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The influence of flush feeding with different nitrogen sources on ovulation and conception rates in Dohne-Merino ewes

By

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PREFACE

The experimental work described in this dissertation was carried out in the Department of Animal and Wildlife Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, from May 2001 to January 2002, under the supervision of Professor Edward C. Webb.

I declare that this thesis/dissertation, which I hereby submit for the degree MSc (Agric) ANIMAL SCIENCE at the University of Pretoria, is my own work and has not previously been submitted for a degree at this or any other tertiary institution.

Signature……

Date……

Abstract

The aim of the present study was to determine if there is a difference in ovulation- and conception rates, in semi-intensively managed Dohne-Merino ewes, flush fed with diets containing different nitrogen sources. Four different nitrogen sources were chosen due to the difference in dietary amino acid composition and cost. In order for a sheep farming enterprise to maximize profitability it is essential to optimize ovulation- and conception rates and to minimize lamb losses in order to increase weaning percentage and therefore profitability. However the cost of dietary supplementation is high and may increase production costs and minimize profitability. One hundred and fourty four (144) Dohne-Merino ewes (age between 14 to 85 months) were included in two dietary supplementation trials (autumn and summer) at the experimental farm of the University of Pretoria in Hatfield. The ewes were divided equally into two trial groups (n=72), with the first trial done in season 1 (started in May 2001, typical breeding season) and the second trial done in season 2 (started in November 2001, out of season; 2nd breeding season). During the day the ewes had ad-libitum access to graze on *Festuca arundinaceace* (Tall Fescue). In both trials the ewes (n=72) were randomly allocated into four dietary supplementation groups, each group receiving a dietary supplement with a different combination of nitrogen sources. The four dietary supplements were formulated on an iso-nitrogen basis, to eliminate the effect of protein level, and to emphasize the possible effect of protein quality (amino acid composition) on ovulation, conception and lambing rates. In both trials the total amount of crude protein intake per ewe was calculated at 256.40g/day, while the total daily allowance of digestible crude protein was calculated at 190g per ewe. The 256.40g crude protein intake per ewe per day is 2 times more than the threshold level of 125g per ewe per day. A minimum daily crude protein intake of 125g is needed for effective rumen functioning, and this together with the interconvertion of energy by the rumen indicates the complex nature of relating dietary differences to physiological responses. These values were kept the same for both the trials in season 1 and 2. The bulk of the 256.40g crude protein per day was obtained from grazing on the *Festuca arundinaceace* pasture. In season 1 the dietary supplement had to provide 40.00g of crude protein per day in order to get to a daily crude protein intake of 256.40g, while in season 2 the provision from the dietary supplement was calculated to be 37.45g of crude protein. The difference in the crude protein level, obtained from grazing of the *Festuca arundinaceace* between season 1 and 2 was due to pasture quality differences. The nitrogen sources used in the trials were urea, sunflower oilcake meal, cottonseed oilcake meal and a mixture of cottonseed oilcake meal and fishmeal. These dietary supplements were fed for a period of 9 days before mating; the weight of each ewe was recorded before the onset of the trial and again on the second day after mating to establish any live weight changes. Synchronization of the ewes was done with Chrono-gest grey sponges (40mg Fluorogestone acetate) from day one and was repeated from day 23. On day 12 each ewe were injected with 1.0ml prostaglandin F2α (Prosolvin,

each milliliter containing 7.5mg Luprostiol). On day fourteen the sponges were removed and two days later all the ewes were checked for cyclic activity with the aid of six vasectomized rams. The six vasectomized rams were introduced to the whole laparoscopy group of 12 ewes, and every ewe that stood twice for mating were identified as cyclic. This practice continued for a period of 30 minutes in the morning and repeated for another 30 minutes in the afternoon up to day 18. The second round of sponges were inserted on day 23 and removed on day 37. Ewes were mated by means of hand mating with two different rams from day 39 to 42. A laparoscopy technique was used on day 45 of the trial to count the number of ovulation points (corpora lutea) on each ovarium of each ewe. The number of fetuses of each ewe was counted on day 90 after mating by means of ultrasound scanning and at birth the number of lambs born was also recorded.

In both these trials dietary supplementation had no significant effect on ovulation, conception and lambing rates. However, looking at the Odds Ratio Analysis for the 144 ewes over the two breeding seasons, the different dietary supplements had a significant influence on the number of ovulation points ($p<0.05$). Compared to urea (dietary supplement 1), the fishmeal cottonseed oil cake mixture (dietary supplement 4) yielded the best results (1.306), followed by the cottonseed oil cake meal (dietary supplement 3) (1.298), and sunflower oil cake meal (dietary supplement 2) (1.050). The same Odds Ratio Analysis showed that the different dietary supplements had a significant effect on the number of lambs born ($p<0.01$). Compared to urea (dietary supplement 1), the fishmeal cottonseed oil cake mixture (dietary supplement 4) yielded better results (1.086), followed by urea (dietary supplement 1) (1.000), and sunflower oil cake meal (dietary supplement 2) (0.801) and lastly cottonseed oil cake meal (dietary supplement 3) (0.784). Breeding season ($p<0.05$) had a significant effect on the number of ovulation points but no difference was observed in terms of the number of lambs born. Age ($p<0.01$) had a significant effect on the number of ovulation points, the number of fetuses counted as well as the number of lambs born. Change in live weight ($p<0.05$) had a significant effect on the number of ovulation points per ewe but as with breeding season it had no significant effect on the number of lambs born. Birth status of a ewe ($p<0.05$), had a significant effect on the number of fetuses and the number of lambs born. The data of both the trials in season 1 and 2 suggests that under the conditions of the study with the odds ratio analyses that the four different dietary supplements had a significantly different effect compared to dietary supplement one on the number of ovulation points and the number of lambs born. However, factors like breeding season, age, change in live weight and birth status of the ewe also had a significant effect on ovulation and conception rates in Dohne-Merino ewes.

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Abbreviations

AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of variance
cm	centimeter
CO_2	Carbon dioxide
CP	Crude protein
DCP	Digestible crude protein
DEI	Daily energy intake
DM	Dry matter
DMI	Dry matter intake
DPI	Digestible protein intake
E	East
FSH	Follicle stimulating hormone
g	Gram
g/day	Gram per day
g/ewe	Gram per ewe
g/kg	Gram per kilogram
h	Hour
ha	Hectare
HCL	Hydrochloric acid
i.e	That is
IVDOM	*In vitro* digestible organic matter
kg	Kilogram
LH	Luteinizing hormone
ME	Metabolizable energy
MHz	Megahertz
MJ	Megajoules
MP	Microbial protein
n	Group size
NRC	Nutrient Requirements of Sheep

NSIP	US National Sheep Improvement Program
OM	Organic matter
p	P-value (significance level)
RDP	Rumen-degradable protein
S	South
SD	Standard deviation
SME	Steroid metabolizing enzymes
UDP	Rumen-undegradable protein
VFA	Volatile fatty acids
°C	Degrees Celsius

CHAPTER 1

1.1 TITLE

The influence of flush feeding with different nitrogen sources on ovulation and conception rates in Dohne-Merino ewes.

1.2 AIM

The aim of the present study was to determine if different nitrogen based dietary supplements had an influence on the ovulation- and conception rates of Dohne-Merino ewes during flush feeding. Four different nitrogen sources were chosen due to the differences in dietary composition (amino acid) and cost. In order for a sheep farming enterprise to maximize profitability it is essential to optimize ovulation- and conception rates with the least cost dietary supplementation.

1.3 MOTIVATION

Sheep are among those farm species whose production methods can be profitably and substantially intensified, right from the point of breeding the ewe through until the time of dispatching the lamb to the abattoir. Some of the most important concerns to South African sheep producers over the years have been to increase the frequency of lambing, the number of lambs born and the weaning percentage of a flock. Low lamb output per ewe is a major factor limiting the energetic efficiency of sheep production and results in low margins and profitability for the producer.

To be economically successful the sheep farmer must manage his sheep in a way to meet the nutrient requirements of the sheep largely from grazing rather than from dietary supplements. Dietary supplementation increases feeding costs, therefore the duration has to be short and it has to have a positive effect on the number of lambs born to make it worthwhile. The response to dietary supplementation or flushing is affected by the season, the age of the ewe, its breed, and body condition, to name a few. Effects of flush feeding or flushing have been recorded as early as 1904, but these results were contradictory and vague (Coop, 1966). Although flushing is a husbandry practice used in major sheep-producing countries, the response to flushing is variable and the practice needs to be evaluated for a particular flock or enterprise, preferably over a number of years to determine its profitability. It is economically of no use to the sheep farmer to increase ovulation rate through flushing, if there is not an increase in the number of lambs born per ewe. Foote *et al*. (1959) reported

that high levels of feeding, which increased ovulation rates, also increased embryonic mortality resulting in a lower lambing rate. If an increase in the number of lambs born per ewe is obtained it is very important to have good flock management in order to capitalize on this advantage to increase profitability.

CHAPTER 2

2.1 INTRODUCTION

One of the most important advantages of sheep production is its high reproductive rate. Photoperiod, temperature and nutrition are three well studied environmental cues that affect reproduction in sheep. The profitability of a sheep farming enterprise depends on a lot of different aspects. Good flock management, optimal use of restricted food recourses and the restriction of livestock losses are some of the most important factors determining the profitability of such an enterprise.

The Dohne-Merino breeding program started at the Dohne Agricultural Research Station near Stutterheim, in the Eastern Cape, in 1939 in South Africa when it became clear that the unique economic and physical environments called for an adapted and more productive Merino-type sheep. The buoyant demand for sheep meat and fine merino wool production favoured the development of new strains combining fine wool production with enhanced fertility and slaughter lamb production. The main criterion used to evaluate animals is their measured relative efficiency in a commercial environment. Selection is therefore aimed at maximum economic return per unit of input in a strictly commercial environment (Campher *et al.*, 1997). With the price ratio of meat to wool close to 1:8 (2006), the weight of lambs produced in woolled sheep flocks is by far the most important factor influencing overall flock income. Independent financial analyses show that meat/wool production systems that currently generate top margins produce by volume, marketable commodities in the ratio of 87% meat and 13% clean wool. This means that for every 4 kg of meat, 1 kg clean wool is produced. Nevertheless wool still has a stabilizing effect on the economy of woolled sheep farming. The Dohne-Merino has an intermediate breeding season. This means that the Dohne-Merino doesn't have distinct peaks in the breeding season like the breeds with a short breeding season.

Breeds or crosses that produce large lamb crops at an early age have the potential to increase efficiency and reduce costs. While ewes bred first at 7 to 9 months are likely to have a greater life-time productivity than those bred first at 12 to 15 months (Hulet *et al.*, 1969), the conflicting nutritional demands for reproduction and body growth in pregnant ewe lambs could depress maternal weight at

lambing, with detrimental effects on lambing ease, lamb birth weight, lamb survival and ewe milk production (Demeke *et al*., 1995). Nutritional demands increase heavily through late pregnancy and lactation, so if nutrient supply, including body reserves, is going to be inadequate, ovulation and conception cycles of the oncoming season may decrease with catastrophic consequences for the ewe and the owner.

The total wellbeing of sheep depends on a host of macro- and micronutrients. Reproduction does not take precedence over processes necessary for survival; hence, reproductive activity ceases well before an animal experience deficiency of a particular nutrient. The response to nutritional treatment of animals depends on live weight, body condition (Nottle *et al.*, 1997; Rhind & McNeilly, 1998; Yildiz *et al.*, 2003), potential reproductive performance and genotype (Abecia *et al.*, 1997; Wilkins, 1997) of animals, and the actual number of ova released at estrus is highly dependent on the nature of the ewe's long-term nutritional regimens (Nottle *et al.*, 1997).

Direct effects of poor nutrition are reflected in reduced conception, embryonic losses, reduced lambing rates (Diskin & Niswender, 1989) and high ewe mortality (Yoder *et al*., 1990). Although, experiments investigating supplementation of the diets during mating have produced conflicting results (Bichard *et al*., 1974; Rhind *et al.*, 1989), it is evident that failure to flush ewes may result in delayed estrus activity and ovulation (Gunn *et al.*, 1979), fertilization failure (Restall *et al*., 1978) and embryonic mortality (Rhind *et al*., 1989). The ewe lamb is less likely to protect the young embryo against fluctuating energy supply and stress than adult ewes (Bichard *et al*., 1974) and therefore flushing is considered important for young ewes.

2.2 NON-NUTRITIONAL FACTORS INFLEUNCING REPRODUCTION

2.2.1 Seasonal breeding of small stock

It is well known that reproduction in sheep is seasonal, at least in breeds originated from temperate climates (Bearden & Fuquay, 1997). Sheep are mainly short-day or autumn breeders. Their breeding season is initiated as day length decreases and night time increases and ends when day length and night time are of similar length. Sheep breeds like Merino, Dorset Horn and Rambouillet that developed in temperate climates are seasonally polyestrous animals with normal ovulatory cycles occurring in the autumn and winter, and a period of seasonal anestrous (non-breeding season) in the spring and summer months (Marshall, 1937; Hafez, 1952). As breeding season approaches, there is an increase in the frequency and amplitude of episodic surges of LH. The retina of the eye is the photic sensor that transmits light signals by way of the retinohypothalmic tract to the suprachiasmic nuclei. Diurnal signals generated by these nuclei are transmitted to the superior cervical ganglia and then to the pineal gland via sympathetic nerves. During darkness, the sympathetic activity increases resulting in the increases secretion rate of melantonin. The pineal gland, through the synthesis and release of melantonin, serves as a mediator between the neural signals induced by changing photoperiod and the endocrine system that regulates cyclic reproductive activity. Through either indirect or direct action on the hypothalamus, melantonin modulates seasonal breeding activity in long-day and short-day breeders (Reiter, 1974).

Intensified management has led to breeding sheep outside the above mentioned natural breeding season when ewes experience seasonal polyestrous activity (Jeffcoate *et al*., 1984; Rawlings *et al*., 1987; Gordon, 1996). The transition from the anoestrus to the breeding season is gradual, with the occurrence of short oestrus cycles, because the first *corpus luteum* often regresses prematurely 5 to 6 days after its formation. Silent ovulations may also occur in some breeds during mid-anoestrus (Ortavant *et al.*, 1988). The duration of anoestrus in ewes varies among breeds. Non-prolific genotypes tend to have a longer anestrous compared to more prolific sheep (Hafez, 1952; Webster & Haresign, 1983; Goodman, 1994; Bartlewski *et al.*, 1998, 2000). There is also tremendous variability in the length of the anestrous period among individual animals within a breed (Webster & Haresign, 1983; Jeffcoate *et al.*, 1984; Bartlewski *et al.*, 1998, 2000).

In a previous study during anestrous in prolific Finnish Landrace ewes in the Northern hemisphere (Bartlewski *et al.,* 2000), it was shown that there were differences in ovarian follicular dynamics and endocrine profiles between ewes going into anestrous early (March – April) or late (May). Finnish Landrace ewes entering anestrous late exhibited greater follicle development and gonadotropin secretion compared with animals that entered anestrous early (Bartlewski *et al.,* 2000). Differences in ovarian and endocrine function in ewes with different durations of anestrous may influence fertility to out-of-season breeding, particularly in non-prolific breeds such as the Western White Face. In a study by Gordon in 1996 the prolific type of ewes showed a greater ability than the non-prolific breeds to maintain cyclic activity after estrous induction, if pregnancy did not occur, which resulted in the higher percentage of treated ewes becoming pregnant to first and second services. Huchkowsky *et al.* 2002 showed that ewes becoming in anestrous early in spring produced fewer antral follicles and the largest follicle recorded each day had a smaller diameter compared with the ewes becoming anestrous late in spring.

Huchkowsky *et al.* 2002 also reported a difference in endocrine parameters between prolific breeds of sheep with a generally short anestrous period and non-prolific genotypes of ewes with longer anestrous; probably reflect varying responsiveness to photoperiodic control of seasonality (McNatty *et al.,* 1984; Goodman, 1994).

Seasonal breeders from high latitudes use changes in photoperiod as a predictor of future conditions and respond to it by restricting ovulation and entering a period of anoestrus. The effect of season on ovulation rates is a complicating factor in experiments (Davis *et al*., 1976; Cumming, 1977). Two experiments were conducted, by Davis *et al*. (1981) with the second experiment later in the breeding season than the first. At similar mean live weights and fed the same basal ration with lupin supplement, ewes had higher ovulation rates in the first experiment than in the second. According to this, there remains a possibility that season could have modulated the response (Rizzoli *et al*., 1976). Moreover, seasonal variations in response to lupine feeding have been shown by Gheradi and Lindsay (1982) and these may reflect seasonal changes in the sensitivity of estrogen. The effects of flushing during the seasonal peak in ovulation rate were less effective than during early or late in the breeding season.

2.2.2 Ovulation in small stock

In female animals the primary determinant of fertility and fecundity is the number of ova shed from the ovaries (the ovulation rate) (McDonald *et al*., 1995). The establishment of cyclic ovarian activity at puberty is important for the formation and release of gametes as well as for the establishment of mature sexual capabilities. Ewes are spontaneous ovulators rather than having ovulation induced by copulation. Sheep can modify their reproductive output as a function of perception of the nutritional environment by modifying the numbers of ova that are shed at each ovulatory cycle (Coop, 1966). Ovulation rate is affected by factors operating up to the time of mating or during the recovery period between lactation and breeding, whereas ova loss or prenatal mortality is affected by nutrition during recovery and also during pregnancy (Coop, 1966). Thus, both the static (during the recovery period) and dynamic (flushing) aspects of nutrition influence lambing rate (Coop, 1966).

In the ewe, it takes approximately 6 months from when follicular growth first commences to when one or more of these undergo final maturation and ovulate (Cahill & Mauleon, 1980; Driancourt & Cahill, 1984). Imposing nutritional handicaps at different stages of folliculogenesis have been shown to influence ovulation rate in the ewe. Fletcher (1974) showed that restricting feed intake 6 months prior to ovulation when those follicles destined to ovulate first commence growing, reduced ovulation rate. According to Nottle *et al.* (1997) restricting nutrition 6 months prior to ovulation may affect the number of follicles that exit the primordial pool and commence growing or inhibit subsequent follicular development, such that those follicles that would normally ovulate are incapable of doing so and are lost through atresia.

It is commonly accepted that all primary follicles are formed during the prenatal period of the female (Bearden & Fuquay, 1997). Gamete production proceeds in the embryonic ovary through mitotic division of the primordial germ cells. Mitosis ceases at birth, with the maximum number of oocytes that a female will ever have being present at this time. Meiosis is soon initiated by factors from the *rete* ovarii but is arrested at the resting stage, with resumption of meiosis not occurring until the onset of puberty (Knobil & Neill, 1988). A primary follicle is a germ cell surrounded by a single layer of follicular cells. It is estimated that approximately 75,000 primary follicles are found in the ovaries of a young heifer (Bearden & Fuquay, 1997). With continual follicular growth and maturation throughout a

female's reproductive life, an old cow may have only 2,500 potential ova. Some potential ova reach full maturity and are released into the duct system for possible fertilization and development of offspring. Most follicles start development and become atretic (i.e., they degenerate) (Bearden & Fuquay, 1997).

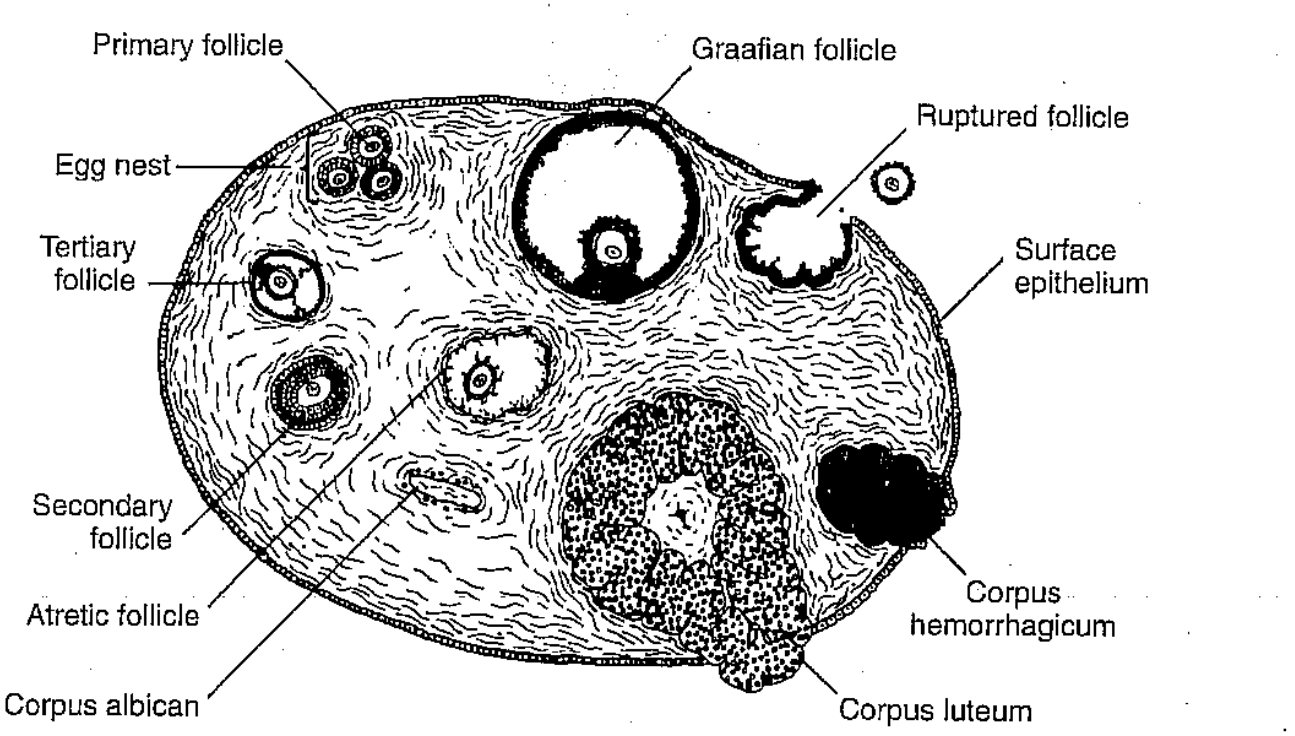

Figure. 1. Diagram of the structures that can be identified in a cross section of an ovary of a reproductively active female. Different maturation stages for follicles and the corpus luteum can be observed. (Adapted from Patten. 1964.)

The different stages of follicular growth and maturation are as follows: 1) Primary follicle, 2) Secondary follicle, 3) Tertiary follicle, 4) Graafian follicle, 5) Ruptured follicle, 6) Oocyte, 7) Corpus hemorrhagicum, 8) Corpus luteum.

The corpus luteum has a grayish white color, is well supplied with blood vessels and is the only ovarian source of progesterone and other progestins. When the corpus luteum regresses, it loses its color, no longer produces progestins and eventually appears as a small white scar on the surface of the ovary, which is called a corpus albicans. It is generally accepted that if an animal is pregnant, the corpus luteum will not regress until late pregnancy (Bearden & Fuquay, 1997). Ovarian follicular

development in ewes is a progressive and recurring process with two to three waves of follicular growth occurring during each cycle of a cow. During each wave, a group of follicles will be recruited and start to grow. Once a follicle has entered the final stages of maturation, it has two alternatives: it will either degenerate through atresia, or it will ovulate. One of these follicles will attain dominance and the subordinate follicles will become atretic. In the last wave of the cycle, the dominant follicle is the ovulatory follicle. Ovulation rate is thus determined more by the number of follicles that escapes atresia than by the number of follicles stimulated to grow and ovulate. Preovulatory gonadotrophin release ends active follicle growth, and a delay in gonadotrophin secretion in animals with high ovulation rates allows other follicles to mature and develop to the point of ovulation. However, this dominance mechanism doesn't have such a big influence in sheep as in cattle and two or three follicles can reach maturity (Driancourt *et al.*, 1991b).

A decrease in ovulation rate has also been demonstrated when nutritional restrictions are imposed immediately prior to ovulation during the antral phase of follicular growth (Coop, 1966; Killeen, 1967; Fletcher, 1971). Driancourt and Cahill (1984) confirmed the findings of Coop (1966), Killeen (1967) and Fletcher (1971), and suggested that undernutrition may increase the incidence of atresia during the antral phase of follicular growth reducing the number of follicles available for ovulation.

Lambing rate is affected by the number of ova fertilized and embryo survival (NRC, 1985) as well as nutritional alterations prior to and during breeding (flushing). Nutritional flushing is defined as the short-term provision of extra feed (energy, protein or both) to raise the plane of nutrition immediately prior, during or after mating. Underwood and Shier (1941) originally argued that flushing stimulates follicular development and favours the maturation of a greater number of ova and this explanation is still valid. The main aim of flushing is to increase the reproductive performance of ewes, and this is normally reflected by high conception and lambing rates.

There are two components of flushing, a static part related to body condition and a dynamic part related to the diet which is distinguishable from and independent of the body weight effect (Coop, 1962; 1966; Tassel, 1967). According to Rattray (1982), the reproductive potential (in terms of multiple ovulations) is increased by an increased body weight during the flushing period. The response to flushing is affected by the age of the ewe (mature ewes show greater response than yearlings), its breed, and the

stage of the breeding season. Currently there is a difference of opinion on the effect of duration of the flushing period, intensity of flushing, body condition of the ewe, effect of season and type of nutrition used for flushing (Coop, 1966; Haresign, 1983; Loubser, 1983; Rhind, 1987). Notter (2000) did a study on the effect of age on prolificacy using records of prolificacy (number of lambs born per ewe lambing) of Targhee, Suffolk and Polypay ewes from flocks participating in the US National Sheep Improvement Program (NSIP) between 1984 and 1994. From these records, prolificacy differed ($p<0.001$) among ewe age groups in all different breeds. Peak prolificacy was generally achieved between four and eight years of age. Exceptions to this generalization include a somewhat sharper peak in prolificacy for the Targhee. Prolificacy of five to six year old Targhee ewes averaged 0.06 higher than the prolificacy of four to seven year old ewes. Ewes that were more than eight years old at lambing had 0.17 to 0.20 fever lambs per ewe lambing than the three to six year old ewes. Thus, prolificacy did not exhibit constant declines until seven years of age. Dickenson & Glimp (1975) used a linear and quadratic regression to evaluate ewe age effects on prolificacy in seven US breeds, and concluded that across all seven breeds, prolificacy was a maximum at 5.9 years of age; this is in line with the results found by Notter (2000).

The ability of nutrition to alter the lambing rate of ewes is well known where a rapid improvement in body weight gain or body condition is associated with an increase in ovulation rate and lambing rate (Nottle *et al.,* 1997b; Rhind and McNeilly, 1998; O'Callaghan and Boland, 1999).

Results from Venter & Greyling (1994) suggest that flushing can increase reproductive performance, but it should not be administered too long prior to synchronization, especially if the ewes have a good body condition. In that particular trial, flushing from 1 week prior to the end of synchronization, for a total period of 3 weeks gave the best results. Much difficulty has been experienced in determining the optimum duration of the flushing period in grazing ewes, since responses do not seem very predictable. The period of flushing is generally accepted as being 2 to 3 weeks prior to mating (Brown & Meadowcroft, 1990). Results from other studies, suggested that the quality of the pasture and supplements is the most important factor, influencing ovulation rate (Knight *et al.,* 1975; Rattray *et al.,* 1980).

Ovulation rate appears to respond to short-term high energy intake only within a specific intermediate range of body condition (2.0-3.0). Wentzel (1986) found an increase in blood glucose concentration of 60% within 48h following flushing, and Russel (1978) and Erasmus (1990) postulated that blood glucose levels could be used as an indicator of the energy status of the animal. According to Venter & Greyling (1994) flushing leads to a higher blood glucose concentration, and it is this higher available energy which is beneficial to the reproductive performance of the ewe. It has been suggested that flushing increases ovulation rate by stimulating the pituitary gland to produce more luteinizing hormone. Thomas *et al.* (1984) reported that a increase in nutrient intake, particularly protein, effectively increases levels of hepatic steroid metabolizing enzymes (SME). These enzymes are thought to be associated with an increased clearance rate of steroids, and a decrease in steroids is associated with an increase in gonadotropins and thus increases ovulation. Another endocrinological explanation of the effect of flushing is that the high plane of nutrition promotes a greater production of insulin, which encourages the uptake of glucose and the synthesis of steroid hormones by the ovary (McDonald *et al.,* 1995).

Positive responses to flushing are only achieved where ewes have a greater appetite drive over mating. Results have indicated that ewes which had been in a lean condition (1.0-2.0) were eating 35% more at mating than those which had been in a fat condition (3.0-4.0) (Haresign, 1983). However results from Pearse *et al.* (1994) indicate that nutritional flushing may increase ovulation rate of ewes regardless of their body condition. The source (Molle *et al.,* 1995; Landau *et al.,* 1996; Branca *et al.,* 2000) and the level (Parr *et al.,* 1987; Rhind *et al.,* 1989; Abecia *et al.,* 1997) or the timing (Molle *et al.,* 1997) of protein or energy supplementation may have critical consequences on the reproductive efficiency in sheep because nutrient requirements for optimum follicle growth may be quite different from those for embryo development (O'Callaghan & Boland, 1999). It has been reported that low feed intake during pre-mating reduces the mean ovulation rate, and during post-mating compromised embryo growth rate and induced a higher rate of ova wastage in sheep (Rhind *et al.,* 1989).

Turner (1966) found that twins could be selected for. Selection for twin-bearing ability was slow but effective because they found a 3% gain per year that represented a gain in lambs born as a percentage of ewes mated as a result of selection. In the same study both flocks showed an increase in multiple births with age of the ewe in the number of lambs born per ewe mated. It is suggested that the

'twinning ability genes' were transmitted by both the ram and the ewe but those in the ewe were only capable of expression; they probably operated by increasing the number of oocytes shed (ovulation rate) (Pacham & Triffitt, 1966).

There is little doubt that stress has a detrimental effect on reproductive competence in farm animals. Most evidence suggests that, although stressors can cause foetal losses in mid-to-late pregnancy, the increased percentage of stress-induced reproductive losses occurs as a result of interference with correct hypothalamus-pituitary function, early embryonic losses result from unsuitable exposure of the ovum to gonadotrophins within the follicle (Staigmiller & Moor, 1984).

2.2.3 Body Condition

Coop (1966) in New Zealand was one of the first to try to define the nutritional effect more precisely; and used the terms static and dynamic to describe the nutritional effects on ovulation and conception rates. The static effect referred simply to body condition, live weight and/or size of the ewe, while the dynamic effect was defined as a change in live weight during the six-week period prior to mating. The liveweight of an ewe is a combination of body size and body condition and as such live weight is not a good measure of an ewe`s body nutrient reserves. In a study by Ducker & Boyd (1977), body size had no effect on mean ovulation rate of ewes that had the same body condition. In Scottish Blackface ewes, ovulation rate was positively related to body condition at mating (Gunn & Doney, 1975). Lindsay (1976) suggested that ovulation rate in ewes is related to what is termed "net nutritional status", which is defined as the sum of nutrients available from body reserves and those absorbed daily from the digestive tract.

Mature Peppin Merino ewes have shown a curvilinear relationship between body weight and ovulation rate with a 2% increase in ovulation rate per kilogram increase in body weight between 37kg and 54kg, and a 4% increase in ovulation rate for each kilogram increase in body weight between 40kg and 48kg (Edey, 1968). In many other trials, researchers in Australia and New Zealand found that for every 1kg increase in body weight there is a linear increase in ovulation rate between 0.8 and 4% (De Haas & Dunlop, 1969; Fletcher, 1971; Killeen, 1972; Cumming, 1972a, 1977; Lindsay *et al.*, 1975; Morley *et al.*, 1978; Knight, 1979; Hinch & Roelofs, 1986). Smeaton *et al.* (1982) and Coop (1962) showed that

an increase in body weight during flushing leads to an increase in the occurrence of multiple ovulations which leads to an increase in the lambing rate. According to Coop (1962) an increase in lambing rate of 5 to 10% could be expected with a 4.5kg increase in body weight. This well established relationship between live weight immediately prior to mating and ovulation rate, of +2% for each additional kilogram, indicates the effect of the ewe's body reserve status, but the 'dynamic' live weight or 'flushing' effect has been much less repeatable and is still a contentious issue (Smith & Stewart, 1990). It follows that heavy ewes given poor feeding may still show a good ovulation rate because they have a reasonable endogenous resource of energy and protein. Ewes in a fleshy condition during breeding, have a significantly higher ovulation rate and greater follicle size, but a lower embryonic survival rate (El-Sheikh *et al.*, 1955). Higher levels of feeding after mating appear to lead to losses of ova by stimulating the metabolism of progesterone, the hormone required for the establishment and maintenance of pregnancy (Pearse *et al.*, 1994). Both severe under nourishment and over nourishment post mating may be associated with ova loss and may have more severe effects than the static intermediate level (Doney & Gunn, 1981).

It is known that there can be certain components of a ewe's nutrition which can have a marked effect on ovulation rate with little change in liveweight (Knight *et al.*, 1975; Smith *et al.*, 1979). Short term lupin feeding prior to ovulation can increase ovulation rate without a measurable change in bodyweight (Lightfood and Marshall, 1974; Knight *et al.*, 1975). A study by Pearse *et al.* (1994) reported that ovulation rate was 64% higher ($p<0.05$) in the lupin supplemented group but was not effected ($p>0.05$) by the condition score of the ewes. These results indicate that nutritional flushing may increase ovulation rate of ewes regardless of their body condition.

2.3 NUTRITIONAL FACTORS INFLUENCING REPRODUCTION

According to Davis *et al.* (1981) the mechanism by which nutrition influences ovulation rate is very complex and unclear. Some studies by Knight *et al.* (1975) suggest that protein rather than energy is involved in acute responses of ewes to nutrition. The partitioning of the different dietary components is very complex in a ruminant; Bergman (1983) found that up to 35% of the animal's requirements for glucose can be met by amino acids and that the ultimate amount of amino acids available to the animal depends on the availability of the dietary protein to escape rumen fermentation. There have been

numerous reports on the positive effect of protein rich supplements on the ovulation rate of ewes (Rhind, 1993). According to Davis *et al.* (1981) it would appear that protein and energy act through separate mechanisms to affect ovulation rate. Feeding supplements of either high protein or high energy content will bring about increases in ovulation rate (Davis *et al.*, 1981). In a report, Bearden & Fuquay (2000) summarized the most common, nutrient related reproductive disorders one may find if a female animal receiving in excess or in short of either energy or protein in its diet. A diet with excess energy can cause reproductive disorders like, low conception, increased abortion, dystocia and retained placenta while a deficiency of energy may cause delayed puberty and suppress oestrus and ovulation. On the other hand a diet with excess protein may cause low conception rate while a diet containing a deficiency in protein may lead to suppressed oestrus, low conception rate, foetal resorption, premature parturition and weak offspring. Due to these unclear findings it is more practical to use Lindsay's (1976) suggestion that ovulation rate in ewes is related to what is termed "net nutritional status", which is defined as the sum of nutrients available from body reserves and those absorbed daily from the digestive tract.

2.3.1 Partition of energy within the ewe

Ruminants derive the major portion of the energy for the performance of metabolic work, growth, repair, secretion, absorption, excretion and mechanical work from volatile fatty acids (VFA), glucose and other monosaccharides, while the dietary carbohydrates that escapes ruminal fermentation plays a secondary role. In adult ruminants only relatively small amounts of dietary carbohydrates escape rumen fermentation. Depending on the diet composition, VFA may contribute up to 80% of the total energy need of the ruminant.

It has been estimated that energy requirements for maintenance of grazing sheep are 60 to 70 percent greater than for comparable pen-fed sheep (Young & Corbett, 1972). The greater need for energy by grazing sheep results largely from the impact of environmental factors and an increased activity increment (NRC, 1985). The use of grain, fed as a supplement to breeding ewes, results in an increase in ovulation rate of up to 20% (Knight *et al.*, 1975). Other reports have not confirmed this magnitude of response and it has been suggested that the time of year may have influenced the magnitude of the response (Rizzoli *et al.*, 1976).

In the female, follicular development and ovulation are not in themselves energetically expensive. Bean & Butler (1997) studied the development of dominant follicles postpartum and found that the follicles are tolerant to periods of energy deficiency, despite a negative energy balance. Grain feeding and increased ewe weight also result in higher plasma glucose levels and greater adrenal and pituitary weight (Bellows *et al.,* 1963; Howland *et al.,* 1966; Memon *et al.,* 1969) and consequently greater total follicle stimulating hormone and luteinizing hormone potency. O'Callaghan *et al.* (2000) found that severe dietary energy restrictions can alter follicle growth characteristics in super-ovulated ewes.

The energy level in the diet has important implications for the metabolism of dietary protein. It is well known that the conversion process of rumen degradable protein into microbial protein is very dependant on the availability of fermentable metabolizable energy. If there is sufficient energy present, microbial protein is formed that is digested further along the digestive tract, if the energy level is insufficient to convert rumen degradable protein to microbial protein, surplus ammonium ions are converted into urea by the liver, and removed from the blood by the kidneys. High dietary protein, resulting in high concentrations of urea nitrogen in plasma and milk has been associated with decreased fertility in dairy cattle (Ferguson *et al.,* 1993). The above findings were confirmed in a study by Butler *et al.* (1996).

In the majority of the reports in which energy has exerted a major effect on ovulation and conception rates, the length of treatment has been about 30 days or more. Feeding a high-energy ration from day 10 of the cycle did not change ovulation rates (Bufour & Matton, 1977). Giger *et al*. (1986) showed that substitution rate, i.e. depression in roughage intake caused by soybean meal and lupin, is lower than by cereal grain. According to Molle *et al*. (1995) herbage intake increased in whole maize grain fed ewes, 3 weeks after the beginning of grain feeding. According to Molle *et al.* (1995) feeding a high-energy ration for less than one oestrous cycle does not increase ovulation rate, but when ewes are fed a high-protein supplement, ovulation rate can increase in as little as 6 days. Successful flushing in grazing sheep may, therefore, be achieved in two different ways: if the flushing supplement does not strongly depress herbage intake, flushing duration may be as short as 6 days (Lindsay, 1976); contrarily, if the flushing supplement decreases herbage intake, ewes should not be mated before herbage intake has recovered, i.e. about 3 weeks after the start of flushing.

2.3.2 Partition of protein within the ewe

Proteins are complex organic compounds of high molecular weight. Proteins are made up of amino acids, the classification of which into indispensable and dispensable. Amino acids are produced when proteins are hydrolyzed by enzymes, acids or alkalis. Certain amino acids can be produced from others by a process known as transamination, but the carbon skeletons of a number of amino acids cannot be synthesized in the animal body and these are referred to as indispensable or essential amino acids. The branched-chain amino acids, Valine, Isoleucine and Leucine forms part of this indispensable amino acid group. However, in the case of the ruminant, all the indispensable amino acids can be synthesized by the rumen microorganisms. In the ruminant, dietary protein can be classified as either rumen-degradable protein (RDP) or as rumen-undegradable protein (UDP). Rumen-undegradable protein passes through the rumen undegraded and is digested in the small intestine into amino acids that is absorbed by the animal. On the other hand rumen-degradable protein is degraded in the rumen and is transformed to either protozoal or microbial protein (MP). This protozoal and microbial protein enters the small intestine and is digested to amino acids that are absorbed by the animal.

When high-bypass protein sources are fed, supplementation with non protein nitrogen (NPN) will be needed to maintain adequate ruminal ammonia levels for microbial protein (MP) synthesis. According to studies by Young *et al*. (1981) and Owens & Bergen, 1983 the increased supply of by-pass dietary protein does not always increase production. This is due to the fact that by-pass protein may be poorly digested post-ruminally, which may lead to a poor balance of amino acids available for absorption from the small intestine. Conversely, if MP is the only protein reaching the small intestine, animal production may not be maximal (Satter *et al*., 1977). However, maximum rates of growth and milk production cannot be achieved without a balanced mixture of MP and complementary dietary amino acids in a suitable form. Most of the nitrogen required by the animal is used for protein synthesis. The crude protein (CP) content of a feedstuff is calculated from its nitrogen content times a factor of 6.25. This CP content is a measure of the nitrogen present in the foodstuff, but gives little indication of its value to the animal. Before the food becomes available to the animal it must undergo digestion, during which it is broken down to simpler substances which are absorbed into the body.

The quality of the protein in a feed is dependent on the amino acid profile as well as the digestibility. On the other hand the protein requirements of a ruminant are dependent on its physiological status and production level. In the ruminant, rumen microbes are the main source of amino acids; ruminants also have the unique ability to minimize protein loss by recycling urea that is normally excreted.

Work by Fletcher (1981) and Davis *et al.* (1981) has indicated significant effects of protein on ovulation rate of ewes. Fletcher (1981) found a response to increased protein only at low energy levels (4 MJ ME/ewe per day). In contrast, Davis *et al*. (1981) found a response to protein at moderate energy levels (11.1 MJ ME/ewe per day) but not at low levels (6.25 MJ ME/ewe per day). Both workers reported a response to increased energy at a constant level of protein intake but each worker found a response at different absolute levels of protein intake.

High protein supplements fed to ewes for 32 days increased ovulation rates above those of ewes receiving less protein, without increasing live weight above that of control ewes (Davis *et al.*, 1981). When the interaction between age and level of protein feeding is considered, it would appear that maiden ewes are more sensitive to inadequate protein feeding and embryonic mortality is greater than for mature ewes (Van der Westhuysen, 1971). This agrees with the finding of Bennett *et al*. (1964) that undernutrition significantly reduced the lambing percentage of 2-year-old primiparous ewes while mature ewes were not affected.

Smith *et al.* (1981) conducted a series of three trials over a two-year period in an attempt to define the effects of protein and energy on ovulation rate. In the first trial, ewes were fed for a period of 19 days at an energy level of 11 MJ ME/kg DM, with different protein levels namely 12, 15, 18, and 22% CP (maize gluten was used as the protein source). The results indicated that as the daily energy intake (DEI) increased the percentage of ewes that had multiple ovulations increased at about 1.5% for each 1 MJ increase. With both groups having the same energy intake the response to protein was discontinuous with the percentage of ewes multiple ovulating 20% on average more for groups where the daily protein intake (DPI) was >125g/ewe per day than for groups whose DPI was < 125g/ewe per day. Trial 2 confirmed the results of trial 1, since an increase in protein intake (from a DPI of 91 to 144) at a similar level of energy (DEI 13MJ) increased the percentage of ewes with multiple ovulations by 20%. Live weight gains with increased energy intake were regulated by the protein content of the

diet in trial 1 and by the level of protein intake in trial 2. This indicates the need for minimum protein intake to enable the full expression of the live weight response to protein intake. The minimum protein intake requirements are needed for effective rumen function and this, combined with the interconversion of energy by the rumen, indicates the complex nature of relating dietary differences to physiological responses. The threshold level of DPI in these trials of approximately 125 g/day is closer to the levels reported by Davis *et al*. (1981) and almost double of that reported by Fletcher (1981). While no clear explanation for these discrepancies was provided, the possibility of differences in the rate of ruminal degradation of protein sources in the different diets must be considered and further explored (Smith *et al.,* 1981). The form of the feedstuff and the frequency of feeding may also influence the response. The daily feeding of fine particulate material in the case of the pelleted diets should result in a more rapid passage through the rumen and thus possibly reduce the extent of protein degradation compared to the more frequent intake of coarser particles in the pasture grazing situation (Beever *et al.,* 1981).

It has been suggested (Knight *et al.,* 1975) that the benefits obtained from lupin and soybean feeding may be due to their lower levels of ruminal degradation (Hume, 1974). This is supported by the failure of increased dietary nitrogen, in the form of urea, to increase ovulation rates (Thompson *et al*., 1973). However, attempts to alter the supply of undegraded protein post-ruminally by formaldehyde treatment of casein were not fully successful in increasing the ovulation rate of Border Leicester and Dorset Horn ewes (Corbett & Edey, 1977). According to Davis *et al.* (1981) protecting casein with formaldehyde against degradation in the rumen, improved casein as a supplement, and resulted in increasing ovulation rate in the ewe. This confirms the results of other studies comparing ovulation rates in ewes fed either protected or unprotected casein (Braden & Mattner, 1970; Corbett & Edey, 1977) in which small, non-lupines and soybeans are protected in part from degradation. However the increases obtained in ovulation rate are so small, it is unlikely that protected casein will have a commercial role in increasing ovulation rates (Davis *et al.,* 1981).

The duration of the period of feeding prior to measurement of response could also be most critical. This has been partially confirmed by Radford *et al*. 1980, Fletcher (1981) and Davis *et al*. (1981), all obtaining responses within one cycle (17 days). Providing excess dietary crude protein (CP) during the 5-8 days before anticipated estrus (i.e. beginning of the mid-luteal phase) increased the ovulation rate

(Smith & Stewart, 1990; Smith, 1998). Increased levels of protein in the diet have, in conjunction with increasing ovulation rates, increased the circulating levels of FSH during the latter half of the estrus cycle (Davis *et al.*, 1981; Knight *et al.*, 1981). Haresign (1981a) suggested that flushing for one cycle may extend its effects by preventing the late atresia of follicles and Lishman *et al*. (1974) reported a greater ovarian follicular response to gonadotrophin in ewes that were on a high plane of nutrition. A pattern of late ovulation suggests higher recruitment of new follicles for ovulation (rather than prevention of atresia of follicles recruited earlier) in ewes fed a maize-gluten meal and a ground-maize grain mixture, as compared with ewes fed soybean meal or maize gluten. According to Davis *et al.* (1981) similar to the reports on lupin grain, other protein supplements did increase ovulation rates of ewes, when fed at supplementation levels sufficient to maintain live weight. In fact, lupin grain had no advantage over field peas, soybeans or protected casein as a means of increasing ovulation rates in ewes, when fed at iso-protein levels. A deficiency in protein might be responsible for low conception, low lambing and low twinning rates in ewes (Salman, 1996; Treacher *et al.*, 1996).

Molle *et al*. (1995) showed that flushing Sarda ewes with soybean meal, while mated on mature grassland, was found to be effective in improving reproductive performance. In particular, ovulation rate increased by 0.40 per ewe ovulating and prolificacy tended to be higher by 0.30 lambs per ewe lambing in flushed ewes, compared with the controls. This study confirms that the advantage of long-term flushing (14 days pre to 21 days post ovulation) in terms of ovulation rate may be partially lost because of the high ova (or embryo) losses. Medium-term flushing (14 days pre to 2 days post ovulation) offers the advantage of higher ovulation rates, (+0.51 corpura lutea per ewe, on average), without having the disadvantage of high ova losses. This may result in a trend for higher prolificacy (+0.38 lambs per ewe lambing), compared with controls. The mechanism through which soybean flushing operates is probably mediated by a short-timed chance on metabolism linked to a corresponding chance in nutrient intake. An alternative is the direct action of branched chain amino acids on the ovary, since protein allowance in excess enhances circulating levels of these acids concomitantly to increase ovulation rate (Downing *et al*., 1995).

Providing excess dietary CP during 5 to 8 days before anticipated estrus (beginning of the mid-luteal phase) increased the ovulation rate (Smith, 1988; Smith and Stewart, 1990). This increase was correlated positively with a change in plasma concentrations of branched-chain amino acids (Waghorn

et al., 1990; Downing *et al.*, 1995a). When ewes are fed excess rumen-degradable protein during the pre-ovulatory period, fluids flushed from the reproductive tract are rich in ammonia and urea. Ewes fed high quantities of urea during the pre-ovulatory period had elevated plasma concentrations of urea and glucose, which were associated with more advanced development of fertilized sheep ova (Mandibela *et al.*, 1995). Ova collected from such ewes metabolize more glucose and are more advanced in their development, but their viability is decreased, compared with ova from ewes fed at maintenance level without excess rumen degradable protein (Mandibela *et al*., 1995). The main source of amino acids in ewes fed rumen degradable protein is microbial protein and is relatively poor in branched chain amino acids (Merchen & Titgemeyer, 1992). High rumen degradable protein has long been suspected to have a deleterious effect on fertility in dairy cows (Ferguson *et al.*, 1986a, 1986b). These high concentrations of urea might be an explanation for the earlier occurrence of ovulation and the larger size of follicles in soybean meal fed sheep. This phenomenon is positive for the enhanced development of the ova, but is highly negative, due to their lower viability, on the number of lambs born.

It is possible to increase the duodenal flow, and hence the absorption of branched chain amino acids, by using feedstuffs rich in branched chain amino acids and of low ruminal degradability, such as maize-gluten meal (Tagari *et al.*, 1995). There are two possible mechanisms by which branched chain amino acids could increase the ovulation rate. First, on the basis of the data from Downing *et al*, (1995), direct effects of changes in plasma concentrations of branched chain amino acids on ovarian function cannot be excluded. Second, insulin may be involved since leucine is the most effective amino acid in stimulating insulin secretion in sheep (Kuhara *et al.*, 1991). Although soybean meal and the maize-gluten meal ground maize grain mixture diets supplied similar amounts of excess protein, feeding ewes with maize gluten meal ground maize grain mixture that was rich in branched-chain amino acids and poorly degraded in the rumen, resulted in an increase in serum concentrations of insulin at estrus, which was not seen in ewes fed with soybean meal (Landau *et al*., 1996).

Ocak *et al.* (2006) demonstrate in their trial that short-term (15-17 days) changes in protein supplementation can have a beneficial effect on non return rate and lambing rate to first estrus and litter size in ewes grazed on rangeland. An increase in non return rate, lambing rate and the mean litter size of 19%, 22% and 0.21 points, respectively, in ewes fed high protein supplemented diets compared to

those fed low protein supplemented diets for a period of 15 days immediately after mating. These may be the result of the beneficial effects of a high protein diet on the non return rate which indicates that embryonic losses are diminished by a high protein diet during the post mating period.

When ewes were fed with soybean meal (twice the protein requirement for maintenance based on NRC, 1985) during an estrous cycle and for the first 5 days after breeding, Berardinelli *et al.* (2001) showed that the transport of ova through the oviduct to the uterus was faster, which may adversely affect development and survival of fertilized ova. Indeed, ewes fed high protein diets during pre- and post-mating returned more frequently to a second estrus than ewes fed a low protein diet during pre-mating. Previous studies reported that high protein supplementation, given in the form of lupin (Downing *et al.*, 1995; Nottle *et al.*, 1997a), soybean meal (Molle *et al.*, 1995, 1997; Branca *et al.*, 2000) and groundnut seed cake (El-Hag *et al.*, 1998) particularly in the pre-mating period, induced an increase in reproductive performance. The effect of supplementary feeding on reproductive performance (Rhind *et al.*, 1989; O'Callaghan and Boland, 1999; Branca *et al.*, 2000) may be the direct or indirect effect through a change in digestibility of poor rangeland, and thus an enhancement in the intake under grazing conditions (Molle *et al.*, 1997; Santra *et al.*, 2002; Chaturvedi *et al.*, 2003).

A study by Ocak *et al.* (2006) showed that a short-term (2 weeks) increase in level of protein supplementation during post-mating period improved reproductive performance in ewes maintained on rangeland. These results suggest that high protein supplementation around the time of mating, especially post mating, and to grazing ewes in autumn can be a practiced way to improve the reproductive performance of ewes when rangeland quality decreases, even after mating.

CHAPTER 3

3.1 MATERIALS AND METHODS

3.1.1 Experimental facilities and duration of experiment

Two trials were carried out at the research farm of the University of Pretoria in Hatfield, (latitude 25º:43' S, longitude 28º: 11' E and ±1500m above sea level) Gauteng in a semi-intensive production system. The first trial (Season 1) started on 3 May 2001, continued for 45 days and ended on 22 June 2001; lambing performance was recorded in spring. The second trial (Season 2) started on 23 November 2001, also continued for 45 days and ended on 11 January 2002, while lambing performance was recorded in autumn.

3.1.2 Experimental animals

In each trial (season 1 and season 2) seventy-two Dohne-Merino ewes (age between 13-85 months) were used. All the experimental animals were weight before the onset of each trial and again on day 44 of the particular trial. In both trials, the 72 ewes were sorted and allocated to 4 different dietary supplementation groups. The 72 ewes were randomly allotted to the four dietary supplementation groups according to their age and fecundity (average body weight per group, 51.58 ± 1.5kg). During the course of each of the two trials the ewes grazed together as a group (n=72) from 08h00 to 16h00. After 16h00 all the ewes were penned separately in the 4 different dietary supplementation groups.

3.1.3 Experimental design

In the procedures described below, all the ewes (n=72) of the four dietary supplementation groups (4 x 18) had to be further subdivided into six groups of twelve ewes each (6 laparoscopy groups). This subdividing was necessary to ensure that there was enough time daily, for each of the twelve ewes in the laparoscopy group, to be subjected to the laparoscopic procedure. In other words, each of the six laparoscopy groups consisted out of twelve ewes or three ewes per dietary supplementation group (6x3x4). The trial for laparoscopy group 1 started on day 1, for laparoscopy group 2 started on day 3,

laparoscopy group 3 started on day 4, laparoscopy group 4 started on 5, laparoscopy group 5 on day 6 and laparoscopy group 6 on day 7. The length of the trial for each group was 45 days, in other words the trial for laparoscopy group 1 started on day 1 and ended on day 45 and that for laparoscopy group 6 started on day 7 and ended on day 51.

The following procedure was followed for each of the 6 laparoscopy groups. On day one progestagen containing intra-vaginal sponges (Chrono-gest grey sponges from Intervet each containing 40mg Fluorogestone acetate) were inserted into the first of six laparoscopy group. On day 12 each ewe were injected with 1.0ml prostaglandin $F_2\alpha$ (Prosolvin from Intervet each milliliter containing 7.5mg Luprostiol). The sponges were removed on day 14 and oestrus was detected with the aid of six vasectomized rams form day 16 to day 18. The six vasectomized rams were introduced to the whole laparoscopy group of 12 ewes, and every ewe that stood twice for mating were identified as cyclic. This practice continued for a period of 30 minutes in the morning and repeated for another 30 minutes in the afternoon up to day 18. From day 18 to 39 of the trial the vasectomized rams grazed with the group of ewes during the day to eliminate the ram effect.

On day 23 each ewe of laparoscopy group 1 was again inserted with a Chrono-gest (40mg Fluorogestone acetate) intra-vaginal sponge which was removed on day 37. The dietary supplementation commenced on day 31 of the trial and continued for 9 days to day 39. The first ewe came into estrus on day 39 and each ewe was mated twice by means of hand mating with two different fertile tested Dohné-merino rams. Each ewe was examined by laparoscopy on day 45 to count the number and measure the diameter of each of the corpora lutea on the ovaries. On day 90, pregnancy of the ewes and the number of lambs was evaluated and confirmed by means of ultrasonography by using an abdominal probe. Finally the number of lambs born per ewe was recorded at birth as well as the bodyweight of the born lambs and the ewes.

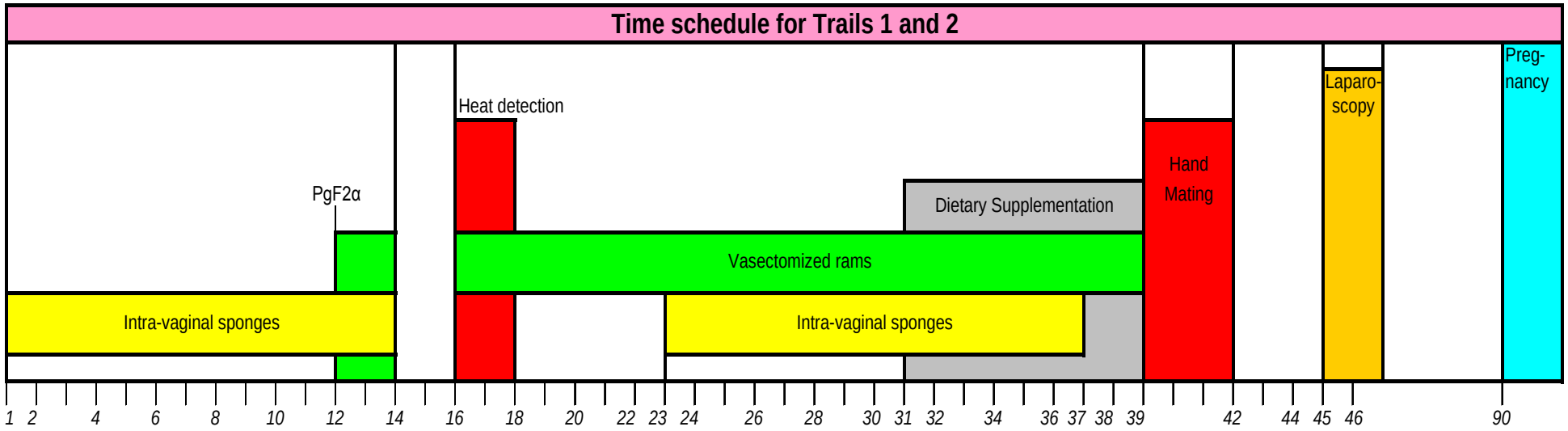

Fig 2. Timeline of the experiments conducted in trial 1 and 2.

3.1.4 Nutritional management

The ewes in both trials in season 1 and season 2 got all their nutrients from the pasture, *Festuca arundinaceae*, and from the dietary supplement. Firstly the CP and DCP contribution of the *Festuca arundinaceae* had to be determined, and then subtracted from the chosen daily allowance in order to get the amount of CP and DCP to be supplemented by the four different dietary supplements. In season 1, the pasture supplied 216.39g crude protein and 166.53g digestible crude protein on a daily DM intake of 1112.88g per ewe. The chosen CP intake per ewe should be approximately 256.4g per day both in season 1 and season 2 while the DCP intake per ewe per day both for season 1 and season 2 should be approximately 190g which is 2 times more than the threshold value of 125g/day for effective rumen functioning according to Waghorn *et al.* (1990).

In both trials *Festuca arundinaceae* (tall fescue) was used as pasture. All the ewes were allowed to graze in a flock from 08h00 to 16h00 every day for the length of the trial. Four plots of 0.5ha each were used in a rotation grazing system. The same plot was grazed for two days every ninth and tenth day. Water was only available from 17h00 up to 08h00 every morning in the different pens. The four different dietary supplements were fed twice a day, two thirds of the daily allowance in the afternoon and one third in the morning. To minimize possible competition between individual ewes, the supplements were evenly spread in troughs, allowing at least 9 cm of eating space per individual ewe.

3.1.4.1 Daily crude protein intake from *Festuca arundinaceae*

Twenty one days before the start of each trial six whethers (average body weight 50 ±3kg) were fitted with harnesses to which faecal collection bags were attached. Over a seven day period the faeces were collected and weighed twice per day in the morning and again in the afternoon to minimize any waste. For each animal the two weights were added to get the total feacal excretion for the day. At the end of the seven day collection period the average feacal excretion per animal per day was calculated. After each collection a representative sample of feaces, from each animal was collected and separately stored frozen prior to bulking for DM analysis.

A random sample of each of the six bulked frozen faecal samples were oven dried, for 24h at 100ºC to constant mass, to determine the dry matter (DM) content of each of the six samples. This percentage DM was used to determine the average daily feacal DM excretion per animal.

To determine the organic matter (OM) content for the feaces of each of the six faecal samples, a duplicate of 1g ± 0.004g of each of the partially dried samples were dried at 60ºC after which each were ashed in porcelain crucibles at 600ºC in a muffle furnace for four hours. This above mentioned organic matter (OM) content is used to calculate the percentage OM in the feaces of each of the six animals.

To determine the *in vivo* digestibility of the pasture one first has to calculate the *in vitro* digestible OM (IVDOM). The *in vitro* technique as described by Tilley and Terry (1963), and modified by Engels & Van der Merwe (1967) was used to determine IVDOM. Three esophageal fistulated whethers were used twice daily (in the morning and in the afternoon) for three days to collect to samples of the *Festuca arundinaceae.* The two samples per day for each animal were added together to form one sample, in other words three samples per animal were collected over the three day period. After each collection the three samples were frozen.

To determine the organic matter (OM) content of each of nine esophageal samples, a duplicate of 1g ± 0.008g of each of the partially dried samples were dried at 60ºC after which each was ashed in porcelain crucibles at 600ºC in a muffle furnace for four hours. The organic matter (OM) content of each of the nine samples for the determination of *in vitro* digestibility were calculated as one hundred minus the percentage ash of each oesophageal sample. For each of the nine samples a 0.2g sample (in triplicate) were incubated in a test tube with rumen fluid, artificial saliva and a urea solution for 48 hours at 39ºC in a water bath. The rumen fluid was obtained from rumen fistulated whethers that was fed on high quality lucerne hay. HCl was added to lower the pH and pepsin was added after which the tubes were again incubated for 48 hours. Afterwards the content were filtered and dried at 100ºC, the residues weighed and ashed at 550ºC for three hours. The digestibility of the organic matter (IVDOM) was calculated as hundred minus the indigestible residue divided by the sample mass times one hundred.

To determine the average daily dry matter intake (DMI/d) per ewe of the *Festuca arundinaceae* the organic matter (OM) content of the collected faeces was calculated and divided by one minus the *in vivo* digestibility of the *Festuca arundinaceae* (AOAC, 1995).

The *in vivo* digestibility of the *Festuca arundinaceae* was determined by means of a regression equation proposed by Engles *et al.* (1981) using the formula:

In vivo digestibility = -11.9725 + (1.1483 x *In vitro* digestibility)

Therefore the average daily intake for each of the six animals were calculated as the average daily organic matter (OM) excretion of the faeces divided by one minus the average *in vivo* digestibility of the nine samples done in triplicate.

The average daily intake over the group of six animals was used as the average daily intake (g/day) per ewe in the trial in season 1 as well as in the trial in season 2.

Table 3.4.1.1 Average daily intake per animal of *Festuca arundinaceae*

	Average daily feacal dry matter excretion (g/animal/day)	Average faecal organic matter content (g/animal/day)	In vitro digestible organic matter	In vivo digestibility	Average daily dry matter intake (g/animal/day)
Season 1	398.36	319.13	72.54	71.32	1112.88
Season 2	416.14	318.90	73.12	71.99	1138.50

According to the above Table 3.4.1.1 the average daily dry matter (DM) intake per ewe grazing *Festuca arundinaceae* from 08h00 to 16h00 was 1112.88g per day in the trial in season 1 and 1138.50g per day in the trial in season 2.

In order to determine the nitrogen content of *Festuca arundinaceae* the nine, above mentioned, oesophageal collected samples were used. The nitrogen content of each sample (in duplicate) was determined by the macro Kjeldahl method according to AOAC (1995) using a block digester and distilling with a Tecator Kjeltec System Model 1002. The average nitrogen content of the 18 samples was used as the nitrogen content, on a wet basis, of the pasture. The percentage crude protein of the pasture was calculated as the average total nitrogen times 6.25.

The percentage crude protein on a DM basis, in grams per kilogram, was used to determine the daily crude protein intake. The daily crude protein intake was calculated as the average daily intake, referred to above, multiplied by the crude protein content of *Festuca arundinaceae.*

In determining the amount of digestible crude protein intake per ewe per day, the daily crude protein intake was converted into daily digestible crude protein intake. This was done using a formula of digestible crude protein divided by crude protein equals a factor, the values of the digestible crude protein and crude protein for *Festuca arundinaceae* were obtained from the NRC (1985). The values used were 147g/kg for digestible crude protein and 191g/kg for the crude protein content. The value of the factor used was 0.7696. Digestible crude protein intake per ewe per day was calculated as the daily crude protein intake multiplied by the above mentioned factor.

Table 3.4.1.2 Daily digestible crude protein intake, per animal, of *Festuca arundinaceae*

	Pasture (DM basis) %N	%CP	Daily dry matter intake (g/day)	Daily crude protein intake (g/day)	Daily digestible crude protein intake (g/day)
Season 1	3.11	19.44	1112.88	216.39	166.53
Season 2	3.08	19.23	1138.50	218.93	168.49

According to Table 3.4.1.2 the intake per ewe of CP from grazing the *Festuca arundinaceae* were 216.39g per day in the trial in season 1 and 218.93g per day in the trial in season 2. In order for the ewe to reach a intake of 256.4g per day of crude protein, CP had to be supplemented through dietary supplementation on a daily basis. In season 1 the shortfall of CP grazed per day was 40.00g and in season 2 it was 37.45g. The digestible crude protein intake per ewe per day in the trial in season 1 was 166.53g and in the trial in season 2 it was 168.49g. In order to supply the needed 190g of digestible crude protein per ewe per day it was necessary to supply 23.47g of digestible crude protein through the dietary supplements in the trial in season 1. For season 2 the supplementation of digestible crude protein was 21.51g through the dietary supplements.

3.1.4.2 Daily crude protein intake from the different dietary supplements

All the raw materials for the dietary supplements were purchased in bulk in enough quantities for both trials. The half of the raw materials not used in the first trial, in season 1, was stored in a cool place to prevent nutrient losses. The materials that were bought for the dietary supplementation trials in season 1 and season 2 included: yellow maize, molasses syrup, urea, sunflower oilcake meal, cottonseed oilcake meal and fishmeal.

The four different dietary supplements were freshly mixed twice daily; two thirds of the daily allowance was given in the afternoon and the other third in the morning. Because of its high palatability, yellow maize was used as the basis for each of the four dietary supplements in both the trials. The same amount of yellow maize (80g per day per ewe) was used in each of the four dietary supplements in both trials in season 1 and 2. The maize was fed in a whole grain form in order to increase intake. Furthermore, exactly the same amount of molasses syrup was used in each of the four dietary supplements, and in both trials in season 1 and 2. Molasses syrup was used as part of the dietary supplement mainly to increase the palatability and intake but also to bind the ingredients in the supplement together to minimize waste. Due to the handling difficulty of molasses syrup, this was diluted with lukewarm water (5:2 molasses/water) to make the mixing process easier and to evenly spread the urea though the mixture. Firstly the urea was diluted in lukewarm water, then the lukewarm water urea mix was mixed with the molasses syrup and then the molasses syrup mix was mixed with the maize and protein source.

Nitrogen sources differ considerately in their quality to the animal for instance there are differences in the crude protein content, the degradability, the digestibility and the amino acid profiles. To test for the different influences nitrogen sources have on production or in this study ovulation and conception rates it was essential to keep some of the variables constant to compare the influence of the quality of a specific nitrogen source on reproduction. In this study it was decided to keep the total daily crude protein intake (256.4g) and the daily digestible crude protein intake (190g) the same in the two seasons (trials). The CP content is merely an indication of the amount of nitrogen in a specific sample but gives no indication of the quality to the animal. Within each season the daily CP content of the four different dietary supplements were kept the same, this was done by

introducing urea as part of each of the four dietary supplements in order to achieve iso-nitogen levels between the four different dietary supplements. Another reason for introducing urea into all four dietary supplements was to ensure that there is enough NPN in the rumen available for optimal microbial functioning. In other words all animals in season one and season two received the same amount of nitrogen (CP) on a daily basis for the length of both trials.

Dietary supplement one consisted only out of yellow maize, molasses syrup and urea. This dietary supplement was chosen to ensure that in one supplement almost all the amino acids (dispensable and indispensable), available to the animal, originated from microbial or protozoal protein. The other three dietary supplements were chosen in such a way that the digestible crude protein content of all three dietary supplements were the same; however there was a difference in the amino acid composition, and therefore a difference in biological value to the animals between the three different dietary supplements. Dietary supplement two contained yellow maize, molasses syrup, urea (for iso-nitrogen levels) and sunflower oilcake meal. The third dietary supplement contained yellow maize, molasses syrup, urea (for iso-nitrogen levels) and cottonseed oilcake meal, while yellow maize, molasses syrup, urea (for iso-nitrogen levels) and a mixture of cottonseed oilcake and fishmeal made up the fourth dietary supplement (Table 3.4.3).

Table 3.4.2 Contents of the different dietary supplements used in trials in season1 and season 2.

Dietary supplement 1	Dietary supplement 2	Dietary supplement 3	Dietary supplement 4
Yellow maize Molasses syrup Urea	Yellow maize Molasses syrup Urea Sunflower oilcake meal	Yellow maize Molasses syrup Urea Cottonseed oilcake meal	Yellow maize Molasses syrup Urea Cottonseed oilcake meal Fishmeal

In dietary supplement two sunflower oilcake meal was used, as shown in table 3.4.3 below this nitrogen source contained only four indispensable amino acids, no BCAA and only one dispensable amino acid and all of them in very small amounts. In dietary supplement three cottonseed oilcake meal was used as the nitrogen source which contained all the indispensable and dispensable amino acids. The levels of certain amino acids like arginine, lysine, methionine, threonine and glycine is

almost the same in sunflower oilcake meal and in cottonseed oilcake meal. In the fourth dietary supplement a combination of cottonseed oilcake meal and fishmeal was used as the main nitrogen source. From table 3.4.3 it is clear that fishmeal is very rich in all the amino acids indispensable as well as dispensable.

Table 3.4.3 Amino acid composition of feeds (Feeds & Nutrition Digest, 1990)

Amino acids		Sources:		Yellow maize	Sunflower oilcake meal	Cottonseed oilcake meal	Fish meal
		DM	%	87.00	91.00	93.00	92.00
		CP	%	10.23	46.15	44.09	69.89
Indispensable amino acids		Arginine	%	0.52	5.98	4.52	34.00
		Histidine	%	0.23		1.15	1.59
		Lysine	%	0.22	1.44	1.72	5.72
		Methionine	%	0.13	0.78	0.61	1.77
		Phenyl-alanine	%	0.52		2.35	3.04
		Threonine	%	0.40	0.89	1.43	2.82
		Tryptophan	%	0.10		0.56	0.82
	BCAA	Iso-Leucine	%	0.46		1.53	3.55
	BCAA	Leucine	%	1.15		2.47	5.33
	BCAA	Valine	%	0.40		2.03	3.41
Dispensable amino acids		Cystine	%	0.13		0.76	0.67
		Glycine	%	0.52	2.77	2.01	4.34
		Serine	%	0.00		1.83	2.63
		Tyrosine	%	0.49		1.04	1.95

To summarize, in dietary supplement one only a very small amount of amino acids were supplied by the yellow maize, all the other amino acids originated from protozoal or microbial protein. In dietary supplement two a small amount of amino acids were available from yellow maize plus five extra amino acids were supplied by the sunflower oilcake meal. (No BCAA were supplied). In dietary supplement three, five amino acids were supplied more or less in the same amounts as in dietary supplement two but nine other amino acids were supplied as well, under which the BCAA (Iso-leucine, leucine, valine). The fourth dietary supplement supplied all the amino acids only in larger amounts than in dietary supplement three. In all the dietary supplements protozoal and microbial protein was also supplied to the small intestine due to effective rumen functioning as a result of NPN inclusion.

A daily shortfall of 40.0g crude protein per ewe in the trial in season 1 and 37.45g crude protein in season 2 had to be supplemented by the each of the four different dietary supplements in order to reach a level of 256.4g crude protein per day. The crude protein content of yellow maize and each of the four different nitrogen sources had to be established. To determine the crude protein content of the yellow maize and that of the four different nitrogen sources, duplicate random samples of the sources were taken and subjected to the macro Kjeldahl method according to AOAC (1995). The percentage crude protein of the samples was calculated as the percentage nitrogen times 6.25. The average percentage crude protein of each duplicate was to be calculated on a DM basis for further use. The digestible crude protein content of yellow maize and each of the nitrogen sources was determined by the crude protein content times a digestible crude protein factor that is specific for each feedstuff. To calculate the above mentioned digestible crude protein factor the formula was used of digestible crude protein divided by crude protein equals this factor, the values of the digestible crude protein and crude protein for yellow maize, sunflower oilcake meal, cottonseed oilcake meal and fishmeal were obtained from the NRC, 1985. For example the specific value for yellow maize used was 77g/kg for digestible crude protein and 102g/kg for crude protein content. The value of the digestible crude protein factor for yellow maize used was 0.755. Digestible crude protein content for yellow maize, sunflower oilcake meal, cottonseed oilcake meal and fishmeal were calculated as the crude protein content multiplied by the above mentioned digestible crude protein factor. The same calculated digestible crude protein content values were used for both the trials in season 1 and season 2.

Table 3.4.4 Crude protein and digestible crude protein content of the maize and the four different nitrogen sources.

	Sample (dry matter basis) % crude protein	Digestible crude protein factor	Digestible crude protein content (g/kg)
Yellow maize	10.101	0.755	76.253
Urea	287.500		
Sunflower oilcake meal	41.576	0.935	388.645
Cottonseed oilcake meal	38.582	0.801	308.994
Fishmeal	70.135	0.969	679.618

Yellow maize was used as the basis of each of the four dietary supplements in both the trials in season 1 and season 2. The amount of yellow maize used in each of the four dietary supplements in both season 1 and season 2 was 80g per ewe per day (DM basis). The crude protein contribution of the 80g of yellow maize per ewe per day, were 8.081g per day. The difference between the daily required supplemented 40.00g crude protein needed and the amount of crude protein supplied by the 80g yellow maize had to come from the different nitrogen sources or combination of nitrogen sources.

In order to compare the possible effect of branched-chained amino acids on conception and ovulation rates it is of the utmost importance to keep the daily crude protein intake from the four different dietary supplements the same. To make this possible it was necessary to use urea in all four dietary supplements to ensure that every ewe got the same amount of crude protein per day.

Table 3.4.5 Composition of the four different dietary supplements in the trial in season 1.

	Season 1		
Supplemented crude protein (CP)		(g/day) 40.000	
	DM basis (g/day)	DCP (g/day)	CP (g/day)
Dietary supplement 1			
Yellow maize	80.000	6.100	8.081
Urea	11.102		31.919
		6.100	40.000
Dietary supplement 2			
Yellow maize	80.000	6.100	8.081
Sunflower oilcake meal	44.689	17.368	18.580
Urea	4.640		13.339
		23.468	40.000
Dietary supplement 3			
Yellow maize	80.000	6.100	8.081
Cottonseed oilcake meal	56.208	17.368	21.686
Urea	3.559		10.233
		23.468	40.000
Dietary supplement 4			
Yellow maize	80.000	6.100	8.081
Cottonseed oilcake meal	44.967	13.894	17.349
Fishmeal	5.111	3.474	3.585
Urea	3.821		10.985
		23.468	40.000

Table 3.4.6 Composition of the four different dietary supplements in the trial in season 2.

	Season 2		
Supplemented crude protein (CP)		(g/day) 37.450	
	DM Basis (g/day)	DCP (g/day)	CP (g/day)
Dietary supplement 1			
Yellow maize	80.000	6.100	8.081
Urea	10.220		29.369
		6.100	37.450
Dietary supplement 2			
Yellow maize	80.000	6.100	8.081
Sunflower oilcake meal	39.639	15.405	16.480
Urea	4.480		12.889
		21.506	37.450
Dietary supplement 3			
Yellow maize	80.000	6.100	8.081
Cottonseed oilcake meal	49.857	15.405	19.236
Urea	3.520		10.134
		21.506	37.450
Dietary supplement 4			
Yellow maize	80.000	6.100	8.081
Cottonseed oilcake meal	39.885	12.324	15.388
Fishmeal	4.534	3.081	3.180
Urea	3.760		10.801
		21.506	37.450

3.1.5 Animal Management

3.1.5.1 Estimation of body condition scores

Body condition scoring (BCS) is a system of describing or classifying breeding animals by differences in relation to body fatness. This is a subjective scoring system but provides a fairly reliable assessment of body composition. The optimum BCS for a ewe to be in before the rams are introduced is between 2½ and 3 (Merrell, 1990). In physiological terms, it is known that ewes in

high BCS (3.0) have larger, estrogenic, ovarian follicles than ewes in low BCS (2.0) (Rhind *et al*., 1989). Before the onset of both the trials every one of the seventy two ewes was condition scored. Condition is assessed by handling the ewe over and around the backbone, in the loin area immediately behind the last rib and above the kidney, using fingers along the top of the backbone and also feel the transverse process in the sheep's loin area. With this scoring system, condition scores range on a scale from 1 (thin) to 5 (very fat) (Church, 1984). The characteristics of a condition score of 2 are that the ewes have a slight amount of fatty tissue detectable between skin and bone. Spinous processes are relatively prominent and rounded and these ewes appear thrifty but have only minimal fat reserves. The body condition of all the ewes in each trial varied from 2 to 4 which indicates the all the ewes were in mint body condition and that there were no extremes.

3.1.5.2 Oestrus Synchronization

On day one the first laparoscopy group of twelve ewes was inserted with a progestagen containing intra-vaginal sponges (Chrono-gest grey sponges from Intervet each containing 40mg Fluorogestone acetate). On day 12 each ewe were injected with 1.0ml prostaglandin $F_2\alpha$ (Prosolvin from Intervet each milliliter containing 7.5mg Luprostiol). On day fourteen the sponges were removed and two days later all the ewes were checked for cyclic activity with the aid of six vasectomized rams. The six vasectomized rams were introduced to the whole laparoscopy group of 12 ewes, and every ewe that stood twice for mating were identified as cyclic. This practice continued for a period of 30 minutes in the morning and repeated for another 30 minutes in the afternoon up to day 18. All the inserted ewes responded and came into estrus between days sixteen and eighteen. From day 18 to 39 of the trial the vasectomized rams grazed with the group of ewes during the day to eliminate the ram effect. The whole insertion process described above was repeated from day twenty three (for the first laparoscopy group) up to day twenty nine (for the sixth laparoscopy group). The first ewe of laparoscopy group 1 came into estrus on day 39 and each ewe was mated twice by means of hand mating with two different fertile tested Dohné-merino rams.

3.1.5.3 General clinical examination

Before the onset of each trial, each one of the seventy-two ewes was clinically examined for physical health. Every ewe was individually marked with a plastic ear tag. The four different ear tag collars were used to identify the different dietary supplementation groups. The ear tags were placed in three different positions in the ear to identify the different laparoscopy groups. All the ewes were divided into the different dietary supplementation groups through a crush.

3.1.5.4 Laparoscopy technique

Laparoscopy is a quick and safe technique that can be used for diagnosing the reproductive status of a ewe. In both these trials this technique was used to determine the ovarian status and ovulation rate on day forty-five. The number of visible corpora lutea on each ovary were counted and measured.

Briefly, the laparoscopic technique entailed depriving the ewes of food and water for a period of 12h prior to examination. Every ewe was secured in a cradle, then the wool from around the udder was shaven and the area sterilized with an antiseptic. Local anesthetic was administered at the sites for trocar penetration. A 7 millimeter cannula was connected via a reducing valve to a CO_2 cylinder (to inflate the peritoneal cavity) and a telescope (6.5 millimeters in diameter and 350 millimeter in length) inserted in this cannula. The light source used was a 150 watt halogen bulb generator with a single outlet. The light source and the telescope were connected via a glass fiber cable (180 centimeter in length and 3.5 millimeters in diameter). The 5 millimeters cannula was inserted to the right of the mid-line, after inflation of the cavity. With the aids of forceps through the 5 millimeters cannula and the telescope, the ovaries were located and inspected. Care was taken throughout the procedure to work as sterile as possible and the wounds were treated following laparoscopy. The trocars and cannulae were soaked in a solution of 70% ethanol with a 5% Savlon concentrate added. The telescope and palpation probe stood in a plastic measuring cylinder, three quarters filled with 70% ethanol. The number of new corpora lutea on the ovaries served as an indicator of the number of ovulations (Evans & Maxwell, 1987). The sites for trocar

penetration were sewed up and the ewes were allowed to recover indoors for approximately 2 hours before allowing them same feed and water.

3.1.5.5 Ultrasound technique

Transabdominal ultrasonography utilized a real time, B-mode scanner (Aloka 500) and a 7.5 MHz stiffened transducer. The ultrasound technique was used for pregnancy detection and the number of fetuses or lambing status on day 90 of each trial.

3.1.6 Parameters measured

Before the onset of each trial and again at the end of it the bodyweight of each ewe was taken. From day thirty-nine up to day forty-four, of each trial, all the cyclic ewes were mated twice by two different rams. Between days forty-five and fifty-one the number of follicles and corpora lutea on each ovary in each ewe were counted by means of laparoscopy. On day ninety the number of fetuses per ewe was counted by means of ultrasonography. Pregnancy duration, lambing status, gender, lamb birth mass and ewe mass at birth were observed at birth of the lamb.

3.1.7 Laboratory methods

Dry matter content

Samples were dried in a force draught oven in aluminium foil containers for 24 hours at 100ºC to constant mass. The aluminium containers were weight directly from the oven. The dry matter content (%) was determined according to A.O.A.C (1995).

Ash and organic matter (OM) content partially dried samples (dried at 60ºC) were ashed in porcelain crucibles at 600ºC in a muffle furnace for four hours, cooled in a desicator and weighed.

% Ash = (Ash mass (g) /Sample mass (g)) x 100

Organic matter (OM) content of samples for determination of in vitro digestibility of OM was calculated as follows:

%OM = (Dry matter (g) – Ash mass (g)) / (Sample mass (g)) x 100

The OM content of the other samples was calculated as follows:
%OM = 100 - % Ash (expressed on a DM Basis)

The in vitro technique as described by Tilley and Terry (1963), and modified by Engels and Van Der Merwe (1967) was used to determine IVDOM. A 0.2g sample (in duplicate) was incubated in a test tube with rumen fluid (obtained from a donor sheep fed on high quality lucerne hay), artificial saliva and a urea solution for 48 hours at 39ºC in a water bath. Samples were continually shaken. After this period HCl (1:4) was used to lower the pH to about 2,0; 3ml pepsin solution was added and the test tubes incubated for another 48 hours. The contents were then filtered through Gooch crucibles using a vacuum pump and dried for 24 hours at 100ºC. The residues were weighed and ashed at 550ºC for three hours and weighed again. The digestibility (D) of organic matter (IVDOM) was calculated as follows:
D = (100 – (Undigested residue (g) / sample mass (g)) x 100
All values are expressed in terms of OM content.
In vitro values were converted to in vivo values as proposed by Engles *et al.* (1981) using the regression equation for samples collected by esophageal fistulated sheep and freeze-dried:
In vivo digestibility of OM = -11.9725 + (1.1483 x IVDOM)

Nitrogen
The nitrogen (N) content of samples was determined by the macro Kjeldahl method according to AOAC (1995) using a block digester and distilling with a Tecator Kjeltec System Model 1002. Percentage crude protein (CP) was calculated as follows:
% CP = % N x 6.25

3.1.8 Statistical Analysis

Analysis of variance (ANOVA) with the GLM model (Statistical Analysis System, 2007) was used to determine the significance (1% and 5%) of supplement effects, birth status, mating season, age and change in liveweight on the number of ovulation points, the number of foetuses and the number of lambs born. Least square means and standard deviations (SD) were calculated. The ANOVA

procedure requires interval or ratio data and the assumption that the k populations are normally distributed.

The nonparametric Kruskal-Wallis test was used to analyze ordinal data as well as with interval or ratio data. The Kruskal-Wallis test is based on the analysis of the independent random samples from each of the k populations, and it does not require normal distribution of populations. Therefore whenever the data from $k \geq 3$ populations are ordinal (categorical data) or whenever the assumption of normality was questionable, the Kruskal-Wallis test was employed as alternate statistical procedure for testing whether the populations were identical.

CHAPTER 4

4.1 RESULTS AND DISCUSSION

In most countries, sheep production is very seasonal, with mutton and wool products becoming available for marketing on a seasonal basis. Such seasonality of output (financial income for the producer) places the sheep at some disadvantage compared to the other farm animals. From a farmer's point of view, the economic return from his sheep will depend primarily on their reproductive efficiency. Increasing the frequency of lambing could increase reproductive efficiency, level out the flow of lambs to the market and utilizing buildings, capital and labour more efficiently (Hulet, 1979). However, because of the seasonal nature of breeding in sheep, any attempt to breed ewes at a greater frequency than once a year is likely to result in at least one mating during or near the sheep's anoestrus in conventional seasonal breeding ewes. Although it is generally accepted that the beginning and end of a sheep's natural breeding season is controlled by natural day length changes, the precise onset of the breeding season in any one year is probably the result of many modifying factors. Factors like breed, age, previous reproductive history of the ewe, changes in the environmental temperature, nutritional status and the sudden introduction of a ram to the ewes can all have a modifying effect on the occurrence of oestrus. The relationship between photoperiodicity and ovarian activity in the ewe is probably more complex than most reports and reviews may indicate. Breeding season in these trials had no significant effect on the number of lambs born (Table 4.1).

Table 4.1 Effect of breeding season on the number of ovulation points, number of fetuses and the number of lambs born. (n=72 in season 1 and n=72 in season 2)

Breeding season	Group size (n)	Number of ovulation points (SD)	Number of fetuses (SD)	Number of lambs born (SD)
1	72	$1.42^{b} \pm 0.52$	$1.29^{a} \pm 0.52$	$1.31^{a} \pm 0.55$
2	72	$1.52^{a} \pm 0.58$	$1.19^{a} \pm 0.60$	$1.39^{a} \pm 0.68$

Means with different superscripts for each trait within the column differ significantly ($P<0.05$).

In these trials the number of ovulation points in breeding season 2 was significantly higher (p=0.02) than the number of ovulation points in breeding season 1. This finding is in contrast with the normal short photoperiod, higher ovulation rate theory commonly accepted, it however supports the theory of Davis *et al.* (1976) and Cumming (1977) that the effect of season on ovulation rate is a complicating factor in experiments. There was also a tendency (p=0.06) for the number of foetuses in breeding season 1 to be higher than the number of foetuses in breeding season 2. Although there was significantly more ovulation points in breeding season 2 compared to breeding season 1 there weren't significantly more lambs born in breeding season 2 compared to breeding season 1. This supports the idea that intensified management has led to breeding sheep outside the above mentioned natural breeding season when ewes experience seasonal polyestrous activity (Jeffcoate *et al*., 1984; Rawlings *et al*., 1987; Gordon, 1996).

In a breeding flock there is almost always a normal distribution of age among the ewes. However, the health and longlivety of each ewe within the breeding flock is essential and plays a very important role not to polarize this normal distribution curve. The total number of ewes in both the trials was one hundred and forty four.

The age of the 144 ewes used in both the trials varied from 14 months up to 85 months. Age of the individuals had a highly significant effect the number of ovulation points, the number of fetuses and the number of lambs born per ewe. Furthermore, a Kruskal-Wallis analysis was done (Table 4.2) to further test possible significant influence of age on the number of ovulation points, number of fetuses and the number of lambs born.

Table 4.2 Effect of age on the different categories of ovulation points, fetuses and lambs born. (n=144)

	Number of ovulation points		**Number of fetuses**		**Number of lambs born**	
	Number of ovulation points	Number of ewes	Number of fetuses	Number of ewes	Number of lambs	Number of ewes
	1	80	**0**	9	**0**	9
	2	60	**1**	91	**1**	78
	3	4	**2**	44	**2**	55
					3	2
Age	p=0.0454		p=0.0035		p=0.0008	
Category	1-2[a]		0-1[b]		0-1[c]	
Category	1-3[b]		0-2[a]		0-2[b]	
Category	2-3[b]		1-2[a]		0-3[b]	
Category					1-2[a]	
Category					1-3[c]	

Categories with different superscripts within the column differ significantly.

[a] $p < 0.05$

[b] $p < 0.10$

[c] $p > 0.10$

This analysis confirmed that age had a highly significant effect on the number of ovulation points (p=0.0454), the number of fetuses (p=0.0035) and the number of lambs born (p=0.0008) per ewe. The mean for variable, age, is 43 months. 32.5% (26/80) of the ewes that had one ovulation points were older than the mean age of 43 months. Age had the most significant influence in the difference

between 1 and 2 ovulation points per ewe. 67.5% (34/60) of the ewes that had two ovulation points were older than the mean age of 43 months, while 50% (2/4) of the ewes that had three ovulation points were older than the mean age of 43 months.

The same phenomenon occurred with the number of lambs born, the biggest influence of age was in the difference between 1 and 2 lambs born per ewe. All nine (9/9) of the ewes that didn't produce any offspring were under the mean age of 43 months. 40% (31/78) of the ewes that produced one lamb were older than the mean 43 months of age, while 60% (33/55) of the ewes that produced twin lambs were older than the mean age of 43 months. Lastly both the ewes that produced three lambs were older than the mean 43 months of age. This coincides with the findings of Gordon, 1967b and Brien *et al.* (1976) that younger ewes tend to have lower ovulation rates and smaller litter sizes than mature ewes with the same liveweight. According to Rattray (1982) the response to flushing is greater in mature ewes than in yearlings.

In sheep, live weight and ovulation rate are usually correlated. Up to a threshold weight which varies with genotype, ewes may not ovulate at all (Fletcher, 1971; Gunn, 1982) but beyond this there is a roughly linear relationship between live weight and ovulation rate (Edey, 1968; Morley *et al.*, 1978) until a plateau is reached which represents the genetic potential of the animal (Gunn, 1982).

Results from this trial (Table 4.3) show that change in live weight had a significant effect on the number of ovulation points per ewe.

Table 4.3 Effect of change in live weight in season 1 and season 2 on the number of ovulation points, number of fetuses and the number of lambs born. (n=144)

	Group size (n)	**Change in live weight**
Season 1	72	$0.32^{b} \pm 2.02$
Season 2	72	$4.89^{a} \pm 2.08$

Means with different superscripts for each trait within the column differ significantly (P<0.05).

In both these trials (season 1 and season 2) a change in live weight had a significant effect on the number of ovulation points (p=0.03) but no significant effect on the number of fetuses (p=0.2) or the number of lambs born (p=0.6). In season two the change in live weight was significantly more (4.89kg) than the change in live weight in season 1 (0.32kg). This huge change in live weight led to the significantly higher amount of ovulation points seen in season two as compared to season one. This result supports the "dynamic effect" theory of Coop (1966).

Ovulation rate in the ewe in the usual breeding season is determined by factors which operate up to the time of mating. Nutritionally the most influential period is probably that between the previous lambing and the time of mating; this is regarded as the recovery period when the sheep's reserves that were depleted during pregnancy and lactation are replenish. There are two considerations in looking at the nutrition of the ewe during this recovery period; the first is the long-term one which largely determines the body condition and the weight of the ewe at mating time, the second is the short-term, "flushing" effect operating at the time of mating. A direct link between body conditions an ovulation rate was established in sheep by Clarke as early as 1934. Coop (1966) in New Zealand was one of the first to try to define the nutritional effect more precisely; he used the terms "static" and "dynamic" to describe the nutritional effects. The static effect was seen as to be a matter of body condition, live weight and size of the ewe while the dynamic effect was defined as a change in live weight during a 6 week period prior to mating. To increase the profitability of a sheep farming enterprise the owner/manager needs to increase ovulation rate, conception rate, the number of lambs born and the number of lambs that survive in order to sell more lambs to increase profitability. Flushing is the short-term supply of extra nutrients before, during and after mating. The main reason for flushing is to stimulate the maturation of follicles in order to increase the number of ova shed from the ovaries. The response to flushing is affected by factors such as age, breed, change in live weight and the stage of the breeding season.

According to Coop (1966) nutrition influences ovulation rate in two ways, the first is the difference in static live weight the so called "static effect" and the second is due to change in live weight the so called, "dynamic effect". The "static" effect deals with the body condition, live weight and the size of the ewe. The "dynamic" effect deals with the change in live weight of a particular ewe. A series of trials with lupine supplements by Knight *et al.* (1975); Lightfoot *et al.* (1976) and Radford *et al.* (1980) showed that the ewe did not had to have change in live weight, as proposed by the "dynamic effect" of

Coop (1966), to get changes in the ovulation rate. In fact, the period of feeding required to increase ovulation rate significantly has been reduced to only 4-6 days prior to mating (Oldman and Lindsay, 1984; Stewart & Oldman, 1986). This findings raises a third possible type of response, a so-called "ultra-short term effect". In both these trials the dietary supplementation was given for a period of 9 days before ovulation and mating which resulted in an increase in live weight. This increase in live weight before ovulation led to the increase in the number of ovulation points (table 4.4) per ewe and this coincides with the findings of Rattray (1982). In this trial, using the Analysis of Variance Statistical System, the four different dietary supplements (Table 4.4) had no significant influence on the number of ovulation points (p=0.46), the number of fetuses (p=0.47) or the number of lambs born (p=0.65).

Table 4.4 Effect of the four different dietary supplementations on the number of ovulation points, number of fetuses and the number of lambs born. (n=36 in each treatment)

Dietary supplement (Treatment)	Group size (n)	Number of ovulation points (SD)	Number of fetuses (SD)	Number of lambs born (SD)
Supplement 1	36	1.47 ± (0.61)	1.28 ± (0.57)	1.33 ± (0.59)
Supplement 2	36	1.44 ± (0.56)	1.25 ± (0.55)	1.36 ± (0.64)
Supplement 3	36	1.50 ± (0.56)	1.14 ± (0.59)	1.25 ± (0.69)
Supplement 4	36	1.47 ± (0.51)	1.31 ± (0.52)	1.44 ± (0.56)

Means with different superscripts for each trait within the column differ significantly (P<0.05).

In both these trials flushing of the ewes led to an increase in live weight and that led to an increase in the number of ovulation points, but the lambing rate couldn't be increased significantly with an increase in the number of fetuses or the number of lambs born. This decrease in the number of fetuses compared to the number of ovulation points in all the four different dietary supplementation groups varied between 11% and 24%. The highest decrease was for dietary supplement group 3 (24%), and the lowest for dietary supplement group 4 (11%). There was also a marked decrease in the number of lambs born compared to the number of ovulation points for all four of the dietary supplements, the decrease varied between 2% (dietary supplement 4) and 16.7% (dietary supplement 3). Although there was more lambs born in dietary supplementation group 4 than in the other dietary supplementation

groups the difference wasn't significant. The increase from the number of fetuses to the number of lambs born could be ascribed to human error while counting the number of fetuses by means of the ultra sound technique. In other words for a decrease in the number of lambs born compared to the number of ovulation points there had to be a high ova loss after the number of ovulation points was counted. It is well established that 20-30% of sheep embryo's die in the first weeks of pregnancy (Edey, 1969; Kelly, 1984). It is also known that progesterone plays a crucial role in maintaining pregnancy in the ewe (Denamur and Martinet, 1955). This ova loss could be due to numerous factors such as environmental, handling and nutritional factors.

In a study by Ulberg and Burfening (1967) it was concluded that if the ambient temperature is high enough to elevate the rectal temperature of ewes near time of breeding by as little as 1ºC there is a marked reduction in conception rate. According to Casau *et al.* (1991) heat stress has adverse effects on ovulation in the ewe with the greatest effect being observed in the first three days after ovulation.

In 1976 Doney demonstrated that the stress of handling ewes in normal husbandry operations may influence ovulation rates adversely and increase the extent of embryo mortality. According to Kilgour and De Lagen, 1970 shearing at or shortly after the mating season, could be regarded as one of the most stressful events that can happen to sheep. The process of shearing involves forcing ewes into strange situations, handling and isolation from other sheep, as well as the stress associated with shearing itself and consequent readjustment in their body metabolism. Welch *et al.* (1979) provided evidence that shearing carried out a week or so after mating exerted a dramatic effect on the lambing pattern subsequently shown by the flock; shearing markedly reduced the proportion of ewes lambing at the expected time, although the sheep become pregnant at a later stage. In both these trials each ewe was examined by laparoscopy on day 45, only 6 days after mating, to count the number and measure the diameter of each of the corpora lutea (ovulation points) on the ovaries. In my opinion if shearing, carried out a week after mating (Welch *et al.*, 1979), had a dramatic effect on the lambing pattern, a process such as laparoscopy must have a more severe stress effect on the ewe, possibly increasing ova or embryo loss. There is ample evidence for the view that any form of stress should be avoided as far as possible during the mating period (Gunn and Doney, 1979); in early pregnancy in the ewe, the indications are that a sustained moderate degree of undernutrition, resulting in a 3-4% loss in live

weight during the first month of gestation is unlikely to have any significant harmful effect in ewes that are in appropriate body condition at mating (Russel *et al.*,, 1969).

According to studies in Australia nutrition in early pregnancy and peripheral progesterone concentrations may be inversely related (Parr *et al.*, 1982; Williams & Cumming, 1982). Later Parr *et al.* (1987) demonstrated that sheep fed high energy rations after mating had reduced progesterone levels and showed an increase in embryo mortality. It is believed that high-plane feeding at mating can increase embryo loss as a result of a higher rate of metabolism decreasing progesterone concentrations. It is known that the liver is a major site of progesterone catabolism (Bedford *et al.*, 1974) and that blood flow to the liver of ewe's increases with feeding (Bensadoum and Reid, 1962). McEvoy *et al.* (1995a) showed that "flushing" the super ovulated ewe before mating did not increase ovulation rate; on the contrary, by adversely affecting preovulatory progesterone levels, high plane feeding potentially incurs serious penalties in terms of embryo development and survival. The avoidance of embryo losses when high preovulatory feeding regimes are employed might be achieved via the use of exogenous progesterone treatment. Bishonga *et al.* (1994) indicated in a study in Aberdeen that high levels of rumen-degradable protein in the form of added urea were associated with reductions in both the rate and the percentage of pregnancies established after auto transfer; it was believed that high levels of plasma urea and ammonia may have adverse effects on early embryo survival.

Looking at the "static" effect it is clear from various reports that body fat content (body condition) directly effects hypothalamic activity and GnRH secretion and that effects on reproductive performance are mediated by way of changes in ovarian hormones or in hypothalamic-pituitary sensitivity of ovarian hormones (Rhind *et al.*, 1989). The effect of body reserves on fertility and litter size in Spanish Manchega ewes managed for three lamb crops in two years on a semi-intensive system has been recorded by Molina *et al.* (1994). Body fat conditions at mating was found to be important; ewes with a score > 3.0 had a significant higher lambing rate (90.8%) than those with a score of < 2.0 (76.6%). In physiological terms, it is known that ewes in high body condition have more and larger, estrogenic, ovarian follicles than ewes in low body condition (Rhind *et al.*, 1989). Ewes in a fleshy condition during breeding, have a significantly higher ovulation rate and greater follicle size, but a lower embryonic survival rate (El-Sheikh *et al.*, 1955). Poor body condition or severe undernutrition during the immediate pre-mating period, irrespective of condition, may delay onset of seasonal oestrus,

lengthen the oestrus cycle, cause ovulation failure, or result in ovulation unaccompanied by oestrus (Doney and Gunn, 1981). Foote and Mathews (1983) reported a very high correlation between body weight and body size (0.999). Changing nutrient intake from a high pre breeding level to a low post breeding level appears to contribute to prenatal mortality. This suggests extremes are to be avoided and that body condition throughout the year is as critical as during a short flushing period.

In both these trials dietary supplementation had no significant influence ($p>0.05$) in ovulation rate. The four different dietary supplements were formulated on an iso-crude protein basis, the difference between the four dietary supplements being the source of protein and the energy content of each protein source. The energy intake level differs from about 1.10MJ/day/ewe in dietary supplement one to 1.70MJ/day/ewe from dietary supplement three. According to Davis *et al.* (1981) the minimum level of crude protein intake per day, for effective rumen functions is approximately 125g/day. In these trials the daily protein intake was about 256.38g/ewe, this is more than double than the threshold level of Davis *et al.* (1981).

Dietary supplement 1 was used to supply excess rumen-degradable protein in the form of urea. As mentioned earlier and according to Mandibela *et al.* (1995) excess rumen degradable protein elevate plasma concentrations of urea and glucose, the ova metabolize more glucose which results in advances development of the ova but the viability of the ova is decreased which results in a lower lambing percentage. Ferguson *et al*. (1986a, 1986b) also concluded high rumen degradable protein to be deleterious for fertility in dairy cows. The main source of amino acids in ewes fed rumen-degradable protein is microbial protein which is relatively poor in branched-chain-amino-acids (Merchen and Titgemeyer, 1992). Dietary supplements 2, 3 and 4 were formulated respectively with sunflower oil cake meal, cottonseed oil cake meal and a mixture of fishmeal and cottonseed oil cake meal as their main crude protein contributors. Urea was used in all four dietary supplements in order to balance the crude protein content between the four different dietary supplements. These different protein sources were chosen in order to supply different amounts of branched-chain amino acids. To increase the duodenal flow, and hence the absorption of branched-chain-amino-acids, feedstuffs rich in branched-chain-amino-acids and of low ruminal degradability, such as maize-gluten meal should be used (Tagari *et al.*, 1995). The difference in the energy intake between the four different dietary supplements could result in an increase of ovulation rate. There are two possible mechanisms by which branched-chain-

amino-acids could increase the ovulation rate. The first mechanism is a direct effect, changes in plasma concentrations of branched-chain-amino-acids, on ovarian function (Downing *et al.,* 1995). Second, insulin may be involved since leucine is the most effective amino acid in stimulating insulin secretion in sheep (Kuhara *et al.,* 1991). Although soybean meal and the maize-gluten meal-ground-maize grain mixture diets supplied similar amounts of excess protein, feeding ewes with maize-gluten meal-ground-maize grain mixture that was rich in branched-chain amino acids and poorly degraded in the rumen, resulted in an increase in serum concentrations of insulin at estrus, which was not seen in ewes fed with soybean meal (Landau *et al*., 1996).

In this trial odd ratios were used (Table 4.5) to compare the effect of the four different dietary supplements on ovulation rate, number of fetuses and the number of lambs born.

Table 4.5 Odds ratio estimate comparison between sunflower oilcake meal, cottonseed oil cake meal, fishmeal cottonseed oil cake meal mixture and urea for both season 1 and season 2.

Comparisons of dietary supplement 2,3,4 : dietary supplement 1	**Number of ovulation points**	**Number of fetuses**	**Number of lambs born**
Sunflower oilcake meal (**2**) : Urea (**1**)	1.085 : 1	0.708 :1	0.801 : 1
Cottonseed oil cake meal (**3**) : Urea (**1**)	1.298 : 1	0.740 : 1	0.784 : 1
Fishmeal, cottonseed oil cake meal (**4**) : Urea (**1**)	1.306 : 1	0.757 : 1	1.086 : 1
	p = 0.0195	p = 0.1442	p = 0.0055

Categories with different superscripts within the column differ significantly.

With comparison to dietary supplement 1 the other three dietary supplements had a significant influence on the number of ovulation points ($p<0.05$) and the number of lambs born ($p<0.01$) per ewe. The reason for the no significant influence on the number of fetuses could be due to the inaccurate counting due to the human error while counting the fetuses by means of the ultra sound technique. With regards to the number of ovulation points the mixture of fishmeal and cottonseed oilcake meal delivered 1,306 ovulation points compared the the 1 of urea. In other words, looking at the number of

ovulation point's dietary supplement 3 and 4 delivered the best results with dietary supplement 2 only marginally better than urea.

According to the results the influence of the dietary supplements on the number of lambs born, were completely different from the effect on the number of ovulation points. The mixture of fishmeal and cottonseed oil cake meal (dietary supplement 4) resulted in just more lambs born than urea. Urea (dietary supplement 1) resulted in more lambs born than sunflower oilcake meal (dietary supplement 2) and cottonseed oilcake meal (dietary supplement 3). The branches chain amino acid content of fishmeal is almost double that of cottonseed oil cake meal, while sunflower oil cake meal and urea has no branch chain amino acids what so ever. This finding supports the findings of Downing *et al*. (1995) and Landau *et al*. (1996) that branched-chain amino acids could result in higher ovulation tempo. In both these trials there were a significant difference between the effects of the different dietary supplements on the number of ovulation points and the number of lambs born which supports the findings of Mandibela *et al.* (1995) and Ferguson *et al.* (1986a, 1986b).

In table 4.6 the effect of the different dietary supplements on the number of ovulation points between the two seasons are compared.

Table 4.6 Odds ratio estimate comparison for the number of ovulation points between sunflower oilcake meal, cottonseed oil cake meal, fishmeal cottonseed oil cake meal mixture and urea for the separate breeding seasons.

Comparisons of dietary supplement 2,3,4 : dietary supplement 1	**Number of ovulation points**	
	Breeding season 1	**Breeding season 2**
Sunflower oilcake meal (**2**) : Urea (**1**)	0.981 : 1	0.902 : 1
Cottonseed oil cake meal (**3**) : Urea (**1**)	1.543 : 1	1.222 : 1
Fishmeal, cottonseed oil cake meal (**4**) : Urea (**1**)	1.044 : 1	1.786 : 1
	p = 0.1901	p = 0.05

According to the table above there was a significant difference in the effects of the dietary supplements on the number of ovulation points in breeding season 2. Breeding season 2 started on 23 November and ended on the 11th January in other words the anoestrus breeding season. The mixture of fishmeal and cottonseed oil cake meal (dietary supplement 4) resulted in the most ovulation points per ewe (1.786), followed by the cottonseed oil cake meal (1.222) then urea (1.000) and lastly sunflower oil cake meal (0.902). The finding also supports the theory that the effect of flushing during the seasonal peak in ovulation rate is less effective than outside the seasonal peak.

The effect of birth status on the number of ovulation points, number of fetuses and the number of lambs born per ewe is shown in table 4.7.

Table 4.7 Effect of birth status of the ewes on the number of ovulation points, number of fetuses and the number of lambs born.

Birth status	Group size (n)	Number of ovulation points (SD)	Number of fetuses (SD)	Number of lambs born (SD)
1	77	1.43 ± 0.52	1.16^{b} ± 0.54	1.26^{b} ± 0.64
2	67	1.52 ± 0.59	1.34^{a} ± 0.57	1.45^{a} ± 0.58

Means with different superscripts for each trait within the column differ significantly (P<0.05).

The birth status of all the ewes used in the trial was either single or twin. Seventy seven of the hundred and forty four ewe's birth status was single and sixty seven was part of a twin. Although the number of ovulation points in the group with twin birth status was higher than the group of single birth status, it was not significant (p=0.6). There was a decrease in the number of fetuses and the number of lambs born compared to the number of ovulation points in both the birth status groups. The decrease was the lowest in the twin birth status group. However birth status of the ewe had a significant effect on the number of fetuses (p=0.04) and an even more significant effect on the number of lambs born (p=0.02). In this trial ewe that was part of a twin gave significantly more lambs than ewes that were born single. The heritability of twining is low and twining seldom occurs in primiparous females. The incidence of twining in sheep increases with age for several years then it declines. Most twins are of dizygous type, in other words they result from ovulation of two oozytes during the same oestrus cycle. These oocytes

are fertilized and eventually implanted in the uterus. Some twins are monozygous, resulting from fertilization of a single oocyte. Monozygous twins are always of the same sex and are genetically and phenotypically identical, except that one is frequently larger than the other.

It is well documented that estimates of heritability of fertility traits in the ewe vary considerably in different breeds of sheep, but they tend to be rather low (Land, 1978), and genetic improvement in fertility by direct selection can generally be expected to be a relative slow process. Selection for increased litter size can be a slow process, with annual improvements of no more than about 2 lambs per 100 ewes (Land, 1978). Heritability is defined as the percentage of total variation that is controlled by the genetic make up of an individual. Direct selection for more prolific ewes within the breed is very important because a small difference in the proportion of ewes carrying multiples may make a large difference to the net income yielded by the flock. Land (1978) reported that there was a dramatic increase in the ovulation rate in one strain of the merino that was subjected to intense selection for prolificy.

CHAPTER 5

5.1 CONCLUSION

In order to maximize the economic return from sheep farming a farmer has to optimize his herds' reproductive efficiency. In weaner lamb production, it is not only essential to achieve high conception rates but it is also important that most ewes produce twins rather than single lambs. A small difference in the proportion of ewes pregnant with twins may make a large difference to the net income yielded by the herd. In order to maximize the number of twins in a herd there is a few managerial alterations that could be enforced.

The ability of a ewe to produce twins is made up of a genetic and an environmental component. From a genetic point of view it is very important to select individual ewes for litter size. In this trial birth status of the ewe had a significant effect on the number of fetuses ($p=0.04$) and an even more significant effect on the number of lambs born ($p=0.02$) per ewe. The ewe that was part of a twin gave significantly more lambs (1.45 vs 1.26) than ewes that were born single. Although this selection is a slow process, due to the low heritability of the fertility trait, it is possible to increase the mean litter size of the herd by 2% per annum or 20% over a period of ten years.

The environmental component can be divided into various factors such as diet quantity and quality, dietary supplementation (flushing), animal health, ewe recovery period, breeding season and age, dynamic and static effects of the ewe, handling of the animals and lastly the effectiveness of the breeding rams.

From this trial, comparing the results over the two seasons, it is evident that dietary supplement 3 (cottonseed oilcake meal) and 4 (fishmeal, cottonseed oilcake meal) resulted in significantly more ovulation points than the sunflower oilcake meal or the urea dietary supplement. However this big advantage wasn't carry through to the number of lambs that was born. An explanation for this high ova loss could be the stress the ewes were subjected to during the laparoscopy process used to count the number of ovulation points. The fishmeal, cottonseed oil cake meal resulted in 1.086 lambs born compared to the urea mixture of 1.000 born, not that big a difference. Looking at breeding season two

again the fishmeal, cottonseed oil cake meal had a significant effect on the number of ovulation points (1.786:1) as compared to the effect of urea. This phenomenon could be due to the fact that dietary supplements 1 and 2 contained no branch chain amino acids and the ewes were totally dependent on the microbial protein synthesized in the rumen, or it could be due to the different energy intake. Unfortunately this did not result in more lambs born which places a question mark on the economical viability of using these particular dietary supplements.

The change in live weight had a significant effect ($p=0.03$) to increase the number of ovulation points but again it failed to increase the number of lambs born. This could be due to high ova loss from the stress from the laparoscopy performed on the ewes only 6 days after mating. It is well known that 20-30% of sheep embryo's die in the first weeks of pregnancy. Therefore it is of the utmost importance to minimize stress of any kind on the ewe. Especially practices such as handling, shearing and immunization should be avoided for at least the first eight weeks after mating. It is of the utmost importance to prevent the ewe flock from losing to much weight and condition during the period from lambing up to mating.

Results from this trial supports the finding that "flushing" during the "off" season (trial 2, November to January) had a significant effect on ovulation rate compared to season 1 (trial 1, May to June). Therefore, in order to increase net income of the farming enterprise, "flushing" should at least be done if in the "off" breeding season.

Results from various studies concluded that younger ewes tend to have lower ovulation rates and smaller litter sizes than mature ewes with the same liveweight. This study supports the conclusion above where age had a significant effect on ovulation rate. The results showed that all 9 ewes that didn't reproduce were ewes under the age of 43 months. The study further showed that 60% of the ewes giving birth to twins were older than 43 months while 60% of the ewes giving birth to a single lamb were under the age of 43 months. The older ewe has a greater the chance for breeding twins. In a breeding flock there is almost always a normal distribution of age among the ewes. However, the health and longlivety of each ewe within the breeding flock is essential and plays a very important role not to polarize this normal distribution curve.

5.2 RECOMMENDATIONS

It was a great privilege to work in the reproduction and nutrition field on sheep. A big advantage of this trial was the practical handling facilities of the small stock unit on the experimental farm of the University of Pretoria. Furthermore it was a great pleasure to work with well educated supervisors as well as well trained assistants on this lengthy trial. However there are a few suggestions if I had to do the trial again. This trial was too long and too many parameters were measured. The amount of ewes used in such a trial must include much more ewes in order to get better reactions on the treatments. I would further break this trial up into at least two trials, the first trial for the period up to ovulation and the second trial from mating onwards up to the birth of the lambs. In order to determine the influence of different nitrogen sources on ovulation rate I would suggest that the dietary supplements should be based on an iso-caloric and a iso-crude protein level and not just on an iso-crude protein level. This would eliminate the compounding effect of energy on the number of ovulation points and the number of fetuses born. That would make it possible to eliminate the urea inclusion, in all four dietary supplements, and furthermore the negative effects of urea on ovulation rate. Care must however be taken in formulating the dietary supplements in a way that the intake of the dietary supplement doesn't substitute the intake from the pasture. I would further try to minimize the stress on the ewes by subjecting fever ewes to laparoscopy, because of its big impact on ova losses. Lastly I would test the rams just to make 100% sure that their semen is of good quality. To the rest of the procedures and the management of the trial I wouldn't change anything.

5.3 CRITICAL OVERVIEW

Due to the degree of difficulty associated with experiments regarding influences on reproduction it is of the utmost importance to carefully plan any experiment in this regard. In order to get a response from dietary supplementation on a reproductive trait it is necessary to use large quantities of animals in a trial and not eighteen animals per treatment as in this trial. Furthermore it is essential to repeat the trial over a few years to test for repeatability of the results before a definite conclusion is to be made. For the best possible results it is very important to include animals that are as uniform as possible in weight, body size and body condition. It is further very important to change the subjective body condition scouring system to a more reliable system. The fact that urea was used as part of all four dietary supplements could have overshadow some of the effects caused by the four different dietary supplements. In order to compare the sole effect of different protein sources it is essential that the amount of energy intake per day must be the same between the ewes. With any dietary supplementation trial care must be taken that the supplement doesn't cause a substitution effect of roughage intake. Reproductive studies have to be broken up into smaller studies, studying only one parameter at a time, keeping all the other parameters constant. It is very important to analyze blood glucose and hormonal levels within each trial because it gives a better understanding of the effect the supplementation (energy and protein levels) had on the ewe. Care has to be taken not to cause excessive stress to animals because stress can ruin the experiment. Also bear in mind that the results obtained from a trial done under a semi-intensively managed farming system may differ significantly from an extensively managed farming system.

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