2. Literature Review

2.1. Glucose

Glucose is the major form of circulating carbohydrate in the blood. It plays an important role in cellular metabolism in that it is an energy source. Only 0 to 6% of glucose turnover in the ruminant is absorbed from the gastro-intestinal tract (Sutton, 1984). The major part of dietary glucose is used as an energy source by the rumen microbes. The rumen microbes are able to break down long chain carbohydrates from the diet into monosaccharides. These monosaccharides are then used to supply the microbes own energy requirements. The microbes catabolise glucose to form pyruvate. The pyruvate is metabolized via three different pathways to form volatile fatty acids (VFA), namely acetate, butyrate and propionate. The volatile fatty acids are absorbed through the rumen wall. These VFA supply between 70 and 80% of the animals total caloric requirements. Propionic acid is carried to the liver where it is converted to glucose. Up to 20% of dietary carbohydrates are converted to propionate and up to 70% are converted to acetate (Lindsay, 1971). Acetic acid largely passes through the rumen wall to enter the blood stream. It is the only volatile fatty acid found in appreciable quantities in the peripheral circulation. It is phosphorylated to acetyl-CoA and enters the TCA cycle. It can also be used directly for the synthesis of milk fat, especially the short chain fatty acids. Butyric acid is absorbed as a ketone body, and is eventually metabolized to acetyl-CoA.

Miettinen and Huhtanen, (1996) conducted an experiment to determine the effects of the rumenal propionate to butyrate ratio on various blood metabolites and on milk production. It was found that increasing the butyrate portion led to decreased milk yield and decreased lactose content in the milk. There was also a decrease in the plasma glucose concentration.

2.1.1.Gluconeogenesis

Gluconeogenesis is the formation of glucose from various precursors. Because very little glucose is absorbed directly from the intestinal tract, the ruminant must depend on other compounds for glucose synthesis and therefore gluconeogenesis is of vital importance. Approximately 85% of glucose is produced in the liver and 15% is produced in the kidneys

(Maynard et al., 1979). The main glucose precursors in the ruminant are propionate, amino acids, glycerol and lactate. Propionate absorption varies with the kind and amount of feed eaten. Twenty to 56% of the total glucose synthesized is derived from propionate and 90% of all the propionate absorbed is removed by the liver for glucose synthesis (Lindsay, 1971; Bergman, 1983). Once the amino group has been removed from the amino acid, the carbon skeletons, which remain are eligible for entry into the tricarboxylic acid (TCA) cycle, to be used as needed. The amino group is converted to urea and excreted. Fifteen to 32% of the total glucose production is derived from amino acids (Lindsay, 1971; Bergman, 1983). The amino acids that have the potential to be converted to glucose via gluconeogenesis are referred to as glucogenic. Other amino acids do not have the potential of forming glucose. These are referred to as ketogenic because they have the potential of forming ketones.

Glycerol becomes an important glucose precursor when propionate absorption becomes negligible. Most of the glycerol exists in combination with fatty acids and is stored as triglycerides in the peripheral tissue. During lipolysis, free glycerol is released along with free fatty acids. Primarily the liver and kidneys remove the free glycerol, where it is used for glucose formation. In fed animals, only 5% of the total glucose production is derived from glycerol but this increases to between 20 and 30% in starved animals (Bergman, 1983).

Lactate originates from two different pathways. Lactate can be produced endogenously or be obtained from exogenous sources. Lactate is produced in the muscle when glycogen stores are catabolised under anaerobic conditions. The lactate is then transported to the liver where it is synthesized into glucose. Endogenous lactate does not contribute to a net increase in glucose synthesis (Murray et al., 1996). Lactate from exogenous sources represents an additional source of glucose. Lactate can contributes up to 5% of total glucose production (Bergman, 1983).

2.1.2. Hormonal Control

Insulin and glucagon are the most important hormones associated with glucose metabolism. Insulin controls a variety of cellular processes, the most important being the stimulation of glucose transport in the cell. Without insulin normal glucose metabolism is virtually

eliminated and the cell must depend on gluconeogenesis for energy. Insulin aids the conversion of glucose to fat, stimulates protein synthesis, reduces ketone formation and increases the storage of glycogen by the liver and muscles. The control of insulin synthesis and secretion depends almost entirely on blood glucose concentration. An increase in the blood glucose concentration stimulates the release of insulin. The insulin in turn lowers the blood glucose concentration, thereby inhibiting further insulin release. In ruminants butyrate and propionate also stimulate insulin release since these are important energy sources. When glucose is injected into the circulation, hepatic glucose production rapidly diminishes and glucose disposal by various routes is accelerated. Only very little glucose is normally excreted in the urine. Glucose infusion at 75% above normal glucose entry rate severely depressed feed intake (Rutter and Manns, 1986). In ruminant, elevated plasma insulin concentration also depressed feed intake, but only when sufficient energy metabolites are available. Therefore it seems likely that the increased plasma insulin, along with a consistently high level of glucose activated the satiety centre(s) (Rutter and Manns, 1986).

Glucagon elevates the blood sugar level by stimulating glycogenolysis, the formation of glucose from glycogen. Glycogenolysis occurs in the liver. Insulin and glucagon are antagonistic. If glucagon cannot raise the blood glucose concentration enough, epinephrine is secreted from the adrenal medulla. The two hormones together lead to increased glycogenolysis and lipolysis. In this way glycerol will be mobilized for gluconeogenesis and the fatty acids that are released become available to supply fuel for oxidation. The result is an increase in blood glucose concentration (Weekes, 1991).

2.1.3. Lactose and glycogen synthesis

In the body glucose is an important precursor for the formation of glycogen and lactose. Glycogen is a complex polysaccharide made up of condensed glucose residues. Glycogen is stored in the liver and the voluntary striated muscle cells. Lactose is produced in large quantities in the mammary gland of lactating animals and makes up nearly half of the solids in milk (Maynard et al., 1979). Lactose does not occur in nature except as a product of the mammary gland. Glucose is the only precursor of lactose. Two molecules of glucose must enter the mammary gland for every lactose molecule formed. The formation of lactose occurs by the condensation of one glucose and one galactose molecule, which has to first be

synthesized from one glucose molecule. Lactose is synthesized in the Golgi apparatus. Amino acids can provide up to 12% of the lactose produced, (Botts et al., 1979).

2.1.4. Glucose metabolism

In non-pregnant and non-lactating animals, between 20 and 30% of the total glucose production is oxidized by the brain. Approximately 10% is converted to glycogen and approximately 30% is deposited as fat (Bergman, 1983; Weekes, 1991). The rest is used as an energy source by the muscles. During pregnancy the fetus can take up to 40% of the maternal glucose production for fetal oxidative metabolism (Bauman and Currie, 1980).

2.1.5. Lactation

Glucose requirements of the mammary gland accounts for 60 to 85% of the glucose used by a lactating ruminant. Lactose synthesis accounts for 50% to 85 % of the total glucose taken up by the mammary gland (Bickerstaffe, Annison and Linzell, 1974). In high producing cows almost all of the available glucose is used by the mammary gland. This suggests that glucose availability may limit milk production in high producing cows (Elliot, 1976). Blood glucose concentration was found to be inversely related to milk production (Kappel et al., 1984). Erfle et al., (1974) found that blood glucose was positively correlated to milk lactose and milk protein concentration.

Lomax and Baird, (1983), found that lactating cows had a lower arterial concentration of glucose and lactate. There was a higher hepatic output of glucose and a higher net hepatic uptake of propionate and lactate.

2.1.6. Reproduction

Dairy cows experience a period of negative energy balance in early lactation. During this time the reproductive functions should also recommence. If the negative energy balance is too great the time from parturition to first ovulation will become greater (Lucy et al., 1992b). This is because lactation has physiological priority over reproduction. The cow's condition at the time of calving therefore plays an important role in the time to first ovulation. If the

animal calves in good condition, she will have body reserves available to supplement her dietary energy supply, thereby keeping the negative energy balance to a minimum.

Chase et al., (1992) found that glucose metabolism changed significantly at different physiological stages. There seemed to be a positive relationship between the growth and progesterone secretion of the corpus luteum and the rate of glucose uptake and metabolism.

2.2. Cholesterol

2.2.1. Origin

Cholesterol is one of the most important animal sterols. It is a structural component of cell walls and is present in the blood and bile. Cholesterol can be synthesized in the body or supplied via the diet. The major precursors of cholesterol are acetyl-CoA, acetoacetyl-CoA and the amino acid leucine. Cholesterol from the diet is absorbed in the small intestine by absorptive mucosa cells and is then transported to the liver by chylomicron carriers. The liver and the small intestine are the major sites of cholesterol synthesis, but almost all cells in the body, except the brain, can synthesize cholesterol. Cholesterol synthesized by the liver accounts for about 50% of the total cholesterol synthesized (Maynard et al., 1979). Both the liver and the small intestines may export cholesterol as a constituent of triglyceride-rich lipoproteins. Cholesterol synthesis in extrahepatic tissues remains relatively constant, while cholesterol in the liver changes with changing rates of cholesterol absorption from the intestine and the overall loss of cholesterol in the bile, feaces, from the skin and that which is converted to hormones. An increase in the cholesterol content of the diet leads to a decrease in the rate of hepatic cholesterol synthesis. If the net loss of cholesterol increases, either by blocking the intestinal absorbance of bile acids or increasing the soluble fiber in the diet, hepatic cholesterol synthesis increases. This is because absolute cholesterol production and absorption must equal cholesterol excretion in the bile, faeces, cholesterol lost from the skin and that converted to hormones. The percentage of cholesterol in triglyceride-rich lipoprotein is low. As the triglyceride-rich lipoproteins are metabolized by lipoprotein lipase and triglyceride is depleted, cholesterol ester content can increase.

Lipids are transported in the blood in association with proteins because of their poor solubility in water. These lipid-protein complexes are called lipoproteins (Puppione, 1978). Lipoproteins are macromolecular complexes of protein, phospholipid, cholesterol, cholesterol ester and triglyceride. Hydrophobic components (tryglicerides and cholesterol esters) are found in the core of the lipoprotein. Components having both hydrophobic and hydrophilic components (cholesterol, phopholipids and proteins) are found on the outer surface. Complexes of different density are formed, depending on the ratio of lipid to protein and the nature of the packaged lipid. These are very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL).

Cholesterol is incorporated into HDLs, LDLs and VLDLs and is transported in this way in the blood. The principal function of VLDLs, which contain free and esterified cholesterol, is the transportation of triacylglycerol from the liver to the peripheral organs for oxidation or storage. During metabolism, the VLDLs are 'emptied' of their core triacylglycerol, leaving a remnant, which is cleared from the plasma by the liver. Cholesterol synthesized in the extrahepatic tissues is transported to the liver by HDL carriers.

2.2.2. Utilization

Cholesterol is a precursor for bile acids, vitamin D and the steroid hormones. Cholesterol is the precursor of five major classes of steroid hormones. These hormones and their production sites are: progestagens are produced in the corpus luteum, estrogens are produced in the ovaries, androgens are produced in the testis, glucocorticoids are produced in the adrenal cortex, and mineralcorticoids are produced in the adrenal cortex.

2.2.3. Influence of Cholesterol on Reproduction

Follicular fluid and granulosa cells are separated from blood circulation by a basement membrane that is impermeable to macromolecular particles in excess of approximately 400000 daltons (Bergman, 1983). HDLs may have a molecular weight less than 400000 daltons, whereas VLDLs and LDLs are unable to transverse the basement membrane when intact. HDLs are therefore the only lipoproteins observed in follicular fluid of bovines (Brantmeier et al., 1987, Grummer and Carroll, 1988). Cholesterol concentration however is lower in HDLs of follicular fluid in those of the plasma. Follicular fluid HDL concentration is

45% of that of plasma or serum HDL cholesterol concentration in cattle. The concentration of HDLs increases as the follicle matures. After vascularization, both LDLs and HDLs bathe luteal cells. LDL cholesterol is preferred as a precursor for ovarian steroid synthesis. The uptake of LDLs by the ovarian tissue occurs by receptor mediated endocytosis. Lipoproteins rather than de novo synthesis from acetate, contribute the majority of cholesterol used for steroid production (Grummer and Carroll, 1988).

The primary source of cholesterol for luteal cell progesterone synthesis is that present in the blood. Therefore, changes in the type and concentration of cholesterol in the blood could play a significant role in regulating steroid hormone biosynthesis by the ovaries (Talavera et al., 1985).

More than one pool of cholesterol seems to exist in the bovine corpus luteum (Mason and Savard, 1964) and it has been noted that in cattle the conversion of cholesterol to pregnenolone is the rate limiting step in progesterone synthesis (Henderson et al., 1981). Other evidence supports the concept that cholesterol availability and not enzymatic activity is the limiting factor in the conversion of cholesterol to progesterone (Henderson et al., 1981). Failure to detect a change in serum estradiol-17ß concentrations after lipid feeding is not surprising because cholesterol is not an immediate precursor of estradiol-17ß. Synthesis of the latter is most likely limited by an enzyme involved in aromatization (Talavera et al., 1985). Progesterone production by cultured bovine granulosa cells is increased markedly in the presence of lipoprotein cholesterol (Savion et al., 1982).

Stage of lactation should be considered when cholesterol related studies are conducted with cycling female cattle (Talavera et al., 1985). Several factors account for the cyclic patterns of serum total cholesterol (TC) during the ovarian cycle in heifers. Firstly, a decline in the serum TC during the luteal phase may result from uptake and utilization by luteal tissue, because cholesterol derived from plasma lipoprotein is the principal source of cholesterol for ovarian progesterone production. Alternatively, luteal phase concentrations of progesterone or some other component may suppress lipoprotein synthesis and (or) release of lipoprotein receptors. Thirdly, estradiol-17ß or another component of the follicular phase may enhance the synthesis and (or) release of lipoprotein receptors.

2.2.4. Influence of Cholesterol on Lactation

Cholesterol concentrations of cows, were found to vary at different stages before and after the onset of lactation (Arave et al., 1975; Kappel et al., 1984; Lean et al., 1992, Ruegg et al., 1992,). It was found that cholesterol concentration decreased before calving. This reflects the nutrient demand of the fetus as well as increased estrogen and progesterone concentrations. The fetus as such is supplied by a rich supply of steroid precursors such as progesterone and pregnenolone from the placenta and does not therefore need the capacity to take acetate or cholesterol through the biosynthetic chain to testosterone (Lamming, 1985).

Cholesterol concentration was lowest at the onset of lactation, increasing as lactation progressed. It is thought that high milk production leading to an increase in thyroid activity may be responsible for this trend (Arave et al., 1975).

There are two thyroid hormones, namely, triiodothyrosine (T3) and tetraiodothyrosine (T4). T3 is three to five times more active than T4 (Ganong, 1995). The thyroid hormones help regulate lipid and carbohydrate metabolism. The thyroid hormones enter the cell and T3 binds to receptors in the nuclei. The thyroid hormone-receptor complex then binds to DNA and increases the expression of specific genes (Ganong, 1995). The resultant mRNA triggers the production of various enzymes that alter the cell function. Thyroid hormones decrease the circulating cholesterol concentration. Plasma cholesterol concentrations drop off before the metabolic rate rises. The decrease in plasma cholesterol concentration is due to increased formation of LDL receptors in the liver, resulting in increased removal of cholesterol from circulation (Ganong, 1995).

Kappel et al. (1984) found that increasing cholesterol concentration during lactation was associated with increased lipoprotein synthesis and changes among the various types of lipoproteins, which are required for lipid transport. There was found to be an increase in the concentration of LDL and HDL_1 (Puppione, 1977). Increased cholesterol during lactation has been associated with increased lipoprotein synthesis and changes among various types of lipoproteins, which are required for lipid transport (Puppione et al., 1978).

There is a decrease in very low-density lipoproteins (VLDL) and an increase in α-low density lipids (αLDL) and high-density lipid-1 (HDL 1) is possibly a response on the part of the cow to enlarge the size of the apoprotein-C reservoir. Apoprotein-C acts as an activator of the enzyme extrahepatic lipoprotein lipase (ELL), (Puppione et al., 1978). Alternatively, the increase in αLDL and HDL 1 may be due to increased VLDL catabolism in the mammary gland.

As cholesterol and lecithin are shed from the VLDL surface during ELL action, the polar lipids are converted by the enzyme lecthin cholesterol acyl transferase (LCAT) to produce more core lipids for HDLs (Puppione et al., 1978). The increase of large α-lipoproteins in the cow during early lactation has been proposed to arise from one or more of the following:

1. Adaptation on the part of the animal to increase the apoprotein-C reservoir. The increased αLDLs should enhance the activation of trigylceride carrier and consequently, the uptake of trigylcerides by the mammary gland.

2. αLDLs are a by-product of VLDL catabolites and LCAT action. This should be reflection of mammary gland activity.

3. αLDLs are a by-product of LCAT action on nascent HDLs. This might serve as an index for the hepatic production of triglycerides for synthesis of milk fat.

Lean et al. (1992) suggests that as cows reach a positive energy balance and become less dependent on mobilized tissue reserves, the production of β-hydroxibutyrate decreases, allowing increased hepatic cholesterol synthesis. This is because the precursors for cholesterol synthesis are directed towards ketone production in early lactation and once the animal enters a positive energy balance the precursors are redirected to cholesterol synthesis (Lean et al., 1992). Cholesteryl esters are taken up by the mammary gland from chylomicrons and the uptake increases ten-fold during lactation. Uptake of unesterified cholesterol from plasma lipoprotein increases considerably during lactation and a substantial portion of milk cholesterol originates from this source, (Botham et al., 1993). The demand for cholesterol by the mammary gland varies according to the stage of lactation.

2.2.5. Diet

It has been found by a number of researchers (Talavera et al., 1985, Grummer et al., 1988, Ruegg et al., 1992) that diets containing a high fat content led to an increase in the plasma cholesterol concentration. Ruegg et al. (1992) found that serum cholesterol concentrations were inversely related to the amount of body condition lost and suggested that this may reflect the availability of body energy sources.

Kronfeld et al. (1982) conducted a study to determine the suitability of various blood parameters for predicting milk production, reproductive efficiency and nutritional status of herds using multiple regressions on blood variables. It was found that cholesterol was one of the best serum predictors for ration variability, but all regression coefficients for cholesterol were negative. This means a high nutritional status was associated with a lower serum concentration of cholesterol.

Serum cholesterol concentrations are genetically based, but are influenced by a wide variety of factors. Along with the above mentioned, cholesterol concentration is also influenced by age, sex, and climate.

2.3. Total Protein

The absorption of intact exogenous proteins does not occur except in limited cases, for example in very young animals. Consequently, virtually all proteins present in the body have been synthesized in the cells from amino acids. There are about 20 amino acids involved in the synthesis of these proteins (Bergman, 1983). Proteins are enzymatically broken down in the digestive tract. The free amino acids which are released with varying efficiency, are readily absorbed under normal conditions (Bergman, 1983).

In ruminants, the microorganisms ferment dietary protein and other nitrogenous compounds. The major product is ammonia. The rate of the fermentation process depends on the solubility and accessibility of the nitrogen compounds and on the time exposed to rumenal fermentation. The ammonia produced may be used by the microorganisms for the synthesis of amino acids. Any excess ammonia is absorbed through the rumen epithelium and carried

to the liver, where it is detoxified to urea. Microbial protein is washed through to the small intestines where they are broken down and the amino acids are absorbed (Perry, 1981). Thus, it is possible for rumen micro organisms to synthesize protein from non-protein nitrogen and potentially provide the animal with a source of amino acids when the diet itself is devoid of protein (Bergman, 1983).

Virtually all nitrogen compounds necessary for the normal functioning of the body can be derived from amino acids. The only exceptions are the nitrogen containing vitamins, niacin, pantothenic acid and riboflavin. Of the 20 amino acid, about half cannot be synthesized by the body and must be obtained from the diet. These are the essential amino acids. The diet must also supply adequate precursors for the synthesis of the other amino acids (Bergman, 1983).

Urea is the major waste product of protein and pyrimidine catabolism. Urea is formed in the liver, passes into the blood stream and is removed by the kidneys for excretion. The amount of urea present in the body and the rate of excretion depends largely on the protein contents of the diet (Bergman, 1983).

2.3.1. Reproduction and Protein

Measurements of serum protein serve as an indicator of normal health. It was found that serum protein was high at estrus and was high up to 48 hours after estrus. It is assumed that this to be due to the fact that elevated estrogen in the blood exerts an anabolic effect, thereby increasing the total serum protein (Ishwar and Pandey, 1994). Sasser et al. (1988) found that restricted protein intake increased the postpartum interval to first estrus, to first service and to conception and it decreased the number of animals that showed estrus and conceived.

Ferguson and Chalupa (1989) found that the effects of protein nutrition on fertility are complex. There are confounding factors such as age, energy, undegraded protein intake and uterine health that influence the responses to varying protein supply. Although protein effects on fertility may be minor within a herd, adverse impacts may occur within specific groups, such as older cows or cows with postpartum complications.

2.3.2. Blood Protein Function

Blood proteins are made up of cellular proteins and plasma proteins. The cellular proteins consist of erythrocytes, leucocytes and platelets. Plasma proteins are made up of albumin, globulin and fibrinogen (Bergman, 1983). Plasma protein constitutes 5 to 7% of the plasma (Kaneko, 1989). Plasma proteins are all manufactured in the liver, except γ-globulin, which is synthesized by the plasma cells. The cellular parts of the blood protein are involved in the coagulation of the blood.

The plasma proteins have various functions. They exert an osmotic pressure. They act as a carrier. Various substances, which are insoluble in water, are solubilized with the help of plasma proteins and thereby easily carried in the plasma, such as iron, thyroxine and cortisol. This carrier function of plasma protein also provides a temporary storage pool of some substances in the plasma, which can then replace the free form in the plasma when the concentration of the free form lowers. Thyroxine, for example, when complexed in equilibrium with plasma protein is inactive, but when it is needed at tissue level, it leaves the carrier protein, becoming active and induces a cellular response (Frandson, 1986).

Plasma proteins act as a buffer. This helps to prevent great changes in the pH of the blood. However, plasma proteins are not the most important buffers in the blood. In some instances plasma proteins are used to produce hormones and may be used to synthesis enzymes (Bergman, 1983). Plasma proteins provide an immune response by either neutralizing antigens or helping to break them down.

Plasma protein deficiency may develop due to insufficient protein production (for example starvation) or due to organ failure (for example, kidney disease leads to large amounts of plasma proteins, especially albumin to be lost in the urine) (Bergman, 1983).

2.4. Blood Urea

Urea is the blood metabolite most obviously and immediately affected by changes in protein intake. Blood urea originates from a number of different sources. Its source can be endogenous, namely from the deamination of amino acids. This process can supply between

30 and 58% of the total urea production (Nolan and Leng, 1982). This occurs under normal circumstances, but increases markedly when fasting or underfeeding occurs. The animal begins to catabolise its body protein reserves and the amino acids become available (Folman et al., 1981). They are then deaminated. The carbon skeleton is used for energy metabolism and the amino group is converted to urea and excreted (Ganong, 1995).

The exogenous supply of urea is from dietary non-protein nitrogen and dietary protein, which is catabolised to ammonia by the rumen microbes. The ammonia is absorbed through the rumen wall. This ammonia has to be detoxified in the liver, where it is converted to urea. This exogenous urea supply can account for 11 to 41% of the total urea production (Nolan and Leng, 1982). Some urea is recirculated via the saliva and the rumen wall, although the quantity is small (Perry, 1981). The rest of the excess urea is excreted in the urine.

There are various reasons for an increase in plasma urea nitrogen. Firstly, protein overfeeding leads to a greater production of ammonia by the rumen microbes because they can not make use of the extra ammonia (Blauwiekel et al., 1986). This is then absorbed through the rumen wall to be detoxified in the liver, leading to an increase in plasma urea nitrogen. Energy underfeeding can increase the blood urea concentration because it leads to reduced protein synthesis by the rumen bacteria, thus producing a surplus of ammonia.

Serum urea nitrogen is a good indicator of rumen ammonia content and its reflective protein intake and solubility. Increased utilization of urea in high producing dairy cows has been the proposed caused of low serum urea nitrogen concentrations. Dietary protein and energy interactions may be reflected in serum urea nitrogen and may have production and health consequences, (Ruegg et al., 1992). It was found that increasing the energy intake of an animal while keeping the protein intake constant led to a decrease in plasma urea nitrogen (Roseler et al., 1993).

Plasma urea nitrogen concentrations fluctuate throughout the day. Generally the minimum concentration occurs before feeding and the maximum approximately four to six hours after feeding.

2.4.1 Urea and lactation

In lactating animals urea diffuses freely across the ducts and tubules of the mammary gland. This leads to a correlation between milk urea concentration and blood urea concentration. Urea makes up 2 to 6% of non-protein nitrogen fraction of milk (Bergman, 1983).

2.4.2. Urea and reproduction

Canfield et al. (1990), found that feeding excess crude protein as rumen degradable protein increased the plasma urea nitrogen concentration and this lead to a decrease in the first service conception rate. Butler et al. (1996) found that plasma urea nitrogen concentration above 19 mg/dl was associated with decreased fertility. Plasma urea nitrogen affects fertility by changing the uterine environment. Increased plasma urea nitrogen leads to a decrease in the pH of the uterus and in this way affects the fertility (Elrod et al., 1993).

2.5. Lactation

Parturition signals a marked and rapid increase in the provision of nutrients to the young as the route of transfer is switched from the placenta to the mammary gland. These changes include enhancing the entry-rate of nutrients to the metabolic pool, decreasing nutrient utilization by tissues non-essential to lactation, augmentation of nutrient partitioning to mammary gland and metabolically related organs and stimulation of mammary substrate uptake by means of membrane located carrier systems.

One of the most notable features of whole-body metabolism in lactating dairy cows is the condition of negative energy balance, which may persist for several weeks postpartum. During this period, energy output in the milk exceeds the energy intake in the food. Body weight is lost as tissue stores are mobilized. Studies with high-producing cows showed that for the first two weeks of lactation 70% of milk fat was derived from adipose tissue stores (Mepham and Kuhn, 1994). This was achieved by reduced lipogenesis and increased lipolysis in adipose tissue, together with increased lipid uptake by mammary cells. Much less is known about repartitioning of protein metabolism at the onset of lactation, but muscle protein appears likely to play a storage role similar to that of adipose tissue (Mepham and Kuhn, 1994).

Nutrient fluxes during lactation are also markedly influenced by increased appetite. In cows where feed intake is the primary limitation to milk production, consumption during lactation may be as much as four times maintenance requirements (Baile and Della-Fera, 1988). Cardiovascular changes, which result in an enhanced supply of substrates to the mammary gland, are an important element of the partitioning of nutrients in lactating animals. Not only is the onset of lactation attended by greatly increased cardiac output (Hanwell and Linzell, 1972), but there are also marked changes in its fractional distribution; viz. the mammary gland, liver and intestinal tract receive larger portions of cardiac output, whereas organs not involved in lactation, such as the skin, receive smaller portions. During established lactation in cows, the mammary gland receives about 16% of cardiac output (Davis and Collier, 1985). From extensive studies in goats it was concluded that at peak lactation, approximately five hundred volumes of blood perfuse the mammary gland for each volume of milk secreted, a Figure which also applies to cows (Peeters et al., 1979). This ratio is only an approximate index.

Availability of substrates for milk synthesis depends not only on their rate of supply in the blood to the mammary gland, but also on their passage across at least two membranes, either or both of which may limit the transfer process. Passage into the interstitial fluid is governed by the capillary permeability and by the concentration gradient across the capillary wall. The method by which the substrate is transported across the membrane also plays a role. The substrates that are transported by passive diffusion, such as glucose, are dependent on the concentration gradient, while those substrates transported by active transport, such as amino acids are independent of concentration.

At any given level of metabolic activity, a web of co-operative and allosteric interactions operates to co-ordinate different parts of the metabolic machinery so as to establish a homeorhetic system. Excessive accumulation or depletion of metabolic intermediates does not occur by virtue of the binding strength and the effect of the binding on enzyme activity that each allosteric site has evolved. The changes that occur in the mammary gland operate over several hours or even days and are probably due to changes in the amount of enzymes

present. At the onset of lactation, major changes occur in the partitioning and utilization of nutrients by various tissues in the body. These changes include:

1. Increase in overall nutrient utilization by the mammary gland.
2. Increased lipolysis and decreased lipogenesis in the adipose tissue.
3. Increased gluconeogenesis and glucogenolysis in the liver.
4. Decreased use of glucose and increased use of lipids as an energy source.
5. Mobilization of protein and catabolism of amino acids in muscle and other tissue (Dhiman et al., 1991).

2.5.1. Glucose

Mammary glucose uptake is essential for milk secretion in that lactation places an additional demand on glucose supply. In goats, glucose flux at peak lactation is about double that in non-lactating animals, and it is thought to be similar in dairy cows (Annison and Linzell 1964). The change is due to a combination of factors, such as reduced glucose uptake by adipocytes, reduced glucose oxidation, increased gluconeogenesis, increased hepatic glucogenolysis and increased intestinal absorption (Bauman and Elliott, 1983). The flux of glucose along the glycolytic pathway provides glycerol-P for milk fat formation and pyruvate for oxidation to yield ATP and acetyl-CoA for lipogenesis. Glucose is also used for the synthesis of lactose, and in the pentose phosphate cycle to generate NADPH required for lipogenesis. It has been found that the mammary gland can not synthesize its own glucose because it lacks the enzyme glucose-6-phosphatase (Threadgold and Kuhn, 1979). Therefore, glucose required for lactose synthesis and other purposes is derived from blood glucose. The rate of lactose synthesis in the mammary gland can be measured from the daily milk yield and the lactose concentration in the milk. The timing of the appearance of lactose around parturition is due to the withdrawal of progesterone, following which prolactin or placental lactogen stimulate the expression of the gene coding for the α–lactalbumin component of lactose synthetase (Turkington and Hill, 1968; Delouis, 1975). The tissue level of galactosyltransferase probably sets the upper limit to the rate of lactose synthesis during established lactation. Actual rates of synthesis during this period are, however, nutritionally controlled through short term factors that may include insulin, but still need to be identified (Henderson et al., 1983). Lactose is synthesized in the Golgi apparatus. Glucose and UDP-

galactose, the precursors of lactose, readily penetrate the membrane of the Golgi apparatus to reach lactose synthetase, but the product, lactose, does not diffuse out again. Lactose is stored in the vesicles and released when the vesicles are osmotically ruptured following exposure to high concentrations of penetrating solutes such as glucose and various sugars (Mepham and Kuhn, 1994).

2.5.2. Lipids

Acetate is a major energy source for the mammary gland and is also the principle precursor of fatty acids of chain length up to C16. Fatty acids of chain length greater than C18 are derived directly from the blood, either from the plasma free fatty acid pool or from plasma lipoproteins following lipolysis in the lumena of mammary capillaries (Mepham and Kuhn, 1994). Fatty acids of chain length C16, C18 and longer are derived from serum lipoproteins (Annison, 1983). Free fatty acids normally show no net artereo-venous differences across the mammary gland, but despite this, their uptake is suggested by a fall in specific radioactivity when isotopically labeled free fatty acids perfuse the gland (Annison, 1983). This can be explained by the fact that free fatty acid uptake is masked by the simultaneous release of fatty acids derived from triacylglycerol lipolysis in the mammary tissue into the venous blood. The complex lipids in plasma are not absorbed directly into the mammary gland. They are hydrolyzed extracellularly. The fatty acids and glycerol are then absorbed and are assembled anew into complex lipids in the cell. Short and medium chain fatty acids are synthesized within the secretory epithelium of the mammary gland. The rest of the fatty acids required are derived from the triacylglycerol component of the plasma lipoproteins. It is through these that the dietary lipids exert an effect on milk fat composition. Milk fatty acids from the plasma are taken up from chylomicrons and very low-density lipoproteins that perfuse the mammary gland. Lactating mammary tissue is very well endowed with the enzyme lipoprotein lipase, whose prolactin dependent induction at the beginning of lactation is a major factor in the redirection of fatty acids away from the adipose tissue to the mammary gland (McBride and Korn, 1963; Mendelson et al., 1977). It is presumed that lipoprotein lipase is synthesized within the epithelial cell. It is then transported to extracellular sites on the lumenal wall of the blood capillaries where it binds and "digests" passing chylomicrons and VLDLs. The precursors for fatty acid synthesis are acetyl-CoA, butyryl-CoA and malonyl-CoA. NADPH is used as a reducing agent. In ruminants, acetyl-CoA arises from free

acetate taken up from the plasma. Because butyryl-CoA can also be used for the initiation of fatty acid synthesis (Nandedkar and Kumar 1969), the ruminant mammary tissue is able to use acetoacetate and 3-hydroxybutyrate. These reach the plasma from the rumen and are reduced to butyryl-CoA (Palmiquist, 1976). In this way, fatty acids are constructed from various precursors drawn from the plasma. NADPH is generally believed to originate from the pentose phosphate cycle.

2.5.3. Proteins

Blood precursors of milk proteins have been a matter of debate. Despite other possible contributions, it remains likely that plasma free amino acids are usually the principle precursors of mammary synthesized protein capillaries (Mepham and Kuhn, 1994). Amino acids are used primarily for the synthesis of milk proteins, but certain types are converted to other amino acids to make up shortfalls. The mammary gland is the only other organ besides the liver to produce substantial amounts of urea. Amino acids are divided into groups sharing affinities for distinct carrier systems. Many animal species have distinct anionic and cationic amino acid transporters. The systems are characterized by properties such as amino acid specificity, sodium dependence or independence and concentrative or exchange capability.

2.5.4. Lactation and Reproduction Interaction

Many researchers have found that high milk production tended to lead to decreased fertility (Butler and Smith, 1989). Hillers et al. (1984) found that cows with higher milk production had longer interval to first service. Conception percentage was less for cows that were inseminated before day 50 compared to cows inseminated after day 50 postpartum.

Nebel and McGillard (1993) found that selection for increased milk yield has increased the blood concentration of somatotrophin and prolactin, which stimulate lactation, and decease the concentration of insulin, a hormone antagonistic to milk production and may be important for normal follicular development. These changes in hormone concentrations promote lactation but may be potentially detrimental to other physiological functions. The timing and the magnitude of negative energy balance interact to determine the extent to which negative energy balance alters the hypothalamic secretion of GnRH and its effect on

gonadotrophin secretion and therefore ovarian secretion of progesterone, which in turn affects the expression of estrus and support of the uterus during early pregnancy.

2.6. Reproduction

In the dairy industry it is desirable for a cow to have an intercalving period of one year. To make this possible it is important that the cow begins to cycle again as soon as possible after parturition. This is because it was found that cows that conceived at first insemination had completed significantly more estrus cycles before insemination than those that did not conceive (Senatore et al., 1996).

There are various factors affecting postpartum infertility. Firstly, a non-involuted uterus may be a barrier to sperm transport and also to implantation. From a practical point of view, very few cows exhibit estrus early enough after calving for uterine involution to interfere with conception (Short et al., 1990). Secondly, short estrus cycles can cause postpartum infertility during the first 30 to 40 days postpartum. Prostaglandin F_2 (PGF_2) appears to be the normal physiological signal whereby the uterus causes regression of the corpus luteum (CL) at the end of the estrus cycle. During the early postpartum period there are higher concentrations of PGF_2 which cause the CL to regress prematurely (Short et al., 1990). Thirdly, anestrus is the most serious problem. Minor factors affecting it are season, breed, age, dystocia, presence of a bull and uterine palpations (Short et al., 1990). The major factors affecting anestrus are suckling and nutrition. Nutritional effects are elicited via a complex interplay among variables such as quantity and quality of feed intake, nutrient reserves stored in the body, and competition of nutrients from other physiological functions (Short et al., 1990).

Increased milk production has been associated with decreased fertility. During early lactation the increase in dietary intake fails to keep pace with rising milk production. The resulting negative energy balance and rate of mobilizing body reserves was found to be directly related to the postpartum interval to first ovulation and lower conception rates (Butler and Smith, 1989). If a cow has a large negative energy balance, the animal will remain in anestrous until the balance begins to go into an upward trend. This is because lactation takes priority over reconception.

2.6.1. The estrus cycle

The estrus cycle is controlled directly by hormones from the ovaries and indirectly by hormones from the pituitary. The estrus cycle is divided into several well-marked phases.

Proestrus: under the stimulation of follicle stimulating hormone (FSH) and some lutienizing hormone (LH), the ovaries produce increasing amounts of estrogen. This causes an increase in the development of the uterus, vagina, oviducts and ovarian follicle. During this stage the follicle grow in size (Frandson, 1986).

Estrus: This is the period of sexual receptivity in the female. During, or shortly after this time ovulation occurs. This is brought about by decreased FSH and increased LH secretion (Frandson, 1986).

Metaestrus: This is the post ovulatory phase during which the corpus luteum functions. During this period there is a decrease in estrogen concentration and an increase in progesterone concentration. Progesterone from the corpus luteum prevents further development of follicles. Another estrus cycle does not occur as long as an active corpus luteum is present. If pregnancy occurs, secretion from a functional corpus luteum is essential for proper implantation of the fertilized ovum (Frandson, 1986).

Di-estrus: This is the comparatively long interval of sexual inactivity between two consecutive estrus periods. In the cow the di-estrus period is an average of 21 days long. During this period, the corpus luteum is active for approximately 15 days, hereby inhibiting the formation of a follicle due to the secretion of progesterone. After this, if conception did not occur, there is a regression of the corpus luteum and a rapid increase in the secretion of FSH. This stimulates the formation of another Graafian follicle, and so initiating the first phase of estrus (van Rensburg , 1973).

Butler and Smith (1989) found that conception rate in cows was directly correlated to the number of ovulations before insemination. It was found that for ovulation to resume postpartum various systems in the body needed to recover from the effects of pregnancy. These are:

1. the brain-pituitary-ovarian system
2. the genital tract

The recovery of these occurs simultaneously and there is definite interaction.

2.6.2. Energy metabolism and reproduction

After parturition, the uterus horn undergoes considerable reorganization. These dramatic changes in growth and size of the reproductive tissue during a key physiological stage implicate high metabolic activity in regards to energy metabolism (Chase et al., 1992). During negative energy balance in early lactation, the rapid increase in the utilization of glucose for milk lactose production results in lower plasma concentrations of both glucose and insulin as compared to later lactation. Although the combined effects of lower glucose and insulin concentrations may play a minor role in stimulating feeding behaviour, the relative lack of insulin would enhance lipolysis in adipose tissue, thereby further increasing appetite via increasing the availability of free fatty acids for hypothalamic oxidation (Butler and Smith, 1989). Butler and Smith (1989) found negative energy balance to be directly correlated to postpartum interval to first ovulation. It was found that the resultant NEB from high milk production determined when ovulation resumed, and the time of first ovulation will in turn determine the number of cycles the animal has before the recommended time of insemination.

Cows with less of a negative energy balance (NEB), expressed estrus earlier than cows with a greater NEB (Spicer et al., 1990). This suggests that energy balance is a regulator of ovarian function. Spicer et al. (1993) conducted an experiment, of which the results obtained support the hypothesis that insulin and IGF-I may have direct local effects on bovine ovarian function, and that these effects are influenced by dose and size of follicle. Stewart et al. (1995) found that IGF-I and insulin may each play a significant role in thecal cell mitogenesis and LH-induced thecal cell steroidogenesis during follicular development in cattle and that glucose enhances these effects. Concentrations of IGF-I in serum of cattle decreased during restricted dietary energy and during NEB associated with early lactation (Vandehaar et al., 1995).

2.6.3. Protein Metabolism and Reproduction

It has been shown that feeding excess protein leads to decreased fertility and increased days to first ovulation (Jordan and Swanson, 1979a; Carroll et al., 1988). Three general theories have been suggested to explain this effect. Firstly, there may be a direct effect on the uterine environment by the altering of the pH. A high protein diets leads to an increase in urea nitrogen in the plasma and in the reproductive tract. The urea decreases sperm viability and is detrimental to the embryo. High protein diets have also been shown to increase vaginal and uterine concentrations of ammonia (Howard et al., 1987; Carroll et al., 1988).

Secondly, high protein diets may alter gonadotrophin secretion. It was found that mid cycle LH concentration was increased but basal estural LH was decreased (Blauwiekel et al., 1986). LH binding to ovarian receptors has been shown to be inhibited by increased urea concentrations and this has been suggested by decreased plasma progesterone in cows fed high protein diets (Jordan and Swanson, 1979b; Jordan et al., 1983). Some studies did not find this however (Blauwiekel et al., 1986; Howard et al., 1987; Carroll et al., 1988).

Lastly, high protein diets may decrease fertility due to a protein:energy imbalance. When protein intake exceeds requirements, a large amount of ammonia is produced and must be detoxified by the liver to urea. This requires energy and can potentially change the animals energy balance status and thereby fertility (Canfield et al., 1990).

2.6.4. Reproductive hormones

2.6.4.1. Follicle Stimulating Hormone (FSH)

FSH secretion leads to an increase in the size of the growing follicle. The result of this is an increase in estrogen secretion from the follicle, which in turn inhibits FSH secretion. As FSH secretion decreases, luteinizing hormone (LH) secretion increases, resulting in the maturing of the follicle and ovulation (Frandson, 1986).

2.6.4.2. Lutienizing Hormone (LH)

LH is released from the anterior pituitary in a pulsitile manner (Rahe et al., 1980). The release of LH is controlled by luteinizing hormone releasing hormone (LHRH) released from the hypothalamus. There are a variety of neuronal inputs conveying information about the animal's internal and external environments that modulate the system, but its exact workings are not known (Schillo, 1992). An increase in luteinizing hormone pulse frequency has been documented in cattle preceding the first ovulation postpartum (Hansel and Convey, 1983). This suggests that a high frequency of pulsitile LH release is critical for stimulating follicle growth and therefore for the induction of estrus and ovulation. Prolonged restriction of dietary energy induces anestrous in sexually mature cattle. This effect is partially attributed to a decrease in LH secretion, (Schillo, 1992). The re-establishment of a normal luteinizing hormone (LH) pulse pattern is the key factor responsible for ovarian follicular development and the initiation of ovarian cyclicity (Schillo, 1992).

Reduction in LH pulse frequency observed during dietary energy restrictions is dramatic and probably represents one of the most important ways by which undernutrition impairs reproductive activity (Imakawa et al., 1986). There are several hypothesizes to explain how energy reserves might regulate LH secretion. Firstly, changes in body fatness have been associated with changes in reproductive activity. Body fat at calving is inversely related to the interval between calving and first estrus (Randel, 1990). Animals that lost the most body fat during the early postpartum period were found to have lower basal concentrations of LH than those that maintained their body weight (Rutter and Randel, 1984). It seems that a reduction in LH pulse frequency is associated with prolonged dietary energy restriction (Richards et al., 1989). Roberts et al. (1997) has indicated that restricted energy intake suppresses the hypothalamic secretion of Luteinizing Hormone Releasing Hormone (LHRH). The mechanism by which this occurs has not yet been determined. In spite of this link between the degree of body fatness and LH pulse frequency, it is doubtful that body fat per se links nutritional status of LH release (Schillo, 1992). Secondly, because nutritional status influences intermediary metabolism is seems possible that nutrition may influence LH secretion via blood borne signals that reflect metabolic

status. It was first suggested by Steiner et al. (1983) that insulin, non-esterified fatty acids (NEFA), and certain amino acids act as such signals. Periods of low nutrition are associated with a decrease in insulin secretion, elevated NEFA concentrations due to enhanced lipolysis and reduced lipogenesis and changes in circulating concentrations of various amino acids (Bergen et al., 1979). Insulin may serve as a nutritional signal influencing LH secretion because the peripheral concentration of insulin is directly proportional to feed intake. Also, insulin passes the blood-brain barrier to influence various functions in the central nervous system (Van Houten et al., 1979). A number of studies have shown the relationship between LH secretion and dietary energy availability (Imakawa et al., 1986; Rutter and Manns, 1986). It has also been suggested that the impaired LH response is due to lower blood glucose concentrations, however, now it is thought to be insulin concentration that affects LH pulsitile release (Butler and Smith, 1989).

2.6.4.3. Progesterone

Growth and development of the corpus luteum (CL) during the estrus cycle is essential to ensure adequate circulating levels of progesterone for the establishment and maintenance of pregnancy. The size and ability of the CL to secrete progesterone change during the estrus cycle (Chase et al., 1992). The physiological state significantly alters the in vivo rates of uptake and metabolism of glucose by the reproductive tissue. There seems to be a positive relationship between development (growth) and function (progesterone content) of CL and the rate of glucose uptake and metabolism (Chase et al., 1992).

2.6.4.4. Prostaglandins (PG)

There are four basic types of natural prostaglandins. They are classed as A, B, E and F. PGF_2 is a natural luteolytic hormone which, in the absence of pregnancy, ends one estrus cycle by destroying the corpus luteum and allows the next one to begin developing. The uterus wall secretes PGF_2 in response to increasing corticosteroid concentration from the fetus, as parturition begins. This leads to myometrial contractions (Frandson, 1986).

2.6.4.5. Somatomedins

Somatomedins or Insulin-like Growth Factors (IGF) have been implicated in many biological processes. These include pre- and postnatal growth, lactation, reproduction and immune functions (McGuire et al., 1992). IGFs are polypeptides predominantly secreted by the liver. They are closely related to insulin (Ganong, 1995). Two different IGFs have been identified, IGF-I and IGF-II. They have been detected in all biological fluids, including milk, colostrum and follicular fluid (McGuire et al., 1992). It was originally thought that somatotrophin stimulated the liver to produce somatomedins, which in turn entered the circulation and were transported to specific target tissues. Subsequent research has showed that in addition to the liver, many tissues contained the message for IGF-I, although the concentrations were much lower than those found in the liver (Murphy et al., 1987). Therefore, in addition to an endocrine function, IGF must have the ability to act in an autocrine and or paracrine manner (McGuire et al., 1992).

It has been found that the majority of IGFs are bound to soluble, high affinity binding proteins (IGFBP). The action of these binding proteins varies from circulatory transport vehicles to retarding IGF degradation, to transvascular IGF movement to the direct modulation of the actions of IGFs at target cells either by enhancing or blocking IGF activity. Six distinct binding proteins have been identified in bovine serum (Cohick et al., 1992).

Spicer et al. (1990) found that an increase in energy balance was associated with an increase in IGF-I concentration in serum during early lactation. Also, the increase in IGF-I concentration was associated with increased progesterone secretion during diestrus of the first and second estrus cycles. Increased milk yield was associated with a decrease in IGF-I concentration in the serum. Spicer et al. (1990) concluded that reduced ovarian activity, that accompanies negative energy balance, might be due in part to the decreased concentration of serum IGF-I. It was found that in invitro studies, (Shams et al., 1988, McArdle et al., 1990), that IGF-I is a potent stimulator of bovine granulosa cells and luteal cell steriodgenesis. Spicer et al. (1990) also found

that IGF-I concentration was negatively correlated to weekly ambient temperature, suggesting that environment may play a role in IGF-I secretion.