# The use of Blood Metabolite Concentrations as Indicators of the Metabolic and Productive Status in Dairy Cows

by

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## DECLARATION

**I DECLARE THAT THIS THESIS FOR THE DEGREE OF MSc (Agric) Production Physiology AT THE UNIVERSITY OF PRETORIA HAS NOT BEEN SUBMITTED FOR A DEGREE AT ANY OTHER UNIVERSITY**

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## ABSTRACT

Twenty-nine dairy cows from three different farms were used in a study to investigate the value of using blood metabolites in determining the metabolic, productive and nutritional status of freshened cows. Blood samples were collected on a weekly basis until nine weeks postpartum. Blood glucose, blood cholesterol, blood total protein and blood urea concentrations were determined spectrophotometrically.

A second trial was conducted with thirty Holstein cows. Their whole blood glucose and cholesterol concentrations were sampled every second week and analyzed immediately with the help of a hand-held glucometer. Fifteen cows were put onto transponders and their feed intake measured.

In these studies it was found that glucose concentration decreased to a certain extent after parturition. With the onset of lactation the increased demand for glucose for the synthesis of lactose is partially responsible for the decrease in glucose concentration as well as the re-organization of the reproductive organs from pregnancy to being able to reconceive. It was also found that during this time there was an increase in the cholesterol concentration. This can be partly attributed to the increase in the mobilization of body reserves and also increased thyroid activity. The mean glucose concentration had an effect on the number of times a cow had to be inseminated before she conceived, with those requiring three inseminations having significantly lower glucose concentrations than those animals only requiring one or two inseminations. The lower glucose concentrations led to an increased interval between calving and reconception, which has an economic impact on a dairy enterprise.

It was concluded that, although the use of blood metabolites to determine an animals metabolic and productive status can be a useful tool, the use of the hand-held glucometer for such determinations is of little value due to the many factors affecting both glucose concentration and cholesterol concentration. The hand-held glucometer can however be used to determine an animal's mean glucose concentration so as to help in keeping it above a certain concentration and in this way keep the period between parturition and reconception to a minimum.

## ABSTRAK

Nege en twintig melk koeie van drie verskillende plase is in hierdie studie gebruik om die waarde van bloedmetaboliete vas te stel vir die bepaling van die koei se metaboliese, produktiewe en voedings status. Bloedmonsters is vanaf kalwing, weekliks geneem vir nege weke. Die bloed glukose, bloedcholesterol, bloed totale protein en bloed ureum konsentrasies is gemeet met die hulp van n' stektrofotometer.

n' Tweede eksperiment was gedoen op dertig Holstein koeie. Die heel bloedglukose en heel bloedcholesterol was elke tweede week gemeet met 'n draagbare glukometer. Hulle bloed was getoets vanaf kalwing tot rekonsepsie. Vyftien van die koeie het transponders aangehad sodat hulle daaglikse voer inname gemeet kan word.

In hierdie studies was dit gevind dat bloedglukose konsentrasie gelydelik afgeneem het na kalwing. Met die begin van laktasie het die behoefte van glukose verhoog vir die sintese van laktose. Dit tesame met die herorganisasie van die reproduktiewe stelsel, wat ook glukose benodig, is gedeeltelik verantwoordelik vir die daling in bloedglukose vlakke. Dit was ook gevind dat gedurende hierdie tydperk daar ook n' verhooging in bloedcholesterol vlakke was. Dit kan gedeeltelik toegeskryf word aan n' verhoging in die mobilisering van ligaams reserwes as ook n' verhoging in skildklier aktiwiteit. Die gemiddelde bloedglukose konsentrasie het ook n' invloed gehad op die hoeveelheid kere n' koeie geinsemineer is voordat sy gevat het. Die koeie wat drie keer geinsemineer was het 'n betekenisvolle laer glukose konsentrasie gehad as die wat net een of twee inseminasies nodig gehad het om te vat. Laer glukose konsentrasies het gely tot n' langer tydperk tussen kalwing en herkonsepsie en dit het 'n ekonomiese uitwerking op n' melkboerdery.

Die gevolgtrekking wat van hierdie studie gemaak kan word is dat, alhoewel bloed metaboliete 'n waardevolle bydrae kan lewer tot die bepaling van 'n dier se metaboliese en produktiewe status, die gebruik van die hand glukometer van min waarde is in die bepaling van hierdie faktor omdat soveel ander faktore bloed glukose en bloed cholesterol konsentrasies beinvloed. Die hand glukometer kan tog 'n waardevolle bydrae maak in die bepaling van 'n dier se bloed glukose konsentrasie sodat die konsentrasie bo 'n sekere vlak gehou kan om sodoende die tydperk tussen kalwing en herkonsepsie tot 'n minimum te beperk.

## 1.0. Introduction

In the dairy industry, milk production and reproduction are the most important economic factors. These factors are influenced greatly by the animal's nutritional status. It is therefore important to judge the animals' nutritional status accurately. In many nutritional studies and under practical farm conditions, changes in weight and milk production are used as indicators of nutrient inadequacy and other metabolic disfunctions. These are often inadequate as a measure of response in that, by the time a change in milk production and/or body weight is noted, the negative effect of the imbalance has already made an economic impact. Since most physiological processes in the animal body involve transport of metabolites by the blood, metabolites should be helpful in detecting changes in types or rate of biochemical processes related to growth or productivity. The value of this information would be determined by the relationship of concentrations of blood constituents to pool size and turnover rate of the metabolites determined (Bowden, 1971).

Genetic improvement in dairy cattle has markedly increased milk yield. The increase in production has been associated with a decrease in fertility (Butler and Smith 1989). Because cows respond to negative energy balance by different combinations of increased feed intake and mobilization of body reserves, neither change in body weight nor milk production are sensitive enough to predict the impact of negative energy balance early enough (Butler and Smith, 1989).

The use of blood metabolites is not a new concept. It started in 1950 (Rowlands, 1980). In the sixties, automated analytical equipment was developed, making the analysis of blood metabolites a routine technique used to assess the metabolic status of an animal. In 1978 Payne put together a group of metabolites into a single package called the "Compton Metabolic Profile Test".

The development of the "Compton Metabolic Profile Test" coincided with an increased intensification of livestock production and with a greater effort being made by the farmer to produce maximum output with minimum costs. This increased output puts strain on the metabolism of the animal, which leads to increased risk of metabolic imbalances. The

"Compton Metabolic Profile Test" was designed to monitor the metabolic health of cows in dairy herds in relation to management, nutrition, milk production, disease and to aid in the diagnosis of metabolic disorders. It does this by comparing the average concentration of blood constituents of a group of cows to the mean concentrations and ranges of the blood constituents taken from cows in many herds. The test was designed to sample cows in three categories, namely: up to 70 days postpartum; up to 150 days postpartum and non-lactating cows.

The blood constituents normally analyzed are: packed cell volume (PCV), hemoglobin, glucose, urea nitrogen, albumin, total protein, calcium, sodium, magnesium, copper and iron. Later, free fatty acids and cholesterol were added to the blood profile because of their relationship to energy status (Ingraham and Kappel, 1988). In 1980, Rowlands introduced the blood profile. This is defined as a set of or a combination of blood metabolites analyzed together in one test. The choice of metabolites depends on factors such as relevance to the problem under investigation, cost, ease of analysis, and stability of the sample in relation to the time in transit between farm and laboratory.

Profiles of blood metabolites have been used widely to identify problem herds and to indicate diertary causes of low production or disease. Concentration of metabolites are of almost no practical use for individual cows due to the fact that there are so many things influencing the blood metabolite concentration, such as diet, environment, time of day, physiological state.

The hand held glucometer was used so as to determine if it is possible to do blood tests on the farm and thereby get immediate results which can help the farmer make the necessary changes immediately. This will then help keep the economic losses at a minimum by halting the various metabolic disorders before they can lead to economic losses. The hand held meter is easy to use and the results are obtained rapidly and it is not very expensive.

# 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.

## 3. Material and methods

This study comprised of two experiments.

### 3.1. Experiment 1: The relationship between various blood metabolites and milk production, milk composition and body condition score of freshened cows

Aim: This experiment was conducted to determine the type of relation between the blood glucose, blood cholesterol, blood total protein, blood urea, milk production, milk lactose percentage, milk protein percentage, milk fat percentage and body condition score from a wide spectrum of cows. Cows of different breeds and from different farms were used to try and obtain a wide range of conditions.

Animals: Initially four Dutch Friesland cows were sampled from farm A but only three cows' data was used because one fell ill. They were fed a total mixed ration twice daily, according to NRC standards for milk production and also had access to grazing. Sixteen Holstein Friesland cows were sampled from farm B. The cows on farm B were fed a total mixed ration twice daily according to NRC standards for milk production. Initially three Dairy Swiss cows were from farm C but only two cows' data was used due to one being culled. They were fed individually three times a day on a commercial ration and had *ad lib.* access to teff hay.

The reason not more cows were taken from farms A and C is that they are both small dairies and more cows were not available at the time of sampling. Due to the influence of various external factors such as weather it was decided not to spread out the period of sampling too much.

Sampling and Procedure: Weekly blood samples were taken from twenty-one cows beginning at prepartum until nine weeks postpartum. The weekly blood samples were taken before feeding and once taken were left to stand at room temperature for three to four hours to allow coagulation, after which the serum was then aspirated and frozen at -15°C until analyzed.

Glucose, cholesterol, total protein and urea concentrations in serum were determined spectrophotometrically. Glucose concentrations were determined by an enzymatic colorimetric method using glucose oxidase and a modified Trinder colour reaction (Reagents Applications Inc., San Diego, California). Cholesterol concentrations were determined using an enzymatic colorimetric method in which cholesterol esters are hydrolyzed to free cholesterol by cholesterol esterase (South African Institute for Medical Research, Sandringham, South Africa). Total protein concentrations were analyzed using an enzymatic colorimetric method based on the Buiret reaction in which cupric ions react in an alkaline solution with all the compounds which have two amides or peptide bonds (Reagents Applications Inc., San Diego, California). Urea concentrations were determined using an enzymatic colorimetric method based on the Berthelot method (Reagents Applications Inc., San Diego, California).

Milk samples were taken every second week and body condition score was determined on weeks alternate to milk sampling. Milk samples were analyzed for fat, protein and lactose content. This was done using infrared to determine the percentages of the various components.

3.2. Experiment 2: Analysis of whole blood glucose concentration and whole blood cholesterol concentration of cows from parturition to reconception with a hand held glucometer.

Aim: The aim of this experiment was to determine the interaction of whole blood glucose, whole blood cholesterol, milk production, feed intake and reconception.

Animals: Thirty Holstein cows from parturition to reconception were used. The cows were fed a total mixed ration according to NRC standards once daily. The diet was composed of whole cotton seed (5%), lucerne (17%), maize silage (34%), eragrostis hay (11%) and a concentrate (33%). The composition of the diet is as follows:

| | |
|---|---|
| Metabolisable energy: | 11.4MJ/kg |
| Crude protein: | 179.0 g/kg |
| Non-protein nitrogen: | 13.57 g/kg |

| | |
|---|---|
| Undegradable protein: | 65.76 g/kg |
| Crude fibre: | 206.33 g/kg |
| Calcium: | 8.54 g/kg |
| Phosphorus: | 4.45 g/kg |
| Fat: | 67.16 g/kg |

Fifteen cows were fed individually using transponder activated feeding bins. The cows used were all calving for at least the second time.

Sampling and procedure: Blood samples were taken on a two weekly basis before feeding from parturition until confirmed pregnant. Whole blood glucose and cholesterol concentrations were analyzed immediately using a hand held glucose and cholesterol meter, (Acutrend GC, Boehinger Mannheim, Mannheim, Germany).

### 3.3 Statistical Analysis

Data was analyzed statistically using SAS (Statistical Analysis System) computer programme (SAS Inc., 1990). SAS PROC GLM was used. The following predictors were used in the model: weeks of lactation, glucose concentration, cholesterol concentration, total protein concentration, urea concentration, body condition score and milk production. All the dependent variables were corrected for cow effect by adding cow as a predictor in the PROC GLM. Wherever necessary, quadratic terms were added to the model to account for curvilinearity.

# 4 Results and Discussion

## 4.1. Blood Glucose

In Experiment 1, various blood metabolites were examined, from five weeks before parturition to nine weeks of lactation, when the cows reached peak production. Twenty one cows at three different farms were sampled, of which three came from farm A, sixteen from farm B and two from farm C. The aim of this experiment was to determine the relationship between the various blood metabolites and production parameters.

In Experiment 2, whole blood glucose and blood cholesterol concentrations were measured in twenty-four animals. Blood was analyzed every two weeks with the help of a hand-held glucometer. The aim of the experiment was to determine the relationship between blood glucose concentration, blood cholesterol concentration and reconception and to determine if the hand-held glucometer could be used to assess the animal's metabolic status more accurately than milk production and body condition score alone.

### 4.1.1. Blood glucose versus weeks of lactation

As the average blood glucose concentration at farms A, B, and C did not differ significantly ($P > 0.05$), data was pooled. Blood glucose concentration decreased over the period sampled ($P = 0.005$).

$$Y = 3.01 - 0.25X + 0.02X^2 - 0.003X^3$$

where $Y$ = blood glucose concentration (mmol/l)

$X$ = week of lactation

$R^2 = 0.651$

The relationship between blood glucose concentration and weeks of lactation is due to the increased demand for glucose for various metabolic functions such as lactation and the involution of the uterus. In Figure 1, the predicted line for blood glucose intercepts the x-axis. This is a mathematical consequence of the model fitted.

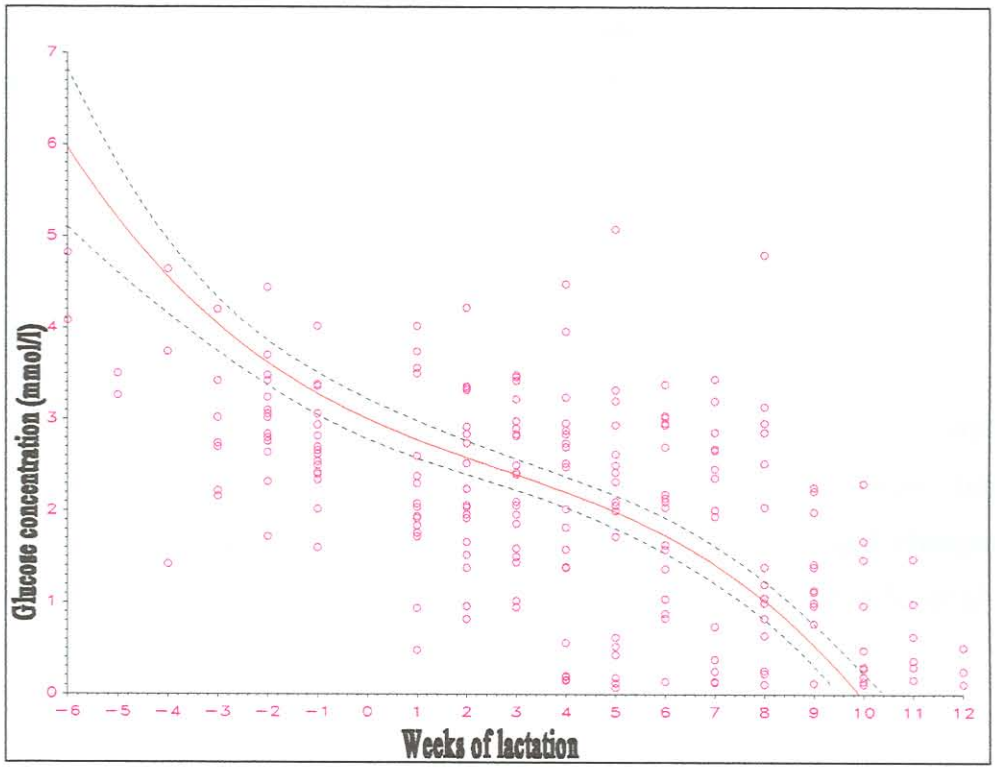


Figure 1. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and weeks of lactation in Experiment 1. The dotted lines represent the 95° confidence intervals.

Table 1. The parameter estimates, standard errors and probabilities of the relationship between blood glucose and weeks of lactation in Experiment 1.

| Variable | parameter estimate | standard error | Probability |
|---|---|---|---|
| intercept | 3.006 | 0.112 | 0.0001 |
| week of lactation | -0.247 | 0.028 | 0.0001 |
| (week of lactation)$^2$ | 0.023 | 0.008 | 0.0035 |
| (week of lactation)$^3$ | -0.003 | 0.0007 | 0.0001 |

This line is expected to rise again after about the eighth week of lactation. This is when milk production has passed its peak and the various metabolic changes relating to the change from pregnancy to lactation have occurred and the demand for glucose begins to decrease.

Rowlands et. al. (1980) found that blood glucose concentration only decreased over the first two weeks of lactation. They found that mean blood glucose concentration before calving was 2.6 mmol/l which decreased to 1.6 mmol/l after two weeks. Cows in Experiment 1

calved with a slightly higher blood glucose concentration, 3.01 mmol/l, but the decrease was greater in magnitude and over a longer period of time. At two weeks postpartum the mean blood glucose concentration was 2.57 mmol/l and at week seven the blood glucose concentration was 1.21 mmol/l. The reason for this discrepancy could be due to management differences such as feeding different rations where the rate of passage through the digestive tract differ and/or environmental differences.

In Experiment 2 there was no relationship between blood glucose concentration and weeks of lactation ($P < 0.05$). During Experiment 1 the weather conditions remained relatively constant, while during Experiment 2 there were numerous rapid changes in temperature, which has been shown to affect blood glucose concentration (Schaffer et al., 1981; Ingraham and Kappel, 1988).

### 4.1.2. Blood glucose versus milk production

Blood glucose concentration was compared to milk production in both Experiment 1 and Experiment 2. There was found to be a relationship in Experiment 1 ($P = 0.027$) but not in Experiment 2 ($P = 0.19$) although the same statistical procedure was used. The reason for this difference in results could be due to the different weather conditions experienced during the two experiments. The weather changes that occurred during Experiment 2 could be the reason for this as it is known that weather has an influence on blood glucose concentration (Schaffer et al., 1981). For Experiment 1 the relationship can be described by the equation:

$$Y = 3.00 - 0.038X$$

where $Y$ = blood glucose concentration

$X$ = milk production

$R^2 = 0.0323$

Table 2. The parameter estimates, standard errors and probabilities of the relationship between blood glucose and milk production of Experiment 1.

| Variable | Parameter estimate | Standard error | Probability |
|---|---|---|---|
| intercept | 3.000 | 0.468 | 0.0001 |
| milk production | -0.038 | 0.0170 | 0.0272 |

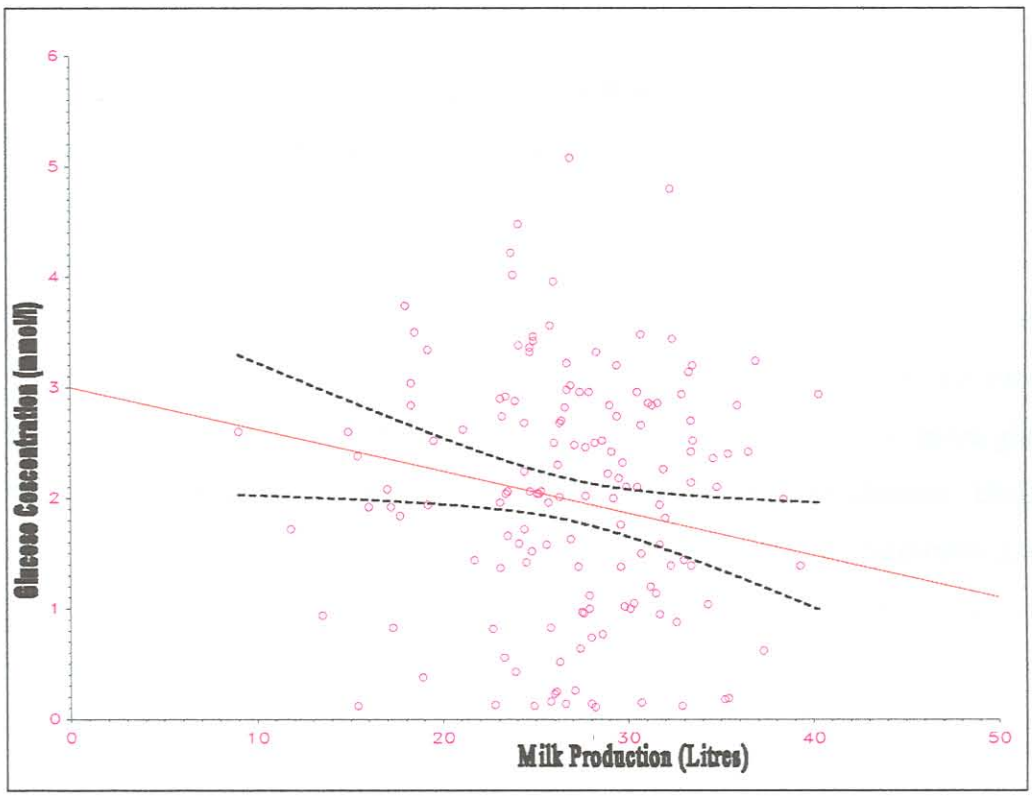


Figure 2. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and milk production in Experiment 1. The dotted lines represent the 95° confidence intervals.

In Experiment 1 the relationship was found to be a negative one. This relationship can be considered negligible ($R^2 = 0.0323$). This can also be seen by the spread of the data in Figure 2. This would indicate that the increased demand for glucose for lactose synthesis does not influence the blood glucose concentration. Amaral-Phillips et al. (1993) found that short term decreases in blood glucose availability did not affect milk production adversely. Kappel et al. (1984), however, found that blood glucose concentration was negatively correlated to milk production up to the sixth week of lactation. They stated that this was due to the demand for glucose exceeding the absorption of glucogenic precursors and the synthesis of glucose. This increased demand for glucose was for the formation of lactose.

In Experiment 1, the blood glucose concentration was then analysed according to milk production groups, by assigning cow records to one of three classes:

High milk production group (average daily production 32 l/day; n=6);

Medium production group (average daily production 27 l/day; n=9)

Low milk production group (average daily production 22 l/day; n=6).

When average glucose concentration of the different production groups was compared, it was found that the blood glucose concentration for the high milk production group was significantly higher than that of the low production group ($P = 0.00316$). There was, however, no significant difference between the high and medium production group nor between the low versus the medium production group ($P > 0.05$), as seen in Table 3 and Figure 3. The high production group had the highest blood glucose concentration; this could be part of the reason for the higher milk production, in that there was more glucose available for the formation of lactose and therefore increased milk production. Bickerstaffe et al. (1974) found that 60 to 85% of blood glucose is taken up by the mammary gland. Of this 50 to 85% is used for lactose synthesis. Therefore, if there is a greater amount of glucose circulating in the blood, more is available for uptake by the mammary gland and for lactose synthesis, thereby increasing milk production.

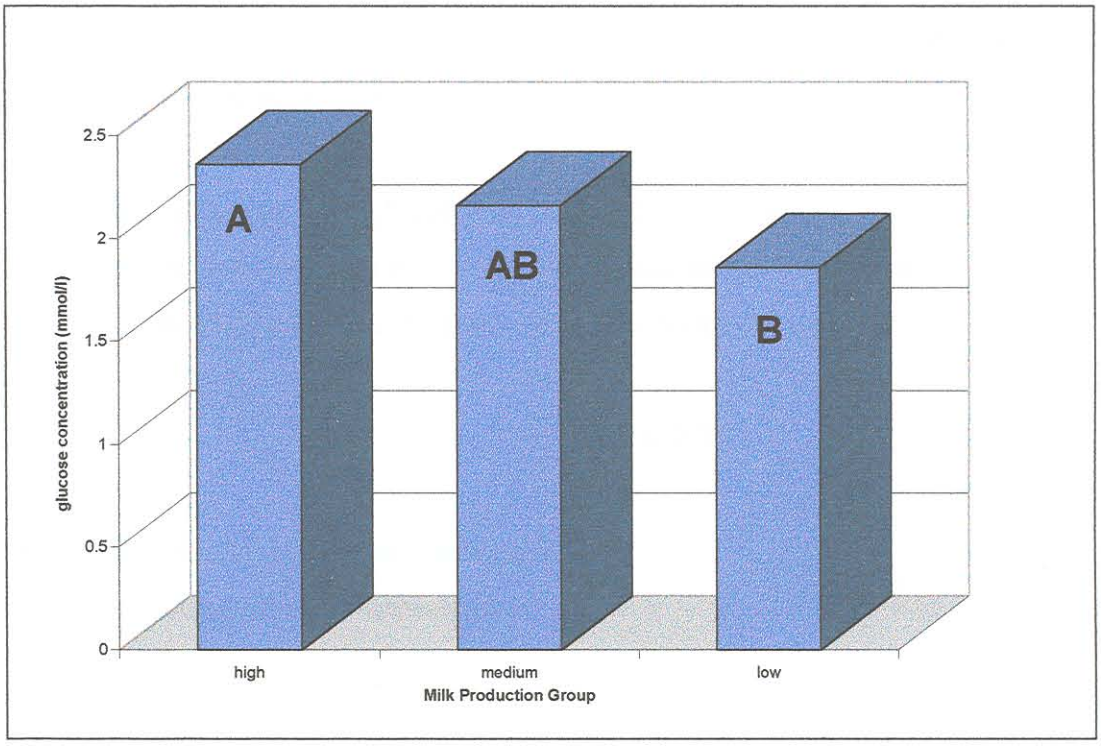


Figure 3. The relationship between blood glucose concentration and high, medium and low production groups of Experiment 1. Means with different letters differ significantly ($P < 0.05$).

Table 3. The relationship of blood glucose concentration between the high, medium and low milk production groups of Experiment 1. Means with different superscripts differ significantly (P<0.05).

| Production group | blood glucose concentration | standard error |
|---|---|---|
| high | $2.356^{a}$ | 0.187 |
| medium | $2.161^{ab}$ | 0.156 |
| low | $1.860^{b}$ | 0.172 |

When blood glucose concentration was compared to milk production, there was only found to be a relationship in the high production group (P = 0.0005; $R^2$ = 0.325). This could be an indication that glucose concentration limits milk production in the high production group but not in the low production group. Thus, glucose availability may limit the amount of milk produced by a high producing cow in a negative energy balance. Due to the weak relationship between blood glucose concentration and milk production ($R^2$ = 0.03), the hand-held glucometer can not be used as management to help determine the cow's energy status and thereby used as an aid to increase milk production.

4.1.3. Blood glucose versus blood total protein concentration

Blood glucose concentration was negatively related to blood total protein concentration (P = 0.0001). This relationship can be described by the following equation:

$$Y = 5.288 - 0.463X$$

where Y = blood total protein concentration
X = blood glucose concentration
$R^2$ = 0.1119

This relationship is due to increased tissue mobilisation. As more tissues are being mobilised due to the increased demand for energy for lactation, the blood total protein concentration increases due to increased mobilisation of body reserves as well as the increased demand for the various carriers for various metabolites. Similarly, Hossaini-hilali et al. (1993) found that

during food deprivation of goats there was an increase in the plasma protein concentration and that the increase in plasma protein indicated mobilisation of body tissue in order to support the high demand for lactation. This does not correspond with the findings of Schrick et al. (1990) who found that although cows on restricted energy diets were nutritionally stressed (losing both body weight and decreasing in body condition score) blood protein concentration was not affected by energy restriction.

Diet can also play a role in the blood total protein concentration. According to Rowlands (1980), in cows on low protein diets, the concentration of albumin and haemoglobin slowly decreases as lactation continues. This is due to the demand for amino acids for the production of milk proteins, which leads to the gradual reduction in the synthesis of other proteins. Caballero et al. (1992) found that an increase in protein intake of ewes led to an increase in blood total protein concentration and urea concentration. For the experiments done here however, this probably not the case because the cows were all fed according to NRC requirements.

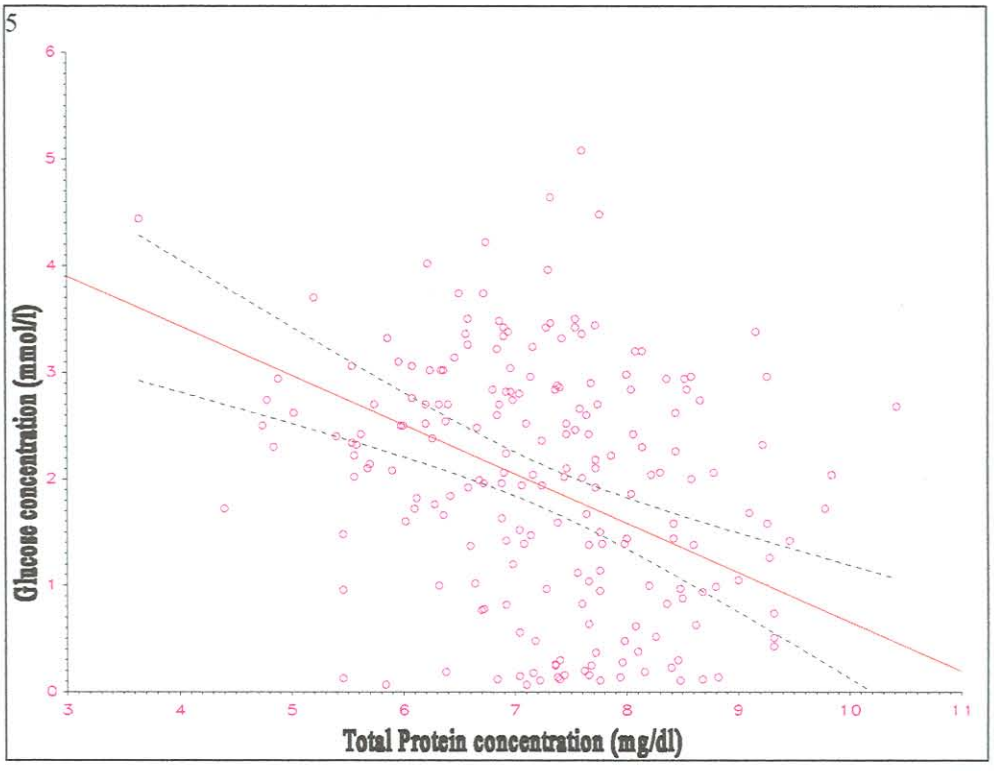


Figure 4. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and blood total protein concentration in Experiment 1. The dotted line represents the 95° confidence intervals.

Table 4. The parameter estimates, standard errors and probabilities of the relationship between blood glucose concentration and blood total protein concentration in Experiment 1.

| Variable | Parameter estimate | Standard error | Probability |
|---|---|---|---|
| intercept | 5.288 | 0.669 | 0.0001 |
| gradient | -0.463 | 0.091 | 0.0001 |

4.1.4. Blood glucose concentration versus body condition score

Blood glucose was found to be related to body condition score (P = 0.0006) as follows:

$$Y = 0.06 + 0.81X$$

where Y = blood glucose concentration

X = body condition score

$R^2 = 0.12$

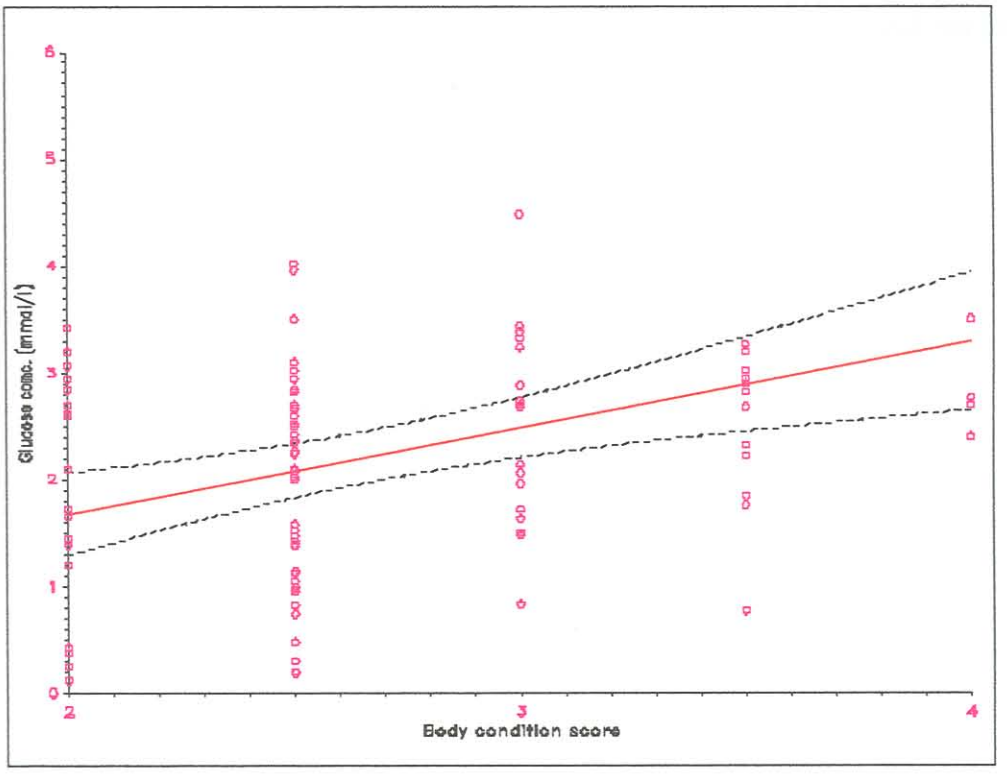


Figure 5. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and body condition score in Experiment 1. The dotted line represents the 95° confidence intervals.

Table 5. The parameter estimates, standard errors and probabilities of the relationship between blood glucose concentration and body condition score in Experiment 1.

| Variable | Parameter estimate | Standard error | Probability |
|---|---|---|---|
| Intercept | 0.0594 | 0.619 | 0.9236 |
| gradient | 0.809 | 0.227 | 0.0006 |

The relationship found indicates that animals with low body condition score had low glucose concentration. This is because animals with a low body condition score had either very little body reserves to mobilise or have already mobilised most of their reserves. This leads to there being few body reserves to mobilise to meet the energy demands of the body which in turn can lead to a low blood glucose concentration.

Blood glucose concentration was positively correlated to body condition score in the low production group ($P = 0.0499$; $R^2 = 0.112$). This relationship was not found for either the high milk production group or for the total group. This could be an indication that the low milk production group did not produce so much milk because they did not have enough body reserves to mobilise to make up the shortfall in energy requirements

4.1.5. Blood glucose concentration versus blood cholesterol concentration

In Experiment 1, blood glucose concentration was correlated to blood cholesterol concentration ($P = 0.001$).

$$Y = 9.78 - 4.11X + 0.594X^2$$

where $Y$ = blood glucose concentration

$X$ = blood cholesterol concentration

$R^2 = 0.377$

Table 6. The parameter estimates, standard errors and probabilities of the relationship between blood glucose concentration and blood cholesterol concentration in Experiment1.

| Variable | Parameter estimate | Standard error | Probability |
|---|---|---|---|
| intercept | 9.78 | 0.503 | 0.0001 |
| blood cholesterol concentration | -4.11 | 0.525 | 0.0001 |
| (blood cholesterol concentration)$^2$ | 0.59 | 0.126 | 0.0001 |

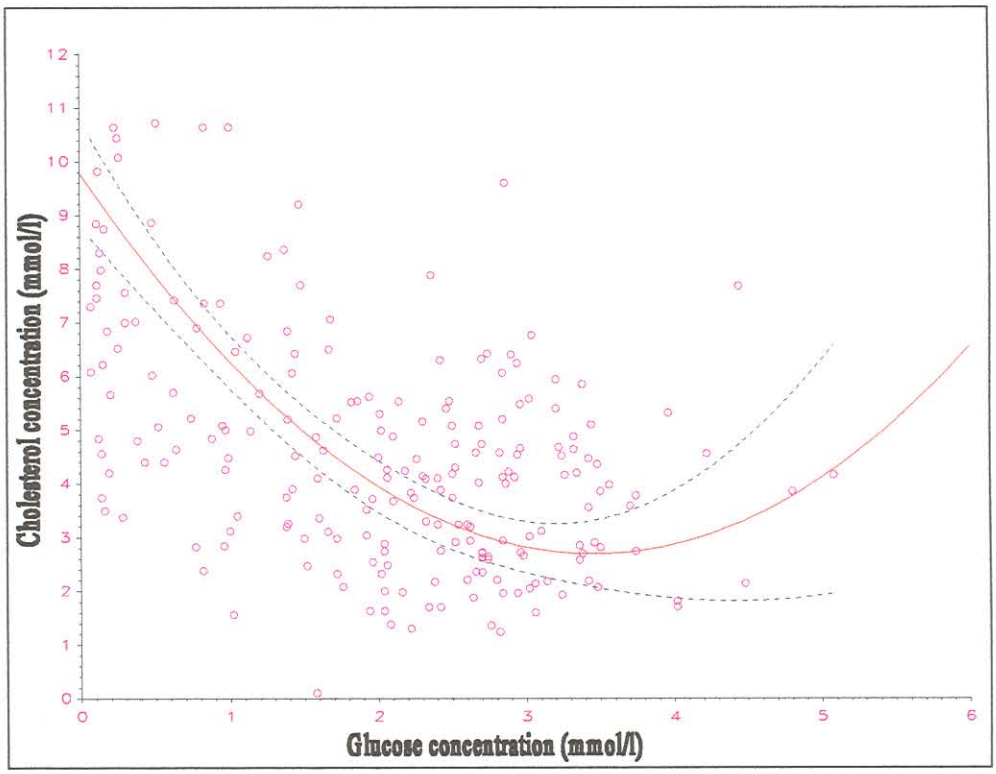


Figure 6. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and blood cholesterol concentration in Experiment 1. The dotted line represents the 95° confidence intervals.

In Experiment 1 blood glucose concentration and blood cholesterol concentration were negatively correlated, although it was not a very strong relationship ($R^2 = 0.38$). When blood glucose concentration decreases there is an increase in lipolysis to meet energy demands. This in turn leads to an increase in high-density lipoproteins (HDLs), which contain cholesterol. This relationship can be seen as an indication of the animal's energy status.

Ruegg et al. (1992) suggests that the relationship between blood glucose and blood cholesterol concentration is an indication of the animals body reserves.

In Experiment 2, blood glucose concentration was found to be correlated to blood cholesterol concentration ($P = 0.015$), which can be described by the equation:

$$Y = 4.233 - 0.238X + 0.0760X^2$$

Where $Y$ = blood cholesterol concentration

$X$ = blood glucose concentration

$R^2 = 0.0501$

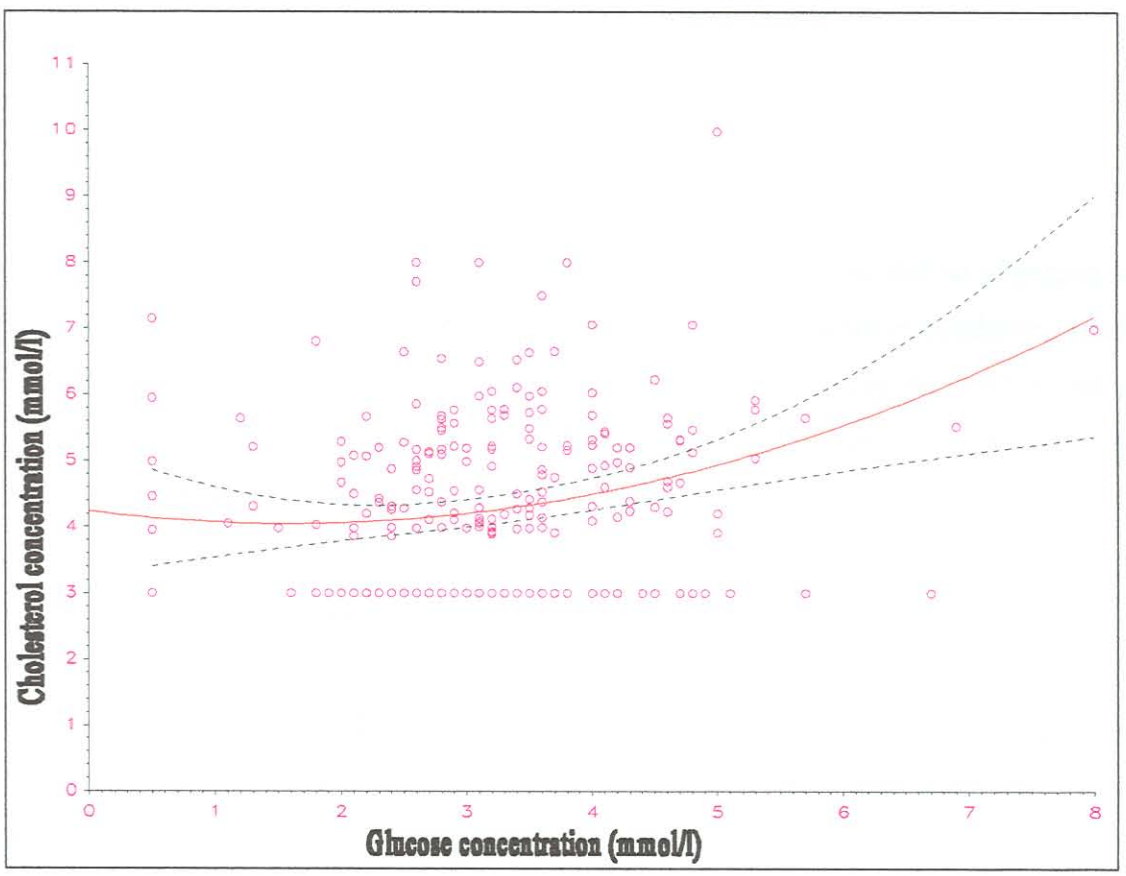


Figure 7. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and blood cholesterol concentration in Experiment 2. The dotted line represents the 95° confidence intervals.

This relationship is so weak ($R^2 = 0.0501$) that is could be seen as insignificant. These results are probably due to other factors having a stronger influence on the glucose concentration and blood cholesterol concentration, such as the weather. This means that the hand-held glucometer is not a very good tool to determine an animal's energy status due to other factors having such a strong influence.

Table 7. The parameter estimates, standard errors and probabilities of the relationship between blood glucose concentration and blood cholesterol concentration in Experiment 2.

| Variable | Parameter estimate | Standard error | Probability |
|---|---|---|---|
| intercept | 4.23 | 0.491 | 0.0001 |
| blood cholesterol concentration | -0.24 | 0.281 | 0.3969 |
| (blood cholesterol concentration)$^2$ | 0.07 | 0.040 | 0.0590 |

The reason for differences in experiments one and two, could be due to changing weather conditions. The cows in Experiment 1 had relatively constant weather conditions while those animals of Experiment 2 had constantly changing weather conditions. Both blood glucose and blood cholesterol concentrations are affected by changing weather conditions (Schaffer et al., 1981; Ingraham and Kappel, 1988).

### 4.1.6. Blood glucose versus insemination number

In Experiment 2, it was found that mean blood glucose concentration of the first, second and third inseminations differed ($P = 0.0581$). Cows that were inseminated three times had lower blood glucose than those cows requiring only one or two inseminations (Figure 8 and Table 8).

Table 8. The mean blood glucose concentrations and standard errors of the different insemination numbers. Means with different superscripts differ significantly (P<0.05).

| **AI Number** | **1** | **2** | **3** |
|---|---|---|---|
| **n** | 22 | 7 | 4 |
| **mean blood glucose concentration mmol/l** | $2.94^{a}$ | $3.88^{a}$ | $1.85^{b}$ |
| **standard error** | 0.23 | 0.43 | 0.80 |

In this Experiment it was found that there was a significant difference between the blood glucose concentration of the first insemination and third insemination and between the blood glucose concentration of the second and third insemination. This indicates that those animals that required three inseminations was at least in part due to their low blood glucose concentration, which was found to play a vital role in conception (Rowlands et al., 1980; Kappel et al., 1984).

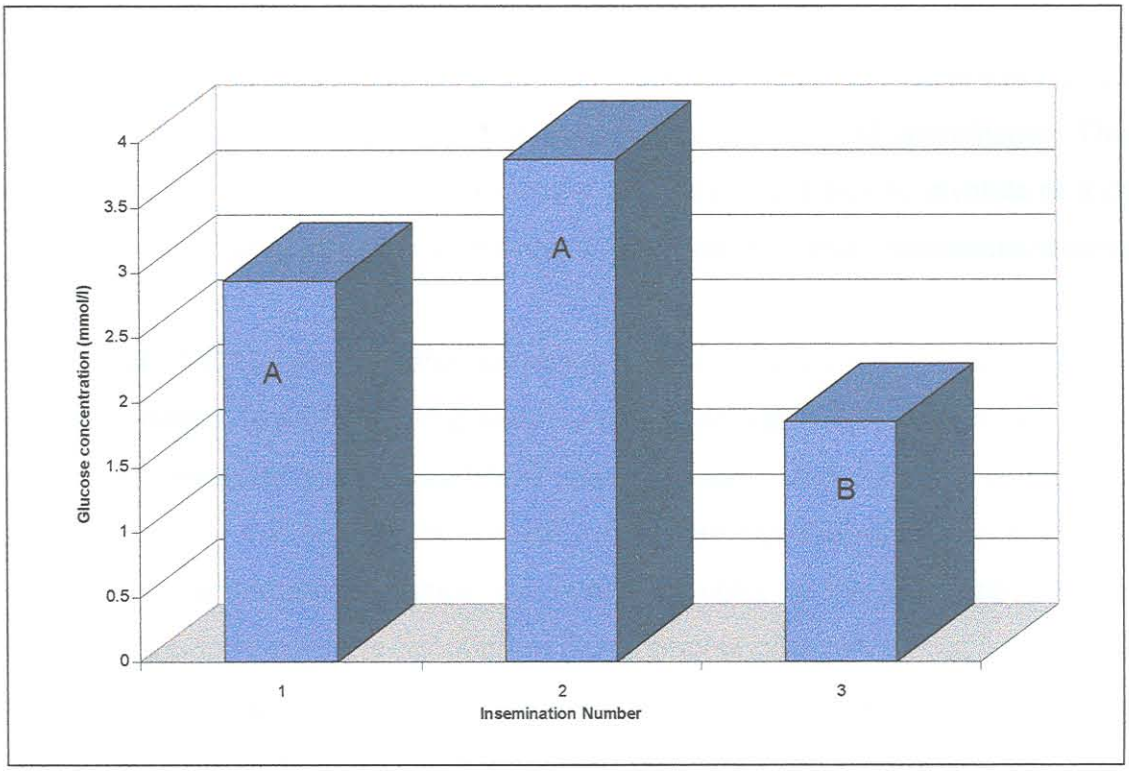


Figure 8. The mean blood glucose concentration of the different insemination numbers. Means with different letters differ significantly (P < 0.05).

Blood glucose plays such a vital role in reconception because glucose is a requirement of the reproductive organs for involution and preparing the uterus for reconception. Because

lactation has physiological priority over reproduction at the stage when reconception should occur (two months after parturition) the reproductive organs may be deprived of glucose, thereby slowing down the rate of involution. Glucose also has a direct effect on the ovarian tissue, although authors differ in the opinion of how glucose affects reproduction. Various authors (Rutter and Manns, 1986; Zurek et al., 1995; Burns et al., 1997) stated that energy restrictions suppressed the secretion of luteinizing hormone and luteinizing hormone releasing hormone. This in turn led to a decrease in progesterone secretion necessary for the formation of a corpus luteum. Spicer et al. (1990) and Kunz et al. (1995) found that the decrease in ovarian activity that accompanied a negative energy balance was in part due to the reduced insulin-like growth factor (IGF-I) concentration, which stimulates bovine granulosa cells and luteal steroidogenesis.

When comparing the blood glucose concentration of the cows that conceived on the first insemination to those that conceived on the second insemination to those that conceived on the third insemination, there was found to be a significant difference between those that conceived on the second versus the third insemination ($P = 0.0556$). There was no significant difference between those conceiving on the first versus the second insemination. This could be due to their reproductive organs requiring a slightly longer time to involute or it could be due to non-physiological factors such as time of insemination and insemination technique.

Allen et al. (1977) found that the percentage of cows that did not conceive at first service increased from 34% where blood glucose concentration was within one standard deviation of the normal mean, to 57% where blood glucose concentration was two or more standard deviations below the mean. It Experiment 2, the mean blood glucose concentration was 3.3 mmol/l and the standard deviation was 0.96. The cows in the group that required three inseminations to re-conceive had a mean blood glucose concentration of 1.85 mmol/l. This means their blood glucose concentration was 1.5 standard deviations below the mean.

Due to the effect of glucose concentration on reproductive function, the hand-held glucometer can be of value because it is fast, accurate and inexpensive. Blood glucose concentration should be monitored from just before calving to peak production on a regular basis. It is necessary to monitor the blood glucose concentration regularly to get an idea of

the cows average blood glucose concentration because it will vary from day to day. Blood glucose concentration should be monitored until after peak production because it is during this time that the cow should re-conceive and it will only happen after the cow has passed her negative energy nadir and the blood glucose concentration is above a certain level. It is therefore vital that the cow's blood glucose concentration be maintained, to obtain maximum milk production and yet still to re-conceive in this time. This is another reason why it is important to monitor blood glucose concentration from the onset of lactation. It is important that blood glucose concentration must be maintained above a certain level for ovulation to occur and thereby increasing the animals number of ovulations before insemination because pregnancy status after inseminations was positively related to the number of ovulations before insemination (Senatore et al., 1996).

## 4.2 Blood Cholesterol

### 4.2.1. Blood cholesterol versus weeks of lactation

In Experiment 1, the mean blood cholesterol concentrations of farms A, B and C did not differ significantly ($P < 0.05$) and therefore the data was pooled. The relationship was found to be curvilinear ($P = 0.0001$).

$$Y = 1.512 + 0.305X + 0.087X^2 - 0.004X^3$$

where Y= blood cholesterol concentration mmol/l

X = weeks of lactation

$R^2 = 0.69$

Table 9. The parameter estimates, standard errors and probabilities of the relationship between blood cholesterol concentration and weeks of lactation in Experiment 1.

| Variable | Parameter estimate | Standard error | Probability |
|---|---|---|---|
| intercept | 1.512 | 0.230 | 0.0001 |
| week of lactation | 0.306 | 0.057 | 0.0001 |
| (week of lactation)$^2$ | 0.087 | 0.021 | 0.0001 |
| (week of lactation)$^3$ | -0.004 | 0.002 | 0.0319 |

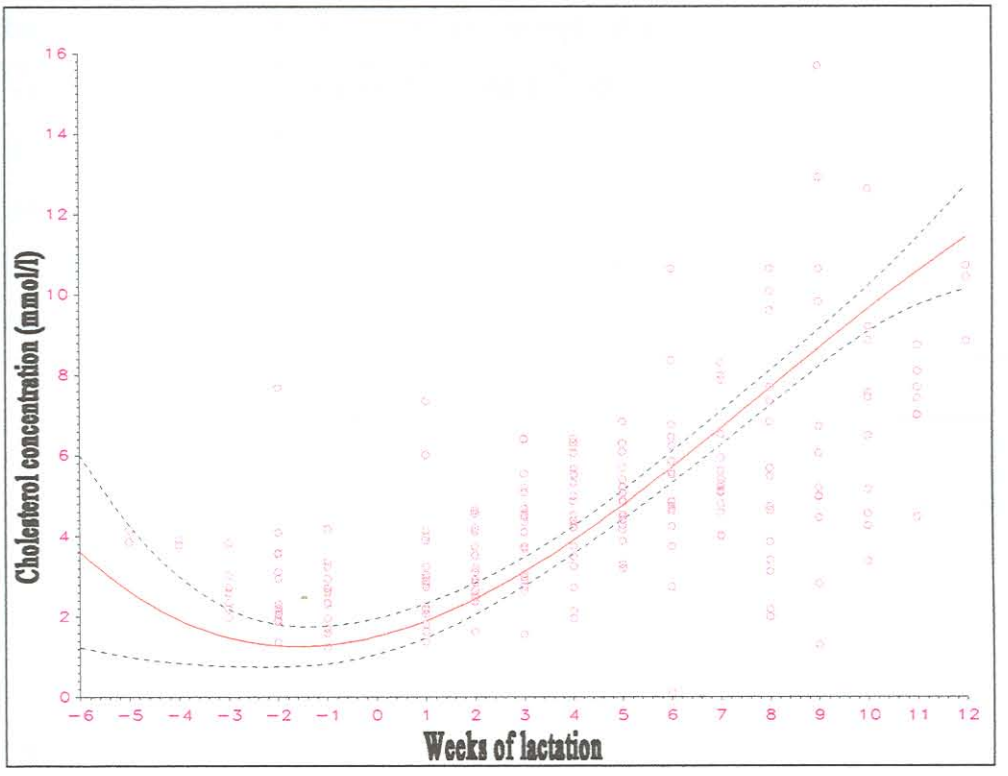


Figure 9. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood cholesterol concentration and weeks of lactation of Experiment 1. The dotted lines represent the 95° confidence intervals.

In Experiment 2, the relationship between blood cholesterol concentration and weeks of lactation was found to be curvilinear (P = 0.0001).

$$Y = 1.723 + 0.421X - 0.018\ X^2 + 0.0003X^3$$

where Y = blood cholesterol concentration

X = weeks of lactation

$R^2 = 0.59$

In both experiments blood cholesterol concentration increased with weeks postpartum. In Experiment 1 the blood cholesterol concentration at parturition was 1.512 mmol/l and increased to approximately 10 mmol/l. In Experiment 2 the blood cholesterol concentration at parturition was 1.723 and increased to approximately 6.5 mmol/l.

Table 10. The parameter estimates, standard errors and probabilities of the relationship between blood cholesterol concentration and weeks of lactation in Experiment 2.

| Variable | Parameter estimate | Standard error | Probability |
|---|---|---|---|
| intercept | 1.723 | 0.208 | 0.0001 |
| week postpartum | 0.421 | 0.057 | 0.0001 |
| (week postpartum)$^2$ | -0.018 | 0.004 | 0.0001 |
| (week postpartum)$^3$ | 0.0003 | 0.00008 | 0.0003 |

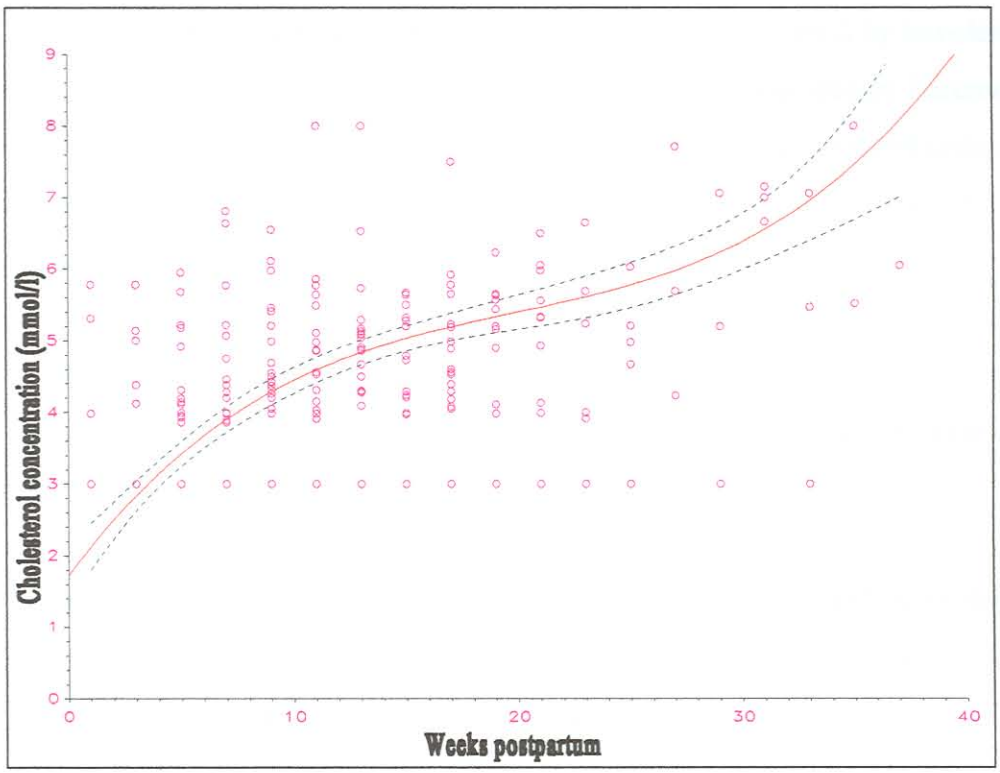


Figure 10. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood cholesterol concentration and weeks of lactation of Experiment 2. The dotted line represents the 95° confidence intervals.

The blood cholesterol concentration of Experiment 1 was quite high. Only Ruegg et al. (1992) found such similarly high concentrations (Table 11). Ruegg et al. (1992) could not explain the reasons for this high concentration but suggested possible reasons to be high fat in lactating cows diet or increased stress due to high milk yields.

Table 11. A comparison of blood cholesterol concentrations found by various authors.

| Author | animal | range (mmol/l |
|---|---|---|
| Arave et al., 1975 | dairy cow | 2.845 - 4.939 |
| Kronfeld, 1982 | dairy cow | 0.802 - 5.586 |
| Kappel et al., 1984 | dairy cow | 1.936 - 5.043 |
| Moody et al., 1992 | beef cow | 2.689 - 4.290 |
| Ruegg et al., 1992 | dairy cow | 2.492 - 10.420 |

The amount and proportion of total blood cholesterol contributed by low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs) varies among species (Grummer and Carroll, 1988). HDL cholesterol predominates in cattle. High blood cholesterol concentration in cattle reflects a high concentration of HDLs (Chapman, 1980). Blood cholesterol concentration gives an indication of the degree to which an animal is mobilising its body reserves. This is because as body reserves are being broken down and used as energy, high-density lipoproteins (HDLs) are being used to transport various energy components. These HDLs contain cholesterol, therefore, with increased lipolysis there is an increase in the HDLs and therefore an increase in cholesterol.

The curvilinear relationship found in both experiments corresponds with the results found by various researchers (Arave et al., 1975; Rowlands et al., 1980; Kappel et al., 1984). Kappel et al. (1984) found that blood cholesterol concentration was lowest at the onset of lactation, increasing to mid-lactation and declining towards the end of the lactation. Low serum cholesterol during the first month of lactation has been attributed to high milk production, which is associated with increased thyroid activity.

Increased thyroid hormone stimulates hepatic glycolysis, increases glucogenesis and gluconeogenesis, enhances lipolysis and decreases sensitivity to the antilypolytic action of insulin. Therefore, an increase in thyroid hormone leads to an increase in fat breakdown, which in turn requires increased lipoproteins, which in turn leads to an increase in the cholesterol concentration of the blood. The hand held glucometer can monitor blood cholesterol concentration, thereby monitoring the amount of body reserves mobilised.

Increased blood cholesterol concentration during lactation has been associated with increased lipoprotein synthesis and change among the various types of lipoproteins, which are required for lipid transport (Puppione et. al., 1978, Kappel et. al., 1984). This is due to increased fat mobilisation to meet the necessary energy requirements. This fat needs to be transported to the various sites in the body where it is required and this is done by the lipoproteins.

### 4.2.2. Blood cholesterol versus milk production

In Experiment 1, serum blood cholesterol concentration was found to be positively correlated to milk production ($P < 0.05$).

$$Y = 0.597 + 0.154X$$

where $Y$ = blood cholesterol concentration

$X$ = milk production

$R^2 = 0.093$

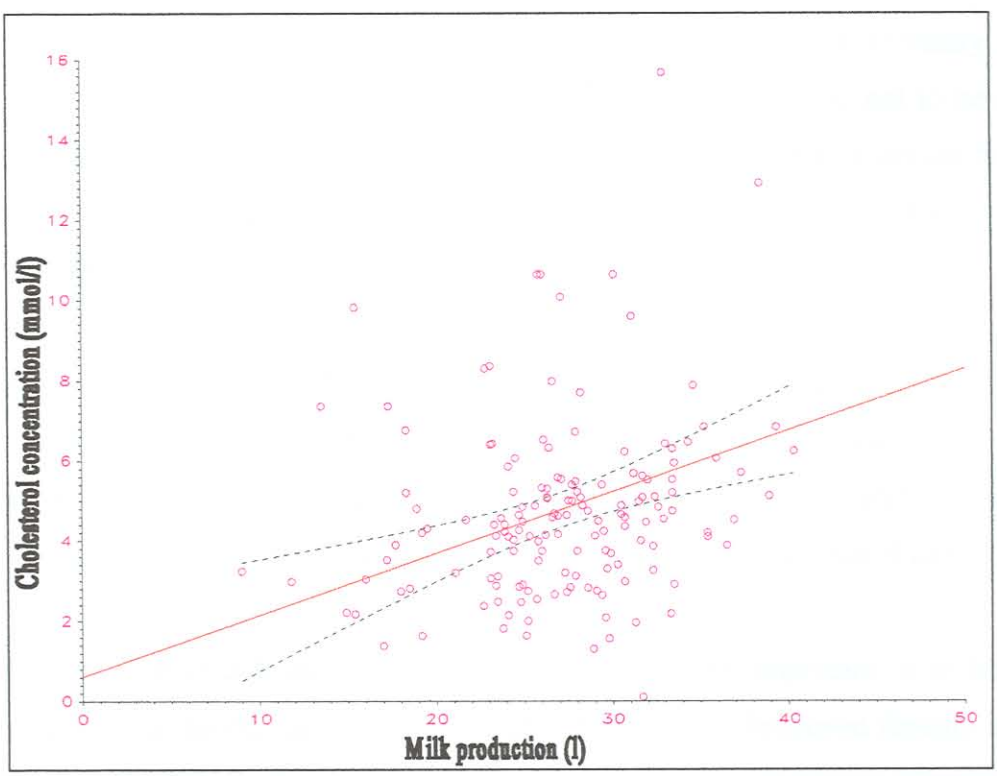


Figure 11. The relationship between the predicted regression (solid line) and the observed data of the relationship between blood cholesterol concentration and milk production of Experiment 1. The dotted lines represent the 95° confidence intervals.

Table 12. The parameter estimates, standard errors and probabilities of the relationship between blood cholesterol concentration and milk production in Experiment 1.

| Variable | Parameter estimate | Standard error | Probability |
|---|---|---|---|
| intercept | 0.597 | 1.084 | 0.5826 |
| milk production | 0.154 | 0.039 | 0.0001 |

In Experiment 2, no relationship (P = 0.1106) was found between whole blood cholesterol concentration and milk production.

This relationship is due to the fact that high milk production leads to increased thyroid activity, which in turn leads to the increased production of thyroid hormones. The thyroid hormones help to regulate lipid and carbohydrate metabolism.

4.2.3. Blood cholesterol versus reproduction

When comparing blood cholesterol concentration to insemination number in Experiment 2, no relation was found. This is an indication that blood cholesterol concentration does not affect the cow's reconception ability. Although cholesterol is a necessary precursor of the various steroid hormones, the demand for cholesterol is such as not to be a limiting factor affecting reproduction. Also cholesterol concentration tends to increase from the onset of lactation peak production as the animal mobilises body reserves, making it unlikely to have any limiting effect.

Rowlands et al. (1980), found that blood cholesterol concentration increased between Week 1 and 6 after calving, with an average increase of 0.54 ± 0.035 mmol/l per week. They also found that cows requiring more inseminations had higher minimum blood cholesterol concentrations but that blood cholesterol concentration was unrelated to conception rate.

It has been found that lipoprotein sterols make an important contribution to ovarian progesterone production (Grummer and Carroll, 1985). Improved fertility in cows has been associated with high circulating concentrations of progesterone (Carstairs et al., 1980; Fonseca et al., 1983). Development of strategies to increase lipoprotein sterol uptake may increase progesterone production and improve conception rate (Grummer and Carroll, 1988).

Several researchers have suggested a relationship between plasma cholesterol concentration and progesterone concentration (Henderson et al., 1981; Talavera et al., 1985). Kappel et al. (1984) has suggested that plasma cholesterol concentration is related to reproductive performance.

In Experiment 1 there was found to be a significant difference between the low production group blood cholesterol concentration and the medium production groups blood cholesterol concentration ($P = 0.049$). There was also a difference between the low and high production groups blood cholesterol concentrations ($P = 0.084$). There was no difference between the high and medium production groups' blood cholesterol concentration (Figure 12)

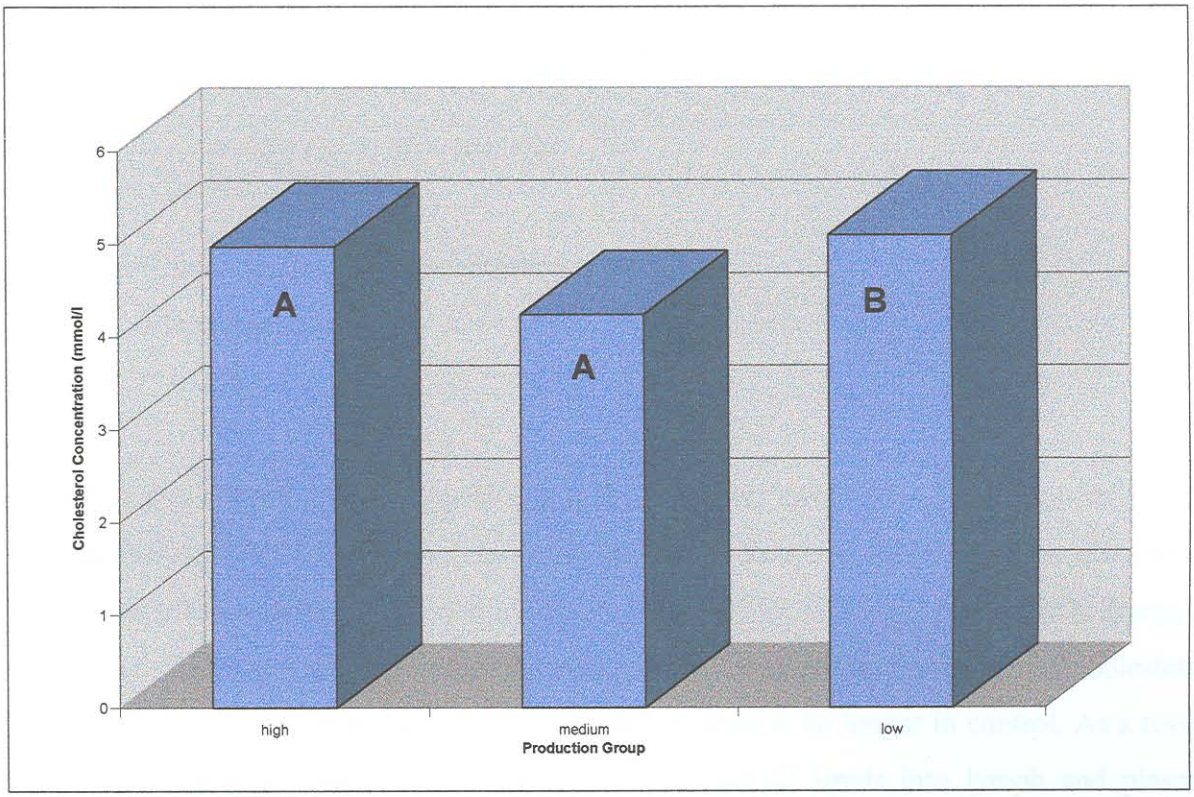


Figure 12. The relationship between blood cholesterol concentration and high, medium and low production groups. Means with different letters differ significantly ($P < 0.05$).

Table 13. The mean values, standard errors and probabilities of blood cholesterol concentration for the different milk production groups. Means with different superscripts differ significantly ($P < 0.05$).

| Group | Mean | Standard error |
|---|---|---|
| high | $4.970^{a}$ | 0.393 |
| medium | $4.241^{a}$ | 0.329 |
| low | $5.102^{b}$ | 0.356 |

4.2.4. Blood cholesterol versus feed intake

In Experiment 2, blood cholesterol concentration was positively correlated to feed intake ($P = 0.030$). The relationship is a very weak one, with only 5% of the change in blood cholesterol being attributed to feed intake. This indicated that blood cholesterol concentration could be considered as having a negligible effect of feed intake.

$$Y = 0.14X - 0.20 + 0.005X^2$$

where $Y$ = blood cholesterol concentration mmol/l

$X$ = feed intake kg/day

$R^2 = 0.056$

Grummer and Carroll (1988) suggested that the form and level of dietary energy influences plasma cholesterol concentration. It has been found that diets with a high fat content led to an increase in plasma cholesterol content (Talavera et al., 1985; Grummer et al., 1988; Ruegg et al., 1992). Under normal conditions a form of homeostasis regulates plasma cholesterol concentration. When dietary fat increases, this mechanism is no longer in control. As a result there is increased synthesis of cholesterol for transport of lipids into lymph and plasma (Talavera et al., 1985).

## 4.3 Blood total protein

### 4.3.1 Blood total protein versus weeks of lactation

In Experiment 1, the mean blood total protein concentration between farms did not differ significantly and therefore the data was pooled. There was found to be a relationship between blood total protein concentration and weeks postpartum (P = 0.005).

$$Y = 5.877 + 0.310X + 0.048X^2 - 0.006X^3$$

where Y = blood total protein concentration mg/dl

X = weeks of lactation

$R^2 = 0.53$

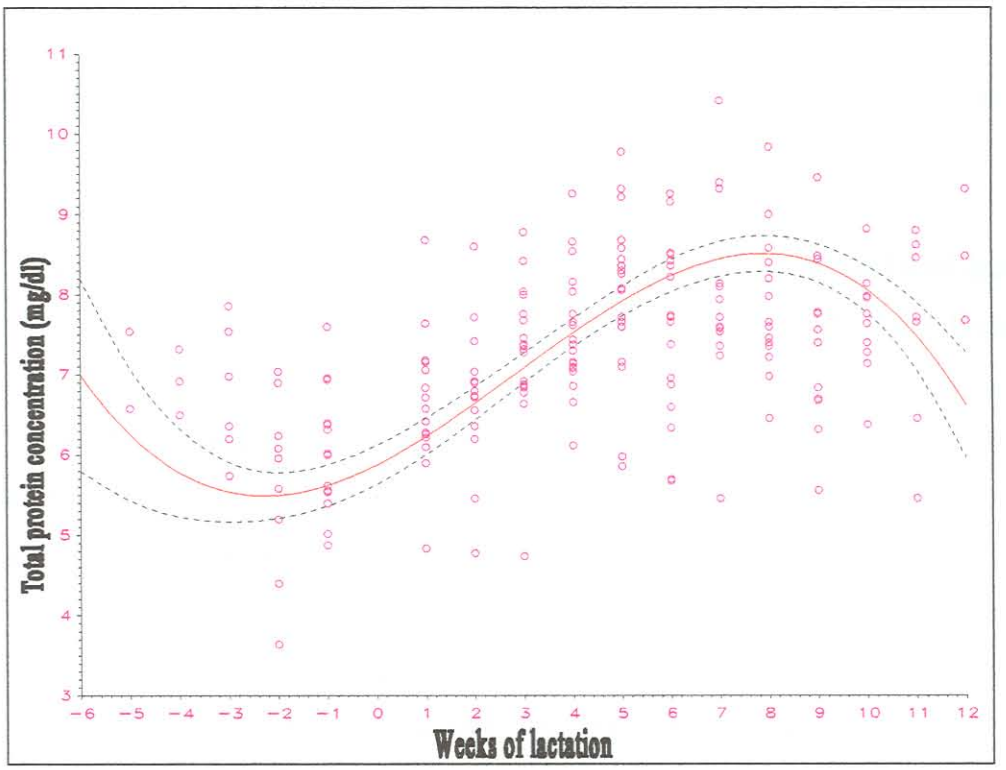


Figure 13. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood total protein concentration and week of lactation of Experiment 1. The dotted line represents the 95° confidence intervals.

Table 14. The parameter estimates, standard errors and probabilities of the relationship between blood total protein concentration and week of lactation of Experiment 1.

| Variable | Parameter estimate | Standard error | Probability |
|---|---|---|---|
| intercept | 5.877 | 0.124 | 0.0001 |
| week of lactation | 0.310 | 0.034 | 0.0001 |
| (week of lactation)$^2$ | 0.048 | 0.010 | 0.0001 |
| (week of lactation)$^3$ | -0.006 | 0.0008 | 0.0001 |

The blood total protein concentration decreases before parturition, is lowest at parturition and increases to peak at approximately eight weeks postpartum. The decrease in blood total protein concentration before parturition is due to the increased requirements of various plasma proteins in the colostrum and also due to the increased demand of protein by the fetus. The peak of blood total protein concentration at peak lactation is due to the fact that many of the plasma proteins are carriers for various metabolic products. At the time of peak lactation there is great metabolic activity due to milk production and mobilisation of body reserves.

Jordan and Swanson (1979a) found that during the first four weeks of lactation the blood total protein concentration increased linearly, $Y = 5.03 + 0.34X$. They found that the blood total protein concentration began at a lower concentration (5.03 mg/dl compared to 5.88 mg/dl) but increased more over the four weeks (6.39 mg/dl compared to 6.80 mg/dl) compared to the results found in Experiment 1. Jordan and Swanson (1979a) also found that after the fourth week the blood total protein concentration plateaued for the next ten weeks.

4.3.2. Blood total protein versus milk production

There was found to be a relationship between blood total protein concentration and milk production ($P = 0.0036$) in Experiment 1.

$$Y = 6.16 + 0.0029X + 0.0018X^2 - 0.00003X^3$$

where $Y$ = blood total protein concentration

$X$ = Milk production

$R^2 = 0.09$

Although it is a very weak relationship ($R^2 = 0.09$) increasing milk production leads in part to an increase in blood total protein concentration. This is due to the increased mobilisation of body reserves requiring more protein carriers in the blood to meet the demand of the metabolites being mobilised.

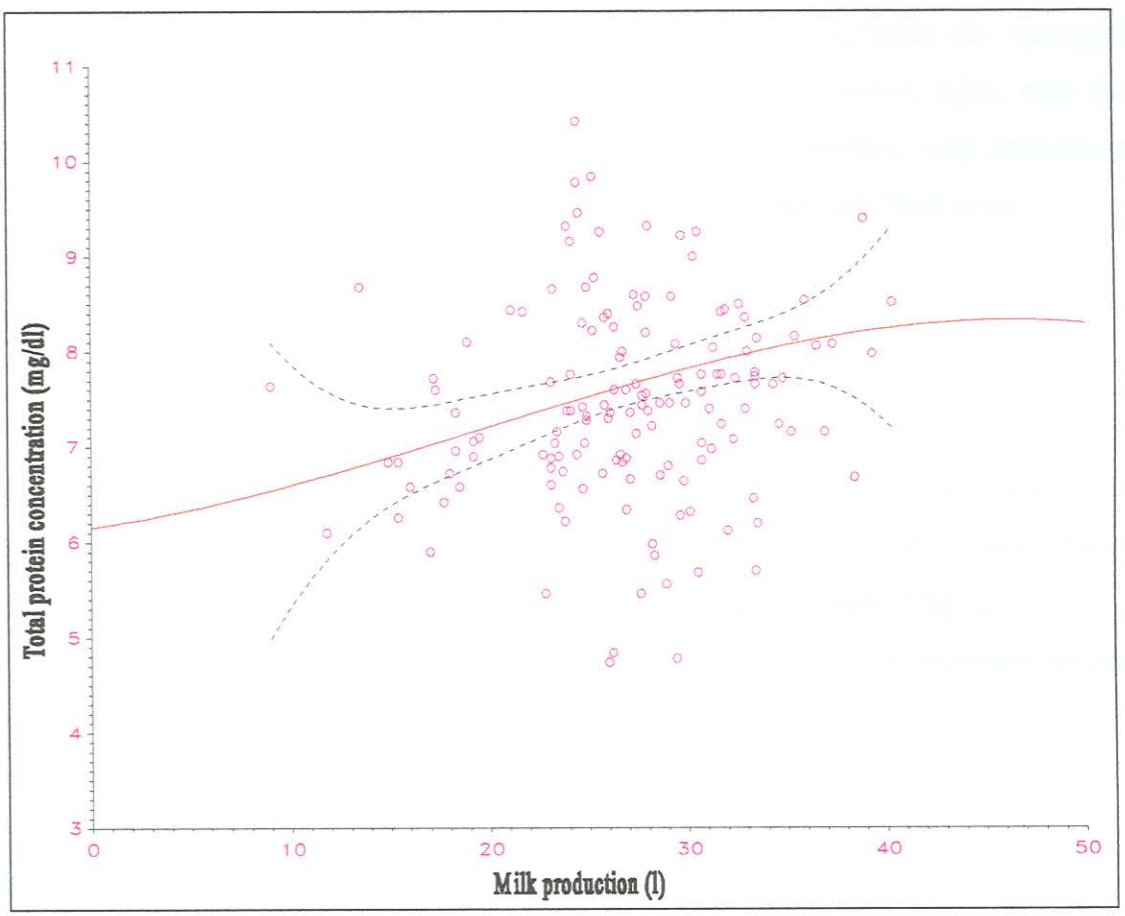


Figure 14. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood total protein concentration and milk production of Experiment 1. The dotted line represents the 95° confidence intervals.

4.3.3. Blood total protein versus body condition score

In Experiment 1 there was found to be a relationship between blood total protein concentration and body condition score ($P = 0.019$).

$$Y = 8.88 - 0.66X$$

where Y = body condition score

X = blood total protein concentration

$R^2 = 0.057$

This is a weak relationship. This is in part due to the fact that blood total protein concentration does not have a direct effect on body condition score, rather, there is a indirect relationship. As the body mobilises reserves to meet the demands for increased milk production there is an increase in blood total protein concentration. Also, with increased mobilisation there is a decrease in body condition score. Therefore, with increasing blood total protein concentration there is an indirect decrease in body condition score.

## 4.4. Blood Urea

### 4.4.1. Farm differences

In Experiment 1 it was found that blood urea concentration differed significantly between farm B and farm C ($P = 0.001$) and also between farm A and farm C ($P = 0.007$). Farm C had significantly lower blood urea concentration than the other two farms (fig. 15).

It has been found that feeding a total mixed ration throughout the day regulated the ammonia concentration and would more likely prevent surges of ammonia that occur when feeding concentrate in two separate portions per day (Wohlt et al., 1976). On farms A and B rations were fed two and three times a day respectively, while on farm C a total mixed ration was fed. Although there were only three cows on farm A and two on farm B, this could be the reason for the significantly lower blood urea concentrations on farm C.

Table 15. The means, standard errors and probabilities of the blood urea concentrations of the different farm groups. Means with different superscripts differ significantly ($P < 0.05$).

| Group | Mean | Standard error |
|---|---|---|
| Farm A | $26.557^{a}$ | 1.527 |
| Farm B | $28.431^{a}$ | 1.704 |
| Farm C | $20.799^{b}$ | 0.641 |

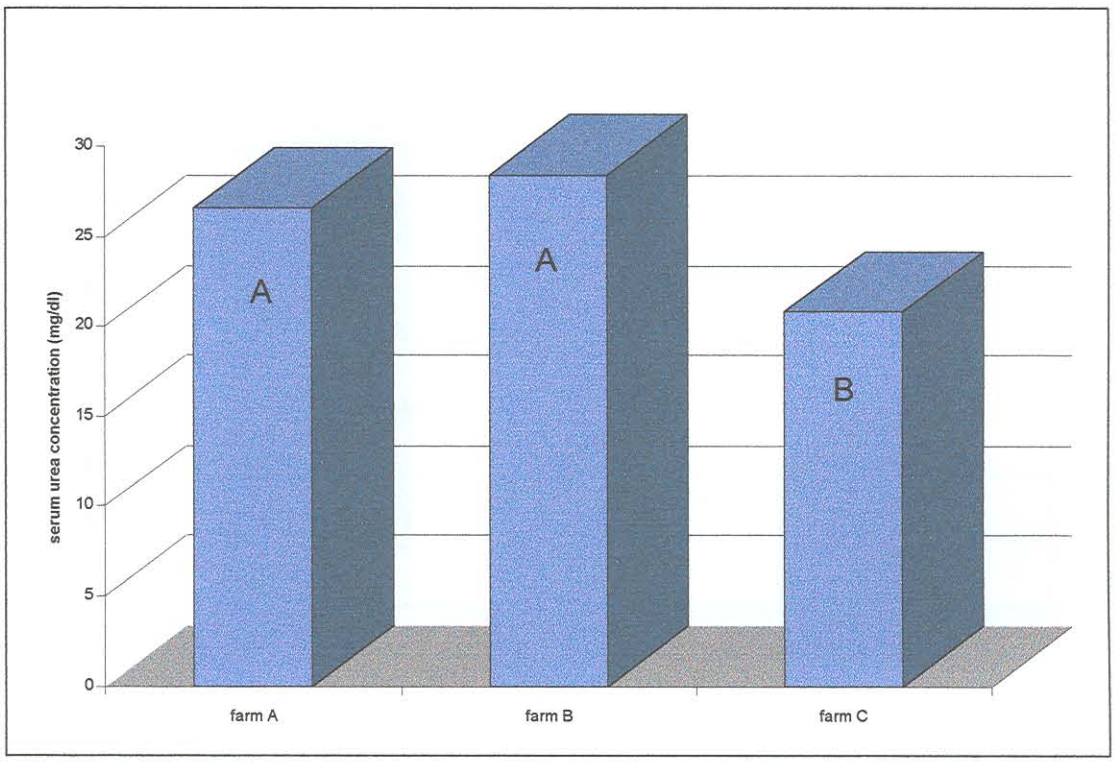


Figure 15. The relationship between blood urea concentration and farms. Means with different letters differ significantly ($P < 0.05$).

When the production groups were compared (Figure 16) it was found that there was significant differences between the high production group and the low production group ($P = 0.0058$) and also between the high production group and the medium production group ($P = 0.0006$).

High blood urea concentrations can be interpreted as an indication of high crude protein intake (Tomlison et al., 1994; Butler et al., 1996) or as an indication of the catabolism of endogenous protein reserves (Bergman, 1983; Amaral-Phillips et al., 1993; Schrick et al., 1996). The high production group had significantly high blood urea concentration than the other two production groups. This could be an indication that there was a greater amount of endogenous protein being broken down in the high production group than in the other groups. This is due to the high production group breaking down more of their protein reserves to supply more substrates to meet the energy demands for milk production.

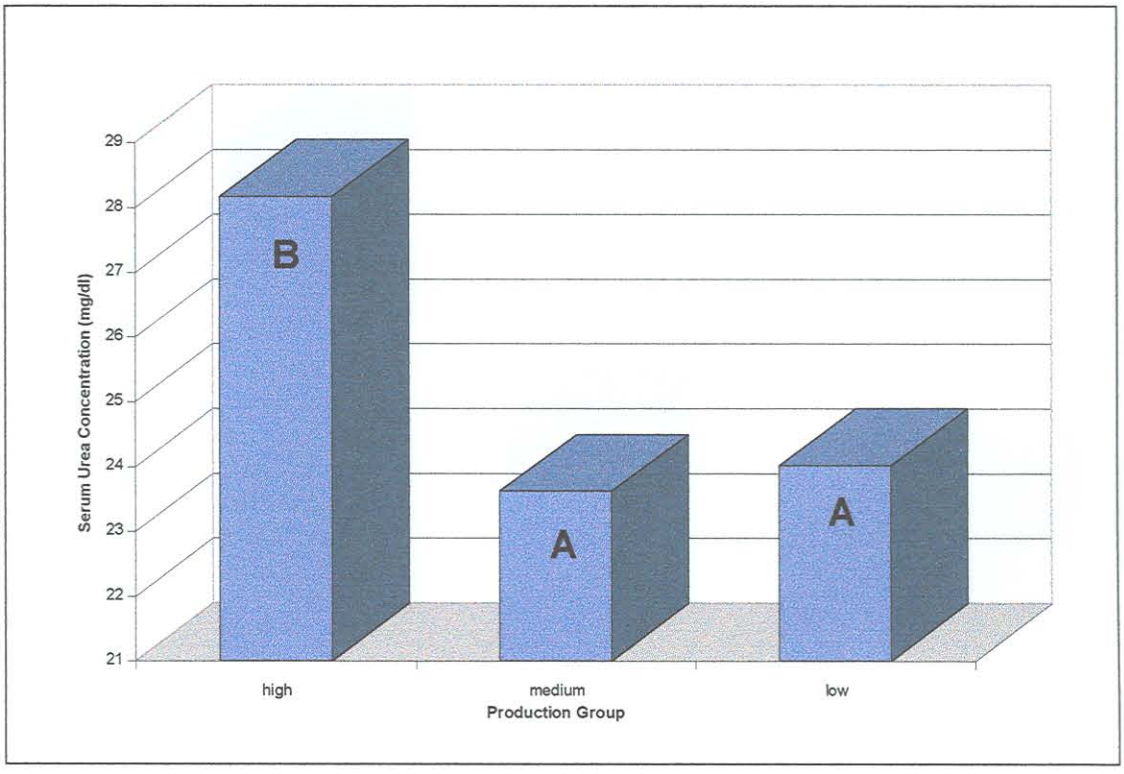


Figure 16. The relationship between blood urea concentration and high, medium and low milk production groups. Means with different letters differ significantly ($P < 0.05$).

## 4.5 Body Condition Score

### 4.5.1. Farm differences

It was found in Experiment 1 that body condition score (BCS) tended to differ between farm A and Farm B ($P = 0.067$) and differed significantly between farm A and farm C ($P = 0.0019$).

Table 16. The means, standard errors and probabilities of body condition score for the different farms. Means with different superscripts differ significantly ($P<0.07$).

| Variable | Mean | Standard error |
|---|---|---|
| Farm A | $3.09^{a}$ | 0.143 |
| Farm B | $2.66^{a}$ | 0.186 |
| Farm C | $2.59^{b}$ | 0.064 |

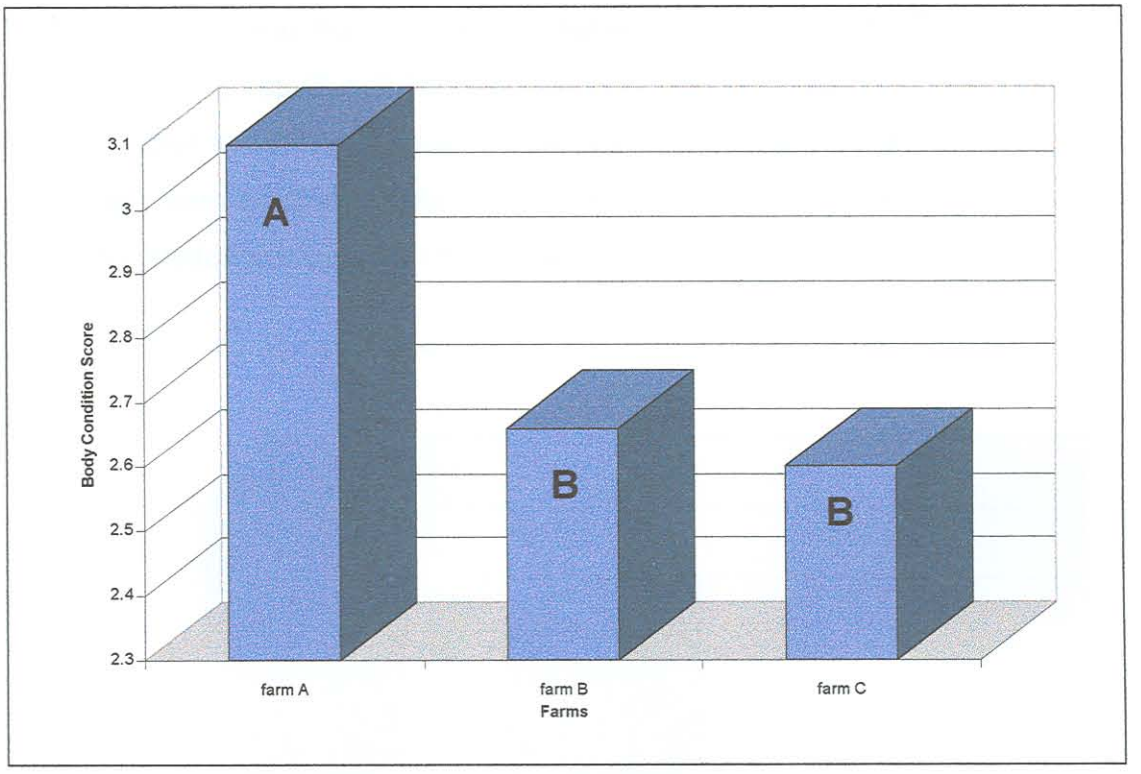


Figure 17. The relationship between body condition score and farms. Means with different letters differ significantly ($P < 0.05$).

It can be seen that the cows in farm A had significantly better body condition than on the other two farms. This could be influenced by the type of animal, in that, the cows on farm a were Dutch-Friesland types, while those on farm B were American Holstein types and the cows on farm C were Dairy Swiss. The Dutch Friesland tends to mobilise less body reserves than the American Holstein type cow. Although no specific conclusions can be drawn due to the low number of animals on farms A and B.

4.5.2. Body condition score versus milk production

Although body condition score is an inaccurate predictor of the animal physiological and nutritional status, it is still an important factor to observe. Cows must calve in a good body condition so as to have adequate body reserves to make up the short fall of energy that the feed can not supply for peak lactation. It is important that the cow is not too fat, otherwise she becomes a possible candidate for fat cow syndrome. A cow that is too lean is also undesirable otherwise there will not be sufficient body reserves and this could lead to ketosis, which has a strong negative effect on production and on reproduction.

In Experiment 1 it was found that body condition score tended to differ between the high and low production group (P = 0.083), with cows in the high production group having a better body condition score than those in the low production group (fig 18). In the low production group there was a correlation between milk production and body condition score (P = 0.0359) but no correlation was found for the high and medium production groups

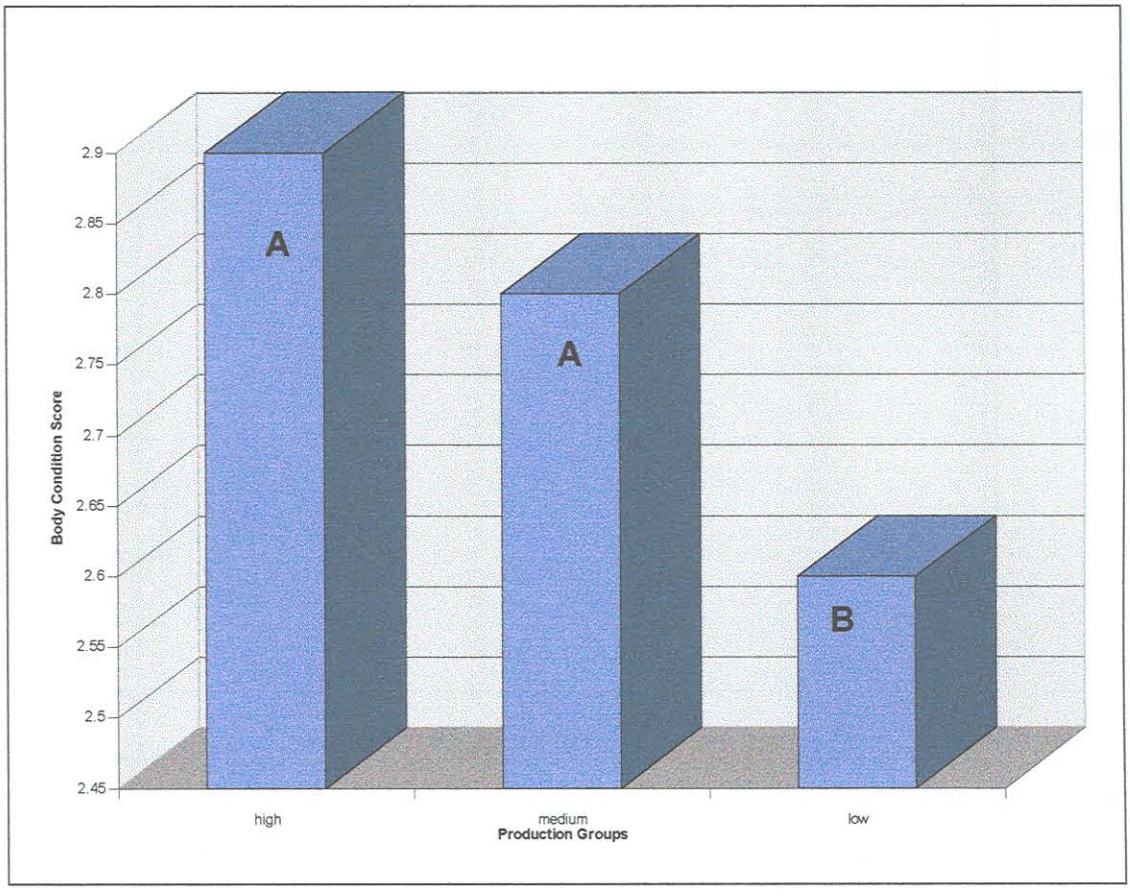


Figure 18. The relationship of body condition score between the high, medium and low milk production groups. Means with different letters differ significantly (P < 0.09).

This could be an indication that body condition influences milk production in the low production group. In cows with good body condition score, the body condition did not influence milk production.

## 4.6 Feed Intake

In Experiment 2, it was found that feed intake was positively correlated with milk production (P = 0.0084).

$$Y = 6.21 + 2.97X - 0.001X^2 + 0.001X^3$$

Where Y= feed intake (kg)

X = milk production (litres)

$R^2 = 0.072$

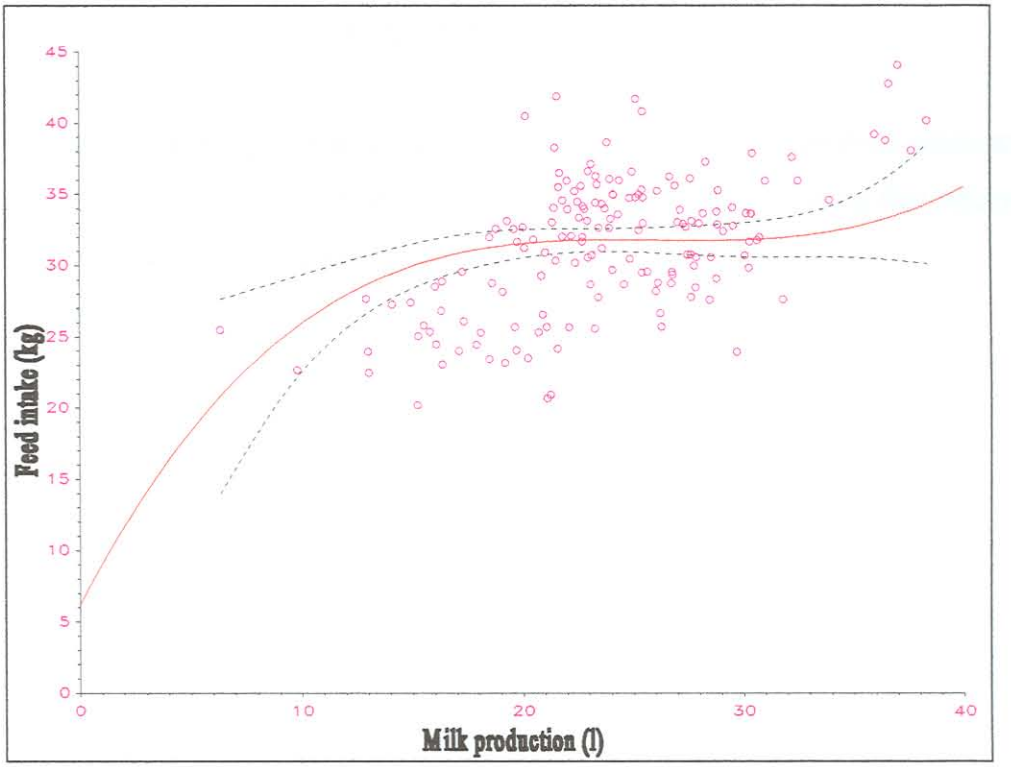


Figure 19. The relationship between the predicted regression (solid line) and the observed data for the relationship between feed intake and milk production in Experiment 2. The dotted lines represent the 95° confidence intervals.

The relationship between feed intake and milk production is a weak one ($R^2$ = 0.072), therefore it can be seen that milk production has little effect on feed intake.

## 4.7. Insemination number

Insemination number was compared to the various metabolites to determine if there was a relationship. There was no relationship found between insemination number and cholesterol. Kappel et al. (1984), however, found there was a relationship between cholesterol and reproductive performance.

There was found to be no relationship between milk production and insemination number. Harrison et al. (1990) found similar results in that there was no relationship between milk production and resumption of postpartum activity in high and average producing cows. Lacasse et al. (1993) found there was a positive relationship between milk production and resumption of ovarian activity. They stated that this was due to the higher dry matter intake of high producing cows leading to the high producing cows having a smaller negative energy balance than the low producing cows.

In this expriment there was found to be no relationship between feed intake and insemination number. Lacasse et al. (1993) found that it was the level of feeding that affected reproduction rather than amount taken in by the cow.

## 5. Conclusion

In this study, blood metabolite concentrations were investigated as an indication of the freshened dairy cows' metabolic, productive and nutritional status. The use of blood metabolites as an indication of the animal's metabolic status has been used before, but there have been arguments against using this for individual animals due to the fact that there are many daily factors and non-nutritional factors affecting the concentration of the various metabolites. However, with regular monitoring and sound advice in the interpretation of the results from an animal scientist and / or veterinarian, the use of blood metabolites is a useful tool in the management of a dairy operation.

A cow will produce well below her capabilities, or she will develop metabolic disorders and health problems if she is unable to alter nutrient intake and whole body metabolism rapidly, or to the extent needed to meet the demands for milk synthesis. Changes in the partitioning of metabolites associated with energy use are of the greatest magnitude at the onset of lactation (Bauman and Currie, 1980). It is important to supply the cow with an energy rich diet during early lactation. This requires the inclusion of a high percentage of grain, but liberal grain feeding can lead to metabolic disorders.

In this study, it was found that blood glucose concentration decreased after parturition and blood cholesterol concentration increased. This is an indication that the animal is mobilising body reserves to make up for the shortfall in the feed. The extent to which the animal mobilises her reserves can influence both her production and reproduction. The use of the hand-held glucometer to monitor this is of little value due to the many factors affecting both blood glucose concentration and blood cholesterol concentration.

Also in this study, it was found that low blood glucose concentration corresponded to retarded conception. This was also found by many other authors ( Rowlands et al., 1980; Kappel et al., 1984, McClure, 1994). It was found that mean blood glucose concentration had an effect on the time it took an animal to reconceive. The hand-held glucometer can be used to determine the mean blood glucose concentration of an animal. This will give an indication of the likelihood that the animal will conceive when she comes on heat. In this way one can have the option of not inseminating when the blood glucose concentration is too low and the

chances of conception are small. Also, with constant monitoring of the blood glucose concentration one can try and keep the blood glucose concentration above a certain level so that when the time comes to inseminate the cow, the chances of conception are high.

Although in this study, blood urea was only examined in Experiment 1 and not in Experiment 2 with respects to the relationship to reproduction, it has been shown by many researchers (Canfield et al., 1990; Elrod et al., 1993; Butler et al., 1996) to affect reproductive function. It was found that a blood urea concentration of above 19 mg/dl had a negative effect on reproduction. Also, researchers have found that blood urea and milk urea concentrations were highly correlated (Bergman,1983). It is therefore of importance to monitor the blood urea concentration via the milk to make certain it does not affect reproduction.

The hand held glucometer can be a useful tool on dairy farms. It can give the farmer an indication of the cow's reproductive status almost immediately. It is a tool that must be used on a regular basis on each animal due to the fact that there are various factors, besides nutrition that affect blood metabolites. The farmer should have the help of a veterinarian and/or an animal scientist to help interpret the results and advise on solutions to any problems. This is due to the fact that interpretation of the results is rarely straightforward. It should be kept in mind that the use of the hand held glucometer is not a simple solution to reproductive problems. This should be considered as an early warning system and used in conjunction with body condition score, milk production, milk urea analysis and general regular visual appraisal of the cows. It should only be used where intense management is present and the solutions suggested can be implemented.

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