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.

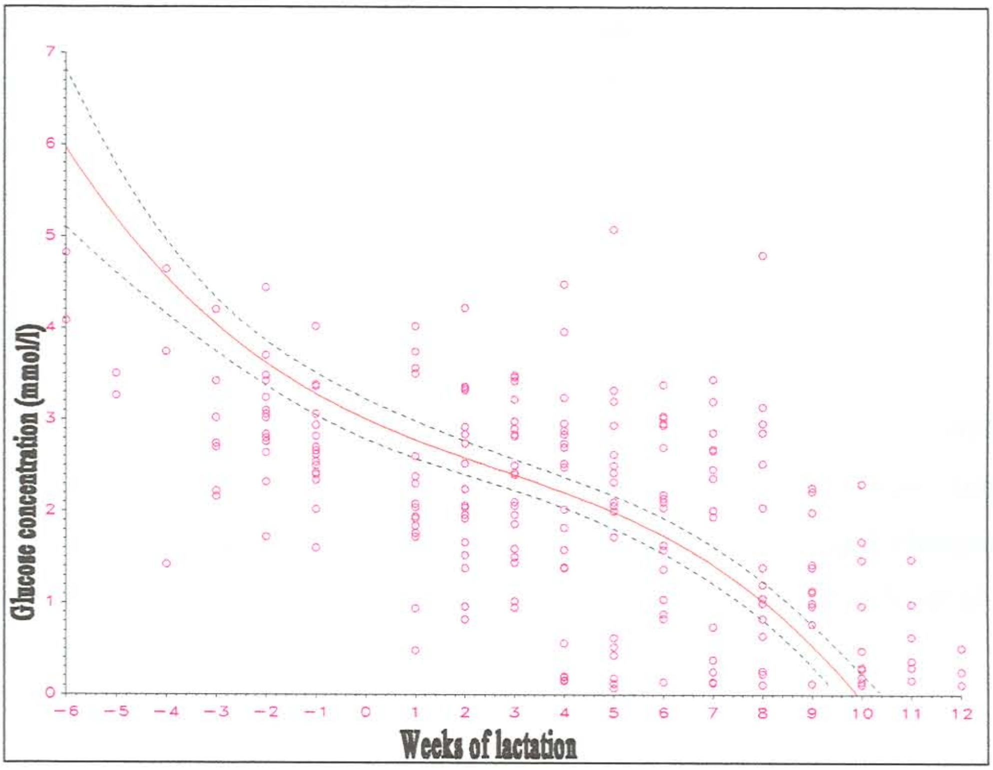

Figure 1. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and weeks of lactation in Experiment 1. The dotted lines represent the 95° confidence intervals.

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

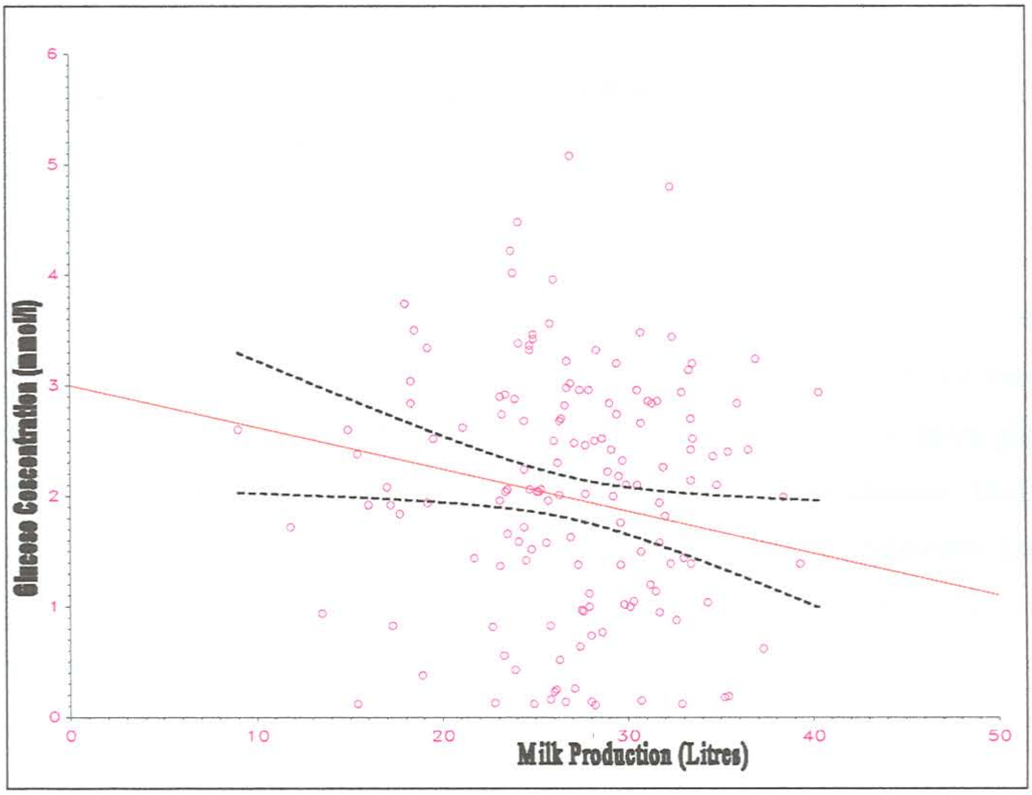

Figure 2. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and milk production in Experiment 1. The dotted lines represent the 95° confidence intervals.

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.

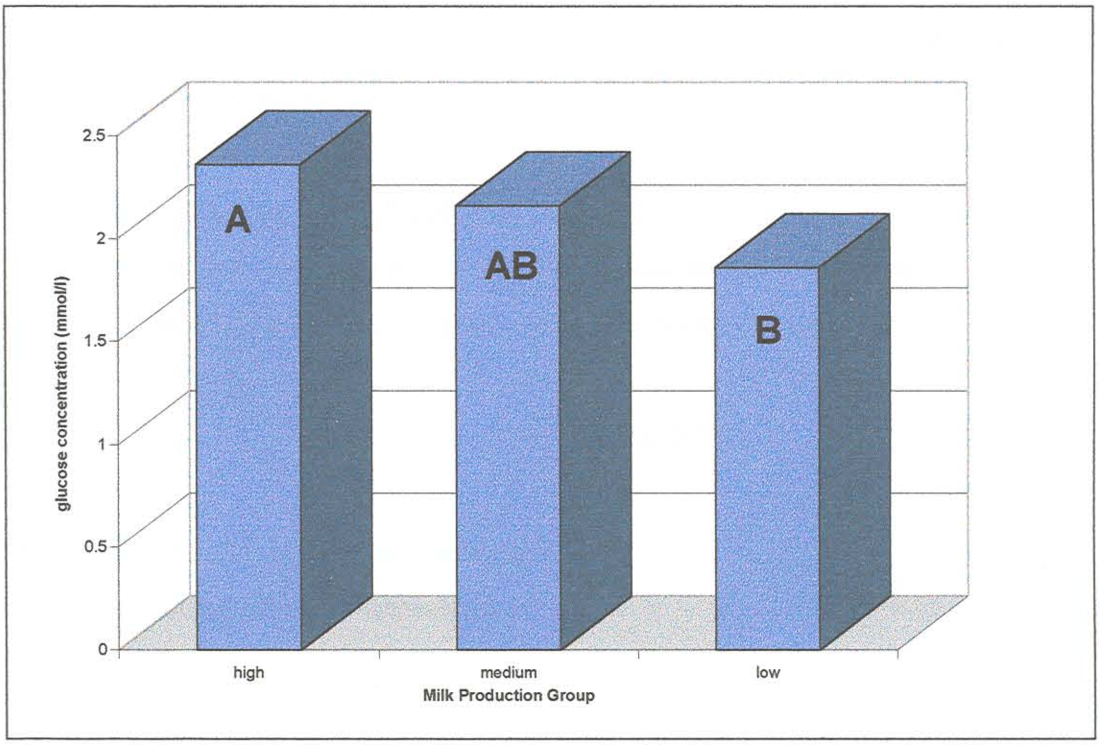

Figure 3. The relationship between blood glucose concentration and high, medium and low production groups of Experiment 1. Means with different letters differ significantly ($P < 0.05$).

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.

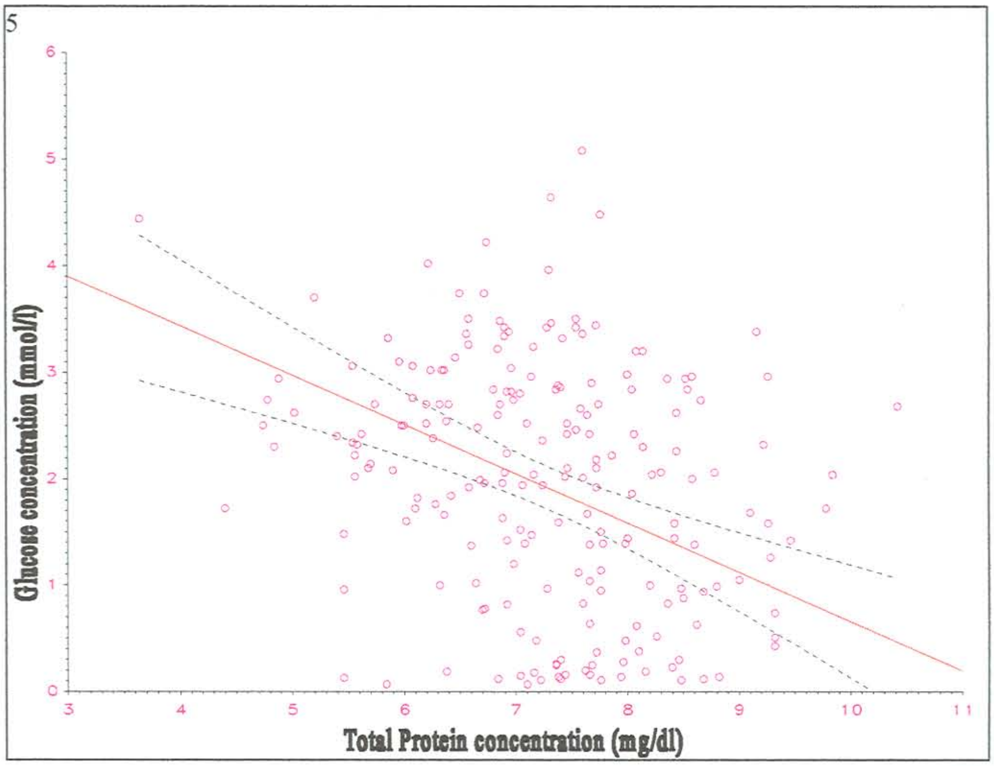

Figure 4. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and blood total protein concentration in Experiment 1. The dotted line represents the 95° confidence intervals.

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

$R^2 = 0.12$

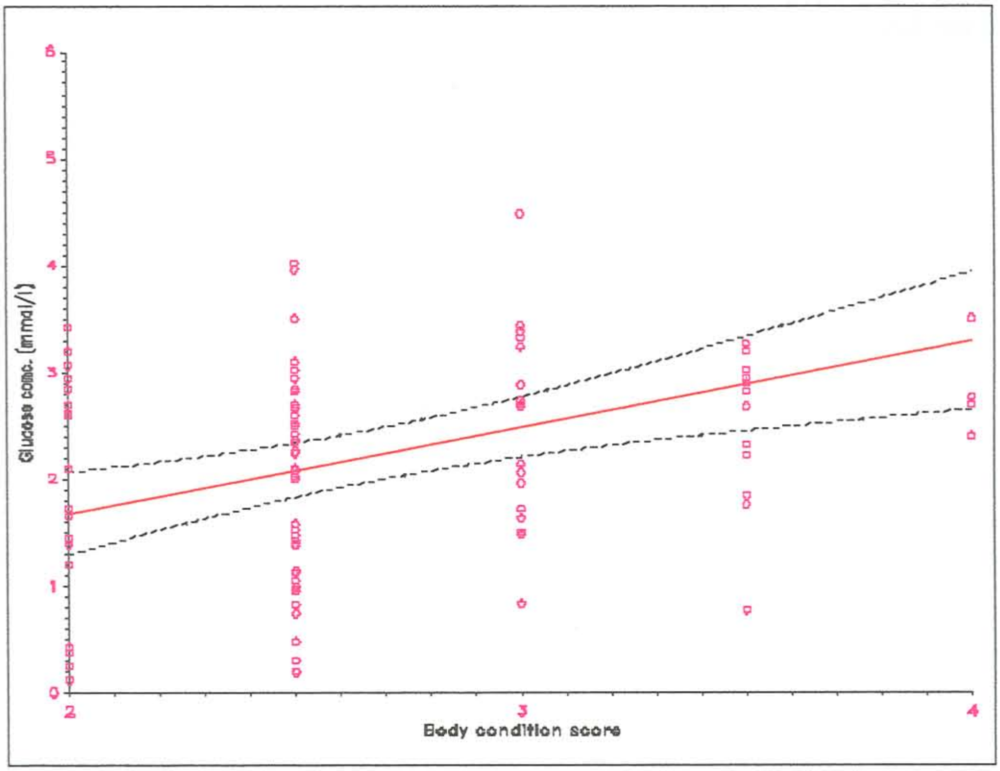

Figure 5. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and body condition score in Experiment 1. The dotted line represents the 95° confidence intervals.

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

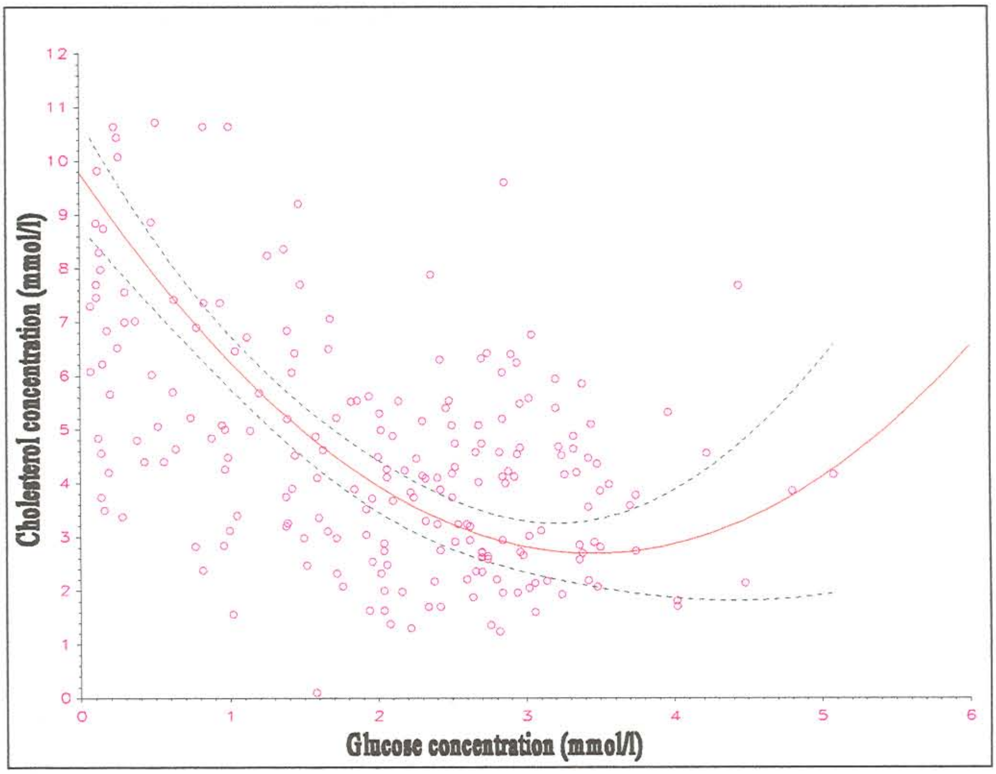

Figure 6. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and blood cholesterol concentration in Experiment 1. The dotted line represents the 95° confidence intervals.

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

$R^2 = 0.0501$

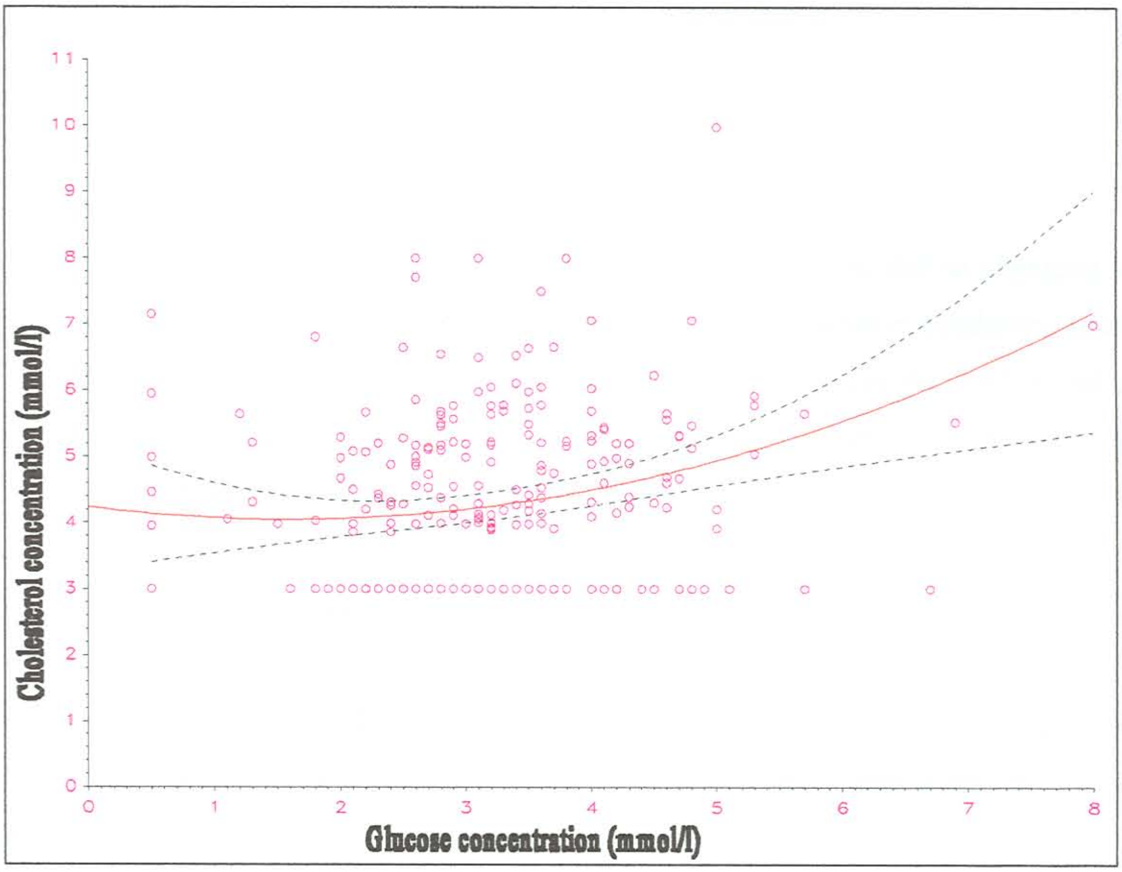

Figure 7. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood glucose concentration and blood cholesterol concentration in Experiment 2. The dotted line represents the 95° confidence intervals.

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).

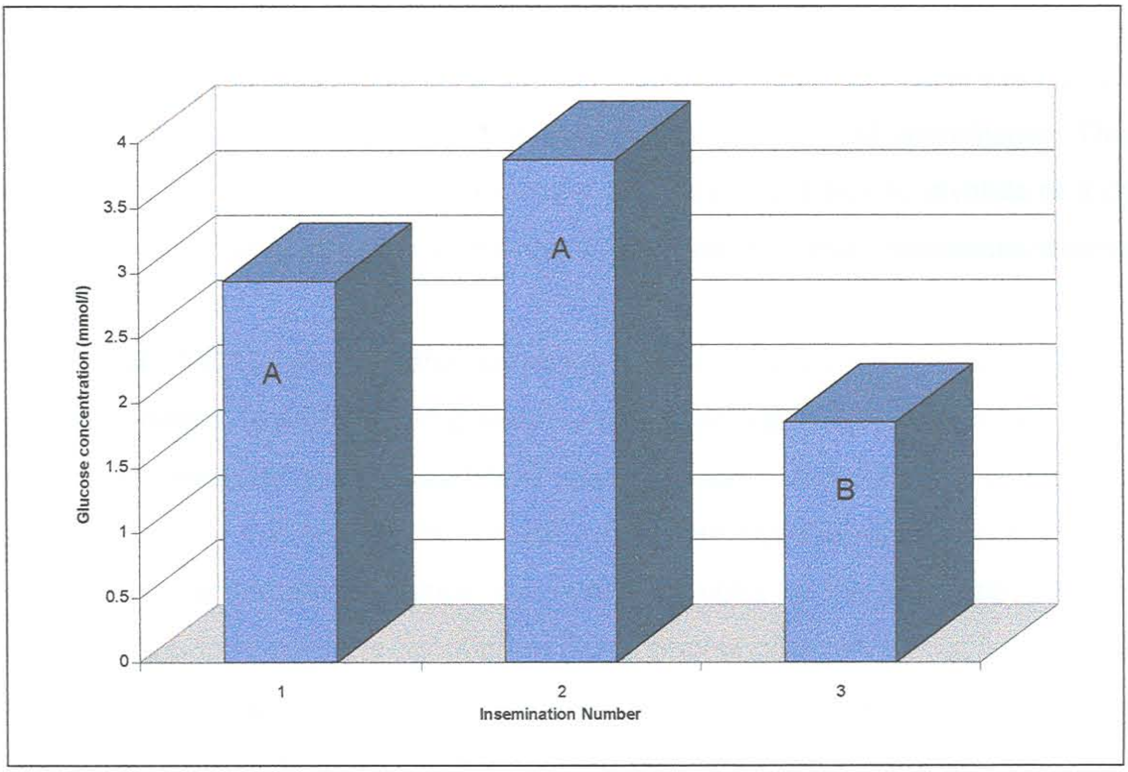

Figure 8. The mean blood glucose concentration of the different insemination numbers. Means with different letters differ significantly ($P < 0.05$).

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

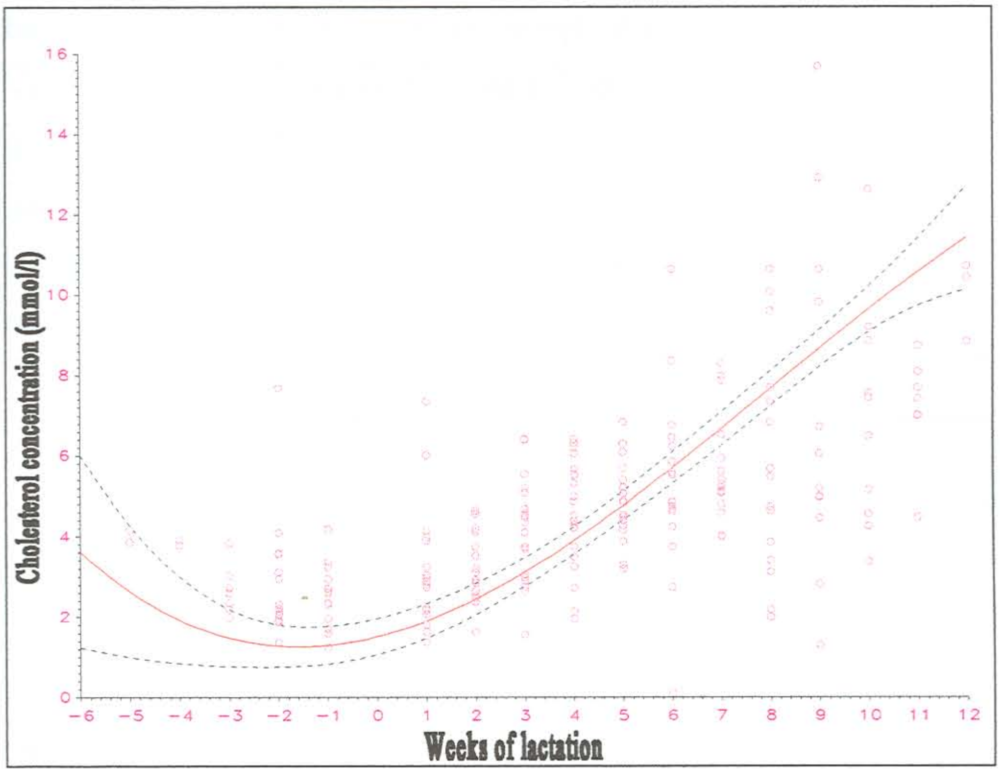

Figure 9. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood cholesterol concentration and weeks of lactation of Experiment 1. The dotted lines represent the 95° confidence intervals.

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

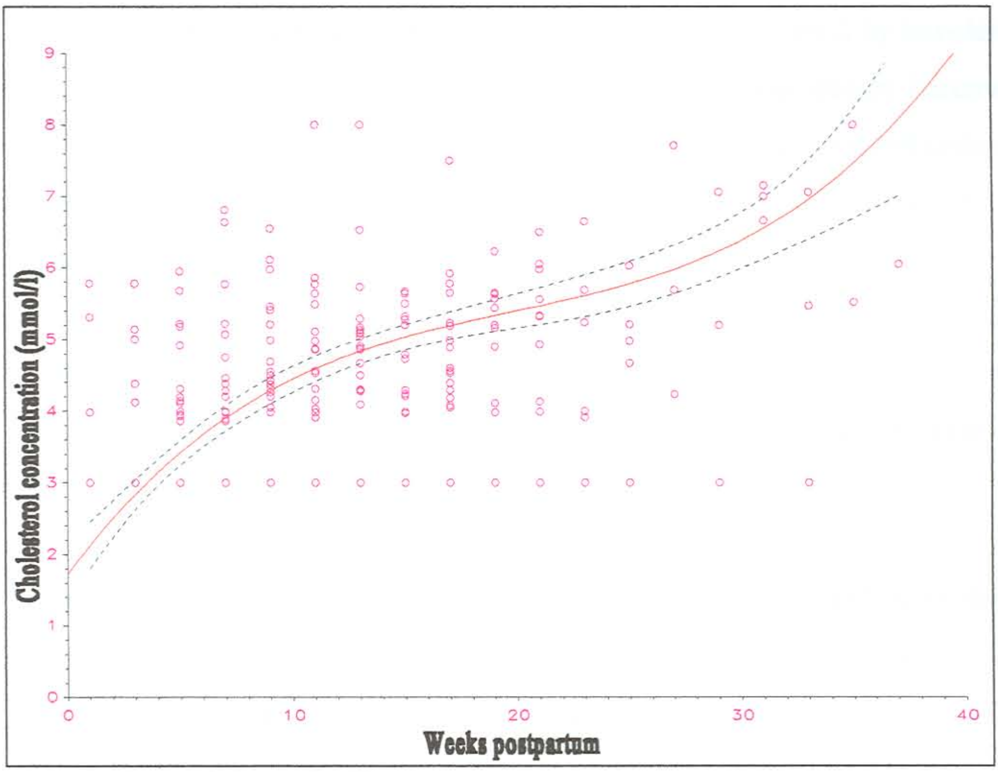

Figure 10. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood cholesterol concentration and weeks of lactation of Experiment 2. The dotted line represents the 95° confidence intervals.

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

$R^2 = 0.093$

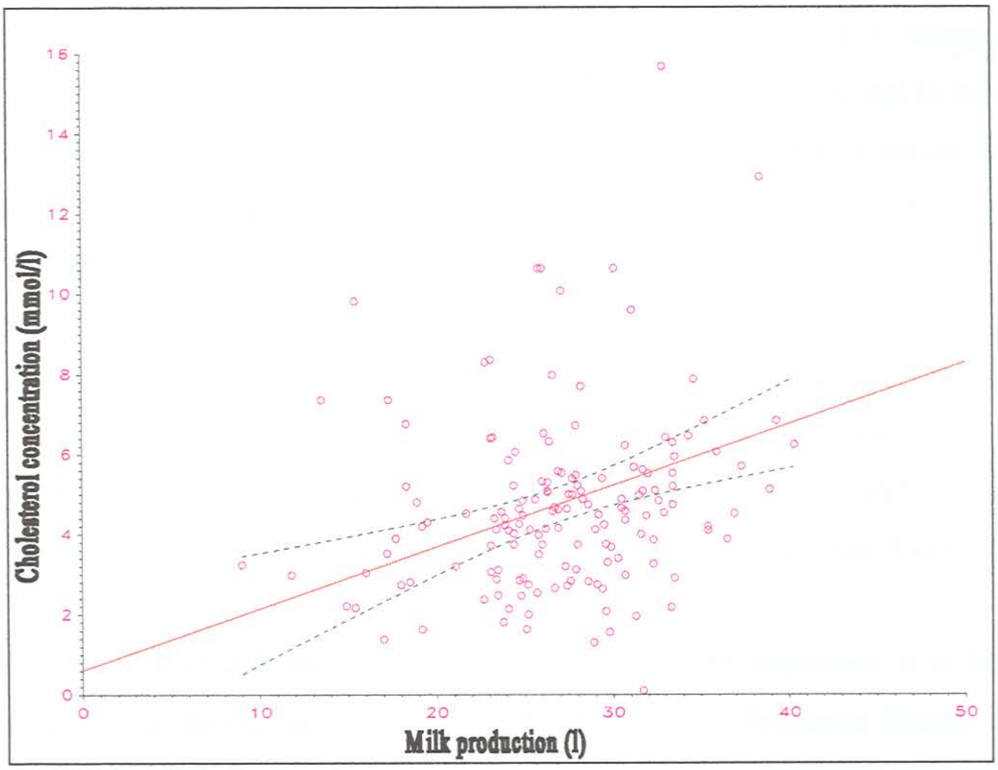

Figure 11. The relationship between the predicted regression (solid line) and the observed data of the relationship between blood cholesterol concentration and milk production of Experiment 1. The dotted lines represent the 95° confidence intervals.

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)

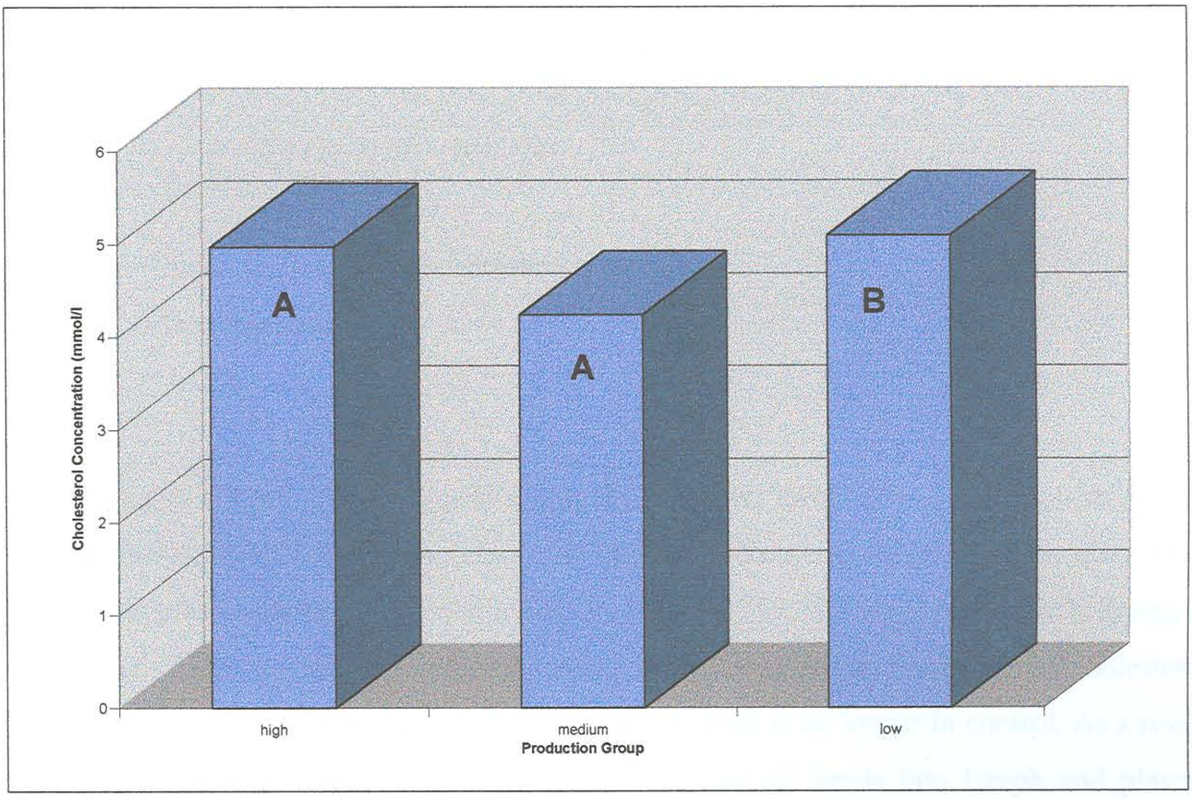

Figure 12. The relationship between blood cholesterol concentration and high, medium and low production groups. Means with different letters differ significantly ($P < 0.05$).

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
$R^2 = 0.53$

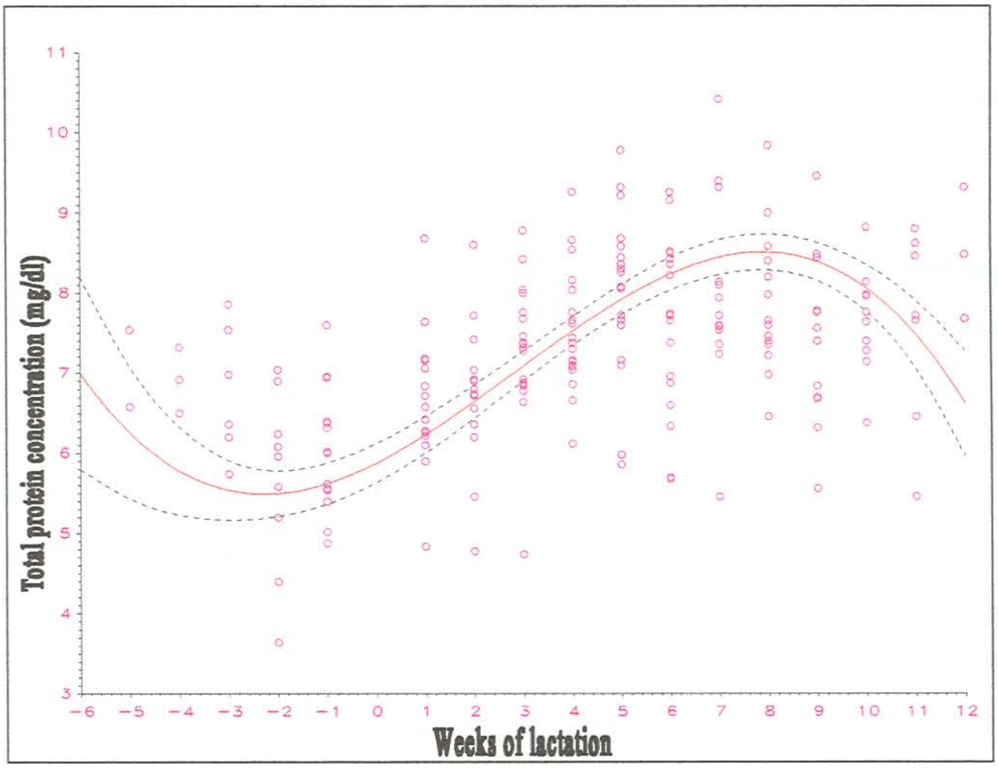

Figure 13. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood total protein concentration and week of lactation of Experiment 1. The dotted line represents the 95° confidence intervals.

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.

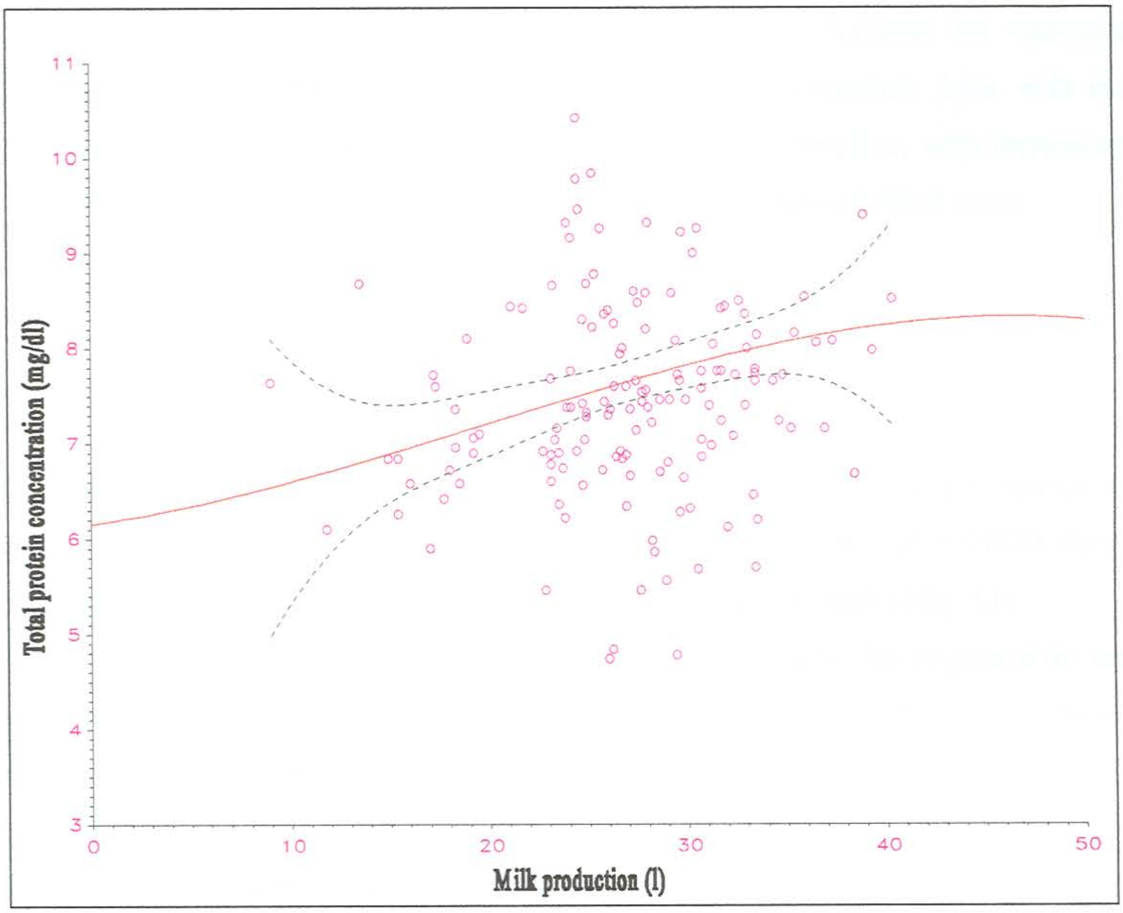

Figure 14. The relationship between the predicted regression (solid line) and the observed data for the relationship between blood total protein concentration and milk production of Experiment 1. The dotted line represents the 95° confidence intervals.

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

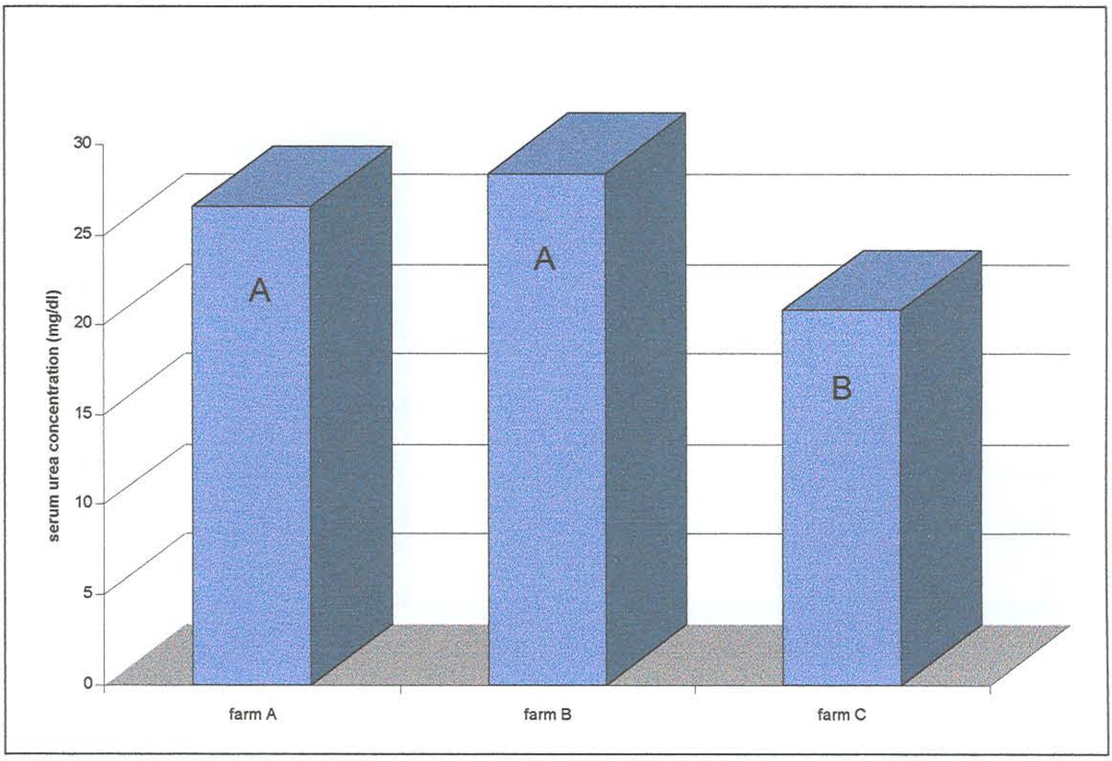

Figure 15. The relationship between blood urea concentration and farms. Means with different letters differ significantly (P < 0.05).

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.

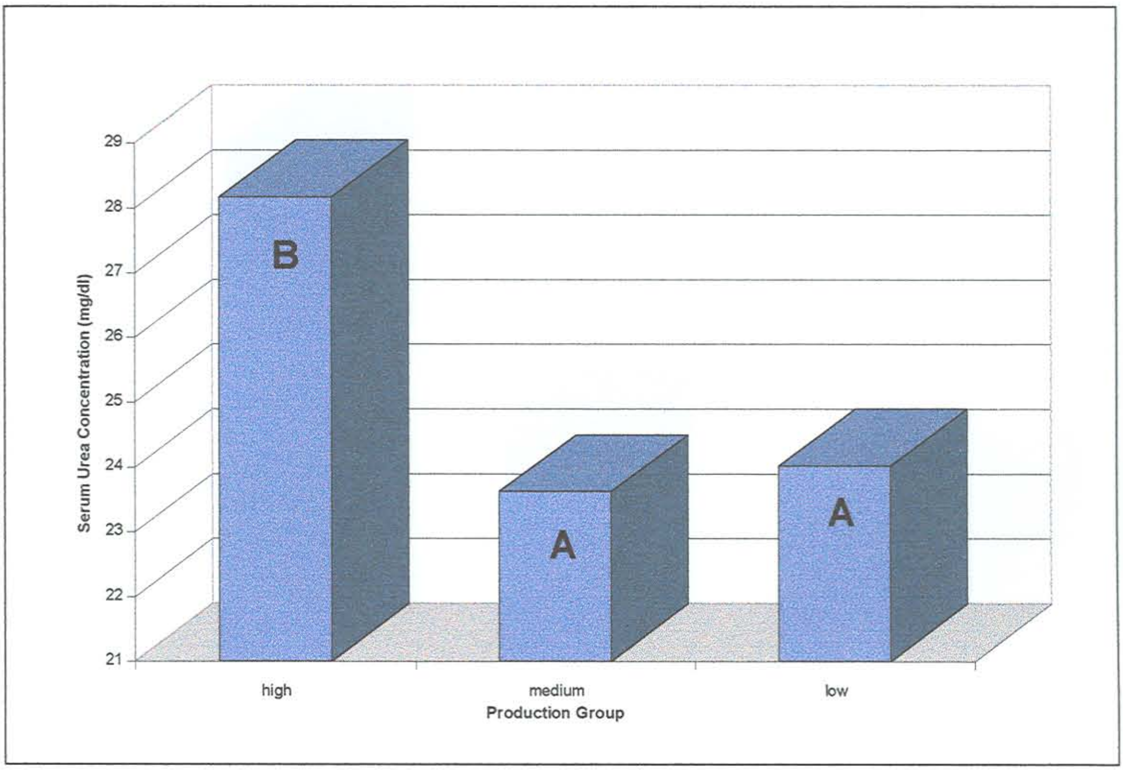

Figure 16. The relationship between blood urea concentration and high, medium and low milk production groups. Means with different letters differ significantly ($P < 0.05$).

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

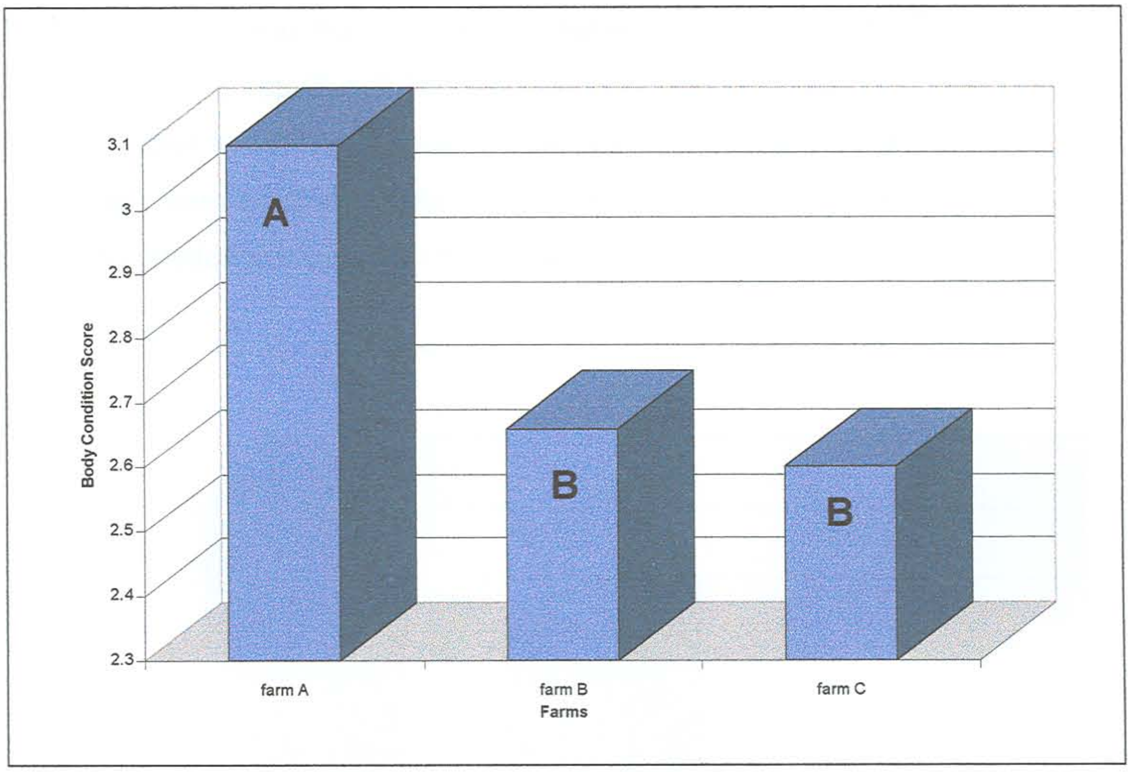

Figure 17. The relationship between body condition score and farms. Means with different letters differ significantly ($P < 0.05$).

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

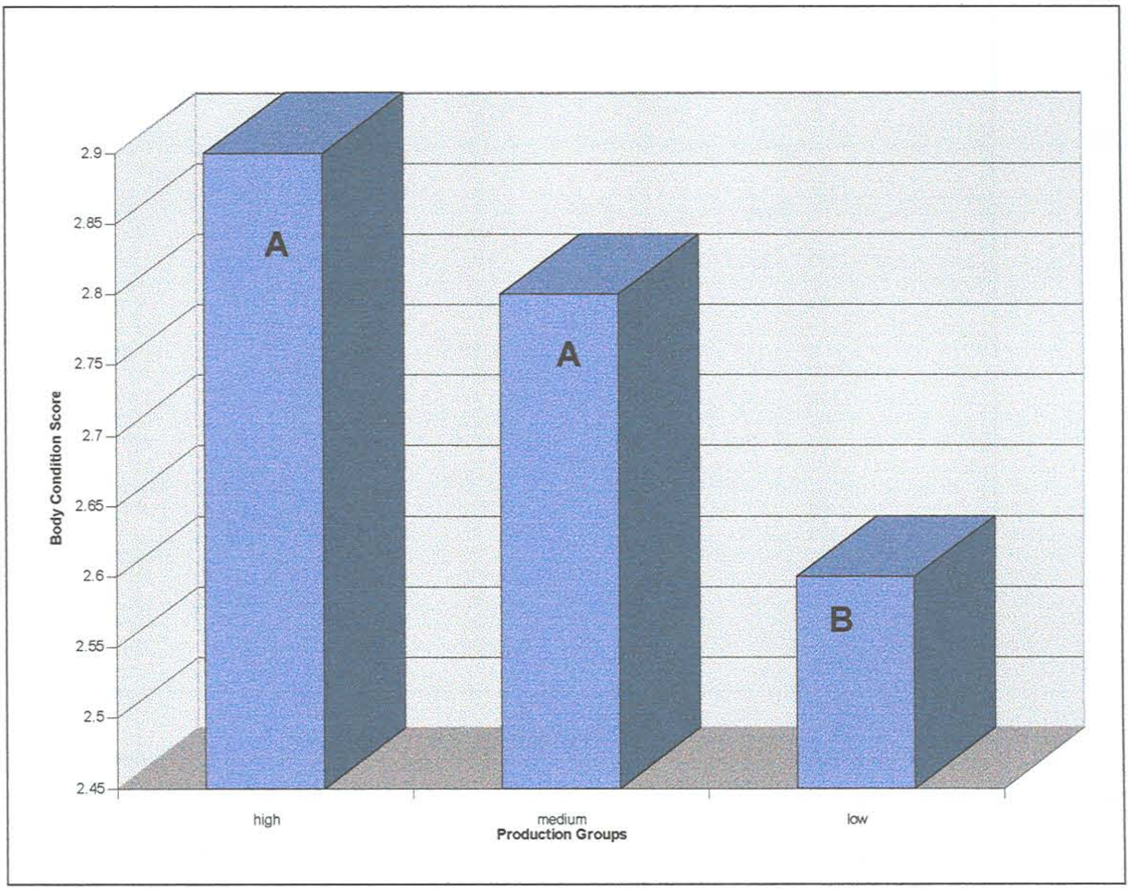

Figure 18. The relationship of body condition score between the high, medium and low milk production groups. Means with different letters differ significantly ($P < 0.09$).

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)

$R^2 = 0.072$

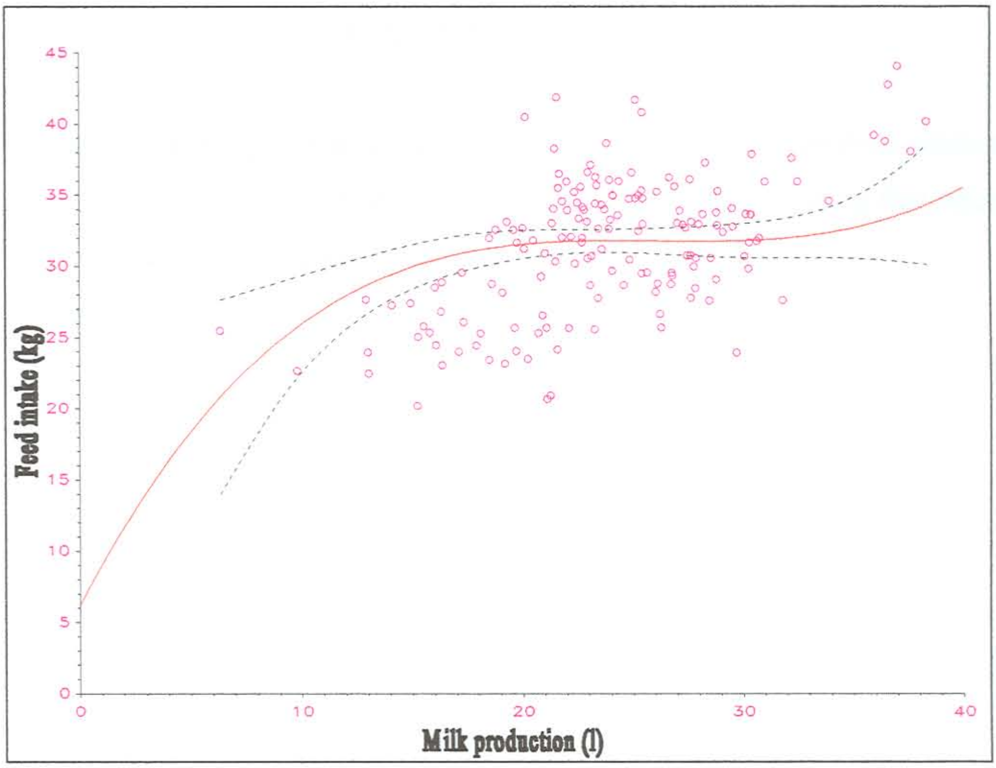

Figure 19. The relationship between the predicted regression (solid line) and the observed data for the relationship between feed intake and milk production in Experiment 2. The dotted lines represent the 95° confidence intervals.

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.