data using generalised linear models of statistical software package REG (Gilmour, 1985). For comparison between means, the least significant difference test was used (Steel and Torrie, 1982). The main effects of this investigation were: breed of the ewes, level of protein pellets supplementation and times of the laparoscopy taken.

Data from laparoscopy were tested for normality and transformed whenever necessary before the analysis (BMDP-2V, 1985). However, all tables and figures of experimental results are presented as untransformed data.

3.4 RESULTS

3.4.1 Cottonseed Pellet intake estimation

Detail estimates of pellet intake prior to commencement of the experiment and three weeks later are shown in Appendix 1, and a summary of the two estimations are given in Table 3.1 and Figure 3.1. Table 3.1 shows the data from individual estimated pellet intake categorised into 10 groups of Li-CSP ingestion (Kahn, pers. comm.). Figure 3.1 illustrates "left-skewed" curve for estimation 1 and shifted to a more normal distribution curve for estimation 2.

Table 3.1 Transformation of pellet intake into pellet categories

No of category	Pellets intake (gram/h)	Number of sheep	
		Estimation I	Estimation II
1	0	50	35
2	0-45	11	17
3	46-90	10	15
4	91-130	6	14
5	131-180	6	11
6	181-220	5	3
7	220-260	5	4
8	260-310	2	1
9	311-350	2	1
10	>350	4	-
Total number of sheep (N)		101	101

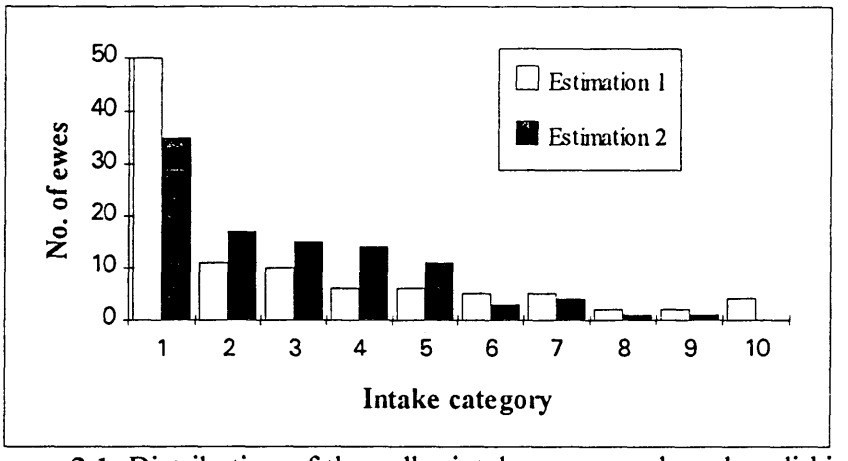


Figure 3.1. Distribution of the pellet intake category based on lithium concentration in blood samples collected 18 h after pellet ingestion.

From the two estimations of pellet intake, the number of ewes that ingested 0 g and more than 350 g Li-CSP between the two measurement periods was reduced from 50 to 35 ewes and from 4 to none, respectively (Table 3.1). Figure 3.2 illustrates the correlation between the first and the second estimation of Li-CSP ingestion. The number of ewes from each pellet intake category significantly increased with time (with coefficient slope of 0.68 and correlation (r) of 0.90; $P<0.001$). Detail of correlation between the first and second estimation and distribution between individual pellet intake and live weight are given in the figure in Appendix 1 and 2.

A significant difference ($P<0.001$) was also noted in the number of ewes ingesting Li-CSP from each intake category between the first and second estimation of pellet intake. More ewes were included in intake categories number 2, 3, 4 and 5 in estimation 1 than in estimation 2.

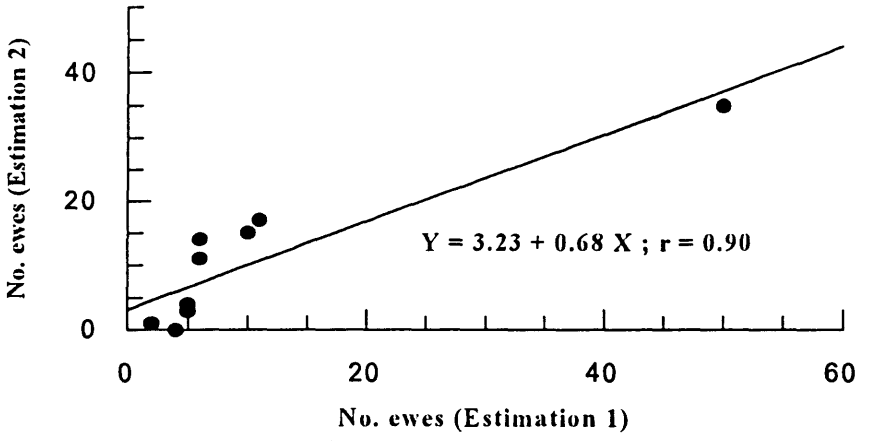


Figure 3.2 The correlation between the number of ewes ingesting Li-CSP between Estimation 1 and Estimation 2. Each (•) symbol represented a pellet intake category.

3.4.2 Effect of cottonseed supplementation on ovulation rate

Results from laparorcopy and live weight are summarised in Table 3.2, which shows neither of the supplementation treatments had a significant effect on the ovarian parameters as compare to the controls. Actual live weight of all experimental ewes were also unaffected by treatments; although a significant difference ($P<0.001$) was found between daily live weight change of different treatment groups. A highly significant difference ($P<0.001$) was found between the Merino and Booroola-Merino strains in the number of CLs, which were 1.09 ± 0.16 and 2.18 ± 0.36 (CL ± sem), respectively.

For overall the experimental ewes there was a significant difference ($P<0.05$) between each period of measurement for ovulation rate: 1.78 ± 0.11, 1.66 ± 0.10 and 1.52 ± 0.09 for the first, second and third laparoscopy, respectively. There was no effect of CSP supplementation found on ovulation rate between the three treatment groups (Figure 3.3). However, there was a tendency ($P<0.08$) for CSP supplementation to result in an increase in the mean proportion of ewes showing oestrus (Table 3.2).

The ovulation rate for each strain, in each observation period is shown in Figure 3.4. A tendency for a lower ovulation rate at the third laparoscopy in the Booroola strain ewes was noted; however this decline was not significant ($P<0.1$). There was no such effect seen in the Merino ewes.

Table 3.2 Mean value (± sem) from three periods of observation by laparoscopy on number of CL (ovulation rate), follicles, active ovaries (CL + F), proportion of ewes showing oestrus and daily live weight change.

Parameter	Group I (Control) n = 33	Group II (100g CSP) n = 33	Group III (200g CSP) n = 33	Probability
Number of CL (ovulation rate)	1.63 ± 0.11	1.57 ± 0.08	1.76 ± 0.12	ns
Follicles (>0.6 mm ∅)	0.57 ± 0.08	0.79 ± 0.09	0.53 ± 0.07	ns
Total stimulation	2.19 ± 0.12	2.35 ± 0.11	2.28 ± 0.13	ns
Ewes showing oestrus (%)	67.7	55.2	68.8	P<0.08
Daily live weight change (g/head/day)	-27.0 ± 4.9	-17.1 ± 3.9	8.8 ± 5.2	P<0.001
Mean live weight (kg)	38.1 ± 0.5	38.4 ± 0.5	39.1 ± 0.5	ns

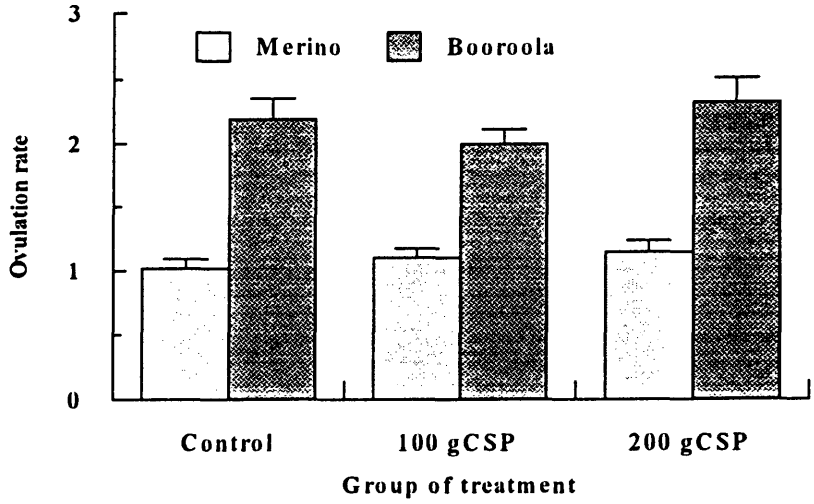


Figure 3.3 Overall means of ovulation rate from each individual group and strain of ewe. (Vertical bar denotes standard error mean).

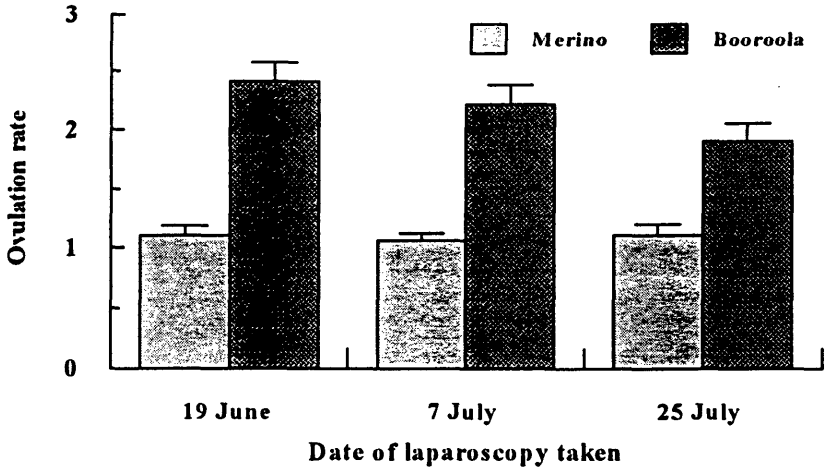


Figure 3.4 Mean ovulation rate at each time for each strain of ewe. (Vertical bar denotes standard error mean).

Although the effect of CSP supplementation on mean ovulation rate was not significant (P<0.6; Figure 3.5), there was an indication of a carry-over effect due to 100 g and 200 g CSP supplementation, with a higher ovulation rate in the supplemented group at the final endoscopy (P<0.1) compare to control ewes (Table 3.3).

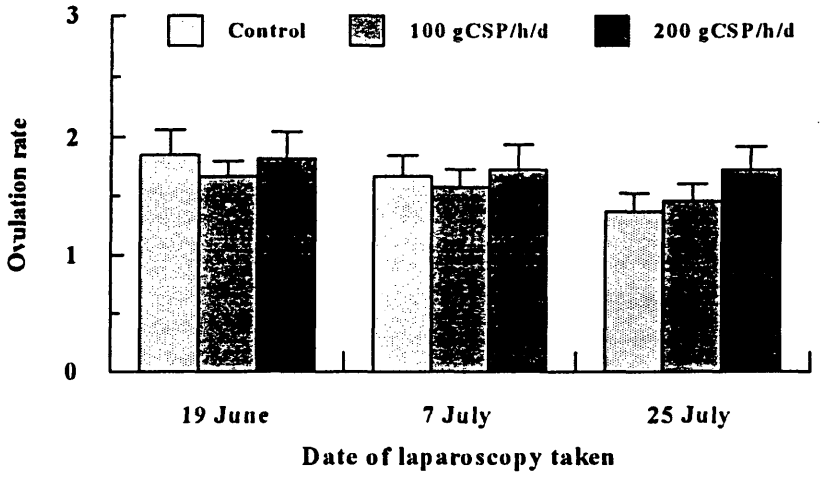


Figure 3.5 Correlation between mean ovulation rate and time during the period of the experiment for each individual treatment group post oestrus synchronisation. (Vertical bar denotes standard error mean).

Table 3.3. Difference in the occurrence of CL treatments during the experiment (T1 and T2) and after supplementation cessation (T3).

	Period of Observation			
	T1	T2	T3	Probability
G1 (Control)	1.80[a]	1.70[a]	1.35[b]	P<0.1
G2 (100g CSP)	1.66	1.55	1.45	ns
G3 (200g CSP)	1.80	1.70	1.71	ns

The pattern of oestrus was similar for all treatment groups in this experiment (Figure 3.6A; 3.6B and 3.6C). No significant difference was noted between Booroola Merino and Merino ewes. Ewes supplemented with 100 g CSP/h/d showed the lowest incidence of oestrus, especially in the first period of observation. The proportion of ewes from all treatment groups that showed oestrus, especially at the first observations, were lower compare than the two later observations; and the proportion of experimental animals that showed oestrus increased over the observation period. There was no indication of a carry-over effect, on the proportion of ewes showing oestrus, after supplementation ceased.

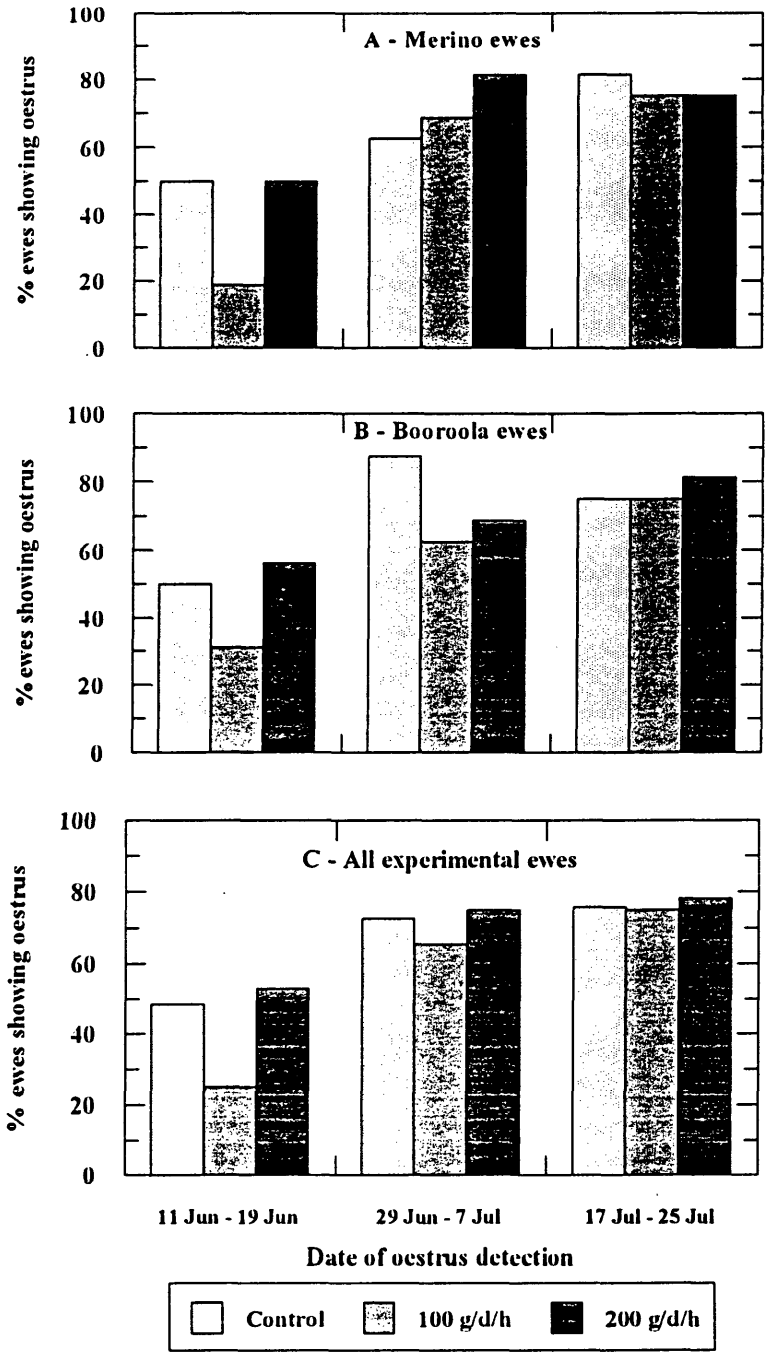


Figure 3.6 The proportion of ewes showing oestrus during each of the 3 oestrous recording periods.

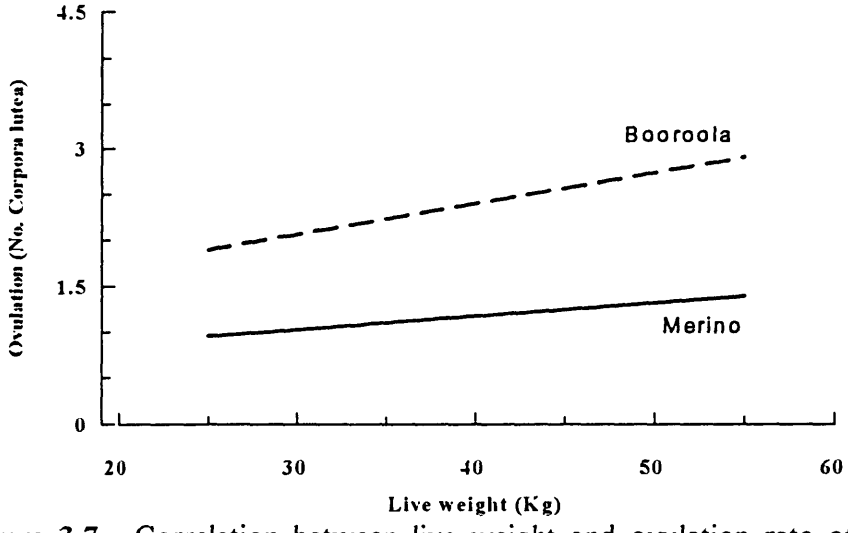


Figure 3.7. Correlation between live weight and ovulation rate of each strain of experimental ewes. Dashed lined (— —) represented Booroola (*F*+) ewes with $Y_B = 0.97 + \mathbf{0.04}X$ (P<0.10), and solid lined (——) represented Merino ewes with $Y_M = 0.6 + \mathbf{0.02}X$ (P<0.07).

Figure 3.7 illustrates the regression between live weight and ovulation rate of each strain of ewe. A positive relationship between live weight and ovulation rate was seen for both Booroola and Merino ewes.

Another approach to test the effect of CSP supplementation is by reallocating the experimental ewes that were not eating the CSP, offered to supplemented groups 100 g CSP and 200 g CSP, and transferring them to the control group (G1). Data were analysed in a similar procedure as before reallocations. Summaries of the result of the reallocation data for ovarian activity is shown in Table 3.4. There was little change in CLs compared to those reported previously (Table 3.2); however, there was some suggestions of an increase in CLs (P<0.16) at the higher level of supplementation.

The effects of supplementation treatment groups interacted with time (group x time) on ovulation rate and the onset of oestrus, after data reallocation, are shown in Figure 3.8 and 3.9, respectively. Figure 3.8 shows the effect of CSP supplementation

tended to remain after supplementation had ceased. However, these differences were not significant between the two observations during the feeding trial nor after supplementation had ceased.

Table 3.4. Ovarian function and live weight change after the reallocation of experimental ewes.

Parameters	Group 1 (Control; n=51)	Group 2 (100g CSP; n=25)	Group 3 (200g CSP; n=23)	Probability
Number of CL (ovulation rate; ± sem)	1.7 ± 0.09	1.6 ± 0.11	1.9 ± 0.11	P<0.16
Follicles (± sem)	0.5 ± 0.08	0.7 ± 0.10	0.6 ± 0.10	ns
Total stimulation (± sem)	2.2 ± 0.12	2.3 ± 0.14	2.5 ± 0.15	ns
Ewes showing oestrus (%)	70.3	61.1	65.0	ns
Mean live weight (kg ± sem)	38.4 ± 0.34[a]	37.9 ± 0.50[a]	39.8 ± 0.50[b]	P<0.02
Daily live weight change (gram/head/day ± sem)	-22.1 ± 0.3[a]	-12.1 ± 0.7[b]	15.4 ± 0.7[c]	P<0.001

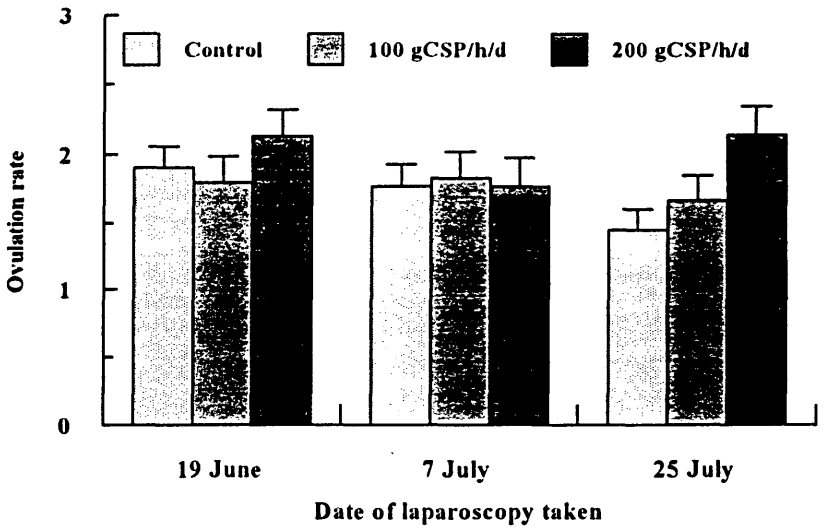


Figure 3.8. Number of CLs found by laparoscopy technique (interpreted as ovulation rate) after the reallocation of experimental ewes. (Vertical bars denoted standard error mean).

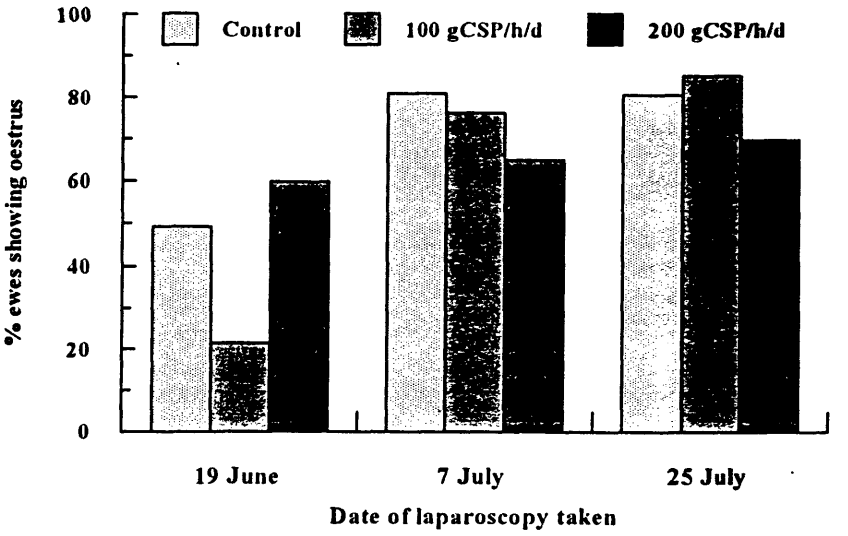


Figure 3.9 Proportion of ewes showing oestrus after the reallocation of experimental ewes.

Figure 3.9 illustrates the proportion of ewes showing oestrus during each of the 3 oestrous recording periods. There is a trend toward a significant difference ($P<0.06$) in the interaction between recording periods and treatment groups. A significant difference ($P<0.001$) in the proportions of 45.0, 73.7 and 80.3 % of ewes showing oestrus for the 19th June, 7th July and 25th July observations, respectively, was also noted.

3.5 DISCUSSION

3.5.1 Lithium-Cottonseed pellet intake

Pellet intake increased over the period of the experiment (Table 3.1 and Figure 3.2), as shown by the reduction in the number of ewes eating 0 gram and >350 gram of Li-CSP during the 3 weeks of feeding trial. This was also found in the studies of Suharyono (1992). The "shifting" pattern of the curve (Figure 3.1) indicates that more ewes became accustomed to the pellet and therefore each individual ewe would be expected to have had equal access to the pellet by the commencement of the experiment. Although the change in motivation of these ewes to eat the pellets was slow, the improvement was highly significant ($P<0.001$) and suggest that the animals were gradually learning to eat as has been previously reported (Llewelyn *et al.*, 1978; Lobato and Pearce, 1978; Lynch *et al.*, 1992). The gradual learning to eat supplements is not unexpected in ewes that have not previously been exposed to supplement (Lynch *et al.*, 1992). The role of learning is similar to that previously reported by Lobato *et al.*, (1980) that familiarisation of dietary supplements to pre-weaning ewes is important as the ewes are then familiar, by sight and/or smell, with supplements offered in subsequent periods. However, there were still ewes that had not learnt in this experiment, as the number of ewes that ingested >350 gram Li-CSP was reduced to zero; but, the proportion of ewes that did not eat the pellet remained at 34.7% of total ewes in estimation 2 compared with 49.5% in estimation 1. The reason for the change in numbers of sheep that accepted the pellets is not clearly understood. It could be due to inadequate quantities of pellets provided (10 kg Li-CSP) at the time of each intake measurement, or due to true aversion of the ewes that were non eaters.

The gradual learning to eat supplements is not unexpected in ewes that have not previously been exposed to supplements (Lynch *et al.*, 1990). The rate of learning is

similar to that previously reported by Lobato *et al.* (1980) for sheep. The fact that some animals were still not eating during the experimental phase of the experiment meant that a reallocation of the experimental animal was necessary to get a true picture of the effects of protein supplementation on individual animal. However, this reallocation did not significantly alter the result (Table 3.4).

3.5.2 Effect of CSP supplementation to ovarian functions

Mean live weight and live weight changes

Mean live weight and live weight changes (Table 3.4) show that supplementation with 100 g CSP/day/head ewes (group 2) and non-supplemented ewes (group 1) were significantly ($P<0.001$) losing more weight during the experimental period as compare to 200 g CSP/day/head fed ewes (group 3) which gained 15.4 gram/head/day. This indicates that the basal level of nutrition from pasture during the experimental period for the two groups was below maintenance. A possible explanation of this might be due to pasture quality which declined during the experimental period because of below zero night temperature. None of the experimental ewes have had access to other basal diets; but supplementation of 200 g/day of CSP was adequate for the ewes to be restored to a positive energy balance.

Correlation of live weight and ovulation rate

It has previously been noted that there is a strong relationship between live weight and ovulation rate (Allen and Lamming, 1961; Coop, 1966; Morley *et al.*, 1978; see Downing and Scarramuzzi, 1991). In the current experiment a positive relationship between live weight and ovulation rate was noted for each strain of ewe (Figure 3.7). The

overall correlation of live weight and ovulation rate for the two breeds of ewes in this investigation shows that there is an increase in the mean of ovulation rate of 2% and 4% per kg increase in body weight for Merino and Booroola Merino (Heterozygous, $F^{B}F^{+}$) ewes, respectively. This result is similar to that reported previously in Merino ewes with an increase of 1.2% and 2% per kg change in live weight (Lindsay *et al.*, 1975; Morley *et al.*, 1978). However, the 4% increases per kg live weight in Booroola ($F^{B}F^{+}$) ewes is lower than the 5.4% reported by Montgomery *et al.* (1983a), but higher than the 2.9% reported by Kleemann *et al.* (1991).

Although a significant difference in the mean live weight was seen between treatment groups, no significant difference in the ovarian function was noted (Table 3.4). This is most likely due to the relative small number of ewes per group. Furthermore, there was no indication of a significant interaction between treatments and period of observation (group x time), especially after the supplementation ceased.

The difference in ovulation rate between Merino and Booroola Merino ewes carrying the fecundity (*F*) gene in this experiment confirms earlier findings by Davis *et al.* (1982) and Montgomery *et al.* (1983b). The overall mean ovulation rate of the *F* gene carriers is 1.24 higher compared to those non-gene carriers (Davis *et al.*, 1982). The Booroola Merino carrier *F* gene of ewes are a highly prolific strain of sheep initially developed from two commercial sheep breeders, the Seears Brothers of 'Booroola', NSW and later by the Divisions of Animal Genetics and Animal Production, CSIRO (Turner, 1982). The Booroola Merino strain of ewes has about 187 % greater ovulation rate then Merino ewes (Bindon *et al.*, 1985); and this is due to a single major gene or closely linked group of minor genes that affecting ovulation rate (Piper and Bindon, 1982).

Onset of oestrus

Although results of ovarian function in table 3.2 show that CSP supplementation resulted in a trend toward a greater proportion of ewes showing oestrus ($P<0.08$), this effect disappeared after the reallocation of animals. This result indicates that CSP supplementation did not alter the onset of oestrus of either the Merino nor the Booroola Merino (*F*+) ewes (Table 3.4).

The average proportion of ewes showing oestrus immediately after the oestrous synchronisation is significantly lower ($P<0.001$; Figure 3.9) in the first observation period as compare to the second and third period of observations; but this was not associated with the occurrence of a CL (Figure 3.8). However, there is no indication that this difference was due to CSP supplementation. No significant difference in ovulation rate between period of observations before and after animal reallocation (Figure 3.5 and 3.8), indicates the possibility that more ewes tended to have a "silent heat" after oestrous synchronisation as previously discussed by Lindsay (1988).

There is evidence that the oestrous response may be influenced by the prostaglandin dose level (Hackett and Robertson, 1980). The onset of oestrous can be effectively synchronised by two doses of 250 μg cloprostenol (ICI 80996) given at 10 days interval, but a lower dose of prostaglandin analogues (125 μg) were often insufficient to induce complete luteolysis (Haresign, 1978; Greyling and Van der Westhuysen, 1979). Also, an endocrine imbalance due to a high level of progesterone or low dose level of prostaglandin may result in a lower oestrous response and fertility of sheep (Haresign, 1978, 1980). These effect may have contributed to the equivocal data on the behavioural oestrus recorded from the first observation in this experiment (Figure 3.9).

Other possibility that may have cause low proportion of ewes showing oestrus after oestrous synchronisation was discussed by Haresign (1978). Haresign explained that the CL of the ewe is only responsive to prostaglandins between day 4 and 14 of oestrus cycle; therefore a single administration of prostaglandin to a flock of ewes, by neglecting

the stage of the oestrus cycle, only 65% of those will respond (Haresign, 1978). However, oestrus synchronisation in the current experiment was using two administrations of prostaglandin which then this may not the caused of the lower proportion of ewes showing oestrus in the first period of observation.

3.6 CONCLUSIONS

Results from this investigation do not support earlier findings that supplementation with bypass protein increases ovulation rate. Possible constraints in this experiment may have been inadequate pasture levels as basal diets during feeding treatment, and therefore an inadequate source of basal energy. However, in this investigation, 200g CSP supplementation did allow an increase in live weight of the ewes maintained on a low quality of pasture.

This experiment has been unable to show the effect of 'long-term' protein-rich pellets (CSP) supplementation on ovulation rate in Merino and Booroola heterozygous ewes, compare with previous studies of 'short-term' (3-6 days) with positive effects on the ovarian activity (Stewart and Oldham, 1986; Nottle *et al.*, 1990). Therefore, the mechanism effect of whereby CSP may alters ovulation rate still requires further elucidated. Adequate energy provided from basal diets fed to the animals may also have to be considered with regard to nutrient balance.

CHAPTER FOUR

EXPERIMENT 2

Effect of *Post partum* Supplementation on Reproductive Activity in Anoestrous (Lactating) Ewes

4.1 INTRODUCTION

Increased pulsating gonadotrophins (LH and FSH) are essential for follicular growth, and it is possible that changes in the secretion of these hormones may mediate the influence of nutrition on ovulation rate and reproductive activity. High protein diets fed *post partum* appears to affect plasma LH and FSH levels in cattle (Rutter and Randel, 1984; Nolan *et al.*, 1988) which may in turn increase the number of ova released from the ovary and increase the number of animals showing oestrus (Sasser *et al.*, 1988).

. Smith (1991) suggested that changes in nutrient intake may alter the rates of neuro-transmitter turnover, and hence regulate the secretion of gonadotrophin from the hypothalamus. Likewise, Pearse *et al.* (1991), using seasonally anoestrous Border

Leicester ewes, argued that the effect of nutrition on ovulation is neural rather than due to changes in the sensitivity of ovarian-pituitary axis via additional exogenous GnRH. However, Downing and Scarramuzzi (1991) could show no effect of nutrient intake on the plasma level of either LH or FSH.

The pulses of LH, in the lactationally anoestrous ewes, are infrequent and hence inadequate to initiate ovarian activity (Lindsay, 1988). Other workers have shown that pituitary responsiveness to GnRH is unrelated to the duration of *post partum* anoestrus in the ewes (Wright *et al.*, 1982; Ainsworth *et al.*, 1982; Crowder *et al.*, 1980), and Crowder *et al.* (1980) suggested that the diminished response to GnRH immediately after lambing was due to a lower pool of LH in blood. There are actually mature follicles present during seasonal anoestrus as well as during the breeding season in ewes (McNatty *et al.*, 1984); and the control of these mature follicles seems to be via both the hypothalamic/pituitary gland secretions and at the ovarian level (Webb and Gauld, 1985).

Detailed knowledge of the pattern of nutrient intake in altering the mechanisms controlling ovarian activity is essential to understand our understanding of how to manipulate reproductive events. The aim of this current study is to obtain information on whether protein supplementation could influence the onset of oestrus in anoestrous ewes. To do so, an assessment of sensitivity of the pituitary to exogenous GnRH was used as a means of assessing the impact of changed nutrient intake.

4.2 MATERIALS AND METHODS

4.2.1 Location

This experiment was carried out at Kirby Research Station, University of New England, 10 km north west of Armidale. All experimental ewes were maintained in a small paddock (± 0.2 ha) with an initial pasture availability of 1.5 tonnes dry matter/ha, as estimated using a pasture probe TM MK-III (Product of Mosaic System Ltd.; Anonymous, 1991). The dominant species were white clover, *Danthonia sp.* and *Poa sp.*; however, all feed was of low quality due to high temperatures and low rainfall during the experimental period.

4.2.2 Animals

Twenty-six Crossbred (Merino x Border Leicester) and 28 Merino multiparous ewes in the stage of early lactation (± 28 days *post partum*) were used. The ewes had lambed between the 17th and 24th December (early summer) and were rearing single or multiple litters. The average liveweight at 2 weeks *post partum* was 52.5 ± 2.1 and 4.5 ± 0.2 kg for ewes and lambs, respectively.

4.2.3 Diets and feeding

The treatments used were similar to those used in the previous experiment (Chapter 3), with supplementation with CSP used as the source of bypass protein, and 3% urea used as the source of non-protein nitrogen.

Each of the breed groups was assigned to one of 3 feeding treatments based on liveweight and CSP intake which had previously been measured by the Li-CSP method described in experiment I (Chapter 3, section 3.2.5). Water was available *ad libitum* during the period of the experiment and each feeding group of animals grazed in small paddocks (0.2 ha) on a rotational basis. The feeding trial commenced when the sheep

averaged 28 days *post partum* (15th January), and continued until 17th March, an overall duration of 60 days. All experimental ewes were additionally supplied with 1 kg of oaten chaff per head per day. Ewes in Group 1 received 1 kg of plain oaten chaff per head per day (0.82% N in the DM feed). The oaten chaff offered to ewes in Group 2 was treated with 3% urea (2.35% N content of DM feed) as the source of non-protein nitrogen, while ewes in Group 3 had access to either 1 kg oaten chaff and an average of 250g of a commercial CSP (protected protein with 32% CP and 2% urea; Millmaster Feeds). Lambs from all ewes were unweaned throughout the experimental period. The feeding treatment and ewe number per group is shown in the Table 4.1 below.

Table 4.1 Feeding treatments on the effects of protein nitrogen (CSP) and non-protein nitrogen (3 % urea) supplementation on reproductive performance of Merino and Crossbred ewes.

	GI (Control) Pasture + 1 kg OC per head **(OC)**	**GII** Pasture + 1 kg OC treated with 3% urea per head **(OC + U)**	**GIII** Pasture + 1 kg OC + 250g CSP (32% CP) per head **(OC + CSP)**
Crossbred	n = 12	n = 9	n = 9
Merinos	n = 11	n = 10	n = 8

4.2.4 Experimental procedure

Initial ewe liveweights were recorded one day prior to the commencement of the experiment and then at each laparoscopy. Lambs were weighed at 2 days of age and ear-tagged. Each supplement group was running with 2 raddle harnessed wethers treated with testosterone (Duratestone, Intervet Ltd.) starting from the 15th January (28 days *post partum*) and throughout the investigation period. Wether teasers were treated every 4 weeks to maintain a stable libido and enable continuous monitoring of oestrus. The colour of the harness raddles was changed following each laparoscopy.

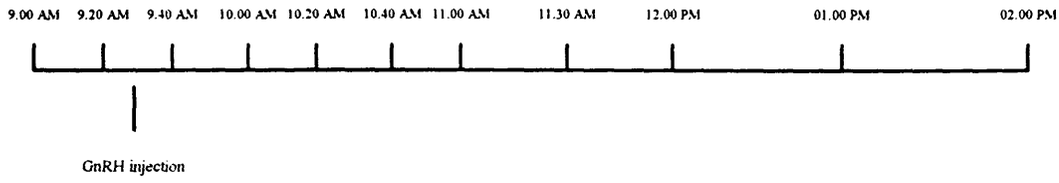
Parameters measured during the experiment included: liveweight for lambs and ewes, the onset of oestrus and ovarian activity. Ovulation rate (OR) was recorded using a mid ventral laparoscopy technique (Killeen and Caffery, 1982; for details see Chapter 3, section 3.2.6). All ewes were supplemented with progesterone using progestogen Repromap intravaginal sponges (with active constituent per sponge medroxyprogesterone acetate 60 mg; Upjohn Pty. Ltd) for 12 days commencing on day 42 *post partum*. Seven days after sponge withdrawal a mid ventral laparoscopy was conducted (day 61 *post partum*). A second laparoscopy was conducted 17 days after the first laparoscopy (day 78 *post partum*). Ten days after the second laparoscopy, 24 animals (8 ewes from each group) were randomly selected for determination of plasma LH concentration.

Supplementary feeding ceased immediately after this bleeding program (day 88 *post partum*) and all ewes were transferred to a pasture of white clover and oats to monitor carry-over effects. A third laparoscopy was conducted at day 108 *post partum*, 20 days after cessation of supplementation.

4.2.5 Plasma LH concentration

A group of 8 ewes was chosen from each of the nutritional treatment groups (4 Crossbred and 4 Merino from each group). Only ewes rearing single lambs were used in this bleeding program.

Each ewe was bled (5 ml) from the jugular on 11 occasions over a period of 5 h. Samples were taken at 20 min. intervals for the first 7 samples and thereafter 2 samples at 30 min. and 2 at hourly intervals. Samples were collected in heparinised tubes and immediately centrifuged (3000 rpm for 10 min.). The plasma was removed and then stored at -20°C until assayed. An intra-muscular (*i.m.*) injection of 50 μg of synthetic GnRH (Fertagyl, Intervet) was given immediately after the second bleeding (20 - 25 min. after first sampling). A diagram of the bleeding protocol is shown in the scheme below:



During the hours of the bleeding program no lambs were allowed to suckle. However, they were all allowed to suckle immediately prior to the commencement of the program to ensure uniformity of suckling stimulus.

Hormone assays

Plasma LH was measured by double antibody radioimmuno assay (RIA). This LH-RIA was earlier described by Gidley and Bindon (1976); however, the current method is a modification by M.A. Hillard (pers. comm., 1994). The method utilised ovine LH, NIH-oLH-S18 as LH standard (at the level of 0, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, and 64.0 ng). Anti-LH antibody used for the assay was rabbit anti-ovine serum (NIDDK anti-oLH.I - $800{,}000^{-1}$K in RIA diluent), and the second antibody was donkey anti-rabbit serum (DARS) and raised against rabbit globulin.

All plasma samples were thawed, and were mixed thoroughly using a vortex mixer for about 5 min. prior to assay. One hundred µl of samples, standards and quality control of samples were pipetted into the correspondingly labelled tubes. A portion of 50 µl anti-sera (NIDDK anti-oLH.I $800{,}000^{-1}$K in RIA diluent) was added to each tube followed by 50 µl tracer (^{125}I.oLH.I3) and 50 µL normal anti-rabbit serum (NRS; 250^{-1} in RIA diluent). All tubes were then mixed with a vortex mixer and incubated for 24 h at 27°C. Following incubation, the second antibody of donkey anti-rabbit serum (DARS; 25^{-1} in RIA diluent) was added to each tube, mixed and incubated overnight at 4°C. After the

second incubation, all tube samples had 300 µl RIA diluent added and were centrifuged for 15 min. at 5000 *g*. The supernatant for each sample was aspirated and tube samples were counted by a gamma-ray emitting counter. Luteinizing hormone levels were expressed as ng NIH-LH-S18 per ml. The sensitivity of the assay was 0.01 ng/ml. The coefficients of variation at 80, 50 and 20 % displacement were 0.42, 2.33 and 13.02 %, respectively, while for intra-assay the coefficient was 8.5 % and 7.3 % for inter-assay.

4.3 DATA ANALYSIS

Reproductive data from this experiment were analysed by analysis of variance and covariance using transformed data (natural logarithm) on the statistical software package BMDP-2V (Jennrich *et al.*, 1981). However, all experimental tables and figures in this chapter are presented as untransformed data. The main effects in this experiment are treatments, breeds, litter sizes and the interaction with time. All reproductive data from animals that show no indications of ovarian activity are excluded from the analysis.

Liveweight and average daily gain data were analysed in a repeated measures design using the statistical software package REG (Gilmour, 1985). For comparison of mean values, least significant difference test based on Steel and Torrie (1982) was applied by using statistical software package NEVA-UNE (Burr, 1980).

4.4 RESULTS

The rainfall pattern during the experimental period is shown in Figure 4.1. There was a heavy fall of rain soon after the commencement of the experiment. Thereafter, rainfall levels were low until day 46 of the experimental period.

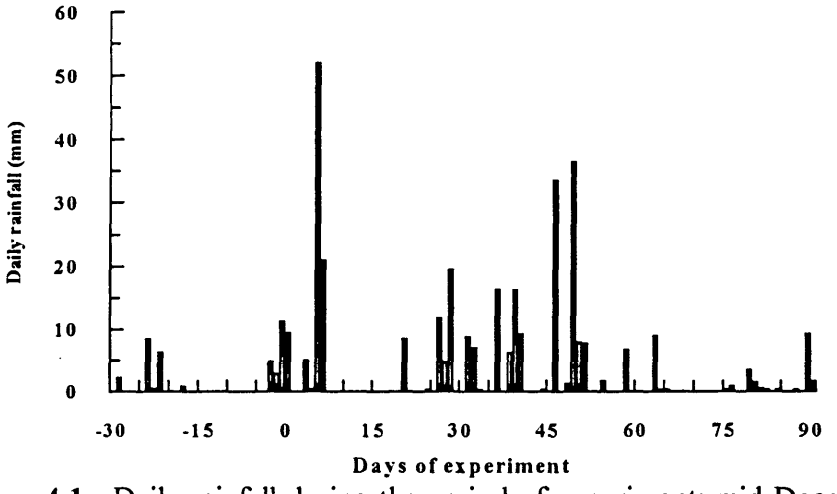


Figure 4.1. Daily rainfall during the period of experiment; mid December 1993 to mid March 1994 (Source: Climate Lab., Department of Agronomy and Soil Science, UNE).

A major proportion of ewes of 65%, 47% and 82%, from G1 (OC), G2 (OC+U) and G3 (OC+CSP), respectively, were marked by the harnessed wethers about 33 days *post partum* (day 33.7 ± 2.2; 33.6 ± 3.3 and 32.0 ± 2.6 for group OC, OC+U and OC+CSP, respectively), or within 5 days after teaser introduction. The first laparoscopy was then carried out to determine whether the onset of oestrus was accompanied by ovulation. This laparoscopy indicated that of the ewes exhibiting oestrous 35%, 32% and 47% had CLs in the control (OC), OC+U and OC+CSP groups, respectively. Records

from the second laparoscopy, 7 days after progesterone pessaries had been removed, showed that the proportions of ewes showing oestrus were significantly (P<0.001) lower, as was those with a CL (P<0.05) compared with the first observation. The treatment groups had: 26%, 5% and 29% showing oestrus and 26%, 16% and 18% ewes ovulated for groups OC, OC+U and OC+CSP, respectively.

Supplementation of OC with the addition of 250 g CSP resulted in a significant (P<0.05) reduction in liveweight loss for both Merinos and Crossbred ewes compared with control and OC+U treatment (Table 4.2). A significant difference (P<0.001) was also found in liveweight loss during the period of the experiment between those ewes rearing a single lamb compared to those rearing multiple lambs: 196 ± 6.0 *v* 145 ± 9.0 g/head/day, respectively. However, there was no interaction between littersize and treatment effect.

Table 4.2 Effects of supplementation treatment on the average daily liveweight loss of experimental ewes and the average daily liveweight gain of lambs (ADG).

Parameter	Animal strain	Group 1 **OC**	Group 2 **OC + U**	Group 3 **OC + CSP**	Probability
ADG lambs (g/d±sem)	Merinos	123.5±17.8	127.6±11.4	156.6±14.4	ns
	Crossbred	133.7±13.9	141.8±10.2	146.8±19.7	ns
	Mean	128.6±10.9	134.7±7.6	151.7±11.9	ns
ADG ewes (g/d±sem)	Merinos	-173.3±20.7	-181.7±21.3	-131.3±35.5	ns
	Crossbred	-210.0±36.8	-217.4±32.1	-140.3±44.0	ns
	Mean	-191.6±20.6[a]	-199.6±19.6[a]	-135.8±24.3[b]	P < 0.05

A total of 9 lambs died during the experiment, 3 from group OC (13.6%), 2 from OC+U (9.1%) and 4 from OC+CSP (18.2%). Supplementation treatments resulted in no significant difference in the ADG of lambs (Table 4.2). A significant difference (P<0.001)

between litter sizes was found in lamb ADG; 163.1 ± 24 *v* 119.2 ± 12.8 g/head/day respectively for single and multiple; however, there was no significant interaction with treatments. Figure 4.2 illustrates the liveweight change for ewes and lambs during the period of the experiment.

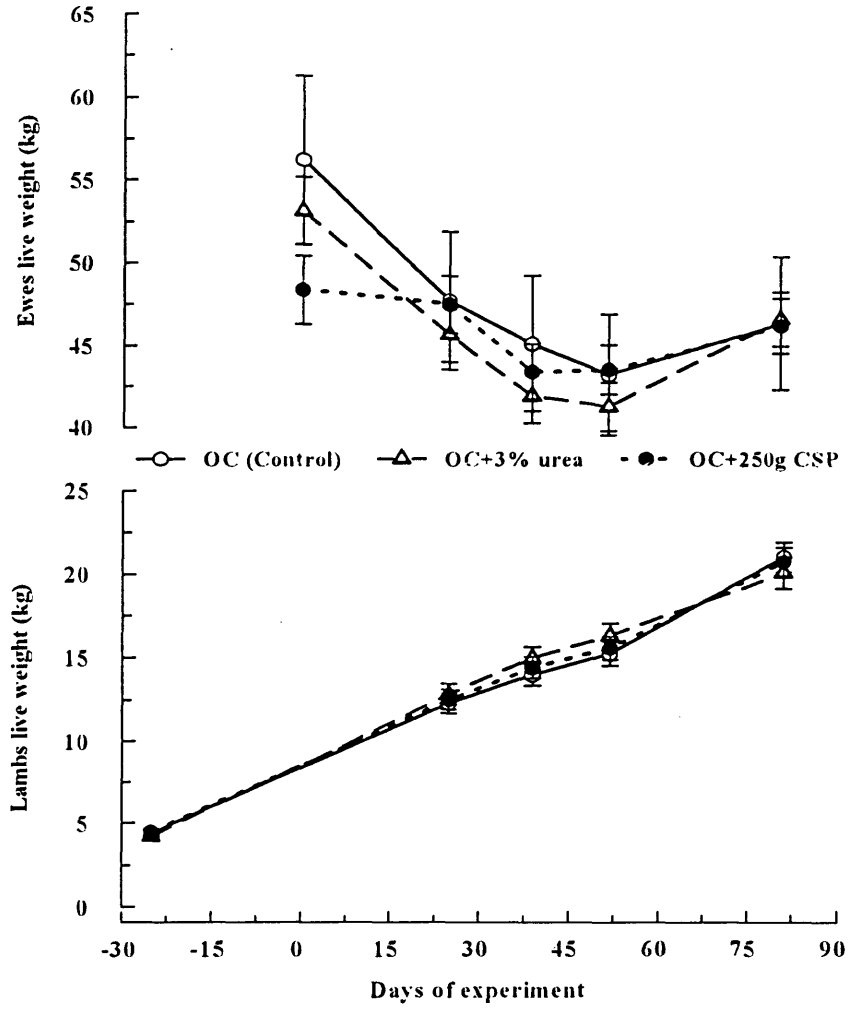


Figure 4.2 Pattern of liveweight loss of ewes from each treatment group (top graph) and liveweight gain of unweaned lambs (bottom graph). Day 0 of experiment indicates the day of experiment commencement and lambs weighed 21 days before day 0 (±2 days of ages).

Other results of this investigation are shown in Table 4.3. Neither OC fortified with 3% urea nor OC+250g CSP/ewe resulted differences in the resumption of ovarian activity *post partum* (ie. OR and total stimulation of ovaries). There was also no significant difference between treatment for OR, nor total stimulation, nor the proportion of ewes showing oestrus, nor in the interaction between effects (ie. breed×treatment) throughout the experimental period.

Table 4.3 Effects of supplementation with OC (Group 1), OC+3% urea (Group 2) and 250 g CSP + OC (Group 3) on the reproductive performance of experimental ewes.

Parameter	Animal strain	Gro up 1 **O C**		Gro up 2 **OC + U**		Gro up 3 **OC + CSP**		Probability
OR* (±sem)	Merinos	1.10 ± 0.30		1.11 ± 0.22		1.00 ± 0.14		ns
	Crossbred	1.50 ± 0.30		1.50 ± 0.16		1.38 ± 0.33		ns
	Mean all		1.26 ± 0.28		1.33 ± 0.16		1.14 ± 0.33	ns
Total stimulation (±sem)**	Merinos	1.73 ± 0.77		2.22 ± 0.58		2.50 ± 0.37		ns
	Crossbred	1.88 ± 0.77		2.00 ± 0.42		2.00 ± 0.86		ns
	Mean all		1.79 ± 0.73		2.10 ± 0.41		2.32 ± 0.52	ns
Proportion of ewes showing oestrus (%)	Merinos	81.8		77.8		85.7		ns
	Crossbred	75.0		66.7		62.5		ns
	Mean all		78.9		71.4		77.3	ns

Note: *) Calculated as the number of total CL found per ewe in one group per number of total ewes in that group.
**) Calculated as the number of total follicles (> 6mm Ø) and CL found per ewe in one group per number of total ewes in that group.
The proportion of ewes showing oestrus is calculated from number of ewes that marked by the harnessed wethers compared with number of ewes that ovulated.

Because this experiment was initiated out of the breeding season and the ewes were suckled *post partum*, the proportion of the flock that had a CL and showed oestrus (CL+O), had a CL but showed no oestrus (CL+nO), had no CL but showed oestrus (nCL+O) and had no CL and showed no oestrus (nCL+nO), from all observation periods,

were examined using chi square (χ^2) analysis. The result of this comparison is shown in Table 4.4. A significant difference was found between the proportion of ewes in each classification of experimental ewes, but no treatment effects were apparent.

Table 4.4 The proportion of ewes within breed classified according to oestrous and ovarian activity for three periods of observation {proportion (number of ewes)}.

Type of Breed	Ewes re-allocations				Probability
	CL+O	CL+nO	nCL+O	nCL+nO	
Merino	0.41 (36)	0.26 (23)	0.08 (7)	0.25 (22)	ns
Crossbred	0.25 (21)	0.21 (18)	0.14 (12)	0.39 (33)	ns
Total	0.33 (57)	0.24 (41)	0.11 (19)	0.32 (55)	P<0.05

Almost half of the blood (the 1st to the 7th for 4 ewes of each treatment group) samples for LH assay were accidentally destroyed during storage. Only 4 ewes from each treatment group had a complete set of samples from the bleeding program; the remaining 4 ewes from each group had only 4 samples (from the 8th to the 11th bleeding). However, there was no significant difference (P>0.50) amongst the animals in individual groups nor between treatment groups (n=8 ewes, respectively), for the last 4 sampling periods of the bleeding program. The plasma LH concentrations presented in Figure 4.3 were from the ewes that had a complete set of samples. The basal and mean concentrations of LH for each treatment group were 0.43 and 22.80, 0.51 and 39.45, and 0.61 and 18.42 ng/ml for control, OC+urea and OC+CSP, respectively. Increases in plasma LH for all the experimental ewes occured with a peak concentration of LH at 100 min. (7th bleeding) after the administration of GnRH. Significant differences (P<0.05) in plasma LH

concentration were found in ewes at 60, 80 and 100 min. after GnRH administration and fed OC fortified with 3% urea as compare to OC+CSP and control fed ewes (Figure 4.3).

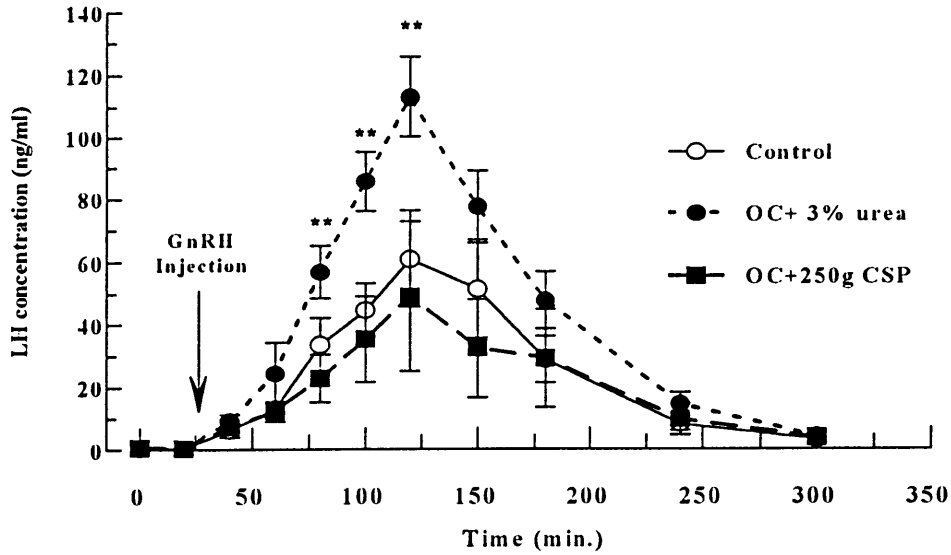


Figure 4.3 Plasma LH concentration for each treatment group after 50 μg synthetic GnRH administration ["**" denotes significant difference P<0.05].

4.5 DISCUSSION

Effects of post partum *supplementation on liveweight*

All experimental ewes lost an average 10 kg of liveweight (Figure 4.2), during the feeding trial, indicating that the ration was not adequate to meet the animals' requirements for lactation. A mean liveweight loss of almost 200 g/day for control and OC+urea fed ewes ($P>0.20$), indicated that these animals were severely underfed (Table 4.2), and significantly more so ($P<0.05$) then the OC+CSP fed ewes. The slightly lower weight loss ($P<0.01$) of the OC+CSP group suggest that the CSP may have stimulated greater pasture intake as reported by Leng (1986). At this stage, when the demand for nutrients is high for milk production and the availability of feed was below maintenance during this time, catabolism of body reserves in required to overcome the deficit and meet the requirements for milk production (Bryant and Smith, 1982; Preston and Leng, 1987). Joseph and Foot (1990) indicated that there is a mobilisation of 3.2 kg of body fat during the first 5 weeks of lactational period in Merino ewes grazing adequate pasture.

It is possible that the environmental factor of rainfall (Figure 4.1) is also contributed in the inadequacy of basal diets from the pasture. There was only 2.80 mm per day of rainfall between days 7 and 45 of the experimental period to support pasture growth. Although additional oaten chaff was offered, the lack of adequate basal diets was reflected in either weight loss and gain of ewes and lambs, respectively.

The demand for amino acids and glucose is an enormous constraint in lactating animals which depend only on rumen fermentation *per se* for their nutrients (Preston and Leng, 1987). The synthesis rate of glucose is often increased by up to three-fold in lactation due to the increased feed intake of lactating cows (Herbein *et al.*, 1978); and 60 to 80 % of the glucose synthesised is apparently taken up by the mammary gland (Annison and Linzell, 1964). The nutrient requirement for lactating ewes is therefore more than double, compared to non-lactating ewes. Thus it is clear that the ration plus the pasture

was inadequate to meet the requirement for milk production during this experimental period.

Although there was no difference in liveweight gain of lambs between treatments (Table 4.2), the results suggest that supplementation with 250g CSP based on OC basal diets on a low quality of pasture results in a significant ($P<0.05$) lowering of the liveweight loss of the ewes.

***Resumption of oestrus* post partum**

The onset of oestrus in a number of the ewes marked by the harnessed wethers, on average 33 days *post partum*, or during the first 5 days of teaser introduction, suggested that the ewes responded to some extent to a "ram effect". The onset of oestrus within 5 days and apparently in response to the rams' introduction has been previously reported (Knight, 1983; Martin *et al.*, 1986; Cushwa *et al.*, 1992) for seasonally anoestrus Merino ewes, and confirms earlier findings on the use of ram or buck to induce behavioural oestrus and ovulation (Oldham *et al.* 1980; Knight, 1983, Knight *et al*, 1983a, 1983b; Martin *et al.*, 1986). Since the ewes in the present study had not seen or smelt the ram during the gestation period, this effect seems to be likely.

The induced oestrus and ovulation associated with the "ram effect" is due to an increased LH secretion immediately after ram introduction (Martin *et al.*, 1980; Knight, 1983; Al-Mauly *et al.*, 1991); LH in turn stimulates follicular growth and oestradiol secretion by the ovaries (Martin *et al.*, 1986), and the oestradiol regulates the expression of sexual behaviour (Scarramuzzi and Martin, 1984). The current investigation confirms those earlier findings which shows that a proportion of ewes showed oestrus immediately after ram introduction, in this case 65%, 47% and 82% for groups OC, OC+U and OC+CSP, respectively; whereas only 35%, 32% and 47% of those ewes exhibiting oestrus had ovulated. The lesser proportion of ewes that had a CL compared with those showing the onset of oestrus indicates that the CL was not maintained or follicle development was

inadequate to respond to the induced LH surge. Knight *et al.* (1983a) and Martin *et al.* (1986) have shown that the CL often regresses within 6 days after ram introduction to anoestrus ewes. It has been suggested, then, that ewes be treated with progestagen pessaries before ram introduction (Knight *et al.*, 1983a; Martin *et al.*, 1986; Pearce *et al.*, 1987) to maintain the CL after ovulation *post partum*.

The significant differences between the first and second observations of ewes showing oestrus ($P<0.001$) and those that had ovulated ($P<0.05$) suggest that there was a diminution of ovarian activity after ram introduction during the *post partum* period. According to Peters and Ball (1987) and Lindsay (1988), the acyclic period in ruminants is of variable length, and is affected by various factors, especially nutritional status, season and the lactational period. Rund *et al.* (1988) indicated that suckling is likely to inhibit the resumption of oestrus and ovarian activity *post partum*; and this was confirmed by Wright *et al.* (1988), who showed that early calf weaning can lead to an increase of LH and FSH secretion from the hypothalamic-pituitary axis. The suckling effect has also been shown to lengthen the anoestrus period of buffaloes (Nordin and Jainudeen, 1992). Rivera *et al.* (1994) have demonstrated that temporary calf removal and additional exogenous FSH advanced the onset of first oestrous and ovulation after parturition as compared to suckled cows. However, there do not appear to be any reports for ewes, possibly because it is unusual for ewes to be lactating at the end of the seasonal anoestrus period.

Supplementation effect on ovarian activity

There was a significant difference (Table 4.4) in the classifications of proportions of CL+O, CL+nO, nCL+O and nCL+nO ewes. However, this difference was not due to supplementation treatments. Difference from this classification indicates that the *post partum* acyclic period influences the regulation of reproductive hormonal status of the ewe during the experimental period. Smith (1991) reported that the resumption of ovarian activity can be stimulated either directly, by increasing the sensitivity of the ovary to FSH,

or indirectly, by increasing oestradiol as the negative feedback mechanism to the hypothalamus, with *post partum* nutrient supplementation. However, the current investigation was unable to demonstrate any such effects of *post partum* supplementation.

Supplementation and GnRH induced LH responses

Supplementation of ewes on low protein forage based diets with non-protein nitrogen and protected protein were unable to produce significant differences in ovarian activity during lactationally anoestrous of ewes as revealed by ovarian characteristics and incidence of oestrus.

The basal and mean concentrations of LH: 0.43 and 22.80; 0.51 and 39.45, and 0.61 and 18.42 ng/ml for control, OC+urea and OC+CSP, respectively, were in a range similar to those reported by McNeilly *et al.* (1985) in anoestrous and Scarramuzzi *et al.* (1970) in oestrous ewes, which were 1.1 and 47.5 ng/ml and 0.35 and 28.45 ng/ml, respectively.

The response of ewe pituitary gland to GnRH as measured by LH during the period of anoestrus has been previously demonstrated by Wright *et al.* (1982) and Brooks *et al.* (1993). In the present experiment the pituitary gland of ewes supplemented with OC+urea significantly responsed to a greater extent to exogenous GnRH compared with control and OC+CSP supplemented ewes ($P<0.05$). However, LH of all ewes from each treatment group are GnRH dose dependent. This result suggests that most of the experimental animals were still under the influence of the factors that induce anoestrous in the out-of-breeding season (see Lindsay, 1988), and which vary according to the genetic background of the sheep (McNeilly *et al.*, 1985), and is also influence by the length of the lactational period (Wright *et al.*, 1988; Pope *et al.*, 1989; Smart *et al.*, 1994).

In the rumen, urea is transformed into ammonia which is utilised by the microbes for growth and improves digestibility on forage diets low in N; this in turn increases energy, VFA and microbial protein available for digestion and absorption.

Supplementation of a low N diet with NPN alters the protein to energy (P:E) ratio in the nutrients absorbed (Preston and Leng, 1987). In contrast, protected protein supplementation (such CSP in this investigation) provides more protein which therefore increases P:E ratio *per se* (Leng *et al.*, 1977). However, the protected protein supplemented group in this experiment did not give any significant increase in responsiveness to GnRH compared with the control ewes. The uncertain effect of protected protein supplemented group, in the present experiment, confirms that the relationship between nutrition, OR and gonadotrophin patterns are difficult to interpret due to inconsistencies in response to nutrition from one experiment to another (see Downing and Scarramuzzi, 1991). If it is true that urea enhances the sensitivity of the pituitary to exogenous GnRH, then, it would be expected that by the third laparoscopy, more ewes supplemented with OC+urea would have commenced cycling. Data from the third laparoscopy showed no significant difference, between treatment groups.

The experiment by Rhind *et al.* (1989) showed that there was no effect of body condition on plasma LH and FSH concentration; ewes in high or low body condition have no differences in FSH and LH pulse characteristics in the luteal or follicular phases of the oestrus cycle. Giving grain to ewes on a basal diet of hay did not increase pituitary gland gonadotrophin concentrations but increased pituitary weight and therefore total LH and FSH content (Bellows *et al.*, 1963; Howland *et al.*, 1966) in oestrous animals. A later experiment by Jabbour *et al.* (1991) also indicated that neither supplementation with lupin grain nor GnRH had an effect on the mean peak concentrations of LH secreted. These studies were confirmed by the finding of Downing and Scarramuzzi (1991) that ovarian response to endogenous gonadotrophin was not increased by the grain supplementation. Likewise, the current study shows that anoestrous ewes that were supplemented with bypass protein source (CSP), had LH concentration that were not significantly different to control ewes after GnRH injection.

Nutrients of feed availability during this study was seems to be inadequate to maintained nutrient requirements of lactational sheep. It would then be possible that larger amount of supplements that supply essential nutrients for rumen microbial growth and for the host, especially in lactation, may overcome the apparent deficiency during this feeding trial. This was already confirm in the discussion of Preston and Leng (1987). Urea supplementation, in this study, did not show any effect in maintaining body condition of the experimental ewes, indicating of a possibility of other nutrients deficiencies besides nitrogen and minerals.

4.6 CONCLUSIONS

Non-protein nitrogen (urea) and protected protein (CSP) supplementation of OC and for pasture based diets for ewes produce no significant increase in ovarian activity in anoestrous ewes. However, the sensitivity of the pituitary gland to exogenous GnRH was increased in pasture fed ewes supplemented with OC contains 3% urea. This difference was not confirmed by changes in ovarian or oestrous activity by the end of the experiment.

Supplementation of CSP, in this investigation, has been able to show little effect on reproductive parameters, ie. lambs daily gain. Possible reasons for this lack of effect may be because of the inappropriateness of the experiment conditions. Leng *et al.* (1977) suggested that responses to protected protein are only possible when the requirements for amino acids are not already being met, and these cannot be expected if the animals are in a low productive stage, whose protein requirements are low, eg. non-lactating, non-pregnant or where energy intake is restricted.

Urea supplementation increases rumen ammonia production (Preston and Leng, 1987) as well as peripheral plasma urea level (Nolan and Stachiw, 1979), and also produces changes in the P:E ratio, which possibly perturb the sensitivity of the pituitary gland of ewe to exogenous GnRH. However, this argument is weakened by the fact that a group of ewes fed additional protected protein supplementation showed no difference from the control treatment group. Therefore, the mechanism which allows urea supplementation to alter the sensitivity of pituitary gland to GnRH stimulation requires further elucidation.