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.