Chapter 1

GENERAL INTRODUCTION

The influence of nutrition on the reproductive and lactation performance of ruminants has long been a matter for concern. The relationship between nutrition and requirement for optimum animal performance are varied over the environmental conditions. Where nutrition is adequate, animal performance may be expected to be optimum but we known little about the specific nutritional requirements of the different phases of the reproductive cycle and this information is vital to the optimisation of reproductive performance with fluctuation nutritional status.

It has been suggested that factors such as the level of body condition at the beginning of pregnancy contribute to feeding effects on reproductive performance. For example, if body condition is maintained during the first month of gestation, then embryo and early foetal losses will be minimised. During mid-gestation, there is rapid growth of the placenta but the growth of the foetus in absolute terms is very small (Robinson *et al.* 1977; McCrabb *et al.* 1992). Over this period it is normal to suggest that animal should not reduce more than 5% of their body weight (Robinson 1985). Finally, there is the phase from 90 days to parturition in which the gain in mass of the foetus. For this period of pregnancy there is general agreement that nutrient intakes should be increased (Robinson 1983).

It is known that severe undernutrition of animals during late pregnancy can depress birth weight and vigour of new born animals, and that twins are likely to be more undernourished and have small birth weights than singles, even when dams have been similarly fed. These differences are also known to remain in early life, associated with lower milk intake of the

smaller offspring and the inadequate maternal nutrition which is likely in such circumstances. The effect of undernourishment of an animal on milk production may be associated with the depression in mammary development as the requirement of nutrients for the conceptus and maternal tissue are increased (Rosso *et al.* 1981; Hall *et al.* 1992).

Nutrition can, for convenience, be defined in terms of energy, protein and specific dietary components such as vitamins and minerals. Measurement of nutritional adequacy in the animal is usually based on the relatively insensitive parameters of liveweight and body condition change, which express nutritional accumulation or loss, mainly of energy (e.g. Oldham and Emmans 1988). Many researchers have suggested the addition of dietary energy intake during the dry period is to attain the greatest milk yield for the subsequent lactation. Feeding during this period is to supply excess of nutrients which can be stored in the body tissue for subsequent mobilisation during a period of reduced dry matter intake.

In addition to energy, it is reported that protein intake (Sasser *et al.* 1988) and source of protein (Hunter and Magnerm 1988) seem to be limiting nutrients for reproductive performance (Clark and Davis 1980). The majority of the protein requirement of ruminants is supplied by microbial protein from the rumen. However, the maximal yield of microbial protein will not meet the net requirements of animals in the final few weeks of pregnancy. This can be supplied by dietary protein which escapes degradation in the rumen. The additional protein source fed to support the conceptus development in late gestation may also improve postpartum performance by decreasing the mobilisation of prepartum maternal body reserve. Many factors may account for the observed effect on reproductive performance and the subsequent milk production, and these will be discussed in the literature review, followed by a study of the effect of feeding different prepartum protein supplements on lactation performance.

Chapter 2

PHYSIOLOGY OF MAMMARY DEVELOPMENT AND MILK PRODUCTION

2.1 INTRODUCTION

Mammary gland development refers to the growth of parenchymal tissues which compound of the amount of secretory tissues. The amount of secretory tissues of mammary gland dictates the potential of lactation for an animal. It is important therefore to understand the normal development and function of the mammary gland and the factors which influence amount of tissue present and its activity. This chapter reviews the literature on the physiological of normal development of mammary gland and its function factors which influence this.

2.2 ANATOMY OF THE MAMMARY GLAND

2.2.1 EXTERNAL APPEARANCE

The mammary gland of all highly developed mammals has the same essential structure. It is found in various sizes, shapes and locations between species, but the function is always the same, to secrete milk for the nourishment of the immature offspring. The udder of the mature cow normally consists of 4 functional glands and similar functional 2 glands in goats and sheep all with independent duct systems. Therefore, milk formed in one gland cannot pass into adjacent glands. The udder is a specialised skin gland and is covered with hair, except for the teats of the bovine.

In cattle, the shape of the udder has been described as being square or as having a more or less rounded saccular appearance. The udder is separated into two halves by a longitudinal groove, the intermammary groove. The fore and rear quarters may be separated by a groove or smoothly joined together; however this characteristic is less obvioused (Holmes and Wilson 1984; Currie 1988). Normally the rear quarters are larger, with 25 to 50% more secretory tissue, and produce approximately 60% more milk than the fore quarters (Smith 1959).

The shape or size of the teat which drains each gland is independent of the shape or size of the udder, and vary from cylindrical to conical. The rear teats are usually shorter than the fore teats. The small canal located in the end of each teat, teat meatus of streak canal, is the only spincter in each gland (Schmidt and Van Vleck 1974). The streak canal is made up of three to five convex epithelial projections that are held closed by involuntary sphincter muscles. The rate of milking of a cow is partially dependent on the size of the streak canal. For example, the faster milking cows usually have a larger diameter of streak canal (Schmidt and Van Vleck 1974).

The weight of the udder varies with the age of cow, the stage of lactation, the amount of milk in the udder, the inherited differences among cows, and the amount of secretory and connective tissue. It is reported that the empty weight of the udder varies from 11 to 27 kg (Smith 1959), while the total weight of the udder and the milk it contains of a high producing dairy cow in some case exceeded to 50 to 68 kg (Smith 1959; Lancelles 1976; Ruckebusch *et al.* 1991).

The weight and capacity of the udder increases with age of animals up to about 6 years of age, with the greatest increase occurring between the first and second lactation. In Schmidt's study once cows had reached 6 years of age, the increase in either weight or capacity of the udders was not significant. The weight decreases with advancing lactation, the most pronounced decrease occurring during the first two months of lactation (Schmidt 1971).

2.2.2 INTERNAL APPEARANCE

It is important for the udder to extent well forward and be large enough to contain the large volume of milk secreted between milking. The principle supporting structures of the udder is provided mainly by the median and lateral suspensory ligaments, and a loose network of connective tissue (Holmes and Wilson 1984). These ligaments attach to the subpubic and prepubic tendons, which in turn have their origin along the ventral surface of the pelvis and anterior border of the pubic bone.

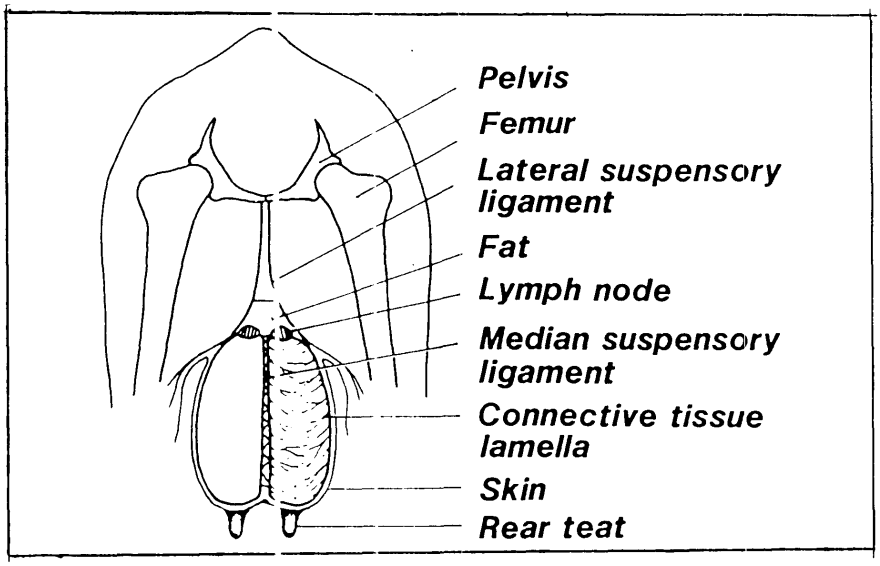

Figure 2.1: Diagram of a cross section of a cow caudal to the rear teats and showing the position of the lateral and median suspensory ligaments (Holmes and Wilson 1984).

The median suspensory ligament is comprised of double sheets of fibro-elastic tissue attached to the midline of the abdomen and separating the udder into right and left halves of the udder. The lateral suspensory ligaments consist of sheets of non-elastic connective tissue. They are located external to the mass of secretory tissue and lie under subcutaneous connective tissue and the skin. The suspensory ligaments of the udder are shown in Figure 2.1. The median and lateral ligaments pass into the udder to form a framework of connective tissue of the gland. It appears that the connective tissue gives support to the mammary gland without compressing the glandular tissue and interfering with blood circulation.

2.3 DEVELOPMENT OF THE MAMMARY GLAND

Milk produced by a cow is a function of the amount of secretory tissue and its secretory activity. The number of potential milk secretion cells is one of the primary determinants for milk production (e.g. Glimm *et al.* 1988; Stelwagen *et al.* 1992). Therefore, it is important to understand the normal growth and development of the mammary gland and the factors which influence the amount of tissue present and its activity. The development of mammary gland tissue is described as "mammogenesis". The normal development of mammary gland can be conveniently divided into five stages.

- Foetal growth
- Growth from birth to prepuberty
- Growth during puberty
- Growth during pregnancy
- Growth during lactation

2.3.1 FOETAL GROWTH

The development of the prenatal mammary gland is reported to begin very early in foetal life, a few weeks after conception (Knight and Peaker 1982a; Currie 1988; Zavizion 1991a,b). The development of foetal mammary gland can be divided into three stages which determine the structure of the mature glands (Knight and Peaker 1982a). Firstly, the proliferation and constriction of ectoderm, which locates on the ventral surface of the embryo and produces the mammary line. Subsequently, the number and location of the mammary glands is determined by the number and position of mammary bud on the mammary line, i.e. inguinal, abdominal, thoracic. Then the bud elongates into the mesenchyme and results in the formation of the primary sprouts and a number of the secondary sprouts arise from them

The mammary buds develop along the mammary line and correspond to the number and accessory glands characteristic of each species. The formation of the mammary buds appear early in the second month of gestation when the embryo is about 2.1 cm long

(Schmidt 1971). Up to this stage the male and female embryos have comparable mammary gland development. The cells continue to proliferate and cause the mammary buds to grow down into the mesenchyme, except for a small opening at the outer pole which will eventually give rise to the teat (Currie 1988).

During the second month of cattle gestation, the embryo is about 8 to 9 cm long, the mesenchyme cells begin to multiply rapidly and the mammary buds go down progressively in the mesenchyme. Mammary buds continue to grow and are carried outwards from the ventral skin surface (Raynaud 1971; Ruckebusch *et al.* 1991). At this time, the formation of the teat begins and the mammary growth rates of the male become slower than that of the female (Schmidt 1971).

The cause of the reduction in mammary growth in the male may be associated with the influence of sex hormones. Studies on the development of mammary gland in male mice have shown that androgen from the gonad of the male foetus is responsible for the destruction of mammary development (Raynaud 1971). This has been confirmed by Kratochwil (1977), using in vitro techniques, who found that the embryonic mammary rudiments become responsive to androgen late on day 13 of foetal life, but are no longer responsive on day 15 or later.

The teat and gland cistern structures start to form during the third month of foetal development. The ectoderm of the mammary buds begin to grow down into the mesoderm to form the primary sprouts (Currie 1988). This primary sprout is important, since the alveolar ultimately develops from it. By this time the embryo is 25 cm long, the teat has a distinct lumen, and the gland cistern gives rise to primitive ducts. Smith (1959) reported that even the teat sphincter has begun to take shape although the papillary duct does not open completely until later.

Essential ancillary structures in the foetal gland: including blood vessels, nerves, lymphatics, connective tissue and myoepithelial cells (e.g. Schmidt 1971; Holmes and Wilson 1984; Currie 1988) also develop at this time. Most of these structures, with the exception of the myoepithelial cells and nerves, arise from the mesenchymal layer which

underlies the mammary epithelium (Knight and Peaker 1982a). The basic vascular system of the udder, the lymphatic system (Schmidt 1971), as well as the myoepithelial cell (Zavizion *et al.* 1991a,b) are comparable to that in the mature gland.

When the bovine foetus is about six months old (50-60 cm stage) the remaining embryonic tissue has differentiated into adipose tissue. Paape and Sinha (1971) reported that this framework of the mammary gland is formed prior to major epithelial cell development and is necessary for the early organisation of mammary epithelium. The fat pad provides the necessary space, support and local control for duct elongation, and ultimately lobulo-alveolar proliferation (Hoshino 1964). It is likely that without the fat pad the mammary epithelium could not proliferate or differentiate (Knight and Peaker 1982a).

Most of the mammary tissue development is completed during the first six months of foetal life (Schmidt 1971). Changes in the foetal mammary gland after this stage are small and the relative growth rate is the same as for the whole body (Cowie and Tindal 1971; Ruckebusch *et al.* 1991). Ordinarily no parenchyma or secretory tissue is developed during the foetal period (Smith 1959). All the non-glandular structures of the udder are rapidly approaching their mature form when foetal life is completed (Holmes and Wilson 1984). The glandular portion, however, is immature (Smith 1959). Thus it is likely that the mammary gland of the prenatal animal develops by morphogenetic movement of cells rather than by proliferation (Knight and Peaker 1982a).

2.3.2 GROWTH FROM BIRTH TO PUBERTY

The development of gland from birth to puberty is mostly due to the increase in connective tissue and deposition of fat in the gland (Schmidt 1971). The immature duct system and stromal portion are proportionately larger than in mature gland (Tucker 1987). It has been found that the extensive growth of fat pad occurs as the result of cell hypertrophy or enlargement rather than hyperplasia or proliferation of cells (Knight and Peaker 1982a) and there is no sign of secretory tissue in the mammary gland (Tucker 1969; Stelwagen *et al.* 1993).

The mammary parenchymal growth consists primarily of the duct structure. The growth of duct system elongates and branches within the fat pad (Sinha and Tucker 1966; Knight and Peaker 1982a; Tucker 1987). Knight and Peaker (1982a) reported that there is an inhibition area around each duct so that other ducts cannot penetrate into it. Therefore, the duct is restricted to grow within the fat pad. Throughout this period the duct system does not function and the growth of ducts are believe to be similar to those of mature animals (Schmidt 1971)

2.3.3 GROWTH DURING PUBERTY

A major development of the mammary gland takes place between birth and the first calving, mainly in the period prior to and during the onset of puberty. Pubertal development of mammary gland is characterised by an increase of parenchyma and also a substantial increase in connective tissue elements (Paape and Sinha 1971).

The udder of the heifer begins to grow at a rate greater than body surface area at the initiation of oestrus cycles at approximately 6 to 7 months of age (Sinha and Tucker 1969a; Swanson and Poffenbarger 1979). Similar patterns of allometric growth are also observed in other species. For example in the sheep at puberty mammary growth is double that found in immature groups (102 vs. 66 mg) (Anderson 1975).

The allometric growth will continue until a plateau is reached at about nine months of age by which time the heifer has passed through several oestrus cycles (Currie 1988; Ruckebusch *et al.* 1991). In sheep, a plateau is reached at 5 months of age (Anderson 1975). After this period the mammary gland growth returns to an isometric pattern until conception. Sinha and Tucker (1969a) reported that the growth of the mammary gland of 16 month old heifers did not posses more mammary DNA than 9-months-old heifers (326 vs. 378 mg/100 kg body weight), indicating that the major portion of mammary growth is completed by nine months old of age (Figure 2.2).

Estimates of the biochemical changes of the mammary gland during various reproductive phases in the female have been reported by Sinha and Tucker (1969a); the mammary

development in heifers is at its lowest level at proestrus (day 20 of the cycle), but greatly increases (118%) at the day of oestrus. This increase is followed by a gradual decrease during and following metestrus (day 2-4 of the cycle) and diestrus (day 7-18 of the cycle) (Sinha and Tucker 1969a).

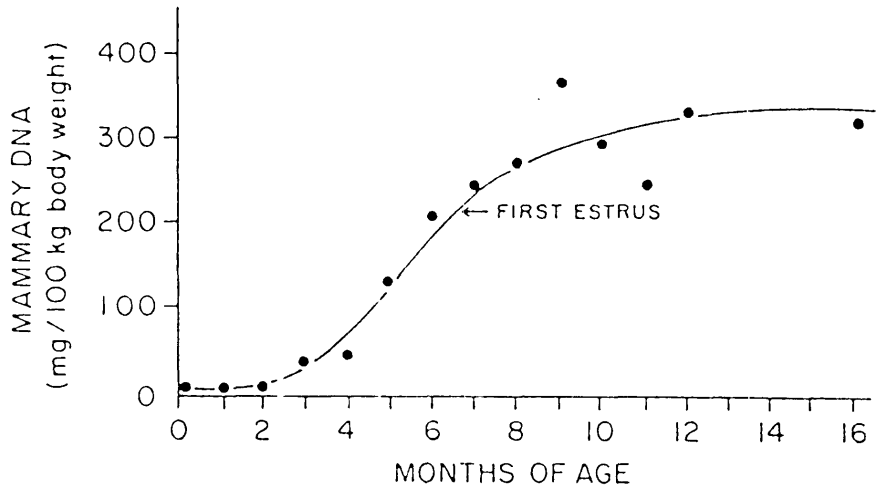

Figure 2.2: Total mammary DNA content in growing heifers (adapted from Tucker 1987).

Sinha and Tucker (1969b) reported that in the first two oestrous cycles the cumulative increase in mammary DNA of rats is initiated at proestrus and continues through metestrus. In the third to fifth cycles the peak of mammary DNA was observed at oestrus and decreased during metestrus. The failure to maintain allometric growth with recurring oestrous cycles is not well understood, but may be associated with the asynchrony between secretion of oestrogen and progesterone during the oestrous cycle (e.g. Knight and Peaker 1982a; Tucker 1987; Nickerson 1992). Therefore, it appears that an increase in mammary development occurs at oestrus but declines during the luteal phase of the oestrous cycle (Sinha and Tucker 1969a; Schmidt 1971; Anderson *et al.* 1982).

The histological appearance of the mammary tissues has been reported to change during this period. Prior to and during oestrus, the alveolar lumina are filled with secretory fluid, and are lined with cubodial epithelial cells. During diestrus, the lobules are relatively

small and the lumina are shrunken with no secretion in them. The epithelial cells are columnar in shape (Sinha and Tucker 1969a; Schmidt 1971). It appears that the cells found at this stage have similar characteristics to those found during pregnancy and lactation (Schmidt 1971). The major ducts have two layers and give rise to a series of branches. These ducts are surrounded by the various thicknesses of connective tissue. For example, thin connective tissue septa separates the alveoli from the lobules, and thick connective tissue septa appears to separate lobes. However, during the pubertal period the mammary tissues do not form the alveolar units. The majority of the tissue within the mammary gland before the first conception is made up of the adipose tissue and a partial duct system (Sinha and Tucker 1969a; Paape and Sinha 1971; Knight and Peaker 1982a; Currie 1988; Ruckebusch *et al.* 1991).

2.3.4 GROWTH DURING PREGNANCY

The majority of mammary development (48-94%) takes place throughout pregnancy (Tucker 1969; Knight and Peaker 1982a). The great change in mammary tissue during pregnancy is associated with proliferation of parenchyma, i.e. duct system, alveoli, but not connective tissue (Tucker 1987; Currie 1988; Ruckebusch *et al.* 1991). Histological appearance of the mammary gland in the first gestation of ruminants have been investigated by a number of workers. There is a substantial increase in the amount of parenchymal and connective tissues during the early stages of gestation (Paape and Sinha 1971; Forsyth 1986; Stelwagen *et al.* 1993). The epithelial structures consist primarily of densely packed tubules with double layers of epithelial cells (Swanson and Poffenbarger 1979). There is a small increase in duct length. The maximum duct length by this time is only 3 cm, while the initial length was 9 cm (Schmidt 1971). The size of gland cistern is still small, and a great increase in size occurs during the fifth and sixth month of pregnancy.

In heifers at 4 months of pregnancy, the secretory tissue is increasing slightly and it is found in the area around the large ducts and also in the gland cistern. The development of mammary tissues has a characteristic appearance comparable to those of the

developed gland of lactating animals, that is the large ducts have the characteristic two layer lining with underlying connective tissue, whereas the smaller ductules and alveoli have a one layer lining of cubodial shaped epithelial cells (Knight and Peaker 1982a).

By the fifth month of pregnancy a small amount of secretory tissue developing from the mammary ductal tree can be observed (Sejrsen *et al.* 1986; Currie 1988). This secretory tissue further proliferates by branching of ducts and the formation of the end bud. Throughout the udder, groups of alveoli begin replacing lipid of the fat pad (Tucker 1969; Swanson and Poffenbarger 1979) and form the first true lobulo-alveolar tissue (Knight and Peaker 1982a; Tucker 1987). The lobules are still small at this time and containing some secretory material (Swanson and Poffenbarger 1979). The various thickness of connective tissue fibres divide the lobules and lobes and are organised into distinct layers (Larson 1985). It appears that the connective tissue and fat pad remain inactive during this rapid growth phase of pregnancy (Paape and Sinha 1971).

The rate of mammary growth derived from the measurement of mammary tissue DNA (e.g. Anderson *et al.* 1981, 1982), and the incorporation of [^{3}H]thymidine into mammary tissue (e.g. Knight and Peaker 1982b) were similar, with an exponential increase during the last half of pregnancy. The alveoli are differentiated from the duct system and increase in size and number (Plaut 1993; Stelwagen *et al.* 1993), but remain small and flattened (Holmes and Wilson 1984). The secretory materials begin to contain numerous fat globules as well as protein-like material (Swanson and Poffenbarger 1979). The gland tissue becomes swollen, because it is surrounded by the adipose tissue, especially in the area around the large ducts and the cistern.

Most of the mammary tissue is laid down and the secretory epithelium of the alveoli begins to function during the last trimester of gestation (Currie 1988; Stelwagen *et al.* 1992, 1993). The epithelial cells are much larger than in previous months and contain the secretion of all products of milk such as fat globules (Schmidt 1971; Swanson and Poffenbarger 1979). In the last month of pregnancy, alveoli double in size and number compared to mid-gestation (Swanson and Poffenbarger 1979). During the last 2 weeks

of gestation, the fully proliferated cells are noticeable with a higher percentage of epithelium and lumen, and a lower percentage of connective tissue (Sordillo and Nickerson 1988).

Mammary epithelial cells obtained from cows 10 days prepartum contained an irregularly shaped nuclei, large nucleus compared to cytoplasm, relatively few large or small lipid droplets randomly located within cells, and fewer casein micelles are evident (Akers and Heald 1978; Akers *et al.* 1981). Cells with these characteristics are classified as immature. At the last week of gestation, the mammary cell cytoplasm enlarges and contains numerous fat droplets at the basal region and accumulates secretory vesicles at the apical region (Sordillo and Nickerson 1988). The lumina of the alveoli and collecting ducts start filling with secretory products: colostrum and milk approximately 0 to 4 days before parturition and extends until a few days postpartum (Swanson and Poffenbarger 1979; Tucker 1981). As mammary fluid accumulates, luminal spaces expand. Expansion of alveolar lumina area lead to compression of surrounding stromal area (Sordillo and Nickerson 1988).

Anderson *et al.* (1981) and Knight and Peaker (1982b) revealed that mammary DNA in small experimental animals has an exponential increase from mid-pregnancy (12 days) to the early lactation. Total DNA in mammary gland (DNA_t) of heifer increases throughout gestation from 24 to 33% (Swanson and Poffenbarger 1979). The normal pattern of cell development is a constant rate of proliferation and a low rate of regression (Knight and Peaker 1982b; Knight and Wilde 1987) with a consequential cells (DNA_t) accumulation as gestation advances (Table 2.1).

In the late month of gestation in the cow the mammary gland tissues contain much secreted products and consequently increases in water content. The change of mammary weight during the second half of pregnancy and throughout lactation is due to the retained fluid (e.g. Anderson 1975). RNA also increases markedly at this stage (Swanson and Poffenbarger 1979; Anderson *et al.* 1981) and there is evidence that total RNA content increases to about 40% above the levels observed during the first few months of gestation (Swanson and Poffenbarger 1979). Therefore as gestation advances, there is a

progressive increase in the RNA : DNA ratio which is a measure of potential synthetic capacity per cell (Hacker and Hill 1972; Swanson and Poffenbarger 1979; Anderson *et al.* 1981, 1982).

Table 2.1: Changes in total amount of mammary DNA (DNA_t) and incorporation of [^{3}H] thymidine into mammary tissue during pregnancy, lactation, and mammary gland involution in mice (adapted from Knight and Peaker 1982b).

Stage	Day	DNA_t (mg)	DNA_t (mg/100g body weight)
Pregnancy	12	1.90	5.38
	16	3.52	8.99
	19	3.85	10.20
Lactation	2	5.39	13.78
	5	7.71	17.95
	10	7.73	16.55
	20	3.41	7.52
Involution	4	2.55	6.77

2.3.5 GROWTH DURING LACTATION

It has been established that mammogenesis of all ruminants (Cowie *et al.* 1980) and some monogastric animals (Hacker and Hill 1972) is complete at parturition. For example, Hacker and Hill (1972) studying the mammary cell proliferation of swine, indicated that the average DNA content is constant in mammary tissue during the second half of gestation. At the last phase of gestation the increase in the number of mammary cells, including DFFT, DNA and RNA, accounted for approximately 100% of the growth. Similarly, Anderson (1975) studied mammary gland development in sheep at various phases of reproduction. These results indicated that approximately 98% of mammary parenchymal DNA (DNA_t) was present during pregnancy and no more than 2% was added during early lactation.

However, results obtained from a number of studies have shown that the proliferation of the mammary parenchyma appears to continues until the end of gestation and overlaps with the onset of lactation (Erb 1977). In cattle, there was a 65% increase in mammary cell numbers between 10 days before and after parturition (Akers *et al.* 1981). The proportion of total alveolar epithelial cells was 11% greater in mammary tissues postpartum than prepartum; however Akers *et al.* (1977) reported that most of the alveolar cells in the bovine mammary gland at 2 days postpartum are not fully differentiated exhibiting minimal proliferation of cellular organelles (Akers and Heald 1978). This indicates that mammary development is not completed immediately after parturition but continues into early lactation (Akers *et al.* 1981). Comparisons of the percentage of mammary development during pregnancy and early lactation in various species is shown in Table 2.2.

Table 2.2: Percent mammary growth during pregnancy and early lactation in various species (adapted from Anderson 1975; Anderson *et al.* 1982; Knight and Peaker 1984).

Species	Pregnancy (% DNA)	Lactation (% DNA)
Rat	60	40
Rabbit	67	33
Mouse	78	22
Hamster	94	6
Sheep	98	2
Goat	80	20

Studies in goats support the observation that the main phase of mammary development (approximately 77% DFFT) occurs during the last trimester of gestation and continues to increase for 2 to 5 weeks before the time of parturition (Wilde *et al.* 1986; Currie 1988). Both milk yield and the mammary cell population increase over the first few weeks postpartum and the increase in DNA content is some 20 to 26% over the first three week of lactation, during which milk yield increased approximately 17% (Knight and Peaker 1984). In the small laboratory species (eg. rat, mouse, guinea pig), the proliferation

activity continues for the first few days or at most 1 or 2 weeks of lactation (Munford 1964; Anderson *et al.* 1981,1982; Knight and Peaker 1982b; Knight and Wilde 1987).

2.4 HORMONAL CONTROL OF MAMMARY DEVELOPMENT AND LACTATION

Mammary growth and development is influenced by the variety of hormones. These include those secreted from the ovary, oestrogen and progesterone; and those from the anterior pituitary gland, namely, growth hormone and prolactin. These hormones are of major importance, as their secretion leads to accelerated growth of mammary tissue (e.g. Sinha and Tucker 1969a; Tucker 1981; Oka *et al.* 1991; Byatt *et al.* 1994). Furthermore, growth factors; epidermal growth factor, insulin-like growth factor-I, -II, transforming growth factor-α, -β and fibroblast growth factor also play an important role in regulating the normal mammary growth and differentiation (e.g. Forsyth 1986; Oka *et al.* 1991; Plaut 1993; Stelwagen *et al.* 1993; Byatt *et al.* 1994).

2.4.1 STEROID HORMONES

2.4.1.1 Oestrogen

The effects of steroid hormones on the mammary gland development in the embryo and foetus have been studied in laboratory species. The growth of mammary gland during the early stage after conception is slow and is not influenced by pituitary hormones (Ceriani 1974). However, it is affected by ovarian (Raynaud 1971) and testicular hormones. Kratochwil (977) reported that when embryos are exposed to the oestrogenic hormones, either directly or via their mother, the development of the mammary gland is inhibited, growth of mammary buds remaining premature development for the nipples or teats in the embryos of both sexes (Raynaud 1971).

The development of the mammary gland during the prepubertal period is related to the concentration of steroid hormones, especially oestrogen. It is reported that ovariectomy reduced the growth of the mammary parenchyma where marked inhibition of ductal and end bud formation (e.g. Paape and Sinha 1971). A reduction of mammary gland growth is associated with the changes in the circulation of oestrogen and growth can be restored by oestrogen administration (Bresciani 1965; Haslam 1988a,b; Forsyth 1989; Woodward *et al.* 1991, 1993).

Development of the mammary gland during postpubertal period involves duct elongation and penetration of the mammary stroma (Linzell 1959; Cowie *et al.* 1980; Tucker 1981). With each oestrous cycle, the ovary releases oestrogen in concert with pituitary hormones, stimulating further growth and development of the mammary gland. During the first half of gestation, oestrogen predominates and is mainly responsible for the ductal growth. By the end of the fourth month, oestrogen levels drop, while progesterone is the dominant hormone. The pattern of the two steroids is reversed after parturition (Knight and Peaker 1982a; Del Vecchio *et al.* 1992). The concentration of ovarian steroid hormones before and after parturition is illustrated in Figure 2.3.

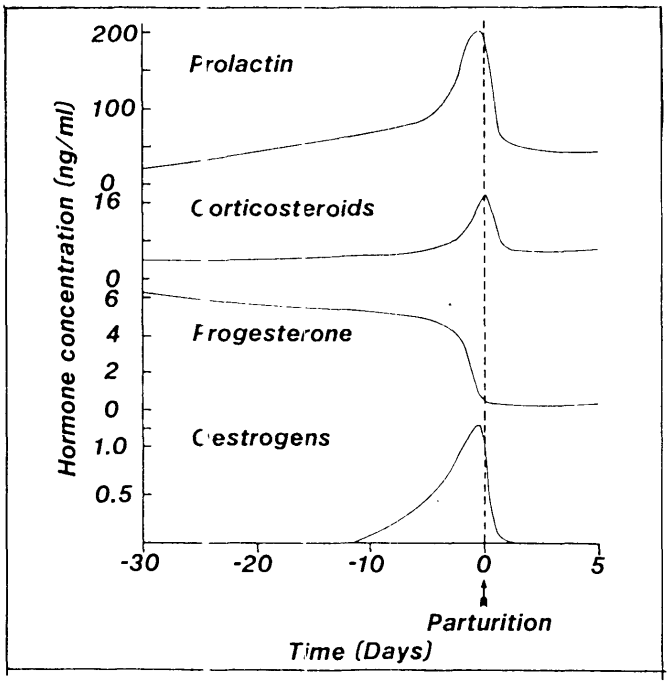

Figure 2.3: The concentrations of hormones for 30 days before and 5 days after parturition in the cow (Holmes and Wilson 1984).

Oestrogen promotes mammary gland growth by mediation at its biological effect via a receptor-mediated mechanism (Haslam and Shyamala 1979b). Oestrogen receptors appear in the adult mammary gland in both mammary epithelium, adipose and connective tissue components (Haslam and Shyamala 1981). Tucker (1981) reported that the proportion of these receptors increase with the increasing weight of the mammary tissue. The mechanism(s) by which oestrogen stimulates cell proliferation remain unknown. Studies in in-vivo and in-vitro have suggested that oestrogen can act directly on the mammary cell, since the proliferation of the mammary epithelial cell appeared in both experimental conditions (Haslam and Shyamala 1979b, 1981; Haslam 1988b).

2.4.1.2 Progesterone

Both in-vivo and in-vitro studies have showed that progesterone is importantly involved in promoting the normal mammary epithelial cell proliferation, particularly in the adult mammary gland (e.g. Wang *et al.* 1990). Progesterone appears to be predominate during the luteal phase and this steroid hormone stimulates the development of lobulo-alveolar cells prior to pregnancy (Nickerson 1992). For example, progesterone given to the adult mice increased in the amount of branching of the ductal tree, indicating an increase of percent labelled DNA synthesis (Tucker 1981; Haslam 1988a).

The role of progesterone in promoting cell proliferation in the mammary gland have been elucidated in a number of studies. Similarly to oestrogen, progesterone also mediates its effects via a receptor-mediated mechanism. Haslam and Shyamala (1979a,b) have demonstrated that progesterone receptors are contained in mammary tissue, and the concentration of these hormone receptors varied depending upon the mammary tissue types relating to the stages of mammary development. A number of workers have reported that a combination of oestrogen and progesterone cause a dramatic increase in synthesis of mammary epithelium over that of progesterone-treated or oestrogen-treated groups (Haslam 1988a; Fowler *et al.* 1991a; Woodward *et al.* 1993). Bresciani (1965) and Tucker (1981) reported similar results with progesterone combined with oestrogen promoting the full development of lobulo-alveolar, indicating a synergism between the two steroid hormones.

In vitro studies on laboratory animals have shown that oestrogen and progesterone stimulate production of epidermal growth factor (EGF) and transforming growth factor (TGF)-α. Gow *et al.* (1991) and Plaut (1993) provided evidence that these growth factors are potent effectors of mammary growth. Both EGF and TGF-α induced a mitogenic response and caused ductal growth and end bud formation. Nevertheless, the response to EGF and TGF-α may be related to the stage of proliferation of the gland and local concentration of the growth factors (Plaut 1993).

2.4.2 POLYPEPTIDE HORMONES

2.4.2.1 Prolactin

The secretion of prolactin is influenced by a numbers of stimuli both from the environment and from endogenous hormones. Environmental stimuli include those of suckling, milking, stress or pain, thermal stimuli and day length (Hart 1976; Hart and Morant 1980; Bruckmaier *et al.* 1992; Sakai 1994). Endogenous hormones levels change in the pattern and concentration according to the stage of reproduction. The pituitary prolactin concentrations of heifers increase 333% between birth to three months of age and is maximum at nine months, associated with the greatest development of mammary gland (Sinha and Tucker 1969a).

Prolactin is necessary for the full lobulo-alveolar development of the mammary gland (Akers 1985), if no other lactogenic hormones are present (Schams *et al.* 1984). Cowie *et al.* (1966) supported this observation reporting that hypophysectomized goats required prolactin for the normal mammary gland development. An intermediate degree of differentiation occurred when cultured mammary tissues, were exposed to various combinations of oestradiol, progesterone, aldosterone, insulin and thyroxine, but not prolactin or growth hormone (Warner 1978). In non-pregnant ewes (Schams *et al.* 1984) lobulo-alveolar development, is restricted if prolactin secretion is reduced (Byatt *et al.* 1994).

However, in pregnant animals, direct infusion of prolactin (Collier *et al.* 1993) or suppression of prolactin secretion by bromocriptine treatment (Schams *et al.* 1984) has no affect on growth of the mammary gland. The lack of mammogenic response to prolactin in the bovine tends to support the idea that prolactin is not directly mammogenic in the bovine mammary gland (Collier *et al.* 1993; Byatt *et al.* 1994). Growth of the mammary gland during late pregnancy is associated with moderate or low concentrations of prolactin in plasma (Delouis *et al.* 1980). Hence, Byatt *et al.* (1994) and Hooley *et al.* (1978) suggested that if prolactin is required for mammogenesis, then the effect of this hormone is maximal at very low concentration. This finding may suggest that a lactogenic hormone produced by the placenta, such as placental lactogen, may play a major role in mammogenesis during pregnancy (Collier *et al.* 1993; Byatt *et al.* 1994).

2.4.2.2 Placental lactogen

Placental lactogen is secreted by the foetal troproblast and is detectable in both the ovine (Martal and Djiane 197?) and bovine (Flint *et al.* 1979). The concentration of placental lactogen in the foetus decreases with advancing gestation (Byatt *et al.* 1992c), while the quantities of placental lactogen in maternal circulation are low during the first half of gestation, increase in mid-gestation, and remain at these levels until parturition (Bolander *et al.* 1976; Buttle and Forsyth 1976; Hayden *et al.* 1979; Mellor *et al.* 1987).

The concentrations of placental lactogen in plasma is related to either foetal number or total foetal weight which are in turn correlated to total placental mass (Hayden *et al.* 1979). In ewes (Bulter *et al.* 1981) and goats (Hayden *et al.* 1979) carrying multiple offspring placental lactogen levels were higher than for those carrying only a single foetus. In dairy and beef cattle, serum levels in twin pregnancies were approximately twice that in animals with a single foetus (Bolander *et al.* 1976).

Akers (1985) reported that this lactogenic hormone is implicated in the preparation of the mammary gland for lactation, stimulation of steroidogenesis, foetal growth, and alteration in maternal metabolism to support the foetus. Thereafter the presence of a

placental lactogen is possibly required for full lobulo-alveolar growth to occur (Hart and Morant 1980). In in-vitro study illustrated that recombinant bovine placental lactogen (rbPL) stimulated significant increases in total mammary DNA above a control group (Byatt *et al.* 1992a, 1994). Vega *et al.* (1989) showed that placental lactogen appeared to be mammogenic for bovine mammary transplants in nude mice. In addition, the mammary tissue from these animals had a high degree of lobulo-alveolar development and contained the secretion within alveoli (Byatt *et al.* 1994). Therefore, it is likely that this lactogenic hormone has a role in stimulating mammogenesis in animals (Schams *et al.* 1984; Byatt *et al.* 1992a,b,c, 1994).

Hayden *et al.* (1979) reported that the concentrations of placental lactogen for pregnant cows remained low throughout the gestation period, whereas Forsyth (1986) reported that higher levels can be measured in late pregnancy. Buttle and Forsyth (1976) and Byatt *et al.* (1992c) reported that the concentration of placental lactogen in cows is about 10- to 1000-fold lower than in sheep and goats. Thus, it seem reasonable to conclude that placental lactogen may have little direct effect on mammogenesis and lactogenesis in cattle (Akers 1985).

2.4.2.3 Growth hormone

Growth hormone (GH), or somatotropin (ST), is secreted by the anterior pituitary gland after stimulation by growth hormone-releasing hormone (GHRH, or GRW) (Ruckebusch *et al.* 1991) There is an obvious increase in plasma GH during gestation and a subsequent decline during the postnatal period. Growth hormone increases again during late prepubertal life, and the adult secretory patterns can be observed after the onset of puberty (Jansson *et al.* 1985). The concentration of growth hormone in ruminants remains low throughout gestation, rising 1 to 2 weeks before parturition (Bassett *et al.* 1970; Oxender *et al.* 1972) (Figure 2.3).

Endogenous bovine growth hormone concentration is positively correlated with the weight of mammary secretory tissue in growing dairy animals. In normal bovine mammary tissue, somatotropin receptor gene expression is primarily localised to the

alveolar epithelial cell region (Glimm *et al.* 1990). The serum concentrations of growth hormone are associated positively with growth of parenchymal tissue, suggesting that growth hormone may play an important role in mammogenesis in young animals at the time of puberty (Sejrsen *et al.* 1983; Johnsson *et al.* 1986).

It has been established that the administration of bST stimulates the growth of mammary parenchyma in primigravid ewes (Stelwagen *et al.* 1993) and heifers (Stelwagen *et al.* 1992) during the latter stage of gestation. This is consistent with the earlier hypothesis regarding the mechanism of action of bovine growth hormone probably enhancing mammary alveolar-lobular development (Asimov and Krouze 1937). Nonetheless, there is no direct evidence to indicate that growth hormone increases the number of secretory cells in the mammary gland of lactating animals (McDowell 1991)

Growth hormone operates on all cells in the body which are GH-dependent; primarily in the liver (Gluckman *et al.* 1987). The direct action of hormones on mammary tissues require the presence of specific high affinity receptors. Mammary cells contain various of hormone receptors, such as oestradiol, progesterone, but apparently not growth hormone (Akers 1985). The results of GH receptor binding studies, using bovine mammary membranes, have lead to the conclusion that growth hormone does not directly regulate mammary development in ruminants (Gluckman *et al.* 1987; Glimm *et al.* 1990; Ruckebusch *et al.* 1991).

There is increasing evidence that many of the actions of growth hormone are mediated by somatomedins, IGF-I and -II (McDowell 1991; Ruckebusch *et al.* 1991). Insulin-like growth factor-I (IGF-I) is produced by the stimulation of the GH binding to specific plasma membrane. Growth hormone and IGF-I are needed for cell proliferation and differentiation (Collier *et al.* 1993). Growth hormone directs the precursor cell to a specific pathway of differentiation while IGF-I enhances growth and replication by either or both autocrine and paracrine effects (Ruckebusch *et al.* 1991). Fibroblast growth factor (FGF), which is known as insulin-like growth factor-II (IGF-II), has been suggested to be mammogenic in this system, but its proliferative activity is most likely due to activation of the IGF-I receptor (Cullen *et al.* 1990, Stelwagen *et al.* 1993).

Therefore, it can be concluded that insulin-like growth factor I acts progressively in the growth of mammary tissue (Gluckman *et al.* 1987; Glimm *et al.* 1990; Stelwagen *et al.* 1992, 1993).

2.5 CONCLUSION

The normal development of mammary gland varies with the reproductive phase of animals. Proliferation of mammary tissues begin very soon after conception. However, the full mammary development only occurs during pregnancy. In late pregnancy, the mammary gland is nearly completely developed and may begin to function. Changes in mammary growth and function are determined as a result of interaction between peptide and steroid hormones, such as oestrogen, progesterone, prolactin, placental lactogen and growth hormone.

Chapter 3

FACTORS AFFECTING MAMMARY DEVELOPMENT AND MILK PRODUCTION

3.1 INTRODUCTION

The output of milk from dairy animals is affected by many factors. These factors can be divided into two broad areas, namely physiological and environmental factors. The physiological or non-nutritional factors include both genetic and non-hereditary factors such as age at calving and lactation number.

3.2 NON-NUTRITIONAL FACTORS EFFECTS ON MAMMARY DEVELOPMENT AND MILK PRODUCTION

3.2.1 PHYSIOLOGICAL FACTORS

3.2.1.1 Genetic selection

The differences in yield and composition of milk between and within breeds are due to genetic variation. Much of this variation is expressed through differences in the amount of secretory tissue present at parturition or its activity (Wilde and Peaker 1990) and is also related to cisternal storage characteristic (Bruckmaier *et al.* 1994; Knight and Dewhurst 1994). Moreover, the genetic control of lactogenesis is signalled to the mammary gland by the endocrine system (Wilde and Peaker 1990; Min *et al.* 1993). Barnes *et al.* (1983, 1985) and Kaemer *et al.* (1986) reported that heifers and cows sired

by bulls with a high genetic potential for milk production had higher concentrations of growth hormone than animals sired by bulls from a randomly bred control population (Beerepoot *et al.* 1991). Thus, it may be possible to use physiological traits as a predictor of milk production (Barnes *et al.* 1985; Klindt 1988).

3.2.1.2 Age at first calving

Age at first calving has been an important management consideration for replacement beef and dairy heifers (e.g. Gill and Allaire 1976; Patterson *et al.* 1992). Heifers are expected to reach puberty at 12 months of age and also to reach a target body weight by this time (182-204 kg for Jersey; 250-273 kg for Friesian; 290 kg for beef heifers) (Swanson *et al.* 1967; Marston *et al.* 1995). Milk production from heifers tend to increase with increasing age at first calving (Gill and Allaire 1976; Lin *et al.* 1988; Moore *et al.* 1991). The possible reason for the higher production may be due to the older heifers being closer to mature body size and thus less likely to divert nutrients into body tissue growth than younger heifers (Holmes and Wilson 1984). However, this advantage is lost in the second and third lactations (Lin *et al.* 1988), since the reduction in the level of milk yield and composition occurs when cows reach their mature age at about 6 years of age and the decline continues throughout the remainder of life (Schmidt and Van Vleck 1974).

3.2.1.3 Stage of lactation

Milk production and composition varies with stage of lactation and lactation number. Milk yield increases rapidly after parturition rising from 50 to 80 percent of the maximum daily yield within a few days, reaching a peak at three to six weeks after calving followed by a gradual decline in yield for the rest of lactation (Hurley 1989). Increases in milk yield occur with successive lactations up to about the fourth or fifth in dairy cows (e.g. Schutz *et al.* 1990; Ray *et al.* 1992) and increase from the first to the third lactation in goats (Bruckmaier *et al.* 1994) and markedly decreased thereafter. The shape of the heifer's lactation curve differs from that of the older cows with peak

production lower and the curve flatter. This may be a result of differences the amount of secretory tissue (Holmes and Wilson 1984).

3.2.1.4 Dry period

The non-lactating period (45-60 days) is an important phase of the lactation cycle of the dairy animal. This is believed to be necessary partly to replenish body reserves, partly to induce both involution and proliferation in the mammary gland (Smith *et al.* 1967; Fowler *et al.* 1991b), and partly to gain maximum benefit from the lactogenic endocrine hormones around parturition (e.g. Knight and Peaker 1982a,b). It is possible that the removal of secretion from the mammary gland in late pregnancy directly affected the normal physiological function of mammary tissue by suppressing involution in the mammary gland (Smith *et al.* 1967; Fowler *et al.* 1991b) with a resultant depression in the mammary secretory tissues.

3.2.2 ENVIRONMENTAL FACTORS

3.2.2.1 Environmental condition

Ambient temperature is the single most important climatic factor that influences animal production, including milk secretion and composition (McDowell 1972). Moore *et al.* (1992) indicated that a maximum temperature above 32°C in the last 60 days prepartum had negative influence on milk and fat production in early and mid-lactation. The effects of high ambient temperature on the physiological function of animals has been the subject of extensive research. Alexander and Williams (1971) and Collier *et al* (1982) indicated that heat stress during pregnancy altered endocrine dynamics and also reduced placental weight and function, and uterine blood flow. As reported earlier placental function is related to the mammary growth and therefore to postpartum milk yield and consequently reduced placental function is likely to depress mammary growth and subsequent milk output (Collier *et al* 1982).

3.2.2.2 Milking practice

Regulation of milk yield by milking frequency has been studied extensively in cows (Hillerton *et al.* 1990; Carruthers *et al.* 1993), goats (Henderson and Peaker 1987), and sheep (Morage 1968). There is evidence to show that more frequent milk removal allows a maximal rate of milk secretion for a greater proportion of the time, whereas less frequent removal causes secretion to be restricted (Hillerton *et al.* 1990; Carruthers *et al.* 1993; Knight *et al.* 1994). This is in agreement with previous studies where the changes in milking frequency increased the activity of existing mammary cells, with further differentiation of these cells, and an increase in the number of mammary cells (Hillerton *et al.* 1990; Wilde and Peaker 1993).

3.3 NUTRITIONAL FACTORS INFLUENCING MAMMARY DEVELOPMENT AND MILK PRODUCTION

Nutrition is another factor which may influence mammary development and subsequently milk production. The effect of nutrition on mammary growth has been reported in several studies; for example, the udder parenchymal tissues tend to increase markedly in feed restricted animals due to the increase in concentration of plasma growth hormone. The effect of nutrition seems likely to be a major factor influencing milk production and composition since milk is produced from nutrients which are extracted from the blood. Animals require five major classes of nutrients for maintenance, growth, reproduction and production. High producing animals have a large demand for these nutrients, particularly energy and protein. A shortage or an imbalance in the supply of energy, protein and vitamins or minerals may subject the animals to nutritional stress, resulting in metabolic disorders or decreased milk production. Requirements for these nutrients depend largely on yield and composition of milk, and body weight and condition of producing animal.

3.4 FEEDING MANAGEMENT FOR THE PREGNANT ANIMAL

3.4.1 FEEDING DURING EARLY PREGNANCY

In early pregnancy (first 15 days), the demand for nutrients for the conceptus is small and is not related to additional requirement for the nutrients of the mother (Robinson 1985). Therefore, the effect of early nutrition on the performance of offspring are unlikely to be direct result of inadequate nutrition at this time.

During the second and third months, animals that are in good body condition at mating can lose liveweight without any adverse effect on placenta and foetal growth (Robinson 1985). However, this is not the case when animals have severely depleted body reserves. Data obtained by McCrabb *et al.* (1990) and Ehrhardt *et al.*(1991) indicated that placental growth is increased to a maximum weight by about mid-pregnancy and there is a decline in weight during the final stage of pregnancy (Barcroft and Kennedy 1939). Undernourished ewes are likely to have impaired growth of the placenta and subsequently reductions in foetal weight (e.g. Clark and Speedy 1980). McCrabb *et al.* (1992) illustrated this in a study where ewes restricted fed during mid-pregnancy had lower body weight and placental weight than well-fed ewes. They concluded that mid-pregnancy is the critical time when the placental growth is sensitive to the maternal undernutrition.

The reason for the reduction in placental size is not fully known, but may be the result of low energy intake providing adequate microbial protein to meet the net protein requirements for tissue maintenance (Robinson 1985). Chronic feed restriction is also associated with a reduction in uterine blood flow which in turn limits the uptake of substrate by the maternal reproductive tissues and the conceptus (Morris *et al.* 1980). Oddy and Holst (1991) also reported that maternal feed restriction during mid-pregnancy alters endocrine factors which mediate the pattern of nutrient use in the dam and may alter the homeorhetic regulation of foetal growth (Bauman and Currie 1980).

3.4.2 FEEDING DURING LATE PREGNANCY

The uptake of nutrients by the uterus increases with advancing pregnancy and it utilised glucose and amino nitrogen throughout gestation (Reynolds *et al.* 1986). Bell (1984) showed that the uptake of glucose by the utero placenta increases 3-fold during the last trimeter of gestation. In addition to the large demands for amino acid some fatty acids are used by the foetus over the last third of pregnancy when most of the foetal tissue is synthesised (Oldham 1986; Preston and Leng 1987). The major source of foetal amino acids is supplied from the maternal dietary protein. It was found by Bassett (1980) that amino acid nitrogen concentrations in foetal blood are higher than those in maternal blood and these amino acids are actively accumulated by foetal placental tissue from the maternal circulation.

Feed intake is usually decreased 30 to 35% during the last 3 weeks prepartum (Grummer 1995), and this results in an increase in the depletion of maternal tissues. Mellor and Matheson (1979) reported that a severe and sudden restriction in feed intake at the beginning of the final month of pregnancy can reduce foetal growth by 30-40% within three days or can cause a complete cessation in growth if this restriction is extended, while the effects of chronic undernutrition influence birth weight by a gradual decrease of prenatal growth (Rattray *et al.* 1974; Mellor and Murray 1981, 1982). Study of Robinson (1977) showed that a delay until last month before lambing in the introduction of a high plane of nutrition resulted in lost of the ewes' body weight and this lead to a reduction in lamb birth weight.

In addition, since most of the lobule-alveolar epithelial cell of the mammary gland in ruminants takes place during late pregnancy, it is clear that undernutrition in late pregnancy reduces mammary development and colostrum production (e.g. Hall *et al.* 1992). Rosso *et al.* (1981) reported that in laboratory animals fed 50% of their normal food intake during pregnancy, mammary DNA was only 70% of that control nonrestricted animals. Pyska and Styczynski (1979) reported a similar result where lower dietary protein in the diet also lead to a reduction in mammary gland growth in virgin and

pregnant animals. Therefore, it seems that higher priority is given to an increase in the level of feeding during this stage of pregnancy.

In general, feeding dairy animals is considered as a simple two compartment system, i.e. if energy intake does not equal energy output, the excess energy is stored in body reserves (fat and protein) and these reserves are subsequently mobilised when the intake of energy does not meet the requirements (Wright *et al.* 1986; Garnsworthy 1988). McCrabb *et al.* (1992) reported that as a result of feeding during the last trimester liveweight of previously feed restricted ewe liveweight increased due in part to repletion of maternal muscle tissue of these ewes. These authors indicated that ewes previously feed restricted were apparently able to utilise additional nutrition during late pregnancy for use by the maternal body and maintaining foetal growth. Kelly *et al.*(1992) observed a similar response in ewes liveweight when they were supplemented during late pregnancy following loss in liveweight during mid-pregnancy.

The body condition and liveweight of animals at parturition is the important factor affecting subsequently milk yield (Grainger and McGowan 1982). Hutton and Peaker (1973) reported that during the last eight weeks before calving cows should not lose body condition. Frood and Croxton (1978) suggested that cows calving with a condition score of 3.5 produced more milk than those calving with a condition score below 2. Studies of Grainger *et al.* (1982) showed that animals in lower body condition partitioned a higher proportion of feed energy at the expense of milk fat production to body condition than those in higher body condition. It is likely that if animals are well fed and are in good condition prior to parturition body reserves will be used to support the energy demand in early lactation when dietary intake is inadequate (Grainger and McGowan 1982; Garnsworthy 1988).

3.4.3 FEEDING DURING THE LACTATION PERIOD (POSTPARTUM)

During the early stages of lactation the demand for nutrients by the mammary gland are extremely high. As the cow goes from a non-lactating state to peak milk production of 35 to 50 kg daily, the nutrient requirement of the cow increases 300-700% as a direct result of the requirement for milk production (NRC 1978). For animals to survive during this period without severe metabolic problems and to attain their peak milk production, it is essential that a diet properly balanced in all nutrients be fed *ad libitum* (Clark and Davis 1980; Bryant and Trigg 1982).

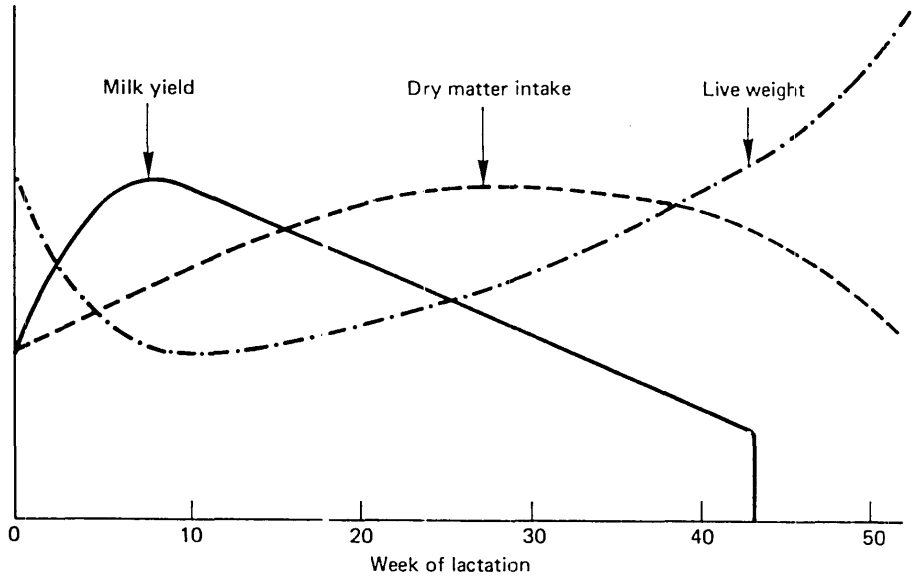

Figure 3.1: The relationships between dry matter intake, milk yield and liveweight (Garnsworthy 1988).

Even when feeding at a level recommended by NRC (1978), lactating cows may suffer from a shortage of energy and protein because maximum intake of dry matter does not occur until after the cow has reached peak milk production (Holmes and Wilson 1984). Bines (1976) found that the peak milk yield occurred between 5 and 7 weeks after calving, whereas the time of maximum food intake was variable, ranging from 5 to 36 weeks (Marston and Lusby 1995). The relationships between voluntary food intake, milk yield and live weight throughout the lactation cycle is shown in Figure 3.1.

Amaral-Phillips *et al.* (1993) reported that cows in early lactation which were in negative energy balance were able to adjust their metabolism by utilisation of body tissues to supply nutrient needs, e.g. glucose. Data obtained by Bryant and Trigg (1982) reported that the mobilised body reserve, represented as a live weight loss, has an energy value 20-30 MJ and is utilised for milk production with an efficiency of 80-85%. Animals may continue to produce milk only as long as they mobilised energy stores. If animals have to rely too heavily upon body stores of energy and protein, either milk production will be held to the level of nutrient availability or they will accede to a metabolic disorder such as ketosis (Clark and Davis 1980; Leng and Nolan 1984; Hutjens 1989).

3.5 NUTRIENT REQUIREMENTS IN LATE PREGNANCY

With the current emphasis on increasing reproductive efficiency, the effect of the timing of supplementary feeding on foetal growth and subsequent milk production is likely to be critical, particularly in those animals kept in adverse environments; however, this is an area that has received little attention. In the available studies (e.g. Robinson 1982, 1983, 1985) have suggested that the pregnant animals must be fed a balanced ration during late pregnancy until the time of parturition in order to maximising body reserve for the subsequent production of healthy young and higher total lactation yield.

The main source of foods for ruminants are pasture grass and by-products of plants. The foodstuffs are comprised of many chemical substances which can be classified into 5 major groupings: water (80-85%), carbohydrates (10%), proteins (3%), fats (1%) and minerals (1.5-2%) (McDonald *et al.* 1995). These components, together with vitamins, supply the animals with their requirement for production either directly or by supplying essential precursors for synthesis. In normal condition most rations provide all of the minerals and vitamins in adequate amounts, therefore the most limiting factors in the feeding rations are energy and protein (e.g. Sklan *et al.* 1991; Cadorniga and Satter 1993; Karunanandaa *et al.* 1994). Very few researchers have investigated the influence of diet during prepartum of cattle on subsequent lactation and reproduction performance. Most information available on prepartum feeding come from sheep studies.

3.5.1 ENERGY REQUIREMENT

The most common manifestation of nutritional deficiency in ruminants is lack of energy which may result from lack of sufficient feed or low on net energy available to the animal from the feed consumed. Inadequate amounts of feed may result from drought or from the low dry matter content of roughages. Digestibility decreases because of the increase in lignification as plants mature, while intake decreases with reduced digestibility, slower passage through the digestive tract, and with a reduction in the palatability of the feed. The energy requirements for animals during the first half of gestation is calculated to slightly exceed those necessary for maintenance. However, during the last month of gestation, energy requirements increase to provide adequate energy for the growth of foetus(es).

McClelland and Forbes (1973) observed that feeding low daily ME intakes for 3 weeks followed by a high level during the last 3 weeks before parturition resulted in better than those fed a constant daily intake of ME throughout the last 6 weeks of gestation. Data obtained from McDonald *et al.* (1995) suggest that the daily requirements for a 55 kg pregnant ewe is 9.9 MJ in week 6 prior to lambing and increase to 12.8 MJ for the last 2 weeks of gestation. Similarly, energy requirement for pregnant cows during the last two month of pregnancy is 72 MJ and rise to 85 MJ in the final month of gestation.

3.5.1.1 Energy nutrition

If prepartum feeding is important, then inadequate nutrition during this period should influence lactation, health and reproductive performance. For example, it is noted that serve undernourishment of dam during late pregnancy has a greater influence on the development of the foetus than on its survival. There are a small number of studies which have specifically examined the influence of dry matter or energy intake during the late pregnancy until the first few weeks of lactation. Consequently, few data are available to document the importance of feeding at this time.

3.5.1.2 Effects of dietary energy

Availability of nutrient reserves to support early lactation depends on late pregnancy diet quality and feeding management. A numbers of experiments have been concerned with energy status of the late gestation diet. Ferguson *et al.* (1990) have summarised the benefits of inclusion of supplementary energy in dairy cattle rations: 1) increased caloric density without compromising fibre (Palmquist and Jenkins 1980), 2) increased energy intake when animals fail to consume sufficient feed as gestation progressed, 3) increased efficiency of energy utilisation (Sklan *et al.* 1991), and 4) enhanced ratio of lipogenic to glucogenic (Kronfeld *et al.* 1980). Kellaway and Porta (1993) reported that feeding a high energy diet such as fat can reduce the disease incidence and under certain circumstance improve reproductive performance.

Steel and Leng (1973a) have noted that the level of dietary energy intake influences quantities of gluconeogenic precursors, particularly propionate, which are available for glucose synthesis. Steel and Leng (1973b) reported that the proportion of glucose may vary between 19 to 62% and is dependent in the rate of propionate production which is significantly affected by plane of nutrition. Because little or no glucose is absorbed from the gastrointestinal tract of the ruminant animal, foetal glucose needs as well as those of the dam must be met by synthesis of glucose from gluconeogenic precursors. It is reported that glycogen is a primary energy reserve in the foetus and may be an importance energy source for new born animals. In early gestation, very little glycogen is present in liver and skeletal muscle of the foetus, but in late gestation, glycogen in the liver increases rapidly and reached at least twice adult concentrations (Prior and Christenson 1976).

Treacher (1970) and Thomson and Thomson (1973) indicated that nutritional treatments resulting in subnormal liveweight gain in the ewes and cows during the last trimester of pregnancy caused a reduction in the birth weight of lambs and calves, particularly multiple offspring. In contrast, Prior and Christenson (1976) estimated that a high level of dietary energy intake by ewes during the last 6 weeks of gestation will result in increase availability of gluconeogenic precursors which accounts for the increased

glucose production and subsequent increased lambing performance. Therefore, it is likely that lambs from ewes on a high plane of nutrition will have more muscle glycogen, an energy source for use immediately after birth and available for maintenance of body temperature (Alexander *et al* 1956) which consequently the improves lamb survival (Christenson and Prior 1976).

During late pregnancy, foetal metabolic rate is approximately twice that of the dam (Reynolds *et al.* 1986) and most of the nutrients required for foetal growth and metabolism is supplied by maternal glucose and amino acids. Increases in maternal requirements for these nutrients is met partly by increased voluntary intake and partly by an array of maternal metabolic adaptations which include increased hepatic gluconeogenesis from endogenous substrates decreased peripheral tissue glucose utilisation and increased fatty acid mobilisation from adipose tissue (e.g. Prior and Christenson 1976; Bell 1995).

Circulating levels of nonesterified fatty acids (NEFA) and ketones tend to be elevated during late pregnancy even in animals fed to predicted energy requirement for conceptus growth and maintenance of maternal tissues (Petterson *et al.* 1994). This event becomes more evident during late gestation and is greatly exaggerated if energy intake is voluntarily or involuntarily restricted (Prior and Christenson 1976; Lee and McIntosh 1982; Grummer 1995). Therefore the metabolic patterns in maternal tissues of well-fed ruminants in late pregnancy are characterised by increased hepatic gluconeogenesis, reduced glucose utilisation in peripheral tissues, unchanged or decreased peripheral utilisation of acetate, and moderately increased mobilisation of NEFA from adipose tissue. Conversely, for undernourished dams to maintain a uterine glucose supply that is adequate to support normal conceptus growth in late pregnancy, this can only occur at the expense of maternal fat reserves (*see* review by Bell 1993, 1995). Similarly, Forbes and Singleton (1964) and Lee and McIntosh (1982) reported that failure to increase energy intakes to meet the needs of the rapidly growing foetus(es) is reflected in either a gradual slowing down of prenatal growth or mobilisation of body reserves of ewes which may result in pregnancy toxaemia.

Increasing dietary energy during late gestation resulted in increases in body weight and body condition score of cows (DelCurto *et al.* 1990; Garnsworthy and Jones 1993; Marston and Lusby 1995). However, Lusby *et al.* (1991) reported that cow BW and BCS were not improved when they were supplemented during lactation with an increased level of energy intake, suggesting that responses in BW and BCS differ with physiological status (Marston and Lusby 1995). Similar observation of liveweight gain is presented by Prior and Christenson (1976) when ewes were fed different levels of dietary energy, 60 (low), 100 (medium) and 140% (high) of the recommended daily digestible energy requirements of ewes, during the last 6 weeks of gestation (NRC 1968). Ewes fed the low energy ration gained less weight than those fed the medium and high energy level.

3.5.2 PROTEIN REQUIREMENT

Robinson (1983) indicated that it is generally accepted that basal feed, i.e. roughages and cereal grains, must contain at least 10 g of crude protein/MJ of ME to provide the maximal synthesis of microbial protein, that is, about 80% of microbial protein is in the form of amino acids and these together with the 20% undegraded protein in the basal feed are available for absorption in the small intestine. This amount of protein is necessary for ewes maintaining their body condition and protein status during pregnancy (Robinson 1985). Ruminants digest and absorb the microbial protein, producing a source of essential amino acids, even when the diets may not contain adequate amounts. The requirements of digestible crude protein (DCP) for ewes during late pregnancy is very variable. Robinson and Forbes (1968) have calculated that the maximum efficiency of utilisation of metabolizable energy (ME) for a 68 kg ewe occurred when the crude protein intake was 81 to 98 g per day, similar to that suggested by NRC (1964). However, successful reproduction can be achieved on much lower protein intakes. Forbes and Robinson (1967) have shown that low intakes of about 45 to 60g DCP per day in pregnancy have little effect on birth weight and early growth of lambs compared with intakes of 90 to 127g.

In addition to the protein accretion in the gravid uterus there is the requirement of protein for the udder and its secretion and this gives a total net protein requirement for pregnancy (NPR_p) (Figure 3.2). Data obtained by Rattray *et al.* (1974) and Robinson *et al.* (1977) show the net protein requirements for pregnancy which indicates the value for the gravid uterus and the udder as shown in Table 3.1. Robinson (1983) indicated that diets containing adequate protein for maximal synthesis of microbial protein (10 g CP/MJ ME) supply a net protein requirement of 5.7 g/MJ of ME, then pregnant ewes that are meeting all their energy requirements from the diet only require an additional supplement of undegraded dietary protein in the last three weeks of pregnancy. This is in agreement with the results reported by Ørskov and Robinson (1981). It should be noted that there is an extremely rapid increase in the net protein requirements in the last week of pregnancy to twice the 5.7g which is provided by microbial protein. This increase is related to the large demand of nutrients for udder growth and colostrum production in the udder (e.g. Robinson 1980).

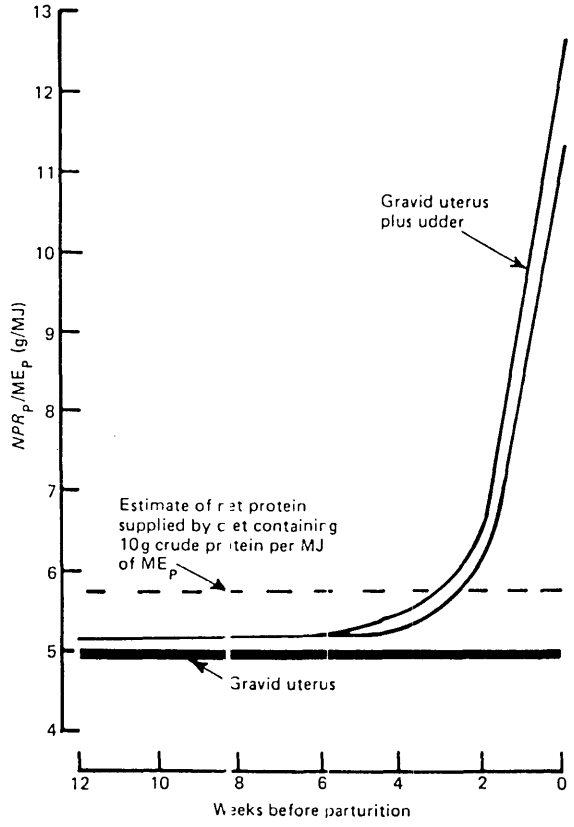

Figure 3.2: The net protein needs of the gravid uterus and udder (NPR_p) in single and quadruple-bearing ewes. Upper and lower lines refer to quadruplets and singletons, respectively (Adapted from Robinson 1985).

Ørskov and Robinson (1981) suggested the use of the net requirement of amino acid nitrogen as an index for expressing the animal's need for protein, that is, the nitrogen required for tissue maintenance and for the net synthesis for tissue, wool and milk. The requirements of amino acid nitrogen for sheep is affected by the physiological state (Figure 3.3).

Table 3.1: The net accretions of protein in the gravid uterus and udder, i.e. net protein requirements for pregnancy (NPR_p) in relation to the corresponding ME requirements ME_p (g/MJ) (adapted from Robinson 1983).

No. of	Stage of gestation (days)						
foetuses	60	74	88	102	116	130	144
1				5.2	5.2	6.0	11.3
2	5.2	5.2	5.2	5.3	5.3	6.1	11.7
3				5.3	5.3	6.2	12.1
4				5.3	5.4	6.2	12.6

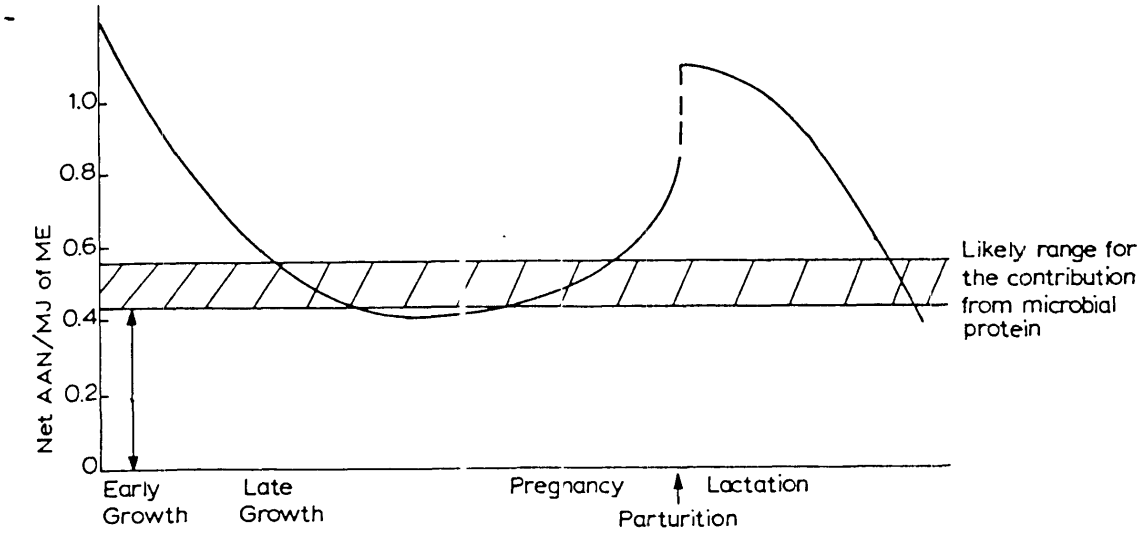

Figure 3.3: An illustration of the effects of age, level of production and physiological state on the net requirements for amino acid nitrogen (adapted from Ørskov 1970).

For example, sheep in late gestation require dietary protein supplements and the concentrations of dietary protein needed to supply a particular net requirement for amino acid nitrogen, depends on the degradability of the protein in the rumen (Figure 3.4) (Ørskov 1977). Therefore, from Figure 3.3 and Figure 3.4, it is important to note that the requirements for dietary protein should be determined in terms of degradable and undegradable protein, rather than total protein. It can be seen from Figure 3.4 that when an animal's requirement for amino acid nitrogen is high, this can be met by the addition of dietary crude protein which is low in degradability.

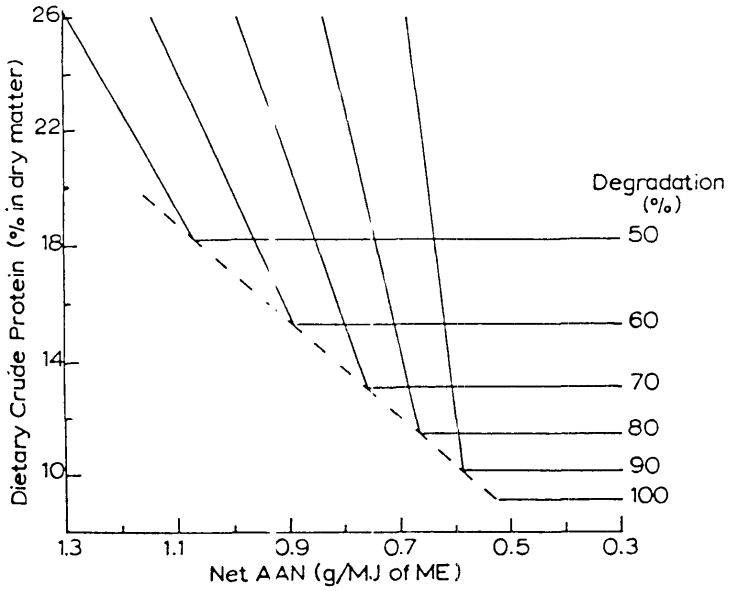

Figure 3.4: The effects of the degradation of protein in the rumen and net requirements for amino acid nitrogen on the crude protein needed in the diet (adapted from Ørskov 1977).

3.5.2.1 Protein nutrition

There is a number of important practical implications of feeding protein during pregnancy as suggested by Ørskov and Robinson (1981) and Robinson (1983). Firstly, during the first three months of pregnancy when it is usual to recommended maintenance levels of energy intake, basal diet which contains less than 10g CP/MJ of ME should be supplemented with urea in order to meet the minimum requirements for rumen degradable protein. Secondly, in the mid-pregnancy period, if the energy intake falls

below maintenance it is necessary to provide a supplement of dietary protein of low degradability in the rumen in order to prevent a loss of protein from the maternal body. Finally, the production requirements for protein in late pregnancy should be considered in relation to production requirements for energy.

3.5.2.2 Use of different sources of protein supplement

3.5.2.2.1 Soluble protein

The addition of grain to high forage diets will increase microbial protein synthesis (Tagari *et al* 1991). Results suggest that protein sources in the grain supplied more nitrogen in the rumen which in turn can increase synthesis of microbial and subsequent improvement in animal performance (e.g. Sánchez and Claypool 1983).

3.5.2.2.2 Protected protein

The bacterial protein synthesis in the rumen, under normal physiological conditions, provides approximately 60-70% of the protein needed to be absorbed from the intestine of high producing animals (Kaufmann and Lüpping 1982). The supplementation of diets for dairy animals with higher amounts of protein to the small intestine is justified because they require more protein than can be provided by rumen microbes (Leng 1992; Aldrich *et al.* 1993; Cadorniga and Satter 1993). The higher the dietary protein intake will be utilised for protein deposition in foetal membranes and fluids and uterine growth and it is possible that simultaneously this will improve postpartum lactation performance by minimising depletion of the maternal protein reserves (Van Saun *et al.* 1993; Metcalf *et al.* 1994).

3.5.2.2.3. Non-protein nitrogen

Non-protein nitrogen (NPN), such as urea, can be used instead of natural protein to provide a source of nitrogen for growth of rumen microbes. The reasons for feeding NPN in the diet is to reduce the need for preformed protein supplements when the supply

is not adequate or because of an economic advantage in the use of NPN (Clark and Davis 1980). According to NRC (1964), the quality of protein, i.e. level and balance of the amino acids, is not an important factor in ruminant nutrition in early pregnancy, with the quantity fed being more important than the source. It has been indicated earlier that ruminants do not efficiently digest the protein in poor quality roughages, however, urea can be used to replace up to one third of the protein equivalent of the ration of low protein and high energy (NRC 1964; Leng 1992). Efficiency of utilisation of NPN decreases as the amount in the ration increases, and as available energy content of the ration decreases (Oldham 1984; Preston and Leng 1987).

3.5.2.3 Effects of dietary protein

In a well nourished foetus, the substrate uptake of amino acids, which was based on measurements of foetal urea production, accounts for 30-40% of foetal oxidation. This is consistent with observations that foetal protein deposition accounts for, at most, 50% of the foetal net uptake of amino acids in sheep (Meier *et al.* 1981) and cattle (e.g. Ferrell 1991). However, data summarised by Bell (1995) suggest that only 32% of amino acid nitrogen taken up in the late gestation bovine foetus is deposited in tissue protein, indicating that the foetal requirement for metabolizable amino acid is approximately three times the net requirement for foetal growth. Bell *et al.* (1992) recently reported that an average rate of crude protein deposition of 74g/d in Holstein foetuses during late pregnancy, the mean birth weight of 45 kg is associated with the 220g/d of metabolizable amino acid requirement for foetal growth.

Ferrel *et al.* (1976) indicated that placental growth of the cow continues into late pregnancy until 230 days of gestation, but that the rate of growth accounts for a net accretion of no more than 7 g/d of CP. On the other hand, the sheep placenta does not grow during late pregnancy and has a net consumption of some amino acids, e.g. glutamine, citrulline, leucine, (Lechty *et al.* 1991; Battaglia 1992) that are used for placental synthesis and released in free form into maternal and foetal circulations (Holzman *et al.* 1977, 1979). Lemons and Schreiner (1983) reported that maternal undernutrition, or at least fasting for 5 days, has little effect on foetal uptake of amino

acids in late pregnant ewes, presumably because the active placental transport of most amino acids is independent of changes in maternal blood concentration (Bell 1993).

The most deficit nutrient during maternal undernourishment is glucose available for oxidation. It is reported that this glucose requirement consists of a component due to increased catabolism of amino acids at the expense of protein synthesis and deposition in foetal tissues. (Lemons and Schreiner 1983). In the studies by Liechty and Lemons (1984), during maternal fasting both maternal tissues and foetal tissues show a net release of amino acids (alanine and glutamine), which may be taken up by the foetal liver and utilised for glucose production. This lead to reduced foetal growth which was associated with increased synthesis and placental excretion of urea (Lemons and Schreiner 1983).

McNeill *et al.* (1994) observed that foetal growth between 110 and 140d was decreased by approximately 20% in twin pregnant ewes a diet low in protein (8% CP) but adequate in energy compared with ewes fed a protein adequate diet (12% CP) over the same period in late pregnancy, implying that the placental capacity to sustain amino acid transport of the maternal supply is limited. These studies indicated that the adverse effect on lamb performance of low plane feeding during late pregnancy is much more pronounced when accompanied by low protein intakes. Kleemann *et al* (1988) confirmed these observations, using highly prolific Booroola × Merino ewes, and reported an increase in birth weight of lambs in response to the prepartum feeding of lupin grain, compared with a cereal grain.

Barry and Manley (1985) have shown that while a high quality pasture could provide the glucose needs for the triple bearing ewes in late pregnancy there was a substantial deficit in the supply of amino acids, resulting in a daily loss of 8g N from the maternal body. Robinson and Forbes (1970) and Sahlu *et al.* (1995) further observed that the ability of milk synthesis in undernourished animals was lower than well-fed animals, and that this was probably due to low body protein reserves resulting from the limitation of protein intake during gestation. Therefore, it is likely that either glucose or amino acids can be

primary limiting nutrients of foetal growth during maternal energy or amino acid deprivation.

Improvement in animal performance determined after feeding a low rumen degradable protein was related to increased flow of protein reaching the small intestine (Kaufmann and Lüpping 1982; Amos 1986). Increasing of dietary protein may result in changes of body protein reserves which could effect milk production (Broster and Oldham 1977). The nutritional significance of reserves is to provide a source of readily available amino acids to animals during protein deprivation or stress.

Allison and Wannemacher (1965) reported that protein reserves have been divided into a highly labile fraction comprising approximately 5% of total body protein and total reserves which can be as great as 50% of body protein. Dairy animals could benefit from a protein reserve due to supplementation of dietary protein for milk production during early lactation. Swick and Benevenga (1977) suggested that the breakdown of muscle protein to provide amino acids for production of milk protein is a mechanism of normal metabolic adaptation. Meanwhile Hunter and Milson (1964) indicated that the body protein reserves not only supply amino acids for synthesis of milk protein but also supply necessary precursors for lactose synthesis up to 12% of the lactose in milk. Results from the studies by Botts *et al.* (1979) have estimated that approximately 25-27% of body protein mass may be capable of being mobilised to supply amino acids for synthesis of milk protein and also for milk lactose.

However, the higher percentages of protein intake that are fed to cows to increase the amount of total protein flowing to the small intestine has not always been advantageous. There is a small positive response in animal performances when a high proportion of protein is added to the diet (Kaufmann and Hagemeister. 1987). Sahlu *et al.* (1993) reported that animals consume excess protein relative to their requirements for maintenance and milk production without danger, but at high percentages of dietary CP the protein is not used. Holmes and Wilson (1984) indicated that in a situation where other form of energy are not available and where dietary protein is in excess of animal requirements, amino acids in tissue protein can be degraded with the accompanying

release of energy. The excess amounts of protein supplements are usually expensive and therefore are uneconomic energy sources. Also, the use of protein as a source of energy is inefficient. Waldo *et al.* (1991) concluded that excess dietary protein will not increase protein deposition unless total energy deposition increases and therefore it seems that fat and protein deposition are negatively correlated.

Aldrich *et al.* (1993) has suggested two plausible explanations for the lack of response to increase undegradable intake protein. First, microbial protein synthesis is reduced because substitution of UDP sources for degradable protein sources deprives rumen microbes of adequate available nitrogen (Armentano *et al.* 1993; Robinson *et al.* 1994). Consequently, the supply of protein to the animals may not be changed. Another explanation is higher UDP fails to increase the flow of essential amino acids, such as arginine and methionine, to the small intestine (Dhiman *et al.* 1993; Karunanandaa *et al.* 1994). Thus animal production may not be expected to increase as a result of inclusion of protein in the diet.

3.5.3 NITROGEN AND ENERGY RATIO (P:E RATIO)

The relationship between the nitrogen and energy requirement is complex and not well understood. Clark and Davis (1980), in summarising data from a number of studies, demonstrated that the optimum ratio of nitrogen to energy for the lactating cow is complicated because 1) there are two requirements, one for the rumen microbes and another for the host animal, and 2) there are many diverse pathways and functions for which nutrients are used including milk production, maintenance, and body tissue loss or gain (Oldham 1984). Furthermore, the mechanism(s) that regulate the partitioning of nitrogen and energy for various body functions are not known.

The ratio of nitrogen to energy has a significant effect on utilisation of both nitrogen and energy in the rumen (Preston and Leng 1987; Aldrich *et al.* 1993). It is reported that a shortage of nitrogen in the rumen will decrease cellulose and fibre digestibility. On the other hand, nitrogen utilisation is reduced when energy is limiting for rumen microbes

(Chowdhury *et al.* 1991; Lobley 1991). Gruber *et al.* (1991) and Dado *et al.* (1993) supported this observation that the microbial growth also requires a source of energy (ATP) usually utilisation of dietary energy for biosynthesis of cell material. To achieve optimal growth, the rate of ATP production (CHO fermentation) must equal its utilisation (Protein synthesis). Therefore, an excess or deficiency of nitrogen in the ration can also cause a reduction in overall efficiency of utilisation of energy by dairy animals.

3.6 CONCLUSION

There are number of factors which influence the output of milk from dairy animals. These can be categorised into two board areas: non-nutritional and nutritional factors. Non-nutritional factors affect both milk production and composition, including difference in genetic potential and physiology of animal before and after gestation. Of these factors, disease (e.g. mastitis) is another factor influencing the normal milk production. The major effects of mastitis include a decrease in milk production and change in milk composition (Houben *et al.* 1993).

It is worthy of note that one of the most significant factors influencing the production and composition of milk is adequate and balanced nutrition. High producing dairy animals have a large demand for the essential nutrients, particularly energy and protein. In order to meet the nutrients demanded of animals, the flow of nutrients from rumen fermentation should be maximised before supplementing the diet with bypass source of protein and energy (Preston and Leng 1987; Aldrich *et al.* 1993). However, animals relying on grazed pasture may not be supplied with adequate amounts of nutrients, therefore the inclusion of supplementary feeds is needed to increase the nutrient levels to meet the animal requirements and consequently improve animal performance. Animals in good condition are able to mobilise their body reserve (fat and protein) to supply the amount of nutrients needed, both energy and protein in the late gestation. However, only a limited number of studies have examined the importance of prepartum feeding on lactation performance particularly the effect of protein(s). The main propose of the following experiment was to examined the effects of prepartum feeding of different protein sources on postpartum milking performance.